

THE EFFECT OF FUNCTIONAL CHEESE NUTRITION FORTIFIED WITH A MIXTURE OF PROBIOTICS AND BIOSTIMULATORS ON SOME CELLULAR AND BIOCHEMICAL PARAMETERS OF EXPERIMENTAL MICE

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This study was designed to evaluate the health effects of functional cheese produced from powdered milk, Landus brand, by adding a mixture of Probiotics, biostimulators inulin and modified starch. The study included a procedure for estimating the logarithmic numbers of *Lactobacillus acidophilus* and *Bifidobacterium* bacteria in functional cheese, the logarithmic numbers for the control cheese treatment reached (8.04, 6.55 logarithm/gm) for the period (1 day and 28 days from storage), respectively, while the logarithmic numbers for cheese treated with the synergistic mixture for the same periods were (11.63, 8.77 logarithm / gm) for *Lactobacillus acidophilus* bacteria, while the logarithmic numbers of *Bifidobacterium* animals for the period (1 day and 28 days from storage) for the control treatment (8.55 and 6.10 logarithm / gm), respectively, while the treatment cheese mixture recorded logarithmic numbers reached (12.15 and 9.49 logarithm / gm) for the period (day and 28 days from Storage), respectively. To achieve the therapeutic goal of synergistic functional cheese consumption, experimental mice that used cheese as an emulsion were used in treatments of five groups of experimental mice, as the group that was fed to cheese added to it combined the mixture of Probiotics and biostimulators together (biological synergy) in most of the results of cellular and biochemical blood tests moral advantage.

KEYWORDS: Synergistic functional cheese, powdered milk, Probiotics.**INTRODUCTION**

Therapeutic cheeses by definition Grattepanche et al., (2008) are cheeses containing strains of biological boosters (Probiotics), which play a role in the manufacture and maturity of cheese, and give health benefits to the host, when there are the recommended numbers 10⁶ units of colony/gm formation as well as the development of sensory properties, by producing Organic acids, stimulation of enzymatic activity and production of bacterial proteins inhibiting the growth of pathogenic microbes.

Shah (2000) indicated that the presence of bacterial numbers, including no less than 10⁶ units of colony formation/gm for the lactic acid bacteria initiator used as a Probiotics is necessary, to achieve the therapeutic goal of its consumption.

Prebiotics are defined as non-digestible food ingredients, which have a beneficial effect in improving the health of the host by stimulating the growth and increase of one activity or a specific number of vital boosters, as by using these aids you can improve the growth of the intestinal bacteria and thus reduce the problems of the digestive system, and from the help of prebiotics used in dairy products (Wilder-Smith et al., 2013), Anulin and

bio-synergy interact in dairy products to achieve improved quality, low fat and effective stability properties (de Souza et al., 2011), interactions between the components reveal the role of *St.thermophilus* in producing quantities of formic acid and carbon dioxide (Mayo et al., 2010) that promote bacterial growth. *Lactobacillus* spp. Modified starch is used as stabilizer or emulsifier, which modifies and stabilizes textures, as well as its functional role as a substitute for milk fat (Abbas et al., 2010; Lobato-Calleros et al., 2014; Morell et al., 2015).

To understand the interrelationship between the microbial groups of the host in the context of nutritional and genetic environments, and various disease phenomena, rodents are widely used for economic and practical reasons (Amato et al., 2015; Nagpal et al., 2018), they represent Non-Human Primates (NHP) because of their genetic and physiological proximity to humans (Nguyen et al., 2015).

To achieve the goal of the study, experimental mice were used to groom functional cheese products and to conduct cell and biochemical tests for blood standards to determine the health benefit that consumption of this type of functional synergistic product gives.

METHODS AND MATERIALS

Reconstituted 12% skimmed milk was prepared (Landus brand, Dutch origin and within the validity period), the method described by Al-Dahan (1983) was used to manufacture cheese with some modification, as the milk was prepared, prebiotics, inulin, and modified starch were added to it at a rate of 2% and mixed well to ensure the homogeneity of the ingredients and their thorough mixing, then the reconstituted milk was treated at 63 °C for 30 minutes and the milk cooled to 31°C, after that, the therapeutic initiator was added by 2%, which was supplied by the French company Danisco, Microbial Meito Renner was added, that prepared by Japanese company MEITO SANGYO CO. LTD, at 1%, and was left until coagulation occurred.

