Effect of Feeding Synbiotic Fermented Milk on Leptin and Insulin Resistance in Obese Rats

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Abstract

The study aimed to investigate the feeding effect of synbiotic fermented milk fortified with Aloe Vera gel (AVG) on leptin concentration and insulin resistance in obese male rats (*Rattus norvegicus*) induced by a high- fat diet (HFD). Twenty-eight rats were divided into 4 groups: control group (SD-C) received the standard diet, control group of HFD (HFD-C) received HFD, and two experimental groups that were given HFD but with an additional oral dose of fermented milk with *Bifidobacterium lactis BB-12* (HFD-B) or yogurt (HFD-Y) which were supplemented with 5% AVG. Body weight, BMI, and Lee index were measured, along with serum levels of some biochemical parameters. The results showed that in comparison to the body weight of the SD-C group (63.91%), there was no significant difference ($p \geq 0.05$) with that of the HFD-B group (66.71%). However, the body weight of the HFD-C and HFD-Y groups increased significantly. This was accompanied by a significant increase in the value of BMI and Lee-Index for the HFD-C group, 0.82 and 0.33 respectively, compared with the rest of the groups, but these values of the HFD-B group did not differ significantly compared to the values of the SD-C group. The serum biochemical parameters of the HFD-B group were the closest to the SD-C group despite being fed on a high fat diet. While they were the highest in the HFD-C group. In conclusion, the synbiotic effect of fermented milk supplemented with 5% of AVG might participate in the increase in insulin sensitivity and decreased leptin resistance, which is promising for future applications in functional food products.

Key Words: Aloe Vera gel, Synbiotic, body mass index, leptin resistance, Adiponectin.

Introduction

Obesity, the accumulation of a mass of adipose tissue in the body, is a global epidemic syndrome (11). This phenomenon almost tripled worldwide between 1975 and 2016, as it is estimated there were more than 1.9 billion people in 2016 who were overweight, and more than 340 million children and adolescents between the ages of 5 and 19 were overweight or obese (32).

A reduction and imbalance of microbial diversity in the gut-microbiome, Dysbiosis, has been linked to a series of metabolic disorders that can lead to several health problems. In addition, obesity and metabolic syndrome are affected by many factors including physiological, diet, lifestyle, genetic and environmental factors. However, diet also plays an important role in the shaping of the intestinal microbiota (14).

Rebalancing the intestinal microbiota is an effective treatment for obesity and other chronic diseases. One of the most important methods of rebalancing is the use of probiotic, prebiotic, and synbiotic foods, which have gained great interest from researchers, producers, and consumers. Previous studies have shown that the effects of using probiotics on body weight and BMI varies depending on bacterial strains and administered dose (3 and 26).

Synbiotic foods, including fermented milk fortified with prebiotics and probiotics, are among the most important functional foods nutritionally. Many starters, consisting of lactic acid bacteria strains, are used in milk fermentation due to their high ability to hydrolyze milk proteins, and produce bioactive peptides capable of inhibiting the activities of α-amylase and glucosidase enzymes, and pancreatic lipase, thus reduced the risk of obesity or diabetes (18).

Leptin, a protein with a molecular weight of 16 kDa, contains 146 amino acids. Leptin is found in the blood at nanomolar concentrations (16). Furthermore, this hormone that is produced by the adipose tissue regulates body weight by controlling food intake and consuming of energy. As a result, leptin plays a key role in energy homeostasis (anti-obesity) and glucose metabolism (21).

People who suffer from obesity tend to display a higher level of leptin in the blood with symptoms of leptin resistance, and it has been proven that levels of leptin in plasma are directly proportional to the percentage of the body fat, so most individuals who suffer from obesity have high concentrations in blood serum or plasma. Leptin resistance may be due to reduced transport of leptin to the central nervous system or downregulation of leptin receptors (31).

There is a close association between obesity, the hormone insulin, and the intestinal microbiota, because diet-induced obesity promotes insulin resistance. One of the mechanisms explaining this relationship is the increased intestinal permeability of lipopolysaccharides (LPS) compounds present in the cell wall of Gram-negative bacteria due to high-fat diets, which leads to the occurrence of endotoxemia, which is associated with insulin resistance (27).

