Effect of some probiotic Lactobacillus spp. on serum lipids of rats

K. A. A. Khater [1,*] and A. R. A. Mohammed [1]

[1] *Dairy Science Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt*

[*] Correspondence: khaterkhater.5@azhar.edu.eg (K. Khater)

Abstract

The main target of the present work is to screen three tested strains of Lactobacillus spp. for functional characteristics for probiotics. The potential probiotic characteristics of three Lactobacillus spp. strains were studied with regard to acid / bile tolerance and surviving in gastric / intestinal juices. Results obtained revealed that all tested lactobacilli strains showed much greater stability at different low pH values (1.5, 2 and 3), and normal growth at bile concentrations up to 1 % w/v, which recommended these cultures to use as probiotic bacteria. Moreover, data obtained declared that all tested Lactobacillus cultures were considered intrinsically tolerant to gastric and intestinal juices. The potential role of these probiotic Lactobacillus cultures on serum lipid of rats was studied. Twenty-five male albino rats were randomly and equally divided into five groups, five rats each. After an adaptation period of 7 days, the first group was fed on basal diet served as control I, the second group was offered basal diet + buffalo's milk plus one of the tested lactobacilli strains. At the end of the 28 days' experimental period, the rats were killed. However, blood samples were collected at the beginning and the end of experiment. From results obtained it could be concluded that supplementation of diets with fermented milk culture with either *L. casei* or *L. rhamnosus* resulted in noticeable decreases in total cholesterol, LDL-cholesterol and triglyceride contents. Moreover, heoretic indexes 1, 2 and l LDL/HDL ratio were markedly reduced in rats received fermented milk as compared with control treatment I (dry diet). In conclusion, fermented milk can decrease in the faecal counts of either coliform or streptococci.

Keywords: Lactobacillus spp; LDL; HDL.

INTRODUCTION

The genus *Lactobacillus* represent one of the major members of the lactic acid bacteria. Also, during the last three decades *Lactobacillus* is considered one of most commonly bacteria used as probiotic. However, probiotics are live microorganisms, which when administered in adequate amounts, confer a health benefit on the host (Sanders *et al.,* 2003). Recently, there has been increasing interest in the use of probiotics to prevent, to alleviate or to treat a variety of infectious and inflammatory conditions. These bacteria, may have several therapeutic functions, including antimicrobial activity, ability to assimilate cholesterol, improved lactose utilization and anti-carcinogenic activity (Chou and Weimer, 1999). Therefore, probiotics should meet criteria as identified by FAO/WHO such as safe for consumption and surviving exposure to low pH and important criteria is the ability to confer a health benefit on a host. Therefore, the main target of the present work is to screen three tested strains of *Lactobacillus* spp. for functional characteristics for probiotics. An additional aim is to evaluate in vivo the impact of feeding rats on fermented milk cultured with different Lactobacillus spp. strains on serum lipids of rats and fecal microflora.

MATERIALS AND METHODS

Materials

Tested strains

Three identified *Lactobacillus* cultures were used in the present study, namly *L. casei* AZ1, and *L. rhamnosis* AZ1 isolated from feces of breast-fed infant, while *L. gasseri* AZ1 isolated from raw milk.

Bile acid

Bile salt was obtained from Difco Laboratories, Detroit, Michigan, USA.

Pepsin and pancreatin

Pepsin from Procine stomach mucosa and Pancreatin from porcine pancreas were delivered from Sigma Chemical Co. Missouri 63103, USA.

Albino rats

Twenty-five mature male albino rates, obtained from the Osman farm animal experiments, with mean body weights of 130 ± 5 gm, were used in the present study.

Basel diet

The chemical composition of basal or control diet was as follows: raw protein, 23%; raw fat, 6.40%; fibers, 3.60% and starch, 67%.

Buffalo's milk

The chemical composition of buffalo's milk obtained from the herd of Faculty of Agriculture, Al-Azhar Univ. Cairo, was as follows: fat, 6.1 %; lactose, 4.3%; protein, 4.1%; solids not fat, 8.9% and salts, 0.5%.

Blood samples

At the beginning of this experiment and after adaptation period, blood samples were drawn from the retrobulber venous plexus of each rate through a capillary glass tube and left to clot at room temperature to obtain a clear serum. At the end of experiment, the rates were killed by withdrawing blood from the abdominal aorta under light diethyl ether anesthesia.

Faeces samples:

Rate faeces samples were collected every week in sterile Petri dishes transferred to laboratory in ice box and immediately subjected to microbiological analysis.

Media

For propagation and maintenance of ***Lactobacillus*** strains MRS broth / medium was used as recommended by De Man Rogosa and Sharp (1960), while for enumeration of coliform count, violet red bile agar (VRBA) medium was adopted as recommended by Klein and Fung (1978). Whereas, the count of staphylococci was determined on Staph 110 media according to APHA, (1992).

Methods

Acid tolerance

Three tested strains were evaluated for their ability to grow in low pH values (1.5, 2 and 3) as described by Pereria and Gibson (2002) with some modifications: MRS broth previously adjusted to pH 1.5, 2 and 3 with HCl, were inoculated with 1 % (v. /v.) of activated tested cultures, the tubes were maintained at 37°C for 3hours. One-milliliter of each samples were taken at various times (0, 60, 120, and 180 min) serially 10-fold diluted and plated into MRS agar, the plates were incubated at 37°C for 48 hours under anaerobic conditions by using double layer technique before enumeration.

Bile tolerance

Bile tolerance was estimated as described by Pereia and Gibson (2002). Overnight tested cultures (1 % (v. /v.) were added into MRS broth with concentrations (0.3, 0.5, and 1 % (w/v.) of bile salts inoculated anaerobically at 37°C for 12h. One-milliliter of each samples were taken at the end of the experiment (12 h), serially diluted, into MRS agar. The plates were incubated at 37°C for 48 hours under anaerobic conditions.

Tolerance of artificial gastric and intestinal juices

Gastric and pancreatic juices were prepared freshly by dissolving pepsin (Sigma) from porcine stomach mucosa (3g/L) and pancreatin (Sigma) from porcin pancreas (1g/L) in sterile saline (5g/L) (Chateris *et al*. 1998). The pHs of the gastric and pancreatic preparations were adjusted to 2.0 and 8.0 with 5 M/L HCl or 1M/L NaOH, respectively.