Then the formed coagulate was cut into cubes and left for 5 minutes. Drain the whey with a cloth and add salt with 2% of the weight of the coagulate. The coagulate was transferred and filled in molds, then pressed and left for 3 hours, then kept at the temperature of the refrigerator, the microbiological evaluation of the days (1, 7, 14, 21, 28) was performed, from storage in the refrigerator. To find out the health benefit of consuming therapeutic cheese containing the mixture of *Lactobacillus acidophilus*, *Bifidobacteria nimals*, and the mixture of biostimulatores, Anulin and modified starch, ALBE experimental mice were used and prepared the standard diet according to (AIN 1993). The therapeutic cheese has been administered to mice, in the form of an emulsion and in sterile conditions, it was prepared according to what mentioned kim *et al.*, (2019).

The dose was at a daily rate of 1 ml/mouse, as the number of mice (60 mice) was divided into (5) groups by (12) mice per group, as follows:

The first group, G1: the positive control group, were fed on standard cholesterol-free feed and distilled water only
The second group, G2: the negative control group, were fed on the diet by adding cholesterol, distilled water, and skim milk.

The third group, G3: was fed on a mixture of biostimulatores, distilled water and standard diet by adding cholesterol.

The fourth group, G4: was fed on a mixture of probiotics, distilled water and standard diet by adding cholesterol.

The fifth group, G5: was fed on the mixture of probiotics, a mixture of probiotics, distilled water, and standard diet with adding cholesterol

The applied steps of the nutritional experiment took place as follows (before the experiment, the first month, the second month), as blood samples were collected for each group in a 1 ml medical syringe and the blood section was divided into two parts. The first section was

placed in the EDTA anticoagulant tubes to estimate the cellular blood characteristics, which included: WBC,RBC,PCV, and Hb, tests were performed using a device Symex -XT-2000i. The second section was placed in the Gel tube tubes and subjected to centrifugation for 15 minutes, at a speed of 50,000 rpm, then the separate serum was transferred to the Abendrov tubes for biochemical blood tests for the determination of each of HDL,LDL, and VLDL, using a device Cobas, integra 400 plus.

Statistical analysis of the studied parameters was done by using a complete randomized design (CRD) to analyze the studied factors and their effects on the different characteristics, and to compare the significant differences between the averages with a test of the least significant difference (L.S.D) ($P < 0.05$) using the ready program (spss 2013).

RESULTS AND DISCUSSION

Figure 1 shows the logarithm of the number of *Lactobacillus acidophilus* in control milk cheese made from whole milk without addition, and the functional Landus milk cheese with its three additions of biostimulatores 2% inulin, modified starch 2% and mixture during storage periods (1,7,14,21,28) respectively, which shows moral superiority of the mixture of biostimulatores and Landus cheese mixtures.