Some probiotic strains have shown a role in regulating the hormone insulin and improving its sensitivity. In a study conducted by Bagarolli *et al*. (8) on the modification of the intestinal microbiota using *Lacticaseibacillus rhamnosus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidumi*, and its effect on improving insulin sensitivity in rats with diet-induced obesity, it was shown that probiotics contributed to improving insulin sensitivity by significantly modifying the intestinal microbiota and treating inflammation caused by HFD.

The aim of this study was to evaluate the effects of two types of synbiotic fermented milk supplement with 5% AVG on body weight,

leptin resistance and insulin resistance in obese rats induced by HFD.

Materials And Methods

Aloe Vera gel (AVG)

Aloe Vera "*Aloe barbadensis miller*" leaves (3 years old) were obtained from the Medicinal Plants Unit of the Faculty of Agricultural Engineering Sciences - University of Baghdad. The gels were prepared according to the method described by Gutiérrez-Álzate *et al*. (13).

Starter Cultures

The starter culture of yogurt (SACCO, Italy) and *Bifidobacterium Lactis* BB- 12® (PROBIOTIC QUEST, UK) were obtained from a local distributor in Baghdad/ Iraq. Before the treatments, the starter cultures were activated and incubated at 42°C and 37°C, respectively.

Preparing yogurt and fermented milk

Yogurt and fermented milk were prepared according to the method described by Chandan and Kilara (12).

Preparing Feed Pellets

The diet was prepared according to their nutritional and physiological requirements as presented by An *et al.* (6): Standard diet (SD) (3750 kcal) and high-fat diet (HFD) (5000 kcal) were used.

Animal experiments

A total of 28 Male white rats (*Rattus norvegicus*) obtained from the animal house of the Faculty of Science - University of Kufa. The rats were 5 weeks old, with an average weight of 105±10 g. The rats were housed individually in special cages made of plastic and provided with food and water, with a controlled temperature at 25 ± 2 °C and maintained on a 12 h light/dark cycle. Animals were acclimatized to laboratory conditions for one week before the experiment as described by Cannella *et al*. (10).

Note: The study was conducted in accordance with bioethical Committee approval at the University of Kufa, Document reference number (6761)

Experiment Design

Male rats (28) were divided into 4 groups (n=7/group). The first group was fed the standard diet (SD) throughout the experiment (negative control), while the other three groups were fed a high-fat diet (HFD). The rats were distributed as following:

Group SD-C was fed SD for the duration of the experiment (negative control). Group HFD-C was fed HFD plus oral gavage of 1.5 ml of distilled water (positive control). Group HFD-Y was fed HFD plus oral gavage of 1.5 ml of yogurt supplemented with 5% AVG. Group HFD-B was fed HFD plus oral gavage of 1.5 ml of fermented milk with *Bif. Lactis* BB-12 supplemented with 5% AVG.

Anthropometrical determination

The weights of the experimental animals were measured using a top-pan scale twice a week. Body length (nose-to-anus) was measured (20).

The body weight and body length were used to determine the following parameters:

- Body mass index (BMI) = body weight (g)/ length^2 (cm^2).
- Lee index = cube root of body weight (g)/ nose-to-anus length (cm).

Blood sample collection

At the end of the experiment (8 weeks), rats in all groups were fasted overnight. Then they were anesthetized using (Ketamine) and (Xylazine) intramuscular injection. Blood samples (3-5 mL) were drawn through Heart Puncture using Syringe, and then the blood was placed into two deferent types of dry, sterile blood collection tubes, with and without coagulant agent. After standing for a while, the tubes were centrifuged to separate the plasma (from free- coagulant agent tubes) and serum (from coagulant agent contained tubes). The clear plasma and serum layer were kept at -18 ºC for screening of biochemical analysis.

Biochemical Examinations

The glucose levels were determined using a rat ELISA kit (ELK6811), with a sensitivity of 0.48 ng/mL and detection range of 1.25–80

ng/mL. Leptin levels were determined using a rat ELISA kit (ELK1244), with a sensitivity of 0.129 ng/mL and detection range of 0.32–20 ng/mL. Adiponectin levels were determined using a rat ELISA kit (ELK2463), with a sensitivity of 1.33 ng/mL and detection range of 3.13–200 ng/mL. Insulin levels were determined using rat ELISA kit (ELK2370), with a sensitivity of 4.93 pg/mL and detection range of 15.63-1000 pg/mL. TNF-a levels were determined using rat ELISA kit (ELK1396), with a sensitivity of 6.1 pg/mL detection range of 15.63– 1000 pg/ml, (ELK Biotechnology Com., China).

