

Effect of Consumption Buffalo's Milk Fortified with Probiotic Yeasts on Rat Serum Lipids

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ABSTRACT

The using of four yeast strains (two cultures belonging to each *S. cerevisiae* and *G. candidum*) to withstand environmental conditions similar to the human digestion tract (probiotic criteria) were examined. The survival of these strains at low pH, in the presence of bile salt, in intestinal juice, also their ability to assimilate cholesterol were followed. However, the obtained results indicate that *S. cerevisiae* AAA3 and *G. candidum* GG1, may be promising candidate strains for use as probiotics. Thus, the potential role of these probiotic yeast cultures on serum lipid of rats was adopted. Twenty-four male albino rats were randomly and equally divided into four groups, six rats each. These rats were acclimatized on basal diet for 7 days before starting the experiment. The first group was fed on basal diet (cont. I), the second group was offered basal diet plus pasteurized buffalo's milk (6.5% fat). The rest two groups were fed on basal diet plus buffalo's milk in addition to either *S. cerevisiae* or *G. candidum*. Blood samples were collected at the beginning of the experiment (after adaptation period) and at the end of experiment. According to the result of serum analysis, total cholesterol and LDL-cholesterol levels in rats fed on *S. cerevisiae* (group 3) were decreased by 23% and 31.59% respectively. While, the corresponding values for rats consumed *G. candidum* were relatively lower, actually 21.46% and 29.12% respectively. Moreover, serum triglycerides concentrations reduced by 4.79% and 5.30% for rats in group 3 and 4, respectively as compared with control 1. Furthermore, data obtained declared that rats fed on either *S. cerevisiae* or *G. candidum* reduced markedly the atherogenic indexes 1 and 2 by mean values of 11.52, 23.86% and 10.1, 21.49% respectively, as compared with control 1. Continuously, rats fed on both yeast strains showed significant antagonistic effect against either coliform or staphylococci, where the reduction in their populations, actually 10.57, 7.42% for group 3 and 8.30, 18.22% for group 4, respectively.

INTRODUCTION

Probiotic yeasts are non-pathogenic strains which have been used as both a preventive and therapeutic agent for the treatment of variety of diseases. A set of selection criteria considered to be relevant for any probiotic microorganism has been proposed by Ouwehand *et al.* (1999). Tolerance to low pH and bile salts are seen as prerequisite for strain survival through the gastrointestinal tract. Probiotics must be able to survive gastric and intestinal juices.

Moreover, one of the most criteria is the ability to confer a health benefits on the host, including the reduction of serum cholesterol levels. Elevated blood cholesterol levels are a major risk factor for coronary artery disease, the leading cause of death worldwide. According to the world Health Organization (WHO), the 20% strokes and 50% of heart attacks are caused by high bad cholesterol (WHO, 2002, Mendis *et al.* 2005 and Roth *et al.* 2011).

Recently, natural dietary supplements can assist in lowering cholesterol and protect against heart disease. However, probiotic bacteria have been investigated as potential cholesterol-lowering therapies, while little studies have assessed the beneficial effect of probiotic yeasts as cholesterol-lowering agent.

Therefore, the aim of the present work was to identify the probiotic criteria of some isolated yeasts. Also, evaluation the impact of feeding rats on buffaloes' milk enriched with either *S. cerevisiae* or *G. candidum* probiotic cultures on plasma lipids and fecal microflora.

MATERIALS AND METHODS

Milk:

Fresh buffalo's milk (6.5% fat) was obtained from the Assiut experimental farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt.

Yeast cultures:

Four isolated yeast cultures namely, *Saccharomyces cerevisiae* AA2, *Saccharomyces cerevisiae* AAA3, *Geotrichum candidum* GG1 and *Geotrichum*

candidum AAA were used in the present study, obtained from Dairy Department, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt.

Bile salt:

The oxgall was obtained from Difco Laboratories, Detroit, Michigan, USA.

Pepsin and Pancreatin:

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

Cholesterol:

Pure cholesterol was obtained from Difco laboratories, Detroit, Michigan, USA.

Albino rats:

Twenty-four mature male albino rats, with mean body weight of 120 ± 5 g. were obtained from the Animal's house, Faculty of Medicine, Assiut University.

