Trakia Journal of Sciences, No 2, pp 101-108, 2014

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

Original Contribution

ENTEROCOCCUS SPECIES AS FEED SUPPLEMENT IN ALBINO RATS

F. Uzokwe[1], C. O. Adegoke[2], S. T. Ogunbanwo[1]*

[1]Department of Microbiology, University of Ibadan, Nigeria
[2]Ogun State College of Health Technology Ilese Ijebu Department of Medical Laboratory Science, Nigeria

ABSTRACT

Enterococcus species has been allied to carry out dual function in fermented food products and can induce diseases in human such as *E. faecium* and *E. faecalis* respectively. Therefore there is need to know which of the *Enterococcus* species is pathogenic and those that are generally regarded as save for human and animal use. The aim of this work is to investigate influence of *Enterococcus* species as dietary supplements in albino rats. The experimental rats were divided into two groups. Test group were fed with standard feeds supplemented with10^8cfu/ml of *Enterococcus* species while control was without *Enterococcus* species. Viable *Enterococci* counts from rectal feacal samples were determined. The weight gained or lost, feed efficiency, behavioral pattern, haemogram and histopathology analysis of the organs and tissues of the rats were carried out. Test group expressed lesions of some parts with specific isolates while others showed no lesion as also seen in the control rats in all the examined parts: ceacum, colon, rectum, kidney, heart and liver. The results indicated that a greater numbers of *Enterococcus* species exist more as commensal in the experimental rats; while *E. ratti* appeared to be a pathogenic strain in this study, *E. avium*, *E. gallinarum*, *E. faecium* and *E. faecalis* were suspicious while E. *porcinus*, *E. dispar*, *E. munditi*, *E. hirae* and *E. cecorum* were considered safe with significant improvement on body weight, feed efficiency ratio, normal haemogram and histopathology observation. Molecular techniques of the above named isolates should be investigated in order to ascertain their safety or probiotic properties.

Key words: Albino Rats, *Enterococcus* species, feeds-supplement, safety.

INTRODUCTION

Enterococus species is in the kingdom Bacteria, division firmicutes, class *Baccilli*, order *Lactobacillales*, Family *Enterococcase* and genus of lactic acid bacteria that are Gram-positive cocci and often occurs in pairs (diplococci) or short chains, and are difficult to distinguish from streptococci on physical characteristics (1, 2, 3). They are facultative anaerobes, chemo-organotrophs with complex nutritional requirements (4; 5). *E. faecalis* and *E. faecium* remain the two most prominent species among the numerous *enterococci* (6).

***Correspondence to**: *Ogunbanwo S.T., Department of Microbiology, Faculty of Science, P.O. Box 22346, University of Ibadan, Ibadan, Nigeria, Tel:+2-8110-11004, Fax:+2-8103-043/8103-118, E-mail: topzybanwo@yahoo.com; st.ogunbanwo@mail.ui.edu.ng*

Enterococci are important groups of bacteria in terms of their interaction with humans (7). Some strains are used for the manufacture of foods while others are associated with foods spoilage especially meats and more importantly certain enterococcal strains cause serious human and other animal infections (8).

Preceding to 1990s enterococci have been recognized as an important cause of bacterial endocarditis for almost a century and in the past decade, increased occurrence of enterococci infections in the hospitals has led to emergence of antimicrobial resistance among such isolates (9). Enterococci were reported as the second most common cause of nosocomial infections in the US and within this group *Enterococcus faecalis* causes the majority of human enterococcal infections. These infections may be

local or systematic and include urinary tract, bacteremia, abdominal and wound infections (10).

The emergence of ampicillin, streptomycin, gentamicin, and vancomycin-resistant enterococci is a cause of concern and difficult to control. Moreover, there can be transfer of resistant gene from enterococci to *Staphylococcus aureus* thereby posing a threat to the patient safety and also challenges for the treating physicians because the gene responsible for this resistance can be transferred easily between the two species carrying pheromone responsive plasmids or conjugative transposons (11).

