SINAI Journal of Applied Sciences 10 (2) 2021 107-120

Available online at www.sinjas.journals.ekb.eg

SCREENED BY iThenticate Professional Plagiarism Prevention

SINAI Journal of Applied Sciences

Print ISSN 2314-6079
Online ISSN 2682-3527

ARU-EGYPT SJAS Trust Science | It Creates

IMPACT OF FEEDING OF BIO-YOGHURT FORTIFIED WITH GLYCOMACROPEPTIDE AND CRUDE VIRGIN OLIVE OIL ON BIOLOGICAL ACTIVITIES OF RATS

Ola F. El-Sayed[*1], H.A. El-Shazly[2], H.A. El-Demerdash[1], M.M.K. Metwally[1]

1. Dept. Dairy and Food Sci., Fac. Environ. Agric. Sci., Arish Univ., Egypt.
2. Dept. Dairy Sci., Food Technol. Res. Inst., Ministry Agric. and Land Reclamation, Egypt.

ARTICLE INFO

Article history:
Received: 20/06/2021
Revised: 28/08/2021
Accepted: 28/08/2021
Available online: 28/08/2021

Keywords:
Yoghurt, *B. bifidum*,
GMP,
olive oil

ABSTRACT

The biological effects of bio-yoghurt fortified with glycomacropeptide (GMP) and crude virgin olive oil (CVOO) and *Bifidobacterium bifidum* Bb12 were investigated on rats. The rats were fed on basal diet and experimental diet in appropriate amounts of basal diet and yoghurt in the ratio 1:1 (W/W) and fresh water were supplied with meal for 21 days compared to control (-) which fed on basal diet only. Rats were arranged in 4 groups, 5 rats each. The results revealed no significant differences between rats' initial body weight whereas a significantly different ($p<0.05$) between rats' final body weight and body weight gain and there is a relative difference in liver, spleen, kidney and heart weights between treated groups and control (-) group (IV), moreover histopathological examination of rats liver tissues indicated the healthful effect of feeding on yoghurt fortified with GMP,CVOO and *B. bifidum* compared to control (-), also all groups fed on yoghurt fortified with GMP,CVOO and *B. bifidum* showed ahipolipidemic action demonstrated by a significant reduction in the concentration of plasma cholesterol, triglycerides and phospholipids and tended to lower plasma low density lipoprotien (LDL) cholesterol concentration and leading to lowering liver enzymes; glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT), moreover the rats fed on yoghurt fortified with food additives and *B. bifidum* had higher counts of lactic acid bacteria and *B. bifidum*, and lower counts of staphylococci and coliforms.

INTRODUCTION

Yoghurt is a coagulated dairy product produced by fermentation of milk with bacterial cultures consisting of a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* **(Gundogdu, *et al.* 2009)**. Addition of these cultures results in acidification of milk and synthesis of aromatic compounds **(Sahan *et al.*, 2008; Sera *et al.*, 2009).** These microflorae have been found to be valuable for human as they help in maintaining health and nutrition. Efforts have been focused on developing yoghurt containing probiotic cultures like *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (**Vinderola and Reinheimer, 2000)**.Probiotic cultures are live microbial food ingredients that are beneficial for human health and improve the intestinal microbial balance resulting in the inhibition of bacterial pathogens, reducing the risk of colon cancer, improving the immune system, lowering serum cholesterol levels **(Saarela *et al.*, 2002)** and alleviation of lactose intolerance and nutritional enhancement **(Alizadeh and Ehsani, 2008)**.

Glycomacropeptide (GMP), arising from cleavage of κ-casein by chymosin or pepsin **(Farrell *et al.*, 2004)**, exhibits several useful biological activities, including binding of cholera toxin and *E. coli* enterotoxins,

*** Corresponding author: E-mail address:** ommoaaz50@gmail.com
https://doi.org/10.21608/sinjas.2021.79960.1025

inhibition of bacterial and viral adhesions, suppression of gastric secretions, promotion of bifidobacterial growth, and modulation of immune responses **(Brody, 2000).** GMP contains nonaromatic amino acids and is therefore used for phenylketonuria (PKU) diets **(Ney *et al.*, 2009).** It is growing interest in exploiting GMP for use in the food industry, GMP provides good palatability and functional properties imparting favorable mouthfeel and flavour to foods, which many existing food preparations used for PKU diets lack **(Marshall, 2004)**.

Olive (*Olea europea* L.) is an evergreen tree that has been traditionally cultivated for olive oil and table consumption. Olive oil is classified as virgin olive oil if it has been extracted exclusively by mechanical or physical procedures such as milling, beating, centrifugation and decantation **(Gandul-Rojas *et al.*, 2000)**. The importance of virgin olive oil is related to its high levels of mono-unsaturated fatty acids (mainly oleic acid) and to the presence of minor components including aliphatic and tri-terpenic alcohols, sterols, hydrocarbons, volatile compounds and several antioxidants **(Ocakoglu *et al.*, 2009)**. Olive oil is rich diet protects human health from cardiovascular diseases, hypertention, inflammation, oxidative stress, obesity, type-2 diabetes and cancer **(Wani *et al.*, 2018)**.

MATERIALS AND METHODS

Materials

Fresh cow's milk was obtained from the herd of Badwy farm of Arish, Egypt. Average chemical composition of milk (3.5% fat, 3.35% protein, 12.6% TS) were determined according to the methods described in **AOAC (2016)**.

Skim milk powder (96% TS, product of Dairy America™) USA, was obtained from the local market of Arish, Egypt.

Direct Vat Starter (DVS) of yoghurt culture was obtained from CHR-Hansen's laboratorie, Denmark, under commercial name type (FD-DVS-YC-X11) containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* ssp. *Bulgaricus.*

Probiotic bacteria strain *Bifidobacterium bifidum* Bb12 was obtained from bafm, Germany.

