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Full Length Research Paper

Biological studies on albino rats fed with *Sorghum bicolor* starch hydrolyzed with α-amylase from *Rhizopus* sp.

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Partially purified amylase was extracted from the culture medium of *Rhizopus* sp. grown in potato dextrose broth for 48 h at room temperature by precipitation with 96.9% ethanol. The enzyme was used to hydrolyze sorghum starch. The hydrolyzed product was afterwards formulated into rat feed, which was fed to albino rats for a period of thirty days. The average daily body weight of the albino rats fed with hydrolyzed formulated feed on the 30th day of the experiment was 131 g while the values recorded for the groups fed with unhydrolyzed and commercial feed were 120 and 97.4 grams respectively. The hematological analysis revealed that the packed cell volume (PCV), Hemoglobin (Hb), red blood cells (RBC), mean cell hemoglobin concentration (MCHC) of the group fed with hydrolyzed formulated feed of 51.8%, 16.9 g/dl, 8.7 × 10^5 µl^{-1} and 32.7%, respectively, were higher than the experimental animals fed with commercial feed with values of 44.2%, 14.4 g/dl, 7.7 × 10^5 µl^{-1} and 32.3%, respectively. The histopathological results shows that there were no lesion present in both the liver and the kidney of the albino rats fed with commercial feeds while lesions were observed in both the liver of the albino rats fed with hydrolyzed formulated feed and unhydrolyzed formulated feed.

Key words: α-Amylase, *Rhizopus* sp., *Sorghum bicolor* starch, hydrolysis, feed formulation, biological studies, albino rats.

INTRODUCTION

Sorghum bicolor and its other species are grown in the Northern states of Nigeria which is one of the five largest producers of the cereal (FAO, 1995). They are grains, which hold a dominant position among arable crops in Africa and other parts of the world. Sorghum and millets have been important staples in the semi-arid tropics of Asia and Africa for centuries. These crops are still the principal sources of energy, protein, vitamins and minerals for millions of people in these regions (FAO, 1995).

The use of these cereals in the developed world has been primarily restricted to animal feed and little work has been done to evaluate them in industrial food systems. Recent work has suggested that these cereals possess unique characteristics that have both nutritional and functional properties that make them relevant to the development of healthy and nutritious foods (Dahlberg et al., 2004). Sorghum and millets are gluten-free, have unique phenolic compounds, which are being identified as having medicinal properties and contain proteins and starch characteristics that make them to be useful as functional foods to impact health. In Africa and Asia, these cereals have been used in traditional food products, but now their use in industrial settings is being explored.

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Sorghum grains are highly nutritious and starchy and are mostly incorporated into man and animal diets. Starch is the major storage form of carbohydrate in sorghum and millets. It consists of amylopectin, a branched-chain polymer of glucose, and amylose, a straight-chain polymer. The digestibility of the starch, which depends on hydrolysis by pancreatic enzymes, determines the available energy content of cereal grain. Processing of the grain by methods such as steaming, pressure-cooking, flaking, puffing or micronization of the starch increases the digestibility of sorghum starch. This has been attributed to a release of starch granules from the protein matrix rendering them more susceptible to enzymatic digestion (McNeil et al., 1975; Harbers, 1975).

However, to derive maximum dietary advantage starch must be broken into smaller units; this helps in easy digestion and absorption of major nutrients contained in the starch inside the digestive system. This process of breaking down starch is known as hydrolysis. In other to make use of the carbon and energy stored in starch, the human digestive system, with the help of α-amylase breaks down the polymer to smaller assimilable sugars, which is eventually converted to the individual basic glucose units. Hydrolysis is the breaking down of starch into smaller units such as oligosaccharides and disaccharide. Enzymatic breakdown of starch has become more popular than acid hydrolysis. This achieves production of broader range of product and enhances productivity in terms of yield. Examples of enzymes used in enzymatic hydrolysis of starch are fungal amylolytic enzyme which can be extracted from *Rhizopus* sp., *Aspergillus* sp. and *Mucor* sp. This work investigates the effect of hydrolyzed starch of *S. bicolor* on albino rats.

MATERIALS AND METHODS

The *Sorghum bicolor* used for this work was obtained from Wazobia market, Sabo, Ogbomoso, Oyo State, Nigeria.