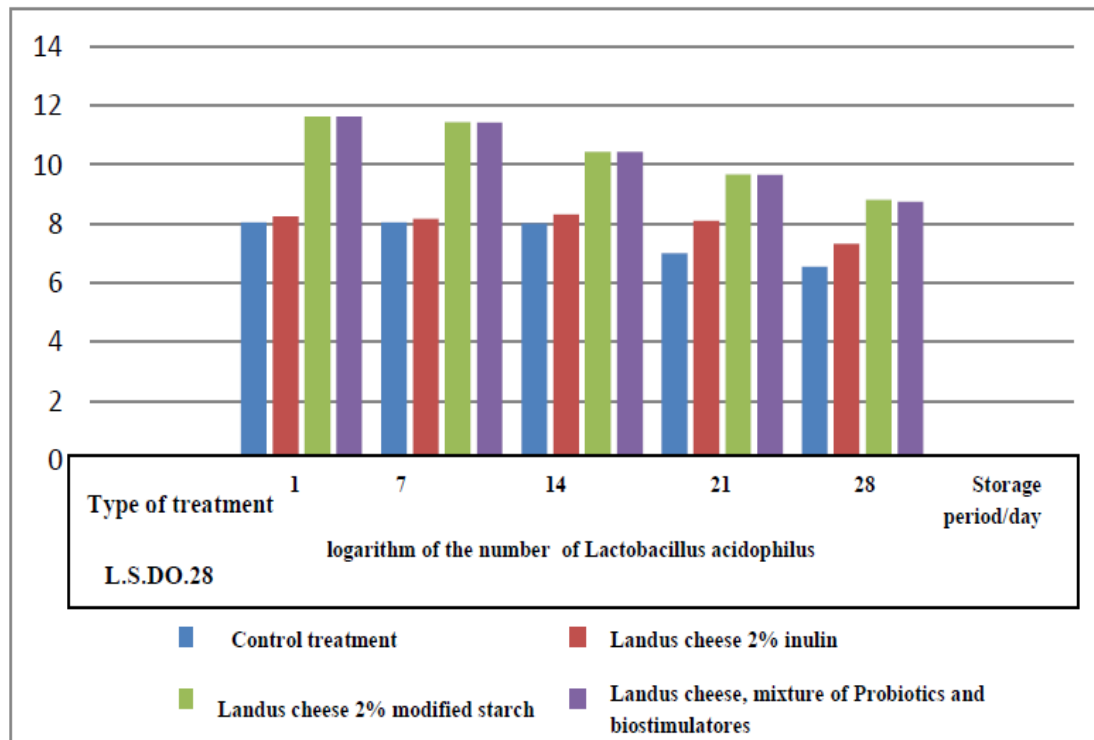


Figure 2: The logarithm of *Bifidobacterium animalis* for processed cheese treatments.

Figure (1) also shows the moral superiority of the mixture of Probiotics and biostimulatores in the logarithmic numbers of the therapeutic bacteria, as it reached at the beginning of storage (1) on 8.04 and 11.63 logarithm / gm for the treatment of control cheese and the cheese of the land of the mixture, respectively, compared to the moral superiority also for the mixture of the milk cheese mixture Landoz in the storage period (28) days compared to the control cheese treatment, as it reached respectively 8.77 and 6.55 logarithm / gm, respectively. As for Figure (2), it shows the logarithmic

numbers of *Bifidobacterium animalis* as it reached in the storage period (1) days for the treatment of control cheese and the mixture the mixture of Landus milk cheese 8.55 and 12.15 logarithm / gm respectively for the treatment of control cheese mixture and Landus milk cheese at a significant level ($p < 0.05$), it was revealed that the moral superiority achieved by mixing the mixture in both types of therapeutic bacteria, despite the low logarithmic numbers of them, but they remained within the range of donors to the health benefits of the host, indicated by scientific studies (Fogarasi *et al.*, 2019)

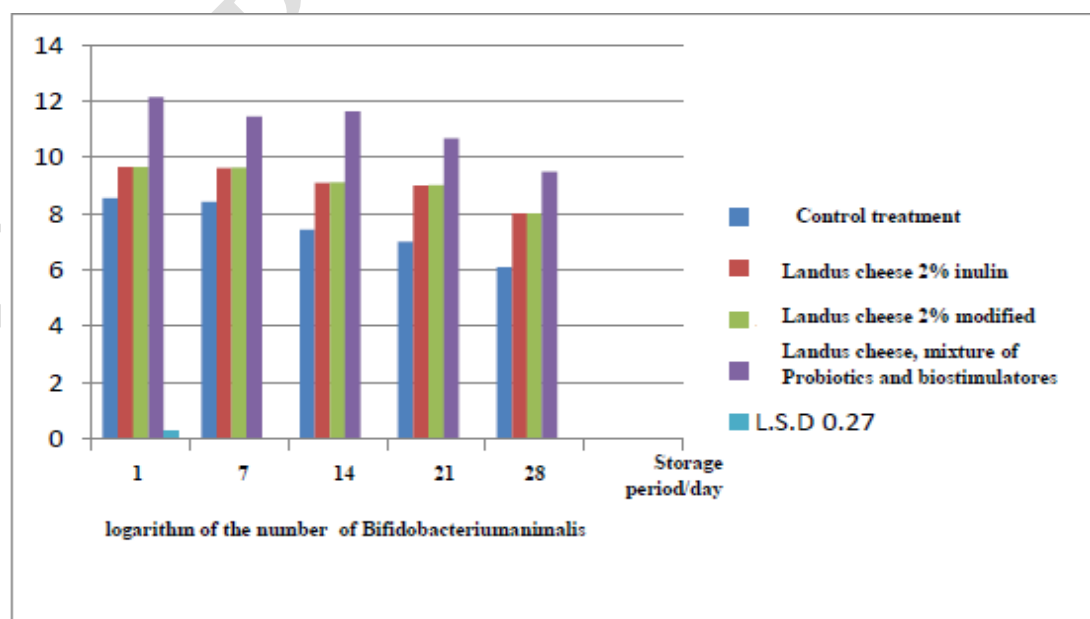


Figure 2: The logarithm of *Bifidobacterium animalis* for processed cheese treatments.