Data Analysis

Data were analyzed according to a complete randomized design (CRD) using GenStat V.12.1 software (VSNi, UK). The arithmetic means of the different parameters were compared using Duncan's Multiple Range test at $p \leq 0.05$. (13)

Results and Discussion

The effect of orally gavaged fermented milk supplemented with 5% AVG on the weight indicators of experimental animals is shown in (Table 1), and it is noted that there are no significant differences at $p \leq 0.05$ between all groups at the end of the acclimation week. However, after feeding the HFD-C group a high-fat diet, an increase in their weights (413.2 ± 24.60 g) was observed, which was significantly greater compared to the SD-C group by 10.45%, which recorded the lowest mean weight (292.2 ± 15.32 g) at the end of the experiment.

This data is consistent with the results reported by Mazloom *et al*, (20) who stated that an increased intake of calories resulted in the accumulation of adipose tissue, an increase in body weight, and an imbalance in the microbiota, which leads to an increase in energy extraction from food components, including the digestion of polysaccharides and the production of short chain fatty acids. Acetate, lactate, and propionate are important energy sources for the host and involved in the synthesis of fats in the liver.

In the same way, the HFD-C group differed significantly compared with all other groups, as it achieved the highest change in the body weight (74.36±2.55%), followed by the HFD-Y group (69.02± 3.15%), while the HFD-B group did not differ significantly (66.71 ± 2.37%) compared to the SD-C group, which was (63.91±1.84%). This indicates that the fermented milk gavage of BB-12 supplemented with 5% of AVG preserved the best reduction in weight increase of experimental animals for controlling obesity caused by HFD, as the weight increase decreased by 7.65% compared with the HFD-C group.

Table 1. Effect of fermented milk supplemented with AVG on weight indicators of obese rats fed a high-fat diet for 8 weeks.

Groups	*Initial weight (g)*	*Final weight (g)*	*Weight gain (g)*	*body weight Change %*	*Body Length (cm)*
SD-C	a 105.5±8.40	a 292.2±15.32	a 186.7±10.07	a 63.91±1.84	a 21.92±0.59
HFD-C	a 105.8±10.76	c 413.2±24.60	c 307.3±23.10	c 74.36±2.55	a 22.42±0.86
HFD-Y	a 106.2±8.25	b 344.3±29.26	b 238.2±28.58	b 69.02±3.15	a 22.92±0.67
HFD-B	a 105.8±9.57	ab 318.5±28.60	ab 212.7±22.80	ab 66.71±2.37	a 22.83±0.93

Mean ± SD (n=7).\ The different small letters within the column indicate a significant difference at (p≤0.05)\ AVG= Aloe Vera gel\ SD-C = fed SD\ HFD-C = fed HFD+1.5 ml of distilled water\ HFD-Y = fed HFD + 1.5 ml of yogurt with 5% AVG\ HFD-B = fed HFD+1.5 ml of fermented milk with *Bif. Lactis* BB-12 supplemented with 5% AVG.

This result is consistent with that reported by Ariyanto *et al* (7) in which the rats given aloe vera supplements lost weight by reducing body fat mass mediated by regulating metabolism and increasing AMP-activated activity in muscle. The effect of AVG at a concentration of 100 and 200 mg/kg on body weight was studied by Rahoui *et al*. (25) They found that it caused a significant decrease in body weight with a decrease in serum glucose, triglyceride, and cholesterol levels. In a recent study, Javaid *et al*. reported that fortifying the diet of obese mice with whole Aloe vera leaf powder at a concentration of 300 mg/kg/day for 40 days led to decrease in the body weight of obese mice (17). It appears that AVG can reduce obesity and its negative effects through a combination of catabolic pathways while increasing energy expenditure and dissipation rather than decreasing appetite and reducing food intake as reported by Shakib *et al*. (28).