Despite their ill effects in clinical their role as food additives cannot be over emphasized. The question about 'dualistic effect' has been raised, enterococci and their metabolic products have become a central issue within different research activities with regards to food safety aspects and their risk or beneficial potential as probiotics or cultures in the food industry, therefore it is necessary to know if only some enterococcal species or strains are harmful (8).

This work is intended to bridge the gap by determining the effect of *Enterococus* species as feed supplement in albino rat.

MATERIALS AND METHODS

Albino Rat and diets

Twenty clinical healthy rats (4 weeks old) were purchased from the veterinary physiology and pharmacology Department, University of Ibadan Nigeria and housed under specific pathogen-free conditions in wire cages for 4 weeks adaptation at experimental house. The rats were fed with standard pellet supplemented with and without Enterococcus species and water was given at adlibitum.

Microorganism

Enterococcus species used in this work were obtained from the culture collection of Department of Microbiology University of Ibadan. It was originally characterized as reported by Ogunbanwo et al. (12). Bacteria were grown in MRS broth at 37°C for 24 h, centrifuged at 400rpm for 25minutes to get the cell which were later rinsed twice by dissolving in phosphate buffered saline (PBS) and then concentrated in 1 mL of PBS. The number of viable cells was determined by agar plate counting.

In vivo Rat Experiment

The experiment was performed as stipulated in the guidelines of the Animal Research Ethic of the University of Ibadan and in accordance with the Helsinki declaration using a modified method of Ogunbanwo et al. (13). The safety effect of rats fed with Enterococci species supplement were determined using eight weeks old Albino rat of an average body weight of 70 to 80g. The twenty rats purchased were grouped into two, ten for the test and ten for control. Enterococcus spp were grown in MRS broth, standardized using spectrophotometer (0.5_{620}) reading and centrifuged at 400rpm for 25minutes to get the cell which were later rinsed twice by dissolving in phosphate buffered saline (PBS) in appropriate stock dilution of 10^8cfu/ml (McFarland std). The appropriate stock dilutions of each of the isolates were mixed with the standard pellets at the rate of 1ml to 200g of standard rat feed pellets.

Oral administration of Enterococcus species containing $5x10^8$cfu or PBS containing 10% sucrose was performed according to Benyacoup et al. (14) by mixing 1ml of the inoculum with the feed. Test rats were fed with standard pellet mixed with each of the ten representative Enterococcus species while control group were fed with the standard pellet without the organisms. All the animals were monitored for clinical sign or death over a period of two weeks determining their weight and feed efficient, haemogram and histological examination of rat's organs. Intra peritoneal inoculation with the Enterococcus species in the rat was done on the third week after feeding (14).

Assessment of haemogram

At the end of the feeding and Intra peritoneal inoculation with the Enterococcus species, blood samples were collected from the media cantus of the eye vein of mice for haematological test by determining the complete haemogram such as the full blood count (FBC), Packed Cell Volume (PVC), platelet, monocytes etc (15).

Histological Examinations of Rat Organs

After sacrificing the rat histological examination of some organs such as the heart, kidney, liver, caecum, colon and rectum were carried out.

Pieces from these organs were taken and fixed in a 10% neutral buffered formalin solution and routinely processed for paraffin embedding. Five micrometer sections were obtained and stained with haematoxylin-eosin for histological examination (16).

Re-isolation of Enterococcus speices from Albino Rat

Faecal samples of each of the treatment were collected from different experimental animal in their respective cage and were serially diluted. Appropriate dilution was inoculated into bile Esculin agar (17) and incubated at 37^{o}C for 48h. Black colonies of the isolates on the medium were counted (18) and expressed in cell/ml.