Cultivation of organism and preparation of cell free extract

The fungus *Rhizopus* sp. used for this work was isolated at Ladoke Akintola University of Technology, Ogbomoso, Oyo-State. The cultivation of the organism and preparation of the cell free extract was as described by Adebiyi et al. (2005).

Mass production of *Rhizopus* sp was carried out using potato dextrose broth. The medium was decanted into 250 ml Erlenmeyer flask covered with cotton wool and sterilized at 121℃ for 15 min in the autoclave. After cooling the flask was inoculated aseptically with spore suspension of *Rhizopus* sp for 48 h this medium was then incubated at room temperature.

At the end of the incubation period, the surface fungal mats were picked from the culture medium using sterilized forceps. The residual waste was filtered and the filtrate was spun at 3,500 × g for 20 min in a centrifuge (Olama et al., 1993). The clear supernatant which was considered as a source of extra cellular enzyme was partially purified by modified method of Kundu and Das (1986). The supernatant was brought to 30% ethanol concentration and centrifuged at 3,500 × g for 20 min (Beckman, Avanti™ J-25 Centrifuge). The supernatant collected was further brought to 70% ethanol concentration and re-centrifuged at 3,500 × g for 20 min. The sediment, that is, the precipitate was taken as the enzyme. For every 150 ml of the solution that was spun in the centrifuge, the pellet recovered was suspended in 5 ml phosphate buffer at pH 6.0.

Preparation of starch and enzymatic hydrolysis of Sorghum bicolor starch

This was carried as described by Adebiyi et al. (2005). Sorghum grains were steeped for 3 - 4 h, grinded and sieved to remove the shaft. The filtrate was allowed to settle and excess water was decanted from it, the starch obtained was dried and kept for further use.

Enzymatic hydrolysis was carried out by suspending 12 g sorghum starch in 100 ml water containing 0.4 g $CaCO_3$ and 0.4 g NaCl. The suspension was then adjusted to pH 6.0 and heated at 72℃ for 2 h in a water bath with continuous stirring. 4 ml of the partially purified enzyme extracted from *Rhizopus* sp. was added when the temperature of the water bath reached 60℃. After 2 h the hydrolyzed product was brought out of the water bath and allowed to cool and sun dried for 2 to 3 days (Linko et al., 1975).

The experimental animal

The feeding experiment was carried out with twenty seven male albino rats which were purchased from the animal house of the University of Ibadan, Oyo-State, Nigeria. The experimental animals were weighed and randomly divided into three groups with nine rats placed in each cage. The albino rats were fed for 30 days. For each diet group, 20 g of feed was given to each albino rats according to their daily allocated ratio. Cool fresh water was supplied regularly and the cages were also cleaned on a regular basis. Body weight changes were monitored daily throughout the duration of the experiment.

Experimental diets

The various treatments used for feeding the rats were;

1. Diet 1 comprises of the hydrolyzed sorghum starch (65.5%) incorporated with some other nutritive supplements of the following composition (%): fish meal 21.0, cellulose 5.0, bone meal 2.5, oyster shell 0.5, premix 0.25, salt 0.25 and palm oil 5.0.
2. Diet 2 comprises of the unhydrolyzed sorghum starch (65.5%) incorporated with some other nutritive supplements of the following composition (%): fish meal 21.0, cellulose 5.0, bone meal 2.5, oyster shell 0.5, premix 0.25, salt 0.25 and palm oil 5.0.
3. Diet 3 is the commercial feed from Ladokun Feeds which was used as the control diet.

Feeding formulation experiments

The three experimental feeding groups were fed with their respective diets and weighed daily for 30 days. At the end of the feeding experiment, blood was collected from rats in each treatment group by cardiac puncture under diethyl ether anesthesia for hematological studies. Group pooled blood was collected into ethylene diamine tetracetic acid (EDTA) tubes for hematological studies.

Packed cell volume (PCV), Hemoglobin (Hb), red blood cells (RBC), total white blood cell (TWBC), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC) and platelets counts were determined as described by Jain (1986).

