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ORIGINAL ARTICLE

Effects of lactic acid bacteria isolated from dairy products on lipid pattern of rats fed with a high fat diet

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ABSTRACT

Background and Objectives: A unique characteristic of probiotics is obesity and fatty liver control. In this study, the effect of lactic-acid-bacteria (LABs) isolated from dairy products was investigated on weight changes, blood biochemical indexes and liver tissue in mice fed a high-fat diet.

Materials and Methods: A total of 49 rats were assigned to 7 groups. The LAB-treated groups received the high-cholesterol diet supplemented with *Lactobacillus reuteri*, *Lactobacillus plantarum*, and *Bifidobacterium animalis* isolated from yogurt. At the end of 4 weeks, body weight changes, lipid factors and liver enzymes as well as liver lipid deposition and adipocyte size were measured.

Results: Serum low-density lipoprotein, total cholesterol, triglyceride levels and hepatic lipid deposition were significantly decreased in rats treated with LABs. The maximum and minimum weights were observed in the first and fourth weeks after treating with *Lactobacillus* and *Bifidobacterium* isolates, respectively. Liver enzymes were significantly decreased by LABs, especially in the group receiving concomitant administration of *Lactobacillus* and *Bifidobacterium*. Fatty liver process was reduced in the fat-fed group treated with *L. reuteri.*

Conclusion: LABs caused decreases in body weight gain, liver function, and adipocyte size. Therefore, coadministration of *Lactobacillus* and *Bifidobacterium* in dairy products can significantly decrease lipid profile.

Keywords: Probiotics; Obesity; Fatty liver; *Lactobacillus*; *Bifidobacterium*

INTRODUCTION

Obesity is one of the health problems worldwide and its increasing prevalence among adults and children has become a global epidemic (1). High-fat diets, which are common throughout the world today, lead to an increase in the prevalence of numerous diseases including type 2 diabetes, irritable bowel syndrome, and gastric cancer, possibly due to imbalances in the existing microbial population (2).

Probiotics are microorganisms that, if used in sufficient quantities, are beneficial to human body health. Although probiotics include yeast, bacteria, or mold species, bacterial species are more predominant (3). In principle, the use of 10^6 to 10^8 probiotics per gram of food a day is essential for the beneficial effects of these organisms (4). Bacterial probiotics have been identified as *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Enterococcus*, *Clostridium*, *Bacteroides*, and *Bacillus* spp., and most of them belong to the genera *Lactobacillus* and *Bifidobacterium*. Probiotic bacteria can have beneficial effects by improving the microbial balance of the native body microflora and therefore can be used in dairy products (5, 6).

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Studies have shown that the intestinal microflora of obese individuals are different from those of lean individuals so the intestinal microbial population of individuals who have lost weight is significantly different from that of normal individuals. Some studies have shown that the use of probiotic products such as cheese containing *L. plantarum* can decrease body mass index and also *L. gasseri* can decrease body fat significantly (7, 8). The positive effect of *Lactobacillus* on obesity control has been shown (9). In addition, *Bifidobacteria* had inhibitory effects on obesity (10). Lactic acid bacteria can have a positive effect on lowering cholesterol levels in mice fed a high-fat diet. Hu et al. (11) in a study showed the effect of two strains of *Lactobacillus* on lipid metabolism. Also, the positive effects of *Lactobacillus* and *Bifidobacterium* on the recovery of paraneoplastic injury in mice have been demonstrated (12). On the other hand, the effects of the combination of these bacteria have been shown for the treatment of non-alcoholic fatty liver disease as well as gastrointestinal tumors (13-15).

This study aimed to investigate the effects of three lactic acid bacteria *L. reuteri*, *L. plantarum*, and *B. animalis* alone and in combination on weight changes, blood biochemical indexes, and liver tissue in mice fed a high-fat diet.

