EFFECTS OF RED BANANA MEAL SUPPLEMENTED WITH VITAMIN ON RATS BIOCHEMICAL INDICES.

[1] Iniubong Abasiodiong Thomas, [2] Nkoyo Isong, [3]M.I. Akpanabiatu, [4]Pat E. Mbah
[1&2] Department of Home Economics, University of Uyo
[3] Department of Biochemistry, University of Uyo
Iniubongabasi02@gmail.com

Abstract
This study aimed at comparing the effects of peeled and unpeeled red banana meal with vitamin C, B_{12} and B complex supplementation on some biochemical indices of different groups of Wistar rats. Forty eight (48) weanling albino Wistar rats weighing between 41 – 57 g were used. They were randomly selected into six (6) groups consisting of eight (8) rats. Coded as Unpeeled Red Banana with Vitamin (URWV), Unpeeled Red Banana without Vitamin (URWO), Peeled Red Banana with Vitamin (PRWV), Peeled Red Banana without Vitamin (PRWV), nutribom and normal rat pellet. They experimental animals were acclimatized for 3days and animals were given food and water for 21days the left overs were recorded daily. At the end of the 21days feeding, animals were anesthetized using chloroform vapour, blood sample taken for biochemical analyses serum AST, ALT, ALP, lipid profile were assayed .The results of the study showed that serum ALT of rat fed with unpeeled red banana with vitamin (URWV) was 26.88 ± 2.65µl/L while rat fed with nutribom (control) was 26.75 ± 1.60 µl/L. The ALP of rats fed with URWV was 239.43 ± 35.25 µl/L which increased significantly ($P < 0.05$) when compared with control (184.11 ± 16.68 µl\L/). Total cholesterol increased significantly in URWV 3.87 ± 0.13 µl/L ($p < 0.05$) when compared with control 1.86 ± 0.17 µl/L, TG & HDL level were not significantly higher than the control in URWV, PRWV and PRWO. Total protein, albumin and globulin in all the groups were not significantly different ($p < 0.05$) when compared with nutribom the control.

Key word – Red Banana, Vitamin, Supplementation, Serum Enzymes, Lipid Profile, Wistar Albino Rats.

Introduction

Banana is the common name for herbaceous plants of the genus Musa and for the fruit they produce banana has become an important economic plant. Banana is one of the oldest cultivated plants. All parts of the banana plant have some medical applications (Sampath Kumar, Butcher, , Katrina, Engelhardt, Heikkonene, Kaski,, Ala-Korpel, and Kovanen, 2012). Red banana is among the local food commodity found in Akwa Ibom State, other parts of Nigeria and in many other countries, though not accepted by many people and given names such as "mboro nkuriku", "adia okpon ekporo" "mboro idat" (mad person fruit) among others but are eaten among the peasant groups. These facts leave us at the mercies of the refined foods which lack adequate nutrient (Udoh, 2019). However, banana is a seasonal fruit and highly perishable due to their high moisture contents.

Fruits and vegetables are an important component of a healthy diet and fruit like banana offer great medicinal benefits (Kashyap, Dodke, Moon, Umak, Kalode, and Jaunjare,2018). Banana aid in the body's retention of calcium, nitrogen and phosphorus all of which work to build healthy and regenerated tissues, Kashyap et al, (2018).

Bananas can be used to fight intestinal disorders like ulcers. It is one of the few fruits ulcer patients can safely consume. Bananas neutralize the acidity of gastric juices, thereby reducing ulcer irritation by cooling the lining of the stomach and promote healing (Kashyap et al, 2018). The study on foods and nutrition is very essential for living. Sampath et al, (2012) also reported that fully ripe banana peel and pulp have antifungal and antibiotic properties. The antibiotics act against mycobacteria and a fungicide in the peel and pulp of green fruits is active against a fungus disease of tomato plants. Norepinephrine, dopamine, and serotonin are also present in the ripe peel and pulp.

Several tropical plants foods have been reported to have hepatoprotective properties (Iweala & Osundiya ,2010). However there are differences in nutritional composition between varieties and hybrids (Amankwah Ayim, Dzisi, Barimiah (2011).