RESULT

Influence of Enterococcus species dietary supplementation on albino rats was investigated. The experimental rats were divided into two groups' ten rats in each group (test and control). Test group were fed with standard feed, supplemented with10^{8}cfu/ml of Enterococcus species. There were increase in weight of both test and control rat groups after two weeks feeding. Test group fed with feed supplemented with Enterococcus species showed remarkable increase in weight from initial weight of 70g to 100g compared to those of control group with initial weight of 70g to 85g (final weight) as shown in **Table 1.**

Table 1. *Influence of Enterococcus species dietery supplementation on growth of Albino Rats*

Test rats fed and inoculated with Enterococcus species								**Control rats (without *Enterococcus species*)**					
Treatment on experimental rats	**Initial weight (g)**	**Weight in two weeks (g)**	**Weight after 24h inoculation in 3rd wk (g)**	**Mean body weight (g)**	**FI (g)**	**FE (g)**	**Behavioral comment**	**Control rats**	**Initial weight (g)**	**Final weight (g)**	**Mean body weight (g)**	**FI (g)**	**FE (g)**
E. faecium (C1R1;221)	80	100	100	93	180	1.935	Normal rat	C01R	70	80	75.0	180	2.400
E. gallinarum (C2R2;188)	80	100	100	93	190	2.043	Normal rat	C02R	80	85	83.5	170	2.036
E. porcinus (C3R3;133)	70	100	100	90	180	2.000	Vibrant rat	C03R	70	80	75.0	180	2.400
E. munditii (C4R4;247)	80	90	80	83	190	2.289	Vibrant rat	C04R	70	75	72.5	170	2.345
E. hirae (C5R5;164)	70	90	70	77	180	2.338	Normal rat	C05R	80	90	85.0	180	2.118
E. avium (C6R6;177)	70	90	70	77	180	2.338	Weak rat	C06R	90	103	96.0	190	1.969
E. ratti (C7R7;198)	70	90	70	77	190	2.468	Weak rat	C07R	70	80	75.0	180	2.400
E. dispar (C8R8;198)	80	100	80	87	170	1.954	Vibrant rat	C08R	80	90	85.0	180	2.118
E. cecorum (C9R9;193)	80	90	80	83	180	2.169	Vibrant rat	C09R	70	75	72.5	170	2.345
E. faecalis (C10R10;178)	70	90	80	80	185	2.313	Fairly weak rat	C010R	70	80	75.0	180	2.400

Meanwhile after 24hours of direct intra peritoneal inoculation of the test rats with *Enterococcus* species on the third week, they showed decrease in weight. Feed efficiency (FE) of both groups fell in the same range (1.935 – 2.468g) and mean body weight (MBW) of test rats ranges between 77g and 93g while that of control rats ranges between 72.5g and 96.5g.

Table 2 shows the viable *Enterococcus* species count obtained from the rectal faecal sample of both test and control rats, before sacrificing and excision. Rat treated with *E. faecalis* had the highest viable *enterococci* count of $6.0x10^{8}$cfu/ml followed by rats treated with *E. gallinarum* ($4.8x10^{8}$cfu/ml) while rats treated with *E. avium* had the least viable count of $2.1x10^{8}$cfu/ml in the test group. Meanwhile, control rats had lower viable count that ranges from $4x10^{7}$ to $1.3x10^{8}$ cfu/ml.

Table 2. *Viable Enterococci count obtained from faecal sample of the experimental rats*

Experimental rats code	*Enterococcus* species	Stool formation	Viable count (cfu/ml)	Control rats code	Stool formation	Viable count (cfu/mil)
C1R1 (221)	*E. faecium*	Normal	8 $X10^7$	CO1R	NWF	$1.2x10^8$
C2R2 (188)	*E. gallinarum*	Normal	$4.8X10^8$	CO2R	Normal	$8x10^7$
C3R3 (138)	*E. porcinus*	Normal	$2.6X10^8$	CO3R	Formed	$1.0x10^8$
C4R4 (247)	*E. munditii*	Normal	$1.6X10^8$	CO4R	NWF	$6x10^7$
C5R5 (164)	*E. hirae*	Normal	$1.6X10^8$	CO5R	NWF	$8x10^7$
C6R6 (177)	*E. avium*	Normal	$2.1X10^8$	CO6R	Normal	$4x10^7$
C7R7 (198)	*E. ratti*	Watery	$4.2X10^8$	CO7R	Normal	$1.2x10^8$
C8R8 (192)	*E. dispar*	Normal	$2.8X10^8$	CO8R	Formed	$6x10^7$
C9R9 (193)	*E. cecorum*	WF	$4.6X10^8$	CO9R	Normal	$1.3x10^8$
C10R10(178)	*E. faecalis*	Normal	$6.0X10^8$	CO10R	NWF	$8x10^7$