Basal diet:

The chemical composition of basal or control diet was as follows: crude protein 21%, fibers 3.50%, crude fat 6.50% and starch 69%.

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.

While, at the end of experiment, the rats were killed by withdrawing blood from the abdominal aorta under light diethyl ether anesthesia. Blood were collected in glass tube and left to clot at room temperature to obtain a clear serum.

Faeces samples:

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

Media:

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 medium according to APHA, (1992).

Methods:

Acid tolerance:

The tested strains were evaluated for their ability to grow in low pH values (1.5, 2.0 and 3.0) according to Chen et al. (2010).

Bile tolerance:

Bile tolerance was estimated as described by Psomas et al. (2001), where two tested concentrations of bil salts, actually 0.3% and 0.5% w/v. were used.

Survival in gastric and intestinal juices:

Gastric and pancreatin juices were prepared by dissolving pepsin from porcine stomach mucosa (3g/L) and pancreatin from porcine pancreas (1g/L) in sterile saline (5g/L). The pHs of the gastric and pancreatic preparations were adjusted to 2.0 and 8.0 with 5M/L HCL or 1M/L Na OH, respectively. The experiment was carried out as described by Charteris et al. (1998).

Assimilation of cholesterol:

The ability of yeast strains to lower cholesterol levels in vitro was determined according to the method of Pereira and Gibson (2002) with some modifications as described by Kourelis et al. (2010).

Feeding experiment:

The rats were randomly and equally divided into four groups, six rats each. The rats were housed in metal cages at room temperature (25±2 °C) and relative humidity (about 55%). Fed and water offered ad libitum to the rats throughout 28 days. The rats were acclimatized on basal diet for one week before starting the experiment. The body weight of each rat was recorded at the beginning and the end of experiment period.

After an adaptation period for 7 days, the first group was fed on basal diet (12 g/rat/day) and served as control (cont.I), while the second group was offered basal diet plus buffalo's milk (and served as control II). The rest two groups were fed on basal diet plus buffalo's milk in addition to either *S. cerevisiae* AA3 or *G. candidum* GG1 probiotic cultures.

Fecal bacterial population:

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

Determination of serum lipids concentration:

Serum lipid concentration was determined according to the method described by Young (2001), by using a commercially available kits (cholesterol, HDL-cholesterol and triglycerides) enzymatic colorimetric method. The developed color was measured at 546 nm.

While, the concentration of LDL- cholesterol could be calculated according to the following formula as follows:

$$\text{LDL-cholesterol} = \text{total cholesterol} - (\text{triglycerides} / 5)$$

Calculation of atherogenic index:

The atherogenic indexes were calculated as LDL-cholesterol / total cholesterol, and (Total cholesterol – HDL)/HDL according to Zommará et al. (2006).

Biological evaluation of rat diets:

Biological evaluation was carried out by determination of body weight gain, food efficiency ratio

and growth rate g/day according to Carthew et al. (2001). Using the following formulas:

$$\text{Body weight gain} = \text{final weight} - \text{initial weight.}$$

$$\text{Growth rate, g/day} = \frac{\text{Body weight (g)}}{\text{Experimental period (day)}}$$

Statistical analysis:

Results were statistically analyzed using one way analysis of variance procedure of ANOVA to determine whether significant (P<0.05) variation occurred among means of each experiment.

RESULTS AND DISCUSSION

Selection and characterization of tested yeasts for use as probiotics:

Four yeast strains were used in this part of study, two cultures belonging to each *S. cerevisiae* (AA2 & AAA3) and *G. candidum* (GG1 & AAA). The tested cultures were examined for their tolerance to low pH (1.5, 2.0 and 3.0) for up to 180 min. Results obtained were presented in Table 1.

