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Evaluation of Mucuna Beans Flour Fermented with *Lactobacillus plantarum* as a Probiotic Food

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOM designed the study, wrote the protocol and wrote the first draft of the manuscript. Author OOA reviewed the experimental design and all drafts of the manuscript. Author SOE managed the analyses of the study. Author AOM performed the statistical analysis. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The aim of the study was to evaluate Mucuna beans flour fermented with *Lactobacillus plantarum in vitro* and *in vivo* for probiotic activities. The *L. plantarum* used was isolated from neonate '*ogi*' made from sorghum thereafter, which was screened for growth and survival in the mucuna beans flour.
Methodology: The methods used involved overnight broth cultures of test isolates *L. plantarum* which were centrifuged at 10,000 rpm for 15 min. The pellets were rinsed out thrice with 10 ml phosphate buffer saline (PBS) into sterilized universal bottles and kept as stock cultures in the refrigerator at 4 ± 2℃. The total viable cells in the stock solution was then determined using serial dilution and pour plate methods. Proximate analysis was carried out on both the fermented and un-fermented samples.
Results: From the results obtained during the study, at the end of 72 hour fermentation at 37℃,

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the *L. plantarum* showed appreciable growth ($8.83x10^6$ cfu/g). After storage for 14 days at refrigeration (4 ± 2℃) and room temperature (25 ± 2℃), there was a considerable increase in the *lactobacillus* found in the products stored at room temperature ($13.67±32.40x10^6$ cfu/g) compared to the one stored at refrigeration temperature ($8.47±2.19 x10^5$ cfu/g). It was observed that there was a steady increase in the total titratable acidity and temperature with concomitant reduction in the pH of samples during the fermentation period. The proximate analysis showed that there was an increase in the protein and moisture contents with decrease in Carbohydrates, fats, fibre and ash contents of the fermented samples compared to the unfermented sample. Furthermore, the body weight of the rats fed with the fermented product was significantly ($p \leq 0.05$) higher than the control group. Also, the Haematological analysis showed that the rats infected with the pathogens and later fed with the fermented mucuna 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.
Conclusion: The results obtained suggest that mucuna beans flour fermented with *L. plantarum* could be used as an ideal probiotic food.

Keywords: Probiotic; pathogens; L. plantarum; mucuna.

1. INTRODUCTION

Fermented foods are of great significance because they preserve wide range of nutritious foods and in the process enhancing the nutritional value, taste, flavour, aromas, shelf-life which enriches the human diet [1]. They are also important vehicle for probiotic foods. Probiotics are live microorganisms (in most cases, bacteria) that are similar to the beneficial microorganisms naturally found in the human gut. These probiotic bacteria are used to prevent and alleviate many different conditions, especially those that cause gastrointestinal disorders. Probiotic bacteria usually form a bioreactor, which facilitates digestion, provides nutrients such as several B vitamins, vitamin K, folate, and some short-chain fatty acids, and helps fortify the immune system [2]. According to [3] up to 10% of human daily energy needs can be derived from the byproducts of the probiotic bacteria in the gut. Furthermore, probiotics can provide multiple benefits for the immune system, when probiotics are abundant in the body, it is harder for pathogenic bacteria to colonise the gut. Some also make bacteriocins, which suppress the growth of pathogenic bacteria. Probiotic bacteria that are selected for commercial use in foods and in therapeutics must retain the characteristics for which they were originally selected [4]. These include growth and survival during manufacture and after consumption, during transit through the stomach and small intestine. Importantly, probiotics must retain the characteristics that give rise to their health effects. Nigeria is endowed with a wide range of fermentable indigenous staple foods that could serve as raw materials for agro-allied industries. A good example is *Mucuna pruriens (L.) DC var. pruriens* a legume consumed and promoted by smallholder farmers in Africa, South America and South Asia as a green manure/cover crop. It is rich in protein (23-35%) and its digestibility is comparable to that of other pulses, like soybean, rice bean and lima bean [5]. Despite its potential, mucuna bean remains a minor food crop. It is poorly adopted in agricultural systems [6]. Velvet bean has gained popularity over the last few years in the natural products market especially the sports nutrition industry. With documented findings it has the ability to increase testosterone and stimulate growth hormone (thereby increasing muscle mass) [7]. It is also showing up as an ingredient in various weight loss, libido, brain/memory, anti-aging, and body builder formulas. The aim of the study is to investigate and assess mucuna beans flour fermented with *Lactobacillus plantarum*, verifying its potential as a probiotic vehicle.

