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Hypolipidemic Potential of *Lactobacillus* and *Streptococcus* sp. from Some Nigerian Fermented Foods

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Abstract: The ability of lactic acid bacteria obtained from some Nigerian fermented foods to influence the levels of total cholesterol (TC), triglyceride (TG), low density (LDL) and high-density lipoprotein cholesterols (HDL) besides the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum of Albino rats was investigated. Following a 14 day feeding trial on pork and its stock, the TC value obtained in the serum of the rats was higher (109 mg dL^{-1}) than in rats fed with the growers mash (100 mg dL^{-1}). Similarly, within the same period, the values recorded in the serum of the experimental rats for TG and LDL levels which were also higher (152 and 53.6 mg dL^{-1}) than those obtained for rats fed on commercial diet (116 and 48.2 mg dL^{-1}, respectively) confirm induced hyperlipidemia. Following hyperlipidemia and continued feeding with probiotes as supplement for another 14 days, the TC values in the serum of the treated rats showed no significant difference when compared to the control while the TG level was below 70 mg dL^{-1}. The TC/HDL ratio in the serum of the rats treated with *L. plantarum* UNAD 0507 and *S. lactis* UNAD 0508 was 1.71 and 2.19, respectively. Similarly, the LDL/HDL ratios obtained with the same treatment were 0.57 and 0.96, respectively. The lower the TC/HDL and LDL/HDL ratios the less atherogenic the lipoprotein profile; an indication of the ability of the organisms to influence hyperlipidemia. In all cases, the activity of AST in both control and experimental rats was usually higher (enzyme activity ranged between 59.9 and 109 IU L^{-1}) when compared to ALT (24.2-35.7 IU L^{-1}). The implications of these findings to human health are discussed.

Key words: Cholesterol, enzymes, high-density lipoproteins, *Lactobacillus* sp., low-density lipoproteins, probiotes, serum, triglyceride

INTRODUCTION

Cholesterol (TC) and other fats like the triglycerides (TG) cannot dissolve in the blood. They have to be transported to and from the body cells by special carriers called lipoproteins; the low-density lipoproteins (LDL) and the high-density lipoproteins (HDL). People with high triglycerides often have a high total cholesterol level (Cooper *et al*., 1982). Two different sources of TC and TG have been established, the liver and the diet (El-Gengaihi *et al*., 2004), which is regarded as the most important environmental variable affecting the serum lipoprotein spectrum. The levels of HDL and LDL cholesterol in the blood are usually measured to evaluate the risk of having a heart attack.

Probiotes can be defined as a food (feed) or drug containing live microbes that when ingested is expected to confer beneficial physiologic effects to the host animal through microbial activities. Bacteria species that have traditionally been regarded as safe are used in probiotics; the main strains used include the lactic acid bacteria and Bifidobacteria.

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The genera *Lactobacillus*, *Leuconostoc* and *Streptococcus* are important members of the lactic acid bacteria (LAB) with the lactobacilli constituting the major group. The metabolic activities of lactobacilli are responsible for their therapeutic benefits. Proteins and complex fats are usually broken down by lactobacilli into easily assimilable components, useful in infants, convalescent and geriatric nutrition (McPherson and Spiller, 1996). However, the activity of these organisms to convert lactose to lactic acid is used in the successful treatment of lactose intolerance (Gilliland and Walker, 1990).

Evidence from preclinical and clinical trials has revealed that lactobacilli can break cholesterol in serum lipids (Rasic *et al*., 1992; De Rodas *et al*., 1996; Noh *et al*., 1997). There is little or no information concerning the ability of some LAB species available in Nigeria to confer such health benefits. For this reason, the aim of the present investigation was to examine whether LAB obtained from locally fermented foods and the intestine of cattle can reduce the high level of lipids induced in the serum of Albino rats. To achieve this aim, the current study examined the effect of feeding Albino rats with fried pork and the pork extract (stock) for fourteen days to induce hyperlipidemia in their serum. Thereafter, the potential of the LAB to influence the levels of total cholesterol, triglycerides, the LDL and the HDL cholesterols, besides the activity of some metabolic enzymes was also examined.