One milliliter of each activated tested cultures was centrifuged 5000 x*g* for 10 min at 4°C and washed three time in sterile PBS. Of each washed cell washed cell suspension 0.2 ml was mixed with 1 ml. of gastric or intestinal juice. After brief vortexing the mixtures were incubated at 37°C. When assaying gastric tolerance aliquots of 0.1 ml were removed after 60, 120 and 180 min for determination of total viable count, while for assaying intestinal tolerance, the sampling times were 60, 240 and 360 min.

Feeding Experiment

Rats were randomly and equally divided into five groups, five rats each. The animals were housed in cages at room temperature (25 ± 2°C) and relative humidity (about 55%) for 28 days. These rats were acclimatized on basal diet for one week before starting the experiment.

After an adaptation period for 7 days, the first group was fed on basal diet (80 g.rat.day^{-1}) and served as control I, while the second group was offered basal diet plus standardized buffalo's milk (40 mL.rat.day^{-1}) and served as control II. The other groups were fed on: 80 g basal diet plus 40 ml. buffalo's milk and one of the tested *Lactobacillus* strains for each rat/day. The animals were weighed at the beginning and the end of the experimental period.

Determination of total cholesterol or triglycerides contents

The total cholesterol or triglycerides concentration were determined in the blood serum by using Spectrum diagnostic kits. The total cholesterol concentration was calculated by using the following equation:

Cholesterol (mg/dL) = (A of the tested sample/A of the standard solution) ×200.

While, triglycerides concentration was calculated using the following formula:

Triglycerides (mg/dL) = (A of the tested sample/A of the standard solution) ×200

where: A= Absorbance at 546 nm.

Determination of High density lipoproteins (HDL) cholesterol content

HDL-cholesterol was determined in blood serum by using Genesis kit, and its concentration calculated by using equation:

Concentration of HDL-cholesterol (mg/dL) = A sample × 570

where: A= Absorbance at 546 nm.

Determination of Low density lipoproteins (LDL) cholesterol content

The LDL-cholesterol was estimated according to the formula of Beena and Prasad (1997) as follows:
LDL-cholesterol (mg/dL) =

Total cholesterol – (HDL + (triglycerides/5)).

Calculation of atherogenic indexes

The atherogenic indexes were calculated as LDL-cholesterol / total cholesterol (index 1) and (total cholesterol – HDL)/HDL (index 2).

Biological Evolution of rat diets

Biological evolution of the different diets was carried out according to Carthew *et al*. (2001) by using the following equations:

Body weight gain = final weight – initial weight

Food efficiency ratio = Daily growth rate/Daily food intake.

Growth rate, g/day = Body weight gain, g / Experimental period long, day**.**

Fecal bacterial population

Appropriate dilution of each fecal sample was plated on either violet red bile agar (VRBA) or staph.110media, plated were either incubated at 37°C for 24 or 48h coliform and staphylococcus, respectively (APHA, 1992).

Statistical analysis

Analysis of variance was computed using the General Linear Model procedure of statistical analysis system (SPSS, 2011). Variable means for treatments indicating significant differences in the ANOVA were compared.

RESULTS AND DISCUSSION

Screening of *Lactobacillus* strains for their probiotic characteristics

Tolerance to acid and bile salts

In the present study, all the tested Lactobacillus spp. were successfully survival in different low pH values (Table 1). They retained varying levels of viability ranged from 100.62 % to 109.04 %. However, pH 1.5 seemed to be more damaging to the tested strains. In this respect, Maffei and Nobrega (1975) stated that the bactericidal effect of acid is evident at pH values below 2.5. Among the tested Lactobacillus strains, *L. gasseri* AZ1 was the most acidic tolerant, while the least survival was observed for *L. rhamnosus* AZ1 strain.

Table 1. Effect of low pH values on viability of tested Lactobacillus spp. strain.

Strains	PH	Incubation time (min)							
		Zero	60	120		180		60	
		Log Cfu/mL	Log Cfu/mL	% increase	Log Cfu/mL	Log Cfu/mL	% Increase	Log Cfu/mL	Log Cfu/mL
L. casei AZ1 KY123805	1.5	12.76	12.89	1.07	12.96	12.89	1.07	12.96	12.89
	2	13.92	14.27	2.53	14.27	14.27	2.53	14.27	14.27
	3	13.33	14.30	7.28	14.13	14.30	7.28	14.13	14.30
L. gasseri AZ1 KY123806	1.5	12.97	13.22	1.92	13.06	13.22	1.92	13.06	13.22
	2	13.62	14.25	4.61	14.04	14.25	4.61	14.04	14.25
	3	13.15	14.34	9.04	13.86	14.34	9.04	13.86	14.34
L. rhamnosus AZ1 KY123789	1.5	12.82	12.90	0.62	12.93	12.90	0.62	12.93	12.90
	2	13.89	14.16	1.93	14.30	14.16	1.93	14.30	14.16
	3	13.21	14.37	8.78	14.16	14.37	8.78	14.16	14.37

cfu: colony forming unit.

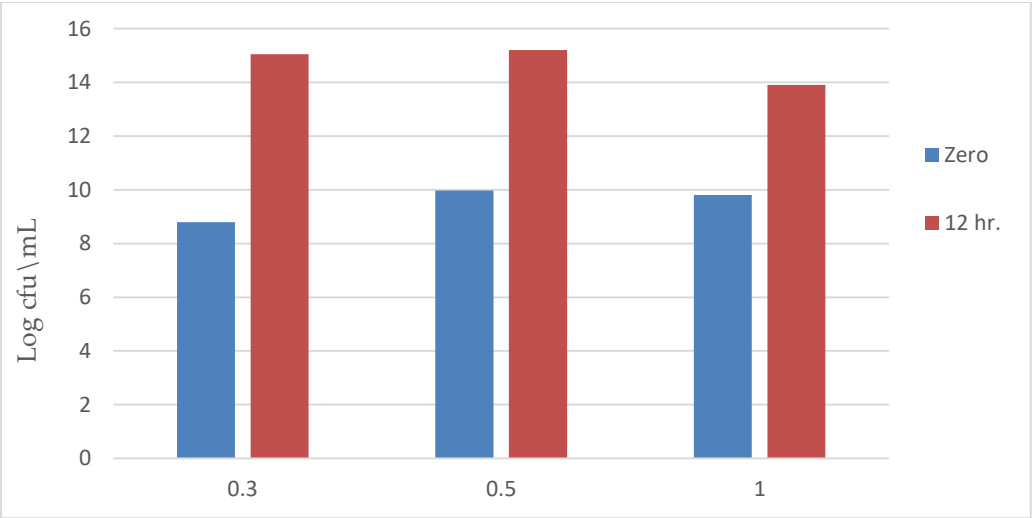

Fig. 1. Bile tolerance of *L. casei* AZ1 KY123805 on different bile salt concentrations.

