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# Hypocholesterolemic Action of *Lactobacillus plantarum* NRRL-B-4524 and *Lactobacillus paracasei* in Mice with Hypercholesterolemia Induced by Diet.

[1]Hanaa A. El-Shafie, [2]Nagwa I.Yahia, [2]Hanem A. Ali, [2]Fatma A. Khalil, [3]Ebtsam M. El-Kady and [1]Yomna A. Moustafa

[1]Microbial Chemistry Department,National Research Centre.
[3]Microbial Biotechnology Department, National Research Centre.
[2]Biochemistry and Nutrition Department, Girls College,Ain-Shams University.

**Abstract:** Cholesterol is known to be a major risk factor for coronary heart disease. Current treatments for elevated blood cholesterol include dietary management, regular exercise, and drug therapy , bile acid sequestrants.Such therapies, however, are often suboptimal and carry a risk for serious side effects. Lactic acid bacteria with active bile salt hydrolase (BSH) or products containing them are suggested to lower cholesterol levels. This study shows the effect of lactic acid bacteria *Lactobacillus plantarum* NRRL B-4524 used single or mixed with *Lactobacillus paracasei* and/or other strains of bacteria in rat diets ( high fat and high cholesterol-enriched) in lowering blood serum cholesterol. Results show that consumption of lactic acid bacteria induced lowering of total serum cholesterol, total lipid, triacylglycerol, LDL-C and atherogenic index and increase in serum HDL-C. All samples experienced a high significant increasing effect for the excretion of free fatty acids and neutral fat in faeces. Mixed groups had a significant decreasing effect on body weight and feed intake/day than did single groups.

**Key words:** Lactic acid bacteria-Anticholesterol-*Lactobacillus plantarum-Lactobacillus paracasei.*

## INTRODUCTION

Coronary heart disease (CHD) is the main cause of death in Canada,US,and many other countries around the world (American Heart Association, 2002;Heart and Stroke Foundation of Canada, 2000).The world Health Organization (WHO) predicts that by the year 2020 ,up to 40% of all deaths will be related to cardiovascular diseases or disease of the heart. Although cholesterol is an important basic block for body tissues, elevated blood cholesterol is a well known major risk factor for CHD (Aloglu and Oner, 2006). Recent modalities for lowering blood cholesterol levels involve dietary management,behavior modification,regular exercise,and drug therapy (Dunn-Emke *et al*., 2001).

Pharmacological agents are available for the treatment of high cholesterol, although they effectively reduce cholesterol levels,they are expensive and are known to have severe side effects (Bliznakov, 2002). Lactic acid bacteria with active bile salt hydrolase (BSH) or products containing them are suggested to lower cholesterol levels through interaction with host bile salt metabolism (De Smet *et al*., 1998). Lactobacilli with BSH activity have an advantage to survive and colonize the lower small intestine where the enterohepatic cycle takes place and therefore BHS activity may be considered an important colonization factor (De Smet *et al*., 1995). In 2000, Sanders proposed mechanism based on the ability of certain probiotic Lactobacilli and Bifidobacteria to deconjugate bile acids enzymatically, increasing their rates of excretion. Cholesterol being a precursor of bile acids, converted its molecules to bile acids replacing those lost during excretion and this lead to reduction in serum cholesterol.This mechanism could be operated in the control of serum cholesterol levels by conversion of deconjugated bile acids into secondary bile acids by colonic microbes.

The use of orally applied microorganisms (probiotics) is a major aim of the concept of functional food. A food can be regarded functional if it is satisfactory demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well being and/or reduction of risk of disease (Lilian and Thompsom, 2001).

Recently, there has been much interest in LAB especially *Lactobacillus plantarum*, due to its beneficial effects in health include anticholesterol; anticarcinogenic properties and stimulation of the immune system

**Corresponding Author:** H.A.El-Shafie, Microbial Chemistry Department,National Research Centre.
E-Mail : Prof_Elshafie@hotmail.com.

(Bengmark *et al.*, 1998 and Mack *et al.*, 1999). *Lactobacillus plantarum*, is the predominating Lactobacillus species on both the oral and intestinal human mucosa. It has the ability to survive the passage through the human gastro-intestinal tract and to establish itself for at least a shorter time in the intestine after consumption (Bengmark *et al*., 1998; Johansson *et al*., 1998 and Niedzielen *et al*., 2001).

The main objective of this work was to study the potential effect of *Lactobacillus plantarum* alone or in combination with other LAB organisms (probiotic mixture) on hypercholesterolaemic rats.