KEY: WF- Well formed stool; NWF=Not well formed stool; ML=Microbial load (N/V);
N=Number of colonies; V=Volume of dilution; R=Dilution factor expressed in cell/mil

The rectal feacal sample from test rats were all formed, except that of the rat fed with *E. ratti* which appeared watery like most of the rats in the control group with unformed rectal faecal sample.

The Erythrocyte value (PCV, HB, and RBC) and indices (MCV, MCHC) of rats inoculated with *Enterococcus* species in the test rats and control rats is shown in Table 3. Rat fed with *E. dispar* had PCV of 58% while Rats fed with *E. cecorum* had 44% of PCV compare with control rats that had 48% PCV. Rats fed with *E. gallinarum* had 7,700 X 10^3µl of WBC count while that of control was 7577 X 10^3µl however low count of WBC ranges from 3200-5400X10^3 µl was recorded in other rats treated with other enterococcus species. There is variations in the levels of other haemograms parameter depending on the species of enterococcus administered **(Table 3).**

Table 3. *Haemogram analyses of rats fed with feed supplemented Enterococcus species*

Heamogram parameter	*E. faecium*	*E. gallinarum*	*E. porcinus*	*E. munditii*	*E. hirae*	*E. ratti*	*E. dispar*	*E. cecorum*	*E. avium*	*E. faecalis*	*Control*
PCV (%)	46	46	47	46	47	46	58	44	48	46	48.0±5.9
HB(g/dl)	15.2	15.4	15.4	14.8	15.6	15.0	17.3	14.5	16.2	15.2	16.1±1.7
RBC($X10^6$µl)	7.68	7.42	7.86	7.46	7.84	7.53	8.92	7.28	8.03	7.56	7.8±0.7
MCV(fl)	60	61	59	61	59	61	65	60	59	60	60.9±3.5
MCHV(%)	33	33	33	32	33	32	29	32	34	33	33.2±1.6
WBC Total($X10^3$µl)	4500	7700	3200	4350	3500	5050	4450	5400	3750	4200	7577.8±3197.7
Lymphocyte(%)	64	73	63	75	72	61	72	80	47	66	64.1±8.3
Neutrophils(%)	32	25	34	21	24	34	24	15	51	29	31.9±8.4
Monocytes(%)	3	-	1	2	4	4	4	3	-	3	2.6±0.5
Eosinophil(%)	1	2	2	2	-	1	-	2	2	2	1.7±0.5
Abs.Lymphocytes(µl)	3410	5621	2016	3262	2520	3080	3204	4320	1762	2772	4751±1995
Abs.Neutrophil(µl)	1104	1925	1088	914	840	1717	1068	810	1913	1218	2424±1484
Abs.Monocyte(µl)	160	-	32	87	140	202	178	162	-	126	188±89
Abs.Eosinophils(µl)	56	154	64	87	-	51	-	108	2	84	133±76
Platelet(µl)	112,000	176,000	82,000	94,000	94,000	138,000	67,000	130,000	30,000	216,000	I70333.33±39474.60

The pathological examination of the experimental rat carried out reveal that control experimental rats had no visible lesion in all the examined organs and tissues of the rats **(Table 4).** However, the kidney of the test rats was not affected when treated with *Enterococcus* species but the liver of the albino rats treated with *E. faecalis*, *E. ratti* and *E. gallinarum* in the same group showed mild centrilobular degeneration of the fatty tissues. The heart of the rats treated with *E. ratti* and *E. avium* showed congested blood vessels. Moreover, the rat treated with *E. ratti* was adversely affected as shown in the intestine which revealed necrosis of submucosal gland and the villi (Plate 1).

