



In Vivo Evaluation of Soya Beans Flour Fermented with Lactic Acid Bacteria as a Potential Probiotic Food

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AM and MY designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. All the Authors managed the analyses of the study. Author SA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This research work was carried out to determine the in vivo antibacterial potential of soya beans flour fermented with lactic acid bacteria. This research focused on the feeding trial of formulated feed made of soya bean fermented for 72 hours with lactic acid bacteria (*Leuconostoc mesenteroides* and *Lactobacillus planetarium*) for albino rats, this is to know the effect of this feed on the rat infected with pathogens, compare with those of control feed. The pathogens used were *Escherichia coli*, *Shigella* sp and *Salmonella* sp. The experiment was divided into eight (8) treatments. Treatments I and II were not infected. Treatment I was fed with normal basal diet while treatment II was fed with the fermented soya bean flour. This was to ascertain the level of existence of the pathogen and the lactic acid bacteria before the introduction the pathogens. Pathogens count in treatment IV, VI, and VIII (rats fed with fermented soya bean flour) decreases as feeding time increases compare to treatment III, V and VII (rat fed with basal diet) which increases with the feeding time. The rats were fed from day 0 to day 56. Lactic acid bacteria commonly used as starter cultures in food technology are known to manufacture antimicrobial products and improve the food

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the organoleptic properties having great potential. Also, the Haematological analysis showed that the rats infected with the pathogens and later fed with the fermented soya beans flour recovered fully since their values are well within the permissible limit and are not significantly ($p \leq 0.05$) different from the control group. *Lactobacillus plantarum* and *Leuconostoc mesenteroids* strains used were able to grow and metabolize during fermentation of soya beans flour. It may be concluded that fermented soya beans flour with these isolates can be used as probiotic food and this in turn can be used in the treatment of infection caused by pathogens. It is recommended that the use of probiotic food for treatments should encouraged as an alternative to the use of antibiotic.

Keywords: Fermented soya beans; flour; probiotic food.

1. INTRODUCTION

Probiotics are defined as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host [1]. Health benefits have mainly been demonstrated for specific probiotic strains of the following genera: *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Bacillus* and *Escherichia coli* [2]. The human microbiota is getting a lot of attention today and research has already demonstrated that alteration of this microbiota may have far-reaching consequences. One of the possible routes for correcting dysbiosis is by consuming probiotics [3-7]. The credibility of specific health claims of probiotics and their safety must be established through science-based clinical studies. As probiotic properties have been shown to be strain specific, accurate identification of particular strains is also very important [8-13]. On the other hand, it is also demonstrated that the use of various probiotics for immunocompromised patients or patients with a leaky gut has also yielded infections, sepsis, fungemia, bacteraemia [14-18]. Although the vast majority of probiotics that are used today are generally regarded as safe (GRAS) and beneficial for healthy individuals, caution in selecting and monitoring of probiotics for patients is needed and complete consideration of risk-benefit ratio before prescribing is recommended [1].

The soya bean (*Glycine max*) is a species of legume native to East Asia, widely grown for its edible bean which has numerous uses. The plant is classed as an oilseed rather than a pulse by the United Nation Food and Agricultural Organization [19].

Fat-free (defatted) soybean meal is a significant and cheap source of protein for animal feeds and many prepackaged meals; soy vegetable oil is another product of soybean crop processing [20-22]. For example, soybean products such as

textured vegetable protein (TVP) are ingredients in many meat and dairy analogues. Soybeans produce significantly more protein per acre than most other uses of land [23].

Traditional non-fermented food uses of soybeans include soy milk, and from the latter tofu and tofu skin while fermented foods include soy sauce, bean paste, natto, and tempeh, among others. The oil is used in many industrial applications [23] The main producers of soy are the United States (36%), Brazil (36%), Argentina (18%), China (5%) and India (4%). The beans contain significant amounts of phytic acid, alpha-linolenic acid, and isoflavones. Soya bean may have been introduced to Nigeria as early as 1908. Nigeria is the largest producer of soybean for food in West and Central Africa [24-29]. The first successful cultivation was in 1937 with the Malayan variety, which was found suitable for commercial production in Benue State, central Nigeria Improved varieties in Nigeria include TGX849-313D, TGX10192EN, TGXg 1447-2E, TGX536-02D, TGX306-036C etc [30].

