

Study Effect of Feeding on Low Fat Labneh Manufactured by adding Oat Beta-Glucan Fat Substitute in some Health and Physiological Indicators of White Mice

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Abstract

This study was conducted to develop non-traditional dairy products with health benefits. The study included the manufacture of labneh from skimmed cow's milk, with the addition of the fat substitute beta-glucan oat at a concentration of 0.4%, for a group of laboratory mice that were fed a diet for 28 days Represented by the L2 treatment to evaluate the performance of beta-glucan fat substitutes, and their contribution to some nutritional and health indicators, where the added beta-glucan had a clear role in reducing the daily and final weight gain of the experimental animals and clearly ($P<0.05$) compared to the group of mice with positive control C+ that were fed a standard diet only. The levels of total cholesterol, triglycerides TG, high-density lipoprotein HDL, low-density lipoprotein LDL and very low-density lipoprotein VLDL showed significant results ($P<0.05$) compared to the positive control C+ treatment.

Keywords:

Oat Beta-glucan , white mice ,T.G, low fat lebneh , cholesterol.

Introduction

It is believed that the consumption of a high-fat labneh has increased significantly worldwide. This diet refers to foods that are prepared quickly, rich in saturated fats, purchased from restaurants that use pre-cooked ingredients, and served in a packaged form. Dietary fats are the most energy-dense nutrients, causing less satiety than carbohydrates or proteins. In addition, it has been observed that eating a high-fat

diet for long periods leads to hyperphagia, increased body weight, fat deposition, and increased levels of glucose, insulin, and triglycerides (TAG) (31), (27).

Fat is also one of the causes of obesity, the risk of which has become increasing not only in Western countries but in most countries of the world, and which can only be controlled through low-calorie diets that have a positive impact on the health of the consumer (10), (8).

Eating large amounts of foods with high levels of saturated fat is associated with increased total blood cholesterol and low-density lipoprotein (LDL) cholesterol (35), which is considered a risk factor for the development of cardiovascular diseases and other metabolic diseases.

Therefore, reducing fat intake, or using fat mimetics in diets, is an effective strategy to reduce the risk of cardiovascular diseases (12).

Fats play a fundamental role in food, as they contribute mainly to highlighting the quality of texture, flavour, texture and consistency. Studies indicate that removing fats from dairy products negatively affects the quality of texture and flavour, and in such cases the problem of texture deterioration arises (24). Fats greatly affect the quality indicators of food products, so reducing their content or excluding them from the composition of food products inevitably leads to a deterioration in their quality, and this in itself is a major challenge for food producers (25). Therefore, researchers have turned to adding some materials as alternatives to fats, as these materials work to improve the rheological properties of food products, and that fat substitutes have a chemical composition that differs from the chemical composition of fats, but they have physical properties similar to those found in fats, so when added to food products, they give a smooth texture and a creamy taste in the mouth, in addition to the viscosity that suits the type of manufactured product, and that fat substitutes are either from a carbohydrate source, such as beta-glucan, inulin, and microcrystalline cellulose, or from a protein source such as sodium caseinate and whey protein concentrates, or from a fatty source, such as olestra (14).

The use of dietary fibers, including beta-glucan, which is characterized by its high

ability to act as prebiotics, reduce cholesterol and blood sugar, and increase the body's immunity, in addition to its effects on weight loss, and it also works to reduce the risk of cancer, as alternatives to fats in some food products (9).

Given the recent trends in the use of fat substitutes in the dairy industry, and their interesting sensory properties, due to their functional properties as well as their nutritional properties, and the fact that some of them contain high levels of biologically active compounds, in addition to their effective role in reducing energy, and obtaining healthy products that play a role in reducing cholesterol and enhancing the immune system (33). Therefore, the current study was conducted and aimed to conduct a nutritional experiment using mice to study the consumption of labneh with the added fat substitute represented by beta-glucan extracted from oats in nutritional and health indicators, which include the weights of mice, and the levels of total cholesterol, TG, HDL, VLDL and LDL.