2. MATERIALS AND METHODS

2.1 Source of Organisms

Lactobacillus plantarum used in this study was isolated from traditionally prepared '*ogi*' made from *Zea mays* and *Sorghum bicolor*. Enterotoxigenic strain of *E. coli* (0157:H7) and *Shigella* (72022) were gotten from the stock culture of Medical Microbiology Laboratory of University College Hospital, Ibadan.

2.2 Source of Mucuna Bean

The Mucuna beans (*Mucuna priuriens*) was gotten fresh from the wild in the outskirt of Owo, Ondo State and was authenticated at the Department of Plant Sciences, Olabisi Onabanjo

University, Ago-Iwoye before it was transferred to the laboratory for analysis.

2.3 Source of Experimental Animals

30 albino rats (*Rattus norvegicus*) aged 6 weeks were sourced from the department of animal production and health Federal University of Technology, Akure. They were housed in stainless steel cages under controlled conditions and placed on a basal diet purchased from Top feeds, Sapele, Delta State, Nigeria.

2.4 Reagents / Chemicals

All reagents and chemicals used were of analytical grade and were obtained from the Department of Microbiology, Federal University of Technology, Akure, Ondo State Nigeria.

2.5 Experimental Design

2.5.1 Culturing and harvesting of *Lactobacillus* cells

Overnight broth cultures of test isolates *L. plantarum* were centrifuged at 10,000 rpm for 15 min. The pellets were rinsed out thrice with 10 ml phosphate buffer saline (PBS) into sterilized universal bottles and kept as stock cultures in the refrigerator at 4 ± 2℃. The total viable cells in the stock solution was then determined using serial dilution and pour plate methods.

2.5.2 Preparation of mucuna flour

Seeds of *Mucuna priurens* were boiled in a pressure pot for 30 minutes to soften the seed and make the seed coats easily removable. Then, the peeled beans were boiled and the water changed three times (at 20 minutes interval) to reduce the toxin content of the seed. Thereafter, the beans were rinsed in distilled water, dried and ground to powder to make the mucuna beans flour.

2.5.3 Fermentation and storage

Mucuna beans flour was mixed with distilled water (1:3) in 3 fermentation jars (LP1, LP2) which were autoclaved at 121℃ for 15 min. Jars were allowed to cool after which each jar was inoculated with 10^7 cfu/g each of the test isolates *L. plantarum* and X was uninoculated serving as the control. After thorough mixing, the properly corked jars were incubated anaerobically at 37℃ for 72 h for fermentation to take place [8]. After, fermentation jar LA1 was stored at 4 ± 2℃ while LA2 was stored at 25 ± 2℃ (room temperature) for 14 days respectively. Viable counts of LAB in the products were determined during the period of fermentation and after storage.

2.6 Microbial Analysis

Samples of mucuna beans flour (10g) which were aseptically collected during the fermentation (at 0, 24, 48 and 72 h) and storage (after 14 days at 4 ± 2℃ and 25 ± 2℃ respectively) were used for bacterial enumeration using serial dilution and pour plate method on De Mann Rogosa and Sharpe Agar. Plates were incubated anaerobically at 37℃ for 48 h to determine the best LAB species in terms of growth and survival in mucuna beans flour.

2.7 Physicochemical Analyses

2.7.1 pH, temperature and total titrable acidity

The pH and Temperature of the mucuna sample was determined using Geirincharz thermos pH meter. A mixture of 10 g of the fermented product was used for pH determination as described in [9]. Total titratable acidity (TTA) was determined by titrating 20 ml of the same sample against 0.1 M NaOH.

2.7.2 Proximate composition

The moisture, crude fibre, fat, protein (N×6.25), ash and carbo-hydrate contents of both the fermented and unfermented samples were determined using the method of [9].