MATERIALS AND METHODS

Source and Feeding of Animals

Two weeks old Albino rats (*Rattus norvegicus*) weighing between 61.2 and 65.3 g were obtained from the Department of Physiology, University of Ibadan, Nigeria and maintained on growers mash (commercial diet) for 48 h.

Effect of an Experimental Diet

The rats were starved for 24 h before separating into 2 major groups for an initial period of two weeks feeding as follows, to induce hyperlipidemia in the rats blood:

Thirty two rats were fed with fried minced pork and the aqueous pork extracts (stock). An average amount of 80 mg pork/g and1 mL of the stock/g body weight of the rats was administered twice daily. Control involved feeding four rats fed with the commercial diet.

The initial and subsequent (every other day) weights of the rats were determined during the feeding period. The colour of the eye, erection of the hair and other parameters were monitored visually.

Effect of Probiotes on the Rats

In another experiment after inducing hyperlipidemia, 32 rats were separated into eight groups and treated as in Table 1. Feeding was carried out as above for another 14 days but supplemented with 1 mL of microbial suspension at different concentrations of the test organisms fed *ad libitum* every alternate 12 h. Rats fed with only pork and its stock served as the control. At the end of the experiment, two rats from each group were sacrificed and the serum analyzed for lipoprotein content and the activity of some metabolic enzymes determined.

Table 1: Concentration of probiotes administered as supplement

Experimental diet, 1 mL (2.39×10^4 cfu mL^{-1}) of *Streptococcus lactis* UNAD 0508
Experimental diet, 1 mL (2.31×10^4 cfu mL^{-1}) of *Lactobacillus delbrueckii* UNAD 0509
Experimental diet, 1 mL (2.44×10^4 cfu mL^{-1}) of *Lactobacillus delbrueckii* UNAD 0501
Experimental diet, 1 mL (2.53×10^4 cfu mL^{-1}) of *Lactobacillus plantarum* UNAD 0507
Experimental diet, 1 mL (2.31×10^4 cfu mL^{-1}) of *Streptococcus cremoris* UNAD 0514
Experimental diet, 1 mL (2.14×10^4 cfu mL^{-1}) of *Lactobacillus thermophilus* UNAD 0517
Experimental diet, 1 mL (2.28×10^4 cfu mL^{-1}) of *Lactobacillus bulgaricus* UNAD 0515

Source of Organisms

The under listed microbes were isolated from different Nigerian fermented foods (Aderiye and Laleye, 2003) and the rumen of cattle as follows: *Lactobacillus delbrueckii* UNAD 0509 and *Streptococcus lactis* UNAD 0508 from *Ogi*, a product of fermented maize; *L. delbrueckii* UNAD 0501 and *Streptococcus cremoris* UNAD 0514 from cooked and uncooked *fufu*, respectively, a product of cassava fermentation; *L. bulgaricus* UNAD 0515 and *L. thermophilus* UNAD 0517 from *wara*, a fermented milk product; and *L. plantarum* UNAD 0507 from the stomach of the cattle. All these organisms were cultured at 25°C and maintained on de Mann Rogosa Sharpe agar (MRSA) slants at 5°C.

Serum Lipid Analyses

Blood was centrifuged for 15 min at 1,500 rpm within 30 min of phlebotomy to separate plasma from the red blood cells (RBCs). Serum total cholesterol, high density lipoprotein cholesterol (HDL) and total triglyceride concentrations were analyzed in quadruplicate with enzymatic kits and standardized reagents using a Reflotron Analyzer (Boehringer Mannheim, Germany). The LDL concentration was calculated as described by Friedewald *et al*. (1972).

Determination of Enzyme Activity

The method of Bergmeyer *et al*. (1986) was employed in the determination of the activity of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST).

Statistical Analysis

The experimental data were presented as the means and standard errors of the means. The statistical significances of difference were evaluated by Students t-test.