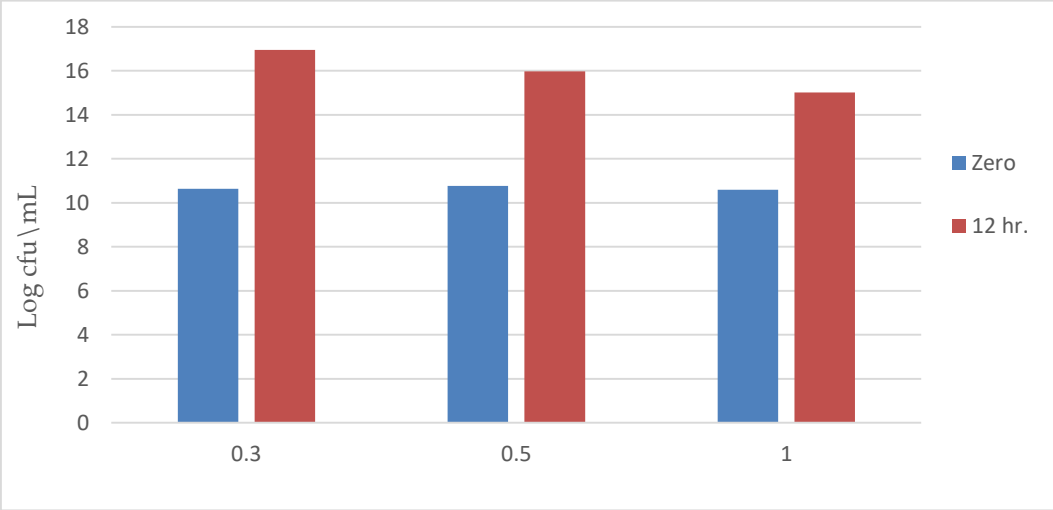

Fig. 2. Bile tolerance of *L. gasseri* AZ1 KY123806 on different bile salt concentrations.

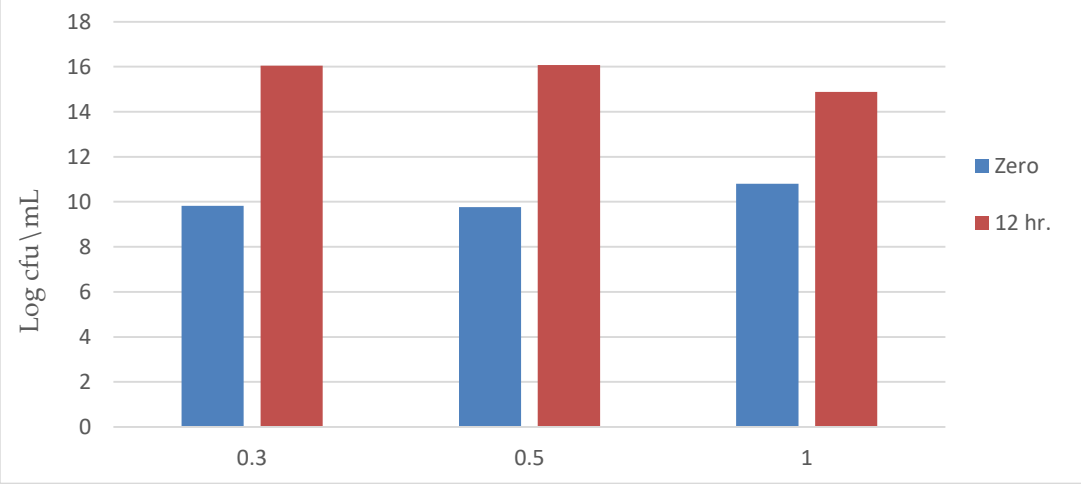

Fig. 3. Bile tolerance of *L. rhamnosus* AZ1 KY123789 on different bile salt concentrations.

Tolerance to bile salts is a prerequisite for colonization and metabolic activity of bacteria in small intestine of the host (Havenaar *et al*., 1992). Once the bacteria reach the intestinal tract, bile entering the duodenal section of small intestine has been found to reduce survival of bacteria. Therefore, probiotics must have an ability to tolerate bile (Kimoto *et al*., 2000).

In agreement with literate recordings, all tested *Lactobacillus* strains exhibited excellent bile tolerance (Figures 1, 2 and 3). In this regard, Oh *et al*., (2000) mentioned that *Lactobacillus* was capable of surviving in the presence of bile due to its ability to deconjugate bile acids bile acids. Also, it was of interested to notice that all tested *Lactobacillus* strains showed varying response toward different bile salt concentrations. In this connection, Gopal *et al*., (1996) reported that the observed differences in tolerance to bile may partly due to the natural differences in growth of individual strains.

Tolerant to simulated gastric and small intestinal juices:

About 2.5 L. of gastric juice and 0.7 L. of pancreatic juice are secreted each day, these secretions present a pH and enzymatic barrier and act in concert with bile to ensure the survival of ingested microorganisms during digestion. Therefore, surviving gastrointestinal transit was found to be an important functional property of tested probiotic bacteria (Succi *et al*., 2005 and Vizoso *et al*., 2006).