It is clear from that the beneficial effects that additives performed on processed cheese have, as additives have a role in improving and promoting the growth of therapeutic bacteria as well as the presence of therapeutic neighborhoods if many studies indicate that the bacteria maintain their vitality during storage, as a small number of them about, a logarithmic cycle (Tharmaraj and Shah 2003; Coeuret et al., 2004; Masuda et al., 2005), in some studies, it was indicated that the number of bacteria increases significantly during storage, approximately two or more algorithmic cycles (Bergamini et al., 2005; Buriti et al., 2005), in a few studies, I found that the number of bacteria can decrease dramatically during storage, by about three logarithmic cycles (Chaluvadi et al., 2012).

Among the factors affecting the viability of the neighborhoods to maintain their vitality during storage, including the added strain of lactobacilli and bifidobacteria in salted white cheese and Pategras Argentino cheese were among the factors affecting making cheese a good or excellent medium (Buriti et al., 2005; De Souza et al., 2008; Milesi et al., 2009), this is consistent with the results of the study, as adding the

mixture of Probiotics to maintaining its vitality throughout the storage period.

The effect of functional cheese nutrition on some cellular blood parameters

Effect of functional cheese nutrition on RBC values $RBC \times 10^6$

Figure 3 shows the effect of functional cheese nutrition on $RBC \times 10^6$ values, which show the superiority of group G5 cheese mixture over control treatment, as it reached 8.5 and 8.3 respectively at the end of the dose period (60) days.

Aboderin et al., (2006) indicated that hematopoiesis consists of two types of components, cells and serum, cells are based on two types of red blood cells and white blood cells. During the process of swallowing foreign bodies as well as the presence of these cells in addition to the epithelial cells of the gastrointestinal tract, this mechanism improves the host's immunity as a result of this interference. The bone marrow is the main organ in the production of single-cell red lymphocytes, lymphocytes and platelets, (Feldman et al., 2000).

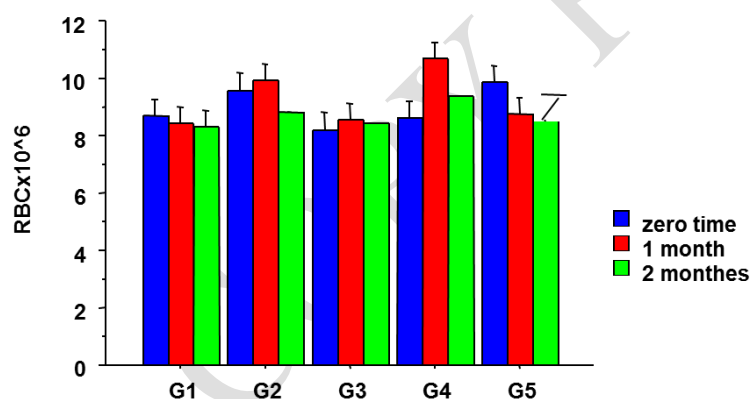


Figure 3: Effect of functional cheese on $RBC \times 10^6$ blood values.

Figure (4) shows the effect of functional cheese nutrition on blood values $WBC \times 10^3$, as it reached group G1 and

group G5 at the time of dose (60) on 9.5 and 5.4×10^3 , respectively.

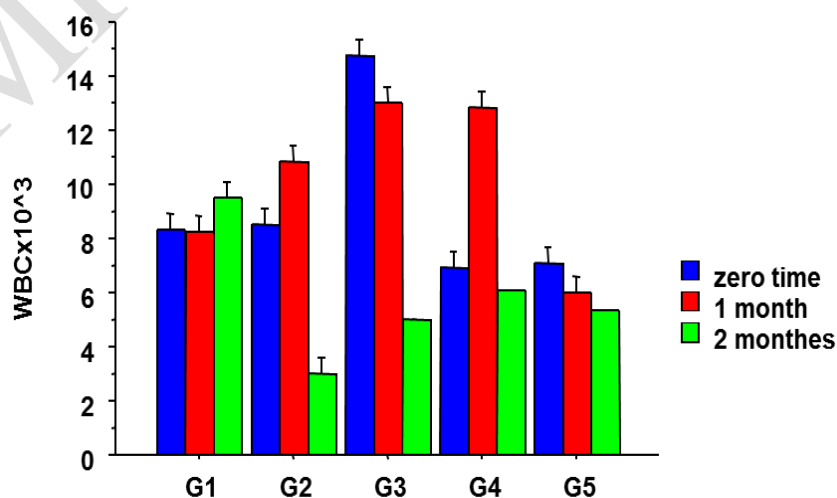


Figure 4: Effect of functional cheese nutrition on $WBC \times 10^3$ blood values for experimental mice.