The preparation of both soybean and locust bean condiments involve the natural fermentation of dehusked cooked seeds [31,32]. The traditional method of preparing soy-iru has been described [33]. The product from such fermentation is sticky with a strong ammoniacal smell. Soy-iru (Soy-dadawa) is a food flavouring condiment prepared by fermenting whole soybean. It is widely consumed by the people of Benue and Plateau States of Nigeria and its consumption is now extending to the southern part of Nigeria [34]. Fermentation can be defined as a complex chemical transformation of organic substances brought about by the catalytic action of enzymes either originally present or secreted extracellularly by the microorganisms fermenting the material [35-38]. The microbiology of fermentation and some biochemical changes occurring during the production of this condiment have been reviewed [2]. Fermentation is a proven method to improve flavour, texture and

nutritional quality of the soybeans. Besides bringing physico-chemical and sensory quality changes, fermentation contributes towards the preservation of food due to release of metabolites that discourage the growth of pathogenic bacteria in foods [39-41]. Fermentation involves a range of microorganisms such as lactic acid bacteria, acetic acid bacteria, yeasts and moulds. It also covers wide range of products such as staples, adjuncts to staples, condiments and beverages that use substrates such as cereals, pulses, soybeans, flowers, milk, meat etc [42-49]. There are many traditional *B. subtilis* fermented soybeans foods in various parts of the world. One of the common examples is *Kinema*, which is traditionally consumed by the non-Brahmin Nepali inhabiting Nepal; Darjeeling and Sikkim of India; and some parts of Bhutan. It is popular among Lepchas and Bhutias who call it *Satlyangser* and *Bari* respectively [48]. Yoshida [49] reported the origin of soybean iru (*Kinema*) in Southern part of China. While it spread, this food settled into a niche as seasonings in East Nepal, North East India, Burma, Thailand and in Japan. Some *Kinema* like fermented soy foods reported in literature are: *Natto* in Japan, *Thua-Nao* in Thailand, *Douchi* in China, *Chungkook-jong* in Korea, *Tao-si* in Philippines, *Dawadawa* in Africa [50]. With its powerful odour and slimy appearance like a rotten food, large numbers of population do not like it, whereas other finds eating soy iru (and similar products) a delightful experience. These foods are eaten in the fresh form or as a fried curry dish along with boiled rice and sometime with boiled rice, and sometimes as soup, pickle or mixed with other vegetables [34,51].

Bacillus spp. is the most dominant naturally fermenting agents in soybeans. These hydrolytic bacteria are associated with utilization and reduction of indigestible oligosaccharides and polysaccharides. The organism has also shown to reduce the activity of anti-nutrients that hinders availability of proteins and phytochemicals present in soybeans [52]. *B. subtilis* fermentation is accompanied by covering intact granules with white-coloured viscous substance, slimy appearance, softer texture, and unique rotten flavour. It also completely removes the beany odour of raw soya beans and increases sensory quality of the product [50].

Chemical studies have been carried out to know the nutritive value of soy-iru, according to [53] fermented soybean has the following chemical composition as expressed by 100 g dry matter,

crude protein 49.51, fat 31.46, crude fibre 3.49, ash 3.97, carbohydrate 15.06 and organic matter 96.03. It also contains appreciable amount of minerals [50], it could be deduced that as much as probiotics performed excellently in the inhibition of diseases causing microorganisms, the use of soya beans and the microbes evolve during fermentation has not been explore and much work has not be carried out on their effectiveness. Therefore, this research work focused on investigation and assessment of soy flour fermented with Lactic acid bacteria and the evaluation of soya beans flour fermented with lactic acid bacteria as a potential probiotic food.

2. METHODOLOGY

2.1 Source of Organisms

Lactic acid bacteria that were used in this study were collected from the stock culture of Microbiology Laboratory of Waziri Umaru Federal Polytechnic, Birnin Kebbi, Kebbi State.

2.2 Collection of Sample

About one hundred kilograms (100kg) of Soya bean (*Glycine max*) seeds were purchased from Central Market, Birnin Kebbi, Kebbi State.

2.3 Preparation of Sample

The soya beans were sorted out for stone, rot and other physical defects. The cleaned beans were dried to obtain about 9.59% moisture content. The beans were roasted on hot- plate until golden brown, the beans were de-hulled immediately after roasting and allow to cool, the roasted beans were milled with Hammer miller (Model GG-300, Henan Gelgoog commercial and Trading co., China) and sieved with 75 micron mesh to obtained the soya bean flour [54].

2.4 Culturing and Harvesting of Lactic Acid Bacteria Cells

Overnight broth cultures of test isolates were centrifuged at 4,000 rpm for 35 min. The pellets were rinsed out thrice with 10 ml phosphate buffer saline (PBS) into sterilized universal bottles and were kept as stock cultures in the refrigerator at $4 \pm 2^{\circ}\text{C}$. The total viable cells in the stock solution were then determined using serial dilution and pour plate methods [55].

2.5 Inocula Development

Species of lactic acid bacteria (*Leuconostoc mesenteroides* and *Lactobacillus plantarum*)

obtained were developed. Pure cultures of the organism were inoculated in sterile saline suspension to make 0.5 McFarland turbidity standards which would be used as inoculums.

