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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Preliminary Study of the Health Promoting Potentials of *Lactobacillus Fermentum* OVL and *Plerotus Sajor caju* Administered to Rats

V.O. Oyetayo¹ and F.L. Oyetayo²

¹Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Nigeria

²Department of Biochemistry, University of Ado Ekiti, Nigeria

E-mail: ovofuta@yahoo.com

Abstract: Preliminary *in vivo* study of the potential synergy of health promoting ability of *Lactobacillus fermentum* OVL and edible mushroom, *Plerotus sajor-caju*, was investigated in rats (*Rattus norvegicus*). From the results obtained, there was no obvious potential synergy between *Lactobacillus fermentum* OVL and *Plerotus sajor-caju* in promoting the growth performance of rats dosed with *Lactobacillus fermentum* OVL and placed on diet compounded from the mushroom, *Plerotus sajor-caju* (MDL). The daily weight gain (DWG), feed conversion ratio (FCR), and protein efficiency ratio (PER) were better in control (PD) placed on diet compounded with casein than in diet compounded from mushroom (MD and MDL). However, there were obvious potential synergy between *Lactobacillus fermentum* OVL and *Plerotus sajor-caju* in stimulating the immune response of the host. The serum globulin in treatment MD and MDL (38.33mg/dl and 46.87mg/dl respectively) were higher and significantly different ($P<0.05$) from control (PD). There was also a potential synergy in the ability of *Lactobacillus fermentum* OVL and *Plerotus sajor-caju* in bringing about an increase in the count of beneficial lactobacilli in the faeces of rats in treatments MD and MDL having faecal lactobacilli count of 6.81 cfu/g and 7.32 cfu/g respectively when compared with control (PD) with 6.66 cfu/g that was lower and significantly different ($P<0.05$).

Key words: Health promoting, synergy, *Lactobacillus fermentum*, *plerotus sajor-caju*

Introduction

Innovative approaches had been tried to maintain intestinal balance and to promote good health in man and animal through the use of probiotics. Probiotics are viable microbial food supplement which beneficially influence the health of the host (Schrezenmeir and DeVrese, 2001). Probiotics had been reported to possess several potential health benefit such as immunostimulation, prevention of gastrointestinal tract (GIT) infections, growth enhancement of farm animals, anticholesterolaemic effects (FAO/WHO, 2001). The genera *Lactobacillus* and *Bifidobacteria* are commonly used as probiotic agents since they form part of the normal intestinal microbiota (Tannock, 1995).

Two main approaches can be used to increase the level of probiotics in the (GIT). It can be by direct dosing of the host through dairy foods such as yoghurts. Another approach to increasing the probiotic bacteria in the GIT is through the use of prebiotics (Crittenden *et al.*, 2001). Prebiotics are food ingredients that are largely undegraded in the small bowel and can beneficially affect the growth and / or activity of one or a number of bacteria (Schrezenmeir and DeVrese, 2001). Examples of prebiotics are specific oligosaccharides, starch fractions (resistant starch), pectins, lactose, lactulose, lactitol, sorbitol and xylo. The consumption of non-digestible ingredients has been demonstrated to alter intestinal bacterial population in particular promoting the

proliferation of bifidobacteria (Van Loo *et al.*, 1999).

Mushrooms are food substances that are rich in non-digestible dietary fibres belonging to B-glucan, chitin and heteropolysaccharides (pectinous substances, hemicellulose, polyuronides etc), making up to as much as 10 - 50% in the dry matter (Mizuno, 1999). Apart from their use as food, there are evidences that suggest that many species contain substances that may prevent or alleviate cancer, heart diseases, viral infections (Oei, 1991, Mizuno, 1999). Thus it is rich in non-digestible fibre, which may selectively enhance the growth of probiotic bacteria in the large intestine.

There exist abundant data in literature on the health promoting effects of *Lactobacillus species* and edible mushroom but this is lacking on the potential for synergy between probiotic *lactobacillus* and edible mushroom *P. sajor-caju*. The current investigation focuses on the health promoting potential of *L. fermentum* OVL and edible mushroom *P. sajor-caju* administered to rats.

Materials and Methods

***Lactobacillus* culture:** *Lactobacillus fermentum* OVL was isolated from Kunnu, a local fermented alcoholic beverage brewed from sorghum. The isolate was characterized using colonial, morphological, and biochemical properties (Parker and Collier, 1990). Preliminary screening show that the isolate has the following probiotic properties of survival at acidic pH,

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resistance to common antibiotics, and growth inhibition of pathogens and food spoilage bacteria.