On the other hand, Figure (5) shows the effect of functional cheese nutrition on the Hb (g/dl) values of experimental mice.

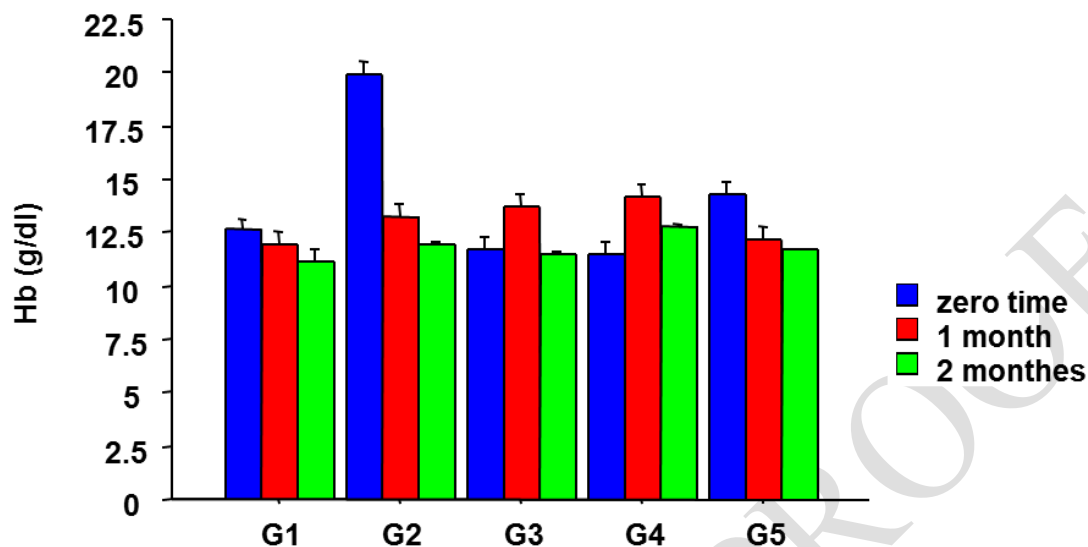


Figure (5): Effect of functional cheese feeding on Hb (g/dl) values of experimental mice.

Figure (5) shows the effect of therapeutic cheese nutrition on the values of Hb (g /dl) during the duration of the dose (60) days as it reached in the group G1, 11.1 and 11.7 (g /dl), respectively, while Figure (6) shows the effect of functional cheese nutrition on PCV% values for experimental mice, as it reached (60) days in the dose period for group G1 and G5, 38.8 and 44.3%, respectively, that shows the superiority of group G5 compared to group G1 at the level of Significant ($P < 0.05$). Several previous studies conducted on mice indicated the effect of the probiotics mixture on the moral height of the percentage of hemoglobin and the size of the packed cells, and it is consistent with the results of this study in the high values of hemoglobin and the volume of packed cells assigned to the group of

biostimulatores mixture and the probiotics mixture treatment group compared to the control group, these results are consistent with AL-Saiad (2012), as there was an increase in RBC, the size of packed cells, and the level of hemoglobin, as the values of RBC in the treatment of the mixture group increased from the control treatment in the current study, this is what studies have shown in the role of Probiotics in influencing the levels and values of white and red blood cells, as well as their role in the volume of packed cells and the percentage of hemoglobin (Candelaria *et al.*, 2005), the examination of hemoglobin and the size of packed pellets shows the state of health of the body (Beard *et al.*, 1996).

