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Effect of Probiotic Hummus on Blood Lipids of Rats

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Abstract

The present study investigates the synbiotic effect of probiotic bacteria and hummus as a prebiotic on blood lipids in Sprague-Dawley rats. A developed probiotic hummus that contained *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bb-12 was added to a previously heated hummus at 75ºC/5 min., followed by an anaerobic incubation at 37ºC/8 h. The experimental diets included cholesterol diet, probiotic + cholesterol diet, hummus + cholesterol diet and probiotic hummus + cholesterol diet. Animals were divided randomly, according to their weights, into four groups (8 rats/group). Each group of the rats was fed one of the four diets for 8 weeks. Inclusion of probiotics to the cholesterol diet showed hypocholesterolemic effect, since it significantly (p<0.05) decreased TC and LDL-C by 14.5% (from 73.38 to 62.75) and 28.5% (from 19.70 to 14.08), respectively, as compared with the control group. No significant effects (p> 0.05) in HDL-C and TG were shown due to this inclusion. The hummus addition to the cholesterol diet caused a significant (p<0.05) reduction of 9.0% (from 73.38 to 66.75), 22.1% (from 19.70 to 15.35) and 14.0% (from 93.88 to 80.75) in TC, LDL-C and TG, respectively. The combined effects of probiotics and prebiotics in probiotic hummus + cholesterol diet caused significant (p<0.05) reductions of 14.1% (from 73.38 to 63.00), 27.5% (from 19.70 to 14.29) and 24.4% (from 93.88 to 71.00) in TC, LDL-C and TG, respectively. It could be concluded that the addition of probiotic hummus to the cholesterol-rich diet caused significant reductions in TC, LDL-C and TG. However, these reductions were not significantly different from those reductions caused by the addition of probiotic or hummus alone except for TG.

Key words: Probiotics, Hummus, Blood lipids, Rats, *Lactobacillus, Bifidobacteria.*

1. Introduction

Widespread interest in the possibility that selected foods might promote health has resulted in the coining of the term "functional foods." Prebiotics and probiotics may positively affect various physiologic functions of the body that will permit them to be classified as functional foods (Douglas and Sanders, 2008). Probiotic bacteria have been the focus of much scientific and commercial interest. This interest is due to a range of possible health effects of these bacteria (O'Bryan *et al*., 2013).

Cardiovascular diseases (CVD) are an important public health concern. In Western countries, they are considered as major causes of mortality and morbidity (Jones *et al*., 2013). In Jordan, the analysis of the mortality data of 2008 showed that the number one killer is CVD with a 36.1 % (Ministry of Health (MOH), 2011). For more than 20 years, the primary focus of public health strategies has been aiming at reducing risk of the coronary heart disease (CHD) and atherosclerosis, by reducing cholesterol concentration in circulating blood (Lye *et al*., 2010).

It was reported that there is a relationship between the consumption of probiotics and the reduction of serum cholesterol levels in human beings (Xiao *et al*., 2003), rats (Liong and Shah, 2006), and hens (Alkhalf *et al*., 2010). Certain strains of probiotic bacteria act directly on bile acids in the gastrointestinal tract (GIT) and are beneficial in reducing serum cholesterol levels (Lye *et al*., 2010).

Legumes, including chickpeas, are one of the most important crops in the world because of their good nutritional quality (Wang *et al*., 2010). Legumes have shown numerous health benefits, e.g., lowering of glycemic index for people with diabetes, increased satiation, cancer prevention and protection against cardiovascular diseases due to their dietary fiber content (Tosh and Yada, 2010). The resistant starch and the raffinose family of oligosaccharides (the α-galacto-oligosaccharides raffinose, stachyose and verbascose), which are found in appreciable concentrations in legumes, are potential prebiotics. The total contents of these a-galacto-oligosaccharides in dry beans, peas, lentils and chickpeas range from 2 to 10 g/100 g dry weight (Tosh and Yada, 2010). The resistant starch content in chickpea is 2.3 g/100 g dry weight (Queiroz-Monici *et al*., 2005).