As shown from Table 2 gastric juice exerted variable influence on the growth of examined cultures. In general, *L. casei* AZ1 strain exhibited more gastric juice resistance than other tested cultures. However, variation in the tolerance to gastric juice was previously reported by Mathara *et al*. (2008) and Kershah (2014). In generally, the three tested Lactobacillus cultures exhibited acceptable levels of survivability and considered intrinsically tolerant to gastric juice, since at least $\geq 10^6$cfu/ml of each strain survived after 180 min of exposure, as previously concluded by Guerra *et al*., (2007).

From the foregoing results, it could be pointed out that all tested strains could be successfully transit the human stomach and reaching the intestinal tract and functioning effectively there.

It was obvious, as the data in Figure 4, that all tested cultures retained viability during growth in simulated intestinal juice and considered intrinsically tolerant to intestinal transit. In this respect, Charteris *et al*., (1998) stated that the majority of probiotic strains were intrinsically resistant to simulated pancreatic juice and showed no reduction in viability up to 4 hours.

According to the obtained results, it might be deduced that *L. gasseri*AZ1 was markedly with regard to intestinal juice tolerance followed by *L. casei* AZ1 strain. However, several authors previously concluded that variations and degree of response to intestinal juice may be strain dependent (Sultana *et al*., 2000; Guerra *et al*., 2007; Kim *et al*., 2008). According to the foregoing results, it could be concluded that the three tested lactobacilli strains may be promising candidate strains for use as probiotics.

Feeding Experiment:

An extensive investigation concerning the potential role of probiotic lactobacilli strains was carried out. Data obtained were summarized in Tables 3, 4 and 5.

The effect of different diets on growth parameters of rats are presented in Table 3. At the end of the experiment (28 days), significant differences in final body weights of all rats were detected, but rats received fermented milk products gained higher body weights than those fed only dry diet (cont. I). Anon (1997) concluded that fermentation of milk by lactic acid bacteria was reported to increased its protein availability and its nutritional value. However, our findings are in agreement with those previously reported by Abd El-Gawad *et al*. (2005), Zommara *et al*. (2006) and Mohamed (2009). Also, as seen from the same table, rats received milk cultured with *L. casie* gained the highest final body weight, being 259.3 g.

In addition, the present results revealed that there were no considerable variations between growth rate or food efficiency among different treatments. This statement is consistent with previous finding of Zommara (2002). Moreover, significant differences were detected among dietary groups in food intake (g/ day), while no-significant differences were observed in body weight gain (g). This finding is in contrary to those reported by Abd El-Gawad *et al*. (2005) and Mohamed (2009).

In order to assess the potential hypocholestrolamic properties of fermented milk made with different *Lactobacillus* strains albino rats were used as an animal model. Results of determination blood serum lipids of rats were presented in Tables 4 and 5.

The results obtained indicated that serum cholesterol level in control II was significantly higher than in control I, by 24.07 % at the end of the experiment. Compared with control group (cont.I), the total serum cholesterol were lowered by 21.39% and 15.74% in rats fed on *L. casei*and *L. rhamnosus*, respectively. However, our results are consistent

with previous reports of Zommara *et al*. (2006), Mohamed (2009), Ying *et al*. (2010), Chuan *et al*. (2104), Song *et al*. (2015) and Bobae *et al*. (2016).

In contrast, *L. gasseri* achieved an opposite trend, in which their final total cholesterol level was markedly increased by 13.27% as compared with control I.

Also, it could be noticed from obtained results that there were significant differences in HDL-cholesterol level between different treatments at the end of the experiment. However, the levels of serum HDL-cholesterol were increased throughout experiment period and the increases values ranged from 4.02% to 29.44%, as compared with their corresponding initial values. Zilva and Mayne (1991) stated that HDL-cholesterol levels are "anti-atherogenic", where their reduced level are associated with increased risk of coronary artery disease.

Since a high blood LDL-cholesterol is associated with increased risk of atherosclerosis and cardiovascular disease, any product that lower this level is of potential value. Therefore, the effect of feeding rats on different lactobacilli strains on LDL-cholesterol level was carried out, results obtained presented in Table (4).

Table 2. Effect of simulated gastric juice on viability of tested *Lactobacillus* spp. strains.

Strains	Incubation time (min)						
	Zero	60		120		180	
	Log cfu/mL	Log cfu/mL	% increase	Log cfu/mL	Log cfu/mL	Log cfu/mL	% increase
*L. casei*AZ1 KY123805	12.77	14.14	10.77	14.75	12.77	14.14	10.77
*L. gasseri*AZ1 KY123806	14.32	14.39	0.50	14.57	14.32	14.39	0.50
L. rhamnosus AZ1 KY123789	14.62	15.65	7.06	15.07	14.62	15.65	7.06

cfu: colony forming unit.

