Effect of Different Lebanese Probiotics on the Growth and Some Biochemical Parameters of the Experimental Rats

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The aim of the present study was to investigate the effect of orally administrated probiotics on the physiological and immunological parameters of Sprague Dawely rats. To improve the efficiency of some local probiotics, dairy products were used as an excellent delivery system for probiotics to the experimental animals. The experimental animals were divided into seven different groups. It was remarkably noticed that the administration of different probiotics resulted in a significant increase in body weight gain in all feeding rats compared with the control group. Feeding rats with different probiotics led to an increase in Hb and PVC in all groups of rats. An increase in the count of both white blood cells and lymphocytes occurred in all feeding rats. A general reduction in ALT, AST and bilirubin was observed in plasma of all feeding rats. A significant decrease was observed in creatinine level of all feeding rats. Concerning urea, a significant reduction in urea level was found in plasma of all feeding rats. Feeding rats with these probiotics showed improvement in lipid metabolism. A significant reduction in cholesterol level was observed in plasma of all feeding rats. An effective decrease in the level of TG, LDL and VLDL and increase in the level of HDL was observed in plasma of all feeding rats compared with the control group. Administration of probiotics showed significant increase in total serum protein especially globulin compared with control group. ELISA analysis showed the presence of marked variation in immunoglobulins level of all feeding rats compared with control group.

Keywords: Probiotics, Immunological, Biological Systems, Experimental Animals.

Probiotics are living microbial cultures or cultured dairy product which beneficially influences the health and nutrition of the host1. *Lactobacillus, Leuconostoc* and *Pediococcus* species have been used extensively in food processing throughout human history, and ingestion of foods containing live bacteria, dead bacteria, and metabolites of these microorganisms has taken place for a long time2. Table 1 listed a few microorganisms which received attention as candidate probiotics3,4. Probiotics are typically provided in products in one of three ways:

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MATERIALS AND METHODS

Milk and dairy product samples used in the present study were freshly collected in sterile jars. Samples were kept under cooling conditions and used in the preparation of probiotics as soon as possible. The organisms used throughout the experiment were two isolates, one bacterial strain namely: *Lactobacillus acidophilus* and was kindly provided by Microbiology Laboratory, Faculty of Science, Alexandria University, and a yeast strain namely: *Saccharomyces cerevisiae* y-1347 was kindly provided by MIRCEN (Microbiological Resource Center Ain Shams University). All the prepared reagents used in the present investigation were provided by Fluka, Germany, and all the kits were obtained from Bicon, Germany. Media used, throughout the present study, were supplied by Oxoid Ltd, United Kingdom. Sixty three male *Sprague Dawley* rats, 3 - months old, weighing 180±10gm were obtained from the animal house, Beirut Arab University, Lebanon. The rats were assigned to seven groups each including nine rats and kept in a cage under conventional conditions of temperature and humidity for 90 days. All groups of rats received the commercial diet and tap water and divided according to the treatment administered daily through a gastric tube needle as follows: Group I: control group; Group II: Goat yogurt group; Group III: Cow yogurt group; Group IV: *Saccharomyces cerevisiae* goat yogurt group; Group V: *Lactobacillus acidophilus* goat yogurt group; Group VI: *Saccharomyces cerevisiae* cow yogurt group and Group VII: *Lactobacillus acidophilus* cow yogurt group. All the rats were acclimatized to the respective diets for one week before the experiment started. Body weight was measured before, during and after the experiment. The body weight was recorded weekly, using manual balance. At the end of the experiment, rats were anesthetized with chloroform, after an overnight fasting, through inhalation. Blood samples were taken from the abdominal aorta of the rats. Fractions of whole blood samples were collected in heparinized tubes (2U/ml) for determination of total blood count, while the remaining samples were collected into sterile tubes and kept at room temperature for 30 min, then centrifuged at 3000Xg for 15 min to obtain sera which were stored at - 20°C for further chemical analysis, which were represented by testing the levels of glucose, total cholesterol, triglyceride, urea, creatinine, SGOT-AST, SGPT-ALT, bilirubin, HDL, total protein albumin and immunoglobulins in the rats’ serum using (Bicon, Germany) kits.