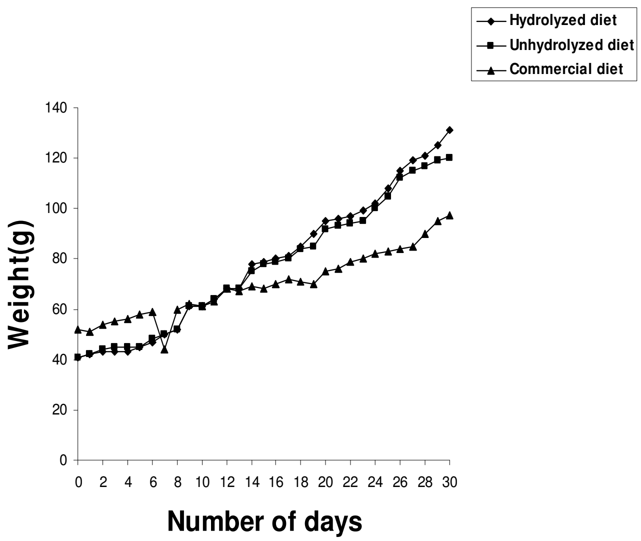

Figure 1. Effects of hydrolyzed, commercial and unhydrolyzed diets on the weight of the albino rats.

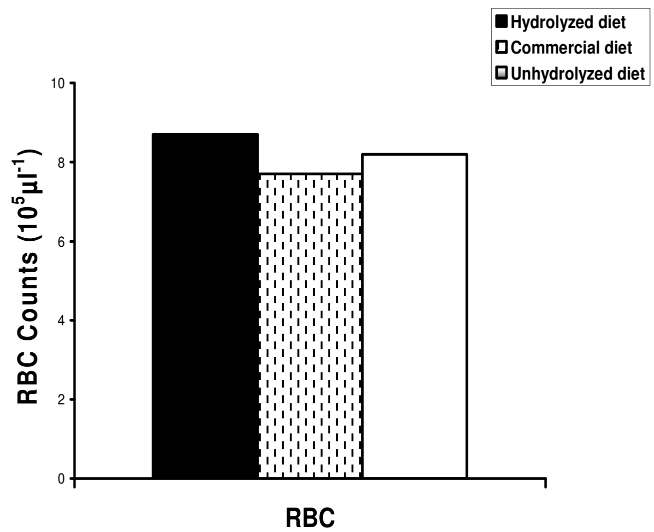

Figure 3. Effects of hydrolyzed, commercial and unhydrolyzed diets on the red blood cells (RBC) of the albino rats.

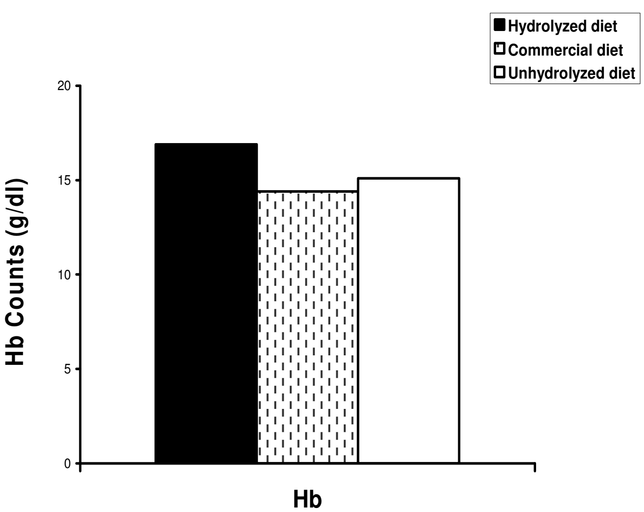

Figure 2. Effects of hydrolyzed, commercial and unhydrolyzed diets on the hemoglobin (Hb) of the albino rats.

The livers and kidneys were harvested for histopathological analysis. The preparation of tissue section was fixed in 10% formaline solution and conventional haematoxyline and eosin (H and E). Staining of the sections was done as described by Baker and Silverton (1980). Photomicrographs of the H and E stained sections on glass slides were taken using an Olympus photomicroscope (Inha, Japan).

RESULTS

The average daily body weight changes for each of the three feeding groups is as shown in Figure 1. The weight of the Diet 1 and Diet 2 groups fed with hydrolyzed and unhydrolyzed feeds increased progressively during the 30 day feeding experiment each having a final value that is greater than that of Diet 3 fed group. Likewise, the weight of the group fed with the commercial feed increased progressively for the first six days having an average daily body weight of 59 g on the 6th day and on the 7th day the group's average weight decreased to 44 g. From the eight day, a progressive increase was recorded however there was a slight decrease in the value on the 19th day. Finally, the Diet 3 fed group has an average body weight of 97.4 g on the 30th day of termination of the experiment.