## MATERIALS AND METHODS

***Materials:***

***Bacterial Strains:***

*Latobacillus paracasei* a LAB strain isolated from yoghurt and identified in our laboratory. *Lactobacillus plantarum* NRRL B- 4524, *Enterococcus faecalis* NRRL B-760, *Lactobacillus acidophilus* NRRL B-4495 lyophilized reference strains purchased from the National Center for Agricultural Utilization Research (USA). *Bacillus subtilus* purchased from the Center of Culture of Microbiological Food, Irradiation Department, National Center for Radiation Research and Technology, Nasr city, Egypt.

***Composition of the High Saturated Fat Diet (g/100g Diet):***

Corn starch, 61.8;Casein (87.5% protein),16; Corn oil, 5; Tallow, 10; Salt mixture, 3.5; vitamin mixture, 1; DL-methionine, 0.3; Choline chloride, 0.2.

***Composition of Mineral Mixture ( g/kg Salt Mix):***

Calcium phosphate dibasic, 500; Sodium chloride, 74.0; Potassium citrate monohydrate, 220; Potassium sulfate, 52; Magnesium oxide, 24; Manganous carbonate, 3.5; Ferric citrate, 6; Zinc carbonate, 1.6; Cupric carbonate, 0.3; Potassium iodate, 0.01; Sodium selenite, 0.01; Chromium potassium sulfate, 0.55; Sucrose finely powdered to make 1.000g (AIN, 1977).

***Composition of Vitamins Mixture per Kg Mix:***

Thiamine HCL, 600 mg; Riboflavine, 600 mg; Pyridoxine HCL, 700 mg, Nicotinic acid, 3 g; Calcium pantothenate, 1.6 g; Folic acid, 200 mg; Biotin, 20 mg; Cyanocobalamine (Vitamin B-12), 1 mg, Retinyl palmitate or acetate, 400.000 IU; - Tocopherol acetate (Vitamin E) , 5000 I; Cholecalciferol (Vitamin D3), 2.5 mg; Menaquinone (Vitamin K), 5 mg; Sucrose, finely powdered to make 1000 g (AIN, 1977).

***Methods:***

***Isolation of Lactic Acid Bacteria1 Strains from Food:***

All samples of yoghurt and milk were held at 4°C for 1 week after the sell - by date. This treatment was designed to enrich lactic acid bacteria, which grow and produce antagonistic substances at lower temperature. 10% of each product was suspended in 0.85% NaCl solution (pH 6.5). 0.1 ml was spread on JP2 medium in Petri dishes. After incubation for 48 hr at 30°C, dishes were exposed to iodine vapor to detect starch hydrolysis areas. Isolated strains were purified by three successive transfers on JP2 medium and cultures were routinely checked for purity by microscopic observation (Vaughan *et al*., 1994).

***Identification of the Selected Strain:***

The isolated strain was examined for catalase production, Gram stain and cell shape and was identified according to its physiological and biochemical characteristics as described by Bergy's Manuals (1994). This strain was characterized using Sugar fermentation reactions (Todorov & Dicks, 2005).

***Preparation of Probiotic Culture:***

A) 200 g of wheat bran flask (obtained from local market) was sterilized by autoclaving for 20 min at 121°C with moisture content 33%.
B) The bacterial strains are grown on MRS broth medium or nutrient broth, incubated at 30°C for 12 hr.
C) Bacterial strains were combined with previously prepared wheat bran and fermented for one week at 37°C (Fukushima and Nakano, 1995).
D) The final proportion of each microbe was adjusted to $10^{7-8}$ colony-Forming units (CFU)/g wheat bran using pure liquid-cultured microbe.
F) 3g fermented wheat bran added to 100g diets in a constant concentration (3 g wheat bran/100g diet).

***Experimental Animals:***

Eighty-eight male albino rats, Sprague Dawley strain (weight range was 105 - 117 g) were obtained from National Research Center, Giza, Egypt. The animals were divided into eleven homogeneous groups each of eight animals, housed individually in stainless steel cages fitted with a wire mesh bottoms and maintained on a 12h light-dark cycle. Room temperature was controlled at 25-30°C with about 50% relative humidity. Then they were allocated to the various experimental diets for 6 weeks. During the experimental period, food and tap water were provided to all animals. The test groups were fed on diets containing 3g of wheat bran/100g diet inoculated with different bacterial strains (Table 1), single or mixed with *Lactobacillus plantarum* and control groups were fed on diets containing the same weight of wheat bran without bacterial inoculation. Body weight gain was recorded every 2 weeks and feed consumption was recorded weekly. The experiment lasted for 6 weeks. 1% cholesterol was added only to the ten number diets. Cholesterol was dissolved in hot corn oil before being thoroughly mixed into the diet. Corn oil for the control diet was heated by the same way.