In a study by Jiwan, Sidhu and Tasleem (2018) observed that bananas are an excellent source of potassium. Potassium is found in a variety of fruits, vegetables and even meats, and a single banana can provide 23 percent of potassium. Potassium helps the muscles as it maintains their proper functioning and prevents muscle spasms. It decreases blood pressure and reduces the risk of stroke.

Kraft (2011) observes their actual potassium content is not high per typical food serving; having only 8 percent of the US recommended Daily Value (DV) for potassium (considered a low level of DV).

Nutrition can also be defined as food at work in the body, the study of science of nutrition deals with what nutrients we need, how much we need, why we need these and where we can get them (Mbah, Orhewere, and Osifeso, (2019).. There is a growing interest in foods rich in bioactive compounds that are used as preventive treatment of disease due to the increase in chronic disease as a consequence of the population eating habits (Urrutia, Ramos, Menegusso, Lenz, Ramos, Tarone, cazarin, cottica, Da silva, Bernardi (2021). Hence nutritionist should be concerned about the populations eating habit by introducing locally available nutritious foods.

Vitamin supplementation is known to impart significant benefits in terms of disease prevention and treatments and has been widely accepted as a measure of control of micro-nutrient deficiencies Eka Idem, Akpanabiatu, Uboh, & Eka, (2007). The addition of vitamins to food upgrade the food value and prevent malnutrition and other non-communicable diseases.

The use of experimental animal model is one of the best approaches for the understanding of pathophysiology of any disease in order to design and develop drugs for the treatment of such diseases (Kruger *et al*, 2012). Sampath et al, (2012) reported that animal studies have shown that banana has the potential to lower cholesterol. It was suggested that the dietary fiber component in banana pulp was responsible for its cholesterol –lowing effect. High level of trigygceride, LDL- cholesterol and VLDL-cholesterol have been associated with heart disease (Iweala et al., 2011). However, consumption of plantain has been shown to reduce triglyceride (Kaimal et al., 2010). Low level serum protein may be seen in severe malnutrition and with conditions that cause malabsorption; such as celioc disease (Roth 2011). Red banana caused significant reduction in the level of total cholesterol, HDL-Cho and VLDL. The American Heart Association recommends an optimal triglyceride level of 100 mg/dl (1.1 mmol/l) or lower to improve heart health (Gill *et al,* 2011). The reference range for total protein is 50 – 85 gl or 5.0 – 8.5 g/dl (Wills and Mehta, 2020). Normal ALP is 44 - 147µl/L elevated levels occur in liver or bone disease such as hepatitis liver damage and liver cancer, bone cancer, healing fractures, oesteomalacia, paget's disease and rickets (Weinstein, 2016). However, elevated levels of ALT do not automatically means that medical problems exist.

Fluctuation of ALT levels is normal over the course of the day and they can also increase in response to strenuous physical exercise (Paul and Giboney, 2013).

Jahoor, Badaloo, Reids and Forrester, (2013) reported that any concentration below the reference range usually reflects low albumin concentration, a condition which can render the body susceptible to liver disease, acute infections and some other health complications. Also any concentration above the reference range may be found in cases such as protein anemia, leukemia.

The objective of the study is to determine and compare the biochemical indies of wistar albino rat fed with red banana meal peeled & unpeeled.

Justification of the Study

Adequate nutrition during infancy and childhood is fundamental to child development, and this requires a good complementary feed which red banana offers. Complementary foods are foods other than breast milk. Red banana is rich in energy, protein, carbohydrate and micronutrient. It is locally available and affordable, easy to prepare by mothers and care givers. This work is justified because red banana may address the needs of people with different aliment and has the characteristics of a good complementary food.