Table 1. Effect of low pH on viability of some selected yeast strains

strain	pH	Incubation time [h]							
		0h		1h		2h		3h	
		Log cfu /ml	Log cfu /ml	% survival	Log cfu /ml	% survival	Log cfu /ml	% survival	
Saccharomyces cerevisiae	1.5	6.34	6.30	99.37	6.08	95.90	5.77	91.01	
AA2	2.0	6.32	6.30	99.68	6.29	99.53	6.20	98.10	
AAA3	3.0	6.35	6.37	100.31	6.39	100.63	6.40	100.79	
Saccharomyces cerevisiae	1.5	6.13	6.05	98.69	5.94	96.90	5.81	94.78	
AA2	2.0	6.29	6.27	99.68	6.27	99.68	6.26	99.52	
AAA3	3.0	6.33	6.40	101.11	6.41	101.26	6.43	101.58	
Geotrichum candidum	1.5	5.95	5.93	99.66	5.88	98.82	5.71	95.97	
GG1	2.0	6.00	6.00	100.00	5.90	98.33	5.86	97.67	
AAA	3.0	5.95	5.95	100.00	5.91	99.33	5.90	99.16	
Geotrichum candidum	1.5	5.90	5.77	97.80	5.14	87.12	4.72	80.00	
GG1	2.0	5.93	5.88	99.16	5.77	97.30	5.74	96.80	
AAA	3.0	5.96	5.95	99.83	5.95	99.83	5.90	98.99	

Cfu = colony forming unit.

It could be gathered from these results that, pH 1.5 seemed to be more damaging to the tested strains, while pH 2.0 showed moderate effect. *G. candidum* GG1 exhibited more acid resistance at pH 1.5 with survival % of 95.97, followed by *S. cerevisiae* AAA3 with corresponding figure of 94.78%, after 180 min. of exposure to acidic condition.

At pH 2.0, *S. cerevisiae* AAA3 ranked the highest survival % actually, 99.52%, after 3 hours of exposure. Hood & Zottola (1988) and Gupta et al. (1996) mentioned that the substantial decrease in the viability of tested strains is often observed at pH 2.0 or below.

Moreover, obtained results revealed that all tested cultures survived better at pH 3.0, up to the end of incubation.

On the other hand, probiotics must have an ability to tolerate bile salts (Kimoto et al., 2000). Therefore, two different concentrations were used in the present study, actually 0.3 and 0.5% (w/v) oxgall, to mimic approximate levels in the intestinal tract as previously reported by Suskovic et al. (1997) and Garriga et al. (1998), they gave a

mean value of 0.3% (w/v) for human gastrointestinal tract bile concentration.

As shown from data which graphically plotted in Figure 1, all tested yeast cultures exhibited excellent bile tolerance. These statement are in complete agreement with those found by Rajkowska and Kunicka-Styezynska (2010) they stated that addition of 0.1% and 1.0% bile did not restrict viability of yeast strains.

In general, *S. cerevisiae* AAA3 exhibited more bile resistance than other tested cultures either at 0.3% or 0.5% bile concentrations after 72 hours of exposure, followed by *G. candidum* GG1.

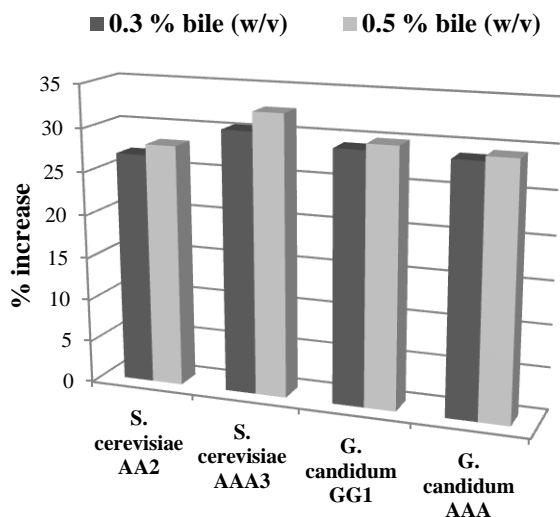


Figure 1. Bile tolerance of different yeast spp. on 0.3 and 0.5% bile salt concentrations (w/v) after 72 hours of incubation.

Continuously, results of yeast survival in either simulated gastric or intestinal juices are graphically presented in Figures 2 and 3. It could be noticed that gastric juice (Fig.2) exerted moderate influence on the growth of all tested cultures. Also, the highest resistance to gastric juice was observed for *S. cerevisiae* AAA3 with population level of 4.85 log cfu/ml and survival rate of 90.65% after 240 min. of exposure. Moreover, relatively high survival per cent was recorded for *G. candidum* GG1, actually 89.40, under the same conditions. This finding is consistent with previous report of Mathara *et al.* (2008) they stated that tolerance to gastric juice was observed among strains showed much higher acid tolerance.