MATERIALS AND METHODS

Isolation and identification of bacteria. In this study, 50 samples of traditional and pasteurized dairy products were collected in Fars province, Iran. Lactic acid bacteria and *Bifidobacteria* were isolated from the samples utilizing MRS agar (Quelab, Canada) and BFM agar (Quelab, Canada), respectively. Incubation was performed in micro-aerobic and anaerobic conditions for 48 to 72 h at 37°C (16). Initial identification of the isolates was done by colony morphology and Gram staining. PCR was carried out utilizing specific primers for *Lactobacillus* and *Bifidobacterium* to confirm the identity of the isolated bacteria (17). For this purpose, DNA was first extracted utilizing a DNA extraction kit (YektaTeb Tajhiiz, Iran). Then the purified DNA (50-100 ng) was added to a 25 µL PCR mixture comprising 0.2 µM specific primers (Table 1), 1X PCR buffer (10 mM Tris–HCl; pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$), 75 µM each dNTPs and 1.5 U Taq DNA polymerase. Then a program was performed which initiated with 94°C for 5 min and followed by 35 cycles including 94°C for 1 min, 58.4°C and 56.4°C for 1 min (*Lactobacillus* and *Bifidobacterium* respectively), and 72°C for 1 min, and ended with 72°C for 5 min. The purity of amplification products was ensured utilizing electrophoresis on 1% agarose gel in TBE 1X buffer (18). Finally, the PCR products were purified using a QIAquick PCR purification kit (QIAGEN, Hilden, Germany). The purified products were sequenced utilizing Sanger method and then database search for sequences of annotated genes corresponding to obtained DNA sequences was carried out using the NCBI nucleotide Blast (http://blast.ncbi.nlm.nih.gov).

Animals. This research was conducted under the permission of the ethics committee of Shiraz branch, Islamic Azad University, Shiraz, Iran with an assigned ethics code 16330507951012. In this study, 35 female Wistar white rats weighing 150-250 g and 6-week-old were obtained from Razi Vaccine and Serum Research Institute (Iran). The rats were housed at 25°C in 50% humidity, in a 12 h light/dark cycle, and were given free access to diet and water.

Experimental grouping. The rats were divided in 4 groups each consisting of 6 rats, including a standard group (group G: healthy rats with standard diet), a positive control without probiotic intake (Group E: rats fed a fatty diet including sheep tail fat), a negative control with probiotic treatment (group F: healthy rats with the standard diet, receiving *L. plantarum*, *L. reuteri*, and *B. animalis*), and treatment groups consisting 4 different groups (A-D) which consumed the fatty diet and *L. plantarum* (group A), the fatty diet and *L. reuteri* (Group B), the fatty diet and *B. animalis* (Group C) and the fatty diet and *L. plantarum*, *L. reuteri*, and *B. animalis* mixed diet (group D). Each bacterial inoculum was prepared in MRS broth with a 1.5×10^8 cfu/ml concentration and 1 ml of the suspension was used each time. Rats were gavaged with bacteria each day for 5 weeks and their body weights were measured weekly.

Blood biochemical analysis. At the end of the experiment, blood samples were taken from all rats to measure changes in liver enzymes including serum alkaline phosphatases (ALK), glutamate pyruvate transaminase (SGPT), and glutamic-oxaloacetic transaminase (SGOT). Cholesterol, triglyceride (TG), low-density lipoprotein (LDL), and low-density lipo-

Table 1. The characteristics of primer pairs which were used for confirmation of the isolated bacteria

Bacterium	Primer name	Primer sequence	Tm	Fragment length
Lactobacillus	Rham1	3′GTCGAACGAGTTCTGATTATTG5′	58.4	520
	RhamR	3′GAACCATGCGGTTCTTGGAT5′	58.4	
Bifidobacterium	BifF	3′ATTTGAGCCACTGTCTGGTG5′	56.4	190
	BifR	3′CATCCGGGAACGTCGGGAAA5′	56.4	

protein (LDL) levels in serum were also detected utilizing analytical kits (Parsazmoon/Iran) in triplicates (19).

