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Potential effect of functional fermented ice-cream on alleviating biochemical complications in obese rats

Hala M. Zeidan[1], Amal S. Abd El-Azeem[2], Amany M. Hegazy[2], Ibrahim H. Badawy[2], Gamal A. Ibrahim[3] and Osama M. Sharaf[3]*

[1]Department of Research on Children with Special Needs, National Research Centre, 33 El Bohouth st., Dokki, Giza, **Egypt.,**
[2]Nutrition & Food Science Dept. National Research Centre, 33 El Bohouth st., Dokki, Giza, **Egypt.**
[3] Dairy Science Dept. National Research Centre, 33 El Bohouth st., Dokki, Giza, **Egypt.**

***Correspondence:** sharafosama@yahoo.com **Accepted:** 26 June 2019 **Published online:** 22 Aug 2019

Obesity is a major public health concern. Focus on the efficacy of probiotics in treating obesity and related disorders increasingly reported. This study was aimed to evaluate the effect of encapsulated *Leuconostoc mesenteroides* as probiotic in functional fermented ice-cream to alleviate the complications associated to obesity. Twenty four rats were divided into three groups. The 1st group (G1) was fed on basal diet and served as negative control (normal rats), the 2nd group (G2) was fed on hyperlipidimic diet to induce obesity and served as positive control and the 3rd group (G3) was fed on hyperlipidemia+ 20% functional fermented ice cream. Feeding period continued for 6 weeks. The results showed that rats in groups 2 gained more body weight than group 1. Serum glucose, malondialdehyde, total lipids, total cholesterol, LDL cholesterol, triglycerides and leptin were all increased in obese rats (G2). The total antioxidant capacity, HDL cholesterol and ghrelin were decreased. Functional fermented ice cream formula caused a marked improvement of all these parameters .The conclusion is that health hazards exerted due to obesity can modulated by probiotics supplementation.

Keywords: Obesity, probiotics, *Leuconostoc mesenteroides*, ice-cream, leptin, ghrelin, lipid profile, insulin, total antioxidant, malondialdehyde

INTRODUCTION

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. A growing number of people, including children, suffer from obesity worldwide (kobyliak et al., 2016).

Imbalance between energy intake and energy expenditure is the most frequent cause which leads to the obesity development. Obesity are affected by many factors including; genetic susceptibility, environmental factors and lifestyle. Recently, great efforts have focused on host and environmental factors that may affect energy balance. Intestinal microflora has received increasing attention due to it considered as a metabolic port between the host and the surrounding environment, in particular with regard to its modulation of inflammation, energy metabolism and body weight homeostasis (Neish, 2009). In humans, overweight/obesity is associated with low faecal bacterial genericness, dyslipidemia, impaired glucose homeostasis and higher low-grade inflammation (Cotillard, 2013 and chatelier et al., 2013). Studies on probiotics belonging to the genus Lactobacillus offers preclinical evidence supporting the “anti-obesity” effects of probiotics. In adddition, other studies have focused on the use of Bifidobacterium strains. Animal and human studies demonstrate that the gut microbiota, are correlated with energy

homeostasis (Ley et al., 2005 and Tremanoli et al., 2012). Indigestible carbohydrates, synthesize short chain fatty acids and amino acids in the gut are fermented by the gut bacteria which possibly share in the host energy supplementation (Nieuwdrop et al., 2014 and Backhead et al., 2005).

By-products from the bacterial fermentation process produce by-products which might also reduce appetite and increase satiety (Coni et al., 2009) and by modulation of bile acid metabolism (Sayin et al., 2013), increased energy expenditure may be the effect of microbiota by which it suppress diet-induced obesity *(*Watanab et al., 2012). In addition, individual's taste and dietary preferences may be affected by the gut bacteria (Alcock et al., 2014). Oral administration of probiotics (viable strains of bacteria) has been suggested as a way to saffect the gut ecosystem to advocacy weight reduction or decrease weight gain. However, the mechanisms by which the supplementation with probiotic may affect the gut microbiota are not yet established (Sanders, 2016).