**Table 1:** Experimental animal design: Eleven experimental diets were prepared as follow:

| | |
|---|---|
| Group 1 | Control negative (high fat diet) |
| Group 2 | Control positive (high fat, high cholesterol diet) |
| Group 3 | High fat, high cholesterol diet inoculated with *Lactobacillus plantarum* |
| Group 4 | High fat, high cholesterol diet inoculated with *Bacillus subtilus* |
| Group 5 | High fat, high cholesterol diet inoculated with *Lactobacillus acidophillus* |
| Group 6 | High fat, high cholesterol diet inoculated with isolated strain (*Lactobacillus paracasei)* |
| Group 7 | High fat, high cholesterol diet inoculated with *Enterococcus faecalis* |
| Group 8 | High fat, high cholesterol diet inoculated with mixed culture (*Lactobacillus plantarum + Bacillus subtilus)* |
| Group 9 | High fat, high cholesterol diet inoculated with mixed culture (*Lactobacillus plantarum + Lactobacillus acidophillus)* |
| Group 10 | High fat, high cholesterol diet inoculated with mixed culture (*Lactobacillus plantarum* + isolated strain (*Lactobacillus paracasei)* |
| Group 11 | High fat, high cholesterol diet inoculated with mixed culture (*Lactobacillus plantarum + Enterococcus faecalis)* |

***Samples Collection:***

At the end of the 6 weeks experimental period the animals were fasted for 12hr. They were anesthetized with diethyl ether. Incisions were made into the abdomen and blood samples were obtained from the portal vein into centrifuge tubes, serum was separated by centrifugation at 3000 rpm. for 15 minutes, and frozen in plastic vials and kept at -20°C for subsequent biochemical analysis.

***Tissues:***

Liver, heart, spleen and kidneys were excised, rinsed in chilled saline solution, then blotted on filter paper, and weighed separately to calculate the absolute and relative organs weight. Liver was stored at - 20°C until analysis. The analysis included the determination of total cholesterol, triacylglycerols and total lipid.

***Extraction of Liver Lipids:***

Lipids were extracted from liver by the method of Folch *et al.* (1957).

***Faecal Samples:***

During the last week, feaces were collected quantitatively from different groups and dried to a constant weight at 55°C, weighed and finally ground in a mill and then stored individually at -20°C until analysis.

***Biochemical Analysis:***

The collected samples were analyzed and the Lipid profile was determined by using enzymatic colorimetric method kits.

***Total Cholesterol (mmol/L):***

Serum and liver cholesterol were determined according to Thomas (1992).

***Triacylglycerols (mmol/L):***

Serum and liver triacylglycerols were determined according to Fossati and Principie (1982).

***Total Lipids (g/l):***

Serum and liver total lipids were determined according to Lars & Martin (2002).

***SerumHDL-Cholesterol (mmol/L):***

HDL-C was determined by using precipitation colorimetric method kits (Richmond, 1973. SerumLDL-SerumLDLCholesterol(mmol/L): LDL-C was calculated according to Lopes *et al*. (1997) using the following formula: LDL - Cholesterol = Total Cholesterol- (Triacylglycerols/2.2)-HDL-Cholesterol.

***Atherogenic Index (AI) in Serum:***

It was calculated according to Igarashi, *et al*. (1997) by the following formula: AI=Total cholesterol –HDL-C /HDL-C.

***Faecal Analysis:***

Lipids extracted from dried faeces were used for the determination of free fatty acids and neutral fat (Kamer *et al.,* 1949).

***Statistical Analysis:***

The experiment followed Complete Randomized Design (CRD). The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1982) using Mstatc Programme. Duncan's Multiple Range Test were used to compare between means of treatment according to Walter and Duncan (1969) at probability 5%.

## RESULTS AND DISCUSSION

***Identification of the Selected Isolated Strain:***

This isolate was rod shaped bacteria; 0.5-1.1 micron in diameter; mostly in small chains; nonmotile; nonspore forming; gram-positive; and catalase-negative, and the cells were of the bacillus type. On the basis of the results presented in Table (2) and the carbohydrate utilization patterns this strain was identified as *Lactobacillus paracasei.* The effect of different probiotic bacteria on final body weight; gain in weight; feed intake/day and feed efficiency ratio is presented in Table (3). It was found that,there was a decreasing effect in final weight, feed intake/day and gain in weight in most groups. Statistical analysis revealed that mixed groups had a significant decreasing effect on final weight, feed intake/day, gain in weight and a non significant effect on feed efficiency ratio compared to single groups. The Effect of supplementing different probiotic bacteria on relative weights of heart, liver, kidney and spleen (g %) are shown in Table (4). Generally it was found that there were significant differences in the mean values of the relative heart, liver and kidney weights, expressed as a percentage of body weight (g of organ weight/100 g of body weight) between different groups of rats while there were non-significant differences in means values of relative spleen weight. The effect of feeding different probiotic bacteria on lipid profile (serum total cholesterol, LDL-cholesterol, HDL-cholesterol, atherogenic index, triacylglycerol, and total lipid) are presented in Table (5). Tested groups showed percentage decrease in serum cholesterol by 19.07 %, 9.02%, 10.89% and 6.86 % for *Lactobacillus plantarum*, *Bacillus subtilis*, *Lactobacillus acidophilus*, *Lactobacillus paracasei* respectively. All groups showed a high significant decreasing effect on LDL–cholesterol except for *E. faecalis*,the effect was non significant. Mixed groups (8, 9, 10, and 11) decreased LDL-cholesterol 27.66 %, 29.34 %, 27.87 % and 26.52 % respectively.