The results showed a significant increase in the BMI of the HFD-C group compared with the rest of the groups, followed by the HFD-Y group. While the HFD-B group did not differ significantly compared to the SD-C group that was fed a standard diet (Figure 1a).

Moreover, the results in (Figure 1b) showed that feeding a high-fat diet for 8 weeks led to an increase in Lee index for the HFD-C group, which indicates body fat accumulation and obesity. While the oral gavage of fermented milk supplemented with 5% AVG reduced the value of the Lee-index in the HFD-Y and HFD-B groups despite being fed the same high-fat diet. The Lee Index did not differ significantly compared to the SD-C group that was fed a standard diet. The results agree with that found by Tada *et al*. (29) who studied the anti-obesity mechanisms of AVG extracts. They observed that administering AVG extracts to male rats fed a HFD for 11 weeks suppressed the increase in body weight and prevented obesity. Also, they confirmed that the presence of aloe sterols regulates the mRNA expression levels of genes associated with the activation of brown adipose tissues (BAT) which store energy in a more compact adipose tissue compared to white adipose tissue (WAT). In the same way, An *et al*. observed that gavage of groups of mice fed HFD with 0.2 ml of a mixture of three strains of *Bifidobacterium* led to a significant decrease in body weight compared to groups of untreated mice that had a similar caloric consumption (6).

The data in (Table 2) represents the results of the oral gavage of fermented milk supplemented with 5% AVG on the levels of glucose, insulin, leptin, adiponectin and TNF-α with percentage changes (increase or decrease) compared with the SD-C-negative and the HFD-C-positive control groups. It is noted that the rats taking a high-fat diet led to a significant increase at p≤ 0.05 in the glucose level for the HFD-C group, as the highest level was 53.01 ± 8.81 ng/ml, with an increase of 27.05% compared to the SD-C group, which had the lowest glucose level 38.67. ±2.74 ng/ml.

These results are consistent with the findings of Al-Samarrai *et al*.(5) who observed an increase in blood glucose level in obese adolescents, with a strong significant association between obesity and high blood glucose.

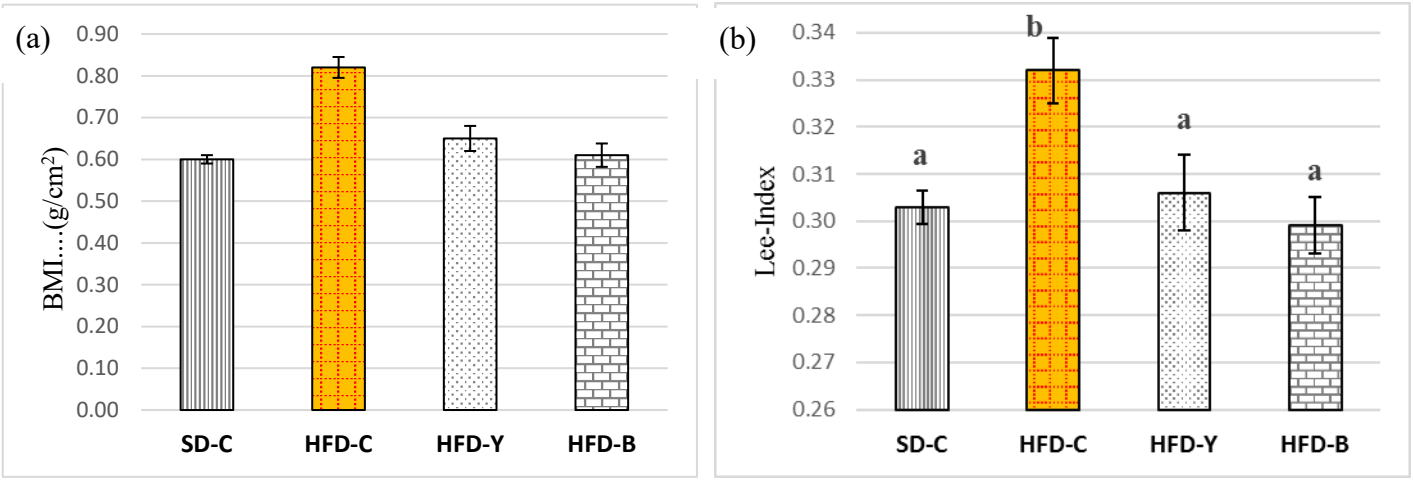

Figure 1. Effect of fermented milk supplemented with AVG on BMI and Lee-Index of obese rats fed a high-fat diet for 8 weeks, n = 7.