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Many traditional foods in Jordan and most Arab countries are based on legumes as a raw material. Hummus, chickpeas paste, is one of the most popularly consumed traditional foods in our region (Fares and Takruri, 2002). With the globalization of the food market, the consumption of hummus has increased dramatically**.** The paste is made of ground re-hydrated cooked seeds of the chickpea legume, to which salt, spices and in many cases sesame seeds paste or 'Tahini' (*Sesamum indium*) are also added (Yamani and Al-Dababseh, 1994). Hummus is considered a relatively cheap protein source and has a nutritive and cultural value (Fares and Takruri, 2002).

Nowadays, there is an increasing trend toward using probiotics in different food systems and in the global market for such functional foods are on the increase (Sudha *et al*., 2009). Probiotics have been proved to be beneficial for health since their use reduced blood cholesterol both in experimental animals and humans; Xiao *et al.* (2003) reported a 22%, 41.2% and 13.2% significant reduction in serum TC, LDL-C and TG concentrations, respectively of rats fed bifidobacterium milk as compared with the control group. Fukushima and Nakano (1996) also found that the rat group receiving the mixture of probiotic bacteria showed a significantly greater decrease in serum TC concentrations (33.2% reduction) than the group receiving *L. acidophilus* bacteria only (21.3% reduction).

Although most of the current probiotic foods are mainly dairy based, there is a growing interest in the development of non-dairy probiotic products due to problems such as lactose intolerance in many people and the unfavorable cholesterol content of fermented dairy products. Additionally, there is an increasing demand for vegetarian probiotic products (Ranadheera *et al*., 2010). This has led to the development of probiotic products from various food metrics including fruits, vegetables, legumes and cereal products (Ranadheera *et al*., 2010). Probiotic fermented barley-based food (Sindhu and Khetarpoul, 2003), probiotic soy cheese, probiotic sausages and other probiotic food products have been developed (Ranadheera *et al*., 2010).

Since there are no studies on developing a "probiotic hummus," the proposed study aims to investigate the synbiotic effect of both prebiotics in hummus and the added probiotics in reducing blood lipids in rats.

2. Materials and methods

This research was approved by the Department of Nutrition and Food Technology Committee for Animal Experimentation and the Faculty of Graduate Studies at the University of Jordan.

Lactobacillus acidophilus NCFM (Danisco, Copenhagen, Denmark) and *Bifidobacterium lactis* Bb-12 (Chr. Hansen, Hørsholm, Denmark) were used after subculturing (for activation) from their freeze dried form. These bacteria are probiotic starter culture used commercially in the production of probiotic dairy products.They were selected after determining their suitability for use as probiotics by performing tests for acid tolerance (Pereria and Gibson, 2002), bile tolerance (Haddadin *et al*., 1997), adhesion to the intestine (Brink *et al*., 2006), antibacterial activity against *Escherichia coli*, *Salmonella typhmarium* and *Staphylococcus aureus* (Mishra and Prasad, 2005), cholesterol assimilation (Gilliand and Walker, 1990) and after the viability in the feed was tested (Al-Awwad *et al*., 2009).

2.1. Propagation and Maintenance of Probiotic Bacteria

The two selected strains were maintained by subculturing in MRS broth, containing 0.05% L-cyseine-HCL (L-cys), using 1% inoculum and 18-20 h of incubation at 37 °C in an anaerobic jar (Oxoid, UK). The cultures were kept in the refrigerator at 4°C between preparation of subcultures. Each isolate was subcultured two to three times prior to every test (Al-Awwad *et al*., 2004).

2.2. Development of Hummus Broth as A Culture Medium for Probiotic Bacteria

Hummus Broth (HB) was developed as a culture medium for probiotic bacteria before their incorporation in hummus to develop probiotic hummus. This broth contained 0.05% L-cys, 0.5% glucose 1.2% yeast extract and 3 % skim milk (Al-Awwad *et al*., 2014).

2.3. Development of Probiotic Hummus

Hummus was prepared under hygienic conditions according to the procedure followed by Yamani and Dababseh (1994). Hummus was heated at 75 ºC/ 5 min, then cooled to 35-40 ºC and inoculated with 10% of hummus broth that contained *B. lactis* and *L. acidophilus*. Probiotic hummus was incubated anaerobically at 37 ºC/ 8 h in an anaerobic jar (Oxoid, UK) using anaerobic kit (Anaerbic Gen Gas-Pack 2.5 L, Oxoid, UK), then it was refrigerated.