Histological analysis. Animal necropsy was done based on animal welfare (20). For surgical treatment, the animals were anesthetized with an intravenous infusion of 100 mg/kg of ketamine hydrochloride and 20 mg/kg xylazine. A midline incision was made in the abdomen. The gastrointestinal tract was moistened with warm saline, covered with gauze, and placed on the outside of the left abdominal cavity. Then the liver was surgically removed and used for histological analysis. Then very thin cuts with a thickness of 5-10 µm were randomly prepared from the tissues using a microtome. Then, following hematoxylin-eosin staining, the signs of fat formation in the cells was observed with a binocular microscope (21, 22).

Statistical analysis. Data were analyzed utilizing SPSS software version 17 (SPSS Inc., Chicago, Illinois, USA). Chi-square test or Fisher's exact test was used to compare the variables. The P values of 0.05 was considered statistically significant.

RESULTS

The isolated bacteria. The results of bacterial culture on MRS and BFM agar media resulted in spherical, white, and soft colonies (Fig. 1). Colonies on BFM agar were smaller in size than the colonies on MRS agar. Gram-positive rods were observed following Gram staining. The results of biochemical tests which were carried out for initial identification of bacteria are shown in Table 2.

PCR amplification of specific targets in 16S rRNA gene revealed a 520 bp fragment in *Lactobacillus* genome and a 190 bp fragment in *Bifidobacterium* genome (Fig. 2). According to the results of sequence analysis, 14 isolates of *B. bifidium*, 9 isolates of *L. casei*, 9 isolates of *L. plantarum*, 1 isolate of *L. rhamnosus*, 1 isolate of *L. sakei*, and 2 isolates of *L. pentosus* were detected.

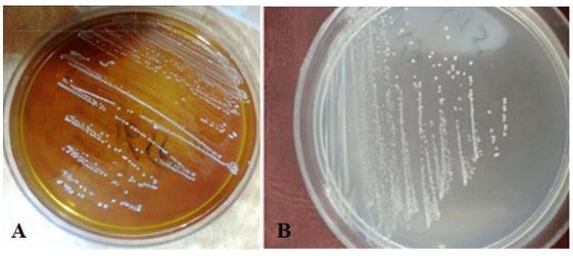

Fig. 1. The bacterial colonies on MRS agar (A) and BFM agar (B)

The effects of treatments on the mice weight. The mice weight changes under treatments are shown in Fig. 3. The results showed that in the group A (rats fed a standard diet and *L. plantarum*), B (rats fed a fatty diet and *L. reuteri*), C (rats fed a fatty diet and *B. animalis*), D (rats fed a fatty diet and *L. plantarum*, *L. reuteri*, *B. animalis*), and F (rats fed a standard diet and *L. plantarum*, *L. reuteri*, and *B. animalis*), the highest and the lowest weights of the mice were detected in the first and fourth weeks, respectively, and also a significant difference was observed in the studied weeks in terms of the weight of the mice (P = 0.000). The results demonstrated that in the groups G (rats fed a standard diet) and E (rats fed a fatty diet including sheep tail fat), the highest and the lowest weights of the mice were detected in the fourth and first weeks, respectively, and also a significant difference was observed in the studied weeks in terms of the weight of the mice (P = 0.000).

The effects of treatments on the biochemical parameters in the mice. The changes in lipid factors and liver enzymes in the studied mice under treatments are shown in Figs. 4 and 5, respectively. The results showed that treatment with *L. reuteri* (group B) had the greatest effect on slowing the fatty liv-

Table 2. The results of the biochemical tests for initial identification of bacterial isolates

Identified bacteria	Catalase production	Oxidase production	Urease production	Indole production	Citrate utilization	Motility	H_2S production	Gelatin hydrolysis	Nitrate reduction	Methyl red	Voges Proskauer
Lactobacillus isolates	-	-	-	-	-	-	-	-	-	-	-
Bifidobacterium isolates	-	-	+	-	-	-	-	-	-	+	-