The isolate was cultured in deMann Rogosa and Sharpe (MRS) broth (LAB M) and incubated at 37°C for 2 days to obtain large cell concentration. The cells were lyophilized using the method described by Fujiwara *et al.* (2001). The concentration of the cell, which was 5.9×10^{13} cfu/g, was determined by serial dilution techniques.

Source of *Plerotus sajor-caju*: Fresh fruit bodies of edible mushroom, *Plerotus sajor-caju*, collected from forest by local farmers were bought at the Oba's Market, Akure. The mushroom samples were oven dried at 60°C and powdered using a Philip blender.

Proximate composition of *Plerotus sajor-caju*: Moisture, crude fat, and ash content of mushroom, *Plerotus sajor-caju* were determined by the method of AOAC (1984). Protein content was determined using the micro-Kjeldahl method. Since mushroom has significant content of non-protein nitrogen, the protein was determined using the adjusted conversion factor 4.38 for mushroom protein (Shashirekha *et al.*, 2002). Total carbohydrate was determined by the method described by Plummer (1971). Proximate composition of *P. sajor-caju* is presented in Table 1.

In vivo feeding trial: Sixteen (16) albino rats (*Rattus norvegicus*) aged 4 - 5 weeks obtained from a research farm in Ile-Ife, Nigeria were used. The rats were acclimated for one week on grower, mash purchased from Bendel feed, Nigeria. The rats weigh 58 - 82g after acclimatization. The rats were divided into 4 groups of rats each. treatment PD (control) were fed on diet composed of (%/diet) casein 10; fats 5; vitamin mineral premix 5; and cornstarch 80. Treatment MD was fed the diet above but the casein was substituted with 10% mushroom (*Plerotus sajor-caju*) protein. The composition of the diets is shown in Table 2. Treatments PDL and MDL in addition to being fed on diet PD and MD respectively were also dosed with 0.3ml of 5.9×10^{13} cfu/g of *Lactobacillus fermentum* OVL. The rats were placed on the diets above for 28 days. Treatments MDL and PDL were orogastrically dosed with the concentration of *Lactobacillus* culture every 7 days. The rats were weighed during the period of experiments and the data was used to determine feed conversion ratio (FCR) and Protein efficiency ratio (PER). The rats were sacrificed by cervical dislocation.

Analysis of serum: The blood samples of the rats were collected into EDTA bottles. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitmans and Frankel (1957). Serum total protein was determined by the biuret method while serum albumin level was

Table 1: Proximate Composition of *Plerotus sajor-caju*

Parameters	*Values (%)
Crude Protein	20.05 ± 0.10
Crude fibre	17.02 ± 0.10
Crude fat	4.60 ± 0.20
Ash	6.52 ± 2.10
Total Carbohydrate	41.10 ± 1.00
Moisture	10.60 ± 0.01

* Values are Means ± SD of three replicates.

Table 2: Composition of Experimental Diets (%)

Ingredients	PD	MD
Corn Starch	80	80
Casein	10	-
Mushroom protein*	-	10
Vegetable oil	5	5
Vitamin/mineral premix**	5	5

*Mushroom (49.89g) was added to diet (MD) to make 10% Mushroom protein in the compounded diet. **Composition of vitamin/mineral premix (A Product of Green Field Nigeria Limited, Lagos): Vit. A (6,000,000 iu); Vit D₃ (1,300,000 iu); Vit E (3,000 iu); Vit K₁ (2000mg/kg); Riboflavin B₂ (4,000mg/kg); Pyridoxine B₆ (1,500mg/kg); Thiamine B₃ (2,000mg/kg); Vit B₁₂ (10mg/kg); Niacin (15,000mg/kg); Pathothenic acid (5,000mg/kg); Folic acid (20mg/kg); Biotin (20mg/kg); Manganese (80g/kg); Zinc (50g/kg); Iron (20g/kg); Copper (5g/kg); Iodine (1.2g/kg); Selenium (200mg/kg); Cobalt (200mg/kg).

assayed by the bromocresol green (BCG) method (Cheesborough, 1991). Serum globulin was determined by deducting serum albumin from serum total protein.

Faecal bacterial count: Faecal samples were collected and investigated on days 0 and 28. Serial dilutions of faeces were plated on MRS agar (LAB M) to select lactobacilli and on eosin methylene blue (EMB) agar (LAB M) to select *E. coli*. The colony forming units growing on the plates were recorded 24 hour after incubation.

Data analysis: Data gathered from the various analyses were processed using one-way analysis of variance and the means were compared using Duncan Multiple range test.