2.4. Diet Preparation and Incorporation Of Probiotic Hummus

2.4.1. Chemical Analysis of Hummus and Probiotic Hummus

Proximate analyses of hummus and probiotic hummus used in the experiment were determined according to the Official Methods of the Association of Official Analytical Chemists (AOAC, 1995).

2.4.2. Counts of the Probiotic Isolates in the Feed

100 g sample of each experimental diet was weighed in separate beakers. 10% (w/w) of probiotic culture in HB, control hummus and probiotic hummus were added and mixed well with each sample. The beakers were kept at room temperature and the total viable count of the bacteria was determined at 24, 48, 72 and 96 h. Consequently, the proper time for changing the rat diets that contained probiotic bacteria was detected in order to keep the probiotic bacterial counts > 10^6 CFU/g in the diet (Tharmaraj and Shah, 2003).

2.5. Preparation of the Experimental Diets

A 1% cholesterol diet was prepared according to AIN – 93 recommendations (Reeves, 1997). Probiotic, hummus and probiotic hummus were added as10% (w/w) to 1% cholesterol diet and blended to obtain three homogenous mixtures. The 1% cholesterol diet was used

as a control. The four experimental diets were kept at 4 °C until used for feeding.

2.6. Animal Experimentation

Animal experimentation was conducted making use of the conditions described by Liong and Shah (2006) and Al-Awwad *et al.* (2004). Initially, 32 male Sprague-Dawley rats were used. Animals were distributed randomly according to their weights (with an average of 219.8 g/ group) into 4 groups (8 rats/ group). They were individually housed in plastic cages with wire mesh bottoms (B. Holden & Crew 2001, North Kent Plastic Cages Ltd) at a temperature of 25 ± 2 ºC with 12 hrs light-dark cycle in the animal room.

Both diet and water were provided *ad libitum.* Total food intake and animal weight were measured once a week throughout the experimental period. At the end of the experiment, after 8 weeks, rats were fasted for 12 hrs and were anaesthetised using chloroform. Blood samples were collected from the right ventricle of each rat heart and were centrifuged at 3200 rpm for 15 min (Hermle, Z 200 A Centrifuge, Germany) to obtain serum. Sera were stored in conical plastic tubes, duplicate for each rat, at – 18 °C for later analyses of serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and, low-density lipoprotein cholesterol (LDL-C).

2.7. Biochemical Tests

The analyses of serum lipids were done in the Medical Laboratories of the Khalidi Medical Center (Amman, Jordan). An automated clinical chemistry analyser, COBAS INTEGRA 400/700/800 system, was used for the analysis. Before performing the analysis tests, calibration of the analyser for TG, TC, LDL and HDL was done according to the manufacturer's instructions.

2.8. Statistical Analysis

The statistical analyses were performed using the Statistical Analysis System, (SAS, 2008) version 9. Analysis of variance (ANOVA) with t -test was used to determine any significant differences between the means (Steel and Torrie, 1980). Values in tables are expressed as mean ± standard error of the mean (SEM). Levels of significance were at (P<0.05).

3. Results

The *L. acidophilus* and *B. lactis* strains used in this study have the characteristics of the probiotics, since they had the acid resistance activity, bile resistance property, cholesterol assimilation ability, adhesion ability, antibacterial activity and they could survive in the feed. These characteristics are related to their intestinal origin.

3.1. Counts of the Probiotic Isolates in the Feed

It was found that the counts of *B. lactis* and *L. acidophilus* in rat's diets were acceptable and more than 10^6 CFU/g until 72 h of keeping the inculated diets under aerobic conditions at room temperature. Accordingly, the probiotic diet and probiotic hummus diet were changed every 72 h.

3.2. Composition of the Experimental Diets

The composition of the diets that were fed to the rats is shown in Table 1 and the proximate analysis of these diets is shown in Table 2.

Table 1. Composition of the four diets (g/kg) which were fed to the rats for eight weeks *

Compositio	Diet Group			
	Cholesterol Group	ProbioticCholesterol Group	Hummus-Cholesterol Group	Probiotic Hummus-Cholesterol Group
Minerals	35	35	35	35
Vitamins	10	10	10	10
Casein **	140	138.93	133.76	133.54
Corn Starch	620.7	521.77	543.84	545
Soybean Oil	30	30	19.2	18.8
Cholesterol 1% †	10	10	10	10
Sucrose	100	100	100	100
Fiber ö	50	50	43.9	43.36
L-Cystine	1.8	1.8	1.8	1.8
Choline €	2.5	2.5	2.5	2.5
TBHQ £	8 mg	8 mg	8 mg	8 mg
Hummus	0	0	100	0
Probiotic Hummus	0	0	0	100
HBSM ¥	0	100	0	0

* Reference: Reeves *et al*. (1997).