2.6 Fermentation and Storage

Soya beans flour were mixed with distilled water (1:3) in 25 fermentation jars which were autoclaved at 121°C for 15 min. Jars were allowed to cool after which each jar were inoculated with the consortium of *Lactobacillus plantarium* and *Leuconostoc mesenteroides*. After thorough mixing, the properly corked jars were incubated anaerobically at 37°C for 72 h for fermentation to take place [56]. After, fermentation the fermented bean was hot air dried and store at 25 ± 2°C (room temperature) for 14 days. Viable counts of LAB in the products were determined during the period of fermentation and after storage.

2.7 In Vivo Studies

Slight modification of the method used by Anthony et al. [55] was used for the *in vivo* study. Earlier to the *in-vivo* studies, the rats were weighed, sorted and allowed to acclimatize to the new environment for 14 days (2 weeks) before the take-off. Isolation and enumeration of the microbial flora in the G.I.T. of apparently healthy albino rats were carried out before the experimental animals were randomly assigned to 8 treatments (I, II, III, IV, V and VI, VII, VIII) of 8 rats each. Treatments I and II were infected, while III and IV were infected with *E. coli* (0.2 ml of 10⁵cfu/g daily for 3 days); V and VI were infected with *Shigella dysenteriae* (0.2ml of 10²cfu/g for 3 days) and VII and VIII were infected with *Salmonella typhi/paratyphi* (0.2ml of 10²cfu/g for 3 days). After which a 4-day post infection period was observed, diet of treatments IV, VI, and VIII were supplemented with 100g each of the fermented sample for 42 days. After feeding on the experimental diet for 6 weeks, all animals were fed with the control (basal diet) for a further 14 days. The total weight gain was observed while bacterial enumeration of fecal samples at 0, 7, 14, 21, 28, 42, 49 and 56 days were also determined using conventional techniques. Approximately 1g of rat faeces was exhaustively extracted with 40 mL of 75% acetonitrile by ultrasonication at room temperature. The supernatant was then allowed to evaporate to dryness by a gentle stream of nitrogen at 37°C. The residue was dissolved by 200 µL of 5% methanol and centrifuged at 15,000 × g for further analysis [55].

2.8 Microbial Analysis

Experimental rat's stool (5g) were collected as labeled during the feeding trials (at 0, 7, 14, 21, 28, 35, 42, 49 and 56 days) were used for bacterial enumeration using serial dilution and pour plate method on selective media. And the bacteria were counted manually and multiply by the inverse of the dilution factors.

2.9 Haematological Analysis

At the end of the study, all rats fasted overnight and blood was collected from the common carotid artery into a heparinized tube for hematological studies. Complete blood count, red blood cell count, platelet count, hemoglobin and packed cell volume were determined using an automatic counter (Sysmex K21, Tokyo, Japan).

2.10 Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS version 15.0. The Duncan's Multiple Range test was then used to separate the means at the 5% level of probability. All results are expressed as means ± SEM of three replicates.

3. RESULTS AND DISCUSSION

The figures below explain the bacteria count of treatment sample during the *in vivo* feeding trial. Fig. 1 and 2 represent Non – infected rats fed with the fermented soya beans and non - infected rat fed with normal basal medium respectively. The vertical axis (0-30) represents the bacteria counts in colony forming units) and the readings are recorded every 7hrs from (0-28hrs).

Prior to the infection of the experimental rats with the pathogens, the rats were fed with the fermented soya beans flour and normal basal food to serve as control in order to confirm the present of the pathogens and the lactic acid bacteria before infection. The results confirm the existence of the pathogens in the rat's stool but not in significant numbers even before the rats were infected with them. The lactic acid bacteria count was determined to confirm their existence as normal flora in the rat (Fig. 1 and Fig.2). The highest number of lactic acid bacteria recorded (2.6 × 10⁶) was achieved when feeding the rats with the fermented soya beans (Fig. 1). This confirms the survival of the lactic bacteria in the

fermented soya bean which confirm the vehicular ability of the bean for the probiotic bacteria. Anthony et al. [55] also confirm the survival of lactic bacteria in Mucuna beans flour. The probiotic potential of the lactic bacteria fermented soya bean flour was confirmed by the reduction in the number of the pathogens as rehearsal time progresses when feeding with the probiotic soya bean food (Fig.1 and 2). De Vrese and Marteau, (2007) confirm the effect of probiotics on diarrhea causing bacteria.

