PROBIOTIC EFFECTS OF FERMENTED FOOD MADE WITH IRISH POTATO (*SOLANUM TUBEROSUM*), RED KIDNEY BEAN (*PHASEOLUS VULGARIS*), MUNGBEAN (*VIGNA RADIATE*), PAPAYA (*CARICA PAPAYA*) AND INOCULATED WITH *LACTOCOCCUS LACTIS* SP. STRAIN IN WEANED RATS

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Abstract
Effects of fermented complementary foods made from Vigna radiate, Solanum tuberosum, Phaseolus vulgaris, Carica papaya and inoculated with Lactococcus lactis sp. were investigated in rats. Colonization of the rat intestine by Lactococcus lactis sp. in vitro was observed by scanning electron microscope (SEM). Healthy males weighing between 45-50 g (21±2 days) were selected and randomly assigned in 4 groups (n=8 rats per group) and fed for four weeks. Group I (control group) was fed with basal diet (BD), group II received basal diet and one ml of culture to make an experimental diet having 1×10^9 cfu/g (BD+P), group III received basal diet mixed with 25 % fermented food without cells culture (BD+FF), group VI was fed with the mixture of basal diet and 25 % of probiotic fermented food to make an experimental diet of 1×10^9 cfu/g (BD+PFF). Diets BD+FF and BD+PFF were called modified diets. The growth performance, hematological parameters and biochemical parameters were evaluated. SEM showed that Lactococcus lactis sp. could adhere to the rat intestine and colonize the intestine wall. In rats that received probiotic and fermented food, there was a significant increase in the white blood cell (WBC), red blood cell (RBC) and hematocrit percentage when compared to the control. There was no significant difference in lipids profile. AST, ALP and ALT activities decreased gradually in rats that received probiotic and modified diets. The results present the importance and benefits of probiotic fermented foods which showed signs of good health in rats.

Keywords: Complementary foods, *Lactococcus lactis sp.*, scanning electron microscope, weaned rats, hematological parameters, biochemical parameters.

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1. INTRODUCTION

The childhood diet must be adequate to support normal growth and development. Several authors reported that in many developing countries, complementary food given to children were made either only with tubers or only with cereals that are processed into porridges which could easily affect the health of children due to their poor nutrients contents. The need for nutritious foods to feed young growing children and avoiding protein/energy malnutrition is now being met through commercially produced foods prepared by extrusion or roller-drying and other high technology processes. However, products in the market are too expensive for the target groups (Gernah *et al.*, 2012). Increasing the variety and quality of food is a challenge. Investigations of simple technologies which are easier to improve the nutritional value of food formulations are still a challenge in developing countries.

The potential of fermented food for reducing or alleviating food relate factors of malnutrition, particularly among weaning age children is important for considering the beneficial properties inherent in these types of food (Lorri, 1993). Some African fermented food products have been reported to have therapeutic values (Aderiye and Laleye, 2003). The fermentation process, with or without the

addition of microorganism to food residue, has been used successfully to preserve food residues (Yang *et al*., 2006). Rather than relying on indigenous microbes, the addition of selected microorganisms helps to ensure that desirable bacteria culture will be the predominant microflora in the feed resulting in a successful fermentation outcome (Kim *et al*. 2011).

Fermented foods have a unique functional property imparting some health benefits to consumers due to the presence of functional microorganisms, which may possess probiotics properties, antimicrobial, antioxidant, peptide production, etc. Some fermented foods are associated with "good bacteria" referred to as probiotics (Patricia *et al*., 2002; Helland *et al*.,2004). The beneficial effects of probiotic foods on human health and nutrition are constantly increasing (Monteagudo-Mera *et al*., 2012), and probiotics are popularly using bio-ingredients in many functional fermented foods (Chávarri *et al*., 2010). The health benefits of some global fermented foods are synthesis of nutrients, prevention of cardiovascular disease, prevention of cancer, gastrointestinal disorders, allergic reactions, diabetes, among other (Tamang *et al*., 2016).