Glycomacropeptide (GMP) was obtained from Ajinomoto Co., Inc.,Tokyo, Japan.

Crude virgin olive oil (CVOO) (*Olea europea* L.) was obtained from Badawy olive press of Arish, Egypt.

Methods

Preparation of probiotic culture

Strains of *Bifidobacterium bifidum* was twice successively activated by inoculating 100 ul of organism in 10 ml of sterilized MRS broth and incubated at 37°C for 16 hours. 10 ml of inoculated MRS broth was added to 100 ml of skim milk (9%) and incubated at 37°C over night, then stored at 5°C until used according to **De Man *et al.* (1960)**.

Yoghurt was manufactured from standardized cow's milk according to **Tamime and Robinson, (1999).** Three treatments of yoghurt were prepared as follows:

Treatment 0 (T0)

Yoghurt without any additives which serves as a control is shown in Diagram A.

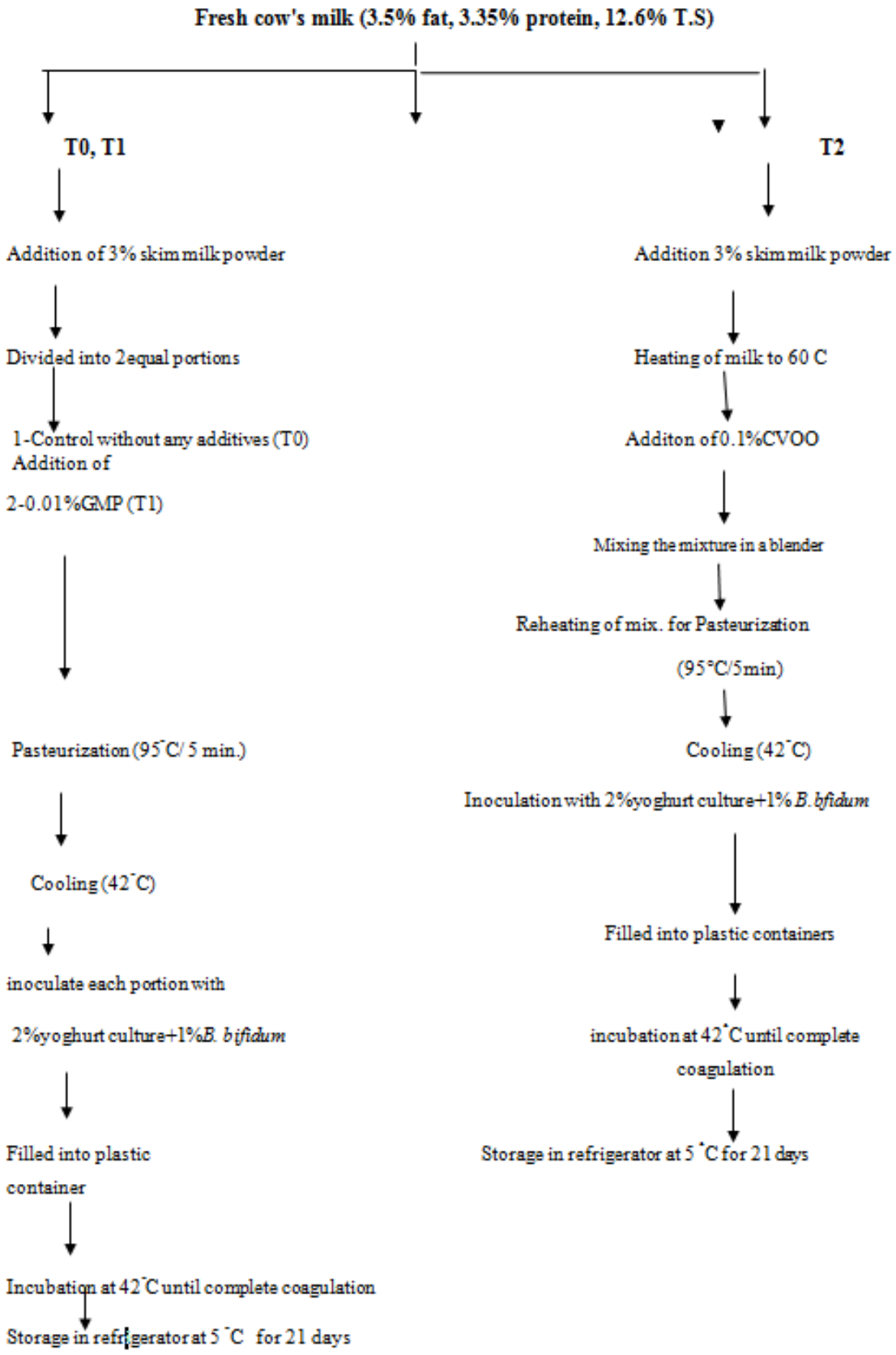
Treatment 1 (T1)

Yoghurt with 0.01% GMP was used in manufacture of yoghurt according to the procedure mentioned by **Tain *et al.* (2015)** is shown in Diagram A.

Treatment 2 (T2)

Yoghurt with 0.1% crude virgin olive oil was used in the manufacture of yoghurt according to the procedure mentioned by **Abbas *et al.* (2015)** is shown in Diagram A.

Diagram (A) – Manufacture of yoghurt



Animals and diets

Four weeks old Albino rats with average weighing between (100–130 g) were purchased from Modern Animal Care Center, National Research Center, Giza, Egypt. The rats were housed individually in an air conditioned room at 21- 24°C with 12 h- light/12 hr dark cycle.The rats were acclimatized on commercial basal diet (Cairo poultry Company,Giza, Egypt) for one week before starting the experiment . The chemical composition of basal diet was as following: 20% casein, 10% safflower oil, 5% vitamins and salt mixture, 5% cellulose, 15% α-corn starch, and 45% sucrose). The rats fed on basal diet and experimental diet and fresh water were supplied with meal for 21 days and the rats' body weight was measured in the beginning and the end of the experimental period.

