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The biological effect of skim milk fortified with *Lactobacillus plantarum* bacteria on biochemical markers in obese rats

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Abstract
This study aimed to evaluate the functional effects of skim milk supplemented with *Lactobacillus plantarum* in the metabolic and biochemical activities of diet-induced obese rats in relation to the dietary intervention and the markers of metabolic disorders. Blood lipid profile, blood glucose, liver enzymes and some kidney function indicators were analyzed in 40 rats from five experimental treatments. The findings indicated that the high fat diet had significantly increased the total cholesterol, triglycerides and low-density lipoprotein (LDL) cholesterol levels, while reducing the high-density lipoprotein (HDL) cholesterol and significantly raised blood glucose and liver enzymes. The probiotic enriched skim milk group on the other hand had a decrease in total cholesterol level from 165.80 to 108.50 mg/dl, triglycerides 142.20 to 92.40 mg/dl, and low density lipoprotein (LDL) 107.96 to 48.52 mg/dl with an increase in high density lipoprotein (HDL) from 41.54 mg/dl compared to the untreated control group. Blood glucose also dropped to 110.03 mg/dL while liver AST and ALT levels were significantly lowered, showing better liver function and lower levels of oxidative stress. In terms of renal function, it was found that creatinine decreased relative to a rise in urea, possibly due to an increased protein metabolism and gut microbial activity. Overall, the results indicate that dietary intervention with probiotics skim milk is a positive factor in decreasing metabolic disorders associated with obesity, and that modifying the gut microbiome could be a key applied factor when developing dietary strategies to decrease associated health risks with metabolic syndrome.

Keywords: *Lactobacillus plantarum*, Probiotics, Skim Milk, Obesity, biochemical markers

1. Introduction

The epidemic of obesity is among the most complicated and pervasive public health threat of the 21st century, and the World Health Organization reports a double in the rates of obesity worldwide. Obesity is not just a risk factor for higher weight, it is a key factor in a group of metabolic diseases called metabolic syndrome. This syndrome involves severe physiological disturbances, most prominently: hyperlipidaemia (high levels of total cholesterol, triglycerides and low-density lipoprotein (LDL)); insulin resistance; and oxidative stress (damage to liver cells, and consequently the development of non-alcoholic fatty liver disease (Boutari, 2022 & Mantzoros) [2].

In recent years, the gut microbiome has been identified as a critical regulator of energy balance and metabolism in its host. Research has already demonstrated that people and animals that are obese have a "microbial imbalance" or gut dysbiosis - a decline in beneficial intestinal bacteria, and an increase in strains that extract more energy from food and induce low-level inflammation in the body (Fan, 2023 & Pedersen) [3]. With this realization, probiotics have become an attractive and safe alternative therapeutic/preventative strategy to reduce obesity-related complications, which is termed microbial modification.

One of the promising bacterial strains is the *Lactobacillus plantarum* with its unique metabolic properties that have attracted a great deal of scientific interest. This strain has been shown to have a high gastrointestinal survival ability and good intestinal mucosa adhesion ability. This bacterium has anti-obesity properties due to several mechanisms, including the production of bile salt hydrolase, which decreases cholesterol absorption in the intestine, and the fermentation of fibre and production of short-chain fatty acids (SCFAs) including butyric acids and propionic acids. These acids are important for enhancing the sensitivity of insulin, controlling hunger hormones, and decreasing fat buildup in the liver (Wang *et al*., 2024) [11]. But the most significant hurdle in using probiotics is getting enough living and viable bacterial cells to the large intestine. This is where the "food matrix" comes in. In obesity cases, skim milk is an excellent and thoughtful choice because it provides both a protein-rich environment (casein and whey) to protect against stomach acid and bile salts and is fat-free (zero-fat), reducing the caloric and fat content of patients or laboratory animals and therefore considered a food medium without additional calories or fat for individuals with obesity disorders (Khorshidian *et al*., 2023) [6]. In addition, skim milk has lactose as a prebiotic, which encourages the activity and growth of the L. plantarum bacteria in the intestine.

While previous studies have explored the use of functional carriers of beneficial bacteria by demonstrating benefits, no studies have examined the exact synergistic effect that the use of skim milk has on blood and liver functional parameters in models of induced obesity in relation to the use of *Lactobacillus plantarum*. Thus, the research problem is the importance of searching for safe and functional dietary alternatives to control metabolic disorders related to obesity. Based on this, this study involves the study and evaluation of the effect of feeding skim milk with *Lactobacillus plantarum* on blood biochemical parameters (total lipid profile, fasting blood glucose), and liver function enzymes (AST and ALT) in laboratory rats induced as obese rats to see the efficacy of feeding skim milk with *Lactobacillus plantarum* as an adjunctive and mitigating treatment of obesity complications.

2. Materials and Methods

2.1. Experimental Animals and Housing

This study was performed with 40 male albino rats weighing 180-200 g at the beginning of the experiments. The seedlings were randomly divided into five treatments, each having eight replicates to eliminate significant differences between the treatments at the beginning of the experiment based on biomass. Rats were kept in sterile plastic cages at room temperature in a normal light/dark cycle (12 hours/12 hours) environment. The rats were allowed to have free access to water and normal pellet food during the one week acclimatization before the experiment was started (Al-Asadi *et al*., 2023) [1].

