

Haematological Studies of Rats Fed Different Doses of Probiotic, *Lactobacillus plantarum*, Isolated from Fermenting Corn Slurry

F.I. Aboderin¹ and V.O. Oyetayo²

¹Haematology Unit, College of Health Sciences, Obafemi Awolowo University, Ileife, Nigeria

²Department of Microbiology, Federal University of Technology, Akure, Nigeria

Abstract: The effect of oral administration of different doses of probiotic, *Lactobacillus plantarum*, isolated from fermenting corn slurry on the haematological parameters of wistar albino rats was investigated. Sixteen (16) rats divided into four groups (A, B, C and D) of four rats per group were used. Group A was placed on the basal diet alone, while group B, C and D were placed on the diet and also dosed with 0.3ml, 0.6ml, and 1.0ml of 10^7 cfu/ml of reconstituted lyophilized culture of *Lactobacillus plantarum* respectively. In rats treated with different doses of *Lactobacillus plantarum* there was a significant increase in the packed cell volume (PCV), haemoglobin and red blood cell (RBC) when compared with the control. Moreover, the differential leucocyte counts reveal an increase in the lymphocyte count of the rats dosed with *Lactobacillus plantarum* when compared to the control except in rats dosed with 1.0ml of 10^7 cfu/ml of the probiotic, which was not significantly different ($P < 0.05$) from the control. There was also a significant increase ($P < 0.05$) in the weight gain by rats fed *Lactobacillus plantarum* when compared to the control. The data obtained showed that *Lactobacillus plantarum* isolated from fermenting corn slurry is safe and it has immunostimulatory effect and can also improve the performance of rats in terms of weight gain.

Key words: *Lactobacillus plantarum*, rats, haematology

Introduction

Probiotic lactobacilli are known to confer an array of health promoting activities on their host after either parenteral or oral administration (Waard *et al.*, 2001; Oyetayo *et al.*, 2003). Some of their beneficial effects include prevention of intestinal infection (Tannock, 1983; Casas and Dobrogosz, 2000), anticarcinogenic activity (Fuller and Gibson, 1997), control of serum cholesterol (Bertazzoni *et al.*, 2001), enhancement of immunity (Aattouri *et al.*, 2001), growth enhancement of animals (Baird, 1977; Chang *et al.*, 2001). The mechanism by which these probiotics affect their host and bring about improvement in the gut barrier can be due to competition for adhesion sites, production of inhibitory compounds, and rebalancing of disturbed gastrointestinal microbial composition and metabolism (Waard *et al.*, 2001, FAO/WHO, 2001).

Lactobacilli have a long history of use as probiotics without established risk to humans (Naidu *et al.*, 1999). No pathogenic or virulence properties had been associated with lactobacilli, bifidobacteria or lactococci (Aguirre and Collins, 1993). There is however reports that under certain conditions some lactobacilli strains have been associated with adverse effects, such as rare cases of bacteremia (Saxelin *et al.*, 1996). It has been observed that lactic acid bacteria may invade the host body by bacterial translocation or other routes causing bacteremia (Berg, 1983; Boersma *et al.*, 2000). However, a study showed that there is no increased incidence or frequency of bacteremia with increased

usage of probiotic lactobacilli (Salminen *et al.*, 2001). The reports above thus make it necessary to ascertain the safety of any organism with probiotic properties. Previous study using this strain of *Lactobacillus plantarum* shows that it can antagonize both pathogenic and food spoilage bacteria, adhere to the ileal epithelial cells of rats, hepatoprotective ability by lowering serum aminotransferase level and anticholesterolaeamic effect (Oyetayo and Osho, 2004). To further confirm the safety of ingestion of this probiotic, a study was designed to investigate the effect of *Lactobacillus plantarum*, on the haematological parameters of healthy rats. A haematological study is a valuable diagnostic tool in evaluating human health (Cheesbrough, 1991). The aim of the present study was therefore to gain insight into the effect of orally administered *Lactobacillus plantarum* on the haematological parameters of rats. Moreover, the effective dose that will bring about health promoting effect in rats was also studied.