2.7.3 *In vitro* studies of gastrointestinal tolerance

Isolate's tolerance to different acidic conditions was tested by centrifuging overnight culture of the test isolate for 10 min at 3000 rpm. The pellet was then resuspended in the same volume of saline solution (9.8 g of NaCl in 1000 ml of distilled water). One milliliter of this dilution (pellet in saline solution) was plated for each of the isolates; this was done so as to estimate the number of viable cells that will be subjected to the acidic pH. Nine milliliter of sterile distilled water that had already been adjusted to pH 2, 3, 4 and 5, using phosphate buffer was transferred into already labeled test tubes, which was done in triplicate for each isolate. Then, 1 ml of the resuspended pellet containing the isolates were

inoculated into the appropriate test tubes, this was shaken and incubated at 37℃ for 3 h. After three hours of incubation, the appropriate dilutions was plated on De Mann Rogosa and Sharpe Agar and incubated anaerobically at 37℃ for 48 h. After subjecting the different isolates to different pH range, the resulting colonies after incubation were counted. The tolerance of the isolates to acidic pH was detected by comparing the number of cfu/g before exposure to the acidic pH with the values after subjection. Also, survival in bile was done by inoculating test isolates into broth containing 10 % of bile which was incubated overnight at 37℃. Then, 1 ml of this culture was plated on MRS agar and incubated for 48 hrs at 37℃, survival in bile is taken as growth on the plates.

2.8 *In vivo* Studies

2.8.1 Experimental animals

The method adopted for the in vivo study was according to [8] with some modifications. 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 6 treatments (I, II, III, IV, V and VI) of 4 rats each. Treatments I and II were not infected, while III and V were infected with *E. coli* (0.3 ml of 10^5 cfu/g daily for 3 days) and IV and VI were infected with *Shigella dysenteriae* (0.2ml of 10^2cfu/g for 3 days). After a 4-day post infection period was observed, diet of treatments IV and VI were supplemented with 10 g each of the fermented sample for 28 days. After feeding on the experimental diet for 4 weeks, all animals were fed the control (basal diet) for a further 14 days. The total weight gain and feacal characteristics (colour and texture), were observed while bacterial enumeration of feacal samples at 0, 7, 14, 21, 28, 35, 42 and 49 days was also determined using conventional techniques. Approximately 1 g of rat feaces was exhaustively extracted with 40 mL of 75% acetonitrile by ultrasonication at room temperature. The supernatant was then evaporated to dryness by a gentle stream of nitrogen at 37℃. The residue was dissolved by 200 µL of 5% methanol and centrifuged at 15,000 × g for further analysis.

2.8.2 Haematological parameters

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).

2.9 Statistical Analysis

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

3. RESULTS AND DISCUSSION

3.1 Physicochemical Changes during Fermentation of Mucuna Beans Flour

There was a significant ($p \leq 0.05$) increase in the total titratable acidity from 0.31% at 0 hr, to 1.98% at 72 hrs for the fermented mucuna beans flour at the end of the fermentation period. While pH values recorded significant decrease ($p \leq 0.05$) in the pH of the sample from 6.75 at 0 hr to 3.75 at 72 hours of fermentation (see Table1). Temperature values ranged between 25 to 27℃. The increase in the acidic contents of the mucuna beans flour during fermentation period which may be as a result of the production of acid by *L. plantarum* this is in accordance with the work of [10] who reported that the production of acids by lactic acid bacteria during fermentation was the major reason for pH lowering in tarhana.

pH of a food has been reported to be important for its keeping quality since low pH discourages the growth of spoilage as well as pathogenic microorganisms [8], therefore the low pH of mucuna beans flour fermented with *L. plantarum* is an indication that it will have a good keeping quality.

3.2 Survival of *L. plantarum* during Fermentation and Storage of Mucuna Beans Flour

During fermentation of mucuna beans flour, the *Lactobacillus* count increased from 61.67 X 10^5 cfu/g (0 hrs) to 88.33 X 10^5 cfu/g (72 hrs), this signifies that the organism thrives well in the flour since the population increased with the time of fermentation (see Table 2).

Table 1. Changes in physicochemical indices of the mucuna flour during fermentation with *L. plantarum* (Values are Mean ± S.E.M)

Time (h)	Temperature	pH	TTA (%)
0	25.17 ± 0.03[a]	6.75 ± 0.02 [c]	0.31 ± 0.00 [a]
24	25.80 ± 0.12[b]	5.55 ± 0.03 [b]	1.25 ± 0.03 [b]
48	26.87 ± 0.03[c]	4.19 ± 0.01 [a]	1.64 ± 0.01 [c]
72	27.07 ± 0.03[c]	3.75 ± 0.02 [a]	1.98 ± 0.02 [c]

Table 2. Survival of the *L. plantarum* in the fermenting of *M. pruriens* flour (Values are Mean ± S.E.M)