There is an increasing interest in the use of health functional materials, especially lactic acid bacteria. *Leuconostoc mesenteroides* is an epiphytic bacterium that is widely spread in the natural environment and used in the production of various useful products in the biochemical and pharmaceutical industries. In addition, it has been used to modify a variety of bioactive substances in an effort to improve their functionality. *Leuconostoc mesenteroides* and *Lactobacillus sakei* were isolated from Kimchi (Lee *et al*., 2018) to investigate their lactic acid bacteria (LAB) supplementation in induced obese mice model. Lee and co-investigators found that these species decreased the serum levels of urea nitrogen, glucose, and triglyceride of these induced obese mice. They also recorded significant decrease of fibrosis, triglyceride, and total cholesterol level in fatty liver tissue by LAB supplementation. These results suggest that alternative nutritional interventions by *L. mesenteroides* and *Lb. sakei* supplementation could be used to decrease symptoms related to obesity in a safe manner.

The body weight is regulated by a complicated system, including both peripheral and central factors. Leptin and ghrelin are two hormones that seem to play an important role in regulation of food intake which in turn affects the body weight (Klok *et al.,* 2007; Pralong and Gaillard, 2001 ; Sahu, 2004). Leptin passes to the circulatory system as a function of the energy stores and can cross blood brain barrier (BBB) producing signals to the brain concerning the body energy stores state. According to the literature, serum and plasma leptin levels are elevated in subjects with higher BMI and percent total body fat. In humans and rodents, these leptin signals to the brain cause a reduction in food intake and an raise in energy expenditure to keep the body fat stores size (Klok et al., 2007).Ghrelin was primarily separated from the stomach, however it has also been specified from other peripheral tissues (i.e. gastrointestinal tract, pancreas, adrenal cortex, and ovary) in addition to ghrelin-producing neurons in the brain (Nussdorfer, 2003; Gaytan et al., 2003 and Date et al., 2002). In addition, ghrelin has also been considered as a target to treat obesity (.Foster-Schubert and Cummings, 2006) due to its association with food intake, adiposity and metabolism regulation (López et al., 2008 and Nakazato et al., 2001). Therefore, the present study was designed to evaluate the role of probiotics in ameliorating hazards resulting from obesity.

MATERIALS AND METHODS

Nutritional Experiment

I. Materials

The ingredients used for preparation of the diet given to the animals were purchased from the local market. These items were corn starch, sucrose, corn oil. Casein was obtained from Sisco Research Laboratories PVT.LTD India.

Salts and vitamins used for the preparation of the salt and vitamin mixtures were obtained from Merk, Germany (AI N95) and prepared according to Reeves et al., 1993.

Kits used for the biochemical analysis were obtained from Biodiagnostic Company Egypt.

Animals used in this experiment were Sprague Dawley albino rats obtained from the animal house of the National Research Center; their body weight ranged between 140-150 g. and comprised both sexes.

II –Methods

1-The standard control diet was prepared according to Revees et al., 1993 as shown on Table (1).

2-The hyperlipidimic diet (for induction of obesity) was prepared as basal diet with addition of 10% sheep fat and it was supplemented with 1%

cholesterol and 0 .25% bile salts (Fukushima et al., 1997).

Preparation of tested formula

Functional fermented ice cream with encapsulated *Leuconostoc mesenteroides* + 0.5 % carboxy methyl cellulose were prepared according to El–Shafei et al.,(2018). Then, 20% of product was added to the hyperlipidemic diet on the expense of starch.

Table (1) Composition of standard, hyperlipidemic and tested formula (g/100g diet)

Ingredient	Group (-ve) Control	Group2(+ve) Control	Group3 (tested diet)
Casein	15	15	15
Corn oil	10	10	10
Lard	-	10	10
Sucrose	10	10	10
Cellulose	4	4	4
Salt mix	4	4	4
Vit mix	1	1	1
Cholesterol	-	1	1
Bile Salt	-	0.25	0.25
Tested formula	-	-	20
Starch	56	44.75	24.75
Total	100	100	100

Design of Animal Experiment

The animal experiment comprised 3 groups each 8 rats

- The 1st group (G1) was fed on basal diet and served as negative control (normal rats).
- The 2nd group (G2) was fed on hyperlipidimic diet to induce obesity and served as positive control.
- The 3rd group (G3) was fed on hyperlipidimic diet + 20% tested formula.

Animals were kept individually in stainless steel cages; deionized water was allowed ad-Libtum.