Probiotic organisms require a vehicle to reach the site of action in an active form. The vehicle is generally a food product, which contains these live bacteria (Agrawal, 2005). Due to the wealth of nutrition and health research, as well as the current prevalence of lifestyle-related diseases, many new food products have been created to target human wellness. A number of new products based on cereals, fruits, vegetables and meats are in the development stage. Fruits and vegetables are healthy foods, because they are rich in antioxidants, vitamins, dietary fibers, and minerals. Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent their use by certain segments of the population (Luckow and Delahunty, 2004).

The aim of this study was to evaluate *in vivo* the beneficial effect of fermented foods made with mungbean (*Vigna radiate*), irish potato (*Solanum tuberosum*), red kidney bean (*Phaseolus vulgaris*) and papaya (*Carica papaya*) in weaned rats.

2. MATERIALS AND METHODS

2.1. Collection of samples

Dry red kidney beans were purchased from a local market of Dschang, Cameroon. Dry dehulled mung beans, potatoes and papaya were purchased from a local supermarket at Mysore, India. The starter used was the stock of *Lactococcus lactis* sp. obtained from the laboratory of Biochemistry, Medicinal plant, Nutrition and Food science (LAPMAN) at the University of Dschang-Cameroon isolated from fermented maize beverage. Before experimental use, *Lactococcus lactis* sp. culture was propagated (1%, v/v) twice in M17 medium incubated at 37 °C for 18 h without agitation.

2.2. Evaluation of the colonization of rat intestine by *Lactococcus lactis* sp.

The intestine of 3-4 months old healthy Wister rats was collected from the Animal house of CFTRI, Mysore, India. The intestine was resected from the viscera content of dissected rat and collected in sterile phosphate buffered saline before it was cut transversely into pieces of 1-2 mm^2. *Lactococcus lactis* sp. of 18 h old broth culture were harvested by centrifugation at 7500 rev min^{-1} for 15 min and washed twice with phosphate buffer saline (0.2 M, pH 7.0). The cells were suspended in 1mL phosphate buffered saline and a piece of the resected rat intestine was added to the suspension. Incubation was done at 37 °C for 18 h.

The piece of intestine was washed to remove none adhering cells and the procedure of scanning electron microscopy was performed on the piece of intestine to reveal adhesion (Ouwehand *et al*., 2002).

2.3. Formulation and preparation

The complementary food were formulated according to the recommended daily allowance to maintain the nutrient composition of the composite paste close to the WHO/FAO standard values for complementary foods, i.e.,

70% carbohydrates, 16% proteins, 7% lipids, 2% ash, and 5% fibers (Ihekoronye & Ngoddy, 1985). The final formulation used was 20 % red kidney beans, 60 % mung bean, 10 % irish potatoes, and 10 % ripe fresh papaya fruits. Red kidney beans were soaked in distilled water for 18 h, removed and dehulled manually; dehulled raw mungbean were soaked in distilled water for 2 h; Irish potato was cleaned, washed, peeled and diced. Papaya fruit was washed, peeled and diced. Raw materials were mixed together with 50 mL of distilled water and ground using Meenũmix model M-86 (Meen Equipment, India) during 2 min. 200 mL of distilled water was added progressively to the paste, homogenized and precooked for 15 min on hotplate. 0.5 g of salt was also added to the paste. Into three 250 mL Erlenmeyer each, 100 mL of paste was introduced, cooked for 1 h to destroy anti nutritional factors and sterilized by autoclaving at 121 °C for 15 min. When the pastes reached room temperature, they were inoculated with different amount of initial inoculums of culture 1×10^6 cfu/g for food 1 and 2×10^6 cfu/g for food 2. Pastes were incubated at selected temperature for 18 h in adequate conditions. Uninoculated paste treated in the same way was used as a control.