The hematological results obtained when the experimental animals were fed with the three diets are shown in Figures 2 - 8. In Figure 2, the albino rats fed with the hydrolyzed sorghum feed (Diet 1) have the highest value of hemoglobin count of 16.9 g/dl while the group fed with the commercial feed (Diet 3) has the lowest value of 14.4 g/dl. Also in Figure 3 the red blood cell (RBC) count of the group fed with the hydrolyzed sorghum feed has the highest value of $8.7 \times 10^5 \mu l^{-1}$ while the albino rats fed with commercial feed has the lowest RBC count of $7.7 \times 10^5 \mu l^{-1}$.

The PCV of the three experimental groups as shown in Figure 4 shows that the group of albino rats fed with hydrolyzed feed has the highest packed cell volume of 51.8% while the commercial diet fed group has the lowest value of 44.2%. In Figure 5 the MCV values of the rats fed with unhydrolyzed sorghum feed has the lowest value of 56.8 μ^3 while those fed with hydrolyzed feed has the highest mean cell volume of 59 μ^3. In Figure 6 as well, the mean cell hemoglobin count of the group fed with the control diet has the lowest value of 32.3%.

Furthermore, Figure 7 shows the histogram for the white blood cell count for the three feeding groups. The experimental animals fed with unhydrolyzed sorghum

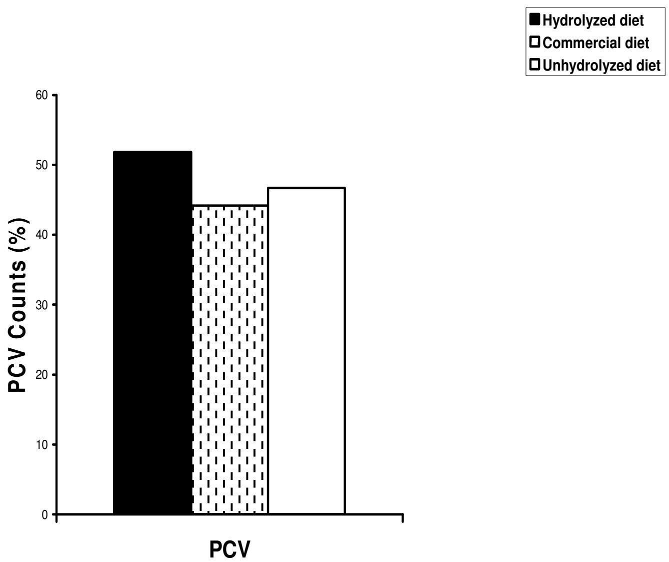

Figure 4. Effects of hydrolyzed, commercial and unhydrolyzed diets on the packed cells volume (PCV) of the albino rats.

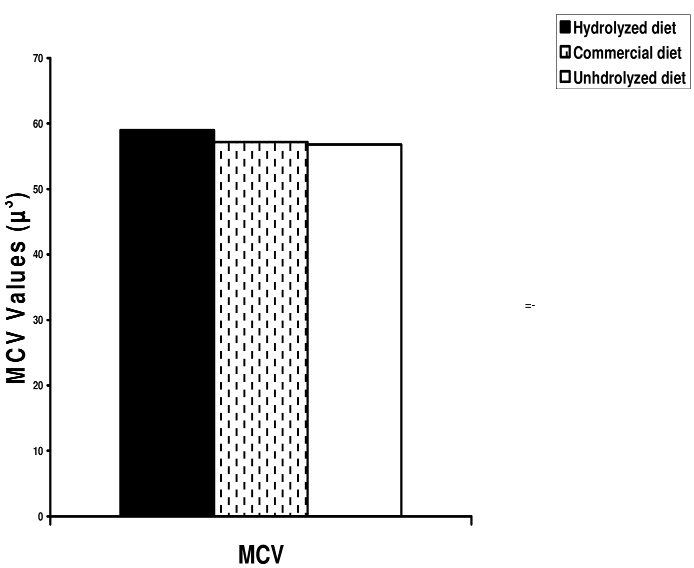

Figure 5. Effects of hydrolyzed, commercial and unhydrolyzed diets on the mean cell volume (MCV) of the albino rats.

starch diet has the highest value of 8167 mm³ while the group fed with hydrolyzed sorghum diet has the lowest value of 7100 mm³. Also the group fed with diet 1 has the lowest platelets count of 184167 while those fed with the commercial feed has the highest platelets count of 292333 (Figure 8).