Time (h)	*L. plantarum*
0	61.67 ± 0.88 [a]
24	68.67 ± 0.33[b]
48	73.67 ± 2.19[b]
72	88.33 ± 0.67 [c]

Table 3. *Lactobacillus* count (x 10^6 cfu/g) in the fermented mucuna beans flour after 14 days storage at different conditions (Values are Mean ± S.E.M)

Day/ condition	Fridge	Room temperature
0	8.83 ± 0.67	8.83 ± 0.67
14	8.47 ± 0.67	13.67 ± 2.40

As shown in Table 3, at the end of the storage period, there was significant increase in the bacterial counts of the fermented mucuna beans flour stored at room temperature which recorded significant increase from 8.83 x 10^6 (day 0) to 13.67 x 10^6 cfu/g (day 14) compared to the one stored at refrigeration temperature which had 8.83 x 10^6 cfu/g on (day 0) to 8.47x10^6 (day 14) cfu/g. According to [11], in order for lactic acid bacteria to exert their probiotic effects on their host, they should be present in sufficient numbers in the vehicle as at the time of consumption. Also, the number of viable probiotic organism needed to confer benefit on the host varies greatly with the food type and strain of the probiotic organism. However, a viable count of 10^7cfu/g of the bacteria has been recommended as the minimal population of probiotics necessary to give a noticeable effect on the host health [12]. The mucuna beans flour fermented with *L. plantarum* stored at room temperature for 14 days had a significant increase in the bacterial growth which recorded 13.67x10^6 cfu/g at room temperature compared to the ones stored at refrigeration temperature (8.47x10^6 cfu/g) at day 14. The *L. plantarum* used was observed to have considerable increase in cell growth after storage, therefore this satisfies the criterion for good probiotic bacteria. However, to guarantee high survival rate of the probiotic bacteria with the sufficient stability of the vehicle, the probiotic product must be cool during storage [12,4]. The viability of the *L. plantarum* after 14 days storage at refrigeration temperature shows that mucuna beans flour may be a good vehicle for this probiotic bacterium. This has been observed earlier by [8], in their investigation of lima beans flour fermented with *Lactobacillus* species.

3.3 Proximate Composition of Mucuna Beans Flour Fermented with *L. plantarum*

In the proximate composition analyses, there was significant increase in moisture content where (LPM) = mucuna flour fermented with *L. plantarum* recorded (18.56%), while (RWM) raw mucuna flour recorded (9.59%). Protein content significantly increased in the fermented sample (LPM) recorded (35.38%), while (RWM) recorded 29.37%. There was significant decrease in fat, fibre, ash and carbohydrate contents when compared to the control (see Table 4). The high moisture content of the fermented product is a precursor to its perishing ability, since most spoilage bacteria survive well at high moisture level. The reduction in the carbohydrate content of the mucuna beans flour after fermentation may be due to the fact that the *Lactobacilli* use them up as source of energy for their growth and this is in agreement with earlier report of [13], who reported that fermentation process reduces carbohydrate contents of cereal and legume blends. Also the significant increase in the protein content of the products may be due to the increase in cellular mass during fermentation. This has been reported earlier by other researchers during fermentation of different types of food [14,15]. The decrease in the ash content in the fermented mucuna beans flour was due to the leakage of some of the water soluble inorganic matter that penetrated the medium used for the fermentation process [16]. The reduction in the fibre content of the fermented mucuna beans flour is desirable because of the attendant drawbacks of high fibre content in foods especially during weaning which include

reduced digestibility, irritation of gut mucosa, reduced minerals and vitamin availability [17].

Table 4. Proximate composition of mucuna beans flour fermented with *L. plantarum* (Values are Mean ± S.E.M)

Parameter	RWM	LPM
Moisture	9.59 ± 0.10	18.56 ± 0.03
Protein	29.37 ± 0.25	35.38 ± 0.81
Fat	7.14 ± 0.08	3.86 ± 0.08
Fibre	6.54 ± 0.08	3.57 ± 0.03
Ash	4.35 ± 0.13	3.97 ± 0.03
Carbohydrate	51.92 ± 0.62	46.35 ± 0.14