2.4. Animal experiments

Thirty two *Wistar* male albino rats (*Rattus nervegicus*) were obtained from Experimental Animal Production Facility of Central Food & Technological Research Institute (CFTRI), Mysore, India. The experimental protocol was approved by the Institutional Animal Ethics Committee, DFRL, Mysore (Reg. No. 28/1999/CPCSEA, dated 11 March 1999). All animals were examined for clinical signs of ill health on receipt and observed within 5 days of arrival. After 7 days of acclimatization, healthy males weighing between 45-50 g were selected and randomly assigned to 10 groups (n = 8 rats per group) and were placed in individual stainless steel cages. Animals were maintained on 12 h light-dark cycle, room temperature was controlled at 25-30 °C with 50 % relative humidity.

The animals were fed with basal diet AIN-93G (1993) (table 1) during one week of acclimatization period. After this period, animals were fed as follow:

Diet 1 or control group was BD (Basal diet) for group 1

Diet 2: Basal diet was mixed with 1 mL of culture to make an experimental diet of 1×10^9 cfu/g (BD+P) for group 2.

Diet 3: Basal diet was mixed with 25 % fermented food without inoculum (BD+FF) for group 3

Diet 4: Basal diet was mixed with 25 % probiotic fermented food to make an experimental diet of 1×10^9 cfu/g (BD+PFF) for group 4.

Table 1: **Composition of the basal diet (BD) (AIN, 1993)**

Ingredient (g/kg)	AIN-93G
Casein	200
Sucrose	100
Corn starch	397.486
Corn oil/Soyabean oil	70
Cellulose	50
Mineral Mix	35
Vitamin Mix	10
Choline Bitartate	2.5
Ethoxyquin/ TBHQ antioxidant	0.014
Maltodextrin	132
L-Cystine	3

Diets 2, 3 and 4 were called modified diets.

The body weight of each rat was measured weekly. The animals were centered in the weighing tray and the weight recorded in grams. The weighing balance was checked and frequently adjusted to zero weight before each measurement was taken. Food intake was monitored weekly, and thus the weight gain and food efficiency ratio (FER) were calculated.

FER = Body weight gain for experimental period (g/day) / (Food intake for experimental period (g/day).

At the end of the feeding period (28 days), food and water were withdrawn from the animals 18 h prior to the termination of the experiment. Rats were anaesthetized with diethyl ether and blood was collected by retro orbital plexus. A portion of whole blood was collected from each

rat and transfer into eppendorf tubes containing EDTA (1 mg/mL) for hematological parameters analysis. The remaining blood samples were also collected in eppendorf tubes without EDTA and allowed to clot for 20 minutes for serum separation.

2.5. Hematological parameters

Hemoglobin (Hb), hematocrit (HCT), white blood cells (WBC), red blood cells (RBC) and platelets (PLT) were analyzed using Sysmex Automated hematology Analyzer XP series.

10 µl of blood was pipetted from the eppendorf tube and analysis was done automatically. Hemoglobin analysis was conducted using a non-cyanide method. White Blood Cells, Red Blood Cells and Platelets were counted using the direct current detection method with coincidence correction. Automatic discriminators separate the cell populations based on complex algorithms. The intensity of the electronic pulse from each of the analyzed cell was proportional to the cell volume. HCT was directly determined based on the red blood cell count and volume detection of each individual RBC. Even with samples at extremely low or unusually high concentrations, the Sysmex cell counters analyze WBC, RBC and PLT with uncompromised precision and accuracy.

2.6. Lipid profile analysis

Serum triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were measured using standard kits (AGAPPE diagnostics) and analyzed automatically using Sysmex Automated hematology Analyzer XP series.

Atherogenic Index (AI) was calculated using following equation:

$$Atherogenic\ Index\ (AI) = \frac{LDL - Cholesterol}{HDL - Cholesterol} \quad (1)$$

2.7. Serum biochemical parameters and serum enzyme analysis

Glucose, bilirubin, urea, Alkaline phosphatase (ALP), Alanine transaminase or ALT and Aspartate transaminase (AST) estimations have been done using commercially available kit by AGAPPE diagnotics and analyzed automatically using Sysmex Automated hematology Analyzer XP series.