The histological results obtained for the kidney and liver of the three experimental feeding groups is as shown in Figures 9, 10, 11, 12, 13 and 14. It was observed that the liver and the kidney of the albino rats fed with the commercial diet have no conspicuous lesions (Figures 9

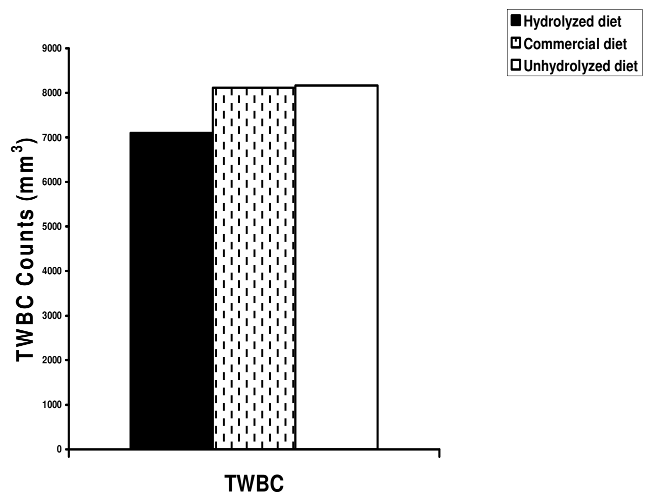

Figure 7. Effects of hydrolyzed, commercial and unhydrolyzed diets on the total white blood cell count (TWBC) of the albino rats.

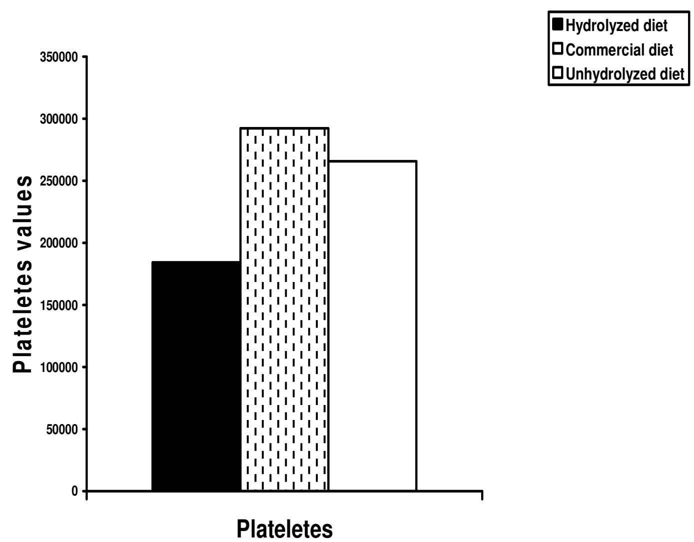

Figure 8. Effects of hydrolyzed, commercial and unhydrolyzed diets on the blood platelets of the albino rats.

and 10). However, focal area of degeneration and necrosis of the tabular epithelial was seen in some of the kidneys of the experimental animals that were fed with hydrolyzed and unhydrolyzed feed (Figures 11 and 12). It was also observed that the livers of the experimental animals fed with Diet 1 and Diet 2 have some lesions (Figures 13 and 14).

DISCUSSION

In this work, it was observed that there was a progressive increase in the weight of the experimental animals that were fed with the feed formulated with hydrolyzed sorghum compared with the animals fed with the commercial

Figure 9. Liver of albino rat fed with commercial diet. No conspicuous lesion seen.

