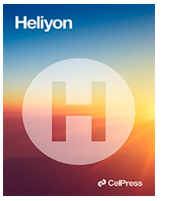
Heliyon 10 (2024) e25981

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Cheese containing probiotic *Lactobacillus brevis* NJ42 isolated from stingless bee honey reduces weight gain, fat accumulation, and glucose intolerance in mice

Nor Hazwani Mohd Hasali [a], Amir Izzwan Zamri [a,b], Mohd Nizam Lani [a,b,c], Vance Matthews [d], Aidilla Mubarak [a,b,c,*]

[a] *Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[b] *Special Interest Group Apis and Meliponi, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[c] *Food Security Research Cluster, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[d] *School of Biomedical Sciences, The University of Western Australia, 6009 Perth, Australia*

ARTICLE INFO

Keywords:
Lactic acid bacteria
Heterotrigona itama
Metabolic syndrome
Obesity
Natural product

ABSTRACT

Background: The high occurrence of metabolic syndrome has driven a growing demand for natural resource-based therapeutic strategies, highlighting their potential efficacy in addressing the complexities of this condition. Probiotics are established to be useful in the prevention and treatment of diabetes and obesity. However, limited exploration exists regarding the application of the isolated *Lactobacillus* strain from stingless bee honey as a probiotic within dairy products, such as cheese. This study investigated the effect of a high-fat diet supplemented with cheese containing probiotic bacteria (*Lactobacillus brevis* strain NJ42) isolated from *Heterotrigona itama* honey (PCHFD) on the symptoms of metabolic disorder in C57BL/6 mice.

Methods and results: Body weight, glucose intolerance, insulin resistance, and fat accumulation were measured during 12 weeks of feeding and compared to mice fed with a normal chow (NC) and high-fat diet (HFD). Over a 12-week feeding period, PCHFD-fed mice exhibited substantial reductions in several metabolic syndrome-associated features. They had a lower rate of weight gain ($p = 0.03$) than the HFD-fed mice. Additionally, they displayed a notable 39.2% decrease in gonadal fat mass compared to HFD-fed mice ($p = 0.003$). HFD-fed mice showed impaired glucose tolerance when compared to NC-fed mice ($p = 0.00$). Conversely, PCHFD-fed mice showed a reduction in glucose intolerance to a level close to that of the NC-fed mice group ($p = 0.01$). These positive effects extended to reductions in hepatic steatosis and adipocyte hypertrophy.

Conclusion: These results indicated that *L. brevis* strain NJ42, isolated from *H. itama* honey, is a prospective probiotic to lower the risk of developing metabolic syndrome features induced by a high-fat diet. These positive findings suggest the prospect of enriching commonly consumed dietary components such as cheese with probiotic attributes, potentially offering an accessible means to alleviating the symptoms of metabolic diseases.

* Corresponding author. Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.
E-mail address: aidilla@umt.edu.my (A. Mubarak).

https://doi.org/10.1016/j.heliyon.2024.e25981
Received 19 June 2023; Received in revised form 29 January 2024; Accepted 6 February 2024
Available online 10 February 2024

1. Introduction

Metabolic diseases have been one of the highest factors of morbidity and mortality throughout the world [1,2]. Obesity is a complex, multifactorial disease determined to be a global public health challenge that increases the risk of noncommunicable diseases, and remains a major cause of disability and death [3]. The global prevalence of diabetes in adults aged 20–79 years is 7.3% and is estimated to reach 8.3% by 2045 [4]. Type-2 diabetes mellitus has become one of the most prevalent global diseases, for which several treatments have been developed. However, current approaches are not completely effective [5], making it difficult to control the hyperglycaemic state even with multiple drugs or non-pharmacological treatments such as dietary modifications, exercise, and weight loss.

The gut microbiota has been suggested to have interactions with the functions of different organs, thus portraying it as a crucial factor for the development of metabolic disorders such as type 2 diabetes and obesity [6]. The gut microbiome can be altered by an obesogenic diet, which results in the activation of proinflammatory mechanisms and metabolic endotoxemia that promote insulin resistance and cardio-metabolic disorders [6]. The high occurrence of metabolic disorders has created an enhanced focus on probiotics, which have been reported to maintain normal gut functions, have an anti-diabetic effect, and promote a reduced risk of diabetes onset [7]. Additionally, more individuals are concerned about food that provides an appropriate amount and quality of nutrients for proper functioning of the body, e.g., functional food, including fermented products with probiotic properties. Previous report has outlined the benefits of regular consumption of probiotics in reducing the risk of cancer, decreasing cholesterol levels, reducing the incidence of diarrhoea, and stimulating the immune system [8,9].

Isolated bacteria, such as lactic acid bacteria from honey and bee bread, have been shown to deliver antimicrobial activity against pathogenic and spoilage bacteria [10,11]. *Heterotrigona itama* is a species of stingless bee native to Southeast Asia and known for its production of unique honey [12]. Its honey is more acidic compared to other honeys, and its dominant microorganisms are those surviving at a very low pH. The honey can act as a new reservoir of *Lactobacillus* that has great probiotic potential to be used in the dairy industry. Dairy products are considered an ideal media for transporting probiotic bacteria into the human gastrointestinal tract. Cheese is a good intermediate to deliver probiotics into the intestine, conferring health benefits to the host due to its ability to create a barrier against the high acidic environment in the gastrointestinal tract making more available environment for probiotic survival [13]. Cheese shows great advantages over other fermented dairy products, such as yoghurt, as the vector for incorporating probiotic bacteria due to its higher pH and buffer capacity, which provide more protection to the probiotic bacteria during storage and transit in the gastrointestinal tract [14]. The ingestion of cheese containing probiotics is reported to have various advantages for human health, which include an enhanced immune system as well as improved oral and intestinal health [15,16].