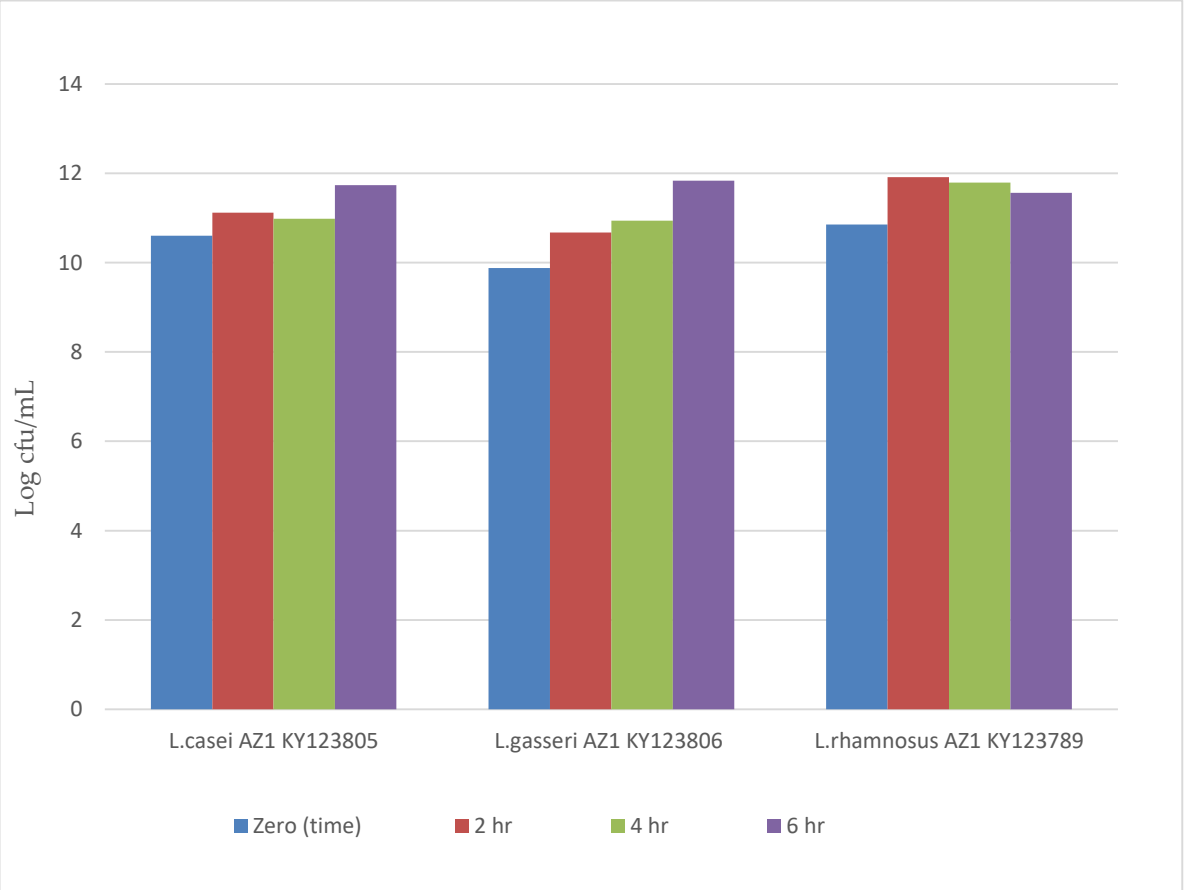

Fig. 4. Viability of tested *Lactobacillus* spp. at simulated intestinal juice.

Table 3. Growth parameters of rats fed on some fermented milk products cultured with *Lactobacillus* spp.

Treatments Parameters	Control I dry diet	Control II B.M. (6.1% fat)	*L. casei AZ1 KY123805*	*L. gasseri AZ1 KY123806*	*L. rhamnosus AZ1 KY123789*	*p*-value
Initial body weight (g)	140.8	141.3	142.2	141.4	139	-
Final body weight (g)	245	256.3	259.3	256.3	257	0.008*
% change to normal control	-	4.61	5.84	4.61	4.90	-
Food intake (g/day)	22.3	21.9	22.7	21.4	22.9	0.030*
Body weight gain (g)	104.2	115.3	117.1	114.9	118.4	0.0483 (n.s)
Body weight gain (%)	74.01	81.39	82.35	81.26	84.89	0.849 (n.s)
Growth rate (g/day)	3.72	4.11	4.18	4.10	4.21	-
Food efficiency	0.169	0.188	0.184	0.192	0.184	-

B.M: Buffalo's milk; n.s: non-significant; *: significant.

Table 4. Levels of serum total cholesterol and HDL-cholesterol in rats fed on some fermented milk products cultured with Lactobacillus spp.

Item Parameters	Treatments Control I dry diet	Control II B.M. (6.1% fat)	*L. casei AZ1 KY123805*	*L. gasseri AZ1 KY123806*	*L. hamnosus AZ1 KY123789*	*p*-value
Initial LDL cholesterol (mg/dL)	24.27	25.62	27.51	29.62	27.03	-
Final LDL-cholesterol (mg/dL)	14.69	33.40	11.42	30.97	8.95	0.170 (n.s)
% Change to normal control	-	(+) 127.13	(-) 22.26	(+) 110.82	(-) 39.07	_
Initial triglycerides (mg/dL)	93.43	95.22	94.36	95.18	93.88	-
Final triglycerides (mg/dL)	97.48	172.53	81.41	126.96	76.04	0.0001**
% change to normal control	-	(+) 76.99	(-) 16.49	(+) 30.24	(-) 21.99	_

B.M: Buffalo's; *: significant; **: high significant; HDL: high-density lipoprotein; (+): increase (-): decrease.

Table 5. Levels of LDL-cholesterol and serum triglycerides in rats fed on some fermented milk products with Lactobacillus spp.

Item Parameters	Treatments Control I dry diet	Control II B.M. (6.1% fat)	*L. casei AZ1 KY123805*	*L. gasseri AZ1* KY123806	*L.rhamnosus AZ1 KY123789*	*p*-value
Initial total cholesterol (mg/dL)	111.36	114.77	112.50	1115.91	113.64	-
Final total cholesterol (mg/dL)	122.73	152.27	96.48	139.02	103.41	0.006**
% change to normal control	-	(+) 24.07	(-) 21.39	(+) 13.27	(-) 15.74	-
Initial HDL-cholesterol (mg/dL)	68.40	70.11	66.12	67.26	67.83	-
Final HDL-cholesterol (mg/dL)	88.54	84.63	68.78	82.65	79.61	0.10*
% change to normal control	-	(-) 4.42	(-) 22.32	(-) 6.65	(-) 10.09	-

B.M: Buffalo's Milk; n.s: non –significant; **: high significant; LDL: low-density lipoprotein (+): increase; (-): decrease.

It might be gathered from the data obtained that buffalo's milk supplementation to dry diet had markedly increase on level of LDL-cholesterol by 127.3 %. Also, it is noteworthy from the same table the rats fed on by fermented milk cultured by either L. *rhamnosus*AZ1or *L. casei* AZ1 possessed the lowest serum LDL-cholesterol concentrations, actually 8.95 mg/dL with the highest reduction

level, actually 39.07 %, while the corresponding figures were 11.42 mg/dL and 22.27 %, respectively for *L. casei*. these results might be considered as promising, because 1 % reduction in cholesterol can reduce the risk of cardiovascular disease for 2 – 3 % (Kourelis *et al*., 2010). In agreement with our finding, previous studies showed that receiving fermented milk products reduce LDL-cholesterol concentration (Abd El-Gawad *et al*. 2005; Zommara *et al*., 2006; Ying Huang *et al*., 2013 and Song *et al*., 2015).