Collection of Samples

The fresh red banana (*Musa acuminata*), crayfish (Cambarus sp), nutribom, iodized salt, vegetable oil (grand), pumpkin leave, mature red banana bunches were purchased from Akpan Andem market in Uyo Local Government Area, Akwa Ibom State, Nigeria. While vitamin B12, Vitamin B Complex and Vitamin C tablets from Emzor company were

purchased from Peke B, Pharmacy Abak Road Uyo, Akwa Ibom State. Other kitchen utensils were camry kitchen weighing balance, hot air oven (Galenkamp BS oven 250, model No. 320) kitchen knife, sieve 5.3 mesh, plates and basin were from food laboratory, Home Economics Department of University of Uyo. The animals were collected from the animal house of Biochemistry in the University of Uyo.

Materials and Methods

Experimental design

The animals were randomized into six groups of eight animals, which were fed with the following diet. Treatment 1 peeled red banana with vitamin (PRWV) unpeeled red banana without vitamins (URWO), peeled red without vitamin (PRWO) unpeeled red banana with vitamins (URWV). Commercial baby food (nutribom) as positive control and normal rat pellet. Daily food consumption weighted and recorded. Lipid profile, total protein, LDL-Chol, HDL-Chol, Triacylglycerol, very low-density lipoprotein Albumin, total protein and globulin. AST, ALP, ALT were analyzed using Randox kit method.

The flow chart for red banana flour production

Matured red banana fruit

↓

Wash

↓

Peeled

↓

Steep in 0.05 % sodium chloride solution (5minute)

↓

Slice 1 mm thickness

↓

Dry in an oven 60 ^{o}C, 3 hours

↓

Milling

↓

Sieve through a (500 mm)

↓

Packaging in an air tight container

↓

Stored at 37 ^{o}C (room temperature)

Figure 1: Flow chart showing the processing of red banana flour production

Composition of feed

Group A, peeled red banana meal fortified with vitamins (PRWV)
Group B, unpeeled red banana meal also fortified with the vitamins (URWV)
Group C, unpeeled red banana meal without vitamins (URWO)
Group D, peeled red banana meal without vitamins (PRWO)
Group E , Normal rat pellets
Group F, Nutribom.

Table 1: Composition of Feed

Group	Flour	Crayfish	Grand oil	Iodize Salt	Pumpkin ml juice	Vit. C	Vit. B_{12}	Vit. B Complex	water
A	900 g	100 g	100 g	3 g	500	200 mg	2 50mcg	2 tablets	
B	900 g	100 g	100 g	3g	500	200 mg	2 50mcg	2 tablets	
C	900 g	100 g	100 g	3g	500				
D	900 g	100 g	100 g	3g	500	-	-	-	
E	Pellet	Pellet	-		-	-	-	-	
F	Nutribom	-	-		-	-	-	-	20ml

Vitamin (C, B_{12} and B-complex)

Experimental Animals and Feeding Protocol

Forty eight (48) weanling albino Wistar rats weighing 41 – 57 g were used in this study. They were randomly selected into six (6) groups consisting of eight (8) rats each. The rats were housed in a wooden cage with stainless steel tops at room temperature. The cages were identified as: PRWV – Peeled red banana with vitamins, PRWO – peeled red banana without vitamins, URWV – unpeeled red banana with vitamins, URWO - Unpeeled red banana without vitamins, Nurtibom, rat pellet. The control group was fed with normal rat pellet. Feeding with the diets and table water were adlibitum for 21 days. The weight of feed given to the animals in each group and the left overs were collected and recorded daily. The animals were acclimatized for 3 days. The respective grammes of feeds were given. This enabled the determination of total amount of food consumed by the experimental animals. Weekly body weights were measured using a chemical balance (Amry model). The animals were maintained under standard laboratory conditions. All animal experiments was carried out in line with the guidelines of Institutional Animal Ethical Committee as approved by the Graduate School, University of Uyo.

Collection of Blood Samples and Analysis

At the end of the feeding experiment, the rats were anesthetized using chloroform vapour, to have easy access to the heart, and blood sample were collected by cardiac puncture with 5 ml syringe. Blood sample were emptied into labeled plain tubes and EDTA bottles for serum preparation and hematological analysis respectively. Serum samples were prepared by centrifugation at 2000 rpm for 5 minutes and sera separated into labeled plain tube and frozen until needed for analysis. Triacylglycerol, total cholesterol, HDL-L, albumin, total protein, alkaline phosphatase, aspartate amino transferase and alanine aminotransferase were assayed spectrophotometrically using reagents from Randox laboratories, UK.