The role of probiotics, a gut flora modulator, in the prevention and treatment of diabetes and obesity has been described in animal and human studies [17,18]. Consumption of fermented foods was reported to lower the risk of developing metabolic syndrome, cardiovascular disease, diabetes, and cancer; relieve lactose intolerance symptoms; and boost immunity and overall health [19]. *L. brevis* OPK-3 from kimchi was previously reported to reduce body weight gain, mass of epididymal fat tissue, hepatic lipid, and decrease the level of lipogenic gene expression in mice [20]. Badehnoosh et al. [17] reported that probiotic supplementation containing *Lactobacillus acidophilus*, *L. casei,* and *Bifidobacterium bifidum* resulted in a reduction of fasting plasma glucose and increase of total antioxidant capacity in women with gestational diabetes mellitus. Animal studies have also shown the capability of probiotics to suppress obesity and diabetes. *L. acidophilus* was documented to be able to prevent obesity, insulin resistance, and hepatic steatosis induced by HFD in mice [21]. Zhao et al. [22] observed a positive impact of *L. plantarum* S9 on the improvement of lipid profile, insulin resistance, and inflammation in high-fat diet-induced metabolic syndrome rats. Additionally, *B. animalis* subsp. *Lactis* LKM512 was reported to reduce inflammation and insulin resistance through modulation of the gut microbiota in HFD-induced obese mice [23]. The positive impact of the specific strains of *Lactobacilli* and *Bifidobacteria* on augmenting the progression of obesity and diabetes suggests that probiotic-mediated modulation of gut flora can be an efficient remedy against metabolic diseases. Currently, there is a substantial research gap pertaining to the utilization of the isolated *Lactobacillus* strain from stingless bee honey as a probiotic agent in dairy products, particularly in the context of addressing metabolic syndrome. We had successfully isolated the *L. brevis* strain NJ42 from *H. itama* honey [24]. Extensive characterization of this strain demonstrated robust probiotic characteristics along with notable antimicrobial properties. Investigating the potential of the isolated *Lactobacillus* strain from stingless bee honey in cheese presents an opportunity to harness its probiotic properties within a widely consumed food item. In the present study, we hypothesised that *Lactobacillus brevis* isolated from *H. itama* honey would be beneficial in improving the features of metabolic syndrome. The aim of this study was to test the effect of probiotic cheese from the *L. brevis* strain NJ42 isolated from *H. itama* honey on glucose intolerance, insulin resistance, and fat accumulation in C57BL/6 mice.

2. Materials and methods

2.1. Production of probiotic cheese

The production of soft cheese using 1 L of skimmed cow milk was conducted following the cheese-making process described previously by Divya et al. [25] and Habtegebriel & Admassu [26], with some modifications. Milk was heated up, and 1% of *L. brevis* strain NJ42 probiotic culture was added as a starter culture, into the milk at 3 h of incubation time and 37 °C during the soft cheese manufacturing process. This strain was obtained from our previous study [24,27], originally isolated from *H. itama* honey. The cheese curd was drained and pressed to remove all the whey, and it was kept at 4 °C prior to incorporation into the tested feed. The utilization of the isolated *L. brevis* in cheese production has the intention of highlighting a promising strategy for developing functional foods

targeting metabolic health. The cheese was therefore incorporated into a high-fat diet to investigate the impact of the probiotic cheese on HFD-induced metabolic disease.

2.2. Experimental animals and diets

Thirty male C57BL/6 mice obtained from the Brain Research Institute Monash Sunway (BRIMS), Monash University Malaysia, were maintained on a normal chow (NC; Teklad Global Diet 2014, Envigo FKA Harlan, USA) until 7 weeks of age. The mice were acclimatised to conditions in the animal holding room, maintained on a 12 h light/12 h dark cycle (24 ± 2 °C). Mice were then allocated to three groups of different diets (n = 10 in each group, 5 mice/cage). The three different feeds were normal chow (NC) as the control, a high-fat diet (HFD; Teklad Custom Diet TD.06415, Envigo FKA Harlan, USA), and a high-fat diet supplemented with 1% probiotic cheese (PCHFD) given to a specific group of mice for 12 weeks. Feed and water were provided to the animals ad libitum. All surgery was performed under anaesthetic drugs, and all efforts were made to minimise suffering. Animal experimental procedures were conducted in accordance with the principles of good laboratory animal care and performed in compliance with the Animal Ethics Committee of Universiti Kebangsaan Malaysia (approval number: UMT/2016/AMIR/23-NOV./804-DEC.-2016-FEB.-2017).

2.3. Body weight, adiposity, and food intake

The body weights of mice were measured weekly throughout the duration of the feeding trial, and the mass of the gonadal fat was measured at the time of sacrifice. Mice were fasted for 5 h prior to sacrifice. The weight of food was measured manually for 11 weeks, and the amount of feed (g) consumed per mouse was determined per week.

2.4. Metabolic assays

The glucose tolerance test (GTT) and the insulin tolerance test (ITT) were performed at weeks 6 and 9, and 7 and 10 of the trial, respectively. Mice were fasted for 5 h prior to the testing by withdrawing the feed from the mice. Tail bleeding was done to obtain blood samples (removal of the very tip of the tail; 1 mm), and subsequent samples were taken from the same cut by gently removing the scab that formed. Glucose levels were measured using a glucometer (Omron: HGM-112; Netherland) immediately before and at 15, 30, 60, 90, and 120 min after an intraperitoneal injection of glucose (1 g/kg of body mass) or insulin (0.5 unit/kg of body mass). The mice were returned to their cages between bleeds and monitored for changes in mobility. After the procedure, mice were given an intraperitoneal injection of 200 μl of saline to aid recovery. The procedure was performed by or under the supervision of staff trained in the techniques required.