** Casein (> 85 % protein), International Ingredient corporation, USA.

† 1 % (w/w) cholesterol (Purity >99%, Bioworld, USA) were added to each cholesterol-enriched diets.

ö Solka-Floc 100 FCC. International Fiber Corporation, USA.

€ Choline bitartarate (41.1 % choline)

£ TBHQ: Tert – Butylhydroquinone (mg)

¥ Every 100 g powder skim milk contains 35.5 g protein. A 100 ml HBSM containing 3 g SM≈1.065 g protein.

Table 2. Proximate analysis of the diets used in the experiment (g/kg (%) wet matter basis) *

* Mean of triplicate with coefficient of variation CV<5

Diets	Moisture	Ash	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract**	Energy ö Kcal (MJ)
Cholesterol	54.0 (5.40)	62.0 (6.20)	119.2 (11.92)	44.8 (4.48)	50.0 (5.00)	669.9 (66.99)	3559.6 (149.05)
Probiotic-Cholesterol	130.9 (13.09)	40.3 (4.03)	123.9 (12.39)	45.7 (4.57)	47.5 (4.75)	611.7 (61.17)	3699.0 (154.84)
Hummus-Cholesterol	104.3 (10.43)	53.1 (5.31)	119.5 (11.95)	42.9 (4.29)	69.1 (6.91)	611.1 (61.11)	3808.5 (138.49)
Probiotic Hummus-Cholesterol	101.7 (10.17)	52.9 (5.29)	110.6 (11.06)	39.3 (3.93)	75.3 (7.53)	620.2 (62.02)	3276 (137.13)

** The values of nitrogen-free extract are calculated by difference

ö Calculated by multiplying grams of crude protein and nitrogen-free extract by 4 kcal and ether extract by 9 kcal; 1 Kcal=4.186 KJ and 1 MJ is equivalent to 1000 KJ.

3.3. Serum Triglycerides and Lipoprotein Cholesterol

Table 3 shows serum lipids and lipoprotein cholesterol values in mg dl^{-1} of rats fed the four experimental diets for 8 weeks. TC and LDL-C values were significantly decreased ($P< 0.05$) in the probiotic + cholesterol diet (62.75 ± 2.02 and 14.08 ± 0.71), hummus + cholesterol diet (66.75 ± 2.20 and15.35 ± 0.76) and probiotic hummus + cholesterol diet (63.00 ± 2.15 and14.29 ± 0.90) groups as compared to cholesterol diet group(73.38 ± 2.19 and 19.70 ± 1.00).

No significant differences were found in the HDL-C values ($P> 0.05$) among the different groups. TG value was greater in cholesterol diet group (93.88 ± 2.19) and significantly higher ($P< 0.05$) than hummus + cholesterol diet (80.75 ± 2.45) and probiotic hummus + cholesterol diet (71.00 ± 2.54) groups but not significantly higher than probiotic + cholesterol diet group.

Table 3. Levels of blood lipids (mg/dl) for rats fed the four experimental diets for eight weeks *, **

Rat groups	Blood Lipids ϕ			
	TC	HDL-C	LDL-C	TG
Cholesterol	73.38 ^{a} ± 2.19	40.78 ^{a} ± 1.13	19.70^{a} ± 1.00	93.88^{a} ± 2.19
Probiotic + cholesterol	62.75 ^{b} ± 2.02	42.76 ^{a} ± 1.17	14.08 ^{b} ± 0.71	88.13 $^{a\,b}$ ± 2.66
Hummus + cholesterol	66.75 ^{b} ± 2.20	40.78 ^{a} ± 1.41	15.35 ^{b} ± 0.76	80.75 ^{c} ± 2.45
Probiotic hummus + cholesterol	63.00 ^{b} ± 2.15	41.99 ^{a} ± 1.42	14.29 ^{b} ± 0.90	71.00 ^{d} ± 2.54

* Each value is represented as mean of eight readings ± SEM.