RESULTS

Inducement of Hyperlipidemia

All the rats fed with both commercial and experimental diets appeared healthy. The colour of the eye was golden and the hair was smooth to touch. The rats' response was sharp and movement swift. The faeces were well formed. The colour and the odour of the urine were normal. Rats fed on the commercial diet recorded a weight gain of about 48.0 g (ca. 73.85% weight increase) within 14 days, whereas an increase of 59 g was observed in the mean weight of the experimental rats (ca. 96.7%) (Fig. 1). Comparatively on the average, a difference of 22.8% weight gain was recorded in rats fed on pork and its stock over those fed on growers' mash after 14 days. The average growth rate of the rats per day when fed the experimental diet was higher (ca. 4.0 g/day) than those fed on commercial diet (3.21 g/day). The feeding of the rats with the experimental diet was to induce hyperlipidemia. However when these data were statistically analyzed there was no significant difference.

The total concentration of cholesterol in the rats fed with commercial diet was 100 mg dL^{-1} (Table 2). After another 14 days of feeding on experimental diet, the TC level in the rats' serum rose by 9% to 109 mg dL^{-1}. There was also an increase of about 31% in the TG level. The LDL content in the serum was found to be 53.6 mg dL^{-1} whereas the HDL level reduced by about 64.2% in the serum of the rats fed on the experimental diet within the same period. The activity of aspartate aminotransferase was much higher (82.4 IU L^{-1}) in the serum of rats fed on pork while alanine aminotransferase exhibited an activity of about 30.4 IU L^{-1} in the serum of rats fed for 14 days on the commercial diet.

Table 2: Concentration of serum lipids and activity of metabolic enzymes in Albino rats after inducement* of hyperlipidemia[I]

Serum lipid/enzyme activity	Commercial diet	Experimental diet*
TC (mg dL^{-1})	100.0	109.0
TG (mg dL^{-1})	116.0	152.0
HDL (mg dL^{-1})	31.6	11.3
LDL (mg dL^{-1})	48.2	53.6
ALT (U L^{-1})	30.4	26.2
AST (U L^{-1})	63.8	82.4

TC: Total cholesterol, ALT: Alanine aminotransferase, TG: Triglyceride, AST: Aspartate aminotransferase, HDL: High-density lipoprotein, *Diet 160 mg minced pork/g body weight daily, LDL: Low density lipoprotein, [I]Feeding period: 14 days

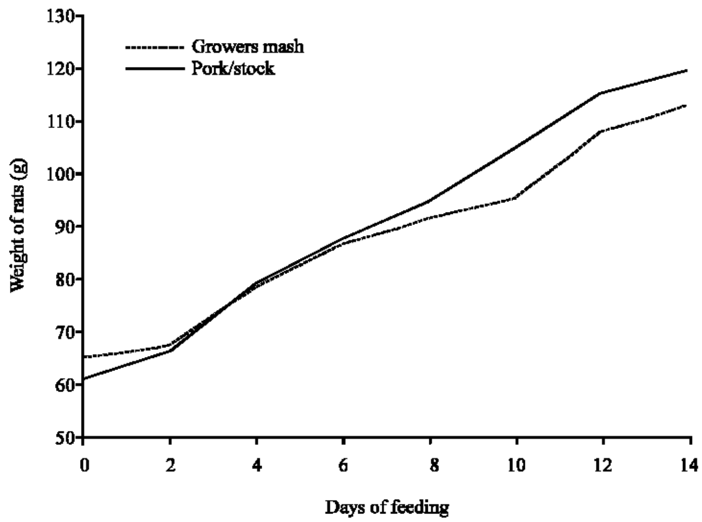

Fig. 1: Weight of rats fed on growers mash and pork/stock

Effect of Administration of Probiotes

In a follow up experiment, *Lactobacillus* and *Streptococcus* sp. were fed as probiotes onto rats *ad libitum* every 12 h in addition to the diet for another 14 days. The average weight gained and the growth rates of the rats are shown in Table 3. The growth rate of the rats treated with *L. delbrueckii* UNAD 0501, *Streptococcus cremoris* UNAD 0514, *L. plantarum* UNAD 0507 was low when compared to those fed *ad libitum* with *L. delbrueckii* UNAD 0509 (2.95 g/day) and *L. bulgaricus* UNAD 0515 (3.33 g/day). The low growth rate had much influence on the weight gained by the rats during feeding.