Furthermore, alternation in triglycerides content was studied and data obtained presented in Table 4. From these results it could be noticed that addition of buffalo's milk to the normal diet (cont. II) markedly increased serum triglycerides by 76.99 % as compared to dry diet (cont. I). In addition, rats received fermented milk cultured by either *L. casei*or or *L. Rhamnosus* ranked noticeable decreases in serum triglycerides content, actually 16.49 % and 21.99 %, respectively as compared to dry diet (cont. I). Recently, several investigators confirmed the reduction of triglycerides concentrations in rats fed different fermented milk products (Ying Huang *et al*., 2013; Xu *et al*., 2013 and Bobae *et al*., 2016).

As a matter of fact, the atherogenic indexesare indication for the susceptibility for atherosclerosis. Therefore, the atherogenic indices were calculated and results illustrated in Table 5. Buffalo's milk supplementation to basal diet (cont. II) led to obvious increases in atherogenic indexes 1, 2 and LDL / HDL ratio by mean values of 82.5 %, 51.69 % and 137.91% respectively as compared with control treatment I. The same trend of result was previously reported by Zommara *et al*. (2006) and Mohamed (2009).

Continuously, the examined *Lactobacillus* spp. showed different results towards atherogenic indices, were rats received fermented product cultured with *L. rhamnosus* AZ1, reduced markedly the atherogenic indexes I, 2 and LDL/ HDL ratio by a mean values of 27.5 %, 22.54 % and 32.25 %, respectively, as compared with control treatment I. In contrast, fermented product cultured with *L. gasseri* showed an opposite trend.

The effect of fermented milk culture products cultured with *Laactobacillus* spp. on rats' intestinal pathogenic microflora had been studied. Data obtained graphically plotted in Figures 5 and 6. Faeces of rats received dry diet or dry diet plus buffalo's milk showed increases in the counts of coliform by 4.5 % and 6.65 % respectively, and in staphylococci counts by 7.12 % and 7.29 % respectively.

In contrast, rats fed on fermented products exhibited highly decreases in the populations of either coliform or staphylococci in their faeces. However, the decrease in coliform counts varied from 3.6 % to 30.65 %, while, the reduction values for staphylococci ranged from 7.95 % to 15.88%. This finding may be due to the double effect of developing acidity and the production of antimicrobial agents which suppress the growth of both pathogens (Kebary, 1995 and Badawi & El-Sonbaty, 1997).

Generally, the obtained results strongly suggest that both *L. casei* and *L. rhamnosus* are promising candidate as they exhibited pronounced hypolipidemic effect and greater antagonistic effect against either coliforms of staphylococci strains.

Table 6. Levels of atherogenic indices in rats fed on some fermented milk products cultured with Lactobacillus spp.

Item	Treatments					*p*-value
Parameters	Control I dry diet	Control II B.M. (6.1% fat)	*L. casei AZ1 KY123805*	*L. gasseri AZ1 KY123806*	*L.rhamnosus AZ1 KY123789*	
Final HDL/total cholesterol (mg/dL)	0.721	0.556	0.713	0.595	0.770	0.018*
Atherogenic index 1	0.120	0.219	0.118	0.223	0.087	0.330 (n.s)
Atherogenic index 2	0.386	0.799	0.403	0.682	0.299	0.018*
% LDL/HDL ratio	16.59	39.47	16.60	37.47	11.24	0.202 (n.s)

*B.M: Buffalo's; n.s: non-significant; *: significant: atherogenic index 1: (LDL/Total cholesterol); atherogenic index 2: (Total cholesterol-HDL)/HDL.*

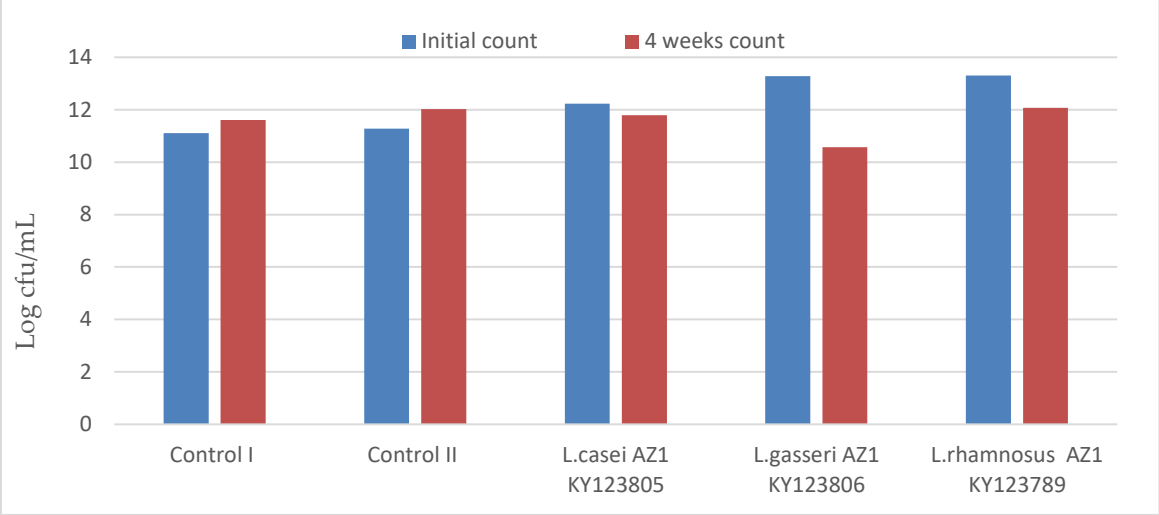

Fig. 5. Effect of fed on fermented milk products cultured with some *Lactobacillus* spp. on fecal coliform population.