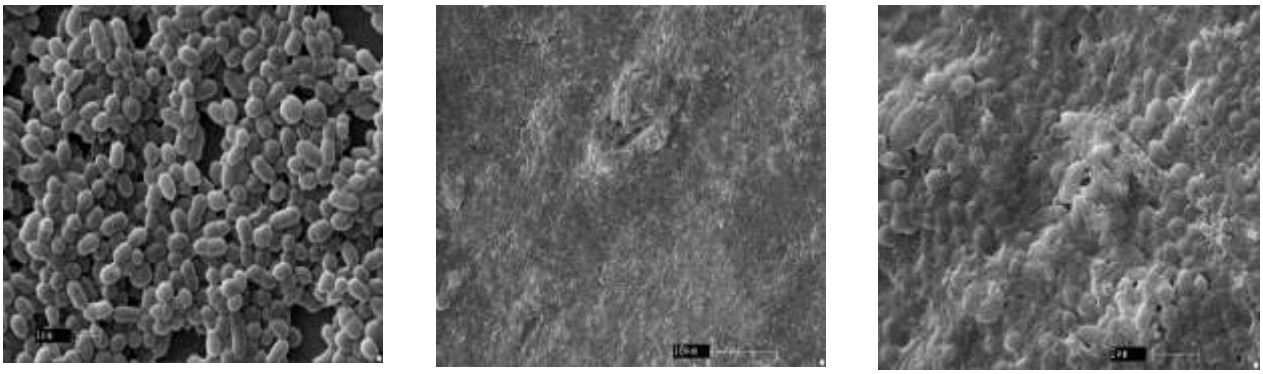
2.8. Statistical analysis

Data collected from triplicate were subjected to statistical analysis. To compare values, analysis of variance (ANOVA) using Microsof Office Excel (Microsoft, Redmond, WA, USA) was used. Pair-comparison of treatment means was obtained by Tukey's procedure at $p < 0.05$, using the Statistical software for Windows (GraphPad InStat).

3. RESULTS AND DISCUSSION

3.1. *Lactococcus lactis* sp. colonization of rat intestine

The scanning electron microscope of *Lactococcus lactis* sp. is represented in figure 1a. Figure 1b shows the scanning electron microscope of rat intestine without cells (control) and figure 1c shows rat intestine with *Lactococcus lactis* sp. On figure 1a we observed that the form of this strain is spherically-shaped and bacteria are grouped in pairs and short chains. On figure 1c we observed that this strain was able to adhere to the intestinal wall of rats and therefore colonize the gut. The ability of cells bacteria to adhere to the intestine may provide beneficial effects, such as the exclusion of pathogens (Ouwehand *et al*., 2002; Lee *et al*., 2003; Kos *et al*., 2003) or host immunomodulation (Schiffrin *et al*., 1995). In the study done by Menad *et al*. (2014) they had also observed the adhesion of *Lactococcus lactis sbsp cremoris* to epithelial cells of the colon Broiler. After adherence, the bacteria can induce now probiotic effects on the host such as increasing the body weight, inhibition of the synthesis of cholesterol, so that cholesterol levels drop and prevent atherosclerosis and coronary heart disease, stimulating the immune system etc. Another important selection criterion for potential probiotic microorganisms is their ability to adhere to the intestinal mucosa for the colonization of the human gastrointestinal tract and prevents their elimination by peristalsis (Kos *et al*., 2003).