2.2. Bacterial Strain and Skim Milk Preparation

A ready-made purified bacterial culture of *Lactobacillus plantarum* (ATCC 14917) was used. For 24 hours, the bacteria were activated in the MRS broth at 37°C. Bacterial cells were then centrifuged at 5000 rpm for 10 minutes, and washed 2 times with physiological saline (PBS). The milk used for the sample was skim milk, which was pasteurized at 85°C for 30 minutes and then cooled to 40°C. To get the final count of 10^9 CFU/ml, the activated bacteria were transferred into the milk. The plate pouring method (Shori *et al*., 2022) [10] was used to confirm the level of the bacteria.

2.3. Induction of Obesity and Diet

In the initial phase, rats were split into two groups: the negative control group: was fed a normal diet (12% fat) and the obesity group: was fed a diet consisting of 12% fat. The obesity group: received a ready-made diet that was used as a high-fat diet (HFD) to induce obesity. This period was 8 weeks until obesity was detected by comparing rat weights and performing body mass index (BMI) calculations (Garcia-Mazcorro *et al*., 2023) [4].

2.4. Experimental Design

The rats were induced to obesity and then randomly allocated into five groups (8 rats in each group) and the treatment was continued for another 6 weeks, as follows: Group 1 (G1): Negative control - standard diet. Group 2 (G2): High-fat diet (HFD) - Positive control. Group 3 (G3): Daily oral dose of unfortified skim milk at a concentration of 2 ml/rat + standard diet. Group 4 (G4): Unfortified skim milk 2ml/rat per day + HFD. Group 5 (G5): Daily oral dose of skim milk fortified with L. plantarum bacteria at a concentration of 10^9 CFU/ml + HFD.

2.5. Blood Sampling

Rats were deprived of food for 12 hours at the end of the 6th week of treatment. After anaesthetised with ether blood samples were obtained by cardiac puncture (5 mL in test tube without anticoagulant). The serum was centrifuged at 3000 rpm for 15 mins and stored at -20°C for biochemical analysis (Zhang *et al*., 2024) [11].

2.6. Biochemical Analysis

Commercial kits and enzymatic-colorimetric method were used to measure the following parameters: Lipid profile: total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL-C). LDL-C calculation: The calculation is done mathematically using the Friedewald formula: LDL-C = TC - HDL-C - (TG/5). The glucose oxidase method is used to measure fasting blood glucose (FBG). Enzymes (ALT, AST) activity was used to evaluate liver cell integrity (Hamed *et al*., 2023) [5].

2.7. Statistical Analysis

The data were statistically analyzed using the SPSS statistical software (version 26). The difference was compared among groups with One-way ANOVA and the means were compared with Duncan's Multiple Range Test. Results are presented as mean ± S.E. and the difference was regarded as significant when ($P < 0.05$).

3. Results and Discussion

Table 1 shows the changes in serum lipid levels of obese rats fed skim milk with addition of *Lactobacillus plantarum*. The findings revealed that the total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL-C) levels were significantly higher ($P < 0.05$) in the G3 and G4 groups as compared to the healthy control groups (G1 and G2) while the high-density lipoprotein (HDL-C) level was significantly lower ($P < 0.05$) in the G3 and G4 groups. This breakdown of lipid profile is directly related to eating a high fat diet with fat accumulation.

However, in group 5 (G5) who were treated with probiotic enriched skim milk, there was significant improvement with a reduction in total cholesterol to 108.50 mg/dL, triglycerides to 92.40 mg/dL, LDL-C to 48.52 mg/dL and an increase in HDL-C to 41.54 mg/dL. The therapeutic effect of lipids lowering is attributed to the ability of L. plantarum strains to modify the gut microbiome and production of short-chain fatty acids (SCFAs) like acetic and propionic acids. The role of these acids in inhibiting cholesterol synthetic enzymes in the liver and decreasing the gene expression of the genes responsible for lipogenesis is very important (Zhao *et al*., 2022) [13]. In addition, these bacteria contribute to increased excretion of bile acids in feces, forcing the liver to utilize cholesterol from the blood to synthesize new bile acids, thus lowering their blood levels (Zheng *et al*., 2023) [14].

Table 2 shows the effect of skimmed milk fortified with *Lactobacillus plantarum* on blood liver function markers (AST and ALT) in obese rats. There was liver damage and impairment in groups G3 (obesity induced) and G4 (obesity induced), as reflected by the significant increase in liver enzymes; the mean AST level was 90.22 U/L and 92.49 U/L, respectively; and the mean ALT level was 78.66 U/L and 79.30 U/L, respectively. These markers were significantly lowered in G5 ($P < 0.05$) and were 62.88 U/L for AST and 43.51 U/L for ALT as compared to G3 and G4. It is a good predictor of non-alcoholic fatty liver disease (NAFLD) mediated by lipotoxicity and oxidative stress from obesity. These enzymes leak into the blood stream if the liver cell membranes are damaged. L. plantarum protects the liver through various mechanisms such as the reduction of hepatic fat, the activation of the antioxidant enzyme system and the decrease of inflammatory cytokine concentrations that cause liver damage and apoptosis (Park *et al*., 2020) [9].