AVG= Aloe Vera gel\ SD-C = fed SD\ HFD-C = fed HFD+1.5 ml of distilled water\ HFD-Y = fed HFD + 1.5 ml of yogurt with 5% AVG\ HFD-B = fed HFD+1.5 ml of fermented milk with *Bif. Lactis* BB-12 supplemented with 5% AVG.

The results of the statistical analysis showed that the gavage of groups of rats with fermented milk supplemented with 5% AVG (HFD-B group) led to a significant decrease by 31.44% at $p \leq 0.05$ in the level of glucose (40.33 ± 5.19 ng/ml) compared with the HFD-C group despite being fed on the same high-fat diet. It was observed that the HFD-Y group, whose glucose level was 45.17 ± 3.84 ng/ml, decreased by 17.36%. The HFD-B group was the closest group to the SD-C group fed on a standard diet.

These results agreed with what was found by Tonucci *et al*. (30) who observed a significant decrease in glycemic concentration in fifty subjects after consuming fermented milk containing *L. acidophilus* La-5 and *Bifidobacterium animalis subsp lactis* BB-12 for 6 weeks. Also, in a recent study by Bonaziz *et al*., it was shown that when obese rabbits, caused by a high-fat diet, were gavaged with 1 ml of *Bifidobacterium animalis subsp. lactis* BB-12® and *Lactobacillus plantarum* 299v® taken separately for 14 weeks, there was a significant improvement in body weight and blood biochemical parameters (9). It also agrees with Afrin *et al*. (2) who reported that consuming AVG reduced the level of glucose in the blood of rats by regulating its absorption in the intestine, as soluble dietary fiber works to prevent and impede the absorption of glucose by trapping it inside the viscous gummy gel and thus reducing the possibility of its arrival to the intestinal wall, which reduces its absorption into the bloodstream, in addition to preventing the digestive enzymes that release glucose from reaching carbohydrates and reducing their effectiveness.

On the other hand, an increase in the level of insulin in the serum of the HFD-C rats of 48.42% was observed, reaching 285 ± 18.71 pg/ml, compared with the SD-C group, which had a value of 147 ± 23.61 pg/ml. While the insulin level decreased to 222.5 ± 16.53 and 160.0 ± 18.74 pg/ml in the HFD-Y and HFD-B groups that were orally gavaged with fermented milk supplemented with 5% AVG. The decrease was 28.09% and 78.13%, respectively, compared to the HFD-C group.

Synbiotic foods can positively modify the host's intestinal microbiota, improving insulin sensitivity and glucose metabolism by reducing Lipopolysaccharide, decreasing inflammation,

intestinal wall permeability, and energy harvesting level as reported by Kim *et al.*(19).

Table 2. Effect of fermented milk supplemented with AVG on weight indicators of obese rats fed a high-fat diet for 8 weeks.

Parameters	Groups			
	SD-C	HFD-C	HFD-Y	HFD-B
Glucose (ng/ml)	38.67±2.74 [a]	53.01±8.81 [b]	45.17±3.84 [a]	40.33±5.19 [a]
Covariance %	--	27.05% A	14.39% A	4.12% A
	-37.08% B	--	-17.36% B	-31.44% B
Insulin (pg/ml)	147.0±23.61 [a]	285.0±18.71 [c]	222.5±16.53 [b]	160.0±18.74 [a]
Covariance %	--	48.42% A	33.93% A	8.13% A
	-93.88% B	--	-28.09% B	-78.13% B
Leptin (ng/ml)	2.25±0.438 [a]	4.44±0.302 [c]	3.11±0.817 [b]	2.49±0.518 [ab]
Covariance %	--	49.32% A	27.65% A	9.64% A
	-97.33% B	--	-42.77% B	-78.31% B
Adiponectin (ng/ml)	25.48±3.66 [b]	12.00±2.27 [a]	15.20±3.18 [a]	22.20±2.53 [b]
Covariance %	--	-112.33% A	-67.63% A	-14.77% A
	52.90% B	--	21.05% B	45.95% B
TNF-a (pg/ml)	145.5±37.62 [a]	301.0±33.29 [c]	211.0±25.77 [b]	174.5±33.72 [ab]
Covariance %	--	51.66% A	31.04% A	16.62% A
	-106.87% B	--	-42.65% B	-72.49% B