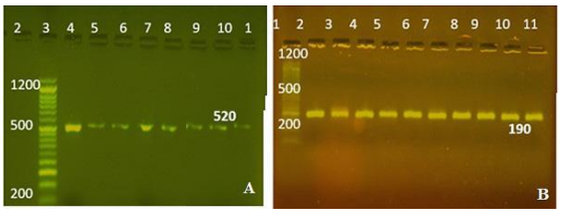

Fig. 2. PCR products visualized by agarose gel electrophoresis. A: confirmation of *Lactobacillus* spp. (column 1: a 50-bp size marker, column 2: *L. reuteri* as positive control, columns 3-11: *Lactobacillus* isolates). B: confirmation of *Bifidobacterium* spp. (column 1: Negative control, column 2: a 50 bp size marker, column 3: *B. animalis* as positive control, columns 4-10: *Bifidobacterium* isolates).

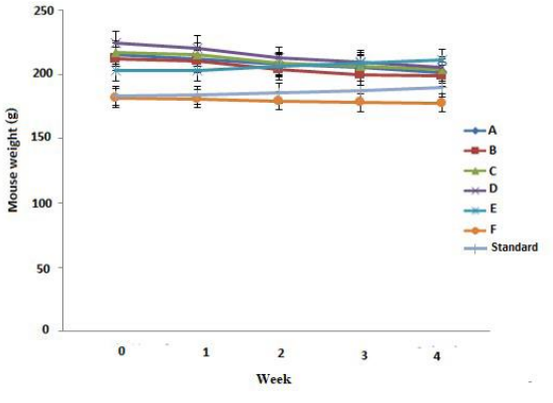

Fig. 3. Changes in the weights of studied mice in different times under treatment

er process in mice. The effect of fatty liver deceleration was observed in groups B, D, A, and C, respectively.

Macroscopic and microscopic changes of liver tissue. Histopathological examination of liver tissues in the positive control group (E) fed the high fat diet showed a microvascular and macrovascular accumulation of fat with hepatocyte enlargement and slightly osteatosis in contrast to the standard (G) (Fig. 6). The results showed that treatment with *L. reuteri* (group

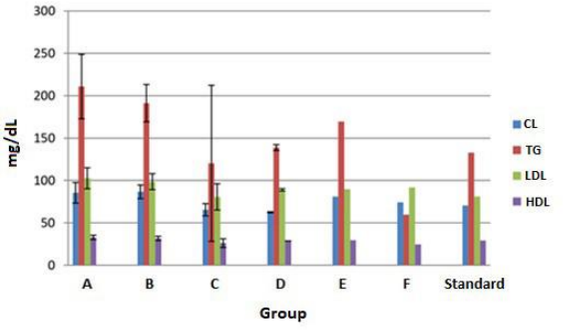

Fig. 4. Changes in lipid factors in the studied mice under treatments. A: rats fed a standard diet and *L. plantarum*, B: rats fed a fatty diet and *L. reuteri*, C: rats fed a fatty diet and *B. animalis*, D: rats fed a fatty diet and *L. plantarum*, *L. reuteri*, *B. animalis*, E: positive control (rats fed a fatty diet), F: a negative control (rats fed a standard diet and *L. plantarum*, *L. reuteri*, and *B. animalis*), G: standard (fed standard diet).

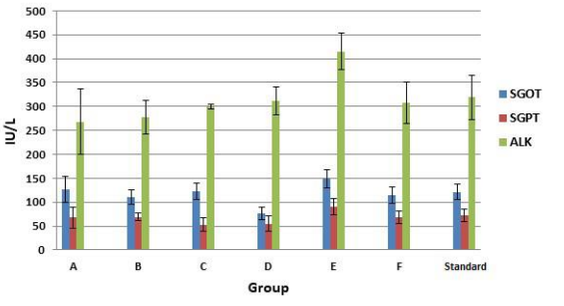

Fig. 5. Changes in liver enzymes in the studied mice under treatments. A: rats fed a standard diet and *L. plantarum*, B: rats fed a fatty diet and *L. reuteri*, C: rats fed a fatty diet and *B. animalis*, D: rats fed a fatty diet and *L. plantarum*, *L. reuteri*, B. animalis, E: positive control (rats fed a fatty diet), F: a negative control (rats fed a standard diet and *L. plantarum*, *L. reuteri*, and *B. animalis*), G: standard (fed standard diet).