Data of the present study were subjected to standard one-way analysis of variance (ANOVA) with probability (P) values ≤ 0.05 considered statistically significant using the COSTAT 2.00 statistical analysis software manufactured by CoHort Software Company.

RESULTS

Figure 1 shows the effect of different probiotic treatments on body weight gain of rats within 90 days. There was a significant increase (P ≤ 0.05) in the body weight gain in all of the experimental animals. Figure 2 shows the effect of different probiotics treatments on hematological parameters of *Sprague Dawley* rats, there was a significant (P<0.05) increase in hemoglobin (Hb)

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Table 1. Microorganisms used as probiotics

| Lactobacillus          | Bifidobacterium | Enterococcus | Others                          |
|------------------------|-----------------|--------------|---------------------------------|
| *L. acidophilus*       | *B. adolescentis* | *E. faecium* | *Streptococcus thermophilus*    |
| *L. plantarum*        | *B. animalis*   | *E. faecalis* | *Streptococcus salivarius*      |
| *L. casei*            | *B. bifermentum*| *E. faecalis* | *Escherichia coli*              |
| *L. rhamnosus*        | *B. breve*      |              | *Bacillus coagulans*            |
| *L. delbrueckii spp. Bulgaricus* | *B. infantis* |              | *Bacillus clausii*              |
| *L. fermentum*        | *B. longum*     |              | *Saccharomyces cerevisiae*      |
| *L. johnsonii*        | *B. lactis*     |              |                                 |
| *L. gasseri*          |                 |              |                                 |
| *L. salivarius*       |                 |              |                                 |
| *L. reuteri*          |                 |              |                                 |
Parameters as bilirubin, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured in the plasma of rats in order to assess the liver function. Figure 3 shows the effect of different probiotic treatments on the liver function of rats. A significant reduction (P<0.05) in alanine aminotransferase (ALT) activity in plasma levels of rats fed with goat yogurt, cow yogurt, *Saccharomyces cerevisiae* goat yogurt, *Lactobacillus acidophilus* goat yogurt, *Saccharomyces cerevisiae* cow yogurt and *Lactobacillus acidophilus* cow yogurt. Also, a significant reduction (P<0.05) in aspartate amino transferase (AST) activity was noticed in the plasma levels of rats in relation to the control. Otherwise, the lowest activity of ALT and AST was found in plasma of rats fed with *Lactobacillus acidophilus* cow yogurt. A significant decrease (P<0.05) in bilirubin concentration was noticed in plasma of all experimental rats. Figure 4 shows a significant decrease (P<0.05) in creatinine levels in plasma of all probiotics fed rats in relation to the control group. Concerning the urea level, results showed a significant reduction (P<0.05) in plasma of rats fed with *Saccharomyces cerevisiae* cow yogurt and *Lactobacillus acidophilus* goat yogurt (59% and 83.2% respectively). The highest reduction of urea level was noticed in the plasma level of rats fed on *Lactobacillus acidophilus* goat yogurt. The present study showed a great effect of different probiotic treatments on the lipid profile in the experimental rats. Results obtained in figure 5 shows a significant decrease (P<0.05) in the cholesterol level, triglyceride level, LDL and VLDL plasma level. On the other hand, a significant increase (P<0.05) in high-density lipoprotein (HDL) level was revealed in plasma of rats fed on different probiotics in relation to the control.

Figure 6 shows the effect of different probiotic treatments on blood glucose level. It has been remarkably noticed that there is no significant (P<0.05) change in the blood glucose level of the experimental animals fed with different probiotics in relation to the control group.

Figure 7 shows the effect of different probiotic treatments on serum proteins. A significant increase (P<0.05) in protein and globulin concentrations were observed in serum of rats fed on goat yogurt, cow yogurt, *Saccharomyces cerevisiae* goat yogurt, *Lactobacillus acidophilus* goat yogurt, *Saccharomyces cerevisiae* cow yogurt and *Lactobacillus acidophilus* cow yogurt in comparison with the control group. The highest decrease (P<0.05) in the albumin concentration was revealed by rats fed on *Lactobacillus acidophilus* cow yogurt.