Materials and methods:-

In this study, 40 laboratory animals of male BALB/C mice of the albino type were used, obtained from the National Center for Drug Research and Control - Baghdad, where they were between 3-4 weeks old, and an average weight of 30-32 g, and were placed in special breeding cages, under controlled conditions such as temperature ranging around $25 \pm 2^{\circ}\text{C}$ and providing the necessary ventilation for them, and water was always available when the animal needed adlabium, while lighting was 12 hours of light and 12 hours of darkness, food was left in the animal cages for 3 days before starting the experiment for the purpose of adapting to the special conditions of the experiment, the experimental animals were randomly distributed into four groups

with 10 mice per group. The effect of feeding processed yogurt containing the oat fat substitute beta-glucan on the rate of increase in body weight, levels of triglycerides (TG), total cholesterol, and lipoproteins (HDL, LDL, VLDL) was studied. The experimental animals and the food consumed were weighed throughout the experiment twice a week for each group.

Experimental design: -

First treatment: continued feeding on a standard diet throughout the experiment period and returned (control treatment C-).

Second treatment: continued feeding on a labneh diet made from whole raw milk and returned (positive control treatment C+).

Third treatment: continued feeding on a functional labneh diet made from skimmed milk only without any addition and returned (treatment L1).

Fourth treatment: continued feeding on a functional labneh diet made from skimmed milk with 0.4% oat beta-glucan added to it and returned (treatment L2).

Table (1) Components and proportions of the standard high-fat feed used in feeding mice (g/100 g)

Ingredients	Standard feed gm/100	High-fat feed gm/100
Casein	20	20
Corn oil	7	7
Cellulose fibers	5	5
Vitamin blend	1	1
metal alloy	3.5	3.5
Colin	0.2	0.2
corn starch	46.8	46.8
Cholesterol	0	2

Note: Complete the feed to 100 g using sucrose.

The diet was prepared to feed the mice according to the above nutritional and physiological requirements.(2)

Sample collection: -

At the end of the experiment, the mice were deprived of food for approximately 8 hours (fasting), after which they were anesthetized with chloroform, then the abdominal cavity was opened to the chest area, and blood was drawn from the heart by cardiac puncture, using a 1 ml syringe and placing the blood from each mouse in a sterile and special test

tube bearing the transaction number, leaving the blood in the refrigerator for 30 minutes to clot, centrifuged at a speed of 3000 rpm (for 15 minutes) to separate the blood serum, placing the serum in Eppendorf tubes in small sizes and storing at a temperature of - 20 C until laboratory tests are performed.

Biochemical analyses:-

The concentration of cholesterol in the blood serum was measured using a kit manufactured and equipped by the Serum and Vaccine Institute. The concentration of cholesterol was extracted according to what was mentioned in (15).

and as in the following equation:-

The concentration of cholesterol in the blood serum (mg/100 ml) = (sample reading) / (standard cholesterol reading) × 200 (concentration of the standard solution).

Measuring the level of triglycerides (mg/100 ml)

The enzymatic method used by (32) was used in the process of measuring the concentration of triglycerides in blood serum, using the analysis kit (Kit) manufactured by Biolabo-France, where the reading was done using a spectrophotometer at a wavelength of 546 nanometers, and the concentration of triglycerides was calculated as in the following equation: -

Triglyceride concentration (mg/100ml blood) = Absorbance of sample / Absorbance of standard solution × 200 (Concentration of standard solution)

Measurement of the concentration of high-density lipoproteins in blood serum (HDL)

The enzymatic analysis method was used to measure the concentration of (HDL) in blood serum according to the method followed by (34), using the analysis kit (Kit) manufactured by Biolabo-France, which is an enzymatic method. The reading was done using a spectrophotometer at a wavelength of 500 nanometers, and HDL was calculated according to the following equation: -

HDL concentration = Absorbance of sample / Absorbance of standard solution × 50 × 10 (mg/100ml blood)

Where:- 50 = concentration of standard solution
10 = dilution factor

Measuring the concentration of low-density lipoproteins (LDL)

The method mentioned in (18) was used in the process of measuring the concentration of low-density lipoprotein (LDL) and according to the following equation: -

LDL concentration (mg/100 ml of blood) = total cholesterol concentration - HDL-VLDL concentration