(A) (B) (C)

Figure 1: Scanning electron microscope. A) Scanning electron microscope of Lactococcus lactis sp. B) Intestine wall of control group. C) Colonization of rat intestine of groups receiving diet with Lactococcus lactis sp

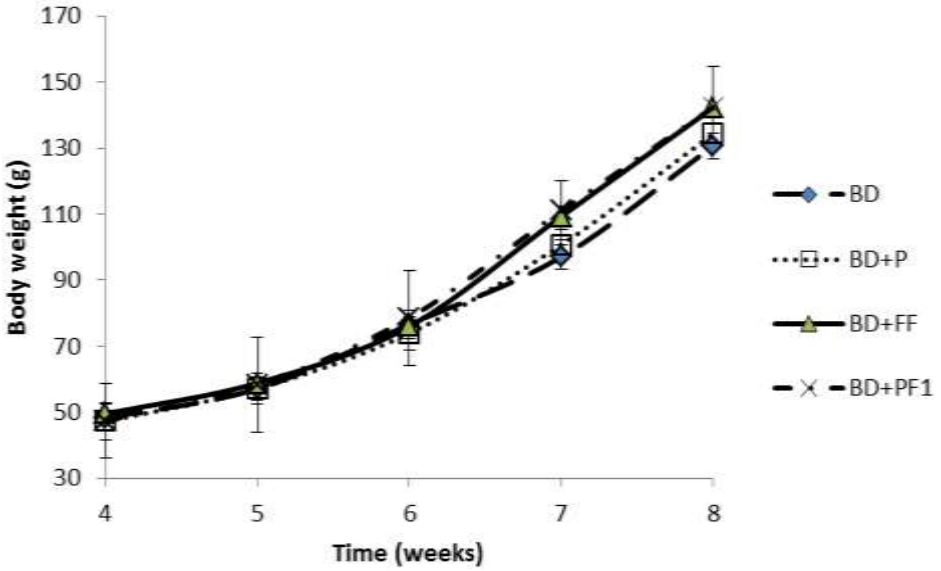


Figure 2: Effect of different diets on growth rate of rats
BD = Basal diet
BD+P = Basal diet mixed with 1 ml of culture to make an experimental diet having 1×10^9 cfu/g
BD+FF = Basal diet mixed with 25 % fermented food without inoculum
BD+PFF = Basal diet mixed with 25 % probiotic fermented food to make an experimental diet having 1×10^9 cfu/g

3.2. Effect of different diets on growth rate of weaned rats

The growth rate of rats fed with basal diet, modified diets containing probiotic, fermented food and probiotic fermented food is depicted in figure 2. Ascending increase in the average body weight of different experimental groups over a period of 28 days was observed. But there was no significant ($p>0.05$) difference in growth curve of rats in different groups. These comparable values suggest that the modified diets supported physiological functions like the control. Modified diets did not affect the final body weight gain. These results obtained agreed with those of Bernardeau, Vernoux & Gueguen (2002) who found that *Lactobacillus acidophilus* added to the drinking water did not change the weight gain, feed intake and water intake in mice; Foo *et al*. (2003) reported that supplementing different levels of fermented fruits (10, 20 %) to the diets of rats did not affect the body weight, live weight gain after four weeks; Loh *et al*. (2009) reported that diets supplemented with 0.25 % and 0.5 % spray-dried metabolite of *Lb. plantarum* had no significantly effect in body weight gain on postweaning rats and Kim *et al*. (2011) also found that rats treated with fermented diets did

not affected the growth rate of animals after the 4-weeks feeding trial.

3.3. Effect of different diets on body weight, food intake and Food Efficiency Ratio

The results of body weight gain (BWG), feed consumption and Food Efficiency Ratio (FER) during the 28 days experimental period are summarized in table 2. No significant difference was observed in final body weight, live weight gain between animals fed with modified diets and basal diet after 4 weeks of experiment. We note that all the modified diets tended to increase average gain in body weight of the rats by 87.04 ± 7.15 to 95.19 ± 8.50 g Hematological studies have been found useful for disease prognosis, for therapeutic and food stress monitoring (Togun and Oseni, 2005). There was no statistical ($p>0.05$) difference in both hemoglobin and platelet between the modified and control fed rats. WBC, RBC and HCT significantly ($p<0.05$) increased in rats receiving diet 4 compared to those of control group. This observation is not similar to those found by Kim *et al*. (2011) who have shown that blood cell components, such as white blood cells, red blood cells and platelet counts, were not affected ($p>0.05$) in rats fed with dry or fermented (aerobically or anaerobically with or without lactic acid bacteria) restaurant food residue mixture-containing diets. The increase in their values after the feeding period may be due to improved nutrition by the fermentation and the presence of lactic acid bacteria. No significant ($p>0.05$) difference was observed in compared with basal diet 83.43 ± 7.63 g. Rats fed with fermented food (diets 3 and 4) consumed significantly higher quantities of food (376.40 ± 4.38, 378.24 ± 5.25 g) probably because of its palatability. FER decreased significantly ($p<0.05$) in group 3 and 4 when compared with to control. Food intake in groups fed with modified diets was higher; in fact probiotics have been reported to increase food intake (Lessard and Brisson, 1987).