Experimental Design

The rats were arranged in 4 groups, 5 rats each. Except for the control (-) diet, all diets were adjusted to contain appropriate amounts of basal diet and yoghurt in the ratio 1:1. The rats' groups were as the following:

Group I: (control +) rats fed on basal diet and yoghurt with mixed culture of (*St. thermophiles, Lb. delbrueckii* ssp. *bulgaricus* and *B. bifidum)*, without any additives.

Group II: rats fed on basal diet and yoghurt fortified with 0.01% GMP and mixed culture of (*St. thermophiles*, *Lb. delbrueckii* ssp. *bulgaricus* and *B. bifidum).*

Group III: rats fed on basal diet and yoghurt fortified with 0.1% CVOO and mixed culture of (*St. thermophiles*, *Lb. delbrueckii ssp. bulgaricus* and *B. bifidum).*

Group IV: (Control -) rats fed on basal diet only.

Tissue sampling and analysis

At the end of the experiment rats were anesthetized with diethyl ether. Blood of each rat was collected by capillary tubes and centrifuged at 3000 rpm for 15 min to separate plasma and red blood cells (RBCs). The erythrocyte lysates (20% V/V) were prepared by lying aliquots of washed RBCs with deionized water as described by **Huang and Fwu (1992)**. Rats' spleen, liver, kidney and heart were removed, washed by physiological saline, dried over filter papers and their weights were recorded. All samples were kept under freezing until analysis.

Histopathological Examination

At the end of the experiment, the lobe of Liver tissues was carefully dissected out, and then fixed instantaneously in 10% formal saline for 24 hr. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax (melting point 55-60^{o}C). Sections of 5 µm thicknesses were prepared and stained with Haematoxylin and Eosin. The cytoplasm-stained shade of pink and red and the nuclei gave blue color **(Sturgill and Lambert, 1997).** Examined by National Research Center, Giza, Egypt.

Determination of liver enzymes

GPT and GOT were determined enzymatically by kits from Medical Device Safety Services (MDSS GmbH), Burckhardtstr.1, 30163 Hannover, Germany.

Determination of plasma lipid

Total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglyceride ad phospholipids were determined ezymatically by kits from Medical Device Safety Services (MDSS GmbH), Burckhardtstr.1, 30163 Hannover, Germany.

Microbiological analysis of feces of rats

Fecal samples were collected from rectum in a sterilized Petri dish and 1.0 g of feces was transferred to 99 ml saline. Samples were analyzed immediately using aseptic sterile dilution technique as described by **Patel *et al*. (1992),** in National Research Center.

Determination of *Lb.bulgaricus* and *St. thermopiles* count

Fecal *Lb. bulgaricus* and *St. thermopiles* were enumerated on bromocresol green whey agar medium. Plates were incubated at 43°C for 48 hr according to **Yamani and Ibrahim, (1996)**.

Determination of *Bifidobacteria* count

Fecal *Bifidobacteria* was enumerated on MRS agar medium. Colonies were counted after anaerobic incubation using a double layer of medium. Plates were incubated at 37°C for 48 hr according to **Dave and Shah (1996).**

Determination of coliform bacterial counts

Appropriate dilutions of fecal samples were plated on MacConky's agar medium. After solidification, plates were incubated at 37°C for 48 hr, the bacterial count was expressed as cfu of coliform /g of fecal as described by **American Public Health Association, (1992)**.

Determination of *staphylococci* bacterial count

The counts of *staphylococci* were determined on Staph 110 media, incubated at 37°C for 48 hr as described by **American Public Health Association (1992)**.

Statistical Analysis

The statistical analysis was carried out using one-way analysis of variance (ANOVA) under significant level of 0.05 for the whole results using the statistical program Costas (Ver. 6.400) and data were expressed as mean ± stander error (SE) with complete randomization design according to **Steel *et al.* (1997)**. To ascertain the significant among means of different samples, least significant difference (LSD) test was applied.

RESULTS AND DISCUSSION

Rats' Growth Properties

Tables 1 and 2 illustrate the effect of feeding on yoghurt fortified with GMP, CVOO and *B. bifidum* of rats' growth parameters after 21 days of feeding.

It is clear from these tables that, there were no significant differences among rats' initial body weight whereas a significant different ($p<0.05$) between rats' final body weight and body weight gain (final body weight – initial body weight) was noted and increased in all rats fed on bio-yoghurt fortified with GMP, CVOO and *B. bifidum* compared to control (+) group (I) and control (-) group (IV) also control (+) group (I) had body weight gain more than control (-) group (IV).

There is a positive correlation between the animal body weight and its organs especially, liver, spleen, kidney and heart **(Michael *et al.*, 2007)**. In this respect, results in Table 2 illustrate relative difference in liver, spleen, kidney and heart weights between treated groups and control (-) group (IV).

These results are in agreement with those obtained by **Hargove and Alford (1978)**, they reported that rats fed yoghurt gained weight faster than those fed unfermented milk.

Histopathology Results

It is clear from the following figures 1:8 Histopathological examination of rats' liver tissues indicated a beneficial physiological effect and possible protective role of feeding on bio-yoghurt fortified with GMP, CVOO and *B. bifidum* on liver tissues damage compared to the control (-) group. Liver is one of the most important vital organs and responsible for many metabolic functions **(Sturgill and Lambert, 1997)**. These findings agreed with **(Hualin wanga *et al.*, 2014)** who findings that olive oil had great protective effect on liver tissue.