** Means with different superscripts within the same column are significantly different (p< 0.05).

ϕ TC: Total Cholesterol, HDL-C: High density lipoprotein- cholesterol, LDL-C: Low density lipoprotein-cholesterol, TG: Triglyceride

4. Discussion

This study aimed mainly at investigating whether there is a synergistic hypocholesterolemic effect of probioitics (*L. acidophilus* and *B. lactis*) when combined with the proposed prebiotic effect of hummus in rat experimental diets containing 1% cholesterol.

The results showed that the incorporation of probiotics (*L. acidophilus* and *B. lactis*) to the cholesterol diet decreased significantly ($p<0.05$) the TC and LDL-C by 14.5% and 28.5%, respectively. An insignificant ($p>0.05$) decrease in the TG of 6.1% was observed. There is an increase in HDL-C value of 4.9% in probiotics + cholesterol group but this increase is not significant as compared with the cholesterol group (Table 3). These effects of probiotic addition on serum TC, LDL-C, HDL-C and TG are in consistence with the results reported by many researchers using rats (Xaio *et al*., 2003; Al-Awwad *et al*., 2004; Huang *et al*., 2013).

The two strains used in the present study have shown a hypocholesterolaemic effect both *in vitro* and *in vivo*. These findings are in agreement with those of other researchers (Taranto *et al*., 2000; Alkhalaf *et al*., 2010; Huang *et al*., 2013) who supported the finding that cultures, actively assimilated cholesterol in the presence of bile salts from a laboratory medium, would function *in vivo* to exert a hypocholesterolaemic effect in the experimental animals.

The hypocholesterolaemic effect can be attributed to the inhibition of exogenous cholesterol absorption from the small intestine by binding of cholesterol and bile acids with the bacterial cells, assimilation of cholesterol, as well as deconjugation of bile in the small intestine due to bacterial bile salt hydrolase activity (Lye *et al*., 2010; O'Bryan *et al*., 2013).

The deconjugated bile acids are not reabsorbed in the large intestine and are excreted through the feces. Excretion of bile acids results in the decrease of the extrahepatic recycling of bile acids, and, thus, cholesterol serum level is decreased due to its uses in *de novo* bile synthesis (Sudha *et al*., 2009; Ooi and Liong, 2010).

Another proposed mechanism of the cholesterol-lowering effect of probiotics is the production of Short Chain Fatty Acids (SCFAs). It is reported that acetate, a SCFA, increases the total cholesterol and decreases the fatty acids, while propionate lowers the hypercholesterolaemic response caused by acetate, which is a precursor of cholesterol (St-Onge *et al*., 2000).

Additionally, the cholesterol lowering effect of probiotic bacteria is due to the inhibition of 3-hydroxy 3-

methyl glutamyl CoA reductase, which is a rate-limiting enzyme and responsible for the endogenous cholesterol biosynthesis in the body (Sudha *et al*., 2009). Hydroxy-methyl glutarate (a 3 HMG-COA reductase inhibitor) was suggested to be an active factor that is produced or enhanced by probiotic bacteria (Sindhu and Khetarpaul, 2003). Lye *et al*. (2010) proposed the conversion of cholesterol to coprostanol by cholesterol reductase produced by Lactobacilli probiotic bacteria.

Taranato *et al*. (2000) proposed that the hypotriglyceridaemic effect of *L. reuteri* might be due to a lowering of intestinal absorption of lipid or to an increase in lipid triacylglcerol synthesis from [^{14}C] acetate with a similar concentration dependency in rat hepatocytes. This inhibition was claimed by Lin *et al*. (1995) to be due to the lowering activity of acytel-CoA synthetase by propionate, or to lowering triglycerides by the production of lipase by probiotic organisms (Sudha *et al*., 2009).

From Table 3, it can be observed that the addition of hummus to the cholesterol diet caused a significant reduction ($p<0.05$) of 9.0%, 22.1% and14.0% in TC, LDL-C and TG values, respectively, as compared with the cholesterol group value. The main constituent of hummus is chickpea to which the obtained results of the hummus + cholesterol group can be attributed. Mathur *et al*. (1984) found that the incorporation of whole chickpea flour (75%) to the rat diet containing 1 % cholesterol caused a significant reduction in TC. Similarly, Sihag and Kawatra (2003) found that rats fed Bengal gram seed coats in 1% cholesterol diet resulted in 35% lower serum cholesterol, as compared to the control group. The researchers attribute this to the high hemicelluloses content of the Bengal gram seed coats.