Statistical Analysis

The results were analyzed by one-way ANOVA for variation within and between groups. $P< 0.05$ shows significant differences between control and experimental groups. Duncan's multiple range test was used to separate the means (Duncan 1955). All values are expressed as mean ± SEM.

Result

The result of lipid profile of the experimental animals (Table 2) shows that animals in group 4 and 1 had significantly ($p <0.05$) lower cholesterol than the other groups. The values of HDL did not change significantly ($P<0.05$) than control. The TG in group 4 was significantly ($p<0.05$) lower than control. Group 3 had significantly ($p<0.05$) higher than control. The VCDL values of group 5 and 6 did not change significantly ($p< 0.05$) while group 2 and 3 were higher significantly than group 4 and control.

The serum total protein, albumin, and globulin result (Table 3) showed no significant different ($p<0.05$) in all the groups than control. The level of total protein was higher in group 1,3, and 5 when compared with the control while globulin had significantly ($p<0.05$) higher level in group 5 than control.

The liver enzymes result (Table 4) shows that group 2 and 6 had significantly ($p<0.05$) lower ALT than the control. The value of ALT in group 2 and 6 was lower than control. AST values of 3,5,6 were lower ($p<0.05$) than 2,4 and control.

Figure 2: Variations in Biochemical indices of wistar albino Rat in response to feed Formula

