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Study the Effect of Feeding on Free Fat Yogurt Manufactured by Adding Beta-Glucan of Barley in Some Health and Physiological Indicators of White Mice

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Abstract

The current study was conducted and aimed at developing unconventional therapeutic dairy products with health benefits. The study included the manufacture of yogurt from cows' skimmed milk with an added of fat replacers beta-glucan at a concentration of 0.4%, an oral dose to a group of experimental mice fed on a diet rich in fat for a period of 28 days represented by the treatment Y2 in order to evaluate the performance of the beta-glucan lipid replacers and its contribution to some nutritional and health indicators where the added beta-glucan had a clear role in limiting the daily and final weight gain of experimental animals significantly ($P \leq 0.05$) compared to a group of positive control C^+ mice fed on a standard diet only. Significantly ($P \leq 0.05$) levels of total cholesterol, triglycerides (TG), low density lipoprotein LDL, very low density lipoprotein VLDL and high density lipoprotein HDL values compared with positive control C^+ treatment.

Keywords: Beta-glucan, free fat yogurt, HDL, LDL, VLDL, white albino mice.

Part of M.Sc. thesis of the 1st author.

Introduction

In recent years in Iraq and most countries of the world, the use of functional foods has become widespread, as it has improved the nutritional value and also improved human health (Martirosyan and Singh, 2015). Functional foods are known as those foods fortified with compounds known to have biological activity that when consumed in sufficient quantities give health benefits. In addition to the important nutritional functions it provides (Brown et al., 2018). The European and American markets are considered one of the most popular markets for selling these foods, as a special system for approving functional foods has been found, known as (FOSHU) for short. Among the most popular functional foods are fermented dairy products, meat products and other fermented foods, as the main goal of using them is to reduce the risk of chronic diseases such as hypercholesterolemia, arteriosclerosis, high blood pressure, heart disease, and to stimulate the immune system (Cara, 2014). Studies indicate that more than 70% of the fatty acids present in milk fat are saturated fatty acids represented by lauric, myristic and

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palmitic acid, which are mainly responsible for atherosclerosis (McSweeny and Fox, 2013). Milk fats are found naturally in the milk of cows and other animals, and they play an important role in the chemical, physical and sensory properties of foods, including the rheological and nutritional properties of dairy products, especially those in liquid or semi-solid form (Nguyen and Prakash, 2020). Studies show that removing fat from dairy products negatively affects texture and consistency, and in such cases the problem of deterioration of the texture emerges (Hakim et al., 2016). Therefore, reducing or completely eliminating fat while preserving quality and sensory characteristics is a major challenge for food producers (Wu et al., 2013). Therefore, fats that improve the rheological properties of products, as fat replacers have a chemical composition that differs from the chemical composition of fats, but they have physical characteristics similar to fats. Which matches the quality of the manufactured product (FSN, 2014). Oligo- and polysaccharide, which may or may not be digested, are good sources of fat replacers. Carbohydrates that are not digested by human enzymes in the small intestine can be well used as fat replacers in the manufacture of functional foods (Sikorski et al., 2008). The grains contain different types of soluble fiber and insoluble fiber, such as beta-glucan, where they are in the form of hydrophilic colloids and have high viscosity and are fermented in the intestine by the intestinal flora, so it is considered one of the good sources for the production of functional food (Marcotuli et al., 2019). Due to recent trends in the use of fat replacers in the dairy industry and the good sensory properties they have interesting due to their functional characteristics as well as their nutritional properties, as some of them contain high levels of bioactive compounds in addition to their effective role in reducing energy and obtaining healthy products for them. A role in boosting the immune system and lowering the level of cholesterol (Vidigal et al., 2014). The current study was conducted and aimed at conducting a nutritional experiment using mice to study the intake of yogurt with added fat replacer represented by beta-glucan extracted from barley in nutritional and immunological indicators that include rat weights, total cholesterol levels, TG, HDL, VLDL and LDL.

Materials and Methods

In this study, 40 laboratory animals were used from male BALB \ C mice of the white albino type, obtained from the College of Veterinary Medicine / University of Fallujah, and the mice were 3-4 months old with an average weight of 30-31 grams, and the effect of feeding on the functional yogurt was studied. The beta-glucan was being studied on the rate of weight gain, levels of triglycerides, total cholesterol and lipoproteins (HDL, LDL, VLDL). The animals were placed in controlled conditions in terms of ventilation and temperatures ranging between 25±2 Cº, and the lighting was 12 hours of darkness and 12 hours of light. The mice were divided into four groups according to the type of diet they would feed on. The mice were placed in their own cages made of plastic. They were provided with food and distilled water always available when the animal needed it (ad-labium). Food was left in their cages for a period of three days before the starting of the experiment in order to adaptation to the conditions of the experiment, and the

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animals and food consumed for each group were weighed along the duration of the experiment (twice a week).