The result in Figs. 3, 4 and 5 above shows the bacteria count of treatment III to VIII during the in vivo feeding trial (10^5 cfu/ml). The rats were fed for 56 consecutive days. The rats were fed from day 0 to day 56. The results show that there are significant differences in bacterial count among the Treatments. Treatment III (*Escherichia coli*

infected rats fed with normal basal diet; i.e control) and treatment IV (*Escherichia coli* infected rats fed with the fermented soya bean flour) (Fig. 3). Treatment V (*Shigella sp* infected rats fed with normal basal diet; that is control) and treatment VI (*Shigella sp* infected rats fed with the fermented soya bean flour) (Fig. 4). These results revealed that the bacterial count decrease close to zero level at the treatment VI, VI and VIII (those fed with the fermented soya bean flour) when compared with the controls treatments (III, V and VII) which indicated that fermented soya bean flour. This study is in accordance with the results of Kebede et al. (2007) who found a higher bacterial activity in the fermented condiment when compared with non-fermented products. Also it corroborates with the report of Bermudez-Brito (2012) who reported the bacterial activity of lactic acid bacteria.

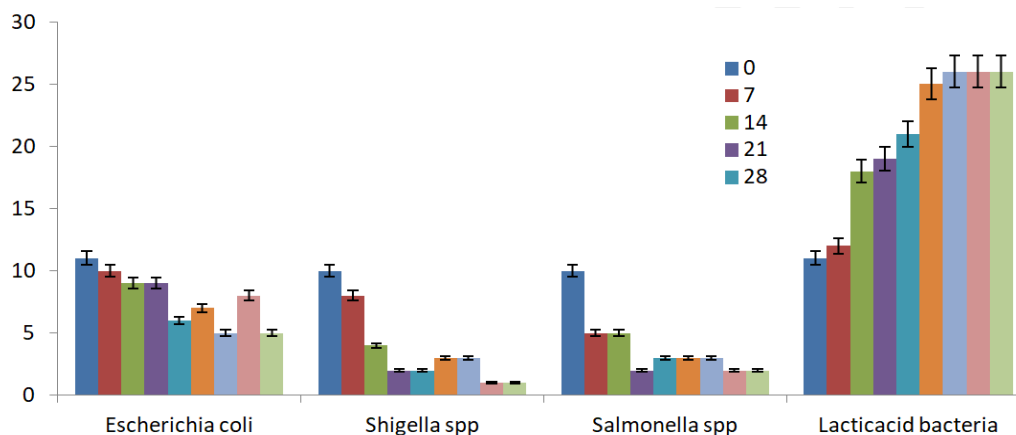


Fig. 1: Bacteria count of treatment I during the in vivo feeding trial (10^5 cfu/ml)
 Key: treatment I =Non – infected rats fed with the fermented soya beans

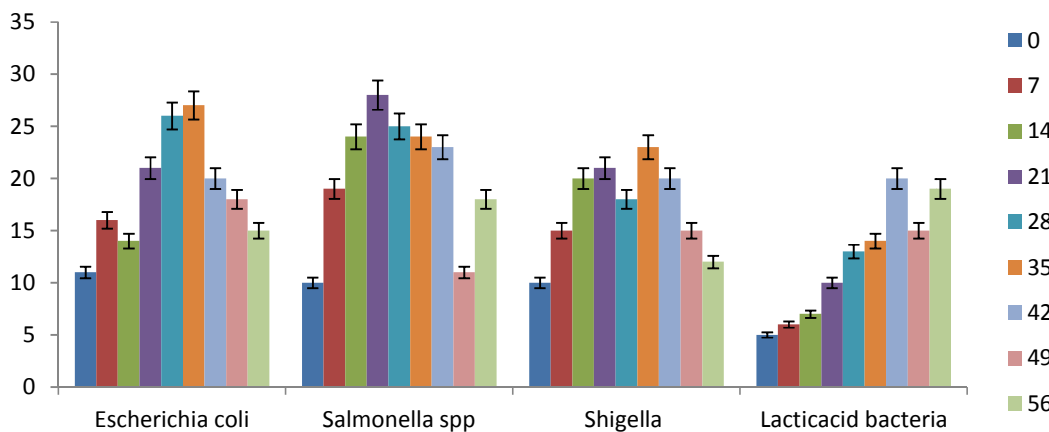


Fig. 2: Bacteria count of treatment II during the in vivo feeding trial (10^5 cfu/ml)
 Key: treatment II =Non – infected rats fed with normal basal diet

