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Faecal Strains of *Lactobacillus acidophilus* Prevents Diarrhoea and Improved the Health of Rats Challenged With Clinical Strain of *Shigella dysenteriae*

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Abstract: The ability of faecal strains of *Lactobacillus acidophilus* source from faeces of albino rat (Lac A), human baby (Lac B) and pig (Lac P) to prevent diarrhea induced by *Shigella dysenteriae* in rats is reported. These faecal strains of *Lactobacillus acidophilus* were found to be effective in preventing *Shigella dysenteriae* induced diarrhea which was obvious in the Control (C) group alone. The probiotic lactobacilli also significantly improve Feed Conversion Ratio (FCR) and Total Weight Gain (TWG) of the rats. In terms of feed consumption, rats treated with *Lactobacillus acidophilus* consumed 11.15 to 17.58% feed less than C to achieve the same weight. The results of serum enzymes also reveal that there was no sign of toxicological effect when probiotic lactobacillus was administered to the rats. The serum Alkaline Phosphatase (ALP) of rats dosed with Lac A, Lac B and Lac P was lower and significantly different ($p<0.05$) when compared with C.

Key words: Faecal strain, *Lactobacillus acidophilus,* diarrhoea, health, *Shigella dyrenteriae*

INTRODUCTION

Gastrointestinal diseases are often triggered when there is disruption of the complex ecosystem of the gastrointestinal tract[1]. The administration of antibiotic and aetiological agents such as toxigenic *Escherichia. coli Salmonella enteritidis, Entamoeba histolytical* and viruses are the most common agents responsible for acute diarrhoea. Infectious diarrhoea is a major world health problem, which is responsible for several million deaths each year[2]. There are reports that by administering probiotics the intestinal flora can be restored to optimal levels by allowing beneficial bacteria to attach to the wall and crowding out harmful bacteria[3,4]. The strongest evidence of a beneficial effect of defined strains of probiotics has been established using *Lactobacillus rhamnosus* GG and *Bifidobacterium lactics* BB-12 for prevention and treatment of acute diarrhoea caused by rotaviruses in children[5-7]. The suggested mechanisms by which probiotics control intestinal pathogens include: production of antimicrobial substances; competition for nutrients; competitive exclusion of pathogens and modulation of the immune system[2].

Shigella dysenteriae has been responsible for epidemics of severe bacillary dysentery in tropical countries[8]. Shigellosis has not attracted the attention that other food-borne enteric pathogens have, but such evidence as available suggests that their survival characteristics are in fact similar to other members of the enterobactericeae[8]. The purpose of this study was therefore to evaluate the efficacy of faecal strains of *Lactobacillus acidophilus* in preventing *Shigella dysenteriae* induced diarrhoea and the improvement of health of rats (*Rattus norvegicus*) infected with the pathogen.

MATERIALS AND METHODS

Lactobacillus strains: Faecal strains of *Lactobacillus acidophilus* were isolated from faeces of human baby, pig and albino rat (*Rattus norvegicus*) on de Mann Rogosa and Sharpe (MRS) agar (Lab M). Rough and crab like colonies were further characterized by cultural, morphological and biochemical properties[9]. Preliminary screening shows that the isolates can inhibit food spoilage and pathogenic bacteria associated with gastroenteritis. The isolates were cultured in MRS broth (Lab M) and incubated at 37°C for two days to obtain large cell concentration. The cells were lyophilized using the method of Fujiwara *et al.*[10]. The concentration of the viable cell (Table 1) was determined by serial dilution techniques[11]. The isolates were designated Lac A (*Lactobacillus acidophilus* isolated from albino rat faeces), Lac B (*Lactobacillus acidophilus* isolated from human baby faeces), Lac P (*Lactobacillus acidophilus* isolated from pig faeces).

Source of *Shigella dysenteriae*: Clinical isolate of *Shigella dysenteriae* was obtained from the culture collection of Obafemi Awolowo University, Ile Ife,

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Table 1: Source/concentration of faecal strains of *Lactobacillus acidophilus*

Isolates	Source/Strain designation	Cell concentration (cfu g^{-1})*
Lactobacillus acidophilus	Albino rat faeces/Lac A	9×10^{10}
Lactobacillus acidophilus	Baby faeces/Lac B	2×10^{10}
Lactobacillus acidophilus	Pig faeces/Lac P	6×10^{10}

*Values are mean of three replicates

Table 2: Composition of growers mash used for acclamatisation

Ingredients	Level in diet
Crude protein	14.5%
Cured fat	4.8%
Crude fibre	7.2%
Crude ash	8.0%
Calcium	0.8%
Phosphorus	0.62%
Lysine	0.6%
Methionine	0.29%
Vit. A	8,000 iu
Vit. D3	2,400 iu
Vit. E	15 mg
Vit. B2	40 mg
Vit.C	50 mg
Manganese	30 mg
Zinc	30 mg
Sodium	0.15%
Metabolisable energy	2,300 kcal kg^{-1}

Source: Product of Bendel feed, Edo State, Nigeria

Nigeria. The culture was kept on fresh nutrient agar (Oxoid) slant and stored in a refrigerator (5°C) before use.