Group 1: A group fed a standard diet and promised a C^- negative control treatment.

The second group: a group that continued to feed on a diet rich in fat and promised a positive C^+ control treatment.

The third group: a group fed on a diet rich in fat +0.2 ml yogurt made from skimmed milk only represented by Y1.

Fourth group: a group fed on a diet rich in fat +0.2 ml yogurt made from skimmed milk with an addition of beta-glucan extract with 0.4% and represented by Y2.

Table (1) components and proportions of the standard, fat-rich diet used to feed the mice (gm / 100 gm).

Ingredients	Standard diet gm \ 100	A diet rich in fat gm \ 100
Casein	**20**	**20**
corn oil	**7**	**7**
Cellulosic fibers	**5**	**5**
Vitamin mixture	**1**	**1**
Mineral mixture	**3.5**	**3.5**
Colin	**0.2**	**0.2**
Corn Starch	**46.8**	**46.8**
Cholesterol	**0**	**2**

Note: The diet are completed to 100 gm by using sucrose.

Prepare a rat feeding diet

The diet for feeding mice was prepared according to the nutritional and physiological requirements before (AIN, 1993).

Sample collection

At the end of the experiment, the mice were prevented from food for about 8 hours (Fasting), then they were anasthized with chloroform, and the abdominal cavity was opened from the lower abdomen to the pharynx. The sterilized blood was left to clot in the refrigerator for 30 minutes, then the serum was collected after central centrifugation at a speed of 2000 x g for 10 minutes. The serum was divided into small volumes in Eppendorf tubes and kept at a temperature of - 20 C^o until use.

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Biochemical analyzes

Measuring the total cholesterol level in the blood serum

The method of enzymatic hydrolysis of cholesterol was followed according to the Young (1995) method developed by the German company (Human) and according to the instructions of the supplying company and according to the following relationship:

Total cholesterol concentration = sample absorbance reading / standard solution absorbance reading x n

Since n (standard solution concentration) = 200 mg / 100 ml.

Measurement of the level of triglycerides in the blood serum

The method of enzymatic hydrolysis of cholesterol was followed according to the Young (1995) method developed by the German company (Human) and according to the instructions of the supplying company according to the following relationship:

Triglyceride concentration = sample absorbance reading / standard solution absorbance reading x n

Since n (standard solution concentration) = 200 mg / 100 ml.

Measurement of high-density lipoprotein (HDL) level in serum

The method of enzymatic hydrolysis (High Density Lipoprotein Cholesterol) was followed by Burstein et al. (1970) developed by the German company (Human) and according to the instructions of the supplying company. The HDL concentration was calculated in 100 ml of serum according to the following relationship:

HDL concentration = sample absorbance reading / standard solution absorbance reading x n

Since n (standard solution concentration) = 200 mg / 100 ml.

Measurement of LDL and VLDL serum levels of low and very low density lipoproteins

The level of both LDL and VLDL was measured by Freid's mathematical equation as mentioned by Shurfani (2006) as follows:

LDL- Cholesterol = Total-Cholesterol - HDL Cholesterol –Triglyceride \ 5

Triglyceride \ 5 = VLDL- Cholesterol

Statistical analysis

The software Statistical Analysis Method- SAS (2012) was used.

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Results and discussion

A study of the effect of free fat yogurt added to it with a fat substitute (beta-glucan) on the average weight of mice

The results presented in Table 2 show the average daily weight gain for groups of experimental mice after 28 days, as it is evident from the results that the highest daily weight gain rate recorded by a group of positive C^+ control mice of 0.2980 g/day and the final weight gain after 28 days was 8.345 g, which is the highest from the results of the negative control group C^- mice which was 0.2189 g/day and 6.130 g for the final increase. The reason for the apparent difference in the final weights rates between treatment C^+ and C^- is due to the nature of the diet presented to each group. In the treatment of positive control C +, the diet was rich in fat Which led to a higher weight gain, this result is consistent with the findings of Al-Badrani (2016) which found that the highest weight gain was after 28 days in the group of mice fed a diet rich in fat of 6.110 gm compared to the group fed on a standard diet of 3.210 gm.

Table 2: Average weight gain of different groups of mice after 28 days.