Concentrations of plasma lipids of rats

Table 3 illustrate the effect of fed on bio-yoghurt fortified with GMP, CVOO and *B. Bifidum* on concentrations of plasma lipids

Table 1. Body weight gain of rats fed on bio-yoghurt fortified with GMP and CVOO

Body weight (g)	Groups				
	I	II	III	IV	LSD 0.05%
Initial	107.35[a]±6.6	110.0 [a] ±7.0	114.0 [a] ±7.0	112.0 [a] ±3.0	22.49
Final	135.0[a]±8.0	149.5 [a] ±10.5	160.2 [a] ±15.9	130.0[b]±5.0	41.26
Gain	28.0[b]±1.0	39.5 [a]±3.5	46.0 [a]±9.0	18.0[c]±2.0	19.84

Group I: (Control +) Rats fed on bio-yoghurt without any additives + basal diet.
Group II: Rats fed on bio-yoghurt fortified with 0.01% GMP + basal diet.
Group III: Rats fed on bio-yoghurt fortified with 0.1% CVOO + basal diet.
Group IV: (Control -) Rats fed on basal diet. Values are means ± SE for 5 rats per group.
Means, in the same row with different letters are significantly different ($p < 0.05$).

Table 2. Growth parameters of rats fed on bio-yoghurt fortified with GMP and CVOO

Growth parameter (g/100g body weight)	Groups				
	I	II	III	IV	LSD 0.05%
Liver (%)	3.36[a]±0.39	3.53 [a] ±0.44	3.70 [a] ±0.46	3.30 [a]±0.36	1.49
Spleen (%)	0.29[a]±0.04	0.34 [a] ±0.04	0.38 [a] ±0.03	0.28 [b]±0.03	0.11
Kideny (%)	0.70[a]±0.03	0.75 [a] ±0.04	0.80 [a]±0.04	0.68[b]±0.03	0.12
Heart (%)	0.35 [a]±0.03	0.38 [a]±0.03	0.41 [a] ±0.03	0.34 [a]±0.04	0.11

* see foot note Table 1

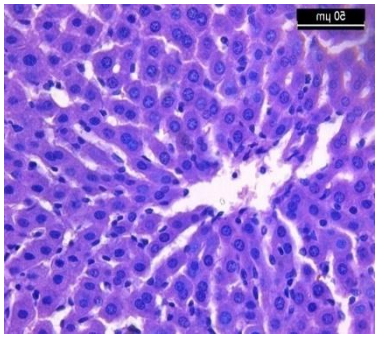


Fig. 1. Hepatic lobule (group I)

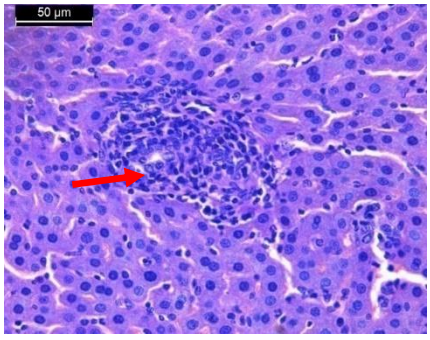


Fig. 2. Portal area (group I)

Light micrograph of a liver section from rats' group I (control +) supplemented with bio-yoghurt without any additives and basal diet showing normal structure of the hepatic lobule and moderate inflammation around the portal area.

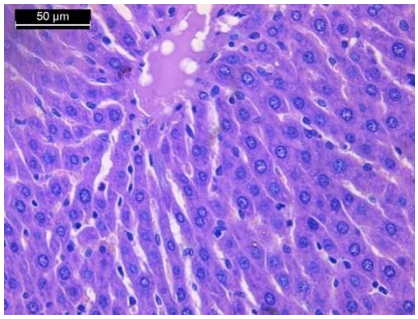


Fig. 3. Hepatic lobule (group II)

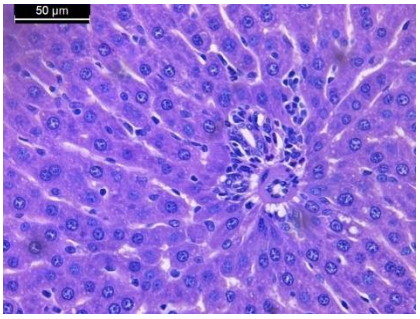


Fig. 4. Portal area (group II) Light micrograph of a liver section from rats (group II) fed on bio-yoghurt fortified with 0.01% GMP and basal diet showing the hepatic lobule appeared nearly normal structure and normal structure of the portal area appeared nearly normal structure.

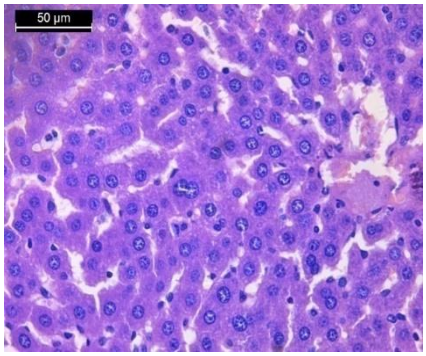


Fig. 5. Hepatic lobule (group III)

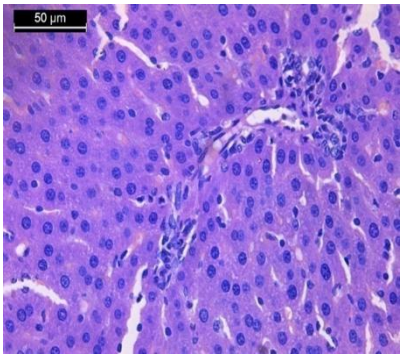


Fig. 6. Portal area (group III)

Light micrograph of a liver section from rats (group III) supplemented with bio-yoghurt fortified with 0.1% crude virgin olive oil and basal diet showing normal structure of the hepatic lobule and portal area.

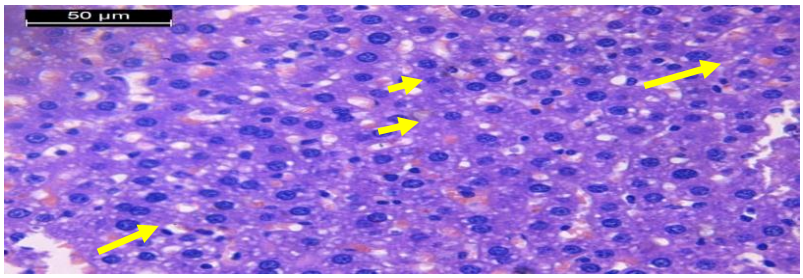


Fig. (7) Hepatic lobule (group IV) Light micrograph of a liver section from rat from (control -) rat group (IV) that fed basal diet only showing disturbance hepatic lobule. Notice a macrovesicular (arrows) and microvesicular (arrowheads) pattern of fatty infiltration.