Figure 8 shows the highest increase in immunoglobulin A (IgA) concentration was recorded in serum of rats fed on *Lactobacillus acidophilus* cow yogurt, followed by rats fed on *Lactobacillus acidophilus* goat yogurt. Concerning the immunoglobulin G (IgG) concentration, a
significant increase ($P<0.05$) was achieved in rats fed on *Lactobacillus acidophilus* goat yogurt and *Lactobacillus acidophilus* cow yogurt respectively.

**DISCUSSION**

Results demonstrated the highest body weight gain was noticed in Sprague Dawley rats.

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**Fig. 2.** Effect of different probiotic treatments on hematological parameters: a, hemoglobin; b, Hematocrit; c, Red blood cells; d, White blood cells and e, Lymphocytes

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fed with *Saccharomyces cerevisiae* goat yogurt and *Saccharomyces cerevisiae* cow yogurt. These results coincide with those reported by\(^9\) who mentioned that *Lactobacillus plantarum* isolated from fermented corn slurry is safe and can improve the performance of rats in terms of weight gain. Probiotics also produce many important enzymes and increase the availability of vitamin B, vitamin K, fatty acids and calcium as well as the enhancement of mineral availability such as iron\(^10\). *Bifidobacterium longum* SPM 1205 have no adverse effect on the general health status, such as behavior and activity, of Sprague Dawley rats\(^11\).

Sprague Dawley rats fed with *Lactobacillus acidophilus* goat yogurt showed significant improvement in the hematological parameters where significant increase in the packed value cells (PVC), hemoglobin (Hb) and RBCs count was recorded in treated rats. These results were similar to those obtained by\(^9\) who revealed that *Rattus norvegicus* rats fed with *Lactobacillus plantarum* showed signs of better health based on their hematological status. Four weeks feeding with *Lactobacillus rhamnosus* HN001 (DR20), *Lactobacillus acidophilus* HN017, and *Bifidobacterium lactis* HN019 (DR10) strains

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**Fig. 3.** Effect of different probiotic treatments on liver function: a, ALT; b, AST and c, Bilirubin
had no adverse effects on mice general health status, hematolology, blood biochemistry, gut mucosa histology or the incidence of bacterial translocation\textsuperscript{12}. Oral ingestion of lactic acid bacteria by \textit{Sprague Dawley} rats increases the proliferation of lymphocytes\textsuperscript{13}. Leucocyte is important in defense mechanism against infection\textsuperscript{14}. The primary role of lymphocyte is in humoral antibody formation and cellular immunity\textsuperscript{14,15}. Feeding with \textit{Bifidobacterium lactis} HN019 resulted in an increase of peripheral blood leucocytes and natural killer cells were active in tumor killing and viral destruction\textsuperscript{16,17}. Such results coincide with that reported by\textsuperscript{18} who found a significant reduction in AST and ALT serum level in rats fed with fermented soybean as probiotics. Serum AST, ALT and total bilirubin concentration showed slight improvement in rats fed with lactulose and probiotics\textsuperscript{19}.

The obtained results coincide with\textsuperscript{20} who observed lower AST and ALT activities in Wistar albino rats fed with \textit{Lactobacillus casei}. Also, significant reduction in bilirubin plasma level was noticed in \textit{Sprague Dawley} rats fed with \textit{Lactobacillus plantarum}\textsuperscript{21}. \textit{Sprague Dawley} rats fed on \textit{Lactobacillus acidophilus} goat yogurt recorded the highest reduction in urea plasma level, however the highest reduction in creatinine level was noticed with \textit{Saccharomyces cerevisiae} cow yogurt and \textit{Lactobacillus acidophilus} cow yogurt fed rats respectively. These results were similar to those obtained by\textsuperscript{22} who found that dietary probiotics decrease urease activity and subsequently reduce ammonia production in the small intestine of \textit{Sprague Dawley} rats. It was also reported that the use of probiotic \textit{Lactobacillus delbrueckii} reduced plasma urea concentration\textsuperscript{23}. The use

\begin{figure}[h]
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\includegraphics[width=\textwidth]{Fig_4}
\caption{Effect of different probiotic treatments on kidney function: a, Creatinine and b, Urea}
\end{figure}