3.4. Effects of different diets on hematological parameters of rats

The effects of different diets on hematological parameters of rats are shown in table 3. group 3 and 4 fed with modified diet containing fermented food. WBC is important in defending the body against infections, it is essential in the detection of myelopoiesis and the identification of leukaemia (Nazaruddin *et al*., 2012). An increased WBC count has been reported in birds given a diet supplemented with probiotics (Rahimi and Khaksefidi, 2006). A red blood cell count is normally used to indicate anaemia. Togun *et al*. (2007) observed that increase in Ht coupled with marginal increase in RBC is indicative of more efficient erythropoiesis in the experimental animals. No significant difference was observed between the hemoglobin concentration of rat fed with probiotic fermented foods (diet 3 and 4). This indicates that supplementing weaning diets with plant protein can maintain adequate levels of hemoglobin in rats and thus human.

Table 2: **Effect of different diets on body weight, food intake and Food Efficiency Ratio (FER)**

Groups	Initial body weight (g)	Final body weight (g)	Gain in body weight (g)	Food intake (g)	FER
1 (BD)	48.37 ± 4.16	131.80 ± 8.62	83.43 ± 7.63	348.29 ± 4.58^{a}	4.19 ± 0.05^{a}
2 (BD+P)	47.67 ± 3.34	134.71 ± 8.57	87.04 ± 7.15	371.35 ± 2.62^{b}	4.27 ± 0.03^{a}
3 (BD+FF)	48.62 ± 4.39	140.85 ± 6.39	92.17 ± 5.51	376.40 ± 4.38^{b}	4.09 ± 0.05^{b}
4 (BD+PFF)	48.01 ± 3.80	143.13 ± 9.36	95.19 ± 8.50	378.24 ± 5.25^{b}	3.97 ± 0.06^{b}

Values represent mean ± STDV, Values bearing different superscripts (a,b,c) differ significantly ($P<0.05$)
BD = Basal diet
BD+P = Basal diet mixed with 1 ml of culture to make an experimental diet having 1×10^{9} cfu/g
BD+FF = Basal diet mixed with 25 % fermented food without inoculum
BD+PFF = Basal diet mixed with 25 % probiotic fermented food to make an experimental diet having 1×10^{9} cfu/g