HDL-cholesterol had opposite trend, there was an increase in HDL-Cholesterol in all tested groups and their effect was non-significant except for G8, G9, G11 it was significant. Single and mixed organisms caused significant decreasing effect on Atherogenic index except for *Enterococcus faecalis*, it had non-significant effect. The highest percentege decrease (35.47 %) was attained by mixed cultures of *Lactobacillus plantarum* and *Lactobacillus acidophilus*.

The tested groups revealed a high significant decreasing effect on total lipid and triacylglycerol except *Enterococcus faecalis* showed high significant increasing effect by 47.89%, 66.05% respectively than did the corresponding control. On the other hand *Lactobacillus plantarum* showed decreasing effect on triacylglycerol and total lipid by 22.96 % and 36.57 % respectively. *Lactobacillus paracasei* had a lower effect on total cholesterol triacylglycerol and atherogenic index than *Lactobacillus plantarum* by 8.3 %, 19.43 % and 25.62 % respectively. Generally, statistical analysis showed that mixed groups versus single one had a high significant decreasing effect on all serum lipid profile except for LDL-C.

**Table 2:** Pattern of fermented carbohydrates of isolated strain (*Lactobacillus paracasei*).

| Code | Carbohydrates | 24 hr | 48 h |
|---|---|---|---|
| 0 | Control | - | - |
| 1 | Glycerol | - | - |
| 2 | Erythritol | - | - |
| 3 | D Arabinose | - | - |
| 4 | L Arabinose | - | - |
| 5 | Ribose | + | + |
| 6 | D Xylose | - | - |
| 7 | L Xyloso | - | - |
| 8 | Adonitol | - | - |
| 9 | Methyl-D-Xylos | - | - |
| 10 | Galactose | + | + |
| 11 | Glucose | + | + |
| 12 | Fructose | + | + |
| 13 | Mannose | + | + |
| 14 | Sorbose | - | - |
| 15 | Rhamnose | - | - |
| 16 | Dulcitol | - | - |
| 17 | lnositol | - | + |
| 18 | Mannitol | + | + |
| 19 | Sorbitol | - | - |
| 20 | -Methyl-D Mannoside | - | - |
| 21 | -Methyl-D-Glucoside | + | + |
| 22 | N-Acetyl-Glucosamine | + | + |
| 23 | Amygdalin | + | + |
| 24 | Amygdalin | + | + |
| 25 | Esculin | + | + |
| 26 | Salicin | + | + |
| 27 | CellobIoBe | + | + |
| 28 | Maltose | + | + |
| 29 | Lactose | - | - |
| 30 | Melibiose | - | - |
| 31 | Sucrose | + | + |
| 32 | Trehalose | + | + |
| 33 | Inulin | + | + |
| 34 | Melezitose | + | + |
| 35 | Raffinose | - | - |
| 36 | Starch | - | - |
| 37 | GlycoGen | - | - |
| 38 | GlycoGen | - | - |
| 39 | Xylitol | + | + |
| 40 | D Turanose | + | + |
| 41 | D Lyxose | - | - |
| 42 | D Tagatose | + | + |
| 43 | D Fucose | - | - |
| 44 | L Fucose | - | - |
| 45 | D ArabitoL | - | - |
| 46 | L Arabitol | - | - |
| 47 | Glueonate | - | + |
| 48 | 2-Keto-Gluconate | - | - |
| 49 | 5-Keto-Gluconate | - | - |

**Table 3:** The effect of supplementing different probiotic bacteria on initial body weight; final body weight; gain in body weight; feed intake/day and feed efficiency ratio of rats.