The room temperature was adjusted at 25°C. The feeding period continued for 6 weeks. During the experimental period the food consumption and body weight of the animals were followed.

At the end of the experimental period (after 6weeks) rats were fasted and blood samples were obtained from the orbital vein and were received into clean dry centrifuge tubes .Serum was separated by centrifugation at 3000 rpm for 15 minutes and kept in deep-freezer at 20 °C until used for biochemical analysis.

The experimental procedure was carried out according to the institutional Animals Ethics Community of the NRC, Egypt.

Biochemical Analysis

Serum total lipids (TL), triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-Ch) and high density lipoprotein cholesterol (HDL-Ch) were determined according to Knight et al., 1972, Fossati and Prencipe 1982, Allain et al., 1974, Levy, 1981, Burstein and Scholnick,1973 respectively.

Glucose was determined calorimetrically (Trinder,1969). Insulin concentration was evaluated by using rat insulin enzyme-linked immunosorbent assay (ELISA) using the instructions available in the manufacturer's kit (G science, Glory Science Co. Ltd, USA).

Lipid peroxidation was measured as malondialdehyde (MDA), the end product of this process following the procedure by Satoh (2004).Total antioxidant capacity(TAC) and catalase activity were determined via colorimetric methods according to Koracevic et al., (2001) and Aebi,1984, respectively.

Leptin serum concentrations for the study groups were measured using *in vitro* rat leptin ELISA (Enzyme-Linked Immunosorbent Assay) kit (ABcam, # ab100773, Cambridge, UK), for quantitative measurement as previously described (Panetta et al., 2017). Ghrelin serum concentration values were assayed using ghrelin (human/mouse/rat) EIA Enzyme Immunoassay kit (BioVision, # K4790, California, USA) for the study groups, according to manufacturer's instructions.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analysis of the data was performed using SPSS-PC software. Unpaired student's t-test was used to compare biological differences. Meanwhile, one-way analysis of variance (ANOVA) was used for comparison of different biochemical values in various experimental groups. It was followed by Duncan's multiple range tests to clarify the significance; p <0.05 was considered significant.

RESULTS

Lipid profile

Table (2) shows serum total lipid, total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol values of the study groups.

The values reported for serum total lipid were 407.25±10.80 mg/dl for negative control group (G1), 748.12±32.16 mg/dl for positive control group (G2) and, 448.75±18.91 mg/dl for rats fed on treated diet (G3) respectively.

Table 2: Lipid Parameters mean values of the study groups.

Parameters Treatment	Total lipid mg\dl	Cholesterol mg/dl	Triglycerides mg/dl	LDL mg/dl	HDL mg/dl
G1	407.25 ± 10.80a	95.31 ± 15.60b	67.50± 10.42ab	38± 1.25b	82.62± 3.08a
G2	748.12± 32.16b	170.10± 3.91a	94.52± 3.13a	60.25 ±± 2.04a	54.12± 1.62b
G3	448.75 ± 18.91a	91.77±20.33b	56.93± 10.24b	32.09 1.50c	87.39± 2.70a

Values are expressed as mean±SE (Standard error). Mean values in the same column sharing the same superscript letters are not significantly different (p< 0.05).

Serum total cholesterol was 95.31±15.60 mg/dl in G1, increased to 170.10±3.91 mg/dl for G2 and 91.77±20.33 mg/dl for group3 respectively.

The values reported for serum triglycerides were 67.50± 10.42, 94.52±3.13, and 56.93± 10.24 mg /dl for the groups 1, 2, 3respectively. The results shows that significant increase in all lipid parameters except HDL-cholesterol in group 2 (positive control) when compared with normal control group (negative control). Lipid metabolism dysregulation was greatly amended as evidenced by reduction in levels of serum total lipid, total cholesterol, triglycerides, LDL-cholesterol and increasing in HDL-cholesterol values in the treated group (3).

Serum glucose and insulin

Mean values of serum glucose and Insulin are presented in Table (3). These values illustrated that serum glucose level was significantly lower (p<0.05) in group 1 compared with obese rats (group 2) (64.62±1.90 and 109.75±2.19mg/dl, respectively). However, treated group 3 which supplemented by diets containing probiotic bacteria exhibited significant decrease (p<0.05) in serum glucose level when compared with group 2 (86.87±2.23, and 109.75±2.19 mg /dl respectively). With regard to insulin results, feeding formula that contains the probiotics bacteria showed noticeable elevation in G3 as compared with the G2(obese rats).