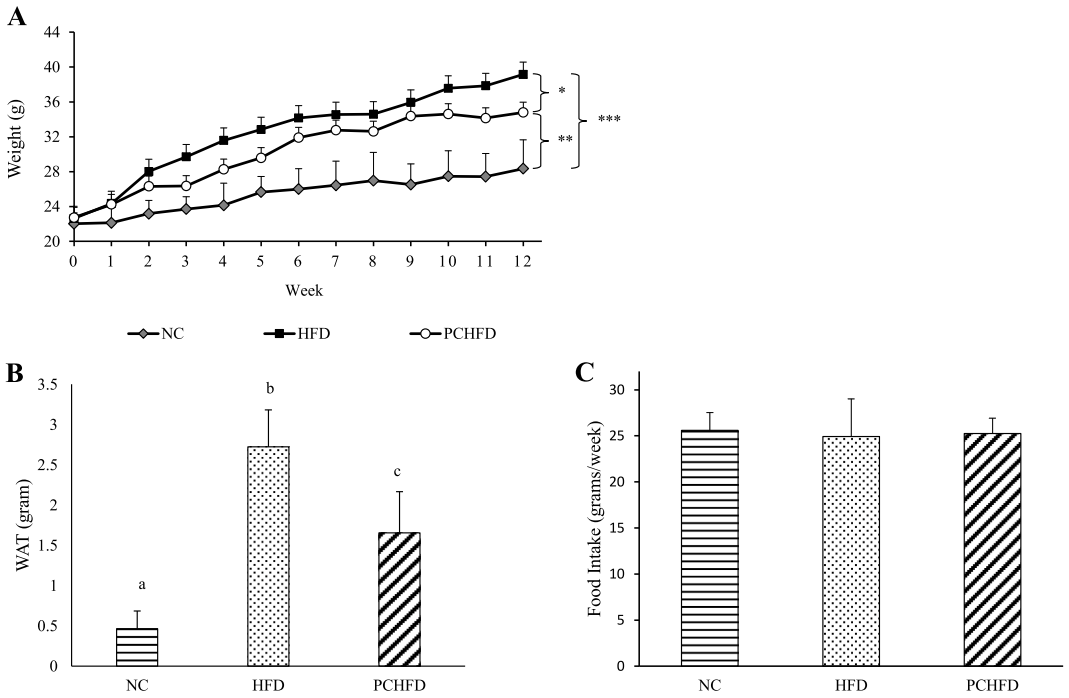


Fig. 1. (A) Body weight gain, (B) gonadal fat pad weights of white adipose tissue (WAT) and (C) food intake in mice fed with normal chow (NC), high-fat diet (HFD) and high-fat diet supplemented with probiotic cheese (PCHFD). Data is presented as mean ± SEM. *, **, ***, and different lowercase superscripts indicate significant differences between groups at $p < 0.05$. Data is presented as mean ± SEM.

2.5. Liver and adipose tissue histology

White adipose tissue and liver samples were dissected from the animals at the end of the trial. The samples were fixed in 4% paraformaldehyde (w/w) overnight prior to being incubated in 50% ethanol (v/v) and then immediately embedded with paraffin. The tissues were thereafter cut into 4 μm sections, stained with haematoxylin and eosin, and then mounted using DPX Mountant (Sigma-Aldrich, USA). A light microscope (Leica TM DM LB2, Germany) and image analyser system (Leica, Germany) was used to visualise and photograph the obtained sections.

2.6. Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 19 (IBM Corporation, Armonk, NY), SAS 9.3 (SAS Institute, Inc., Cary, NC), and Matrix Laboratory (MATLAB 2014, The MathWorks, Inc., USA). Results are expressed as the mean ± standard error of the mean (SEM). Statistical significance was accepted at $p < 0.05$. For data with repeated measures over time (weight, GTT, and ITT), random-effect linear models were fitted in SAS using the PROC MIXED command to observe data for each variable (weight and glucose). The models contained fixed effects for treatment group (diet) and time as a categorical variable. The area under the curve (AUC) for the metabolic assay was calculated using MATLAB 2014.

3. Results

3.1. Effect of a high-fat diet supplemented with probiotic cheese containing L. brevis strain NJ42 on fat accumulation in C57BL/6 mice

The body weight of mice in three different groups (normal chow-fed group, NC; high-fat diet-fed group, HFD; and high-fat diet supplemented with probiotic cheese-fed group, PCHFD) within a 12-week trial is depicted in Fig. 1A. HFD and PCHFD-fed mice showed a significantly higher rate of body weight increase throughout the study when compared to NC-fed mice ($p = 0.00$). Interestingly, when the two high fat diet groups are compared, PCHFD-fed mice showed a lower rate of weight gain ($p = 0.03$).

This observation was supported by the mass of the gonadal fat that was measured at the end of the study period (week 12). HFD-fed mice displayed the highest mass (2.73 g), followed by PCHFD-fed mice (1.66 g) and NC-fed mice (0.46 g) (Fig. 1B). There was a 6.5-fold and 4-fold increase in the mass of gonadal fat in HFD-fed and PCHFD-fed mice, respectively, when compared to those fed with NC ($p = 0.00$). Both data on body weight and mass of the gonadal fat established that the high-fat diet induced obesity in the C57BL/6 mice. The incorporation of cheese containing probiotic bacteria, *L. brevis* strain NJ42, into HFD was able to reduce the fat mass by 39% compared to those fed a high-fat diet only within 12 weeks of feeding ($p = 0.003$).

The estimated food intake in the NC, HFD, and PCHFD groups was also measured. As presented in Fig. 1C, no difference was

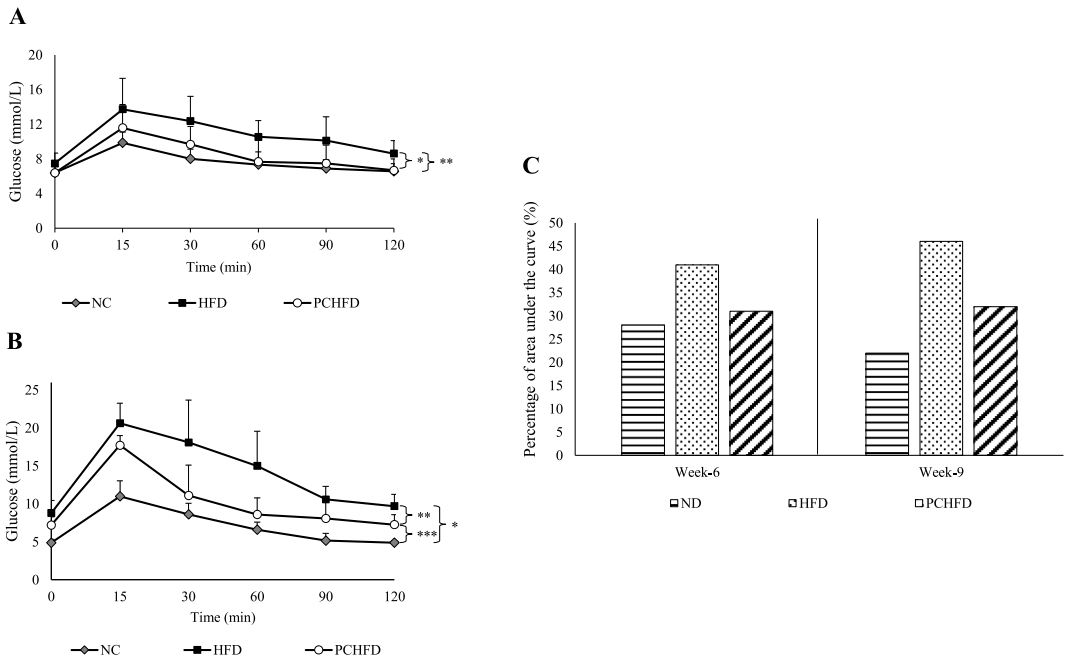


Fig. 2. Glucose tolerance test (GTT) at (A) week 6 and (B) week 9 in mice fed with normal chow (NC), high-fat diet (HFD) and high-fat diet supplemented with probiotic cheese (PCHFD), (C) percentage of area under curve (AUC) of glucose tolerance test for week 6 and week 9. Data is presented as mean ± SEM. *, ** and *** indicates significant differences at $p < 0.05$.

observed among the three different feeding groups ($p = 0.72$). The average food intake of the NC, HFD, and PCHFD mice groups was 25.6 g, 24.9 g, and 25.2 g per week, respectively, for the entire experimental period.