As seen in Fig.3, it was evident that strain *S. cerevisiae* AAA3 was slightly better with regard to intestinal juice tolerance than other tested cultures, where it population reduced by only 6.75% after 240 min. of exposure to intestinal juice.

In general, from the obtained results, it was of interest to notice that all tested strains retained approximately normal viability during growth in simulated small intestinal juice. In this respect, Charteris *et al.* (1998) mentioned that the majority of probiotic strains were intrinsically resistant to simulated pancreatic juice.

Data of cholesterol assimilation are shown in Table 2 and Figure 4. It was clear from data obtained that all tested cultures had the ability to reduce cholesterol

concentration, approximately in equal percent. Also, it might be gathered that *G. candidum* AAA strain assimilate more cholesterol %, actually 26.31%, as compared with other tested strains. However, our data confirmed the fact that assimilation of cholesterol into yeast cells was the only mechanism by which yeast strains removed cholesterol from the growth medium.

Table 2. cholesterol assimilation by different selected yeast strains

Strains	% cholesterol	% assimilation of cholesterol
<i>Saccharomyces cerevisiae</i> AA2	37.42	23.68
<i>Saccharomyces cerevisiae</i> AAA3	36.77	25.00
<i>Geotrichum candidum</i> GG1	36.77	25.00
<i>Geotrichum candidum</i> AAA	32.90	26.31

$$\% \text{cholesterol} = \frac{A(\text{sample})}{A(\text{stander})} \times 100$$

$$\% \text{Assimilation cholesterol} = \frac{(\% \text{cholesterol of control} - \% \text{cholesterol of strain})}{\% \text{cholesterol of control}} \times 100$$

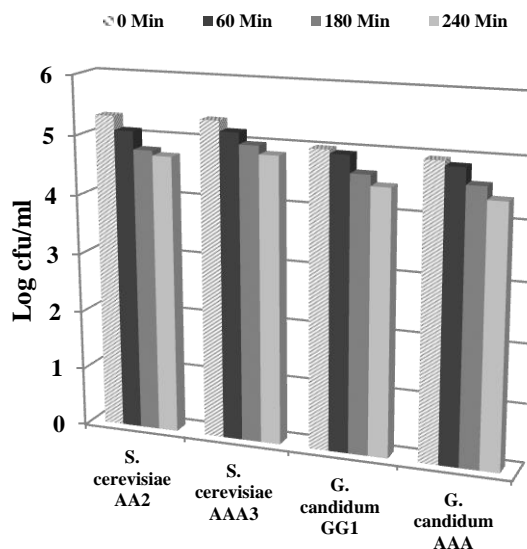


Figure 2. Viability of tested yeast spp. in simulated gastric juice.

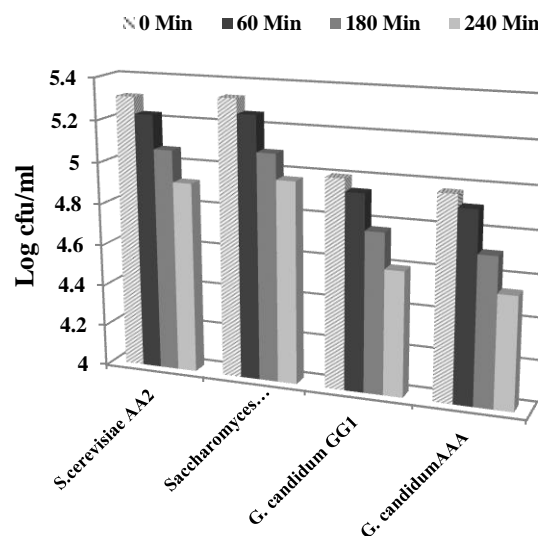


Figure 3. Viability of tested yeast spp. in simulated intestinal juice.

Finally, from the previous results, it might be deduced that *S. cerevisiae* AAA3 and *G. candidum* GG1 may be promising candidate strains for use as probiotics.