B) had the greatest effect on slowing the fatty liver process in mice (Fig. 7). Fatty liver deceleration was observed in groups B, D, A, and C, respectively.

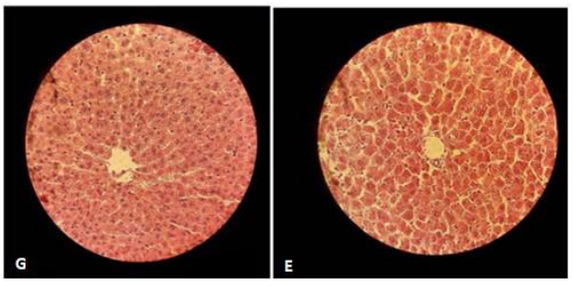

Fig. 6. Microscopic view of the liver tissue of the standard group (G) which shows normal hepatocytes and liver tissue and adipose tissue accumulation and hepatocyte enlargement to some extent which is seen in the group E.

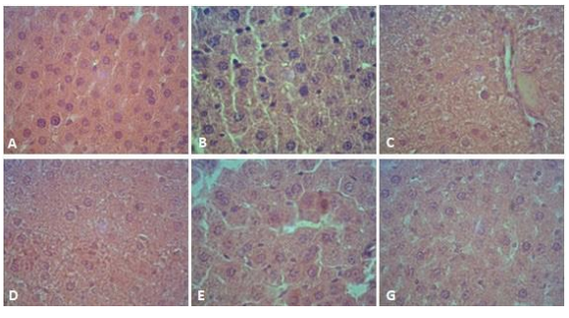

Fig. 7. Effect of treatment with bacteria on slowing the fatty liver process in tissue in comparison with the standard (G) and the positive control (E). The greatest effect was shown in treatment group with *L. reuteri* (group B).

DISCUSSION

Nowadays, the effects of probiotics on human health have received much attention. Results from numerous studies have shown that in mice with a high-fat diet, consumption of probiotics such as *Lactobacillus* and *Bifidobacterium*, either alone or in combination, can lead to obesity (23-25). However, the mechanisms of action of these probiotics are still unclear. Due to the cholesterol-lowering effect of lactic acid bacteria, several isolates of them were obtained from yogurt samples and their effect on lipid metabolites, weight changes, liver enzymes and liver tissue was investigated. Comparing the effects of the isolates with each other and with the control group, all isolates were able to control high-fat diet effects in the treated mice. In this regard, a significant decrease in cholesterol, triglyceride, LDL, and HDL levels was seen in the serum of the gavage rats given the isolated stains.

The reduction of lipid metabolites by probiotic bacteria may be due to the activity of hydrolase enzymes produced by these bacteria. These enzymes are conjugated in the gut-liver cycle and thus affect the metabolism of bile salts resulting in a decrease in serum cholesterol and LDL levels (26). In addition, short fatty acids produced by probiotics can inhibit enzymes such as hydroxymethyl glutarate coenzyme reductase. This enzyme plays an important role in the cholesterol synthesis pathway in the liver, thereby reducing cholesterol production by the liver (27).

Also, the consumption of certain species of *Lactobacillus* and *Bifidobacterium* in mice for a long time leads to reduced body fat (28-30). However, Salazar et al. (31) did not observe weight loss in mice treated with a *B. animalis* isolate, possibly due to the short-term use of probiotics in mice, as the use of probiotics has been proposed to be continued for a long time (3 to 18 weeks) (32, 33). Therefore, it can be said that the anti-obesity effects of probiotics can only be caused by some strains of lactic acid bacteria and are partly dose-dependent. As shown in this study, strains of *B. animalis*, *L. reuteri*, and *L. plantarum*, had a greater reduction effect than other bacteria and simultaneous use of all strains resulted in the least weight changes. Probiotic bacteria can be isolated from dairy products and consumption of these products can be considered an effective factor in lowering blood lipids and cholesterol as well as controlling cardiovascular diseases. Previous studies have shown a decrease in blood cholesterol after the use of probiotic products. In most studies high doses of dairy products were able to lower blood cholesterol and normal doses had such effect as well (34-37).