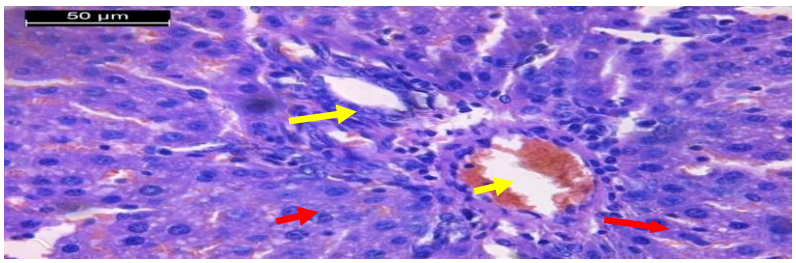


Fig. (8) Portal area (group IV) Light micrographs of liver of a section of liver from (control -) rat group (IV) that fed basal diet only congested portal area that associated with lymphocytic infiltration (arrow) in the portal and periportal areas (arrow). Notice the dilated sinusoids (arrowhead). Some nuclei showed hyperchromasia (red arrowhead) and other showed pyknosis (red arrow).

Table 3. Concentration of serum lipids of rats fed on bio-yoghurt fortified with GMP and CVOO

Parameter (mg/dl)	Groups				
	I	II	III	IV	LSD 0.05%
Cholesterol	90.2^{a} ±9.8	85.15 ^{ab}±05.15	78.0 ^{b} ±9.4	98.25 ^{a} ±4.75	1.49
Triglycerides	70.0^{a} ±12.0	62.5 ^{ab} ±14.5	45.0 ^{b} ±6.0	74.0 ^{a} ±13.0	0.11
Phospholipids	146.0^{ab} ±6.0	141.0 ^{ab} ±6.0	118.8 ^{b} ±19.2	153.5 ^{a} ±3.5	0.12

* see foot note Table 1

of rats. It is clear from this table that all groups fed on bio-yoghurt fortified with GMP, CVOO and *B. bifidum* showed ahipolipidemic action demonstrated by a significant reduction in the concentration of plasma cholesterol, triglycerides and phospholipids more than control (+) group (I) and control (-) group (IV) and all results was in the normal range. The highest value of plasma lipids was recorded in each of control (-) group (IV) while the lowest values recorded in group (III) fed on bio-yoghurt fortified with CVOO and this might be attributed to its high content of polyphenols which act as antioxidants and had role in reducing cholesterol **(Estruch *et al.*, 2013).** These results are in agreement with those obtained by **Kheadr *et al*. (2000)** they demonstrated the hypocholesterolemic effect of yoghurt culture in human and laboratory animals.

Concentrations of Serum HDL and LDL Lipoprotein Cholesterol of Rats

Table 4 shows plasma lipoprotein (HDL, LDL) concentration and atherogenic indices of rats fed on bio-yoghurt fortified with GMP, CVOO and *B. bifidum* and it was clear that, all groups fed on bio-yoghurt fortified with GMP, CVOO and *B. bifidum* tended to lower plasma LDL cholesterol concentration which had harmfull effects in the health more than control (-) group (IV). Results showed no significant differences in HDL cholesterol among all rats' groups and all results were in the normal range. The highest value of HDL-cholesterol was recorded in group (II) fed on GMP while, the lowest was recorded in control (+) group (I). Whereas there were a significant different ($p < 0.05$) among groups in LDL cholesterol and the highest value was recorded in each of control (-) group (IV) followed by control (+) group (I) while the lowest value was recorded in group (III) fed on CVOO. This reduction may be attributed to the reduction of total cholesterol **(Kheadr *et al.* (2000)**. Also, there were significant differences ($p < 0.05$) among groups in the ratio of HDL cholesterol to total cholesterol and the highest value was recorded in group (III) fed on CVOO while, the lowest value was recorded in control (-) group (IV). LDL cholesterol to total cholesterol (the atherogenic index) is an indication for susceptibility for atherosclerosis **(Kawase *et al.*, 2000)** was comparable among all groups and the highest value was recorded in control (-) group (IV) followed by control (+) group (I) while the lowest value was recorded in group (III) fed on CVOO and this might be attributed to its high content of phenolic compounds display a broad spectrum of health promoting characteristics, including lipid-improving, anti-oxidant, anti-inflammatory, anti-atherogenic, anti-thrombotic, anti-mutagenic, anti-microbial effects **(Gorzynik *et al*., 2018)**.

Activity of Liver Enzymes (GOT, GPT) in Rats' Serum

Table 5 show activity of GOT and GPT in rats serum fed on bio-yoghurt fortified with GMP, CVOO and *B. bifidum* resulted in lower activity of these enzymes compared to control (-) group (IV) that were in the normal range. GPT was found in significant quantities in liver, kidney and skeletal muscle, in decreasing order when liver cells are damaged, GOT and GPT levels rise especially early in the disease (**Othman and El-Missiry, 1998).** These results may suggest a beneficial physiological effect and possible protective role of feeding rats on bio-yoghurt fortified with GMP, CVOO and *B. bifidum*. There is significant different ($p < 0.05$) among groups and the highest value was recorded in control (-) group (IV) followed by control (+) group (I) whereas the lowest value was recorded in group (III) fed on yoghurt fortified with CVOO. These results are in agreement with those obtained by **Caramia *et al.* (2012**).