Thus, extensive investigation concerning the potential role of probiotic yeasts on serum lipid in rats fed on diets containing one of the promising strains either *S. cerevisiae* AAA3 or *G. candidum* GG1 was carried out. Results obtained were summarized in Tables 3 and 4.

From these results, it could be seen that no significant differences in initial body weights between all tested rats, while, high significant differences in average body weight at the end of the experiment were detected. Moreover, rats consuming *S. cerevisiae* gained the highest body weight, actually 197.35 g, while those feeding *G. candidum* ranked the least weight, being 190, 53 g. The same trend of results was previously reported by Alfitori et al. (2013). Conversely to our results, Aloglu et al. (2015) and Ryan et al. (2015) mentioned that no significance difference observed in rats weight between different treatments during feeding period.

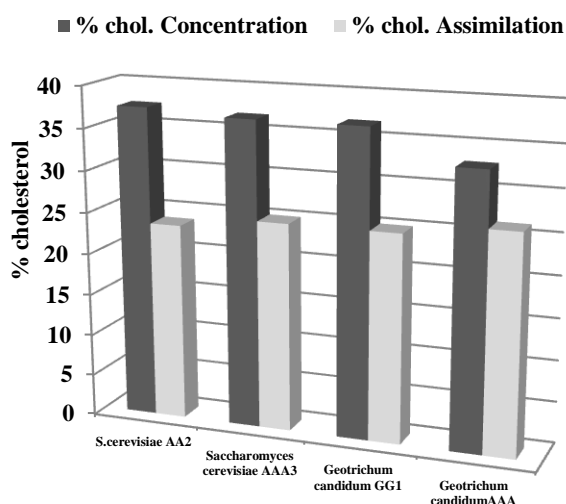


Figure 4. Cholesterol assimilation by different yeast spp.

In addition, high significant differences were found among dietary groups in body weight gain. In contrast, no

significant differences were detected in food intake among different dietary groups. Moreover, the present results declared that there were considerable variations between growth rate or food efficiency among different treatments. This statement is consistent with previous finding of Zommara (2002) and Mohamed (2009).

At the end of the experimental period, the rats were sacrificed and internal organ were excised immediately and weight. Data obtained presented in Table 3. As shown from these results, there were high significant differences found among dietary groups in liver and kidney weights, while only significant differences found among different treatments in spleen weight.

Also, it was of interest to notice that the internal origins in rats fed on yeast cultures were noticeably higher than those consumed dry diet (control I). Similar trend of results was previously reported by Abd El-Gawad et al. (2005).and Mohamed (2009).

Biochemical indices of different treatments are summarized in Table 4. It was evident from the obtained data that the total cholesterol levels were significantly reduced in both groups fed on yeast strains. This finding is consistent with previous reports of Alfitori et al. (2013) and Aloglu et al. (2015). From the same table, it could be also observed that rats fed on *S. cerevisiae* was the most effective group in lowering serum cholesterol, with reduction level of 23%. In this respect, Kannan et al. (2005), Shrivastava & Jha (2010) and Glip & Seyidoolu (2012) stated that there was a noticeable decrease in cholesterol levels by consumed dietary yeast.

However, rats fed on either dry diet (cont. I) or dry diet supplemented with buffalo's milk (cont. II) had no hypocholesterolaemic effect. This finding is consistent with previous report of Alfitori et al. (2013).

As seen in Table 4, the levels of HDL-cholesterol in rats group fed on yeast strains showed no statically significant change. This statement is consistent with previous finding of Alfitori et al. (2013). However, HDL-cholesterol are "anti-atherogenic", in which reduced their levels are associated with increased risk for coronary artery diseases (Zilva et al. 1991).