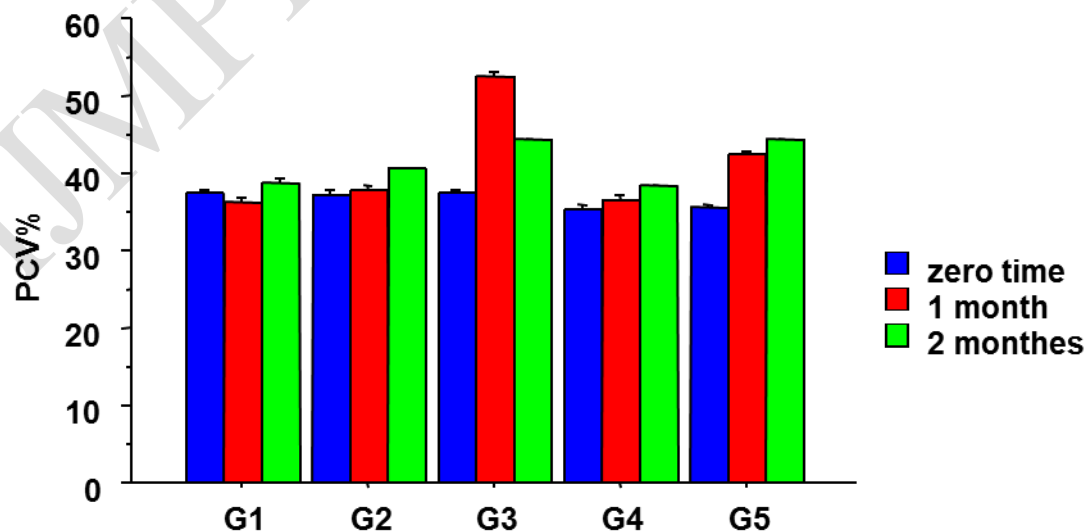


Figure 6: Effect of functional cheese nutrition on PCV% values of experimental mice.

Figure 6 shows a significant difference in the PCV% values of the G5 group over the G1 group, as it reached in the duration of the dose (60 days) on 44.4 and 38.8%, respectively, at a significant level ($P < 0.05$), it is what was observed in this study, as the treatment of the group that was fed on the mixture of Probiotics and biostimulatores was superior to that of the control group, which explains the readiness of the nutrients provided by the daily dose of emulsified soft cheese, which was positively reflected by the rest of the blood traits, the study also found that the synergistic action of Probiotics

and biostimulatores reflected its positive effect in two important directions RBC and hemoglobin, which is appropriate for increasing the bioavailability and availability of nutrients (Lafta and Abbas 2017).

Effect of functional cheese nutrition on some biochemical standards

Figure (7) shows the effect of functional cheese feeding on LDL values (LDL) in experimental mice, as it reached (60 days) in the dose period in group G1 and G5 7.0 and 6.0 (g/dl) respectively,

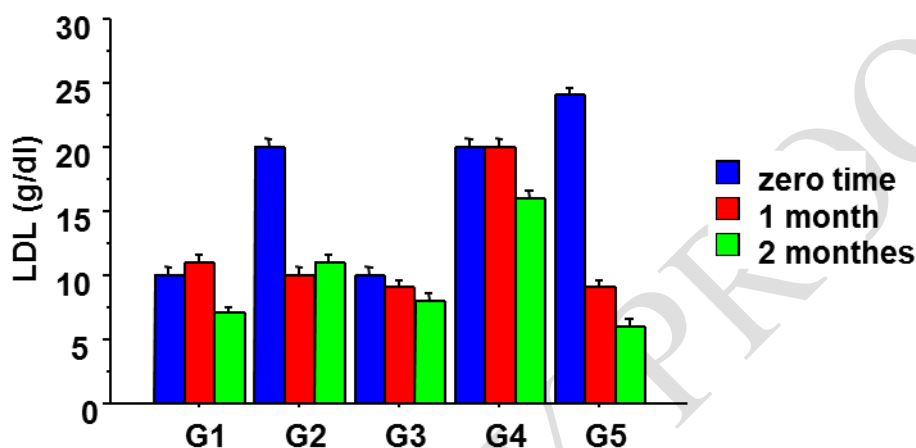


Figure 7: Effect of functional cheese nutrition on LDL (g /dl) values for experimental mice.

the effect of functional cheese nutrition on HDL (g/dl) values for experimental mice was also shown, as in the dose period (60 days) the group G1 and G5 respectively reached 32.0 and 70.0 (g/dl), as for Figure (8), it shows the effect of therapeutic cheese feeding on the VLDL values for the experimental mice, as they were in the G1 and G5 groups 68.0 and 74.0 (g/dl) respectively.

to the group G1 as the levels of packed cells and the level of hemoglobin, as the values of red blood cells in the treatment of the mixture group exceeded the control treatment in the current study, which was shown by studies in the role of Probiotics in influencing the levels and values of white and red blood cells, as well as their role in the size of packed cells and the percentage of hemoglobin (Candelaria *et al.*, 2005).