Source of experimental rats: Sixteen four weeks old albino rats (*Rattus norvegicus*) were obtained at the Department of Physiology, University of Ibadan, Nigeria. The rats placed on commercial feed (Table 2) for a week to acclimatize. At the end of the acclimatization period, the rats weighed between (60-80 g).

***In vivo* feeding:** The rats were divided into 4 groups of 4 rats each. Groups Lac A, Lac B and Lac P were respectively orogastrically dosed with 0.3 mL of the cell concentration of the culture in Table 1 and in addition 0.3 mL of 10^5 cfu mL^{-1} of *Shigella dysenteriae*. The control, group C was orogastrically dosed with 0.3 mL of 10^5 cfu mL^{-1} of *Shigella dysenteriae* alone. The treatment above was repeated the second day. The rats were further fed for 18 days after the treatment above. The initial and final weights of the rats were recorded and the data gathered was used to calculate final weight gain and feed conversion ratio. The rats were slaughtered by cervical dislocation and the blood collected into an EDTA bottle for analysis of serum enzymes.

Analysis of serum enzymes: Serum Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel[12]. The Bessy Lowry and Brock alkaline phosphate method was used for the analysis of serum alkaline phophatase[13].

Faecal characteristics of rats: Faecal characteristics such as colour and texture were observed in the faeces of rats in the different treatments. The faecal samples were collected prior to ingestion (day 0) and up to 5 days after ingestion.

Data analysis: The data gathered from weight measurement and serum biochemical enzymes were processed using one-way analysis of variance (ANOVA). Means were compared using Dunnet t-tests ($p<0.05$)

RESULTS

The results of Table 3 showed that the serum ALP of rats in the control (C) group that was experimentally infected with *Shigella dysenteriae* was higher (150 iu L^{-1})

Table 3: Level of serum enzymes in rats

Treatments	ALP	AST	ALT
C	150.00±0.00*	36.47±1.99*	19.63±2.50
Lac A	56.67±3.10	20.00±7.63	21.57±7.01
Lac B	30.00±5.00	22.23±7.13	12.00±1.90
Lac P	25.00±5.00	30.63±7.45	23.23±6.53

Values are mean and SEM of four replicates. * Values along column are highed and significantly different ($p<0.05$) from other treatments

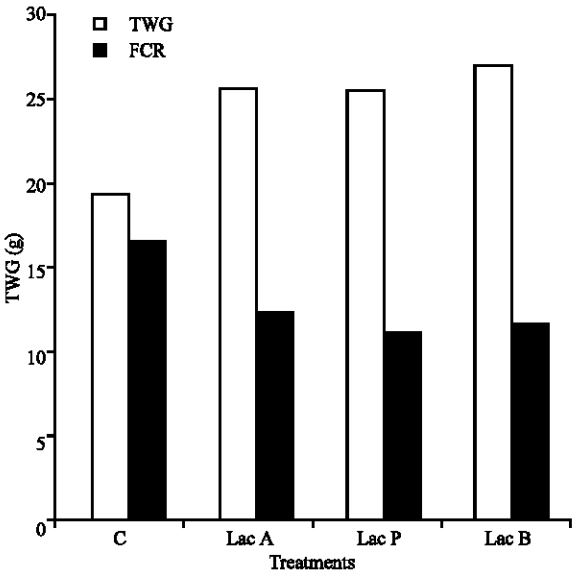

Fig. 1: Growth performance of rats fed faecal strain of *Lactobacillus acidophilus* and simultaneously infected with clinical strain of *Shigella dyseteria* (TWG: Total Weight Gain; FCR: Feed Conversion Ratio)

Table 4: Faecal characteristics of rats within the first five (5) days of treatments