Table 3. Growth parameter and internal organ weights of rats fed on basal or/and yeast supplemented diets

Treatments parameters	Group 1 (control I) dry diet	Group 2 (control II) (B.M 6.5%fat)	Group 3 (S. cerevisiae)	Group 4 (G. candidum)	Significance (P<0.5)P value
Initial body weight (g)	119.32	120.69	120.84	120.40	0.5408 (N.S)
Final body weight (g)	175.11	190.84	197.35	190.53	.0001 **
Feed intake (g/day)	11.88	12.08	12.38	11.62	0.6889 (N.S)
Body weight gain (g)	55.79	70.15	76.51	70.13	.0001**
Body weight gain (%)	46.76	58.12	63.32	58.25	.0001**
Growth rate (g/day)	1.99	2.51	2.73	2.50	-
Food efficiency	0.167	0.207	0.220	0.215	-
Liver (g)	4.500	4.597	4.820	4.613	.0001**
Relative organ weight (r)	2.57	2.39	2.44	2.42	-
Kidney (g)	1.058	1.213	1.240	1.225	.0001**
Relative organ weight	0.60	0.63	0.62	0.64	-
Spleen (g)	0.418	0.430	0.468	0.427	0.2832*
Relative organ weight (r)	0.238	0.225	0.237	0.224	-

B.M: buffalos milk *: significant **: high significant N.S: non-significant r: organ weight/final body weight x 100

Since a high blood LDL-cholesterol is associated with increased risk of atherosclerosis and cardiovascular diseases, thus any product lower this level is of potential value. The data in Table 4 revealed that there were high significant differences in plasma LDL-cholesterol concentrations between different treatments at (P < 0.001). However, additions of buffaloes' milk (6.5% fat) markedly increase LDL-cholesterol concentrations by 5.86%, as compared with control I (dry diet), while rats fed on either *S. cerevisiae* or *G. candidum* reduced LDL- cholesterol concentrations by 31.59% and 29.12% respectively. Similar trend of results was previously reported by Galip & Seyidoolu (2012) and Alfitori *et al.* (2013). In general, for each 1 mg/dl decrease in LDL-cholesterol concentration in plasma there is approximately a 2% decrease in mortality.

Furthermore, the alternations in triglyceride contents were carried out and results obtained illustrated in Table 4. It could be gathered from these data that addition of buffaloes' milk (Cont. II) markedly increase serum triglycerides concentration by 13.85% as compared to dry diet (Cont.I). This statement was previously confirmed by Alfitori *et al.* (2013) and Aloglu *et al.* (2015). On the other hand, the yeast groups (Group 3 and 4) significantly decrease triglyceride contents as compared with hyperlipidemic group (Cont. II), where the reduction levels attained 4.79% and 5.30% respectively. In this respect, Aloglu *et al.* (2015) give higher value for triglyceride reduction, actually 25%.

Table 4. Biochemical indices of rats fed on basal or/and yeast supplemented diets

Treatments parameters	Group 1 (control I) dry diet	Group 2 (control II) (B.M 7%fat)	Group 3 (<i>S. cerevisiae</i>)	Group 4 (<i>G. candidum</i>)	Significance (P<0.5) P value
Initial total cholesterol (mg/dl)	229.92	230.30	234.09	234.09	0.9969 (N.S)
Final total cholesterol (mg/dl)	264.33	282.46	203.51	207.60	.0001**
Initial HDL-Cholesterol (mg/dl)	36.40	36.18	36.51	36.56	0.9558(N.S)
Final HDL-Cholesterol (mg/dl)	40.79	42.09	39.87	39.38	0.6869 (N.S)
Initial LDL-cholesterol (mg/dl)	157.07	158.18	161.75	162.22	0.9558 (N.S)
Final LDL-cholesterol (mg/dl)	185.20	196.05	126.69	131.26	.0001**
Initial triglycerides (mg/dl)	182.29	179.69	179.17	179.56	0.9362 (N.S)
Final triglycerides (mg/dl)	194.61	221.57	185.29	184.30	.0001**
Final HDL/total-cholesterol	0.154	0.149	0.196	0.190	0.0006**
Atherogenic index 1	0.703	0.794	0.622	0.632	0.8779 (N.S)
Atherogenic index 2	5.49	5.78	4.18	4.31	0.0002**

B.M: buffalos milk N.S: non-significant **: high significant HDL: high-density Lipoprotein LDL: low-density lipoprotein
 atherogenic index1: LDL/total cholesterol Atherogenic index 2 : (total cholesterol-HDL)/HDL

Table 5. effect of selected probiotic yeasts strains on fecal coliforms and staphylococci population