Liver enzymes are indicators of liver damage and impaired serum concentrations of these enzymes in liver patients. In the present study, the consumption of probiotic bacteria was able to decrease the serum level of these enzymes in the serum of treated mice with a significant difference compared to the control group. Decreased liver enzymes such as ALT and AST have also been previously reported in patients treated with probiotic bacteria (38-40). Examination of liver enzymes after treatment with isolated probiotics in this study showed that these isolates are capable of reducing liver enzymes and improving liver tissue. Research has shown that long-term (1 month) probiotic intake can decrease AST enzyme levels in mice with liver disease (41). However, some studies have also reported no effect of some probiotics on liv-

er enzymes (39-41) indicating that these effects are observed by some strains of lactic acid bacteria and may be dose-dependent. In this regard, in our study, the bacteria were not equally effective in reducing liver enzymes. One possible mechanism for these effects of probiotics may be due to their effect on reducing inflammation of the liver tissue (42), which was also observed in this study. The main limitations of this study included the cost, low number of animals and the short duration of observations.

CONCLUSION

Treatment of mice fed a high-fat diet with probiotic bacteria isolated from dairy products resulted in a decrease in lipid profile and weight loss. We also demonstrated that the isolated probiotic bacteria were able to decrease liver enzymes and decrease inflammation in liver tissue. It can be concluded that probiotic dairy products can be effective in the management of liver and cardiovascular diseases as well as in weight control. The bacteria isolated in the present study can be considered in the next studies for producing probiotic dairy products.

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REFERENCES

1. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in underweight and obesity from 1990 to 2022: a pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults. *Lancet* 2024; 403: 1027-1050.
2. Qian L, Gao R, Huang J, Qin H. Supplementation of triple viable probiotics combined with dietary intervention is associated with gut microbial improvement in humans on a high fat diet. *Exp Ther Med* 2019; 18: 2262-2270.
3. Maftei NM, Raileanu CR, Balta AA, Ambrose L, Boev M, Marin DB, et al. The potential impact of probiotics on human health: an update on their health-promoting properties. *Microorganisms* 2024; 12: 234.
4. Champagne CP, Ross RP, Saarela M, Hansen KF, Charalampopoulos D. Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices. *Int J Food Microbiol* 2011; 149: 185-193.
5. Gerritsen J, Smidt H, Rijkers GT, de Vos WM. Intestinal microbiota in human health and disease: the impact of probiotics. *Genes Nutr* 2011; 6: 209-240.
6. Tegegne BA, Kebede B. Probiotics, their prophylactic and therapeutic applications in human health development: A review of the literature. *Heliyon* 2022; 8(6): e09725.
7. Sharafedtinov KK, Plotnikova OA, Alexeeva RI, Sentsova TB, Songisepp E, Stsepetova J, et al. Hypocaloric diet supplemented with probiotic cheese improves body mass index and blood pressure indices of obese hypertensive patients-a randomized double-blind placebo-controlled pilot study. *Nutr J* 2013; 12: 138.
8. Jung SP, Lee KM, Kang JH, Yun SI, Park HO, Moon Y, et al. Effect of *Lactobacillus gasseri* BNR17 on overweight and obese adults: a randomized, double-blind clinical trial. *Korean J Fam Med* 2013; 34: 80-89.
9. Million M, Maraninchi M, Henry M, Armougom F, Richet H, Carrieri P, et al. Obesity-associated gut microbiota is enriched in *Lactobacillus reuteri* and depleted in *Bifidobacterium animalis* and *Methanobrevibacter smithii*. *Int J Obes (Lond)* 2012; 36: 817-825.
10. Lee PH, Macfarlane DJ, Lam TH. Factors associated with participant compliance in studies using accelerometers. *Gait Posture* 2013; 38: 912-917.
11. Hu X, Wang T, Li W, Jin F, Wang L. Effects of NS *Lactobacillus* strains on lipid metabolism of rats fed a high-cholesterol diet. *Lipids Health Dis* 2013; 12: 67.
12. Liboredo JC, Anastácio LR, Pelúzio MD, Valente FX, Penido LC, Nicoli JR, et al. Effect of probiotics on the development of dimethylhydrazine-induced preneoplastic lesions in the mice colon. *Acta Cir Bras* 2013; 28: 367-372.
13. Wang W, Shi LP, Shi L, Xu L. Efficacy of probiotics on the treatment of non-alcoholic fatty liver disease. *Zhonghua Nei Ke Za Zhi* 2018; 57: 101-106.
14. Yang Y, Xia Y, Chen H, Hong L, Feng J, Yang J, et al. The effect of perioperative probiotics treatment for colorectal cancer: short-term outcomes of a randomized controlled trial. *Oncotarget* 2016; 7: 8432-8440.
15. Liang S, Xu L, Zhang D, Wu Z. Effect of probiotics on small intestinal bacterial overgrowth in patients with gastric and colorectal cancer. *Turk J Gastroenterol* 2016; 27: 227-232.
16. Karimi G, Jamaluddin R, Mohtarrudin N, Ahmad Z, Khazaai H, Parvaneh M. Single-species versus dual-species probiotic supplementation as an emerging therapeutic strategy for obesity. *Nutr Metab Cardiovasc Dis* 2017; 27: 910-918.