Key: LPM= Mucuna flour fermented with L. plantarum, RWM= Raw mucuna flour

3.4 Enumeration of Faecal Analysis of Microorganisms

At the end of the 49 days of feeding, results obtained from the faecal analysis shows that there was significant increase ($p \leq 0.05$) in the number of *E. coli*, *Enterobacteria* and *Lactobacilli* in groups fed with the experimental diet (Fig. 1) while the number of *Shigella* significantly decreased. Fig. 2 recorded an increase in *Lactobacilli, E. coli* and *Enterobacteria,* while *Shigella* showed significant decrease ($p \leq 0.05$). Meanwhile, Fig. 3 showed difference in the bacterial count where there was significant increase ($p \leq 0.05$) in the number of *E. coli*, *Enterobacteria* and *Lactobacilli*, but *Shigella* significantly decreased ($p \leq 0.05$). In Fig. 4, at this point the forth treatment applied during the in vivo feeding trial showed significant increase ($p \leq 0.05$) in the number of *Shigella* and *E. coli,* and a decrease in *Lactobacilli* when compared with the control groups (I, II and III). During the fifth treatment applied in the in vivo feeding trial period (Fig. 5), *Lactobacilli*, *E. coli* significantly increased ($p \leq 0.05$) while the number of *Enterobacteria* decreased ($p \leq 0.05$) significantly compared with the control groups (I, III, IV).

At the end of the sixth treatment, (Fig. 6), results obtained showed significant increase in *Lactobacilli*, *Shigella*, *Enterobacteria* and *E. coli* significantly increased ($p \leq 0.05$). Moreover, rats infected with the pathogens without giving them experimental diet showed the symptoms of wet-loose faeces and weakness and bloody diarrhea throughout the experimental period whereas in the groups infected with pathogen then fed with fermented mucuna beans flour diet showed those symptoms only during the first 17 days and 21 days of infection which disappeared rapidly after they were given the probiotic food. There were no symptoms of diarrhea in the rats fed with the basal diet and those fed with the experimental diet alone throughout the experiment period. This is in line with previous report of [18] who reported the use of some probiotic *Lactobacillus* strains in the dairy industry to reduce the incidence of traveller's diarrhea and to promote recovery from acute diarrhea. The lower number of *E. coli* and *Shigella* dysenteriae as well as other *Enterobacteriaceae* observed in this study was from the microbial analyses of the faecal samples of the rats showed that their growth might have been inhibited by the presence of the *Lactobacillus* species. Also, they may have actually been killed by secretions from the *Lactobacillus* species as most of them have been reported to be capable of producing biocidal substances.

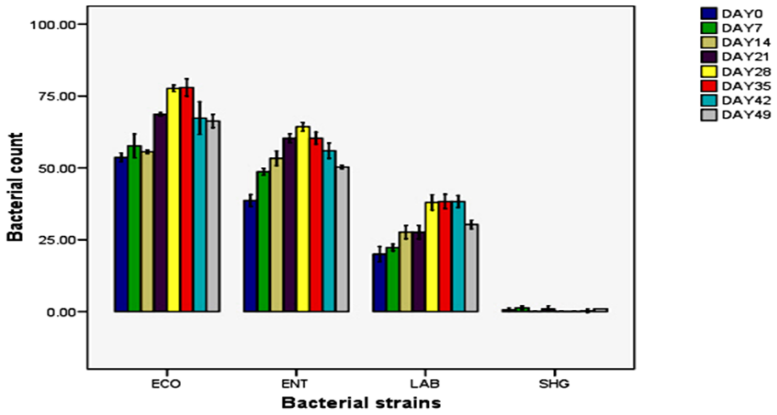

Fig. 1. Bacterial count of treatment I during *in vivo* feeding trial (10^5 cfu/ml)

Key: I= rats fed with basal diet only, LAB= Lactobacillus sp, ECO= E. coli, ENT= other Enterobacteria, SHG= Shigella dysenteriae