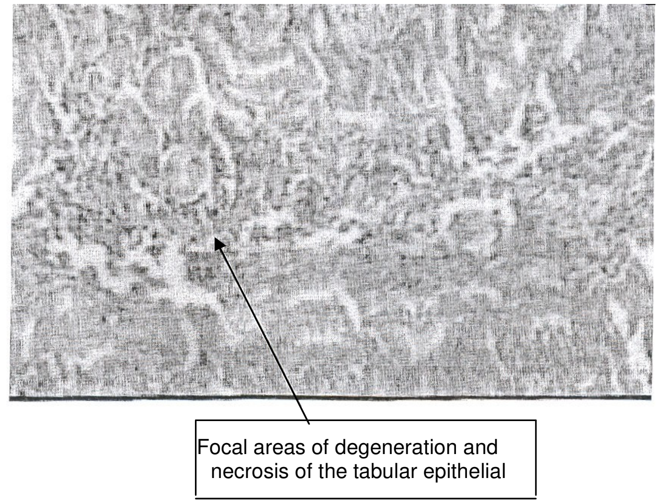

Figure 12. Kidney of albino rat fed with unhydrolyzed diet.

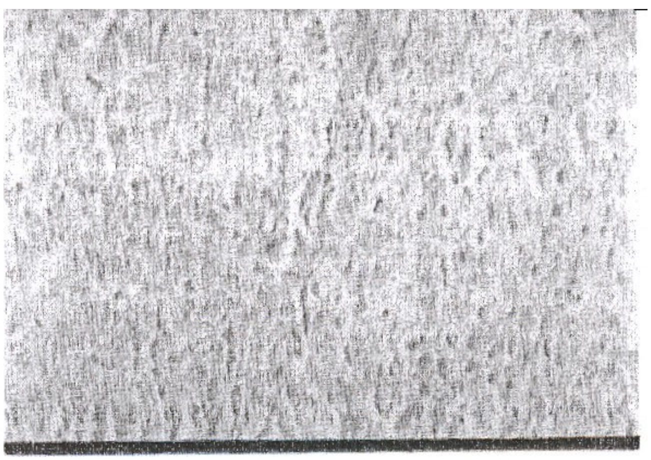

Figure 10. Kidney of albino rats fed with commercial diet. No conspicuous lesion seen.

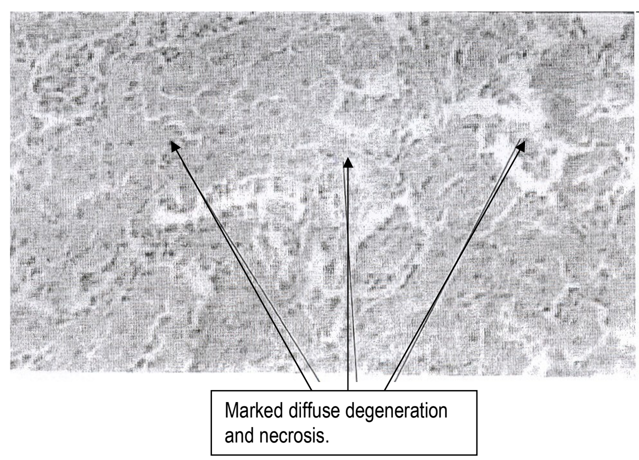

Figure 13. Liver of albino rat fed with hydrolyzed sorghum starch diet.

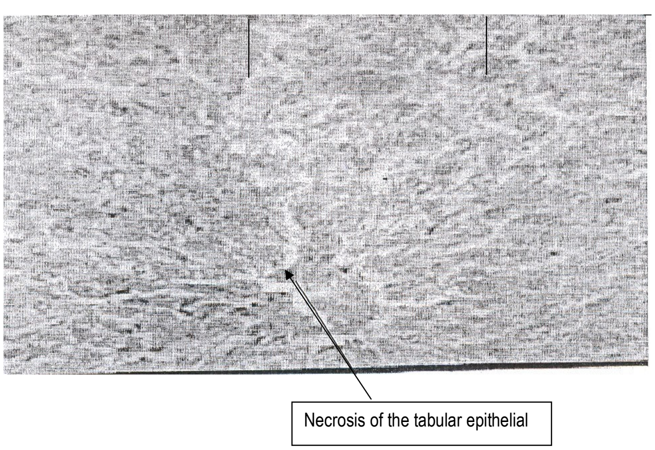

Figure 11. Kidney of albino rat fed with hydrolyzed sorghum starch diet.

diet whose weight reduced at a time during the feeding experiment. This increase in weight may be due to the ease of absorption of the important nutrients present in the diet. Hydrolysis of starch produces disaccharides and oligosaccharides. Oligosaccharides are easily absorbed than mere starch and quickly digested. They are also now being incorporated into commercially prepared foods and medicines due to their advantage of being low in calories, non-carcinogenic, bifidogenic and being able to prevent constipation (Barreteau et al., 2006). Therefore, the hydrolyzed sorghum may be useful in the preparation of baby foods and healthy foods for the diabetics. Oligosaccharides are useful alternatives to sugar especially for people with diabetes such foods are known to be low in calories (Alexandra, 1992). In addition, the hydrolyzed product may be of benefit in the confectionery industry.