| Parametrs<br>----------<br>Groups | Initial weight (g) | Final weight (g) | | Gain in weight (g) | | Feed intake /day (g) | | Feed efficiency ratio |
|---|---|---|---|---|---|---|---|---|
| G1 (Control negative) | 117.31 | 296.80 | a | 179.44 | a | 16.88 | ab | 0.254 |
| G2 (Control positive) | 112.38 | 290.10 | ab | 177.75 | a | 17.38 | a | 0.245 |
| G3 (*L. plantarum*) | 108.00 | 275.30 | abcd | 167.25 | bc | 16.00 | abc | 0.250 |
| G4 (*B. subtilius*) | 110.63 | 270.90 | bcd | 160.25 | cdf | 16.00 | abc | 0.237 |
| G5 (*L. acidophillus*) | 108.75 | 270.40 | bcd | 161.63 | cd | 15.25 | cde | 0.251 |
| G6 (*L. Paracasei*) | 108.50 | 284.00 | abc | 175.50 | ab | 16.63 | abc | 0.250 |
| G7 (*Enterococcus faecalis*) | 110.75 | 293.00 | ab | 182.25 | a | 16.63 | abc | 0.260 |
| G8 (*L. plantarum +B. subtilius*) | 110.50 | 276.90 | abcd | 166.38 | bc | 15.75 | bcd | 0.251 |
| G9 (*L. plantarum +L. acidophillus*) | 106.38 | 271.40 | bcd | 165.00 | c | 14.50 | de | 0.268 |
| G10 (*L. plantarum +L. Paracasei*) | 107.25 | 260.60 | cd | 153.38 | de | 14.13 | e | 0.261 |
| G11 (*L. plantarum +Enterococcus faecalis*) | 105.88 | 256.5 | d | 150.63 | e | 13.88 | e | 0.256 |
| G2 versus G3 | N.S | N.S | | S | | N.S | | N.S |
| Single Versus mixed | N.S | S | | S | | S | | N.S |

-Statistical analysis of results were done according to Duncan's multiple range test.
-Means with different letters within each column are significant at 5% level and means without letters are not significant.
- S = Significant - N.S = Not significant

**Table 4:** The effect of supplementing different probiotic bacteria on relative organs weight (g %).

| Group | Relative heart weight | | Relative liver weight | | Relative kidney weight | | Relative spleen weight |
|---|---|---|---|---|---|---|---|
| G1 (Control negative) | 0.334 | b | 3.6 | bc | 0.569 | cd | 0.455 |
| G2 (Control positive) | 0.329 | bc | 4.24 | a | 0.701 | a | 0.39 |
| G3 (*L. plantarum*) | 0.369 | a | 3.83 | b | 0.676 | ab | 0.448 |
| G4 (*B. subtilius)* | 0.331 | bc | 3.5 | bc | 0.611 | bcd | 0.423 |
| G5 (*L. acidophillus*) | 0.317 | bcd | 3.56 | bc | 0.636 | abc | 0.353 |
| G6 (*L. Paracasei*) | 0.303 | bcd | 3.68 | bc | 0.582 | cd | 0.397 |
| G7 (*Enterococcus faecalis*) | 0.306 | bcd | 3.78 | b | 0.637 | abc | 0.401 |
| G8 (*L. plantarum + B. subtilius)* | 0.286 | d | 3.62 | bc | 0.56 | d | 0.375 |
| G9 (*L. plantarum + L. acidophillus*) | 0.307 | bcd | 3.45 | bc | 0.604 | cd | 0.438 |
| G10 (*L. plantarum + L. Paracasei*) | 0.294 | d | 3.3 | c | 0.547 | d | 0.388 |
| G11 (*L. plantarum +Enterococcus faecalis*) | 0.3 | cd | 3.45 | bc | 0.596 | cd | 0.398 |
| G2 versus G3 | S | | S | | N.S | | N.S |
| Single Versus mixed | N.S | | N.S | | S | | N.S |

-Statistical analysis of results were done according to Duncan,s multiple range test.-Means with different letters within each column are significant at 5% level and means without letters are not significant.-S = Significant
-N.S = Not significant

**Table 5:** The effect of supplementing different probiotic bacteria on serum total lipid, total cholesterol, triacylglycerol, HDL-cholesterol, LDL- cholesterol and atherogenic index.