In a study on human beings, Pittaway *et al*. (2007) found that there were reductions in TC of 0.25 mmol/L and in LDL-C of 0.20 mmol/L following the consumption of the chickpea diet for five weeks, as compared to wheat diet. In a meta-analysis of eleven clinical trials (Anderson and Major, 2002), it was concluded that the sum of the whole rather than individual components (soluble dietary fiber, oligosaccharides, isoflavones, phospholipids and PUFAs, phytosterols, saponins and vitamins and minerals) were responsible for the hypocholesterolaemic effect of legumes.

Prebiotics contribute to hypocholesterolaemia via two mechanisms: decreasing cholesterol and bile acids absorption accompanied by enhancing their excretion via feces (Lin *et al*., 1995), and the production of SCFAs upon selective fermentation by intestinal bacterial microflora. Prebiotics are fermented in the colon by large bowel bacteria, yielding SCFAs such as butyrate, acetate and propionate (Ooi and Liong, 2010).

The hypotriglyceridaemic effect of prebiotics is mostly due to the decrease in the *de novo* lipogenesis in the liver (Ooi and Liong, 2010) and to the increase in TG-rich lipoprotein catabolism (Delzenne and Kok, 2001). Furthermore, propionate was also reported to inhibit fatty acid synthesis, thereby lowering the rates of triacylglycerol secretion (Ooi and Liong, 2010).

In the present investigation, total cholesterol of the probiotic hummus + cholesterol group was significantly ($p<0.05$) lowered by 14.1% as compared with TC of the cholesterol group. However, the reduction of TC in this group was not significantly ($p>0.05$) different from that of the probiotic + cholesterol and hummus + cholesterol groups. The LDL-C of the probiotic hummus + cholesterol group was significantly ($p<0.05$) lowered by 27.5% when compared with the cholesterol group, but it was not significantly ($p>0.05$) different from LDL-C of the cholesterol + probiotic and hummus + cholesterol groups.

The HDL-C value of the probiotic hummus + cholesterol group shows a non-significant ($p>0.05$) difference as compared with the cholesterol, probiotics + cholesterol and hummus + cholesterol groups. The TG of the probiotic hummus + cholesterol group was significantly ($p<0.05$) lowered by 24.4%, 19.4% and 12.1% when compared with the cholesterol, probiotics + cholesterol and hummus + cholesterol groups, respectively.

Little information is available on the effective synbiotic dosage of probiotics and prebiotics needed to exert their hypocholesterolaemic effects) (Ooi and Liong, 2010). Kikuchi-Hayakawa *et al*. (1998) found that the addition of Bifidobacterium-fermented soya milk to 0.5% cholesterol-enriched diet decreased the levels of plasma TC, TG and VLDL + LDL-C in hamsters. Liong and Shah (2006) concluded that the synbiotic effect of *L. casei*, fructooligosaccharide, and maltodextrin that were incorporated into rat diet, that contained 1% cholesterol, significantly lowered serum TC and TG levels. However, The HDL-C and LDL-C levels were not affected by this synbiotic effect.

To the best of our knowledge, this is the first time in Jordan a probiotic product based on the traditional food "hummus" is produced; it is also the first study in Jordan, to the best of our knowledge, that studies the synbiotic effect of this product in reducing blood lipids. However, further studies are needed for optimizing probiotic hummus production, studying its shelf-life and using the "Tetra Pak" technique to maintain the anaerobic conditions for extending it. Also, there is a need for finding the suitable methods or techniques for administering probiotic bacteria in hummus, such as the microencapsulation technique.

5. Conclusion

According to the results of this study, it could be concluded that the addition of probiotic hummus to the cholesterol-rich diet caused significant reductions in TC, LDL-C and TG. However, these reductions were not significantly different from those reductions caused by the addition of probiotic or hummus alone except for TG.

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Conflict of Interest

The authors have equally contributed to the work and agree to submit it for consideration to the ***Jordan Journal of Biological Sciences,*** and they also declare that they do not have any conflict of interest.

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