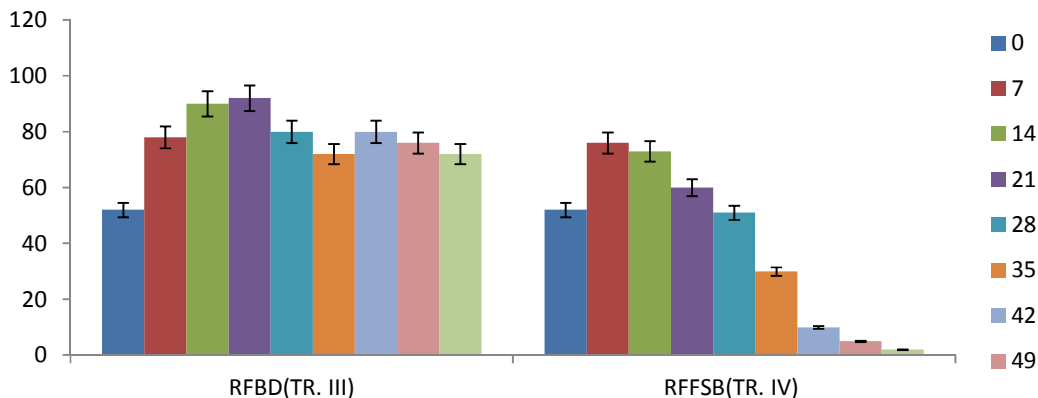


Fig. 3: Bacteria count of treatment III and IV during the in vivo feeding trial (10⁵cfu/ml)
 Key: treatment III = *Escherichia coli* infected rats fed with normal basal diet (control); treatment IV = *Escherichia coli* infected rats fed with the fermented soya beans; RFB= rat fed basal diet; RFFSB= rat fed fermented soya bean flour; TR = Treatment

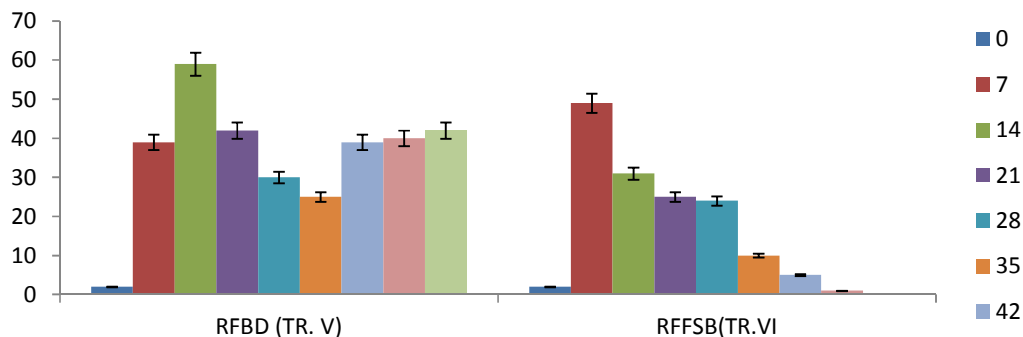


Fig. 4: Bacteria count of treatment V and VI during the in vivo feeding trial (10⁵cfu/ml)
 Key: treatment V = *Shigella spp* infected rats fed with normal basal diet (control); treatment VI = *Shigella spp* infected rats fed with the fermented soya beans; RFB= rat fed basal diet; RFFSB= rat fed fermented soya bean flour; TR = Treatment

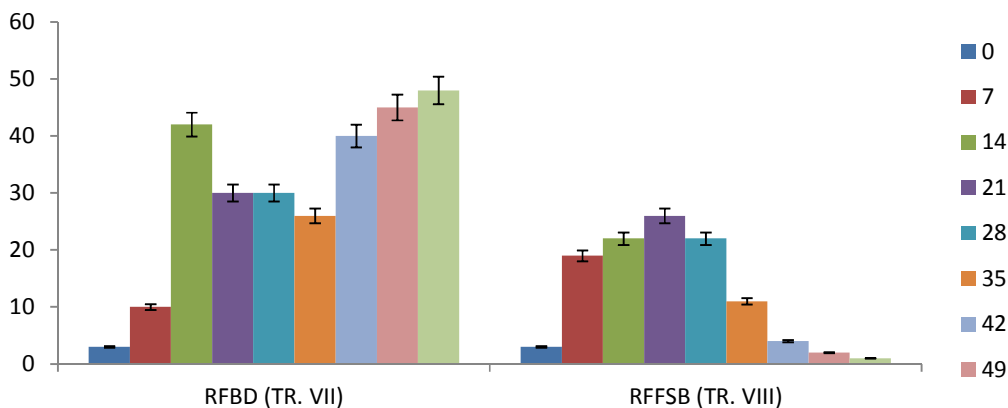


Fig. 5: Bacteria count of treatment VII and VIII during the in vivo feeding trial (10⁵cfu/ml)
 Key: treatment VII = *Salmonella spp* infected rats fed with normal basal diet (control); treatment VIII = *Salmonella spp* infected rats fed with the fermented soya beans; RFB= rat fed basal diet; RFFSB= rat fed fermented soya bean flour; TR = Treatment