Materials and Methods

Source of *Lactobacillus plantarum*: *Lactobacillus plantarum* was isolated from fermenting corn slurry on deMann Rogosa and Sharpe (MRS) agar (LAB M). The isolate was characterized using colonial, morphological and biochemical properties (Parker and Collier, 1990), and tentatively identified as *Lactobacillus plantarum*. The isolate was cultivated in MRS broth (LAB M) for 2 days and lyophilized using the method described by Oyetayo *et al.* (2003).

Aboderin and Oyetayo: Assessing the safety of *Lactobacillus plantarum* using haematological parameters

Table 1: Composition of basal diet

Ingredients	Level in diet
Crude protein	14.5%
Crude 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.	8,000iu
Vit. D ₃	2,400iu
Vit. E	15mg
Vit. B2	40mg
Vit.C	50mg
Manganese	30mg
Zinc	30mg
Sodium	0.15%
Metabolisable energy	2,300kcal./kg.

Source: Product of Bendel feed, Edo State, Nigeria.

In vivo feeding trial: Sixteen (16) wistar albino rats (*Rattus norvegicus*) aged 5 to 6 weeks with average weight of 85g were obtained from the animal section of Faculty of Health Sciences, Obafemi Awolowo University, Ile Ife, Nigeria. The rats were acclimatized on basal diet (Table 1) for one week *ad libitum* before treatment. The rats were randomly assigned to four groups (A, B, C and D) of four rats per group. Group A was placed on the basal diet alone, while group B, C and D were placed on the diet and also dosed with 0.3ml, 0.6ml, and 1.0ml of 10^7 cfu/ml of reconstituted lyophilized culture of *Lactobacillus plantarum* respectively. The treatment above was repeated every seven 7days for a period of 4 weeks (28 days).

The daily weight gain and final weight gain of the rats were monitored throughout the experimental period after which the rats were killed by cervical dislocation.

Blood analysis: Blood samples were collected from rats by cardiac puncture into EDTA bottles. Auto haematological analyzer, ADVIA 60 (Bayer Bayer, Germany) was used to analyse the following blood parameters: Packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), platelets (Plts), red blood cell (RBC), differential leucocytes (neutrophil, eosinophil, basophil, monocytes and lymphocytes), mean cell haemoglobin concentration (MCHC), mean cell volume (MCV) and mean cell haemoglobin (MCH).

Statistics: Data gathered from haematological parameters and weight gain were analysed using one way analysis of variance. Means were compared using Duncan multiple range test. All statistical analyses were performed with SPSS software version 10.

Results

Full blood count: The full blood count of rats is presented in Table 2. The PCV of rats dosed with *Lactobacillus plantarum* (B, C, D) was higher and significantly different ($P < 0.05$) when compared to the control (A). The same trend was also observed in the Hb and RBC. However, the WBC count was highest in control (A) and significantly different from the other treatments. There was no significant difference in the Plts count in treatments A and B however, the Plts counts were higher and significantly different ($P < 0.05$) in treatments C and D when compared to the control.

Differential leucocyte count: The differential leucocyte count is presented in Table 3. The neutrophil count in the control (A) was higher and significantly different ($P < 0.05$) when compared to the rats treated with *Lactobacillus plantarum*. Moreover, the eosinophil count of rats in control group (A) was the same with group C but higher than what was observed in group B. The highest eosinophil count was however recorded in group D. There was no significant difference in the basophil and monocytes counts in all the groups. The lymphocyte counts were however, higher in the rats fed *Lactobacillus plantarum* (B and C) when compared with the control (A).

Total weight gain (TWG): Fig. 1 shows the TWG of rats. The TWG of the rats was highest in group D. The TWG was higher in the rats orogastrically dosed with *Lactobacillus plantarum* when compared with the control (A).

Discussion

There has been a number of studies that reveal the probiotic potential of lactobacilli as health promoting bacteria in man and animals (FAO/WHO, 2001). In the last two decades the potential of probiotic lactobacilli to restore gastrointestinal health has received interest. *Lactobacillus plantarum* is one of the bacteria associated with the fermentation of corn in the production of *Ogi*, a fermented corn product that is popularly consumed in Nigeria (Akinrele *et al.*, 1970). Locally, in the southern part of Nigeria, water obtained from the fermenting corn slurry is used in treating incidences of diarrhea in goats (Oyetayo and Osho, 2004).