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of probiotic bacteria is reported to decrease blood urea and serum creatinine levels in rats\textsuperscript{24,25}. Probiotics showed an excellent effect on lowering the level of ammonemia and endotoxemia of rats\textsuperscript{26}. In the present investigation, it was revealed that probiotic administration has modulated the lipid metabolism of all fed rats. These data were similar to those presented by\textsuperscript{27} who stated that probiotic intervention lead to changes in serum global lipid profile. A significant reduction in the plasma cholesterol concentration was observed in rats treated with \textit{Lactobacillus plantarum} I-UL4. The results indicated that the metabolite produced by \textit{Lactobacillus plantarum} I-UL4 had lowering effect on plasma cholesterol in rats\textsuperscript{28}. Sprague Dawley rats fed on \textit{Lactobacillus acidophilus} cow yogurt revealed a significant reduction in serum LDL and VLDL. Similar results were obtained by\textsuperscript{29} who recognized low LDL level in rats fed on fermented soybean. Fermented milk produced from \textit{Lactobacillus gasseri} significantly reduced LDL of rats\textsuperscript{30}. In the present study, Sprague Dawley rats fed on \textit{Lactobacillus acidophilus} cow yogurt showed a significant elevation in HDL level. Remarkable increase in HDL level was noticed in rats fed on yogurt supplemented with \textit{Bifidobacteria}\textsuperscript{31}. \textit{Lactobacillus sporogenes} improved HDL level in blood\textsuperscript{32}. Upon investigating the influence of probiotics on glucose blood level it has been found that there is no significant change in the blood glucose level of all fed \textit{Sprague Dawley} rats. These findings were inconsistent with that obtained...
by 33 who reported that probiotics had no effect on healthy rats but reduced the blood glucose level in diabetic rats. Probiotics can obviously improve the glucose absorption in craniocerebral injury rats where results showed reduction in glucose serum level as well as glucose urine level 34. The use of Saccharomyces cerevisiae goat yogurt and Saccharomyces cerevisiae cow yogurt was considered the best treatment for the stimulation of the immune system; these results were in agreement with that obtained by 35 who revealed that the use of probiotics was associated with higher levels of total serum protein due to increased globulin levels. Probiotic bacteria have a possible competition for nutrients with pathogens in the digestive tract 36 or the hypothetical stimulation of the immune system, as the activation of macrophage 37.

The lowest albumin concentration was achieved by rats fed on Lactobacillus acidophilus cow yogurt. These data coincide with that obtained by 38 who found Wistar albino rats’ serum albumin was significantly reduced with Lactobacillus acidophilus, Bifidobacterium bifidum and Enterococcus faecium. Similar findings were observed by 39 who found that probiotic treatments activated protein metabolism in animal, redistributed protein fractions for raising globulin content and decrease albumin concentration in serum.

In the present study, it was revealed that Sprague Dawley rats fed on Lactobacillus acidophilus cow yogurt recorded the highest increase in immunoglobulin A (IgA) concentration as well as immunoglobulin G (IgG) concentration. Probiotics also enhance humoral immune responses by increasing Immunoglobulin A (IgA) producing cells and stimulating antibody responses to some specific antigens 40. Rats of AGUS strain fed with Lactobacillus plantarum showed increased IgG levels 41. Probiotic-supplemented milk formula (milk supplemented with Bifidobacteria) were not significantly affecting IgG antibody titers 42.
Fig. 6. Effect of different probiotic treatments on blood glucose level of *Sprague Dawley* rats.

Fig. 7. Effect of different probiotic treatments on Plasma proteins: a, Total protein; b, Albumin and c, Globulin.
Fig. 8. Effect of different probiotic treatments on the immunoglobulin concentration of Sprague Dawley rats: a, IgG and b, IgA.

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