Table 4. Concentration of serum HDL and LDL lipoprotein cholesterol and atherogenic indices of rats fed on bio-yoghurt fortified with GMP and CVOO

Parameter (mg/dl)	Groups				
	I	II	III	IV	LSD 0.05%
HDL Cholesterol	65.1^{a}±1.3	71.8^{a} ±10.4	70.2^{a} ±9.6	69.2^{a} ±9.8	31.88
LDL Cholesterol	24.6^{ab}±7.2	11.0^{ab}±4.2	7.5^{b}±4.1	29.2^{a}±5.0	18.75
HDL/Total cholesterol	0.73^{b}±0.06	0.84^{ab} ±0.07	0.90^{a}±0.02	0.69^{c}±0.07	0.18
Atherogenic index	0.26^{ab}±0.05	0.12^{b} ±0.04	0.09^{b} ±0.04	0.30^{a} ±0.07	0.17

* see foot note Table 1

Table 5. Activity of liver enzymes (GOT and GPT) in serum of rats fed on bio-yogurt fortified with GMP and CVOO

Parameters (U/dl)	Groups				
	I	II	III	IV	LSD 0.05%
GOT	76.2^{ab}±11.6	67.6^{b} ±11.2	53.3^{b} ±7.4	103.8^{a}±9.6	35.46
GPT	16.1^{ab}±1.4	14.6^{b} ±0.8	12.7^{b} ±0.9	24.6^{a}±2.2	4.38

* see foot note Table 1 * GOT: glutamic-oxaloacetic transaminase. *GPT: glutamic-pyruvic transaminase.

Effect of Fed on Bio-Yoghurt Fortified with GMP and CVOO on Fecal Bacterial Population of Rats

Table 6 illustrate the effect of fed on bio-yoghurt fortified with GMP, CVOO and *B. bifidum* on fecal bacterial population of rats. The results indicated that, there were considerable variations among rat groups in their fecal content of LAB, *B. bifidum*, staphylococci and coliforms. The rats fed on bio-yoghurt fortified with GMP, CVOO and *B. bifidum* had higher counts of LAB and *B. bifidum*, On the other hand the number of staphylococci and coliforms bacterial counts significantly reduced in the feces of these groups compared to control (-) group. The highest count for each of LAB and *B. bifidum* was recorded in group (II) fed on bio-yoghurt fortified with GMP followed by group (III) fed on bio-yoghurt fortified with CVOO, while the lowest count was recorded in control (-) group (IV). The highest count for each of staphylococci and coliforms bacteria was recorded in control (-) group (IV) whereas, the lowest count was recorded in each of group (II) and group (III). These results are in agreement with those obtained by **Misra and Kulla (1994)** who found that dietetic yoghurt supplemented with *B. bifidum* creates favorable conditions for proliferation of beneficial intestinal microorganisms and discourage the growth of harmful ones. Also, GMP enhances the establishment of a healthy intestinal microbiota and prevents pathogenic bacteria colonization in mice after 15 days of daily treatment with GMP and using fluorescence in situ hybridization, a significant increasment in each of the number of Lactobacillus and Bifidobacteria was observed, together with a significant decrease in Enterobacteriaceae and coliforms on fecal samples of mice (**Chen *et al*., 2012**).

Table 6. Effect of fed on bio-yoghurt fortified with GMP and CVOO on fecal bacterial population of rats

Parameters (log cfu/g)	Groups				
	I	II	III	IV	LSD 0.05%
Lactic acid bacteria	$8.4^{b}\pm0.3$	$10.3^{a}\pm0.3$	$10.2^{a}\pm0.3$	$5.4^{c}\pm0.2$	1.09
Bifidobacteria	$7.58^{b}\pm0.3$	$9.28^{a}\pm0.1$	$9.23^{a}\pm0.1$	$5.18^{c}\pm0.1$	0.93
Staphylococci	$2.78^{b}\pm0.2$	$2.17^{c}\pm0.1$	$2.28^{bc}\pm0.1$	$3.67^{a}\pm0.3$	0.57
Coliforms	$2.80^{b}\pm0.2$	$2.46^{b}\pm0.1$	$2.28^{b}\pm0.2$	$3.69^{a}\pm0.4$	0.77

* see foot note Table 1

Conclusion

Feeding bio-yoghurt fortified with glycomacropeptide, crude virgin olive oil and *B. bifidum* improve the growth parameters of rats and have a healthful effect on rats liver tissues and showed ahipolipidemic action demonstrated by a significant reduction in the concentration of plasma cholesterol, triglycerides and phospholipids and tended to lower plasma LDL cholesterol concentration which had harmful effects in the health and leading to lowering liver enzymes; GPT and GOT, moreover the rats fed on yoghurt fortified with GMP, CVOO and *B. bifidum* had higher counts of lactic acid bacteria and *B. bifidum*, and lower counts of staphylococci and coliforms more than control (-) group.

REFERENCES

Abbas, H.M.; Hassan, F.A.M.; Kassem, J. M.; Rasmy, N.M.; El-Kaluobi, M. H. and El-Okaby, M.F. (2015). Preparation of cheese yoghurt using extracted high virgin olive oil. Int. J. Dairy Sci., 10 (6): 288-296.

Alizadeh, A. and Ehsani, M.R. (2008). Probiotic survival in yoghurt made from ultrafiltered skim milk during refrigeration storage. Res. J. Biol. Sci., 3: 1163-1165.

American Public Health Association, (1992). Standard Method for the Examination of Dairy Products. 14th Ed. Washington, USA.

AOAC (2016). Association of official Analytical Chemists. Official Methods of Analysis. 17th Ed., Gaitherburg, M D, USA.

Brody, E.P. (2000). Biological activities of bovine glycomacropeptide. Br. J. Nutr., 84: 39-46.

Caramia, G.; Gori, A.; Valli, E. and Cerretani, L. (2012). Virgin olive oil in preventive medicine: From legend to epigenetics. Europ. J. Lipid Sci. and Technol., 114 (4): 375-388.