Table 3 shows that L. plantarum fortified skim milk supplementation had no significant effect on blood glucose and kidney function markers in obese mice. The fasted blood glucose (FBG) was high in obese group G3 (150.27 mg/dL) and G4 (152.45 mg/dL). Meanwhile, L. plantarum showed a significant effect in reducing the FBG level to 110.03 mg/dL in group G5. This is a significant improvement in the insulin sensitivity of cells and a decrease in insulin resistance, as a result of the control of systemic metabolic pathways and low-grade inflammatory condition of the organism which affects insulin sensitivity of cells (Zhao *et al*., 2022) [13].

Regarding sort function, the dossier revealed a important decrease in creatinine levels in group 5 (G5), arriving 0.69 mg/dL, distinguished to the contaminated rats camp 3 (G3), that written 0.80 mg/dL. This decrease indicates the act of probiotics in asserting glomerular filtration rate (GFR) and lowering oxidative stress on sort fabric (Lin and others. 2021). As for urea levels, the results show a surprising variation; group 5 (G5) written an important increase of 73.58 mg/dL, distinguished to the not cooked corpulent group (G3), that recorded 43.47 mg/dL. Scientifically, likely the upgraded creatinine index, this increase in urea concede possibility not certainly develop kind failure or damage. Rather, it is more likely the result of two pertain determinants: raised total protein content from graze milk, accompanying raised bacterial effervescence of amino acids in the intestines for one additional microorganisms. This leads to greater levels of liquid that the liver subsequently converts to urea. This end demands further metabolic research to question allure exact pathways. Additionally, L. plantarum microorganisms grant permission play a role in lowering kind fabric damage (Kim and others. 2025) [7].

Table 1: Effect of skimmed milk fortified with *Lactobacillus plantarum* on the serum lipid profile of obesity-induced rats.

Groups	Biochemical Parameters			
	TC (mg/dL)	**TG (mg/dL)**	**HDL-C (mg/dL)**	**LDL-C (mg/dL)**
G1	82.14a	74.50a	48.30a	18.94a
G2	84.50a	76.20a	47.17a	22.16a
G3	155.00c	135.60c	32.15c	95.78c
G4	165.80c	142.20c	29.40c	107.96c
G5	108.50b	92.40b	41.54b	48.52b
MSE	3.00	5.05	3.12	3.50
P. Value	0.001	0.001	0.001	0.002

G1: Negative Control; G2: Positive Control (Skim Milk); G3: Obese rats (HFD); G4: Obese + Skim Milk; G5: Obese + Skim Milk + L. plantarum. MSE: Mean square error. Note: Values are expressed as Means; different superscript letters (a, b, c) within the same column indicate significant differences ($P < 0.05$).

Table 2: Effect of skimmed milk fortified with *Lactobacillus plantarum* on serum liver function markers (AST and ALT) in obesity-induced rats

Groups	Biochemical Parameters	
	AST (U/L)	**ALT (U/L)**
G1	57.14^{a}	35.11^{a}
G2	58.95^{a}	35.87^{a}
G3	90.22^{c}	78.66^{c}
G4	92.49^{c}	79.30^{c}
G5	62.88^{b}	43.51^{b}
MSE	4.31	1.29
P. Value	0.041	0.003

G1: Negative Control; G2: Positive Control (Skim Milk); G3: Obese rats (HFD); G4: Obese + Skim Milk; G5: Obese + Skim Milk + L. plantarum. MSE: Mean square error. Note: Values are expressed as Means; different superscript letters (a, b, c) within the same column indicate significant differences ($P < 0.05$).

Table 3: Effect of skimmed milk fortified with *Lactobacillus plantarum* on blood glucose levels and kidney function markers in obesity-induced rats

Groups	Biochemical Parameters		
	FBG (mg/dL)	**Urea (mg/dL)**	**Creatinine (mg/dL)**
G1	89.52a	32.20a	0.40a
G2	92.63a	35.80a	0.41a
G3	150.27c	43.47c	0.80c
G4	152.45c	45.12c	0.85c
G5	110.03b	73.58b	0.69b
MSE	2.31	1.85	0.22
P. Value	0.001	0.004	<0.001

G1: Negative Control; G2: Positive Control (Skim Milk); G3: Obese rats (HFD); G4: Obese + Skim Milk; G5: Obese + Skim Milk + L. plantarum. MSE: Mean square error. Note: Values are expressed as Means; different superscript letters (a, b, c) within the same column indicate significant differences ($P < 0.05$).

4. Conclusion

Using glance milk as a a ship that carries airplanes for *Lactobacillus plantarum* bacteria is a very productive working food strategy. This study manifested that this collaboration not only supplies a fat-free digestive medium but also energetically adjusts the metabolic disorders guide obesity by threatening hurtful lipid levels, reconstructing insulin sympathy, enhancing kind function, and lowering liver stress**.**

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