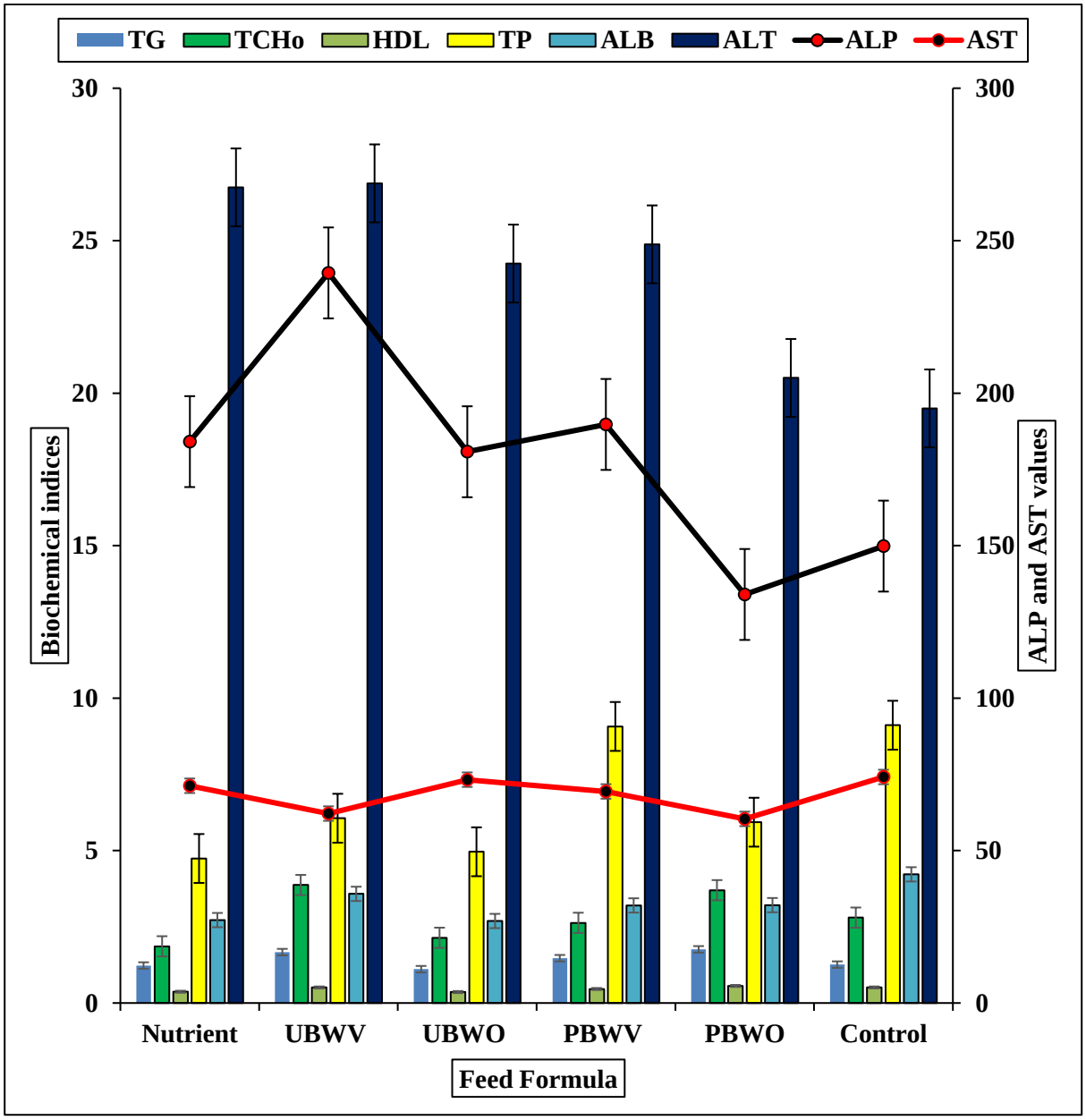

Table 2: Results of the effect of red banana meal (*Musa acuminata*) on lipid profile of albino Wistar rats

Group	Meal / Formular	Cholesterol (mmol/L)	Triacylglycerol (mmoI/L)	HDL-HDL-Chol (mmoI/L)	LDL-C (mmoI/L)	VLDL-C (mmoI/L)
1	NUTRIBOM	1.86 ± 0.17^{a}	1.23 ± 0.02^{ab}	0.37 ± 0.03^{a}	0.18 ± 0.58^{a}	0.75 ± 0.13^{b}
2	Rat pellet	2.80 ± 0.27	1.26 ± 0.10	0.51 ± 0.04	0.45 ± 0.12	1.22 ± 0.12
3	URWV	3.87 ± 0.13^{e}	1.67 ± 0.12^{cd}	0.51 ± 0.02^{bc}	0.89 ± 0.10^{e}	1.75 ± 0.06^{d}
4	URWO	2.14 ± 0.17^{b}	1.11 ± 0.03^{a}	0.36 ± 0.03^{a}	0.46 ± 0.14^{c}	0.49 ± 0.02^{a}
5	PRWV	2.63 ± 0.20^{c}	1.47 ± 0.08^{bc}	0.46 ± 0.02^{b}	0.27 ± 0.07^{b}	0.82 ± 0.07^{c}
6	PRWO	3.70 ± 0.27^{d}	1.76 ± 0.14^{d}	0.56 ± 0.04^{c}	0.65 ± 0.12^{d}	0.82 ± 0.07^{c}

Keys: URWV = Unpeeled Red Banana with Vitamins; **URWO =** Unpeeled Red Banana without Vitamins; **PRWV** = Peeled Red Banana with Vitamins; **PRWO** = Peeled Red Banana without Vitamins. a = $p < 0.05$ (all groups compared with control). b = $p < 0.05$ (groups URWO, PRWV, PRWO compared with URWV). C = $p < 0.05$ (groups PRWV, PRWO, Nutribom compared with URWO).
Note: the superscript "a" is the lowest and "d" the highest

Table 3: Result of the Effect of Red Banana Meal (musa acuminata) on Serum Total Protein, Albumin and Globulin of Albino Wistar Rats