Mean ± Sd (n=7).\ The different small letters within the row indicate a significant difference at (p≤0.05)\ A= Covariance ratio compared to SD-C group\ B= Covariance ratio compared to HFD-C group\ AVG= Aloe Vera gel\ SD-C = fed SD\ HFD-C = fed HFD+1.5 ml of distilled water\ HFD-Y = fed HFD + 1.5 ml of yogurt with 5% AVG\ HFD-B = fed HFD+1.5 ml of fermented milk with Bif. Lactis BB-12 supplemented with 5% AVG.

The highest leptin level (4.44 ± 0.302 ng/ml) was observed in the HFD-C group, which differed significantly compared to all other groups. Since the level of leptin in blood is directly proportional to the amount of adipose tissue, the high level of leptin (hyperleptinemia) is closely related to obesity. A significant decrease at p≤ 0.05 was found in leptin levels collectively when body weight decreased as a response to the oral gavage of fermented milk supplemented with 5% AVG in the HFD-Y and HFD-B groups, (3.11 ± 0.817 ng/ml and 2.49 ± 0.518 ng/ml) respectively, with a decrease of 42.77% and 78.31% for the groups above, compared to the HFD-C group. From the results, the HFD-B group was closest to the SD-C control group, which was 2.25 ± 0.438 ng/ml, where they did not differ significantly at p≤ 0.05.

Adiponectin plays an important role in many metabolic and cellular functions, with its main function being to increase insulin sensitivity and anti-inflammatory effects, as lower levels of adiponectin are associated with increased rates of obesity according to Aljutaily *et al*. (4). The results revealed that the HFD-C and HFD-Y groups showed a significant decrease at p≤ 0.05 in the level of adiponectin (12.00 ± 2.27 and 15.20 ± 3.18 ng/ml) respectively, as they decreased by 112.33% and 67.63% compared to the SD-C group which recorded the highest level (25.48 ± 3.66 ng/ml), while no significant difference was observed for the HFD-B group (22.20 ± 2.53 ng/ml) compared to the SD-C group at the same probability level.

Leptin exerts various functions in the innate immune system. In monocytes, leptin stimulates the release of pro-inflammatory cytokines, such as TNF-α and induces expression of cell surface markers which are important for activation of resting monocytes according to Poetsch *et al.* (23). There was a significant increase at p≤ 0.05 in the level of TNF-α in the serum of the HFD-C group (301.0 ± 33.29 pg/ml) compared to the

SD-C, HFD-Y and HFD-B groups, which were 145.5 ± 37.62, 211.0 ± 25.77 and 174.5 ± 33.72 pg/ml, respectively, the differences for these groups were -106.87, -42.65% and -72.49%, respectively, compared to the HFD-C group, while no significant difference was observed between the SD-C and HFD-B groups.

Obesity-associated enlargement of adipocytes results in enhanced leptin secretion and therefore higher serum leptin levels, which may also result from chronic hyperinsulinemia and increased cortisol turnover. In addition, some free fatty acids, estrogens and TNF-α may stimulate leptin secretion as reported by Poetsch *et al*. (23).

Procaccini *et al.* reported that obesity is associated with low-grade chronic inflammation, which results from changes in both the innate and adaptive immune system (24). Elevated levels of leptin are associated with insulin resistance and hypothalamic inflammation, which are considered as risk factors for the development of metabolic syndrome and other cardiovascular diseases as reported by Abella *et al*. (1).

Conclusion

It can be concluded that the use of AVG in the preparation of Synbiotic fermented milk contributed to reducing obesity caused by a high-fat diet in rats, further increasing insulin sensitivity, decreased leptin resistance, and reduced low-grade inflammation caused by obesity. Therefore, the utilization of AVG as a prebiotic is promising in the preparation of synbiotic foods that have anti-obesity, leptin resistance and insulin resistance effects. However, future studies are required for a longer period of treatment.

Conflicts of Interest

The authors declare no conflicts of interest.

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