Measuring the concentration of very low-density lipoproteins (VLDL)

The concentration of very low-density lipoproteins in the blood serum was calculated according to the method mentioned in (16) and according to the following equation: -

VLDL (mg/100 ml blood) = triglycerides / 5

Statistical analysis:-

Uses of the statistical analysis method of the SAS program (2012) (30)

Results and Discussion:-

Effect of adding beta-glucan on the average body weight and daily weight gain of experimental mice

The results of Table (2) show the average daily and final weight gain of the experimental mice groups after 28 days, where it was shown that the group of positive control mice C+ that were fed on a diet rich in fat recorded the highest daily weight gain rate, reaching 0.2902 g/day, while the final weight gain reached 8.127 g. This result is consistent with what was found by (20), who indicated an increase in the final weight rate of the group of treatment mice fed on a diet rich in fat, and

also consistent with what was found by (6), who concluded that the average weight of the positive treatment mice that were fed on a diet rich in fat increased.

The daily and final weight gain rate of the control group C-, which was fed a standard

diet, was 0.2114 g/day and 5.921 g, respectively. The reason for the difference in the final weight rate between the control treatment C+ and C- is due to the type of diet provided for each treatment. In the positive control group, the diet was rich in fat, so a higher weight gain occurred.

Table (2): Effect of beta-glucan on body weight rate and daily weight gain of treatment groups of mice after 28 days

Treatments	Body weight (g)		Body weight gain after 28 days (g)	Daily rate of increase in body weight (g)
	Initial weight rate	Final weight rate		
positive control Treatment C ⁺	31.487	39.614	8.127	0.2902
Negative control Treatment C ⁻	30.327	36.248	5.921	0.2114
Treatment L1	31.645	38.714	7.069	0.2524
Treatment L2	31.713	34.641	2.928	0.1045
L.S.D Value	2.185 NS	4.247 *	4.059 *	0.115 *
* (P≤0.05) ,NS: Non-Significant				

These results are consistent with what was found by (4), who indicated that the highest weight gain after 28 days was for the group of mice fed a diet rich in fat, reaching 6.110 g/day, compared to the group fed a standard diet, which reached 3.210 g.

The daily weight gain rate for the L1 mice group fed on a diet of labneh made from skimmed milk was 0.2524 g/day, and the final weight gain was 7.069 g. while the daily weight gain rate for the L2 mice group fed on a diet of labneh made from skimmed milk with 0.4% beta-glucan added was 0.1045 g/day, and the final weight gain rate was 2.928 g, which is the treatment that excelled in the sensory evaluation experiment.

When comparing these results, we find that the highest weight gain was in the positive control treatment, followed by the L1 treatment, then the C-control treatment, and the L2 treatment. This means that beta-glucan has contributed to reducing the rate of weight gain, and that the reason for this is due to the role played by dietary fibers, including cellulose, in surrounding nutrients, primarily fats, which reduces their absorption and excretes the largest amount of them with waste, in addition to reducing the duration of food remaining in the digestive tract and facilitating its excretion with the stool, which reduces the chances of fat absorption.

These results are consistent with what was reached by (3), who indicated that the rate of weight gain decreased after 28 days for the

group of mice that were fed a diet rich in fat with mozzarella cheese to which 0.2% of the fat substitute beta-glucan was added, as it reached 2.701 g compared to the positive control group, which reached 8.029 g. We note that the lowest weight gain was in group L2, which was fed on the labneh diet with the added fat substitute beta-glucan, compared to the other treatments. This is due to the presence of fibers that enhance the feeling of satiety, and the lack of need to add more calories to the diet, thus reducing the amount of food consumed, which then causes weight loss (7). It is noted from the results of the statistical analysis that there were no significant differences ($P < 0.05$) in the weights of the treatment mice during the adaptation period. It is also noted that there were significant differences in the daily weight gain rate between the control treatment C+ and C-, as well as between treatment L1 and L2, and also between treatment C+ and L2. It is also noted that there were no significant differences between treatments C+ and L1.

The effect of adding beta-glucan on the level of total cholesterol, triglycerides, HDL, LDL and VLDL lipoproteins in groups of different treatment mice.