Table 4. *Histopathology examination of the rats fed with feed supplemented Enterococcus species*

Treatment	Identified Part	Comment
E. faecium+ C_1R_1	Heart, Kidney and Liver Intestine	No leision loss of Villi
E. gallinarum+ C_2R_2	Heart, Kidney, Rectum and Cecum	No visible leision
	Liver	
	Colon	Centrilobular fatty degeneration
E. porcinus+ C_3R_3	Kidney, Heart, Liver and Intestine	No leision
E. munditti+ C_4R_4	Kidney, Heart, Liver and Intestine	No leision
E. hirae+ C_5R_5	Kidney, Heart, Liver and Intestine	No leision
E. avirum+ C_6R_6	Kidney, Liver and Intestine	No leision
	Heart	Congested blood vessels.
E. ratti+ C_7R_7	Kidney	No leision
	Liver	Fatty degeneration focal
	Heart	Congested blood vessel
	Intestine	Necrosis of the submucosal gland and the villi.
E. dispar+ C_9R_8	Kidney, Heart, Liver and Intestine	No leision
E. cecorum+ C_9R_9	Kidney, Heart, Liver and Intestine	No leision
E. feacalis+ $C_{10}R_{10}$	Liver	Mild centrilobular partal fatty degeneration
		No leision
	Kidney, heart and Intestine	
Control Rats (Co_1toCo_{10})	Intestine, Kidney, Heart and Liver	No visible leision

Key: Intestine=Cecum, Colon and Rectum, C_1R_1 to $C_{20}R_{20}$ stands for rats 1 to 20 in group A, while CO_1 to CO_{20} stands for control rats.

DISCUSSION

There was increase in weight of both test and control rats after two weeks of feeding. Test group had higher weight gained when fed with feed supplemented with *Enterococcus* species compared to those of control after two weeks. Meanwhile after 24h of direct intra peritoneal inoculation of the rats in the test group with *Enterococcus* species on the third week, they showed decrease in weight. This could be attributed to stress or sudden shock, since none of the rats died before sacrifice. Feed efficiency (FE) and mean body weight of both groups fell within the same range.

Rats treated with *E. faecalis* had the highest viable *Enterococci* count while rats treated with *E. avium* had the least viable count. Viable *enterococci* count from both the control and test rats is an indication that the strains are normal commensal. The rectal faecal sample from test rats were all formed, except that of the rat fed with *E. ratti* which appeared watery like most of the rats in control group with unformed rectal faecal sample.

The erythrocyte values of rats inoculated with *Enterococcus* species in test rats showed little or no different from those of the control rats, conversely, rats inoculated with *Enterococcus species* had lower value of WBC counts compare to the control. This is an indication that they were leucopaenic compared to the control rats. The leucopaenia was due to lower absolute neutrophil and percentage monocyte counts in test group than control group. According to Dukes, (19) neutrophil usually appears at sites of inflammation in large numbers. However, increase or decrease in the full blood count (FBC) is not enough to establish a bacterial infection.