3.2. *Effect of a high-fat diet supplemented with probiotic cheese containing L. brevis strain NJ42 on the improvement of glucose intolerance and insulin resistance in C57BL/6 mice*

A positive effect of cheese containing probiotic bacteria, *L. brevis* strain NJ42, on delaying the progression of type-2 diabetes in C57BL/6 mice was observed. Intra-peritoneal GTT (at weeks 6 and 9) and ITT (at weeks 7 and 10) were performed after the start of the respective feeding regimes. Supplementation of the cheese into the high-fat diet was found to delay the development of metabolic syndrome features.

The results of the GTT for both week-6 and week-9 are presented in Fig. 2A and B. After 6 weeks of feeding (Fig. 2A), HFD-fed mice had significantly impaired glucose tolerance with abnormally high glucose levels compared to NC-fed mice ($p = 0.00$). In contrast, PCHFD-fed mice had an improvement in glucose tolerance ($p = 0.01$), with glucose levels lowering closer to the NC-fed mice. The effect on GTT was further illustrated through the percentage of area under the curve (AUC), as shown in Fig. 2C. The AUC of the HFD group was higher (41%) than that of the NC group, which was 28% (AUC), while the PCHFD group had a reduced glucose intolerance (32% AUC).

Additionally, at week 9 of feeding, HFD consumption resulted in highly impaired glucose tolerance compared to NC-fed mice (Fig. 2B) ($p = 0.00$). Incorporation of the probiotic cheese in the high-fat diet clearly showed a significant positive improvement in glucose intolerance when compared to the HFD group ($p = 0.003$). The AUC for HFD-fed mice was higher (46%) compared to NC-fed mice (22%) (Fig. 2C), while the PCHFD group displayed a reduction in glucose intolerance (33% AUC) to a level closer to that of the NC group.

The results from the insulin tolerance test (ITT) on week-7 and week-10 are illustrated in Fig. 3A and B, respectively. The data demonstrated that PCHFD-fed mice had slightly less insulin resistance compared to mice fed with HFD-fed mice, although no significant difference was observed at both week-7 and week-10 ($p = 0.34$ and $p = 0.40$, respectively). The effect on ITT was further illustrated through the percentage of area under the curve (AUC), as shown in Fig. 3C. The AUC of the PCHFD group was observed to be slightly lower compared to HFD at both week-7 and week-10.

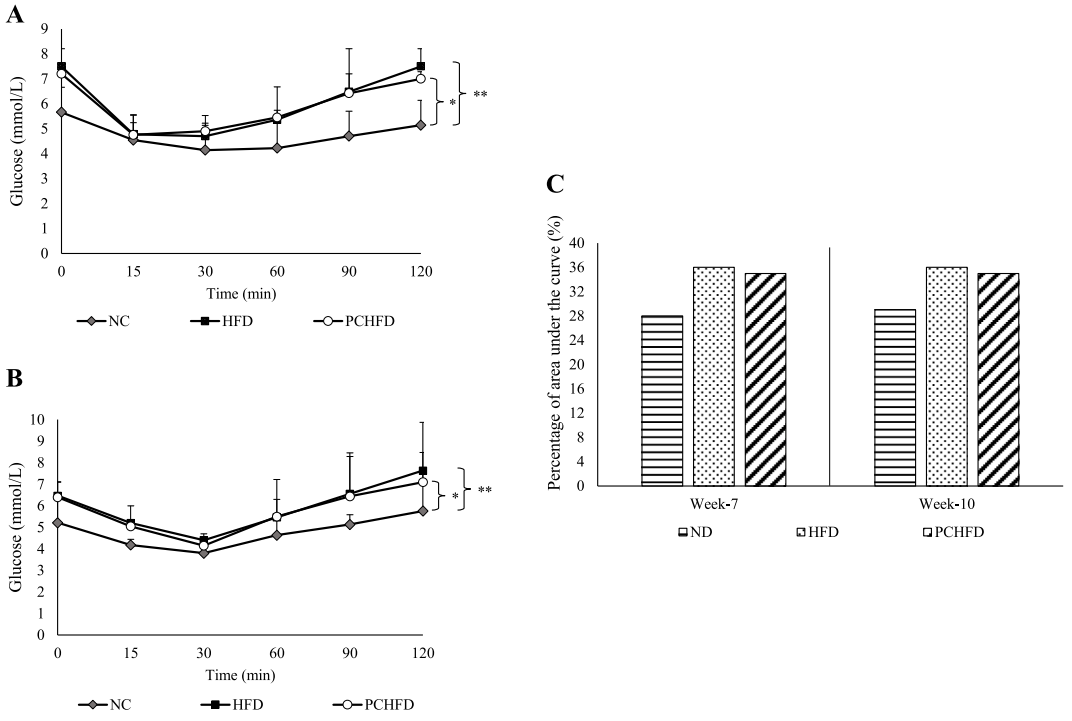


Fig. 3. Insulin tolerance (ITT) test at (A) week 7 and (B) week 10 in mice fed with normal (NC), high-fat diet (HFD) and high-fat diet supplemented with probiotic cheese (PCHFD), (C) percentage of area under curve (AUC) of ITT for week 7 and week 10. Data is presented as mean ± SEM. * and ** indicates significant differences at $p < 0.05$.

3.3. Effect of a high-fat diet supplemented with probiotic cheese containing L. brevis strain NJ42 on reducing hepatic steatosis and adipocyte hypertrophy in C57BL/6 mice

In the present study, histological examination was performed on the liver and white adipose tissue to examine fat accumulation related to high-fat diet-induced obesity in C57BL/6 mice. Fig. 4A illustrates the haematoxylin and eosin staining of the liver tissue sections from NC, HFD, and PCHFD-fed mice. It was observed that more steatosis was present in the liver of HFD-fed mice when compared to NC-fed mice. However, PCHFD-fed mice displayed markedly reduced hepatic lipid accumulation.