Table (3) shows the values of total cholesterol, triglycerides, HDL, LDL and VLDL for the groups of mice treated with +C, C-, L1 and L2, where the total cholesterol value for the group of mice treated with positive control was recorded at 158.83 mg/100 ml, and this value is high compared to the total cholesterol value for the group of mice treated with negative

control C-, which was 138.78 mg/100 ml, and the reason for this is that the mice in the positive group were fed a diet rich in fat. This is consistent with what was found by (20) and (4) who indicated an increase in the levels of total cholesterol, LDL, VLDL and triglycerides, and a decrease in good HDL in the group of mice fed a diet rich in fat.

The liver is the main organ responsible for the process of cholesterol synthesis, breakdown and balancing in the body, as bile acids are synthesized when cholesterol is consumed. Since beta-glucan in oats has the ability to bind bile acids and fill the deficiency of this acid, this helps to consume larger amounts of cholesterol from the body, and thus form or synthesize bile acids again, and by this mechanism the level of cholesterol in the blood decreases (17). Also, the cholesterol content in the liver is always identical to the cholesterol levels in the blood serum, meaning that when serum cholesterol levels rise, the cholesterol content of the liver will also be high (1). The cholesterol value for treatment L1 was 150.48 mg/100 ml, which is a high value when compared to treatment L2, which was 130.67 mg/100 ml, due to the effect of beta-glucan in the diet of this group of mice. This is consistent with what was found by (28) and (23), who indicated that beta-glucan reduces cholesterol levels in the blood. It is also consistent with what was found by (1) and (3), who indicated that beta-glucan reduces the level of cholesterol in the serum, by encouraging the excretion of bile salts, and thus increasing the amount of cholesterol converted into salts.

Table (3): The effect of adding beta-glucan on the level of total cholesterol, triglycerides, HDL, LDL and VLDL lipoproteins for groups of experimental mice after 28 days.

Treatments	Total cholesterol mg/100 ml	Triglycerides TG mg/100 ml	High Density Lipoprotein HDL mg/100ml	Low Density lipoprotein (LDL) mg/100ml	Very low Density lipoprotein (VLDL) mg/100ml
Positive control Treatment C ⁺	158.83	144.87	56.23	78.42	31.17
Negative control Treatment C ⁻	138.78	127.63	69.41	45.12	25.34
Treatment L1	150.48	136.93	58.76	69.22	28.31
Treatment L2	130.67	112.41	77.92	33.84	21.89
L.S.D Value	12.85 *	11.93 *	7.442 *	11.02 *	5.438 *
*(P≤0.05)					

It is noted from these values that the value of the positive control treatment C⁺ is higher due to the high fat content resulting from this treatment because the labneh was made from whole milk of the group of mice in this treatment. It is also noted that the value of treatment L2 decreased compared to the positive treatment C⁺, as well as when compared with treatment C⁻ and L1, in which the values of triglycerides increased compared to treatment L2. The reason is that this treatment was made from skimmed milk with beta-glucan added to it at a rate of 0.4% as a substitute for fat. This is consistent with what was mentioned by (19) and (29), who indicated a decrease in triglycerides when taking doses of beta-glucan ranging between 3 and 6 g/person/day for two weeks.

It is also consistent with what was found by (5), who stated that total cholesterol and triglycerides decreased when beta-glucan extracted from wheat was added to the rats' diet. (21) showed that the reason for the decrease in the level of triglycerides is due to the reduction in the process of building

fats in the liver from its new sources, as a result of reducing the activity of enzymes that stimulate the building of fats. Also, the decrease in triglycerides is associated with an increase in the process of demolition of lipoproteins rich in them, in addition to the important role played by both glucose and insulin in the process of regulating fatty acids and triglycerides.

It is noted from the results of the statistical analysis that there are significant differences between the positive control treatment C⁺ and the negative control treatment C⁻, as well as significant differences between the L2 treatment on the one hand and all treatments on the other hand, which enhances and confirms the important role played by fat substitutes in reducing triglycerides.