Group	Meal / Formular	Albumin (mmol/L)	Total Protein (mmol/L)	Globulin (mmo1/L)
1	NUTRIBOM	2.72 ± 0.06^{b}	4.74 ± 0.18^{a}	$2.34^{b} \pm 1.13^{a}$
2	Rat pellet	4.22 ± 0.44	7.11 ± 0.43	2.89 ± 2.03
3	URWV	3.58 ± 0.19^{d}	6.06 ± 0.58^{b}	2.47 ± 1.44^{b}
4	URWO	2.69 ± 0.07^{a}	4.96 ± 0.31^{a}	2.43 ± 8.9^{a}
5	PRWV	3.20 ± 0.14^{c}	7.07 ± 1.07^{b}	3.65 ± 69.72^{d}
6	PRWO	3.21 ± 0.16^{c}	5.93 ± 0.23^{b}	2.58 ± 1.35^{c}

Keys: UBWV = Unpeeled Red Banana with Vitamins; **URWO =** Unpeeled Red Banana without Vitamins; **PRWV** = Peeled Red Banana with Vitamins; **PRWO** = Peeled Red Banana without Vitamins
Note: Values are expressed as mean ± standard deviation, n = 8
a = $p < 0.05$ (all groups in comparism with control).
b = $p < 0.05$ (Groups URWV, PRWV compare with PRWO).
C = $p < 0.05$ (groups PRWO, PRWV compare with nutribom).
Note: the superscript "a" is the lowest and "d" the highest

Table 4: Results of the Effect of Red Banana (*Musa acuminata*) meals on liver enzymes of Wistar Rats

Group	Meal/ Formular	ALT (µl/L)	ALP (µl/L)	AST (µl/L)
1	NUTRIBOM	26.75 ± 1.60^{c}	184.11 ± 16.68^{ab}	71.25 ± 5.68^{c}
2	Rat Pellet	19.50 ± 2.78	149.84 ± 14.64	74.13 ± 8.10
3	URWV	26.88 ± 2.65^{d}	239.43 ± 35.25^{b}	62.13 ± 6.13^{a}
4	URWO	24.25 ± 1.62^{ab}	180.78 ± 17.91^{c}	73.25 ± 6.42^{d}
5	PRWV	24.88 ± 1.48^{ab}	189.74 ± 27.04^{ab}	69.38 ± 4.24^{b}
6	PRWO	20.50 ± 2.02^{a}	133.98 ± 5.39^{a}	60.38 ± 7.59^{a}

Keys: URWV = Unpeeled Red Banana with Vitamins; **URWO =** Unpeeled Red Banana without Vitamins; **PRWV** = Peeled Red Banana with Vitamins; **PRWO** = Peeled Red Banana Without Vitamins
Note: Values are expressed as mean ± standard deviation, n = 8.
a = $p < 0.05$ (all groups in comparism with control).
b = $p < 0.05$ (Groups URWV, PRWV compare with PRWO).
C = $p < 0.05$ (groups PRWO, PRWV compare with nutribom).
Note: the superscript "a" is the lowest and "d" the highest

Discussion

In Akwa Ibom State, Nigeria, banana is a staple food used in the preparation of complementary weaning food, food for nursing mothers and for the prevention of medical conditions such as malnutrition. Banana is able to satisfy hunger and also meet nutritional needs of individual (Umar, I. A, Sidu, Y. Lawal, M and Malgandi S. A (2010). Albino rats are most frequently used experimental paradgims to assess the effect of food in animals. This work reported the performance of red banana based meal in biochemical indices of weanling albino rats. The result of our study showed that albumin, total protein and globulin of the experimental groups were within the same range. Triglyceride concentration was significantly increased ($p < 0.05$) in PRWO compared with the control. High level of triglyceride, LDL- cholesterol and VLDL-cholesterol have been associated with heart disease (Iweala et al., 2011). However, consumption of plantain has been shown to reduce triglyceride (Kaimal et al., 2010). LDL- cholesterol was significant in URWV. The nutrient composition of diet with addition of vitamins were improved compared with those without vitamins and nutribom (complementary baby formula). Red banana caused significant reduction in the level of total cholesterol HDL-Cho and VLDL, nutribom had the lowest the lowest value of LDLC. The American Heart Association recommends an optimal triglyceride level of 100 mg/dl (1.1 mmol/l) or lower to improve heart health (Gill *et al,* 2011).