Treatments parameters	Group 1 (control I) dry diet	Group 2 (control II) (B.M 6.5%fat)	Group 3 (<i>S. cerevisiae</i>)	Group 4 (<i>G. candidum</i>)	Significance (P<0.5) P value
Coliforms count (log cfu/g)					
Initial count	4.82	4.80	4.83	4.82	0.0432*
4 weeks count	4.93	5.02	4.32	4.42	.0001*
% increase /decrease	(+) 2.28	(+) 4.58	(-) 10.56	(-) 8.30	-
Staphylococci count (log cfu/g)					
Initial count	4.84	4.82	4.85	4.83	0.0278*
4 weeks count	5.20	5.22	4.49	3.95	.0001**
% increase / decrease	(+) 7.44	(+) 8.30	(-) 7.42	(-) 18.22	-

B.M: buffalos milk (-): decrease (+): increase *: significant **: high-significant

As a matter of fact, the atherogenic index is an indication for the susceptibility for atherosclerosis. Therefore, the atherogenic indexes and the ratio between LDL or HDL- cholesterol and total cholesterol were calculated and data obtained plotted in Table 4. These data indicated that feeding on either *S. cerevisiae* or *G. candidum* reduced markedly the atherogenic indexes 1 and 2 by mean values of 11.52%, 23.86% and 10.1, 21.49% respectively as compared with control treatment (Cont. 1). However, the reduction in the atherogenic indexes would be expected as these parameters were calculated from the values of total, LDL and HDL-cholesterol which were pronounced affect by dietary probiotic yeasts.

Additionally, the effect of probiotic yeasts on rats intestinal pathogenic microflora which are reflected on their feces had been studied where the population of

staphylococci and coliform bacteria were estimated, data presented in Table 5 and Figures 5 & 6. It was obvious from the obtained data that the counts of either coliform or staphylococci were reduced by a mean value of 10.56% and 7.42% for rats consumed *S. cerevisiae* (group 3), and 8.30% and 18.22% in group 4, respectively. In contrast, the corresponding counts of both pathogens in rats feces feeding on control treatments (I and II) were noticeably increased at the end of feeding period by 2.28% and 4.58% for coliforms, 7.44% and 8.30% for staphylococci, respectively. However, the reduction in pathogens counts in Group 3 and 4 may be due to the production of antigungal agents by probiotic yeast cultures which suppress both pathogens (Castgiuolo *et al.*, 1989 and Zbar *et al.* 2013).

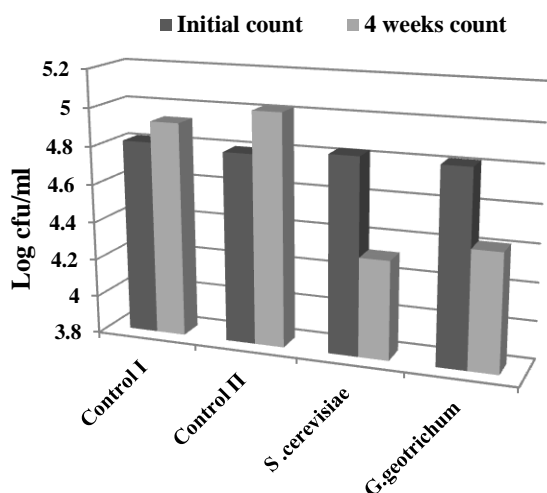


Figure 5. Effect of selected probiotic yeast strains on fecal coliform population.

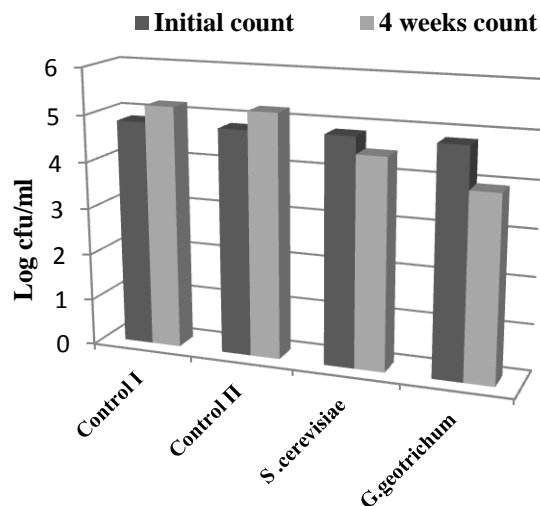


Figure 6. Effect of selected probiotic yeast strains on fecal staphylococci population.