| Parameters<br>----------<br>Groups | Total lipid in serum (g/l) | Total cholestrol in serum (mmol/l) | Triacyl glycero in serum (mmol/l) | lHDL-cholestrolL in serum (mmol/l) | DL-cholestrol in serum (mmol/l) | Atherogenic index |
|---|---|---|---|---|---|---|
| G1 (Control negative) | 11.490 b | 3.585 bc | 1.497 b | 1.529 a | 1.375 b | 1.349 e |
| G2 (Control positive) | 11.810 b | 3.803 a | 1.603 b | 1.231 e | 1.844 a | 2.151 a |
| G3 (*L. plantarum*) | 10.970 bcd | 3.268 e | 1.235 c | 1.356 bcde | 1.351 b | 1.417 dc |
| G4 (*B. subtilius)* | 11.560 b | 3.460 bcde | 1.452 bc | 1.271 cde | 1.529 b | 1.746 bcd |
| G5 (*L. cidophilus*) | 10.050 de | 3.389 cde | 1.379 bc | 1.350 bcde | 1.412 b | 1.520 cde |
| G6 (*L. Paracasei*) | 10.310 cde | 3.542 bcd | 1.475 b | 1.296 cde | 1.576 ab | 1.780 bc |
| G7 (*Enterococcus faecalis*) | 19.610 a | 3.652 ab | 2.366 a | 1.251 de | 1.326 b | 1.967 ab |
| G8 (*L. plantarum + B. subtilius)* | 11.100 bc | 3.366 cde | 1.418 bc | 1.388 bcd | 1.334 b | 1.429 cde |
| G9 (*L. plantarum + L. cidophilus*) | 10.270 cde | 3.321 de | 1.375 bc | 1.393 bc | 1.303 b | 1.388 e |
| G10 (*L. plantarum + L. Paracasei*) | 9.729 e | 3.309 de | 1.441 bc | 1.324 bcde | 1.330 b | 1.529 cde |
| G11 (*L. plantarum +Enterococcus faecalis*) | 10.270 cde | 3.513 bcd | 1.588 b | 1.437 ab | 1.355 b | 1.453 cde |
| G2 versus G3 | N.S | S | S | S | S | S |
| Single Versus mixed | S | S | S | S | N.S | S |

-Statistical analysis of results were done according to Duncan's multiple range test.-Means with different letters within each column are significant at 5% level and means without letters are not significant.
- S = Significant
- N.S = Not significant

Statistical analysis for the effect of feeding different probiotic bacterial strains ( single and mixed) on liver total lipid, total cholesterol and triacylglycerol in comparison to positive control revealed that there was a higher significant decreasing effect for all groups. Meanwhile mixed groups showed higher decreasing effect than single groups on total lipid and triacylglycerol,but concerning total cholesterol single groups prevailed (Table 6).

**Table 6:** The effect of supplementing different probiotic bacteria on liver total lipid, total cholesterol and triacylglycerol (mg/g tissue)

| Parametrs<br>----------<br>Group | Total lipid in liver (mg/g tissue) | | Total cholestrol in liver (mg/g tissue) | | Triacylglycerol in liver (mg/g tissue) | |
|---|---|---|---|---|---|---|
| G1 (Control negative) | 49.22 | cd | 18.18 | b | 19.01 | b |
| G2 (Control positive) | 54.36 | a | 20.4 | a | 20.15 | a |
| G3 (*L. plantarum*) | 49.82 | c | 12.59 | de | 12.69 | g |
| G4 (*B. subtilius)* | 51.87 | b | 10.92 | f | 18.43 | b |
| G5 (*L. acidophillus*) | 49.97 | c | 11.66 | ef | 13.35 | fg |
| G6 (*L. Paracasei*) | 50.07 | c | 13.28 | d | 14.51 | de |
| G7 (*Enterococcus faecalis*) | 50.2 | c | 11.92 | ef | 17.37 | c |
| G8 (*L. plantarum + B. subtilius)* | 48.57 | d | 12.23 | e | 15.25 | d |
| G9 (*L. plantarum + L. acidophillus*) | 49.61 | c | 16.8 | c | 14.39 | def |
| G10 (*L. plantarum + L. Paracasei*) | 49.71 | c | 16.66 | c | 13.84 | ef |
| G11 (*L. plantarum +Enterococcus faecalis*) | 50.03 | c | 17.19 | c | 16.68 | c |
| G2 versus G3 | S | | S | | S | |
| Single Versus mixed | S | | S | | S | |

-Statistical analysis of results were done according to Duncan's multiple range test.
- Means with different letters within each column are significant at 5% level and means without letters are not significant.
- S = Significant -N.S = Not significant

All samples (single or mixed) experienced a high significant increasing effect on the excretion of free fatty acids and neutral fats in faeces. Statistical analysis revealed that mixed groups dominated than single groups (Table 7). Results show that *Lactobacillus plantarum* NRRL B-4524 used single or mixed with *Lactobacillus paracasei* and/or other strains of bacteria in rat diets (high fat and high cholesterol-enriched) had a high significant decreasing effect on all lipid profile and as a result, can establish a basis for their use in lowering blood serum cholesterol.