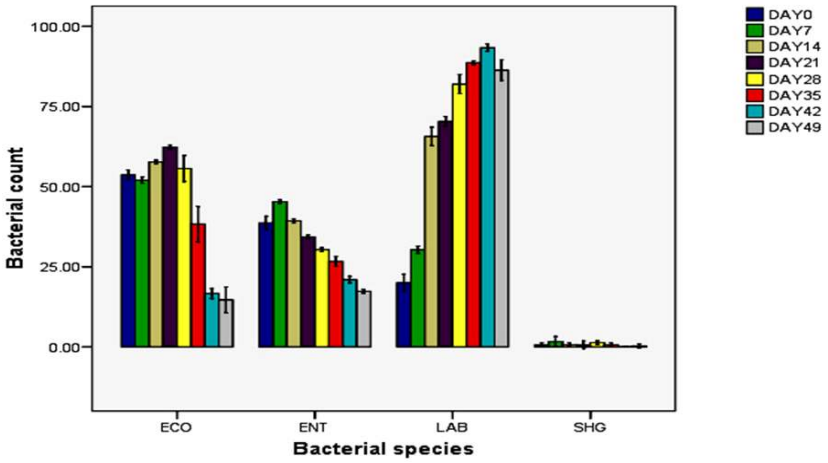

Fig. 2. Bacterial count of treatment II during *in vivo* feeding trial (10^5 cfu/ml)
Key: II= rats fed with fermented mucuna beans only, ECO= E. coli, LAB= Lactobacillus sp, SHG= Shigella dysenteriae, ENT= Other Enterobacteria

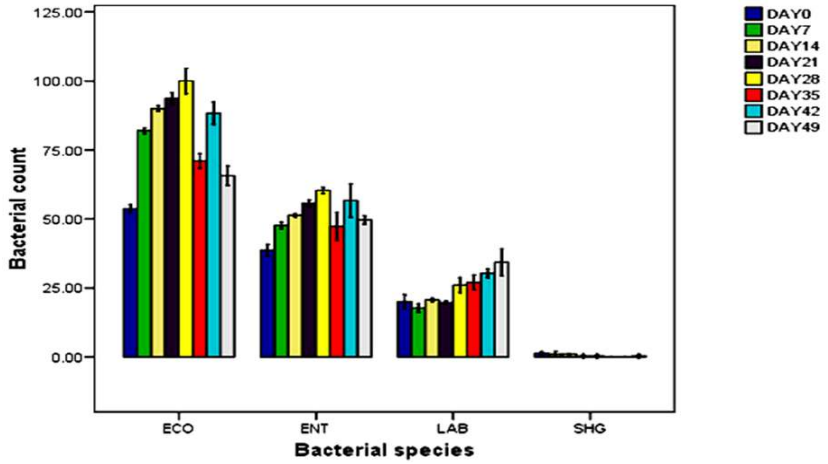

Fig. 3. Bacterial count of treatment III during *in vivo* feeding trial (x10^5 cfu/ml)
Key: III= Rats infected with E. coli without further treatment, ECO= E. coli, ENT= Other Enterobacteria LAB= Lactobacillus sp, SHG= Shigella dysenteriae

Table 5. Weight of rats fed with mucuna beans flour fermented with different strain of Lactic acid bacteria (Values are Mean ± S.E.M)

Groups /Days	I	II	III	IV	V	VI
0	71.00 ± 0.00	71.00 ± 0.00	71.67 ± 0.33	72.00 ± 0. 00	73.00 ± 0.00	74.33 ± 0.33
7	84.33 ± 0.33	84.00 ± 0.00	85.33 ± 0.33	85.67 ± 0.88	86.00 ± 1.15	87.33 ± 0.67
14	98.00 ± 0.58	100.33 ± 0.58	82.67 ± 0.33	75.67 ± 0.33	82.00 ± 1.53	75.00 ± 1.00
21	110.67 ±0.88	127.33 ± 1.20	74.67 ± 1.20	70.33 ± 0.33	89.67 ± 0.33	83.67 ± 0.88
28	138.67 ±1.33	160.33 ± 1.45	69.33 ± 0.33	67.33 ± 0.33	97.67 ± 0.33	92.67 ± 0.33
35	161.00 ±0.58	181.67 ± 1.45	77.33 ± 0.33	75.67 ± 0.88	116.67±0.67	117.33±1.20
42	172.33 ±1.33	198.33 ± 0.33	96.00 ± 1.15	92.00 ± 1.53	148.33±0.33	140.67±0.33
49	192.33 ±1.45	220.00 ± 1.53	112.33±0.88	105.33 ±2.33	171.67±0.67	166.33±0.88

Key: I= rats fed basal diet only, II= rats fed with fermented mucuna flour only. III= rats infected with E. coli without treatment. IV= rats infected with Shigella without treatment. V= rats infected with E. coli and then fed with L. plantarum fermented mucuna flour, VI= rats infected with Shigella and then fed with L. plantarum fermented mucuna flour