Table 3: Serum glucose and insulin levels of different experimental groups.

Parameters Treatment	Glucose mg/dl	Insulin (mu/L)
G1	64.625 ±1.90c	3.175±0.45a
G2	109.75± 2.19a	2.925± 0.06a
G3	86.87± 2.23b	3.25 ±0.72a

Values are expressed as mean± SE (Standard error). Mean values in the same column sharing the same superscript letters are not significantly different (p < 0.05).

Malondialdehyde, Total antioxidant capacity and catalase activity:

Table (4) presented serum mean values of malondialdehyde, total antioxidant capacity and catalase of normal and obese rats .These data clarify that malondialdehyde serum level of group 1 (normal rats) was significantly lower (p<0.05) when compared with obese rats (group 2) (3.79±0.07 and 4.82±0.11nmol/ml; respectively). Meanwhile, group 3 which supplemented by diet containing probiotic bacteria showed significant decrease (p<0.05) in serum MDA level in comparison with the positive control (group 2) (3.36±0.13, and4.82±0.11 nmol/ml; respectively). With regard to the effect of feeding diet containing probiotic bacteria on serum total antioxidant capacity (TAC) level in obese rats; data of the present study showed that total antioxidant capacity was significantly decreased in positive control group (1.55±0.11 mM/L) in comparison with the negative control (1.93±0.067 mM/L). Significant differences in total antioxidant level were observed in obese group fed on treated diet as compared to the positive control (1.87±0.08 and 1.55±0.11 mM/L respectively). Concerning catalase level, data revealed that significance increase in positive control group in comparison with the negative control (458.12±20.50 and 252.39 ±42.83 U/L; respectively). Adding the probiotic bacteria to the diet of obese rats (group3) decreased the level of catalase significantly when compared with the positive control (128.98 ±32.95, and 458.12±20.50 U/L; respectively).

Serum leptin and ghrelin:

Table (5) shows the body weight gain, leptin and ghrelin concentrations among the study groups.

As shown in Table (5) leptin concentration was elevated in positive control compared with normal group, but formula group have lower leptin concentration than that of normal rats.

Table (4): Malondialdehyde, Total antioxidant capacity and catalase activity of the different experimental groups

Parameters Treatment	Malonaldhyde (nmol/ml)	Total antioxidant (mMl/L)	Catalase(U/L)
G!	3.79 ± 0.07b	1.93± 0.067a	252.39 ± 42.83b
G2	4.82 ± 0.11a	1.55± 0.11b	458.12 ± 20.50a
G3	3.36 ± 0.13c	1.87±0.08a	128.98 ±32.95c

Values are expressed as mean± SE (Standard error). Mean values in the same column sharing the same superscript letters are not significantly different (p <0.05).

Table (5): Leptin and Ghrelin concentrations in the study groups.

Group	Leptin (pg/ml)	Ghrelin (pg/ml)	Body weight gain
Normal	1702.5±197.33^{b}	563.25±76.48^{b}	92.875±9.474^{b}
PositiveControl	3252.5±523.85^{a}	457±79.70^{c}	141.375±6.99^{a}
Formula	1146.75±162.45^{b}	978.25±77.95^{a}	111.5±14.19^{b}

Values are expressed as mean± SE (Standard error). Mean values in the same column sharing the same superscript letters are not significantly different (p< 0.05).

On the other hand, formula group has higher ghrelin concentration compared with normal and positive control groups.

Concerning leptin values, no statistical significance difference was found between normal and formula groups. As regard to ghrelin concentrations, significant difference was found between normal and formula groups. However, significance differences were observed (p< 0.05) between positive control and formula groups concerning both leptin and ghrelin concentrations. Body weight gain was not significantly different between normal and formula groups, but significance difference was seen (p< 0.05) between positive control and formula groups.

DISCUSSION

Obesity is a major public health concern (Shin et al., 2018). Genetic background, physical activity grade, cultural and environmental determinants are important factors affect obesity. In addition, energy metabolism dysregulation help in intracellular Lipid accumulation and overabundant storage of adipose tissue, resulting in difficulties in locomotion and association with comorbidities such as hypertension, dyslipidemia, and diabetes (Jung et al., 2014; Janghorbani et al., 2012).