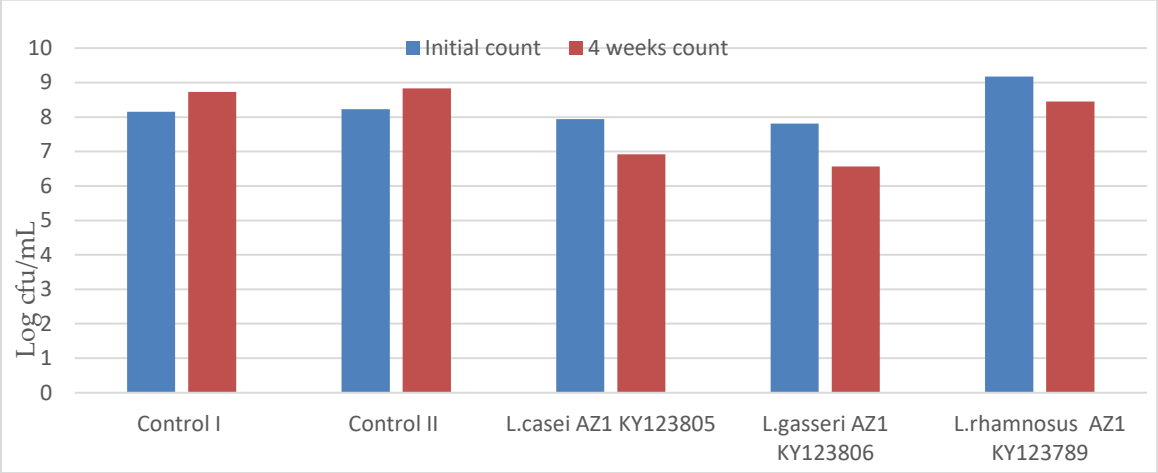

Fig. 6. Effect of fed on fermented milk products cultured with some *Lactobacillus* spp. On fecal staphylococci population.

CONCLUSION

The total serum cholesterol levels were lowered by 21.39 % and 15.74 % in the group fed *L. casei* KY123805 and *L. rhamnosus* KY123789, respectively. Contrarily, to *L. casei and L. rhamnosus*, *L. gasseri* KY123806 achieved opposite trend. Plasma HDL-cholesterol levels were increased at the end of the experiment as compared with control I.

REFERENCES

Abd El-Gawad, I.A.A., El-Saved, E.M., Hafez, S.A., El-Zeini, H.M., Saleh, F.A. 2005. The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. Int. Dairy J. 15 (1), 37-44.

Anon, O., 1997. Nutritional benefits of zabady and other fermented milk products. Natl. Dairy Counc. Trop. 8, 1.

APHA, American Public Health Association. 1992. Methods for Examination of Dairy products. 16th Edition, American Public Health Association, Washington.

Badawi, R.M., El-Sonbaty, A.H., 1997. Viability of *Staphylococcus aureus* and *Escherichia coli* in zabady made with bifidobacteria. Egypt. J. Dairy Sci. 25, 217-234.

Beena, A., Prasad, V., 1997. Effect of yogurt and bifidus yogurt fortified with skim milk powder, condensed whey and lactose-hydrolysed condensed whey on serum cholesterol and triglycerides levels in rats. J. Dairy Res. 64 (3), 453-457.

Bobae, K., Park, K. Y., Ji, Y., Park, S., Holzapfel, W., Hyun, C. K., 2016. Protective effects of *Lactobacillus rhamnosus* GG against dyslipidemia in high-fat diet-induced obese mice. Biochem. Biophys. Res. Commun. 473 (2), 530-536.

Carthew, P., Baldrick, P., Hepburn, P.A. 2001. An assessment of the carcinogenic potential of shea oleine in the rat. Food Chem. Toxicol. 39 (8), 807-815.

Charteris, W. P., Kelly, P.M., Morelli, L., Collins, J.K., 1998. Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human gastrointestinal tract. J. Appl. Microbiol. 84 (5), 759-768.

Chou, L., Weimer, B., 1999. Isolation and characterization of acid and bile-tolerant isolates from strains of *Lactobacillus acidophilus*. J. Dairy Sci. 82, 23-31.

Chuan, L., Nie, S.P., Ding, Q., Zhu, K.X., Wang, Z.J., Xiong, T., Xie, M.Y., 2014. Cholesterol-lowering effect of *Lactobacillus plantarum* NCU116 in a hyperlipidaemic rat model. J. Funct. Foods. 8, 340-347.

DeMan, C., Rogosa, M., Sharp, M.E., 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 22, 130–136.

Gopal, A., Shah, N.P., Roginski, H., 1996. Bile tolerance, taurocholatede conjugation and cholesterol removal by *Lactobacillus acidophilus* and *Bifidobacterium* spp. Milchwissenschaft. 51, 619-623.

Guerra, N.P., Bernárdez, P.F., Méndez, J., Cachaldora, P., Castro, L.P., 2007. Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. Anim. Feed Sci. Technol. 134 (1), 89-107.

Havenaar, R, Brink, B.T., Huis In 't Veld, J.H.J., 1992. Selection of strains for probiotic use. In: Fuller, R., (ed), Probiotics: The scientific basis. Springer Netherlands, Dordrecht, pp. 209-224.

Kebary, K.M.K., 1995. Production, partial purification and stability of anti-microbial substances produced by. *Bifidobacterium bifidum* DI. Egypt. J. Dairy Sci. 23, 151-166.

Kershah, T.A., 2014. Studies on probiotic bacteria. M.Sc. Thesis, Al-Azhar Univ., Cairo, Egypt.

Kim, S.J., Cho, S.Y., Kim, S.H., Song, O.J., Shin, I.S., Cha, D.S., Park, H.J., 2008. Effect of microencapsulation on viability and other characteristics in *Lactobacillus acidophilus* ATCC 43121. LWT-Food Sci. Technol. 41 (3), 493-500.

Kimoto, H., Ohmomo, S., Nomura, M., Kobayashi, M., Okamoto, T., 2000. *In vitro* studies on probiotic properties of lactococci. Milchwissenschaft. 55 (5), 245-249.

Klein, H., Fung, D.Y.C., 1978. Identification and quantification of fecal coliforms using violet red bile agar. J. Milk Food Technol. 39 (11), 335-341.

Kourelis, A., Kotzamanidis, C., Litopoulon-Tzanetaki, E., Scouras, Z.G., Tzanetakis, N., Yiangou, M., 2010. Preliminary probiotic selection of dairy and human yeast strains. J. Biol. Res. 13, 93-104.

Maffei, H.V., Nobrega, F.J., 1975. Gastric pH and microflora of normal and diarrhoeic infants. Gut. 16 (9), 719-726.