Treatments	Colour	Texture	Appearance	Comment
C	Light brown	Wet, loose	Watery	Sign of diarrhoea
Lac A	Light brown	Soft, moist	Pelleted	No sign of diarrhoea
Lac B	Light brown	Soft, moist	Pelleted	No sign of diarrhoea
Lac P	Light brown	Soft, moist	Pelleted	No sign of diarrhoea

and significantly different ($p<0.05$) from the other treatments that were orogastrically dosed with faecal strains of *Lactobacillus acidophilus* in addition to experimental infection with *Shigella dysenteriae*. The rats treated with *Lactobacillus acidophilus* (Lac A, Lac B and Lac P) had a serum ALP that ranges between 25.0 to 56.67 μL^{-1}. Similarly; the serum AST of C was higher and significantly different ($p<0.05$) from the other treatments (Lac A, Lac B and Lac P). However, the serum ALT of treatments Lac A and Lac P were slightly higher than C but not significantly different ($p>0.05$). Serum ALT of Lac B was the lowest (12.0 μL^{-1}). The Total Weight Gain (TWG) of rats dosed with *Lactobacillus acidophilus* was higher though not significantly different from what was observed for C (Fig. 1). The same trend was observed in the Feed Conversion Ratio (FCR) that was better in rats dosed with *Lactobacillus acidophilus* (Lac A, Lac B and Lac P) than what obtains in C.

DISCUSSION

A number of studies exist suggesting the desirable effect of probiotic lactobacilli on the health of animals and man. Most of these trials only measure growth stimulation, ability to prevent diarrhoea caused by *Salmonella* sp., *E. coli* and rotaviruses[1,7]. However, there is little or no report on the ability of *Lactobacillus* species to prevent gastroenteritis as a result of *Shigella dysenteriae*. This pathogen has been reported as the major cause of severe bacillary dysentery in tropical countries[8]. This study was carried out in order to assess the efficacy of faecal strain of *Lactobacillus acidophilus* in preventing diarrhoea induced by clinical strains of *Shigella dysenteriae* in rats. Monitoring of the growth performance and some serum biochemical enzymes of the treated rats was also used to assess the probability of conferring any health benefit.

The results of biochemical enzymes of rats' serum show that the serum ALP of rats challenged with clinical isolates of *Shigella dysenteriae* (C) was higher and significantly different ($p<0.05$) when compared with rats that were challenged with the pathogen and simultaneously dosed *Lactobacillus acidophilus*. Serum ALP levels are known to be elevated in diseases of bone, placenta and intestine[13]. The observation above is an indication that faecal strains of *Lactobacillus acidophilus* may be effective in preventing disorders that are associated with elevated serum ALP.

Serum AST was also higher in C (36.47 iu L^{-1}) and significantly different ($p<0.05$) from other treatments (Lac A, Lac B and Lac P) that had between 20.00 to 30.63 iu L^{-1}. AST is an enzyme that increases in activity in diseases such as severe bacterial infections, malaria, pneumonia, pulmonary infarcts and tumours of organs such as heart and muscle[13,14]. A lower serum AST observed in rats dosed with faecal strains of *Lactobacillus acidophilus* confirms its ability to suppress the pathogenic activity of *Shigella dysenteriae* in the intestine of the rats in treatments Lac A, Lac B and Lac P. A further confirmation of this was observed in rats that were challenged with the pathogen, *Shigella dysenteriae* (C). The stool of rats in this treatment (C) was watery within the first 5 days (Table 4). The serum ALT in all the treatments was not significantly different ($p>0.05$). ALT is principally found in the liver and is regarded as being more specific than AST for detecting liver cell damage[13,14]

The rats treated with faecal strains of *Lactobacillus acidophilus* performed better in terms of TWG and FCR (Fig. 1). Feed Conversion Ratios (FCR) of these rats were 12.38, 11.66 and 11.16 for treatments Lac A, Lac B and Lac P, respectively when compared with C (16.57). This implies that in terms of feed consumption, the rats treated with probiotic *Lactobacillus acidophilus* consumed 16.15% (Lac A); 17.58% (Lac B) and 16.67% (Lac P) feed less than C to achieve the same weight. Earlier reports by Francisco *et al.*[15] and Oyetayo[16] shows that probiotic lactobacilli had an increasing effect on body weight and feed FCR in piglets and albino rats.

These results show that faecal strain of *Lactobacillus acidophilus* can prevent gastroenteritis induced by clinical strain of *Shigella dysenteriae* and also improve the performance of the rats challenged with the pathogen. There was no sign of pathogenic/toxicological effect when these strains of *Lactobacillus* were administered to the experimental rats. The ability of these faecal isolates to prevent *Shigella dysenteriae* induced gastroenteritis may suggest their suitability as an effective bio-therapeutic agent in place of antibiotics in the prevention and treatment of bacillary dysentery caused by *Shigella dysenteriae*.

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