Results

The results of the biological evaluation of the nutritional quality of the different diets PD and MD and those dosed with *Lactobacillus fermentum* OVL, PDL and MDL are presented in Table 3. There were significant differences ($P < 0.05$) in PER, FCR, daily weight gain (DWG) and total weight gain (TWG) in all the treatments. However, there were no significant differences ($P < 0.05$) in the daily feed consumed (DFC).

Table 4 shows the results of the analysis of serum from rats in the different treatments. There was no significant

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Table 3: Biological Evaluation of the Protein Quality of Experimental Diets

Treatments*Parameters	PD	PDL	MD	MDL
PER	0.43 ^b ±0.03	0.54 ^c ±0.04	0.09 ^a ±0.02	0.08 ^a ±0.02
DWG(g)	4.24 ^b ±0.26	5.37 ^c ±0.41	0.92 ^a ±0.22	0.82 ^a ±0.23
TWG (g)	43.80 ^b ±1.74	59.33 ^c ±3.61	7.43 ^a ±1.19	6.33 ^a ±2.40
FCR	2.75 ^a ±0.22	2.22 ^a ±0.18	17.94 ^b ±5.58	17.79 ^b ±3.96

*Values along row with the same superscript are not significantly different (P>0.05)

Table 4: Effects of Experimental Diets on Blood Proteins (mg/dl) of Rats Treatments*

Parameters	PD	PDL	MD	MDL
Total Protein	66.4 ^a ±0.57	91.2 ^c ±11.30	68.00 ^a ±5.74	83.15 ^{ab} ±0.57
Albumin	31.57 ^a ±0.61	39.43 ^c ±3.75	29.67 ^a ±1.89	36.33 ^{ab} ±0.94
Globulin	34.83 ^a ±0.87	51.77 ^c ±7.60	38.33 ^{ab} ±3.90	46.87 ^{ab} ±0.90

*Values along row with the same superscript are not significantly different (P>0.05)

difference (P<0.05) in the total protein and albumin of rats in treatments PD and MD; however, these were significantly different (P>0.05) in treatments PDL and MDL when compared with PD (control). There was a significant difference (P<0.05) in the globulin level of all the treatments. Treatment PD had the lowest globulin level of 34.83mg/dl) when compared with the other treatments.

The results of liver enzymes, AST and ALT are presented in Table 5. There was a significant difference (P<0.05) in the serum AST and ALT in all the treatments. The serum AST of treatment PD was higher and significantly different (P>0.05) from the other treatments. However, the ALT of treatment PDL was higher (16.33iu/l) and significantly different (P<0.05) from other treatments.

The results of faecal bacterial count are presented in Table 6. Faecal lactobacilli counts were higher and significantly different (P<0.05) in treatments PD on the 28th day. Moreover, faecal *E. coli* counts were lower and significantly different (P<0.05) in treatments MD, PDL, and MDL when compared to treatment PD.

Discussion

The beneficial effects of *Lactobacillus species* as probiotics had been well documented by various researchers. *Lactobacillus* had been found to have several potential benefits such as growth enhancement of farm animals, protection from pathogens, alleviation of lactose intolerance, anticholesterolaemic and immunostimulatory effects (FAO/WHO, 2001). Edible mushrooms had also been associated with several health promoting effects such as alleviation/prevention of cancer, heart diseases, viral infections, anticholesterolaemic and immunostimulatory effects (Oei, 1991; Mizuno, 1999).

In the current investigation, a probiotic *Lactobacillus fermentum* OVL was used to compliment diet compounded from edible mushroom, *Plerotus sajor-caju*, which has the potential of a prebiotic based on the high fibre content (17.02%). Mizuno (1999) had earlier reported that edible mushroom had dietary fibre content

of 10 to 50% in the dry matter.

Biological evaluation of rats fed the different diets shows that rats placed on mushroom diet (MD and MDL) had lower PER, DWG, and TWG when compared with rats placed on casein diet (PD and PDL). Protein efficiency ratio (PER) indicates how much of a certain type of protein food is needed to promote growth (McLaughan, 1975). This observation indicates that more mushroom protein may be needed for better growth performance. The rats fed protein diet (PD and PDL) had a better feed conversion ratio (FCR) than rats fed from diet compounded from mushroom (MD and MDL). This result indicates that in terms of feed consumption, treatments MD and MDL will consume 58.75 and 52.33% feed respectively to attain the same daily weight (4.24g) as treatment PD (control). There was no obvious effect of *Lactobacillus fermentum* OVL in terms of weight gain, FCR, PER in rats in treatment MDL, however, in treatment PDL there was an obvious effect of the probiotic, *Lactobacillus fermentum* OVL, in the parameters above.