The values of high-density lipoprotein (HDL) for the control treatments C⁺, C⁻, L1 and L2 were .2356, 69.41, 58.76 and 77.92 mg/100 ml, respectively. The statistical analysis results show significant differences in HDL values between treatment C⁺,

treatment C-, treatments L1 and L2. The HDL values for treatment C+ were lower than for treatment C-, which is attributed to the higher weight gain of the group of mice in the positive treatment C+, which is matched by a decrease in this type of lipoprotein. This is consistent with what was stated by (22) who indicated that there is a correlation between a decrease in the level of HDL lipoprotein and weight gain in experimental animals. Therefore, it is noted that the final weights of the positive control group of mice increased due to the decrease in the values of good fats HDL in them compared to the negative control treatment, and this is consistent with what was found by (20)) who indicated a decrease in the levels of good fats HDL in the blood plasma of the group of mice fed on a diet rich in fat content, and stated that the reason is related to the decrease in the activity of the enzyme lipoprotein lipase and lecithin cholesterol acyl transferase, and it is also noted that the values of HDL increased in the L2 treatment fed on yogurt containing 0.4% oat beta-glucan compared to the positive control treatment and the negative control treatment, and this is considered a positive effect of these materials represented by beta-glucan, which is an indicator of good health.

It is noted from the results of the statistical analysis that there are significant differences between the L2 treatment and the rest of the different treatments, which indicates the positive role of the added fat substitute.

The values of low-density lipoprotein LDL for treatments C+ and C- and treatments L1 and L2 were 78.42, 45.12, 69.22 and 33.84 mg/100 ml, respectively. These values indicate that the value of the positive control treatment is higher than that of the negative control treatment. It is also noted that the value of treatment L2, which consists of manufactured yogurt with the addition of the fat substitute beta-glucan, is lower.

This is a good indicator of the effect of this additive, as this type of lipoprotein LDL is considered a bad fat in the blood, so its increase poses a health risk. This is consistent with what was found by (26), who indicated that the viscosity of β -glucan interacts with bile acids, thus preventing their reabsorption in the large intestine, which leads to increased secretion of bile acids in the stool, thus increasing the need to synthesize bile acids from cholesterol, which is the mechanism that reduces harmful cholesterol. It also agrees with what was found by (29), who indicated that adding beta-glucan led to a decrease in the value of low-density lipoprotein LDL.

As for the values of very low-density lipoprotein (VLDL), the highest level was recorded in the positive control treatment C+, which was fed on a fat-rich diet, reaching 31.17 mg/100 ml, while the negative control treatment and L1 treatment reached 25.34 and 28.31 mg/100 ml, respectively. It is noted that the value of the positive control treatment increased compared to the negative control treatment, while the L2 treatment recorded a significant decrease, reaching 21.89 mg/100 ml, which is the lowest value compared to the rest of the treatments, in which the mice of this group were fed a diet containing labneh fortified with the fat substitute beta-glucan. The results showed that the L2 treatment was superior even to the control treatment C-. The results of the statistical analysis showed that there was a significant difference ($P < 0.05$) in the VLDL values between the positive treatment C+, the negative treatment C-, and the L2 treatment, and that there was no significant difference between C- and L1, the final results reached and through Table (3), it is clear to us that the high levels of total cholesterol, triglycerides (TG), LDL and VLDL in the group of positive treatment mice that were fed a diet rich in fat, is the main reason

responsible for weight gain and obesity in the individuals of this group, and other disorders and problems associated with fat (22) and (3).

We conclude from this the importance of the fat substitute represented by beta-glucan, which worked to maintain the levels of total cholesterol and fats within normal limits, reduce weight gain, increase the percentage of good lipoprotein HDL and reduce the levels of harmful lipoproteins LDL and very harmful VLDL. This is consistent with what was mentioned by (11), who indicated that beta-glucan works to improve the level of

fats, by increasing the conversion of cholesterol into bile acids, which enhances the reduction of cholesterol levels in the enterohepatic circulation.

It is also consistent with what was found by (13), who indicated that beta-glucan oats have a high molecular weight and are also characterized by high viscosity. These properties make it have additional health benefits such as controlling and controlling the levels of total cholesterol and (LDL) compared to fibers that contain a low molecular weight.

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