Table 3: **Hematological and biochemical parameters**

	Foods			
Parameters	BD	BD+ P	BD+ FF	BD+ PFF
WBC ($\times 10^3/\mu l$)	3.47 ± 0.32^a	3.78 ± 0.33^{ab}	4.03 ± 0.08^b	3.93 ± 0.13^b
RBC ($\times 10^6/\ \mu l$)	7.78 ± 0.40^a	8.08 ± 0.36^a	8.41 ± 0.60^{ab}	8.91 ± 0.44^b
Hg (g/dL	14.2 ± 0.84	14.5 ± 0.54	15.1±0.77	16.3 ± 0.59
HCT (%)	42 ± 2.86^a	44.5 ± 1.43^a	45.2 ± 2.52^{ab}	46.8 ± 2.08^b
PLT ($\times 10^3/\ \mu l$)	651 ± 155	657 ± 248	806±161	853 ± 223
Total cholesterol (mg/dL)	111.61 ± 8.55	105.20 ± 3.27	107.19 ± 6.76	106.93 ± 9.99
HDL-cholesterol (mg/dL)	54.34 ± 6.27	54.03 ± 3.87	56.98 ± 7.18	58.52 ± 7.33
VLDL+LDL (mg/dL)	55.05 ± 10.71	51.16 ± 2.67	46.41 ± 9.53	48.12 ± 9.32
Triglyceride (mg/dL)	91.89 ± 21.19	83.28 ± 23.51	90.49 ± 10.40	77.96 ± 7.88
Atherogenic index	1.01 ± 0.31	0.95 ± 0.15	0.81 ± 0.21	0.82 ± 0.21
Glucose (mg/dL)	97.95 ± 6.81^a	82.45 ± 5.38^b	75.64 ± 6.75^{bc}	70.09 ± 5.89^c
Urea (mg/dL)	20.40 ± 2.35^a	17.94 ± 2.24^a	15.93 ± 1.64^b	16.93 ± 2.71^{ab}
Bilirubin (mg/dL)	0.59 ± 0.06^a	0.49 ± 0.06^a	0.45 ± 0.08^b	0.38 ± 0.07^b
ALP (Alkaline Phosphatase) (U/L)	39.74 ±4.59	40.43 ±1.84	37.82 ±3.99	36.92 ±3.48
ALT (Alanine Transaminase) (U/L)	23.04 ±4.56	20.59 ± 4.31	20.94 ±4.09	18.76 ±5.40
AST (Aspartate Transaminase) (U/L)	79.48 ± 4.34^a	71.11 ± 8.09^{ab}	67.97 ± 6.56^b	63.57 ± 6.72^b

Values represent mean ± STDV, Values bearing different superscripts (a,b,c) differ significantly ($P<0.05$)
BD = Basal diet
BD+P = Basal diet mixed with 1 mL of culture to make an experimental diet having 1×10^9 cfu/g
BD+FF = Basal diet mixed with 25 % fermented food without inoculum
BD+ PFF = Basal diet mixed with 25 % probiotic fermented food to make an experimental diet having 1×10^9 cfu/g

Some reports indicated an increase in the iron bioavailability during the fermentation. Iron is necessary in the formation of hemoglobin (Nazario, 2011). Iron is concomitantly ingested with some vitamin C or lactic acids, which are both enhancers of iron absorption (Branca and Rossi 2002, Teucher *et al*., 2004). Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of the blood of vertebrates and other animals (Eshaghian *et al*., 2006). Hemoglobin transports oxygen from the lungs or gills to the rest of the body, such as to the muscles, where it releases this oxygen. The absence of iron decreases heme synthesis and red blood cells production.

3.5. Effect of different diets on blood lipid profile

The highest total cholesterol value (111.61 ± 8.55 mg/dL) was observed from rats fed with basal diet which numerically reduced to 105.20 ± 3.27, 107.19 ± 6.76 mg/dL respectively for rats fed with diet 2 and diet 3; whereas the lowest value (106.93 ± 9.99 mg/dL) was obtained from rats fed with probiotic fermented food (table 3). Apart from the highest HDL-Cholesterol value (58.52 ± 7.33 mg/dL) obtained from weaned rats fed with modified diet containing probiotic fermented food (diet 4), VLDL+LDL, Triglyceride values and Atherogenic index were lowest in this group than in other groups. But there was no significant difference in blood lipid profile from all groups.

Total plasma concentration of cholesterol and triglycerides can give a clear insight into hyperlipidaemia arising either from excessive dietary intake or genetic disorders. High dietary intake of saturated fat for instance has been shown to initiate LDL-Cholesterol synthesis by the liver. There was no significant difference in serum lipid in this study. These results obtained here agreed with those found by Kim *et al.* (2011) in rats fed with fermented diets where the growth rate of animals after the 4-weeks feeding trial was not affected. Contrary to Loh *et al.* (2009) who had reported that rats (postweaning rats) fed with supplemented 0.25 % and 0.5 % spray-dried metabolite of *Lb. plantarum* had a significantly ($p<0.05$) lower

plasma cholesterol concentration than those of the control group; Salahuddin *et al.* (2013) and Zambou *et al*. (2013) also reported that a diet rich in probiotics decreases total cholesterol and LDL cholesterol concentration in blood plasma in mice and rabbits respectively. The non-significant of most of the serum lipid profiles may be due to the use of different probiotics strain, raw materials used for fermented foods, age of rats (weaned rats), different experimental condition, time and duration of study.