Chen, Q.; Cao, J.; Jia, Y.; Liu, X.; Yan, Y. and Pang, G. (2012). Modulation of mice fecal microbiota by administration of casein glycomacropeptide. Microbiol. Res., 3, e3.

Dave, R.I. and Shah, N.P. (1996). Evaluation of media for selective enumeration of *Streptococcus thermopiles, Lactobacillus delbrueckii* subsp. *Bulgaricus, Lactobacillus acidophilus* and bifidobacteria. J. Dairy Sci., 79: 1529.

De Man, J.C.; Rogosa, M. and Sharp, M. E. (1960). A medium for the cultivation of lactobacilli. J. Appl. Bacteriol., 23: 130-136.

Estruch, R.; Ros, E.; Salas-Salvado, J.; Covas, M.I.; Corella, D. and Aros, F. (2013). Primary prevention of cardiovascular disease with a Mediterranean diet. The New England J. Med., 368 (14):1279-1290.

Farrell, H.M.Jr.; Jimenez-Flores, R.; Bleck, G.T.; Brown, E.M.; Butler, J.E.; Creamer, L.K.; Hicks, C.L., Hollar, C.M.; Ng-Kwai-Hang, K.F. and Swaisgood, H.E. (2004). Nomenclature of the proteins of cows' milk—Sixth revision. J. Dairy Sci., 87, 1641–1674.

Gandul-Rojas, B.; Cepero, M.R.L. and Minguez-Mosquera, M.I. (2000). Use of chlorophyll and carotenoid pigment composition to determine authenticity of virgin olive oil. J. Ame. Oil Chem. Soc., 77: 853-858.

Gorzynik-Debicka, M.; Przychodzen, P.; Cappello, F.; Kuban-Jankowska, A.; Marino Gammazza, A. and Knap, N. (2018). Potential health benefits of olive oil and plant polyphenols. Int. J. Molec. Sci., 19 (3): 686.

Gundogdu, E.; Çakmakçi, S. and Dagdemir, E. (2009). The effect of garlic (*Allium sativum* L.) on some quality properties and shelf–life of set and stirred yoghurt. Turkish J. Vet. and Anim. Sci., (33): 27–35.

Hargove, R. E. and Alford, J. A. (1978). Growth rate and feed efficiency of rats fed yoghurt and other fermented milk. J. Dairy Sci., 61:71.

Hualin Wanga, B.; Wat-Hung, S.; George Lim, T. and Jennifer M.F. W. (2014). Differential protective effects of extra virgin olive oil and corn oil in liver injury: A proteomic study. Food and Chem. Toxicol., 74:131-138.

Huang, C.J. and Fwu, M.L. (1992). Protien insufficiency aggravates the enhanced lipid peroxidation and reduced activities of antioxidative enzymes in rats fed diets high in polyunsaturated fat. J. Nutr., 122: 1182-1189.

Kawase, M.; Hashimoto, H.; Hosada, M.; Morita, H. and Hosono, A. (2000). Effect of administration of fermented milk containing whey protein concentrates to rats and healthy men on serum lipids and blood pressure. J. Dairy Sci., 83: 255.

Kheadr, E.E.; Abd El-Rahman, A.M. and El-Soukary, F.A. (2000). Impact of yoghurt and probiotic strains on strains on serum cholesterol and lipoprotein profile in rats. Alex. J. Agric. Res., 45: 81-100.

Marshall, K. (2004). Therapeutic applications of whey protein. Altern. Med. Rev., 9: 136–156.

Michael, B.; Yano, B.; Sellers, R.S.; R. Perry; Morton, D.; Roome, N.; Johnson, J.K. and Schafer, K. (2007). Evaluation of organ weights for rodent and non-rodent toxicity studies: A review of regulatory guidelines and a survey of current practices. Toxico. Patho., 35:742.

Misra, A.K. and Kulla, R.K. (1994). Use of *Bifidobacteria bifidum* for the manufacture of Bio-yoghurt and fruit Bio-yoghurt. Indian J. Dairy Sci., 47: 199.

Ney, D.M.; Gleason, S.T. and Van Calcar S.C. (2009). Nutritional management of PKU with glycomacropeptide from cheese whey. J. Inherit. Metab. Dis., 32: 32–39.

Ocakoglu, D.; Tokatli, F.; Ozen, B. and Korel, F. (2009). Distribution of simple phenols, phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for two harvest years. Food Chem., 113: 401-410.

Othman, A.I. and El Missiry, M.A. (1998). Role of selenium against lead toxicity in male rat. J. Biochem. Mol. Tox., 12: 345.

Patel, J.R.; Dave, J.M.; Dave, R.I. and Sannabhadti, S.S. (1992). Effect of feeding milk fermented with mixed culture of human strains of Lactobacilli on a faecal Lactobacilli and Coliform counts in human test subjects. Indian J. Dairy Sci., 45: 379-382.

Saarela, M.; Lahteenmaki, L.; Crittenden, R.; Salminen, S. and Mattila-Sandholm, T. (2002). Gut bacteria and health foods-the European perspective. Int. J. Food Microbiol., 78: 99-117.

Sahan, N.; Yasar, K. and Hayaloglu, A. A. (2008). Physical, chemical and flavor quality of non-fat yoghurt as affected by a β-glucan hydrocolloidal composite during storage. Food Hydrocolloid, 22, 1291:1297.

Sera, M.; Trujillo, J.A.; Guamis, B. and Ferragut, V. (2009). Flavour profiles and survival of starter cultures of yoghurt produced from high-pressure homogenized milk. Int. Dairy J., 19: 100-106.

Steel, R.; Torrie, J. and Dickey, D. (1997). Principles and Procedures of Statistics: A Biometrical Approach, 3rd Ed., McGraw-Hill, New York, NY.