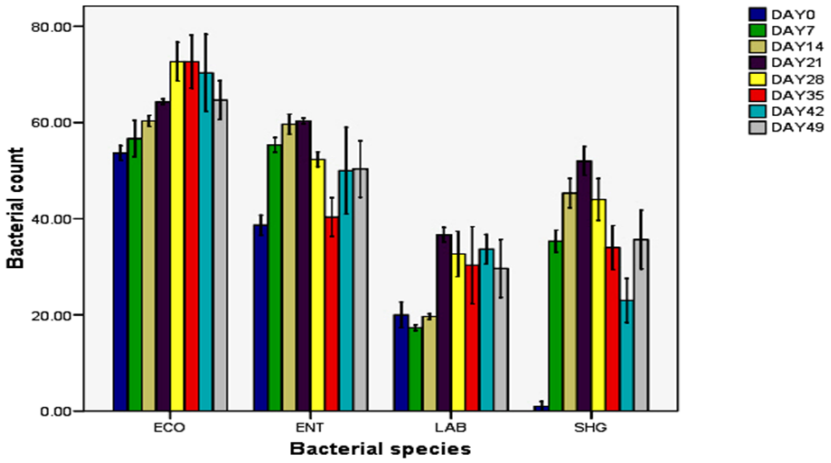

Fig. 4. Bacterial count of treatment IV during *in vivo* feeding trial (10^5 cfu/ml)

Key: IV= Rats infected with Shigella dysenteriae without further treatment, ECO= E. coli, ENT= Other Enterobacteria LAB= Lactobacillus sp, SHG= Shigella dysenteriae

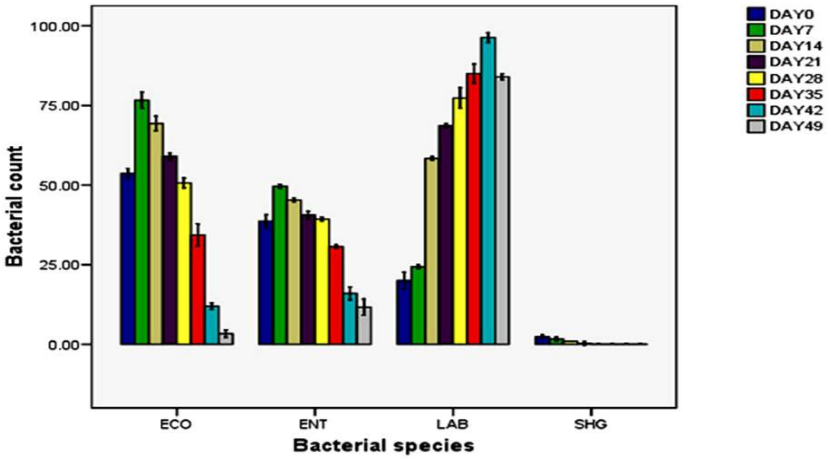

Fig. 5. Bacterial count of treatment V during *in vivo* feeding trial

Key: V= Rats infected with E. coli and then fed with mucuna beans flour fermented with L. plantarum, ECO= E. coli, LAB= Lactobacillus sp, SHG= Shigella dysenteriae, ENT= Other Enterobacteria

Table 6. Haematological parameter of rats fed with mucuna beans flour fermented with *L. plantarum* (Values are Mean ± S.E.M)

Treatment	RBC	WBC	PCV	Haemoglobin	Platelet
I	6.84 ± 0.04	8.58 ± 0.08	39.20 ± 0.35	12.80 ±0.40	167.33 ± 2.73
II	8.65 ± 0.23	7.20 ± 0.11	44.83 ± 1.04	15.27 ± 0.23	264.00 ± 9.29
III	5.95 ± 0.15	18.12 ± 0.44	29.60 ± 0.25	10.23 ± 0.26	133.67 ±2.60
IV	5.63 ± 0.20	22.56 ± 0.52	33.17 ± 0.52	9.50 ± 0.15	137.33 ±8.41
V	6.97 ± 0.03	11.51 ± 0.13	35.47 ± 0.83	12.00 ± 0.15	173.33 ±9.28
VI	6.87 ± 0.06	12.06 ± 0.16	37.63 ± 0.12	11.73 ± 0. 09	180.00 ±11.59

Key: I= rats fed basal diet only, II= rats fed with fermented mucuna flour only. III= rats infected with E. coli without treatment. IV= rats infected with Shigella without treatment. V= rats infected with E. coli and then fed with L. plantarum fermented mucuna flour, VI= rats infected with Shigella and then fed with L. plantarum fermented mucuna flour