Table 1. Weight of rats subjected to different feeding trials

Treatment/Days	0*	7*	14*	21*	28*	35*	42*	49*	56*
I	70.29±5.50 ^a	83.28±2.04 ^a	92.42±4.70 ^c	111.80±6.40 ^c	134.12±10.30 ^d	160.34±3.90 ^d	174.29±4.60 ^d	189.48±6.80 ^e	190.22±0.00 ^d
II	70.28±3.40 ^a	84.56±2.20 ^b	100.42±5.90 ^d	126.42±4.79 ^d	162.10±6.30 ^e	182.63±3.50 ^e	199.12±4.40 ^e	222.00±8.10 ^f	222.56±0.00 ^e
III	70.59±4.00 ^a	85.24±7.90 ^c	81.84±9.00 ^b	72.56±8.50 ^a	69.28±3.60 ^a	69.56±2.00 ^a	70.26±2.70 ^a	81.20±5.05 ^a	90.22±0.00 ^a
IV	71.00±5.30 ^b	85.67±4.50 ^c	75.22±3.70 ^a	70.10±3.80 ^a	60.20±3.00 ^a	72.20±5.00 ^{ab}	88.56±2.40 ^{ab}	92.42±6.22 ^b	102.43±0.00 ^b
V	71.24±6.06 ^b	86.22±3.00 ^d	81.90±5.04 ^b	88.58±2.00 ^b	98.20±4.90 ^b	119.20±5.00 ^c	148.19±3.03 ^c	162.00±4.20 ^d	173.56±0.00 ^c
VI	71.21±5.33 ^b	86.90±3.00 ^d	74.67±3.40 ^a	82.95±7.30 ^b	92.76±5.40 ^b	117.31±9.00 ^c	141.20±5.70 ^c	180.15±3.20 ^e	168.23±0.00 ^{bc}
VII	71.18±1.50 ^b	84.76±3.50 ^b	75.42±2.10 ^a	70.32±4.08 ^a	67.33±4.20 ^a	75.76±1.60 ^b	92.10±5.06 ^b	98.56±8.30 ^b	105.43±2.40 ^b
VIII	70.90±4.05 ^a	86.56±5.40 ^d	74.76±2.00 ^a	82.56±4.80 ^b	91.90±3.40 ^b	115.25±2.80 ^c	142.50±5.30 ^c	150.25±7.70 ^c	170.20±5.10 ^c

Key: The results in the table above were presented in means±standard deviation. The superscripts indicate the ranking of the post hoc test using Duncan Multiple Range Test. The means with different superscripts were significantly different from one another down the column while those with the same superscripts were similar. The asterisks indicate an experimental set up with significantly different outcomes from the Analysis of Variance (ANOVA)

Table 2. Haematological parameter of rats subjected to different feeding trials

Treatment		RBC*	WBC*	PCV*	HAEM*	PLATELET*
I	Non – infected rats fed with the fermented soya beans	7.25±2.00 ^c	8.48±1.70 ^a	38.59±1.40 ^b	13.21±1.06 ^c	168.20±3.40 ^b
II	Non – infected rats fed with normal basal diet	8.24±0.40 ^d	7.52±0.65 ^a	43.98±2.55 ^c	16.00±1.11 ^d	265.22±2.90 ^d
III	<i>Escherichia coli</i> infected rats fed with normal basal diet	6.20±0.09 ^b	17.90±1.43 ^c	28.50±1.75 ^a	9.89±2.20 ^a	131.76±2.90 ^a
IV	<i>Escherichia coli</i> infected rats fed with the fermented soya beans	5.60±0.50 ^a	22.20±2.00 ^d	34.17±4.04 ^{ab}	9.50±3.04 ^a	136.79±1.07 ^a
V	<i>Shigella spp</i> infected rats fed with normal basal diet (control); treatment	6.79±0.10 ^b	11.49±1.06 ^b	36.20±2.31 ^b	12.67±2.05 ^{bc}	172.90±5.20 ^{bc}
VI	<i>Shigella spp</i> infected rats fed with the fermented soya beans	6.87±0.68 ^b	12.00±1.58 ^b	38.73±3.66 ^b	11.74±0.88 ^b	180.22±3.00 ^c
VII	<i>Salmonella spp</i> infected rats fed with normal basal diet	5.60±0.33 ^a	21.90±0.44 ^d	33.72±1.00 ^{ab}	9.48±2.40 ^a	135.20±2.10 ^a
VIII	<i>Salmonella spp</i> infected rats fed with the fermented soya beans	7.20±0.12 ^c	11.59±1.22 ^b	39.00±0.83 ^b	12.00±1.00 ^{bc}	181.00±2.80 ^c