The previous forms that represented some biochemical criteria in which the group G5 was significantly superior

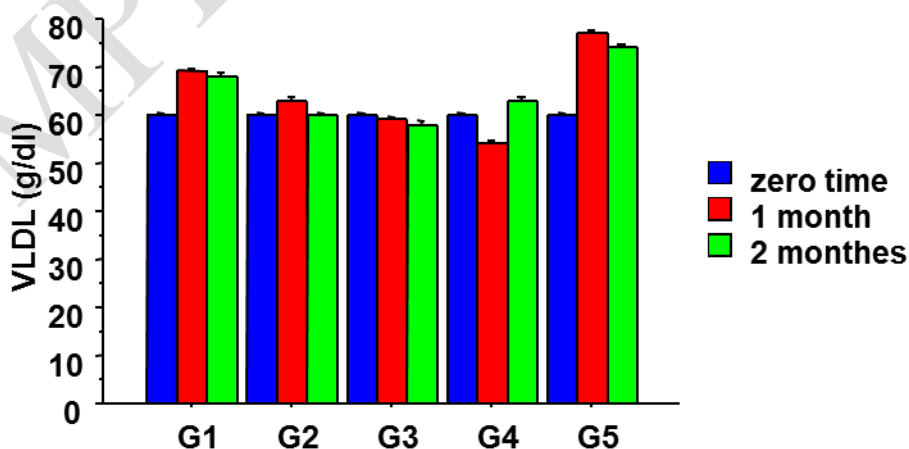


Figure 8: Effect of therapeutic cheese nutrition on VLDL (g/dl) values for mice

As it was observed in this study, the treatment of the G5 group that was fed on the mixture of probiotics and

biostimulatores was superior to that of the control group, which explains the readiness of the nutrients provided by

the daily dose of soft emulsified cheese, which was positively reflected by the rest of the blood traits. In two important directions are RBC and hemoglobin, which is appropriate for increasing the bioavailability and availability of nutrients (Lafta and Abbas 2017).

The results of this study showed that therapeutic cheese backed by a mixture of probiotics and biostimulators had a significant effect on reducing LDL and low-density lipoproteins, which is consistent with Taranto *et al.*, (1998) about the role of *Lb.reuteri* bacteria in reducing total cholesterol in the serum of mice fed on food supported with *Lb.reuteri* CRL 1098 strain at a rate of 104 cells/day for 7 days compared to the control group, the results of the study also agreed with (Stolarczyk *et al.*, 2002), noting that the feeding of experimental mice with rhamnosus, *Bifidobacterium inulin* and biostimulators resulted in a decrease in cholesterol and blood pressure.

It is believed that lactic acid bacteria possess an effective system in reducing the level of cholesterol BSH Bile Salt Hydrolase, in which it binds the bile salts through their interaction with the metabolism of the bile salts of the host, which results in compounds that are bound with the bile acids in a way that prevents their reabsorption into the hepatic intestinal circulation, which causes an increase in demanding cholesterol as a precursor to the creation of bile salts leading to a reduction in its level in the blood and these free salts do not function functionally as in the associated form and thus prevent it from being absorbed and it results in reducing the cholesterol concentration in the blood serum (De Smet *et al.*, 1998), as well as the effect that the beneficial synergistic action of the mixture biostimulators gave on cholesterol levels in rat serum (Ooi and Liang 2010). The decrease in HDL values that occurred in the group that was given the synergistic mixture, is consistent with the results of many studies. Hussein (2004) found that the use of cultures of a mixture of curative bacteria and the yoghurt starter led to control of total cholesterol and LDL in the serum of experimental animals, and also caused throughout the consumption period to raise levels of HDL. Abdullah (2018) found that Synbiotic yogurt, biostimulators inulin at a concentration of 2.5% caused a significant decrease in total cholesterol and LDL levels and increased HDL rates during the feeding period. Among the possible reasons for this role are played by the biological synergies. Practically, the cheese and milk consumed use selected strains of lactic acid bacteria, and the probiotic regeneration of fermented products from the *Lactobacillus* and *Bifidobacterium* strains is added (Mozaffarian *et al.*, 2011).