17. Sul S-Y, Kim H-J, Kim T-W, Kim H-Y. Rapid identification of *Lactobacillus* and *Bifidobacterium* in probiotic products using multiplex PCR. *J Microbiol Biotechnol* 2007; 17: 490-495.
18. Kramer M, Obermajer N, Bogovič Matijašić B, Rogelj I, Kmetec V. Quantification of live and dead probiotic bacteria in lyophilised product by real-time PCR and by flow cytometry. *Appl Microbiol Biotechnol* 2009; 84: 1137-1147.
19. Atwa H, Labib J, Abd-Allah H, Ibrahim A. Clinical and biochemical predictors of Non-Alcoholic fatty liver disease in obese children and adolescents. *Int J Pediatr* 2022; 10: 15583-15593.
20. Brownlie HW, Munro R. The veterinary forensic necropsy: a review of procedures and protocols. *Vet Pathol* 2016; 53: 919-928.
21. Sanchez M, Darimont C, Drapeau V, Emady-Azar S, Lepage M, Rezzonico E, et al. Effect of *Lactobacillus rhamnosus* CGMCC1. 3724 supplementation on weight loss and maintenance in obese men and women. *Br J Nutr* 2014; 111: 1507-1519.
22. Lopez-Yus M, Hörndler C, Borlan S, Bernal-Monterde V, Arbones-Mainar JM. Unraveling adipose tissue dysfunction: molecular mechanisms, novel biomarkers, and therapeutic targets for liver fat deposition. *Cells* 2024; 13: 380.
23. Druart C, Alligier M, Salazar N, Neyrinck AM, Delzenne NM. Modulation of the gut microbiota by nutrients with prebiotic and probiotic properties. *Adv Nutr* 2014; 5: 624S-633S.
24. Kobyliak N, Conte C, Cammarota G, Haley AP, Styriak I, Gaspar L, et al. Probiotics in prevention and treatment of obesity: a critical view. *Nutr Metab (Lond)* 2016; 13: 14.
25. Dahiya DK, Renuka A, Puniya M, Shandilya UK, Dhewa T, Kumar N, et al. Gut microbiota modulation and its relationship with obesity using prebiotic fibers and probiotics: a review. *Front Microbiol* 2017; 8: 563.
26. Kimoto H, Ohmomo S, Okamoto T. Cholesterol removal from media by lactococci. *J Dairy Sci* 2002; 85: 3182-3188.
27. Tang H, Huang W, Yao YF. The metabolites of lactic acid bacteria: classification, biosynthesis and modulation of gut microbiota. *Microb Cell* 2023; 10: 49-62.
28. Stenman LK, Waget A, Garret C, Klopp P, Burcelin R, Lahtinen S. Potential probiotic *Bifidobacterium animalis* ssp. lactis 420 prevents weight gain and glucose intolerance in diet-induced obese mice. *Benef Microbes* 2014; 5: 437-445.
29. Sung HK, Youn SJ, Choi Y, Eun SW, Shin SM. Body fat reduction effect of *Bifidobacterium breve* B-3: A randomized, double-blind, placebo comparative clinical trial. *Nutrients* 2022; 15: 28.
30. Ma Y, Fei Y, Han X, Liu G, Fang J. *Lactobacillus plantarum* alleviates obesity by altering the composition of the gut microbiota in high-fat diet-fed mice. *Front Nutr* 2022; 9: 947367.