**Table 7:** The effect of supplementing different probiotic bacteria on faecal free fatty acids and neutral fat (g/100 g dry faeces).

| Parametrs | Free fatty acids | | Neutral fat | |
|---|---|---|---|---|
| Groups | | | | |
| G1 (Control negative) | 1.325 | b | 17.61 | bc |
| G2 (Control positive) | 0.941 | d | 17.34 | d |
| G3 (*L. plantarum*) | 1.58 | a | 17.71 | ab |
| G4 (*B. subtilius)* | 1.028 | d | 17.61 | bc |
| G5 (*L. acidophillus*) | 1.006 | d | 17.72 | ab |
| G6 (*L. Paracasei*) | 1.536 | a | 17.69 | ab |
| G7 (*Enterococcus faecalis*) | 0.967 | d | 17.49 | c |
| G8 (*L. plantarum + B. subtilius)* | 1.152 | c | 17.75 | a |
| G9 (*L. plantarum + L. acidophillus*) | 1.464 | a | 17.74 | a |
| G10 (*L. plantarum + L. Paracasei*) | 1.591 | a | 17.74 | a |
| G11 (*L. plantarum +Enterococcus faecalis*) | 1.019 | d | 17.61 | abc |
| G2 versus G3 | S | | S | |
| Single Versus mixed | S | | S | |

- Statistical analysis of results were done according to Duncan's multiple range test.
- Means with different letters within each column are significant at 5% level and means without letters are not significant.
-S = Significant - N.S = Not significant

***Discussion:***

Nutrition should be an elementary factor extending psycho-physical condition and satisfaction of man's life becomes more and more important.The above aim is achieved in many ways. One of them is the use of robotic bacteria in the production of food of increased nutritional value, high therapeutic quality and desired sensory characteristics. A large number of experiments lengthened the list of useful microorganism used in the production of robotics, which were originally employed in man's diet. Dairy products based on lactic fermentation bacteria characteristic for their robotic features are currently easily accessible on the market (Goderska *et al.,* 2002).The most popular today are the dairy products with Lactobacillus and Bifidobacterium bacteria which play an important role in food fermentation processes, and they are generally recognized as safe (GRAS). This work describes the identification of a yoghurt isolated strain which was gram-positive,catalase negative, non-motile and the cells were of bacillus type. The carbohydrates utilization patterns of the isolated strain coincided with *Lactobacillus paracasei*. In 1996, Fukushimo and Nakano proved that Lactic acid bacteria are characteristic for anti-cholesterol features. As a result *Lactobacillus plantarum* NRRL-B-4524 was used single or mixed with *Lactobacillus paracasei* to study their effect as probiotic mixture on controlling hypercholesterolemia in rats. Statistical analysis revealed that there were non-significant difference between treatments concerning feed efficiency ratio and significant differences between treatments concerning final weight; gain in body weight and feed intake/day among different groups of rats as compared to the positive control group. These results may be due to the positive effect of *Lactobacillus plantarum* in improving intestinal health, its high digestive capacity particularly for the breakdown of proteins and its ability to eliminate protein wastes from the intestine before they enter the bloodstream. Mack *et al.* (1999) reported that *Lactobacillus plantarum 299v* had the ability to inhibit entero-pathogenic *Escherichia coli* adherence to intestinal epithelial cells by inducing intestinal mucin gene expression in the cells.

Adawi *et al*. (1997) demonstrated that, administration of *Lactobacillus plantarum* 299v can increase the total load of lactobacilli in the intestine and suppress bacterial groups with adverse effects. Similarly, Herías (1999) found that, *Lactobacillus plantarum* was able to keep the balance within the intestinal environment through competition with *E. coli* for intestinal colonization and can influence intestinal and systemic immunity. Also, Bengmark *et al*. (1998) demonstrated that, *Lactobacillus plantarum* has the ability to reduce and eliminate potentially pathogenic microorganisms both *in vitro* and *in vivo*.

Mixed groups showed a significant decreasing effect on gain in body weight; final weight and feed intake/day more than single groups did. But the effect was non-significant for feed efficiency ratio. Fukushima and Nakano (1995) reported that, there were non-significant differences between probiotic groups and control group concerning final weight and feed intake/day.

However, in 1996 Fukushima and Nakano demonstrated that feed intakes of mixed groups were significantly lower than those of the single groups and the feed efficiency of mixed-organism groups tend to be higher than those of the single-bacteria groups. Effect of supplementing different probiotic bacterial strains on serum and liver lipids profile showed that diet containing *Lactobacillus plantarum*, promoted a high The effect of feeding different probiotic bacterial strains on relative organs weight revealed that, there were significant differences between treatments concerning relative weight of all organs except relative weight of spleen. The comparison of mixed groups versus single groups cleared that, mixed groups had a non-significant effect on relative weight of heart; liver and spleen and significant decreasing effect on relative weight of kidney.

In this connection, Bakry (2002) and Fukushima&Nakano (1996) found that, there was significantly decrease in liver weight of rats fed on the hyperlipidemic diet compared with other treatment groups. However, Fukushima and Nakano (1995), Endo et al. (1999) reported that, there was a non-significant effect for probiotic on relative weight of liver fed on a high-fat, high-cholesterol diet containing the probiotic. Significant decrease in serum total cholesterol; triacylglycerol; LDL-C and atherogenic index. It also caused a high significant decrease in liver total cholesterol; triacylglycerol and total lipid but the effect on serum total lipid was non-significant. However, it had high significant increasing effect on HDL-C as compared to positive control. These results may be related to the ability of *Lactobacillus plantarum* to increase the total level of carboxylic acids in faeces mainly acetic acid and propionic acid (Johansson *et al*., 1998).