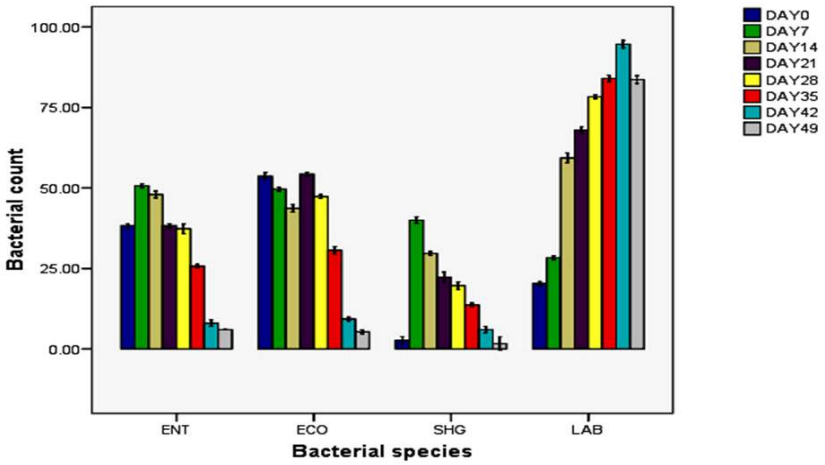

Fig. 6. Bacterial count of treatment VI during *in vivo* feeding trial (10^5 cfu/ml)

Key: VI= Rats infected with Shigella dysenteriae and then fed with mucuna beans flour fermented with L. plantarum, ECO= E. coli, LAB= Lactobacillus sp, SHG= Shigella dysenteriae, ENT= Other Enterobacteria

3.5 Weight Performance and Haematological Parameters of Rats Fed with Mucuna Beans Flour Fermented with *L. plantarum*

The results of the effect of the fermented mucuna beans flour on the body weight of the animals compared with control is shown in (Table 5). There were significant differences (P≤ 0.05) in the weight of animals in treatment (II) compared to the control group (I) while there was significant increase in the weight of the rats infected with pathogens and then fed with the fermented mucuna beans flour compared to those infected without control. Also, the haematological analyses of the rats blood showed significant (p≤0.05) difference between the WBC counts of the group infected without further treatment (III, IV) compared to the other groups. Also, the RBC, Platelet, PCV and haemoglobin count of the infected group without further treatment was significantly different at (p≤0.05), which was observed to be lower than that of the other groups (Table 6). Furthermore, all the internal organs of the infected rats without further treatment (III, IV) were significantly (p≤0.05) lower in weight than the other groups. This is in agreement with the work of [19] who reported that that *L. plantarum* increases the levels of α-3 unsaturated fatty acids in foods thus protecting such foods from multiplication of harmful bacteria. Another report by [8] supported the fact that it might be responsible for faster increase in the body mass in animals fed with fermented products. Haematopoeitic system is one of the most important indexes of physiological and pathological status in man and animals [20]. The haematological parameters of all the groups infected with the pathogens and then fed with the experimental diets as well as those fed with basal diet, those fed only with experimental diet falls within the laboratory permissible limits compared to the groups infected with the pathogens and not fed with the experimental diet which is far outside the permissible limits. This shows that the mucuna beans flour fermented with *Lactobacillus* species helps in quick recovery from infections caused by these organisms.

4. CONCLUSION

The application of probiotics as growth enhancer in prevention intestinal disorders as well as disease control has been in existence from early periods [21,22]. Probiotics are now used as replacement for the widely used antibiotics and synthetic chemical feed supplements. This is because, of their ability to inhibit the growth of pathogenic organisms and also by enhancing absorption of nutrients [23]. From the results of this study, there is great amount of evidence suggesting that *L. plantarum* showed good growth and survival in mucuna beans flour. Moreover, it had positive effect on the intestinal flora balance of the rats as well as enhancing their growth as revealed in their body weight gain and haematological parameters. Therefore, mucuna beans flour fermented with *Lactobacillus plantarum* could serve as an ideal probiotic food.

DISCLAIMER

The title of this manuscript was presented in the conference "2nd International Conference on Infectious Diseases & Diagnostic Microbiology". Available link is: "http://diagnosticmicrobiology.conferenceseries.com/abstract/2015/evaluation-of-mucuna-beans-flour-fermented-with-lactobacillus-plantarum-as-a-probiotic-food" Sep 11-13, 2017 Dallas, USA.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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