Probiotics are live microorganisms that having benefits to their hosts. Their efficacy results in treating obesity and related disorders are increasing exponentially. Variances of obese human hosts intestinal microbiota may affect their energy metabolism (Cani and Delzenne, 2009). Probiotic therapy has an advantage that it may be a hopeful treatment to invert the hosts biological-associated alterations in their metabolism that are associated with obesity and its related disorders (Shin et al., 2018) .

The results reported from this study show that most of the lipid parameters of the obese rats were disturbed .It was noticed a significant increase in the level of total Lipids, LDL-ch, total cholesterol and triglycerides and reduction in HDL-ch concentration .The improvement of the levels of lipid parameters may be related to the role of probiotics bacteria in modulating metabolic parameters in high fat diet induced obese rats. Recently, in high-fat diet (HDF) induced obese mice, *Bifidobacterium* was shown to amend metabolic parameters through altering the expression of fatty acid and cholesterol synthesizing enzymes and hepatic lipid and glucose levels (Moya et al.,2014). Therefore, probiotics could be a charming treatment choice throughout their beneficial interactions with the microbiota and/or epithelial cells in the intestine of their hosts. In animal models, many strains of *Lactobacillus* and *Bifidobacterium* (the major constituents of probiotics) have been reported to exert useful effects on obesity and/or its associated metabolic complications (e.g., dysregulated lipid profile and increased glucose level) (Takemura et al., 2010 and Yin et al., 2010)

Our data are also in consistent with (An et al., 2011) who indicated that probiotic including *Bifidobacterium longum*, *Lactobacillus acidophilus*, are able to reduce cholesterol in either human and rat. Moreover, results declared that the hypolipidmic effect of probiotic bacteria may be attributed to the role of lactic acid bacteria fermentation products in the inhibition of cholesterol synthesis enzymes that result in reduction in cholesterol synthesis, cholesterol elimination in feces that facilitated by probiotic

bacteria which inhibit the absorption of cholesterol back into the body by binding with cholesterol, and the bacteria interfere with the recycling of bile salt to facilitate its elimination, which raises the demand for bile salt made from cholesterol and in turn results in body cholesterol consumption .

Many studies have reported anti-obesity effects of some bacterial strains such as *Lactobacillus* spp. and *Bifidobacteriu*m spp. (Lee et al., 2009 and Yun et al., 2009). In this study ,it was observed that feeding of a high fat diet for 5 weeks produced significant increases in body weight, and administration of lactobacillus(LAB) reduced body weight gain and fat weight.

Serum glucose level was significantly increased in G2 (obese rats) compared to normal group. The treated group with probiotics showed significant improvement.

Concerning serum insulin results, there were also an improvement but it was non-significant. (Cusi, 2010) focused on the link between metabolic disturbances in adipose tissue during obesity and the development of insulin resistance and type 2 diabetes mellitus (T2DM).He demonstrated that obesity caused adipocyte hypertrophy, macrophage infiltration,and adipocyte insulin resistance via pancreatic β cell dysfunction.). Although we could not clarify the mechanism involved, we speculated that probiotics exerted anti-obesity effects by preventing metabolic disturbances in adipose tissue.

As mentioned previously, Lee et al., (2018) suggested that LAB dietary supplementation with *L. mesenteroides* and *Lb. sakei* may be act as effective and safe nutritional supplementations to decrease complications associated with obesity.

Also our results are in agreement with (Yu et al., 2013) who proved that probiotics (*Bifidobactrium* and *Lactobacillus acidophilus*) had lowering effect on fasting glucose and insulin sensitivity index.

(Kumar et al.,2012 and Mazloom et al.,2013) stated that probiotic containing foods may reduce the levels of fasting and postprandial blood sugars in human.

Moreover, Yun et al., (2009) reported a significant reduction in fasting and decreasing glycolated hemoglobin in probiotic treated rats.

Antioxidants play an important role in scavenging free radicals and upregulating antioxidant genes thus maintaining body balance (Hegazy et al., 2019).