The effect of ingestion of the probiotes on the level of the different serum lipids in the Albino rats is shown in Table 4. There was no drastic change in the total TC level in the experimental rats except those treated with *L. delbrueckii* UNAD 0501. Meanwhile, the TG level in all the treated rats fell below 70 mg dL^{-1}. The high-density lipoprotein cholesterol levels (HDL) in the rats fed with *L. delbrueckii* UNAD 0501 (21.8 mg dL^{-1}) and *S. cremoris* UNAD 0514 (15.6 mg dL^{-1}) were the lowest. Meanwhile, LDL level in rats treated with these microorganisms was the highest (73.8 mg dL^{-1}).

The activity of AST in rats fed on the experimental diets was usually higher (enzyme activity ranged between 59.9 and 109 IU L^{-1}) when compared to that of ALT which ranged between 22.3 and 35.7 IU L^{-1} (Table 4). The activity of AST was the highest in rats treated with *S. cremoris* UNAD 0514.

Table 3: Effect of administration of probiotes on weight and growth rate of Albino rats

Probiotes/diet	Weight gain* (g)	Weight gain (%)	Growth rate (g/day)
Lactobacillus bulgaricus UNAD 0515	46.6^{c}	43.8	3.33
Lactobacillus delbrueckii UNAD 0501	33.2^{a}	30.4	2.37
Lactobacillus delbrueckii UNAD 0509	41.3^{b}	29.1	2.95
Lactobacillus plantarum UNAD 0507	36.5^{a}	35.8	2.61
Lactobacillus thermophilus UNAD 0517	39.0^{b}	34.5	2.79
Streptococcus cremoris UNAD 0514	36.8^{b}	27.2	2.57
Streptococcus lactis UNAD 0508	36.8^{b}	28.4	2.63
Experimental diet	39.1^{b}	26.4	2.79

*Values with different superscripts are significantly different

Table 4: Effect of administration of probiotes on serum lipids and some metabolic enzymes of Albino rats

	Serum lipid (mg dL^{-1})				Enzyme activity (U L^{-1})	
LAB cells/diet	TC	TG	HDL	LDL	ALT	AST
Lactobacillus bulgaricus UNAD 0515	110	67	29.1	70.9	24.5	59.9
Lactobacillus delbrueckii UNAD 0501	105	66	21.8	73.8	35.7	99.6
Lactobacillus delbrueckii UNAD 0509	117	60	29.1	73.0	24.5	105.0
Lactobacillus plantarum UNAD 0507	110	68	64.2	36.8	32.6	97.8
Lactobacillus thermophilus UNAD 0517	112	60	42.3	59.3	24.2	93.6
Streptococcus cremoris UNAD 0514	110	66	15.6	73.8	35.4	109.0
Streptococcus lactis UNAD 0508	114	65	52.2	50.2	29.6	83.3
Experimental diet	113	156	30.5	71.0	22.3	92.5

Table 5: Effect of administration of probiotes on serum TC/HDL and LDL/HDL ratios of hyperlipidemic Albino rats

Probiotes/diet	TC/HDL	LDL/HDL
Lactobacillus bulgaricus UNAD 0515	3.78	2.44
Lactobacillus delbrueckii UNAD 0501	4.81	3.39
Lactobacillus delbrueckii UNAD 0509	4.02	2.51
Lactobacillus plantarum UNAD 0507	1.71	0.57
Lactobacillus thermophilus UNAD 0517	2.65	1.40
Streptococcus cremoris UNAD 0514	7.05	4.73
Streptococcus lactis UNAD 0508	2.19	0.96
Experimental diet	3.70	2.33

Very high TC/HDL and LDL/HDL ratios were recorded in the serum of rats treated with *S. cremoris* UNAD 0514 (7.05, 4.73) and *L. delbrueckii* UNAD 0501 (4.81, 3.39). However, very low TC/HDL ratio was observed in rats treated with *L. plantarum* UNAD 0507, *S. lactis* UNAD 0508 and *L. thermophilus* UNAD 0517 (1.71, 2.19 and 2.65, respectively). Similarly, the LDL/HDL ratios obtained with the same test organisms were also low, 0.57, 0.96 and 1.40, respectively (Table 5).