Also it may be contributed to its ability for stimulation of the epithelial mucin production which is supported by an increased number of bacterial taxable to produce acetic acid and propionic acid leading to a higher amount of fermentable material in the colon which can be converted to short-chain fatty acids which have positive effects on the lipid metabolism in the liver (Mack *et al*.,1999 ).In this connection Bukowska *et al.*(1998) demonstrated that *Lactobacillus plantarum* had the ability to decrease the levels of fibrinogen and LDL-cholesterol in serum of men with moderately elevated cholesterol concentrations .

Our results showed that diets containing separate groups of *L. acidophillus*, *L. paracasei* and *Enterococcus faecalis* or their mixed groups caused a significant decreasing effect on serum total lipid except for *Bacillus subtilus* the effect was non-significant. LDL-C provides significant effect for all tested groups except *Lactobacillus paracasei*. However, serum triacylglycerol caused a non-significant effect for all groups except *Enterococcus faecalis* . The undesirable increasing effect of *Enterococcus feacalis* on serum triacylglycerol was in contrast with the results obtained by Danielson *et al.* (1989) and in accordance with Toit *et al.* (1998) who reported that, this effect could have been due to the extremely long period of feeding the high-cholesterol, high-fat diet to reach a definite steady state of elevated cholesterol levels. Also, Endo *et al. (*1999) stated that the incorporation of *Enterococcus feacalis* decreased the total cholesterol levels.

All the treatments revealed high significant decreasing effect on liver total cholesterol; triacylglycerol and total lipid. The mechanism of cholesterol reduction caused by the three Lactobacillus strains (*Lactobacillus plantarum*; *Lactobacillus acidophillus or Lactobacillus paracasei)* may be related to the convertion of bile salt to free bile acid through an enzymatic activity that hydrolyze conjugated bile acids in the intestinal lumen. Conjugated bile acids are important in the emulsification, digestion and absorption of lipids, but are much less efficient in the unconjugated form and excreted in faeces. This lowers the bile acid content and causes the liver to use cholesterol to produce more bile acids, which is converted later to bile salt to meet the demands of lipid digestion and absorption. Thus, the outcome is reduction in body cholesterol (Chiu *et al*., 2005).

The decreasing effect of *Lactobacillus acidophillus* on serum cholesterol level may be attributed to cholesterol assimilation of this strain beside the deconjugation of bile salts through interference with the enterohepatic cycle. These results are in agreement with the results obtained by De Rodas *et al.* (1996). In this respect, Pereira and Gibson (2002) found that, the hypercholesterolemia effect of probiotic bacteria are due to their ability to ferment food-derived indigestible carbohydrates to produce short-chain fatty acids in the gut, which can then cause a decrease in the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis and/or redistributing cholesterol from plasma to the liver.

The hypocholesterolaemic effect of the probiotic mixture may be also due to the decrease in synthesis of a polipoprotein B-l00 (which is the major protein component of circulating LDL) in the liver and small intestine, or the decrease of transfer of cholesterol ester from HDL to LDL as a result of feeding the probiotic (Suzuki *et al.,* 1991).

In 1999 Usman & Hosono reported that the differences between groups in their hypocholesterolemic effect are related to the mechanism of cholesterol binding to bacterial cell walls which varies between bacterial species. The binding differences of the different bacterial species are due to chemical and structural properties of their cell wall and even killed cells may have the ability to bind cholesterol in the intestine.

The effect of feeding different probiotic bacterial strains on faecal free fatty acids and neutral fat revealed that, rats fed on diet containing probiotic bacterial strains single or mixed had a high significant increasing effect on free fatty acids and neutral fat excretion in faeces as compared with control group. Lactic acid bacteria (LAB) with active bile salt hydrolase (BSH) are suggested to lower cholesterol levels through an interaction with host bile salt metabolism that bind to bile acids and prevent their being reabsorbed into the enterohepatic circulation, thereby resulting in increased demand for cholesterol as a precursor of bile salt synthesis. Also deconjugated bile salts do not function as well as conjugated forms in the solubilization of cholesterol and therefore prevent it from being absorbed, which could then reduce serum cholesterol concentrations (Begley *et al.*, 2006).

At last we can suggested that the ingestion of lactic acid bacteria (LAB) containing active BSH might be regarded as a biological alternative to common medical or surgical interventions to treat hypercholesterolemia and that *Latobacillus plantarum* had a decreasing effect on hypercholesteraemia and its effect increased when mixed with other LAB strains.

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