Data reported from our study showed that serum malondialdehyde and catalase levels were significantly increased in the obese rats as compared to control normal rats. Also the total antioxidant capacity level was significantly decreased in the previous obese group. The disturbance in the antioxidant state was improved in the group treated with the formula containing probiotic bacteria.

Many researchers have investigated that mannan oligosaccharides a component of probiotic bacteria stimulate the mechanism of oxidative defense and protect the gastrointestinal tract in ways other than just removing undesirable bacteria (Ognik and Krauze 2012&Aluwong et al.,2013).In addition, (kogan et al.,2008) suggested that yeast cell wall β-glucans may have antioxidant activity. Moreover, probiotics can also stimulate the antioxidant system of the host and elevate the activities of antioxidants efficiently. Studies in pigs showed that dietary Lactobacillus fermentum supplementation could increase serum super oxide dismutase (SOD) and glutathione peroxidase (GPx)and enhance hepatic catalase (CAT), muscle SOD, and Cu and Zn-SOD compared to the control group (Wang et al.,2009).

Probiotics are considered as potential targets for obesity therapy but the anti-obesity mode of action of probiotics is still unknown (Chen et al., 2017). In addition, Leptin and ghrelin, hormones are critical for the neuroendocrine control of energy homeostasis. Obesity is characterized by the development of resistance to leptin and ghrelin. Therefore, leptin and ghrelin can be exploited as targets for pharmacological management of obesity (Cui et al., 2017). The present study demonstrated that body weight gain and leptin concentration were markedly elevated in positive control group compared with normal rats. This finding is in agreement with (Friedman and Halaas, 1998) that is leptin is produced by adipose tissue to circulate into the bloodstream in a direct proportional manner with adipose mass. However, body weight gain and circulating leptin concentration were greatly reduced upon co-administration of formula containing probiotics and mannitol to the study group as shown in Table(5). This finding is in a good harmony with the results observed by Kang et al., 2013 upon using a probiotic strain that isolated from human breast milk *Lactobacillus gasseri* BNR17. This may due to the reduction of fat mass by co-administration of formula that could effect on the energy balance in a negative manner which is resemble the calorie restriction and/or excessive exercise situation. In addition, calorie restriction and/or excessive exercise stimulate the affinity to eat and

conserve energy (Leal-Cerro et al., 1998 and Maffei et al., 1995) which is not observed in rats' co-administered with the formula that contains probiotics, as reduced body weight gain was recorded for the formula group. These findings could be attributed to the antiobesity effects of probiotics (i.e. specific lactic acid bacteria), as suggested previously (Yoda et al., 2015; Qiao et al., 2015; Karimi et al., 2015; Stenman et al., 2014; Fak and Backhed, 2012).

On the other hand, Table (5) shows that ghrelin concentration was reduced as body weight gain increased in positive control group. These results are in accordance with findings reported in the literature where there is an inverse relationship between ghrelin and BMI where it is up-regulated in under-nourished states and is down-regulated within positive energy balance states, such as obesity (Otto et al., 2005; Tschop et al., 2001 and Otto et al., 2001). In addition, co-administration of the formula containing probiotics, increased ghrelin concentration and decreases the body gain weight in the studied rats. Although, there is little evidence exists to associate between the acute hunger signal in the pre-prandial period and inducing feeding effect that attained by high levels of ghrelin (Schmid et al., 2005). Therefore, the actual mechanism by which the formula decreased body weight gain, decreased leptin and increased ghrelin concentrations in the study group needs further investigations.

CONCLUSION

Our data have shown that probiotic bacteria can ameleorate blood glucose level, insulin, lipid profile, leptin and ghrelin, and decreasing oxidative stress associated to obesity. It's thus recommended that it can be safely using functional fermented ice-cream containing low calorie sugars produced by lactic acid bacteria to relief many of the complications that occur due to obesity.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

HMZ participated in biochemical analysis, leptin and ghrelin measurements, data analyses and writing the manuscript.
ASA and AMH, collecting the literatures, designed and performed the animal experiment and biochemical analysis, did the statistical analysis and also wrote the manuscript.
IHB revised the manuscript
GAI and OMS conceived the research idea, prepared the encapsulated probiotic bacteria, manufacture functional fermented ice cream and participated in the writing and revision of the manuscript.

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