Keys: The results in the table above were presented in means± standard deviation. The superscripts indicate the ranking of the post hoc test using Duncan Multiple Range Test. The means with different superscripts were significantly different from one another down the column while those with the same superscripts were similar. The asterisks also indicate an experimental set up with significantly different outcomes from the Analysis of Variance (ANOVA)

These results shows that there was a significant difference between basal diet and fermented soya bean flour feeding trials the rat fed with fermented soya bean flour are more effective than the one fed with basal diet. Which indicates that soya bean flour is a good vehicle for lactic acid bacteria and are very effective when used as probiotic in the control of the pathogens. Mucuna beans as also been used as a vehicle for probiotic bacteria [55]. According to [57] a viable count of 10^7 cfu/g of the bacteria has been recommended as the minimal population of probiotics necessary to give a noticeable effect on the host health.

The weight of the rats was measured in replicates per treatment and an Analysis of variance using the post hoc test to determine the particular treatment that was responsible for which significant difference was observed at 95% confidence level.

The feeding treatments had significantly different effect ($p < 0.05$) on the rats' weight from the control to the 56th day of the treatment. While the trend analysis shows the progression of the weight of the rats at varied days of feeding, the ANOVA reveals the differences or similarities between the treatments for each day of sampling and allows analyzing the effect of the body weight of the rats. Treatments V, VI and VIII had similar effect on the weight of the test rats from the beginning (i.e: 0) to the end (i.e: 56) as they were ranked in the same homogenous subset in the inferential analysis. By the 14th day of treatment, the rats treated with feed II had the highest recorded weight while those infected with Shigella and fed with fermented soya bean flour (TVI) yielded the least weight increase. On the other hand, the 14th through the 56th days of treatment, healthy rats, uninfected and fed basal diet had the highest recorded weight in the experiment.

As can be seen in Table 2, the body weight of the healthy animals, corresponding to the controls (TI and TII), increased throughout the time of the experiment. From day 7, the body mass values determined in the healthy animals and fed the basal diet (TII) were significantly ($p < 0.05$) higher than those of the healthy animals and fed with fermented soya bean flour (TI), as well as that of infected animals (all other treatments: TIII, TIV, TV, TVI, TVII and TVIII). From day 14 to day 56, the values obtained in the determinations made in healthy animals fed with fermented soya bean flour (TI). On the other hand, comparing the change in body mass

[difference between the mass determined at the end of the experiment and the mass at the beginning (day =)] resulting from the effect of the diet (BD and fermented soya bean flour) administered to the infected animals (according to the pathogen used, namely: TIII vs TIV; TV vs TVI and TVII vs TVIII), it is observed that the animals infected with E. coli fed with fermented soya bean flour (TIV) gained mass, 16%, compared to that of those fed BD (TIII); that the animals infected with Shigella (TV and TVI, fed with BD and fermented soya bean flour, respectively) presented similar values ($p < 0.05$) for the body mass corresponding to day 0, as well as for those of the body mass determined at the end of the period of time analyzed (day 56), so it could be said that the gain in body mass was similar for these animals. Finally, in relation to the animals infected with Salmonella (TVII and TVIII), those fed with fermented soya bean flour (TVIII) tripled their body mass compared to the animals fed BD (TVII). In summary, it can be said that the presence of fermented soya bean flour in the diet supplied to animal infected with E. coli (TIV) or with Salmonella (TVIII) favored the gain in body mass compared to those of their peers fed the basal diet (TIII and TIV), respectively); while it had no effect on Shigella-infected animals.

The increase in body mass observed in animals fed with fermented soya bean flour is attributed to the antimicrobial properties of BAL-fermented soya beans. Afolabi and Adewolu [30] reported a steady increase in the weight of rats in a similar experiment conducted by them and attributed it to a better immune response against pathogens in vivo. The resistance of rats to physiological stress and the potential pathology of Salmonella spp was described as almost very substantial in a similar experiment (Dixi et al., 2011), although the fermentation period of soybeans and the starter used were not the exact ones used in this study.

The effect of the treatments on the hematological parameters of the rats were also tested to enable a comparative examination of the varied effects of the feeds on the rats such that inferences can be made in terms of application of the feeds. The RBC level was most favored by feed II while VII had the highest WBC counts. Apart from the two controls that were not infected prior the trial Probiotic feeding, The PVC count, HAEM and Platelet count were optimum when fed with Treatment VI, V and VIII respectively. The magnitude and comparative presentation of the significantly different effect of the feed treatment

was shown in the bar chart (Fig. 6). In other words, the experimented treatment effects were not due to chance.