31. Salazar N, Neyrinck AM, Bindels LB, Druart C, Ruas-Madiedo P, Cani PD, et al. Functional effects of EPS-producing *Bifidobacterium* administration on energy metabolic alterations of diet-induced obese mice. *Front Microbiol* 2019; 10: 1809.
32. Bagarolli RA, Tobar N, Oliveira AG, Araújo TG, Carvalho BM, Rocha GZ, et al. Probiotics modulate gut microbiota and improve insulin sensitivity in DIO mice. *J Nutr Biochem* 2017; 50: 16-25.
33. Ray M, Hor PK, Ojha D, Soren JP, Singh SN, Mondal KC. Bifidobacteria and its rice fermented products on diet induced obese mice: Analysis of physical status, serum profile and gene expressions. *Benef Microbes* 2018; 9: 441-452.
34. Nabavi S, Rafraf M, Somi MH, Homayouni-Rad A, Asghari-Jafarabadi M. Effects of probiotic yogurt consumption on metabolic factors in individuals with nonalcoholic fatty liver disease. *J Dairy Sci* 2014; 97: 7386-7393.
35. Greany KA, Bonorden MJ, Hamilton-Reeves JM, McMullen MH, Wangen KE, Phipps WR, et al. Probiotic capsules do not lower plasma lipids in young women and men. *Eur J Clin Nutr* 2008; 62: 232-237.
36. Ataie-Jafari A, Larijani B, Alavi Majd H, Tahbaz F. Cholesterol-lowering effect of probiotic yogurt in comparison with ordinary yogurt in mildly to moderately hypercholesterolemic subjects. *Ann Nutr Metab* 2009; 54: 22-27.
37. Sadrzadeh-Yeganeh H, Elmadfa I, Djazayery A, Jalali M, Heshmat R, Chamary M. The effects of probiotic and conventional yoghurt on lipid profile in women. *Br J Nutr* 2010; 103: 1778-1783.
38. Aller R, De Luis DA, Izaola O, Conde R, Gonzalez Sagrado M, Primo D, et al. Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. *Eur Rev Med Pharmacol Sci* 2011; 15: 1090-1095.
39. Karahan N, Işler M, Koyu A, Karahan AG, Cırış IM, Sütçü R, et al. Effects of probiotics on methionine choline deficient diet-induced steatohepatitis in rats. *Turk J Gastroenterol* 2012; 23: 110-121.
40. Bhathena J, Martoni C, Kulamarva A, Tomaro-Duchesneau C, Malhotra M, Paul A, et al. Oral probiotic microcapsule formulation ameliorates non-alcoholic fatty liver disease in Bio F1B Golden Syrian hamsters. *PLoS One* 2013; 8(3): e58394.
41. Esposito E, Iacono A, Bianco G, Autore G, Cuzzocrea S, Vajro P, et al. Probiotics reduce the inflammatory response induced by a high-fat diet in the liver of young rats. *J Nutr* 2009; 139: 905-911.
42. Kelishadi R, Farajian S, Mirlohi M. Probiotics as a novel treatment for non-alcoholic Fatty liver disease; a systematic review on the current evidences. *Hepat Mon* 2013; 13(4): e7233.