DISCUSSION

Dietary cholesterol and triglycerides are mainly obtained from eating animal products and saturated fats. In this study, the fried pork and its extract (stock) used as the experimental diet contained high protein and fat contents (data not shown in this report). McPherson and Spiller (1996) showed that some specific nutritional factors responsible for altering human lipoprotein metabolism include the content and composition of the dietary fats.

Rats fed on the experimental diet showed a higher body weight gain than those fed on the commercial diet. This difference in body weight gain may be attributed to increased fat and protein contents in the diet when compared to those rats fed on the commercial diet. This agrees with an earlier finding of Edem *et al*. (2003) where the increased fat and protein contents in meals supplemented with high percentage of oil were reported to be responsible for the body weight disparity in rats. Mihoko *et al*. (2003) also reported that rats' receiving the Lactobacillus GG cells showed a lower body weight gain than those of the control and cholesterol groups, but there was no significant difference.

The increased levels of serum triglyceride, total and LDL cholesterols in the experimental rats during the first 14 days of feeding may be caused by an enhancement of the intestinal absorption of these lipids (Mihoko *et al*., 2003), which also confirmed the inducement of hyperlipidemia in the rats serum.

With the administration of probiotes, obvious changes were noticed in the weights of the rats fed with lactobacilli and the experimental diet. This observation coupled with the low TC and TG values as in Table 4, showed the ability of some of these microbes to reduce the level of some lipoproteins in the rat serum. Tsimikas and Reaven (1998) showed that the fatty acid composition of serum lipoproteins of rats was influenced by the rate and extent of oxidation of the fatty acids. Some studies on the hypocholesterolemic effects of LAB have also supported the hypothesis that bacterial cells with high cholesterol-binding capacity could inhibit the absorption of cholesterol in the small intestine and thereby decrease the serum cholesterol level (Bernard and Carlier, 1991; Ikedia *et al*., 1991; Tso *et al*., 1995; De Rodas *et al*., 1996). This may be so in this study especially where the concentration of TC and TG reduced following inducement of hyperlipidemia and subsequent administration of LAB cells in the rats.

High levels of blood cholesterol and LDL have been reported to influence the incidence of atherosclerosis in man (Mancini and Parillo, 1991; Carpentier, 1997). The atherogenecity resulting from increased levels of serum lipoproteins depends on its distribution between HDL and LDL. The lower the LDL/HDL ratio, the less atherogenic the lipoprotein profile (EI Gengaihi *et al*., 2004). Similarly the LDL/HDL ratio of 0.96 recorded by *S. lactis* UNAD 0508 was very low compared to those obtained from rats treated with other LAB cells.

The high HDL level and the corresponding low LDL value in rats fed with *L. plantarum* UNAD 0507 gave an indication of hypolipidemic potential of the organism.

The ALT and AST are enzymes located in the liver cells which are spilled into the blood stream when the liver cells are injured. The high concentrations of AST in the rats serum recorded in this study indicate that there is a leakage from the damaged cells of some organs other than the liver. The AST is a mitochondrial enzyme that is also present in the heart, muscle, kidney and brain. The ALT level is generally increased above the normal values in situations where there is damage to the liver cell membranes (Arias *et al*., 2001). In many cases of liver inflammation, the ALT and AST activities are estimated roughly in a 1:1 ratio. However, our report showed very high ALT: AST ratios of between 1:2 and 1:4 in the different vital organs.

It is usually accepted that live bacteria in the human gastrointestinal tract (GIT) need to reach a minimal concentration of 10^5 units per mL in the small intestine and 10^7 units mL^{-1} in the colon to express potential normal intestinal micro flora, the gastric acid secretion, intra luminant bile acids probiotic activities. The survivability of the LAB cells may have been influenced by interactions with the immune systems and the mobility functions of the rats GIT. In spite of these host factors and the low concentration of 10^4 cfu mL^{-1} of the different LAB species administered every 12 h, only *L. plantarum* UNAD 0507 and *S. lactis* UNAD 0508 showed some promising hypolipidemic potentials in the serum of Albino rats studied.

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