The enzymes of these two strains of bacteria have efficacy that leads to cholesterol precipitation by dissolving it from the bile salts and not absorbing it (Homayouni *et al.*, 2012), the reason may also be attributed to the decrease in lipids associated with low-density lipoproteins (LDL), this is reflected in the

decrease in total cholesterol. Turroni *et al.*, (2015) indicated that *Bifidobacterium* bacteria show metabolic capacity that can significantly increase beneficial bacteria and metabolic activity when they are present in the culture media. Among the possible factors in the observed decrease in this study is the effect of modified starch in host microbes as a result of short-chain acids affecting immune metabolism, which agreed with what was indicated by Upadhyaya *et al.*, (2016). In addition to the role of inulin in increasing the rate of short-chain fatty acids such as butyric and propionic and its effect in reducing liver cancer cells, which is reflected in the health effects of it and in *in vivo* experiments, inulin has had a role in reducing fat cells (Lnage *et al.*, 2015). The results of the study also agree with Zhou *et al.* (2015) on the effect of modified starch in reducing fat accumulation, inulin works as a vital catalyst for lowering LDL, VLDL, and triglycerides (Balcazar-Munoz *et al.*, 2003), these results were not consistent with any previous study regarding the effect of the inulin stimulus mixture and the modified starch in group treatment (G3) with control treatment (G1). The values of LDL (7.0, 8.0 g/dl) respectively in the last period of 60-day trial compared to TG values for the same period in which two groups (G1, G3) were treated respectively (69.0, 79.0 g / dl). The results agreed with Mao *et al* (2018) regarding the decrease in the treatment values of the group (G3) from the control treatment (G1) in the VLDL as it reached in the period of (60 days) (58.0, 68.0 g / dl), and on the role of modified starch and inulin with the mixture of Probiotics significant decrease in total cholesterol and triglycerides was observed in mice.

The explanation of the beneficial effects of the synergistic mixture in reducing the triglycerides observed in the study of the group (G5) compared to the control group (G1) is attributed, as found by (Sharma and Puri 2015), to inhibit the activity of most liver enzymes that stimulate fat building, this is reflected in the decrease and less activity of the process of building fat in the liver, and this is consistent with the current study, as well as the results of the study Al-Hamdani (2019), which found a significant decrease in triglycerides for the treatment of the group of rats fed to the biostimulators XOS and skim milk compared to the control group, Higgins (2014) and Keenan *et al.*, (2015) linked the lower body fat of experimental animals and their nutrition to resistant starch and the small size of the adipose cell.

The results of the study are consistent with (Pouyamanesh *et al.*, 2016), indicating a decrease in cholesterol and blood glucose in experimental mice fed on inulin for a period of 8 weeks compared with the treatment of feeding control on a high-fat diet, as it was found that the biological synergy is enhanced efficiently when both of probiotics and biostimulators work together, recent studies have found a significant decrease in total cholesterol after consuming resistant starch in food for 12 months (Dodevska *et al.*, 2016), as for the

effect of resistant starch on triglycerides and their concentration Bodinham *et al.*, (2014) and Gargari *et al.*, (2015), found that triglyceride concentrations decreased by 15%, as resistant starch had a role in reducing triglycerides.

Figuro *et al.*, (2011) and Al-Hamdani (2019), found that the synergy mixture of skim milk and the biostimulatores Xylooligosacchrieds had an effect in improving absorption by increasing the length of villi and depth of crypts that represented the nucleus of absorption in the intestine.

The production of bacterial metabolic products, including organic acids and bacteria, from beneficial bacteria that carry out biological activity in the host are characterized by inhibitory properties towards pathogens (Islam 2016).

CONCLUSION

It is concluded from this study that dosing of experimental mice with functional cheese made from powdered milk Landus type with a mixture of biostimulatores 2% inulin and 2% starch-modified characterized by containing the highest logarithmic values of bacteria numbers *Lactobacillus acidophilus* Bifidobacterium, during the 28-day storage period compared to the control treatment, as the dose resulted in beneficial health effects in both cellular and biochemical blood parameters, this may be due to the role of probiotics bacterial and that the range 10^6 is a range that achieves health and therapeutic benefits.

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