According to the design of the experiment, the healthy animal fed with the basal diet (TII) is used as a healthy control (we consider it as healthy), so the RBC value correspond to the normal value for this animal and under these conditions of testing. Now, in Table 2 it is observed that the RBC value for TII is higher ($p < 0.05$) than the corresponding values obtained in the samples of all the other treatments (TI, TIII, TIV, TV, TVI, TVII and TVIII), would indicate that these animals deteriorated their health (the treatments affected health, possible anemia). Further studies are required, in addition to the corresponding microscopic analysis. The function of white blood cells is to defend the body. When analyzing the white blood cell count (WBC), it is observed that the values found in healthy animals (TI and TII) are similar ($p < 0.05$), that is, they would correspond to the normal value, for these animals and under the conditions of the essay; on the other hand, these values are lower ($p < 0.05$) than those obtained in all the treatments in which the animals were infected with the pathogens under study (*E. coli*, *Shigella* and *Salmonella*). Higher values would indicate infection, which is attributed precisely to the exogenous bacteria incorporated in each treatment.

Hemoglobin (HAEM) is responsible for transporting oxygen to the tissues. Table 2 shows the normal HAEM value for the animals considered as healthy control (TII corresponding to healthy animals and fed the basic diet). In all

the other treatments tested, the HAEM values were lower ($p < 0.05$) than those of the healthy control, this would indicate that their tissues would receive less oxygen, that is, the healthy condition would be affected. It would appear that the presence of the fermented soya bean flour in the diet with which healthy animals (TI) were fed would affect the capacity to transport oxygen to the tissues of these animals, compared to the normal value obtained for those fed the base diet (TII).

Platelets are associated with primary hemostasis; the highest ($p < 0.05$) value was found in healthy animals, fed the basic diet (TII), therefore, for these animals and under the test conditions, it is considered a normal value. As can be seen in Table 2, in the platelet count, all the other animals (TI, TIII, TIV, TV, TVI, TVII and TVIII) presented lower values ($p < 0.05$) than normal; that is, they present what is called thrombopenia. Thrombopenia is caused by a variety of agents, including viruses and bacteria. It is not clear why in TI the platelets value was lower ($p < 0.05$) than in TII, it would seem that the presence of the fermented soya bean flour in the diet of healthy animals affects the capacity for primary homeostasis.

Hence the weight gain may be related and influenced by many other factors that were not considered in this experiment. However, the observed improvement in hematological parameters such as (V, VI and VIII) may be better if the period of feeding and quantity fed were varied and tested for optimization (Berthier, 2003).

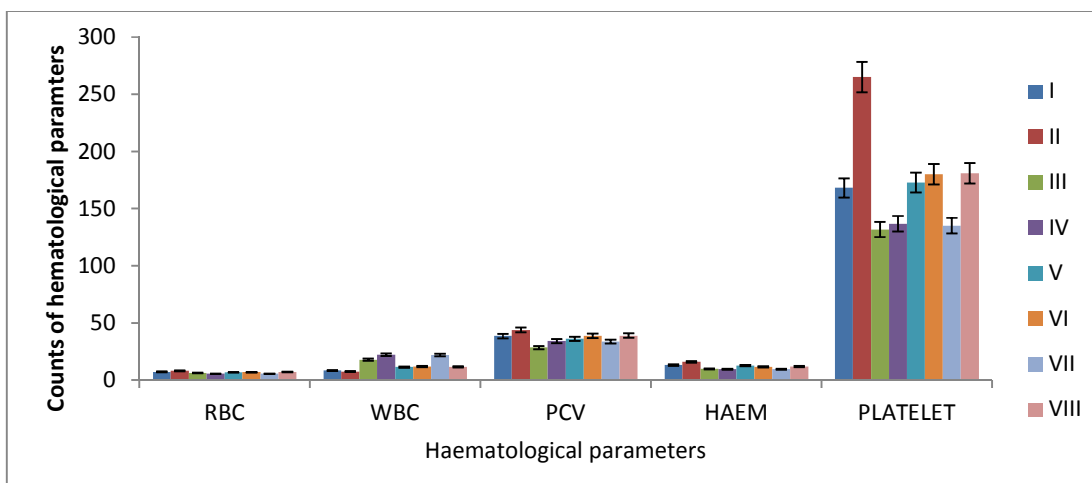


Fig. 6. Hematological parameter of rats subjected to different feeding trials
RBC: Red blood cell, WBC: White blood cell, PCV: Packed cell volume, HAEM: Haemoglobin

4. CONCLUSION

According to the result obtained from this research work. Lactic acid bacteria may be used for treatment of certain infection caused by these pathogens. *Lactobacillus plantarum* and *Leuconostoc mesenteroides* strains used were able to grow and metabolize during fermentation of soya beans flour. It may be concluded that fermented soya beans flour with these isolates can be used as probiotic food

5. RECOMMENDATION

It is recommended that the use of probiotic food for treatments should be encouraged as an alternative to the use of antibiotics.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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