Fig. 4B illustrates the haematoxylin and eosin staining of the white adipose tissue sections from NC, HFD, and PCHFD-fed mice. Significant adipocyte hypertrophy was observed in HFD-fed mice compared to NC-fed mice. Interestingly, PCHFD-fed mice had smaller-sized adipocytes.

4. Discussion

The observations gained in this study support our hypothesis that cheese containing probiotic *L. brevis* strain NJ42 from *H. itama* honey is beneficial in reducing the progression of type 2 diabetes and obesity in C57BL/6 mice. Supplementing probiotic cheese into a high-fat diet (PCHFD) was observed to reduce body weight gain and mass of gonadal fat in the mice by 11% and 39%, respectively. This observation is in line with various reports on the ability of probiotic bacteria to prevent obesity [18,21,28]. Rather et al. [29] also observed a reduced body weight gain and fasting blood glucose levels following consumption of yoghurt containing *L. casei* NCDC 19. Similarly, Kim et al. [30] and Park et al. [20] reported a reduction of body weight and white adipose tissue following supplementation with *L. rhamnosus* 4B15 and *L. brevis* OPK-3, respectively, in HFD-fed mice. Chen et al. [31] examined an anti-obesity effect of *L. rhamnosus* LRH05 on diet-induced obese mice, where there was a reduction in body weight and white adipose tissue. Additionally, Akram et al. [32] reported that the probiotic-fermented milk containing *L. fermentum* NCDC 400 and *L. rhamnosus* NCDC 610 reduced the body weight of mice fed with an obesogenic diet. Kim et al. [33] also reported the positive outcome of *L. gasseri* BNR17 in reducing visceral fat and waist circumference in obese humans. The anti-obesity effect of probiotics may be due to the expression of adiponectin, which regulates fuel metabolism to control fatty acid oxidation and decreases the storage of fat in adipose tissue [29]. In addition to adipokine, probiotics may also modulate signalling pathways like AMP-activated protein kinase (AMPK), which affects lipid metabolism [34]. Furthermore, probiotic bacteria can produce conjugated linoleic acids that reduce body fat and exert beneficial effects on diet-induced obesity in mice [35]. The probiotic cheese tested in this current study might have improved gut barrier function, reducing circulating lipopolysaccharides and subsequent fat accumulation [36].

Probiotics have been reported for their potential in the prevention of metabolic syndrome [37]. The present study reports that supplementing cheese containing probiotic bacteria from *L. brevis* strain NJ42 was able to delay the development of diabetes influenced by the high-fat diet given to C57BL/6 mice. The GTT clearly showed that the progression of diabetes in PCHFD-fed mice was

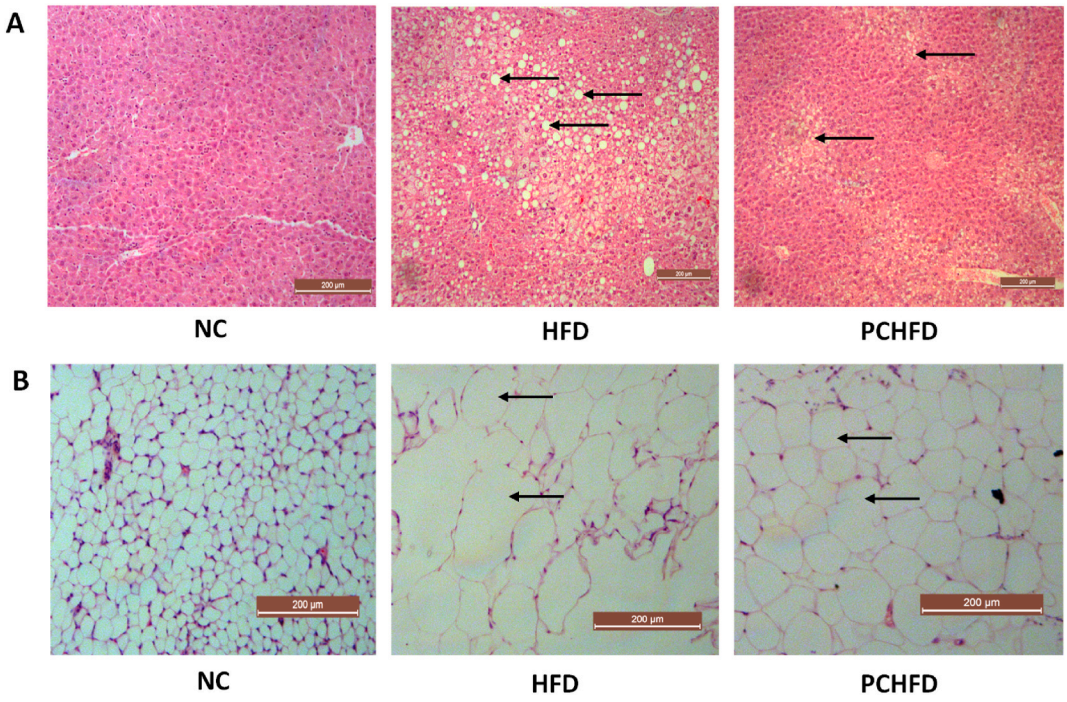


Fig. 4. (A) Liver steatosis and (B) white adipocyte hypertrophy in mice fed with normal chow (NC), high-fat diet (HFD) and high-fat diet supplemented with probiotic cheese (PCHFD). Arrows indicate steatosis in A, and adipocyte hypertrophy in B.

delayed compared to HFD-fed mice. To our knowledge, our study is the first to report on the effect of the probiotic bacteria, *L. brevis* strain NJ42, on glucose tolerance in high-fat-diet- induced obese C57BL/6 mice. Chen et al. [38] reported an increase in the glucose transporter type-4 (GLUT4) expression in *L. reuteri* 263 of high-energy-diet-fed rats. GLUT4 is the principal glucose transporter protein, which enhances glucose removal from the circulation. The study suggested that *L. gasseri* BNR17 provides anti-diabetic activity by elevating the expression of fatty acid oxidation-related genes and increasing GLUT4 expression. This mechanism may explain the improvement of glucose tolerance in PCHFD-fed mice observed in this study. Body weight gain and insulin resistance can be suppressed using probiotics by modulating gut flora composition and stimulating the formation of gut hormones [39].