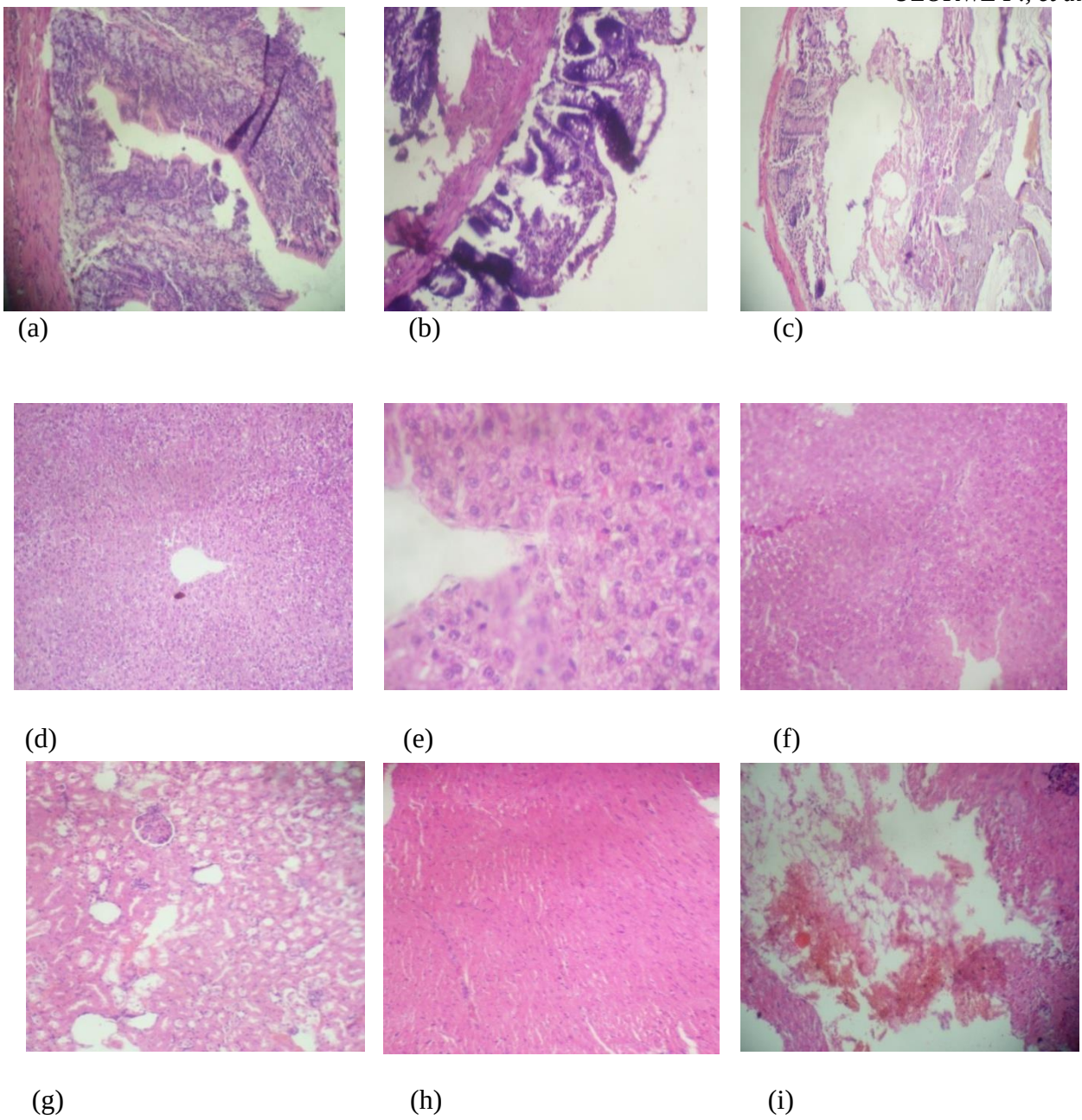

Fig 1a-i: Photomicrograph of the tissues & organs of albino rats treated with *Enterococcus* species
(a) The photomicrograph of the intestine of rats infected with *E. hirae, E. porcinus, E .munditti, E. cecorum, E. dispar,* also seen in group B rats showing villi with no visible lesion (b)Intestine of rat treated with *E. gallinarum* showing stunting and matting of the villi with vacuolations of the submucosal glands. (c) Rat intestine with *E. ratti* showing marked desquamation of the villi. (d) Rat liver treated with *E. ratti* showing hepatic degeneration with vacuoles in the hepatocytes. (e) Rat liver treated with *E. feacalis* showing moderate hepatic degeneration with vacuoles in the hepatocytes (f) Liver treated with *E. dispar* which appeared like grp.B rats. (g) Kidney of exp.rats (A&B) no lesion. (h) Rat heart with *E. faecium* like those of group B with no visible lesion (i) Rat heart treated with *E. ratti* and similar to that of *(E. avium* with congested blood vessel) showing hemorrhage.