Lower serum protein had been associated with protein deficiency (Pond *et al.*, 1980). The total serum protein of rats in treatment MD (68.00mg/dl) was higher though not significantly different (P<0.05) from treatment PD (66.40mg/dl). However, rats in treatment MD and PDL had a higher total protein of 83.15mg/dl and 91.20mg/dl respectively. This may indicate a positive effect of *Lactobacillus fermentum* OVL on the rats. Serum albumin level in treatment MD was not significantly different (P<0.05) from casein diet (PD). One important observation in this study is the increase in serum albumin of rats in treatments PDL (39.43mg/dg) and MDL (36.33mg/dl). Albumin is a major protein, which is formed by the liver, and chronic liver disease causes a decrease in the amount of albumin produced (American Liver Foundation, 1995). A higher serum albumin observed in treatment MDL especially may be an indication of a potential synergy between *Lactobacillus fermentum* OVL and edible mushroom, *Plerotus sajor-caju* in the diet.

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Table 5: Serum Aminotransferase level (iu/l) of Rats Fed Experimental Diets Treatments*

Parameters	PD	PDL	MD	MDL
AST	34.33 ^c ±1.18	31.00 ^{bc} ±0.00	27.00 ^a ±1.63	28.33 ^{ab} ±0.94
ALT	6.67 ^a ±0.47	16.33 ^d ±0.47	15.00 ^c ±0.00	9.00 ^b ±0.41

*Values along rows with the same superscript are not significantly different (P>0.05)

Table 6: Faecal Bacterial Counts (cfu/g) of rats fed Experimental Diets

Day	Lactobacilli Counts*				E. coli Counts*			
	PD	PDL	MD	MDL	PD	PDL	MD	MDL
0	6.94 ^b ±0.01	6.85 ^a ±0.01	6.87 ^a ±0.01	6.90 ^a ±0.01	6.98 ^b ±0.00	7.06 ^d ±0.01	6.87 ^c ±0.01	6.93 ^a ±0.01
28	6.66 ^b ±0.07	7.29 ^a ±0.00	6.81 ^a ±0.01	7.32 ^a ±0.05	7.05 ^c ±0.05	6.37 ^a ±0.04	6.78 ^b ±0.02	6.44 ^a ±0.06

*Values along rows with the same superscript are not significantly different (P>0.05)

Serum globulin was higher in treatment MD (38.33mg/dl) when compared with treatment PD (34.83 mg/dl). Serum globulin was higher in treatments MDL and PDL (Table 5). An increase in serum globulin is an indication of immunostimulation (Baron *et al.*, 1994). A higher serum globulin observed in treatments MD, MDL and PDL may indicate immunostimulatory effect associated with edible mushroom *Plerotus sajor-caju* and *Lactobacillus fermentum* OVL. Bohn and Bemiller (1995) and Aattouri *et al.* (2002) had reported the immunostimulatory properties of mushroom and lactic acid bacteria respectively.

Serum AST level of rats in treatments MD and MDL was lower and significantly different (P<0.05) compared to treatment PD (control). However, serum ALT was lowest in treatment PD when compared with other treatments. The reason for the decrease in the serum ALT of treatment PD could not be categorically stated. Serum AST and ALT tests are simply markers of liver or biliary tract disease (American Liver Foundation, 1995), hence they lack some sensitivity in detecting chronic liver disease (Johnston, 1999). A observation of the gross morphology of the rats in all treatments however do not show any sign of injury.

The ability of a probiotic strain to balance intestinal microflora can be assessed by detecting the size of harmful population e.g. *E. coli*, and of beneficial bacteria especially lactobacilli in the faeces (Mitsuoka, 1992). Higher counts of faecal lactobacilli and a concomitant decrease in faecal *E. coli* counts were observed in rats in treatments MD and MDL. This may indicate a potential synergy between lactobacilli and mushroom. The higher lactobacilli counts recorded for MD and MDL when compared with PD, may be due to the fibre content of *Plerotus sajor-caju* (17.13%). Dietary fibre had been reported to promote the growth of fermentative species such as *Lactobacillus species* (Walker and Duffy, 1998). The higher faecal lactobacilli in treatment PDL may be as a result of the probiotic, *Lactobacillus fermentum* OVL, orogastrically administered to rats in this treatment. The results of the present investigation demonstrate that there is no obvious potential synergy between *Plerotus*

sajor-caju and probiotic, *Lactobacillus fermentum* OVL, in the biological evaluation of the rats in treatments MD and MDL. However, there was obvious potential synergy in the immunostimulatory effects and in increasing the count of beneficial lactobacilli in the faeces of rats in treatments MD and MDL when compared with treatment PD that serves as control.

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