To assess the safety of this probiotic bacteria, *Lactobacillus plantarum*, it was fed to wistar albino rats and the haematological parameters of the rats was observed.

The results of the haematological parameters shows that rats dosed with *Lactobacillus plantarum* show signs of better health based on their haematological status and performance in terms of weight gain. In a previous study, Zhou *et al.* (2000) reported that 4 weeks consumption of *L. rhamnosus* HN001 (DR20), *L. acidophilus* HN017, and *Bifidobacterium lactics* HN019

Aboderin and Oyetayo: Assessing the safety of *Lactobacillus plantarum* using haematological parameters

Table 2: Full Blood Counts of Rats Orogastrically Dosed With Different Concentrations of *Lactobacillus plantarum* *

Full Blood Count	A	B	C	D
PCV (%)	33.33 ^a ±2.45	44.10 ^b ±1.11	43.00 ^b ±2.68	40.00 ^{ab} ±2.55
Hb (g/dl)	10.75 ^a ±0.48	14.25 ^c ±0.48	13.65 ^b ±0.69	13.18 ^b ±0.69
WBC (mm ³)	5.98 ^c ±1.43	3.45 ^{ab} ±0.62	3.18 ^a ±0.34	3.43 ^{ab} ±0.36
Plts (mm ³)	294.75 ^a ±22.37	293.50 ^a ±51.94	408.50 ^{ab} ±28.69	456.75 ^b ±34.26
RBC (mm ³)	7.21 ^a ±0.09	10.15 ^c ±0.16	8.34 ^{ab} ±0.79	9.04 ^{bc} ±0.64
MCH (l/pg)	17.88 ^a ±0.43	17.78 ^a ±0.20	17.95 ^a ±0.36	17.25 ^a ±0.10
MCV (l/m ³)	60.00 ^b ±1.83	54.00 ^a ±0.41	55.75 ^a ±1.11	53.50 ^a ±0.65
MCHC (g/dl)	31.40 ^a ±0.21	32.23 ^{ab} ±0.35	32.30 ^{ab} ±0.09	32.98 ^b ±0.61

*Values are means and standard errors for 4 rats per treatment. Means along the row with different superscript are significantly different (P < 0.05).

Table 3: Differential Leucocytes Counts of Rats Orogastrically Dosed With Different Concentrations of *Lactobacillus plantarum* *

Differential Leucocytes counts (%)	A	B	C	D
Neutrophil	15.75 ^b ±1.49	7.75 ^a ±0.85	12.00 ^{ab} ±0.31	13.75 ^{ab} ±3.28
Eosinophil	0.25 ^{ab} ±0.25	0.00 ^a ±0.00	0.75 ^{ab} ±0.25	2.00 ^b ±1.08
Basophil	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00	0.00 ^a ±0.00
Monocytes	1.25 ^a ±0.48	0.75 ^a ±0.25	0.50 ^a ±0.00	1.50 ^a ±0.29
Lymphocytes	82.75 ^a ±1.80	91.50 ^b ±0.65	86.75 ^{ab} ±0.83	83.75 ^a ±2.43

*Values are means and standard errors for 4 rats per treatment. Means along the row with different superscript are significantly different (P < 0.05).

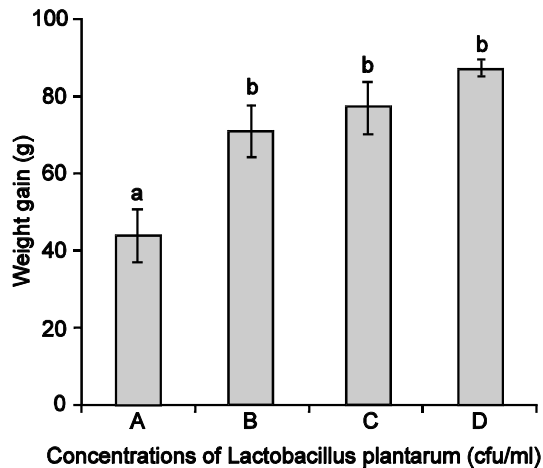


Fig. 1: Weight gain of rats orogastrically dosed with different concentrations of *Lactobacillus plantarum*. Bars with different superscript are significantly different (P<0.05)

(DR10) strains had no adverse effects on mice general health status, haematology, blood biochemistry, gut mucosa histology or the incidence of bacterial translocation.