The decrease of MCV and MCHC implies iron deficiency; likewise decrease in PCV and HB is an indication of reduced food intake. Murray et al. (20) on the other hand, reported that the plasma of patient with liver disease often show a decrease in albumin to globulin ratio, the production of albumin decreases relatively in conditions of protein malnutrition. According to Benyacoub et al. (14) the ability of *E. faecalis* to stimulate the immune system at both mucosal

and systemic level highlight the mechanisms by which it could be used as probiotic to antagonize pathogens in vivo. This work highly recommends the 1gA, 1gG and 1gE determination on further studies for effective labeling of probiotic *enterococci*.

The histopathology examination of the experimental rat carried out revealed that control experimental rats had no visible lesion in all examined organs and tissues. However, the kidney of the test rats was not affected when treated with *Enterococcus* species but the liver of the rats treated with *E. faecalis*, *E. ratti* and *E. gallinarum* in the same group showed mild centilobular degeneration of the fatty tissues. The heart of the rats treated with *E. ratti* and *E. avium* showed congested blood vessels. Moreover, the rat treated with *E. ratti* was adversely affected as shown in the intestine which reveals necrosis of submucosal gland and the villi. Tissues and organs subjected to histological examination in this study include the colon, cecum and rectum as the down part of the GIT that collects the faecal materials ready for re-absorption and where the bulk waste are gathered, liver as the center for metabolism, kidney major organ for absorption and removal of waste and the heart, major organ for blood circulation.

Considering the test rats treated with *E. porcinus*, *E. dispar*, *E. cecorum*, *E. hirae* and *E. munditti* revealed no visible lesion in some of the examined organs and tissue like those of control rats. However, *E. hirae* and *E. porcinus* had negative gelatinase reaction and are non haemolytic. They all induced effective growth (weight gained) on the rats they fed with. With good feed efficiency, vibrant (energetic) and healthy looking with well formed stool indicated that the *Enterococcus* species might not be pathogenic strains. *E. avium*, *E. gallinavum*, *E. faecium* and *E. faecalis* also increased the weight of the fed rats but had mild reaction on the examined parts of the rats. With protease and gelatinase activities of these organisms they could induce dental caries, Su et al. (21) reported gelatin as bioactive peptidase while Gold et al. (22) established gelatin liquefying human oral *E. faecalis* isolates to induce caries formation in germ free rat. Moreover, *E. ratti* isolated in this work might be pathogenic strain because not only that it induces diarrhea and weakness to the recipient rat it also haemolysed red blood cell completely, had protease and gelatinase enzymatic activity (12). *E. ratti* revealed fatty degeneration of the liver to the fed rats. It showed congested blood vessels of the heart and necrosis of the sub mucosal gland and the villi of the intestine in the experimented rats. Hence it could be one of the cariogenic *Enterococcus* strains in dental plaque, which can demineralise enamel and dentin being produced as a by-product of the carbohydrate metabolism.

From this study it can be inferred that some of the enterococcus species showed evidence of being pathogenic while others could be regarded as safe. However, to really distinguish between the pathogenic and non pathogenic species requires a further genomic study as empirical evidence of their pathogenicity.

ACKNOWLEDGEMENT

The authors are grateful for the technical assistance of Laboratory Scientist in the Department of Veterinary, University of Ibadan, Nigeria and Dental Center Dugbe Ibadan, Nigeria.

REFERENCES

1. Schleifer, K.H. and Kilpper-Balz, R., Transfer of *Streptococcus faecalis* and *Streptococcus faecium* to the Genus *Enterococcus* norn. rev. as *Enterococcus faecalis* comb. nov. and *Enterococcus faecium* comb. nov. *Inter. J. Systematic Bacteriol.* 34 (1): 31-34, 1984.
2. Willey, J., Woolverton, C. and Sherwood, L.**,** Prescott's Principles of Microbiology Publisher McGraw Hill Higher Education ISBN 9781259009532, 2008.
3. Gilmore, M. S.; The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance. Washington, DC: American Society for Microbiology, 2002.
4. Diebel, R.H., The Group D Streptococci. *Bacteriol. Rev.* 28:330–366, 1964.
5. Hardie, J.M., Whiley, R.A., Classification and overview of the genera Streptococcus and Enterococcus. *J. Appl. Microbiol. Symp. Suppl*. 83, 1S–11S, 1997.
6. Franz C M A P, Holzapfel W H, and Stiles M E (1996). Enterococci at the crossroads