Mathara, J.M., Schillinger, U., Guigas, C., Franz, C., Kutima, P.M., Mbugua, S.K., Holzapfel, W.H., 2008. Functional characteristics of *Lactobacillus* spp. from traditional Maasai fermented milk products in Kenya. Int. J. Food Microbiol. 126 (1), 57-64.

Mohamed, Y.A., 2009. A Study on Yoghurt and Yoghurt Derivatives. Ph.D. Thesis, Al-Azhar Univ., Cairo, Egypt.

Oh, S., Kim, S.H., Worobo, R.W., 2000. Characterization and purification of a bacteriocin produced by a potential probiotic culture, *Lactobacillus acidophilus* 30SC. J. Dairy Sci. 83 (12), 2747-2752.

Pereira, D.I., Gibson, G.R., 2002. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. Appl. Environ. Microbiol. 68 (9), 4689-4693.

Sanders, M.E., Reid, G., Gaskins, H.R., Gibson, G.R., Mercenier, A., Rastall, R., Klaenhammer, T.R., 2003. New Scientific paradigms for probiotics and prebiotics. J. Clin. Gastroenterol. 37 (2), 105-118.

Song, M., Park, S., Lee, H., Min, B., Jung, S., Kim, E., Oh, S., 2015. Effect *of Lactobacillus acidophilus* NS1 on plasma cholesterol levels in diet-induced obese mice. J. Dairy Sci. 98 (3), 1492-1501.

SPSS, 2011. IBM SPSS Statistics for Windows, release 20.0.0. Chicago, IL.

Succi, M., Tremonte, P., Reale, A., Sorrentino, E., Grazia, L., Pacifico, S., Coppola, R., 2005. Bile salt and acid tolerance of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese. FEMS Microbiol. Lett. 244 (1), 129-137.

Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P., Kailasapathy, K., 2000. Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. Int. J. Food Microbiol. 62 (1-2), 47-55.

Vizoso, P.M.G., Franz, C.M., Schillinger, U., Holzapfel, W.H., 2006. *Lactobacillus* spp. within vitro probiotic properties from human faeces and traditional fermented products. Int. J. Food Microbiol. 109 (3), 205-214.

Xu, H., Wang, T., Li, W., Jin, F., Wang, L., 2013. Effects of NS *lactobacillus* strains on lipid metabolism of rats fed a high-cholesterol diet. Lipids Health Dis. 12 (1), 67.

Ying, H., Wang, J., Cheng, Y., Zheng, Y., 2010. The hypocholesterolaemic effects of *Lactobacillus acidophilus* American Type Culture Collection 4356 in rats are mediated by the down-regulation of Niemann-Pick C1-Like 1. Br. J. Nutr. 104 (6), 807-812.

Ying, H., Wang, X., Wang, J., Wu, F., Sui, Y., Yang, L., Wang, Z., 2013. *Lactobacillus plantarum* strains as potential probiotic cultures with cholesterol-lowering activity. J. Dairy Sci. 96 (5), 2746-2753.

Zilva, J.F., Pannall, P.R., Mayne, P.D., 1991. Clinical Chemistry in Diagnosis and Treatment. 5th Edition. ELBS with Edward Arnold Publisher, London, P. 233.

Zommara, M.A., El-Baz, A.M., Rashed, M.A., Mansour, A.A., 2006. Health promoting effects of mixed Zabady and Bifidobacteria fermented milks fed to rats. Egypt. J. Dairy Sci. 34 (1), 47.

Zommara, M.A., 2002. Impact of feeding rats on whey and fermented milk whey products on plasma cholesterol and fecal sterol secretion. J. Agric. Res. Tanta Univ. 28 (2), 327-340.

تاثير بعض المدعمات الحيوية على ليبيدات الدم في الفئران

خاطر عبد الفتاح احمد خاطر[1]، احمد ربيع عبده محمد [1]

[1] قسم الالبان، كلية الزراعة، جامعة الازهر، القاهرة

الملخص العربي

درست خصائص مدعمات الحياة Probiotic لثلاثة سلالات من .*Lactobacillus* spp تمت دراسة قدرة السلالات علي تحمل الحمض واملاح الصفراء والبقاء على قيد الحياة في العصائر المعدية / المعوية. اوضحت النتائج المتحصل عليها ان جميع سلالا ت lactobacilli المختبرة اظهرت ثباتا كبيرا على قيم الـ pH المختلفة (١,٥و٢ و٣)، والنمو الطبيعي بتركيزات املاح الصفراء التي تصل الى ١٪ w/v، واوصت النتائج باستخدام هذه السلالات كمدعمات حياة. كما اظهرت النتائج المتحصل عليها ان جميع سلالات *Lactobacillus*المختبرة تعتبر متاصلة في جوهرها لعصير المعدة والامعاء. تمت دراسة الدور المحتمل لسلالات *Lactobacillus* المعوية على ليبيدات الدم في الفئران. حيث تم تقسيم ٢٥ من الفئران الذكور من نوع الالبينو، حيث قسمت الى خمس مجموعات، كل منها يحتوي علي خمسة فئران، اخضعت هذه الفئران لفترة تاقلم مدتها ٧ ايام تتغذى خلالها الفئران علي العليقة الجافة فقط، اجريت التجربة لمدة ٢٨ يوما وتم تغذية المجموعة الثانية علي العليقة الجافة + لبن جاموسي بالاضافة الى احدى سلالات *Lactobacillus* المختبرة، وبعد ذبح الفئران تم جمع عينات الدم في بداية ونهاية التجربة.اظهرت النتائج المتحصل عليها ان روث العلائق من الوجبات الغذائية مع اللبن المتخمر بواسطة *L. casei* او *L. rhamnosus* ادى الى انخفاض ملحوظ في مستوي الكوليسترول الكليوLDL-chol والجلسريدات الثلاثية. وعلاوة على ذلك، انخفض مؤشر تصلب الشرايين ١، ٢ ونسبة LDL / HDL بشكل ملحوظ في الفئران التي تغذت على اللبن المتخمر مقارنة بعينة الكنترول I (النظام الغذائي الجاف). كما اوضحت النتائج انه عند تغذية الفئران على اللبن المتخمر ادي ذلك الى انخفاض كبير في بكتيريا القولون والبكتيريا الكروية في براز الفئران في نهاية التجربة.