Sturgill, M.G. and Lambert, G.H. (1997). Xenobiotic-induced hepatotoxicity: mechanisms of liver injury and methods of monitoring hepatic function *Clinical Chemistry*, 43 (8): 1512–1526.

Tain, Q.; Wang, T.T.; Tang, X.; Hang, M.Z.; Leng, X.J. and Mao, X.Y. (2015). Developing a potential prebiotic of yoghurt: growth of *Bifidobacterium* and yoghurt cultures with addition of glycomacropeptide hydrolysate. Int. J. Food Sci. and Technol., 50: 120–127.

Tamime, A.Y. and Robinson, R.K. (1999). Yoghurt. Science and Technology. London, UK: Wood head Publishing.

Vinderola, C. G. and Reinheimer, J. A., 2000. Enumeration of *Lactobacilii casei* in the presence of *L. acidophilus, Bifidobacteria* and lactic starter bacteria in fermented dairy products. Int Dairy J., 10: 271- 275.

Wani, I.A. (2018). Olive oil and its principal bioactive compound: Hydroxytyrosol–A review of the recent literature. Trends in Food Sci. and Technol., 77: 77-90.

Yamani, M.I. and Ibrahim, S.A. (1996). The differential enumeration of *lactobacillus delbruekii* subspecies *bulgaricus* and *Streptococcus salivarius* subspecies *thermopiles* in yoghurt and labneh using an improved whey medium. J. Soc. Dairy Technol., 49: 103-108.

الملخص العربي

تأثير التغذية بزبادي حيوي مدعم بالجليكوماكروبيتيد وزيت الزيتون الخام البكر على الأنشطة البيولوجية للفئران

علا فتحى السيد[1]، هويدا عبدالله الشاذلى[2]، حسن عبدالمنعم الدمرداش[1]، ممدوح مصطفى كمال متولى[1]

1. قسم علوم وتكنولوجيا الاغذية والألبان، كلية العلوم الزراعية البيئية، جامعة العريش، مصر.
2. قسم بحوث الألبان، معهد بحوث تكنولوجيا الأغذية، مركز البحوث الزراعية، وزارة الزراعة واستصلاح الأراضي، مصر.

تم دراسة التأثيرات الغذائية والبيولوجية للزبادي المدعم ب (0,01% جليكوماكروبيتيد، 0,1% زيت الزيتون الخام البكر) والـ *B. bifidum* باستخدام فئران التجارب كنموذج بديل عن تغذية الانسان حيث تم تقسيم فئران من نوع Albino عمرهم حوالى 4 اسابيع الى 4 مجموعات ووزعت الفئران الى خمسة فئران لكل مجموعة بحيث كانت متساوية تقريبا في الوزن (بدون فروق معنوية احصائيا). وتم أقلمة الفئران لمدة اسبوع غذيت خلالها على عليقة تجارية وبعد انتهاء فترة الأقلمة تم تغذية 3 مجموعات من الفئران على الزبادي بالإضافة الى العليقة التجارية باستثناء المجموعة الرابعة وهى مجموعة المقارنة تم تغذيتها على العليقة التجارية فقط لمدة 21 يوم، ويمكن تلخيص النتائج المتحصل عليها كما يلى: أدت التغذية على الزبادى المدعم ب (0,01% جليكوماكروبيتيد، 0,1% زيت الزيتون الخام البكر)وال *B. bifidum* الى زيادة معدلات نمو الفئران مقارنة بمجموعة المقارنة بينما لم تكن هناك اختلافات فى الاوزان النسبية لكبد وقلب الفئران من المجموعات المختلفة ولكن توجد اختلافات نسبية فى اوزان الطحال والكلى للفئران بين المعاملات ومجموعة المقارنة. كما اظهرت دراسة فحص نسيج الكبد للفئران ان انسجة كبد الفئران المغذاة على الزبادي المدعم ب (0,01% جليكوماكروبيتيد، 0,1% زيت الزيتون الخام البكر) وال *B. bifidum* افضل اصلاحا فى انسجة الكبد حيث اظهرت الصور المجهريه الضوئية أن تركيب فص الكبد والمنطقة البابية للكبد في الفئران طبيعي يليهم مجموعة الفئران المغذاة على زبادى الكنترول حيث اظهرت الصور المجهرية الضوئية ان تركيب فص الكبد طبيعي مع ملاحظة ظهور التهاب معتدل حول المنطقة البابية للكبد بينما كانت مجموعة المقارنة المغذاة على العليقة التجارية فقط اقلهم فى الحالة الصحية لنسيج الكبد، ولوحظ ان كل معاملات الزبادي المدعم ب (0,01% جليكوماكروبيتيد، 0,1% زيت الزيتون الخام البكر) وال *B. bifidum* تأثيرا خافضا للكوليستيرول والجلسريدات الثلاثية والفسفوليبيدات في بلازما الدم، كما ادت الى خفض البروتينات الدهنية منخفضة الكثافة (LDL) الضارة صحيا في بلازما دم الفئران لنسبة اقل نسبيا من مجموعة المقارنة، وادت التغذية على جميع معاملات الزبادي المدعم ب (0,01% جليكوماكروبيتيد، 0,1% زيت الزيتون الخام البكر) وال *B. bifidum* الى خفض انزيمات الكبد (GPT, GOT) مقارنة بمجموعة المقارنة، كما كانت الفروق معنوية بين المجموعات ($p < 0.05$). كما انه مقارنة بمجموعة المقارنة احتوت عينات البراز للفئران المغذاة على الزبادي المدعم ب (0,01% جليكوماكروبيتيد، 0,1% زيت الزيتون الخام البكر) وال *B. bifidum* على زيادة ملحوظة في اعداد بكتريا حامض اللاكتيك وسلالة الـ *B. bifidum* ، بينما انخفضت اعداد بكتريا القولون والبكتريا العنقودية.

الكلمات الاسترشادية: زبادي، *B. bifidum*، جليكوماكروبيتيد، زيت الزيتون.