of food science. Int. J. Food Microbiol. 47: 1-24.
7. Facklam, R., Carvalho, Teixeira, L., History, taxonomy, biochemical characteristics, and antibiotic susceptibility testing of enterococci. The enterococci: pathogenesis, molecular biology, and antibiotic resistance. M. S. Gilmore. Washington, ASM Press, 2002.
8. Franz, C., Stiles, M.E., Schleifer, K.H. and Holzapfel, W.H., Enterococci in foods--a conundrum for food safety. *International Journal of Food Microbiology*, 88(2-3):105-122, 2003.
9. Low, D.E., Keller, N., Barth, A., Jones, R.N., Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis*; 32 (Supply 2): S133-45, 2001.
10. Schaberg,D.R., Culver, D.H., Gaynes, R.P., Major trends in the microbial etiology of nosocomial infection. *Am J Med*; *91* : 72S-75S, 1991.
11. Seema, S., Meenakshi M., Das, B.K., and Arti K., Enterococcal infections and antimicrobial resistance, *Indian J Med Res* 128: 111-121, 2008.
12. Ogunbanwo, S.T., Uzokwe, F., Emikpe, B.O., Oni, L.O., Adegoke, C.O., Prevelence and characterization of antibiotic resistance Enterococci from human with dental plague. *Life Sci J*; 9(1s): 57-67, 2012.
13. Ogunbanwo, S. T., Sanni, A. I. and Onilude, A. A., Influence of bacteriocin in the control of *Escherichia coli* infection of broiler chickens in Nigeria. *World Journal of Microbiology and Biotechnology* 20: 51 – 56, 2004.
14. Benyacoub, J., Prez, P.F., Rochat, F., Saudan, K.Y., Reuteler ,G., Antille, N., Humen, M., De Antonio, G.I., Cavadini, C., Blum, S. and Schiffrin, E.J., Prevention of Girdia intestalis infection in mice by feeding with *Enterococcus faecium* strain SF68 *J. Nutr.* 135: 1171 – 1176, 2005.
15. Kenneth, Ryan, C. George, Ray, Nafees Ahmad, W. Lawrence Drew, James Plorde Sherris Medical Microbiology, Fifth Edition McGraw Hill Professional, pages 1040, 2010.
16. Ochei, J. and Kolhatkar, A., Medical Laboratory Science Theory and Practice. Tata McGraw-Hill Publishing Company Limited New Delhi New York Pp 1337, 2000.
17. Denis, D.G., Mater, Philliope, L. Gerad, C. and Maria, J. F., Evidence of Vancomycine resistance gene transfer between *enterococci* of human origin in the gut of mice harboring human microbiota, 2005.
18. Frazier, W. C. and Westhoff, D. C. (1985). *Food microbiology*. Tata McGraw-Hill Publishing Co. Ltd, India Bombay. 540 pp.
19. Dukes, H.H., Physiology of domestic animals. 11th ed. M. J. Swenson, W. O. Reece Editors. Cornell University Press. Ithaca and London, 962 pp, 1993.
20. Murray, R.D., Downham, D.Y., Clarkson, M.J., Faull, W.B., Hughes, J.W., Manson, F.J., Merritt, J.B., Russell, W.B., Sutherst, J.E. and Ward, W.R., Epidemiology of lameness in dairy cattle: description and analysis of foot lesions. *Vet. Rec*. 138:586-591, 1996.
21. Su, Y.A., Sulavik, M.C., He, P., Makinen, K.K., Makinen, P., Fiedler, S., Wirth, R., Clewell, D.B., Nucleotide sequence of the gelatinase gene (geIE) from *Enterococcus faecalis subsp. Liquefaciens*. Infec. Imun.59: 415-420, 1991.
22. Giold, O.G., Jordan, H.V., Van Houte, J., The prevalence of *enterococci* in the human mouth and their pathogenicity in animal model. *Arc. Oral Biol.* 20: 473-477, 1975.