The gut microbiome has been regarded as the impelling cause of the pathogenesis of metabolic diseases [40]. *Lactobacilli* is one of the well-studied probiotics that act as gut-flora modulators owing to their preventative effect on metabolic disorders, particularly diabetes and obesity [39]. Yadav et al. [41] demonstrated that administration of a yoghurt containing *L. acidophilus* and *L. casei,* disrupted the onset of glucose intolerance, hyperglycaemia, hyperinsulinaemia, and dyslipidaemia and decreased oxidative stress in rats. Li et al. [42] also observed an improved glucose tolerance from *L. plantarum* HT121 supplementation in HFD mice. The potential mechanisms underlying the reduction of glucose intolerance in mice following the administration of *L. brevis* via the consumption of PCHFD may involve gut microbiota modulation. *Lactobacillus* probiotics have the potential to modulate gut flora, which impacts metabolic processes, including glucose metabolism [43]. The probiotic functions to regulate gut barrier integrity and reduce intestinal inflammation. By enhancing the expression of tight junction proteins and reducing gut permeability, these probiotics may restrict the passage of bacterial substances such as lipopolysaccharides (LPS) into the systemic circulation, thereby diminishing systemic inflammation and insulin resistance [36]. Probiotics also enhance the production of short-chain fatty acids (SCFAs), which can regulate energy homeostasis, glucose metabolism, and lipid storage, potentially impacting glucose tolerance and fat accumulation [44]. This resonates with our findings on reduced body weight gain and mass of gonadal fat, as discussed earlier.

The present study also showed reduced hepatic steatosis in liver tissue sections and smaller-sized adipocytes in the white adipose tissue sections through histological assessment. This finding suggests that lipid metabolism in adipose tissues from probiotic cheese supplementation is increased compared to mice fed a HFD only. These results are also in accordance with our finding on the highest total gonadal fat mass in HFD-fed mice, followed by PCHFD and NC. *L. brevis* supplementation in the HFD might contribute to mitigating gut microbiota-induced inflammation, which thus limits macrophage infiltration into adipose tissues. Consequently, this suppression of inflammatory responses might deter adipocyte proliferation and differentiation [36], leading to the reduced adipocyte size observed in this current research. Jang et al. [18] reported that hepatic lipid accumulation was reduced in probiotic *L. rhamnosus* GG-fed mice compared to the control group. They also found that *L. rhamnosus* GG reduced lipid uptake in the intestine, and this suggested a therapeutically potent effect of well-known probiotics against diet-induced obesity, non-alcoholic fatty liver disease, and related metabolic diseases. Another study by Kim et al. [45] showed that probiotic treatment with *Bacillus* spp. was able to reduce the deposition of fat in hepatocytes in comparison to HFD-fed control mice. This finding was also in agreement with Choi et al. [46], which indicated that the probiotic *L. reuteri* MG5149 and *Weissella cibaria* MG5285 were able to reduce fat accumulation in the liver and adipose tissue of high-fat diet-induced obese C57BL/6J mice. Additionally, Mu et al. [47] also reported reduced hepatic steatosis in mice receiving *L. plantarum* KFY02 isolated from naturally fermented yogurt. *L. rhamnosus* 4B15 was also found to reduce the expression of genes related to fat synthesis and inflammation in adipose and hepatic cells. Probiotics have been reported to affect various signalling pathways involved in lipid metabolism and inflammation, such as AMPK and peroxisome proliferator-activated receptors (PPARs), which regulate lipid oxidation, synthesis, and storage in the liver [47]. The reduction of LPS levels through modulation of the gut microbiota could also reduce hepatic lipid accumulation [42]. According to Jang et al. [18], accumulation of fat in the liver is an early stage in the progression of non-alcoholic fatty liver disease (NAFLD) that is related to disruption of lipid and glucose metabolism. The probiotics from *Lactobacillus* species were reported to protect mice from NAFLD induced by HFD, and improve gut permeability, inflammation, and modulation of gut flora in diet-induced obesity in C57BL/6J mice. These mechanisms potentially contribute to the reduction of hepatic steatosis and smaller-sized adipocytes observed in the PCHFD-fed mice in this current study.

5. Conclusion

The data obtained in this study show the ability of the *L. brevis* strain NJ42, isolated from *H. itama* and incorporated into cheese, to reduce body weight gain and white adipose tissue mass in mice fed a high-fat diet. This probiotic was also able to reduce glucose intolerance, hepatic fat accumulation, and adipocyte hypertrophy. The probiotic also tended to reduce insulin resistance; however, measuring blood insulin levels in the future will help to ascertain this observation. The findings highlight the importance of the bacterial strain in lowering the risk of developing features of the metabolic syndrome in a mouse model. These positive results recommend the potential use of the *L. brevis* strain NJ42 probiotic for managing metabolic syndrome-associated complications through a less radical approach compared to drugs or hormone therapy. These findings emphasise the benefits of enriching cheese with probiotics. Further studies are required to understand the precise cellular mechanisms leading to their ameliorating effects on the symptoms of metabolic syndrome.

Ethical approval

Animal experimental procedures were approved conducted in accordance with the principles of good laboratory animal care and performed in compliance with the Animal Ethics Committee of Universiti Kebangsaan Malaysia (approval number: UMT/2016/AMIR/23-NOV./804-DEC.-2016-FEB.-2017).

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

CRediT authorship contribution statement

Nor Hazwani Mohd Hasali: Writing – original draft, Visualization, Validation, Investigation, Formal analysis. **Amir Izzwan Zamri:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Mohd Nizam Lani:** Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Vance Matthews:** Writing – review & editing, Visualization, Methodology, Data curation. **Aidilla Mubarak:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Amir Izzwan Zamri reports financial support was provided by Malaysia Ministry of Higher Education. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Authors acknowledge the financial support from the Ministry of Higher Education, Malaysia through Fundamental Research Grant Scheme (FRGS/1/2014/STWN03/UMT/02/1). Authors thank the staff at the Faculty of Fisheries and Food Science and the Institute of Marine Biotechnology for their technical assistance.