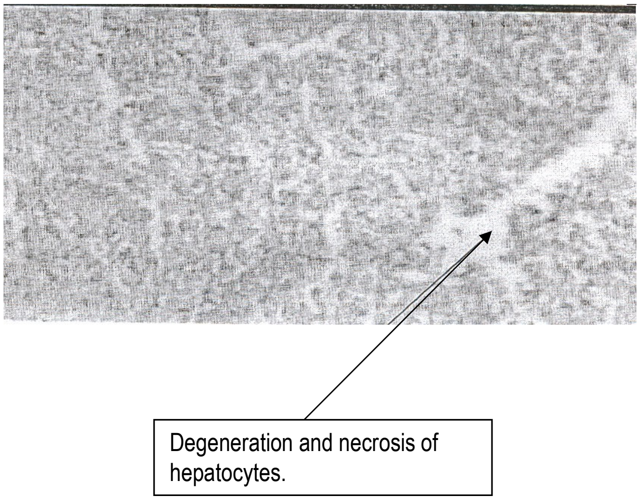

Figure 14. Liver of albino rat fed with unhydrolyzed formulated feeds.

Furthermore, hematological result revealed that the albino rats that were fed with the hydrolyzed feed have the highest packed cell volume, MCV, hemoglobin (Hb) count and red blood cells (RBC). This may be due to the fact that the feed has some nutrients like minerals and vitamins that help in the formation and maintenance of the blood. This suggests that the hydrolyzed diet actually improved the RBC and Hb which is the oxygen-carrying compound present in the red blood cells. The total Hb concentration depends primarily on the number of red cells in the blood sample. PCV and Hb are used for monitoring quantitative changes in red blood cells (Adebiyi and Oloke, 2002). Also, the MCV values of the rats fed with the hydrolyzed diet is higher than that of the commercial diet. A decreased MCV value suggests a microcytic red cell which is common with iron-deficiency anaemia (Odutola, 1992). This indicates that the hydrolyzed sorghum diet performed better than the commercial diet. Ohta et al. (1995) discovered that fructose oligosaccharide feeding increased the hematocrit (PCV) ratio. The concentration of hemoglobin and the apparent absorption of Fe were also increased by fructose oligosaccharide - feeding. This seems to be a similar experience with the increase in the PCV and Hb concentration of the group fed with hydrolyzed diet. Previous work by Adebiyi et al. (2005) has shown that hydrolyzed sorghum starch consists of oligosaccharides, vitamins, essential amino acids, minerals and free of toxins. Hence, it may be useful in production of food and feed.

Furthermore, according to Schalm (1970) blood is ever changing with cells being continuously added and removed in healthy state; however, a remarkable balance of normal range is established. Nutritional deficiency, organic breakdown or penetrations of foreign materials do result in the alteration of blood composition. Therefore, hematological values and blood chemistry could be useful indices in appraising nutritional status of an animal. In this work, the hematological parameters are within the normal range for rats (CCAC, 1980).

The white blood cells and platelets count results showed that the group fed with the hydrolyzed feed had a good amount present. The white blood cells in the body are a major component of the host defense system. They fight against diseases present in the body system. Hydrolyzed feed formulae are now recommended by most nutritional committee in Europe for partially breast fed infants with high risk of allergies (Host et al., 1999). Functional foods is now generating a global market of 33 billion US dollars, therefore food rich in oligosaccharides could play a major role as functional food ingredients (Barreteau et al., 2006).

In conclusion, the hydrolyzed feed has been shown to improve the experimental animals' blood formation and increase their weight. Thus the hydrolyzed sorghum starch may be of health benefits in malnourished children and those who have health challenges of this kind. Further work could be carried out on the histopathological aspect to know the cause of some lesions which were seen in the liver and the kidney of the experimental animals since the hydrolyzed product may be of tremendous health benefits.

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