Treatment	Body weight (gm)		Body weight gain after 28 days (gm)	Average daily increase in body weight (gm)
	Average starting weight (gm)	Average final weight (gm)		
C^-	30.479	36.609	6.130	0.2189
C^+	31.593	39.938	8.345	0.2980
Y1	31.253	38.715	7.462	0.2665
Y2	31.688	34.735	3.047	0.1088
L.S.D value	2.49 NS	3.93 *	2.09 *	0.114 *

NS (non-significant difference). (* P <0.05) significant difference.

The average daily increase for the Y1 group members fed on a diet rich in fat with the oral dose in yogurt made from skimmed milk without any addition which amounted to 0.2665 gm/day and the final weight gain was 7.462 gm. As for the Y2 group members fed on a diet rich in fat with the oral dose with added yogurt added fat replacer for beta-glucan with 0.4% for the treatment that outperformed in the sensory orthodontic experiment, the weight gain and the final were 0.1088 and 3.047 gm respectively. When comparing these results, we find that the higher weight gain was in the positive control treatment, followed by the Y1 treatment and then the C^- control treatment and the Y2 treatment. This means that the beta- glucan fat replacer contributed to reducing the weight gain rate. These results are consistent with what Al-Azzawi (2018) found which indicated a decrease in the rate of weight gain after 28 days of treating the group of mice fed on a diet rich in fat with mozzarella cheese with added beta-glucan by 0.2%, reaching 2.701

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gm, compared with the group of positive control rats of 8.029 gm. When comparing the rate of weight gain of the treatment group to which the beta-glucan fat replacer was added to the rest of the treatments, we find that the least weight gain was in the Y2 group fed on a fat-rich diet with the yogurt added to it beta-glucan and the reason for this is the presence of fibers that give a feeling of satiety, which reduces the amount food intake delays the feeling of hunger and thus reduces the amount of energy supplied to the body, resulting in a decrease in weight (Al-Hasani, 2007). It is noticed from the results of the statistical analysis that there are no significant differences ($P \leq 0.05$) in the weights of the mice during the adaptation period. It is also noticed that there are significant differences in the rate of daily increase between the control treatment C^+ and C^- as well as between the treatments Y1 and Y2 on the one hand and the factors C^+ and Y2 on the other hand, it should be noted that there are no significant differences between the C^+ and Y1 transactions.

The effect of added free fat yogurt (beta-glucan addition) on total cholesterol, triglyceride, HDL, LDL and VLDL levels

Table 3 shows the values of total cholesterol, triglycerides, HDL, LDL, and VLDL in the previously mentioned C^+, C^-, Y1 and Y2 mice, where the total cholesterol value for the positive control subjects was 160.07 mg / 100 ml and this number is high compared to the total cholesterol value of the treatment C^- which amounting 139.58 mg / 100 ml and this is consistent with what Al-Badrani (2016) found, which indicated high levels of total cholesterol, LDL, VLDL and triglycerides, and decrease HDL in the group of mice fed on a high-fat diet. The liver is the main organ responsible for the process of cholesterol synthesis, demolition and balance in the body, and the cholesterol content in the liver is always identical to the levels of serum cholesterol, meaning that when serum cholesterol levels are high, the liver's cholesterol content is also high (Abou-zeid, 2016). As for the value of cholesterol for treatment Y1, it reached 152.23 mg / 100 ml, which is a high value compared with treatment Y2, which reached 130.84 mg / 100 ml due to the effect of beta-glucan inside feeding this group of mice, and this is consistent with what Al-Azzawi (2018) and Abou-Zeid (2016) found, whom indicated that beta-glucan works to reduce the level of cholesterol in the serum by encouraging the elimination of bile salts and thus increase the amount of cholesterol converted to salts.

Table 3: Total cholesterol, triglyceride, HDL, LDL, and VLDL ratios for different treatment mice groups after 28 days.

Treatment	Total cholesterol mg / 100 ml	Triglycerides TG mg / 100 ml	High-density lipoproteins (HDL) Mg / 100 ml	Low-density lipoproteins, (LDL) Mg / 100 ml	Very low-density lipoproteins (VLDL) Mg / 100 ml
C^-	139.58	129.21	68.37	44.68	26.42
C^+	160.07	146.46	55.88	82.54	32.36

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Y1	152.23	138.76	58.93	73.12	29.00
Y2	130.84	113.81	80.62	34.61	22.74
L.S.D value	33.02 NS	28.19 *	9.56 *	11.84 *	7.59 *

NS (non-significant difference). (* P <0.05) significant difference.