There was a general increase in the counts of Hb, PCV and RBC in rats dosed with *Lactobacillus plantarum* (groups B, C and D) when compared to the control (A) (Table 2). A high level of Hb, PCV and RBC is an indication that the rats are not anaemic while a lower level is a sign of anaemia (Schalm *et al.*, 1975; Cheesborough, 1991). The WBC (leucocytes) counts was however higher in the control (A) when compared to

the other treatments dosed with *Lactobacillus plantarum*. White blood cell (WBC) is important in defending our body against infection (Schalm *et al.*, 1975). The leucocytes counts however cannot give specific information and this necessitated the differential leucocytes counts. In the differential leucocytes count, the neutrophil was higher in the rats of the control (A) group when compared to groups B, C and D. Neutrophil is majorly responsible for phagocytosis of pathogenic microorganisms during the first few hours after their entry into tissues. There was however no significant difference in the basophil and monocytes counts in all the treatment groups. Basophil counts increases upon sensitization to an antigen (or allergen) while monocyte is responsible for defense of tissue against microbial agents (Schalm *et al.*, 1975; Cheesbrough, 1991). The lymphocyte counts of rats dosed with *Lactobacillus plantarum* was higher and significantly different (P<0.05) in groups B and C except group D when compared with the control (A). The primary role of lymphocytes is in humoral antibody formation and cellular immunity (Schalm *et al.*, 1975; Baker and Silver, 1985). In essence, the increase in the lymphocyte count observed for rats in group B and C shows sign of immunostimulatory effect. Aattouri *et al.* (2001) had earlier reported that oral ingestion of lactic acid bacteria by rats increases lymphocyte proliferation and interferon- α production.

The total weight gain in rats that consumed *Lactobacillus plantarum* was higher and significantly different (p<0.05) from the control. Probiotics had been used as growth promoters due to their ability to suppress the growth and activities of growth depressing microflora and their ability in enhancing absorption of

Aboderin and Oyetayo: Assessing the safety of *Lactobacillus plantarum* using haematological parameters

nutrients through the production of digestive enzymes (Fuller and Gibson, 1997).

The data gathered from these study shows that *Lactobacillus plantarum* isolated from fermenting corn slurry is safe, has immunostimulatory properties due to raised lymphocyte count in rats and can enhance better performance of animals in terms of weight gain. Overview of the whole haematological parameters shows that rats in group B dosed with 0.3ml of 10^7 cfu/ml concentration of *Lactobacillus plantarum* (B) had the highest PCV, Hb, RBC and lymphocyte counts, though the weight gain by rats in this group was not as high as what was obtained in C and D.