A total cholesterol reading can be used to assess an individual risk of heart disease (Sihr *et al,* 2012). In the study by Mosa et al., (2015) they observed that triglyceride was 72.50 ± 1.36 mg/dl and total cholesterol was 116.65 ± 4.38 mg/dl.

The reference ranges for total protein is from 5.6 – 8.5 g/dl (Wills et al., 2022). In this study it was within acceptable range for healthy animals. The reference value for albumin in wistar rats is 2.5 – 5.5 g/dl. (Wills et al, 2022) Albumin was within acceptable limit in all the groups. The lack of adequate protein would deplete immunity and microbial action because globulin principally responsible for both natural and acquired immunity that an individual has against invading organism, low level serum protein may be seen in severe malnutrition and with conditions that cause malabsorption; such as celioc disease (Roth 2011).

Umar et al (2010) reported that serum albumin showed slight significant ($p < 0.05$) difference in all the groups when compared with their control. Also reported that the animals on corresponding mixture diet were greater than the reference range. This may be due to the decrease in the synthetic function of the liver. They also reported serum globulin content of rats fed with millet and maize were significantly ($p < 0.05$) difference when compared to animals on frisocream diet these may indicate that the animals were free from infection.

The references ranges of ALT, ALP, AST were 10 to 40 µ/l, 30 to130 µ/l and 50 to 150 µ /l respectively (Sharp and La Regina (1998). Apartate amino-transferase (AST) ALT, ALP. provide insights into the disease process by diagnosis prognosis and assessment, (Akpanbiatu, 2019). The difference observed between the AST, ALP and ALT activities of the animals compared with control showed slight variation which may be due to the nutrient in the unpeeled banana. AST level observed in these studies were within normal physiological range. Buncharoen et al 2012 also reported AST, ALP and ALT of the same range.

This result is higher than the one obtained by Mosa et. al., 2015 which had a range of 120.3± 0.14 µl to 129.1± 0.18µl. Normal ALP is 44 - 147µl/L elevated levels occur in liver or bone disease such as hepatitis liver damage and liver cancer, bone cancer, healing fractures, oesteomalacia, paget's disease and rickets (Weinstein, 2016). However, elevated levels of ALT do not automatically means that medical problems exist. Fluctuation of ALT levels is normal over the course of the day and they can also increase in response to strenuous physical exercise (Paul and Giboney, 2013).

Iwela et al, (2011) in their study the liver enzymes were significantly altered except AST that was significantly increased in hepatotoxic control group. The liver enzymes. AST and ALT play a role in the metabolism of amino acids. There was also a reduction of AST in the non-hepatortoxic groups fed with musa paradisioca – supplemented diet which showed that plantain may possess protective effects on the liver. Some alterations seen may be due to bioactive ingredient contained in the peeled of red banana.

Any concentration below the reference range usually reflects low albumin concentration, a condition which can render the body susceptible to liver disease, acute infections and some other health complications. Also any concentration above the reference range may be found in cases such as protein anemia, leukemia etc (Jahoor, Badaloo,

Reids and Forrester, 2013). Red banana has ability to reduce the levels of total cholesterol, LDL cholesterol and VLDL. Although the values on total cholesterol, LDL-C and VLDL –C were lower ($p > 0.05$) in the control group. The URWO was low in triacylglycerol ($p > 0.05$) than other groups.

In conclusion, this study revealed that red banana meal is a good complementary food and may be used as infant weaning diet, suitable meal for the family and prevention and treatment of some non-communicable disease. Fortification of meals with vitamins is also essential considering the decreased in nutrient due to method of foods processing. Red banana may reduce the risk of malnutrition as observed in the study. Albino rats are most frequently used to assess the effect of food in animals, hence the use of rat.

Recommendation

1. The knowledge of the nutritional composition of local food commodities & application of same may increase utilization & improve health benefit.
2. Red banana is a seasonal food people should be trained on suitable methods of processing, packaging and storage for availability during scare season.
3. Farmers need advocacy through local gathering in churches, market, village squares and household on red banana as a local food available in the community, promote cultivation and compliance in its usage as meals for the family.

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