As for the triglyceride values for C^-, C^+, Y1 and Y2 treatments, they were 129.21, 146.46, 138.76 and 113.81 mg / 100 ml respectively. It is noticed that these values are higher for the positive control treatment because the mice of this treatment were fed a diet rich in fat, and it is also noticed that this value was decreased for treatment Y2 compared to treatment C^+ as well as for treatment C^-, as well as for treatment Y1 in which the triglyceride values increased compared to treatment T2, which added beta-glucan as a replacer for fat, this is consistent with what Al-Azzawi (2018) found, who indicated that the level of triglycerides decreased in a group of mice fed on a diet rich in fat, with an oral dose of a mixture of mozzarella cheese with beta-glucan added to it for 28 days, It reached 111.61 mg / 100 ml compared to the triglyceride level of a group of positive control mice of 145.85 mg / 100 ml, and also agrees with Al-Hasani (2007) who found that adding beta-glucan extracted from wheat to the diet of mice reduced total cholesterol and triglycerides. Kofuji et al., (2012) attributed the reason for the decrease in the level of triglycerides is to reduce the process of building fats in the liver from its new sources, by reducing the activity of enzymes that stimulate the building of fats, also lack of triglycerides is also associated with increased breakdown of lipoproteins rich in them, in addition to the important role that insulin and glucose play in the regulation of fatty acids and triglycerides. It is noticed from the results of the statistical analysis that there are significant differences between treatment C^+ and C^-, as well as the presence of significant differences between treatment Y2 on the one hand and between all treatments on the other hand, and this reinforces the important role that beta-glucan fat replacer plays in reducing triglycerides. As for the HDL lipoproteins values for control C^- and control C^+, Y1 and Y2 treatments, they were 68.37, 55.88, 58.93, and 80.62 mg / 100 ml respectively. It is noted from the results of the statistical analysis that there are significant differences in the HDL values between the C^+ treatment and the C^- treatment, and the treatments Y1 and Y2. It is noted that the values of these lipoproteins are lower for the C^+ treatment compared to the C^- treatment. The reason for the weight gain of the C^+ group is due to the decrease of this type of lipoproteins, and this is consistent with what was mentioned by Kok et al. (1996), it is noticed that the final weights of the positive control group members increased for the low values of HDL in them compared to the negative control treatment, and the result is consistent with what he found by Kalaivanisailaja et al. (2003), who indicated a decrease in the levels of benign HDL in the blood plasma of mice fed a diet with high fat content and attributed the reason to the decreased effectiveness of the enzyme lipoprotein lipase and lecithin cholesterol acyl transferase. Beta-glucan by 0.4% compared to the positive control treatment C^+ as well as the negative control C^- treatment, this is a positive effect of the beta-glucan fat replacer, which is an indicator of good health. As for the LDL values for the C^- and C^+ and the Y1 and Y2

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treatments, they were 44.68, 82.54, 73.12 and 34.61 mg / 100 ml respectively, as it is noticed that this value is higher for the positive control treatment compared to the negative control treatment. It is also noticed that these values are low for the treatment Y2 supported by its diet in yogurt contains the beta-glucan fat replacer, and this is a good indication for the action of this added replacer. As for the level of VLDL, the highest level was recorded in the group of C^+ mice fed on a diet rich in fat amounting to 32.36 mg / 100 ml. It was noted from the results that a significant decrease in the VLDL value of the group of Y2 treated mice fed on a diet rich in fat was given with the oral dose of yogurt added to it beta-glucan and reached 22.74 mg / 100 ml. As for C^- and Y1 treated mice, it reached 26.42 and 29.00 respectively. It is evident from the results that the lowest value was recorded by treatment Y2, as it surpassed even treatment C^-. The results of the statistical analysis indicate the presence of a significant difference ($P \leq 0.05$) in the VLDL values between treatment C^+ and treatment C^- and Y2, there were no significant differences between C^- and treatment Y1. From the overall results that are presented in Tables 2 and 3, it can be said that the fat-rich diet on which the C^+ positive control mice were fed led to high levels of total cholesterol, TG, LDL and VLDL, which are directly responsible for the occurrence of obesity and the weight gain of the mice of this group and other disorders. Lipid related (Kok et al., 1996; Al-Azzawi, 2018). Hence, it becomes clear that the beta-glucan fat replacer, which was introduced into the treatment diet Y2, has a significant effect in preventing excessive weight gain, reducing levels of TG and total cholesterol, bringing them within normal limits, increasing the proportion of good fats (HDL), and reducing levels of harmful fats LDL and VLDL. This is in agreement with what was mentioned by Bach Knudsen et al., (2017) who indicated that beta-glucan is of great importance due to its cholesterol-lowering properties and that one of the possible mechanisms includes the sticky properties of beta-glucan that reduces the reabsorption of bile acids from the small intestine and thus interferes with the intestinal and hepatic circulation of the bile acids that help remove the cholesterol accumulated in the body.

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