References

- Aattouri, N., M. Bouras, D. Tome, A. Marcos and D. Lemonnier, 2001. Oral ingestion of lactic acid bacteria by rats increases lymphocyte proliferation and interferon α production. Br. J., 87: 367-373.
- Aguirre, M. and M.D. Collins, 1993. Lactic acid bacteria and human clinical infection. J. Appl. Microbiol., 75: 95-107.
- Akinrele, I.A, O. Adeyinka, C.C.A. Edwards, F.O. Olatunji, J.A. Dina and A.O. Koleoso, 1970. The development and production of Soy-Ogi: a corn based complete protein food. Federal Institute of Industrial Research, Oshodi (FIRO), Research Report Number, 42.
- Baird, D.M., 1977. Probiotics help boost feed efficiency. Feed stuffs, 49: 11-12.
- Baker, F.J. and R.E. Silver, 1985. Introduction to Medical Laboratory Technology, Macmillan Press 6th Edition, 320-328.
- Berg, R.D., 1983. Translocation of indigenous bacteria from the intestinal tract. In Human intestinal microflora in health and diseases ed Hentges. D.J. London: Academic Press, pp: 333-352.
- Bertazzoni, M.E., A. Benini, M. Marzotto, H. Hendriks, A. Sbarbati and F. Dellaglio, 2001. Preliminary screening of health-promoting properties of new *Lactobacillus* strain: *in vitro* and *in vivo*. HEALFO abstracts, Italy.
- Boersma, W.J.A., M. Shaw and E. Claassen, 2000. Probiotic bacteria as live oral vaccines. *Lactobacillus* as the versatile delivery system, p: 234-270. In R. Fuller and G. Perdigon (ed.), Probiotics 3: immunomodulation by the gut microflora and probiotics. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Casas, I.A. and W.J. Dobrogosz, 2000. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad spectrum protection against disease in humans and animals. Microbial Ecology of Health Diseases, 12: 247-285.
- Cheesborough, M., 1991. Medical laboratory manual for tropical countries. 2nd edition Tropical Health Technology and Butterworth Scientific limited. Vol. 1, pp: 494-526.
- Chang, H., J. Kim, H. Kim, W. Kim, Y. Kim and W. Park, 2001. Selection of potential probiotic lactobacillus strain and subsequent *in vivo* studies. Antonie van Leeuwenhoek, pp: 193-199.
- de Waard, R., J. Garssen, J. Snel, G.C.A.M. Bokken, T. Sako, Huis in 'T Veld, J.H.H and J.G. Vos, 2001. Enhanced antigen-specific delayed-typed hypersensitivity and immunoglobulin G2b responses after oral administration of viable *Lactobacillus casei* YIT9029 in wistar and brown Norway rats. Clinical and Diagnostic Laboratory Immunology, 8: 762-767.
- FAO/WHO, 2001. Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria (LAB). October. 2001.
- Fuller, R. and G.R. Gibson, 1997. Modification of the intestinal flora using probiotics and prebiotics. Scandinavian J. Gastroenterology, 32 (Supplement 222), 28-31.
- Naidu, A.S., W.R. Bildlack and R.A. Clemens, 1999. Probiotic spectra of lactic acid bacteria (LAB). Cr. Rev. in Food Sci. Nutr., 39: 1-126.
- Oyetayo, V.O. and B. Osho, 2004. Assessment of probiotic properties of a strain of *Lactobacillus plantarum* isolated from fermenting corn slurry. J. Food, Agri. Environ., 2: 132-134.
- Oyetayo, V.O., F.C. Adetuyi and F.A. Akinyosoye, 2003. Safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent *in vivo*. Afr. J. Biotech., 2: 448-452.
- Parker, M.T. and L.H. Collier, 1990. Streptococcus and *Lactobacillus*. In Topley and Wilson's Principles of Bacteriology, Virology and Immunity, 8 ed. Vol. 2, pp: 148-156.
- Salminen, M.K, A. Jarvinen, M. S. Saxelin, Tynkkynen, H. Rautelin and V. Valtonen, 2001. Increasing consumption of *Lactobacillus GG* as probiotic and the incidence of lactobacilli bacteremia in Finland. Clinical Microbiology and Infection, 7: (suppl. 1) 802.
- Saxelin, M., H. Rautelin, S. Salminen and P.H. Makela, 1996. The safety of commercial products with viable *Latobacillus* strains. Infectious Disease and Clinical Practice, 5: 331-335.
- Schalm, O.W., N.C. Jain and E.J. Carrol, 1975. Veterinary Haematology 3rd Edition. Lea and Febiger, Philadelphia, pp: 421-538.
- Tannock, G.W. 1983. The effect of dietary and environmental stress on the gastrointestinal microflora In Health and Disease. ed. D.J.Hentges. New York: Academy press, pp: 517-539.
- Zhou, J.S., Q. Shu, K.J. Rutherford, J. Prasad, M.J. Birtles, P.K. Gopal and H.S. Gill, 2000. Safety assessment of potential probiotic lactic acid bacterial strains *Lactobacillus rhamnosus* HN001, *L. acidophilus* HN017, and *Bifidobacterium lactis* HN019 in BALB/c mice. Int. J. Food Microbiol., 56: 87-96.