In conclusion, from the foregoing results, it could be stated that selected probiotic yeasts had a marked effect on reducing the levels of plasma lipids (total, LDL-cholesterol and triglycerides) also reducing the populations of either coliform or staphylococci organisms in rats intestinal tract.

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تأثير إستهلاك اللبن الجاموسي المدعم بخمائر المدعمات الحيوية على ليبيدات الدم في الفئران خاطر عبدالفتاح خاطر¹، ياسر عبدالقادر أحمد² وأحمد عبدالدايم أحمد² ¹ قسم الألبان- كلية الزراعة- جامعة الأزهر بالقاهرة- مصر ² قسم الألبان- كلية الزراعة- جامعة الأزهر بأسبوط- مصر

تمت دراسة استخدام أربع سلالات من الخمائر (إثنان من كل من: *S. cerevisiae* & *G. candidum*) لتحمل الظروف البيئية المشابهة للقناة الهضمية للإنسان؛ وتمت دراسة مقدرة تلك السلالات على النمو في pH منخفض. وكذلك تحملها لأملح الصفراء في العصير المعوي. وأيضاً مقدرتها على تمثيل الكوليسترول. وأوضحت النتائج المتحصل عليها أن كل من *S. cerevisiae* AAA و *G. candidum* GGI مرشحان للاستخدام كمدعمات حيوية. وتمت دراسة دور مزارع الخمائر الحيوية على مصلي ليبيدات دم الفئران. حيث أجريت تجربة تغذية باستخدام 24 ذكور فئران الألبينو؛ التي اختيرت عشوائياً وقسمت إلى أربع مجموعات؛ كل منها تحتوي على ستة فئران. أخضعت هذه الفئران لفترة تأقلم مدتها 7 أيام تتغذى خلالها الفئران على العليقة الجافة فقط قبل بداية التجربة. وأجريت التجربة لمدة 28 يوماً على النحو التالي: المجموعة الأولى: يتم تغذيتها على العليقة الجافة فقط (الكنترول الأول) المجموعة الثانية: يتم تغذيتها على العليقة الجافة + لبن جاموسي مبستر (6,5% دهن) (الكنترول الثاني) ، بينما المجموعتين الثالثة والرابعة فقد غذيت على العليقة الجافة + لبن جاموسي + إحدى سلالاتي الخميرة إما *S. cerevisiae* أو *G. candidum*. جمعت عينات من دم الفئران في بداية التجربة (بعد أسبوع الأقامة) وفي نهايتها. حيث أظهرت نتائج تحليل سيرم الدم ما يلي: إنخفاض مستوى الكوليسترول الكلي، والكوليسترول منخفض الكثافة في مجموعة الفئران المغذاة على *S. cerevisiae* (المجموعة الثالثة) بنسب 23%، 31,59% على الترتيب. بينما القيم الممتثلة في المجموعة الرابعة للفئران المغذاة على *G. candidum* كانت 21,46% و 29,12% على التوالي وذلك مقارنة بعينة الكنترول الأول. وعلاوة على ذلك حدث انخفاض في تركيزات الجلسريدات الثلاثية بنسب 4,79% و 5,30% في المجموعتين الثالثة والرابعة على التوالي مقارنة بعينات الكنترول الأول. كما أوضحت النتائج المتحصل عليها أن تغذية الفئران على *S. cerevisiae* و *G. candidum* أحدثت خفصاً في مؤشر تصلب الشرايين 1 و 2 حيث كانت نسبة الإنخفاض 11,52%، 23,86% و 10,1%، 21,49% على التوالي. مقارنة بالكنترول الأول. أظهرت النتائج أيضاً أن تغذية الفئران على كل من سلالاتي الخميرة أحدثت تأثيراً مضاداً Antagonistic على بعض أنواع البكتيريا غير المرغوب تواجدها staphylococci أو coliforms حيث تناقصت أعدادها بنسب 10,57%، 7,42% في المجموعة الثالثة و 8,30%، 18,22% في المجموعة الرابعة على التوالي.