3.6. Effect of different diets on serum biochemical parameters and serum enzymes activities

Results of serum biochemical parameters and serum enzymes activities are presented in table 3. This indicated that serum glucose concentration of rats in group 4 decreased significantly ($p<0.01$) when compared with rats fed with basal diet (group 1), and ($p<0.05$) when compared to group 2 which received basal diet plus probiotic; while there was no statistical ($p>0.05$) difference between group 3 and 4.

The control experimental rats gave the highest value for the urea test and bilirubin concentration. Except in group 3 where there was a significant ($p<0.05$) decreased in urea concentration compared to group 1 and 2, no statistically difference was observed between the others groups. There was no significant ($p>0.05$) difference in urea and bilirubin concentration of rats fed with fermented foods (group 3 and 4).

The significant reduction in glucose, urea and bilirubin concentration in rats fed with fermented foods (diet 3 and 4) could be due to the effect of fermentation and probiotic bacteria. Serum urea level use as an index of renal function, it is a useful test of renal excretory functions because it correlates well with the clinical consequence of retained metabolite in renal dysfunction. Bilirubin is the end product heme breakdown (Sticova and Jirsa, 2013). About 80 % of bilirubin originates from degradation of erythrocyte hemoglobin in the reticuloendothelial system; the remaining 20 % comes from inefficient erythropoisis in bone marrow and degradation of other heme proteins (Tenhunen *et al*., 1968). This suggests that modified diets might not promote the degradation of red blood cells.

There was a significant ($p<0.05$) decrease in AST activity in groups fed with diet 3 and 4 when compared with the control. ALP and ALT activities decreased gradually in rats that received modified diets containing probiotic, fermented food and probiotic fermented food, but there was no significant different between all groups.

Transaminases (AST and ALT) are involved in amino acid metabolism. AST is present in liver, kidney, cardiac and squeletic musles; whereas ALT is present principally in the liver. The concentration of ALT and AST in the blood vessels will increase if any abnormalities in liver function occur. In this study, except in control group which had a highest ($p<0.05$) AST value when compared to other groups, the activities of the serum enzymes (transaminases, alkaline phosphatase) in both the control and modified diets fed rats were comparable. No toxicity levels were shown with respect to ALT, AST and ALP in the blood because the results obtained were still in the range of normal *Wistar* rats which are around 17.5-30.2, 45.7-80.8, 56.8-128 U/L respectively throughout the trial period according to Welfensohn and Lloyd (2003). This suggests that the formulated diets did not cause functional impairment due to nutritional stress or leaking of enzymes into the blood by hepatocellular injuries. These observations attest to the safety of the local formulated food to support normal biochemical functions. Results obtained here are similar to those reported by Khalil *et al*. (2015) in rats fed with high-dosage dietary groups supplemented with LAB (T200-LL and T200-PA), who found that the supplementation reduced the serum activity levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) by 11.3, 11.9 and 32%, respectively after 3 weeks.

4. CONCLUSION

Coming to the end of this study, we have to note that *Lactococcus lactis* sp. was able to adhere and colonize the rat intestine. Data obtained proved that fermented complementary food formulated are safe and have improved the growth performance of rats in terms of weight gain; increased the value of some hematological parameters such RBC and HCT; and reduced the serum biochemical parameters. Diet 4 show better results on all the parameters tested which indicated that this diet may sustain effective growth and development in children. It can be concluded that no safety concerns were identified, this means that the probiotic formulated fermented food could be used to feed children.

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