References

[1] World Health Organization, Taking action on childhood obesity report. https://www.who.int/end-childhood-obesity/publications/taking-action-childhood-obesity-report/en/, 2018. (Accessed 24 May 2019).
[2] N. Chew, C.H. Ng, D. Tan, G. Kong, C.X. Lin, Y.H. Chin, R. Foo, M. Chan, M. Muthiah, Global burden of metabolic diseases: data from Global Burden of Disease 2000-2019. A consortium of metabolic disease, Eur. Heart J. 44 (2023) 168–169.
[3] World Health Organization. WHO European regional obesity report 2022, World Health Organization. Regional Office for Europe, 2022, pp. 1–25.
[4] International Diabetes Federation, The global picture, in: IDF Diabetes Atlas, eighth ed., 2018. https://www.diabetesatlas.org. (Accessed 12 March 2019).
[5] Z. Sun, X. Sun, J. Li, Z. Li, Q. Hu, L. Li, X. Hao, M. Song, C. Li, Using probiotics for type 2 diabetes mellitus intervention: Advances, questions, and potential, Crit. Rev. Food Sci. Nutr. 60 (2020) 670–683.
[6] T.P. Scheithauer, E. Rampanelli, M. Nieuwdorp, B.A. Vallance, C.B. Verchere, D.H. Van Raalte, H. Herrema, Gut microbiota as a trigger for metabolic inflammation in obesity and type 2 diabetes, Front. Immunol. 11 (2020) 571731.
[7] M.K. Salgaço, L.G.S. Oliveira, G.N. Costa, F. Bianchi, K. Sivieri, Relationship between gut microbiota, probiotics, and type 2 diabetes mellitus, Appl. Microbiol. Biotechnol. 103 (2019) 9229–9238.
[8] S. Manzoor, S.M. Wani, S.A. Mir, D. Rizwan, Role of probiotics and prebiotics in mitigation of different diseases, Nutrition 96 (2022) 111602.
[9] V. Subramaniyan, S. Chakravarthi, R. Jegasothy, W.Y. Seng, N.K. Fuloria, S. Fuloria, I. Hazarika, A. Das, Alcohol-associated liver disease: a review on its pathophysiology, diagnosis and drug therapy, Toxicol Rep 8 (2021) 376–385.
[10] S.M. Mohammad, N.K. Mahmud-Ab-Rashid, N. Zawawi, Probiotic properties of bacteria isolated from bee bread of stingless bee *Heterotrigona itama*, J. Apicult. Res. 60 (2020) 172–187.
[11] E. Lashani, A. Davoodabadi, M.M. Soltandallal, Antimicrobial effects of *Lactobacillus plantarum* and *Lactobacillus paracasei* isolated from honey against *Staphylococcus aureus*, J. Babol Univ. Medical Sci. 20 (3) (2018) 44–49.
[12] L. Tuksitha, Y.L.S. Chen, Y.L. Chen, K.Y. Wong, C.C. Peng, Antioxidant and antibacterial capacity of stingless bee honey from Borneo (Sarawak), J. Asia Pac. Entomol. 21 (2) (2018) 563–570.
[13] A. Homayouni, F. Ansari, A. Azizi, H. Pourjafar, M. Madadi, Cheese as a potential food carrier to deliver probiotic microorganisms into the human gut: a review, Curr. Nutr. Food Sci. 16 (1) (2020) 15–28.
[14] F. Cuffia, C. Bergamini, M. Candioti, Probiotic soft sheep's cheese: evaluation of probiotic survival and its influence on proteolysis and organoleptic characteristics, Int. Food Res. J. 25 (2018) 399–407.
[15] S. Tewari, J. David, A. Gautam, A review on probiotic dairy products and digestive health, J. Pharmacogn. Phytochem. 8 (3) (2019) 368–372.
[16] E.G. Sarmento, D.E. Cesar, M.L. Martins, E.G. de Oliveira Góis, E.M.F. Martins, A.N. da Rocha Campos, A. Del'Duca, A.D. de Oliveira Martins, Effect of probiotic bacteria in composition of children's saliva, Food Res. Int. 116 (2019) 1282–1288.
[17] B. Badehnoosh, M. Karamali, M. Zarrati, M. Jamilian, F. Bahmani, M. Tajabadi-Ebrahimi, P. Jafari, E. Rahmani, Z. Asemi, The effects of probiotic supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes, J. Matern. Fetal Neonatal Med. e31 (2018) 1128–1136.
[18] H.R. Jang, H.J. Park, D. Kang, H. Chung, M.H. Nam, Y. Lee, J.H. Park, H.Y. Lee, A protective mechanism of probiotic *Lactobacillus* against hepatic steatosis via reducing host intestinal fatty acid absorption, Exp. Mol. Med. 51 (2019) 1–14.
[19] M.D.C.G. Peluzio, M.D.M.E. Dias, J.A. Martinez, F.I. Milagro, Kefir and intestinal microbiota modulation: implications in human health, Front. Nutr. 8 (2021) 638740.
[20] J.E. Park, S.H. Oh, Y.S. Cha, *Lactobacillus brevis* OPK-3 from kimchi prevents obesity and modulates the expression of adipogenic and pro-inflammatory genes in adipose tissue of diet-induced obese mice, Nutrients 12 (3) (2020) 604.
[21] Y. Kang, X. Kang, H. Yang, H. Liu, X. Yang, Q. Liu, H. Tian, Y. Xue, P. Ren, X. Kuang, Y. Cai, M. Tong, L. Li, W. Fan, *Lactobacillus acidophilus* ameliorates obesity in mice through modulation of gut microbiota dysbiosis and intestinal permeability, Pharmacol. Res. 175 (2022) 106020.
[22] L. Zhao, Y. Shen, Y. Wang, L. Wang, L. Zhang, Z. Zhao, S. Li, *Lactobacillus plantarum* S9 alleviates lipid profile, insulin resistance, and inflammation in high-fat diet-induced metabolic syndrome rats, Sci. Rep. 12 (2022) 15490.
[23] L. Ma, A. Zheng, L. Ni, L. Wu, L. Hu, Y. Zhao, Z. Fu, Y. Ni, *Bifidobacterium animalis subsp. lactis* lkm512 attenuates obesity-associated inflammation and insulin resistance through the modification of gut microbiota in high-fat diet-induced obese mice, Mol. Nutr. Food Res. 66 (3) (2022) 2100639.

[24] N.H. Hasali, A.I. Zamri, M.N. Lani, A. Mubarak, Antibiotic susceptibility, antibacterial activity and probiotic characterisation of isolated *Lactobacillus brevis* strains from *Heterotrigona itama* honey, Malays. Appl. Biol. 47 (2018) 105–112.
[25] K.H. Divya, M.C. Varadaraj, Response surface plots for the behavioral pattern of Yersinia enterocolitica in chocolate milk as affected by trans-cinnamaldehyde, a spice essential oil constituent, Food Bioprocess Technol. 5 (2012) 498–507.
[26] H. Habtegebriel, S. Admassu, Optimization of the production process of soft cheese from camel milk using linear programming technique, Optimization 49 (2016) 35–41.
[27] N.H.M. Hasali, A.I. Zamri, M.N. Lani, A. Mubarak, Z. Suhaili, Identification of lactic acid bacteria from Meliponine honey and their antimicrobial activity against pathogenic bacteria, Am.-Eurasian J. Sustain. Agric. (AEJSA) 9 (6) (2015) 1–7.
[28] T. Cerdó, J.A.G. García-Santos, M. Bermúdez, C. Campoy, The role of probiotics and prebiotics in the prevention and treatment of obesity, Nutrients 11 (2019) 635.
[29] S.A. Rather, R. Pothuraju, R.K. Sharma, S. De, N.A. Mir, S. Jangra, Anti-obesity effect of feeding probiotic *dahi* containing *Lactobacillus casei* NCDC 19 in high fat diet-induced obese mice, Int. J. Dairy Technol. 67 (2014) 504–509.
[30] S. Kim, J.Y. Joung, D. Kang, N.S. Oh, Y. Yoon, Anti-obesity effects of *Lactobacillus rhamnosus* 4B15, and its synergy with hydrolysed lactose skim milk powder, Int. Dairy J. 123 (2021) 104997.
[31] Y.T. Chen, S.Y. Chiou, A.H. Hsu, Y.C. Lin, J.S. Lin, *Lactobacillus rhamnosus* strain LRH05 intervention ameliorated body weight gain and adipose inflammation via modulating the gut microbiota in high-fat diet-induced obese mice, Mol. Nutr. Food Res. 66 (1) (2022) 2100348.
[32] M. Akram, S.A. Ali, P. Behare, G. Kaul, Dietary intake of probiotic fermented milk benefits the gut and reproductive health in mice fed with an obesogenic diet, Food Funct. 13 (2022) 737–752.
[33] J.M. Kim, M.K. Yun, O. Kim, B. Kwon, Cho Lactobacillus *gasseri* BNR17 supplementation reduces the visceral fat accumulation and waist circumference in obese adults: a randomized, double-blind, placebo-controlled trial, J. Med. Food 21 (5) (2018) 454–461.
[34] L.C. Lew, Y.Y. Hor, M.H. Jaafar, A.S.Y. Lau, B.K. Lee, L.O. Chuah, K.P. Yap, A. Azlan, G. Azzam, S.B. Choi, M.T. Liong, *Lactobacillus* strains alleviated hyperlipidemia and liver steatosis in aging rats via activation of AMPK, Int. J. Mol. Sci. 21 (16) (2020) 5872.
[35] S.C. Ribeiro, C. Stanton, B. Yang, R.P. Ross, C.C. Silva, Conjugated linoleic acid production and probiotic assessment of *Lactobacillus plantarum* isolated from Pico cheese, Lebensm. Wiss. Technol. 90 (2018) 403–411.
[36] H.I. Kim, J.K. Kim, J.Y. Kim, S.E. Jang, M.J. Han, D.H. Kim, *Lactobacillus plantarum* LC27 and *Bifidobacterium longum* LC67 simultaneously alleviate high-fat diet-induced colitis, endotoxemia, liver steatosis, and obesity in mice, Nutr. Res. (N. Y., NY, U. S.) 67 (2019) 78–89.
[37] S. Torres, E. Fabersani, A. Marquez, P. Gauffin-Cano, Adipose tissue inflammation and metabolic syndrome. The proactive role of probiotics, Eur. J. Nutr. 58 (2019) 27–43.
[38] L.H. Chen, Y.H. Chen, K.C. Cheng, T.Y. Chien, C.H. Chan, S.P. Tsao, H.Y. Huang, Antiobesity effect of *Lactobacillus reuteri* 263 associated with energy metabolism remodeling of white adipose tissue in high-energy-diet-fed rats, J. Nutr. Biochem. 54 (2018) 87–94.
[39] R.G. Kerry, J.K. Patra, S. Gouda, Y. Park, H.S. Shin, G. Das, Benefaction of probiotics for human health: a review, J. Food Drug Anal. 26 (2018) 927–939.
[40] J. Wu, K. Wang, X. Wang, Y. Pang, C. Jiang, The role of the gut microbiome and its metabolites in metabolic diseases, Protein Cell 12 (5) (2021) 360–373.
[41] H. Yadav, S. Jain, P.R. Sinha, Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats, Nutrition 23 (2007) 62–68.
[42] X. Li, Y. Huang, L. Song, Y. Xiao, S. Lu, J. Xu, J. Li, Z. Ren, *Lactobacillus plantarum* prevents obesity via modulation of gut microbiota and metabolites in high-fat feeding mice, J. Funct.Foods 73 (2020) 73.
[43] G. Wang, G. Zhu, C. Chen, Y. Zheng, F. Ma, J. Zhao, Y.K. Lee, H. Zhang, W. Chen, *Lactobacillus* strains derived from human gut ameliorate metabolic disorders via modulation of gut microbiota composition and short-chain fatty acids metabolism, Benef. Microbes 12 (3) (2021) 267–281.
[44] R. Bohan, X. Tianyu, Z. Tiantian, F. Ruonan, H. Hongtao, W. Qiong, S. Chao, Gut microbiota: a potential manipulator for host adipose tissue and energy metabolism, J. Nutr. Biochem. 64 (2019) 206–217.
[45] B. Kim, J. Kwon, M.S. Kim, H. Park, Y. Ji, W. Holzapfel, C.K. Hyun, Protective effects of *Bacillus* probiotics against high-fat diet-induced metabolic disorders in mice, PLoS One 13 (2018) e0210120.
[46] S.I. Choi, S. You, S. Kim, G. Won, C.H. Kang, G.H. Kim, *Weissella cibaria* MG5285 and *Lactobacillus reuteri* MG5149 attenuated fat accumulation in adipose and hepatic steatosis in high-fat diet-induced C57BL/6J obese mice, Food Nutr. Res. 65 (2021).
[47] J. Mu, J. Zhang, X. Zhou, Z. Zalan, F. Hegyi, K. Takács, A. Ibrahim, S. Awad, Y. Wu, X. Zhao, M. Du, Effect of *Lactobacillus plantarum* KFY02 isolated from naturally fermented yogurt on the weight loss in mice with high-fat diet-induced obesity via PPAR-α/γ signaling pathway, J. Funct.Foods 75 (2020) 104264.