Research Journal of Biological Sciences 3 (12): 1376-1380, 2008
ISSN: 1815-8846

Study on the Effect of Consuming Different Amount of Fermented Milk with *Lactobacillus casei* 01 on Haematological Parameters in Rats

[1]Hamid Mirzaei, [2]Bahram Amouoghli Tabrizi, [2]Ali Hasanpour, [3]Alireza Babapour and [4]Guity Karim
[1]Department of Food Hygiene, [2]Department of Clinical Science, [3]Graduated of Veterinary Medicine,
Faculty of Veterinary Medicine, Islamic Azad University, Tabriz Branch, Tabriz, Iran
[4]Department of Food Hygiene, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran

Abstract: The potential use of probiotics in restoring the urogenital and gastrointestinal health has received tremendous interest in the last decade, while few safety concerns are still being debated. Effect of oral administration of different doses of probiotic *Lactobacillus casei* 01 on the haematological parameters, serum protein and albumin of albino rats was investigated. Thirty two rats divided into four groups (A, B, C and D) of 8 rats per group were used. Group A was placed on the basal diet with pure milk alone, while group B, C and D were placed on the diet and also treated with 32, 64 and 96 mL of 10^7 cfu mL^{-1} of milk cultured of *Lactobacillus casei* 01, respectively for 45 days. Blood samples were collected by carotid artery cannulation for terminal bleeding into potassium EDTA containers. Parameters were determined using autohaematological analyzer. Results show there are significantly increase ($p<0.01$) in PDW, PMV and lymphocytes% by control group rats when compared to the other group rats. In rats treated with lowest doses of *Lactobacillus casei* 01 there were significantly increase ($p<0.01$) in Total protein, albumin, WBC and Mid amount when compared with the control, also amount of Granulocytes, HGB, RBC count and HCT in the highest dose group of *Lactobacillus casei* 01 are significantly highest ($p<0.01$) except RDW-CV that it was significantly increase ($p<0.01$) to the C group rats when compared with control group. In other parameter was not found meaningful different ($p<0.05$). The data obtained showed that milk fermented *Lactobacillus casei* 01 is safe and it has immunostimulatory effect. Its effect on haematological parameters was dose dependent.

Key words: *Lactobacillus casei*, rats, haematological parameters

INTRODUCTION

Friendly bacteria, called probiotics call our intestinal tract home. The reason they are called probiotic bacteria because the term probiotic means for life, as opposed to the term antibiotic which means against life. Our bodies are actually designed to have a harmonic relationship with these probiotic bacteria. They help us digest the food, kill harmful microorganisms and keep us functioning properly in a number of ways. The rationale for the ingestion of microbes in fermented Food products elucidated by Elie Metchnikoff's hypothesis, have received tremendous attention in recent times. Probiotics is defined by the Food and Agriculture Organization of the United Nations and the World Health Organization as live microorganisms which when administered in adequate amounts confer a health benefit to the host. To date, probiotic products have mainly been delivered as freeze-dried cells or fermented foods, restricted to improving the health and well-being of the intestinal tract of humans, particularly in prevention and treatment of diarrhea. Probiotic lactobacilli are known to confirm an array of health promoting activities on their host after either parenteral or oral administration (DeWaard *et al*., 2001; Oyetayo *et al*., 2003; Mirzaei, 2004). Some of their beneficial effects include prevention of intestinal infection (Mirzaei *et al*., 2006), anti carcinogenic activity (Fuller and Gibson, 1997), control of serum cholesterol (Bertazzoni *et al*., 2001), enhancement of immunity (Aattouri *et al*., 2002), growth enhancement of animals (Bomba *et al*., 2002; Chang *et al*., 2001). The mechanism by which these probiotics affects their host and bring and bring about improvement in the gut barrier can be due to competition for adhesion site, production of inhibitory compounds and rebalancing of disturbed gastrointestinal microbial composition and metabolism (De Waard *et al*., 2001; FAO/WHO, 2001). Lactobacilli have a long history of use as probiotics without established risk to humans (Naidu *et al*., 1999).

Corresponding Author: Hamid Mirzaei, Department of Food Hygiene, Faculty of Veterinary Medicine, Islamic Azad University, Tabriz Branch, Tabriz, Iran

No pathogenic or virulence properties had being associated with lactobacilli, bifidobacteria or *lactococci* (Aboderin and Oyetayo, 2006). There is however, repots that under certain condition some *lactobacilli strains* have been associated with adverse, such as rare cases of bacteremia. It has been observed that lactic acid bacteria may invade the host body by bacterial translocation or other routes causing bacteremia (Aboderin and Oyetayo, 2006; Berg, 1999). However, a study showed that there is no increased incidence or frequency of bacteremia with increased usage of probiotic lactobacilli (Salminen *et al*., 2002). The reports above thus, makes it necessary to ascertain the safety of any organism with probiotic properties. In the other study effects of probiotic *Lactobacillus casei* 01 in the enable to adhesion and transiently colonies in the gastro-intestinal tract investigated (Ouwehand *et al*., 1999). Effect of *lactobacillus plantarum* with differential doses in haematological parameter studied and result showed that *Lactobasillus plantarum* has immunostimulatory effect and can also improve the performance of rats in terms of weight gain and of course improve haematological parameters in treatment groups (Aboderin and Oyetayo, 2006). Promoting growth effect, composition of gastrointestinal micro flora and blood parameters in newborn calves with probiotic yeasture studied and there are no any significantly different between treatments and control groups. To further confirm the safety of ingestion of this probiotic, a study was designed to investigate the effect of *Lactobacillus casei* 01, on the haematological parameters of healthy rats. A haematological study is a valuable diagnostic tool in evaluating human health (Cheesborough, 1991).

The aim of the present study was therefore to gain insight into the effect of orally administered *Lactobacillus casei* 01 on the haematological parameters of rats. Moreover, the effective dose that will bring about health promoting effect in rats was also studied.

MATERIALS AND METHODS

Source of *Lactobacillus casei* 01: Strain of *Lactobacillus casei*, which was received from Chr-Hansen (Horsholom, Denmark) institute, was being added in sterilized and homogenized milk with 1.5% of fat containing 1 mL of solution of probiotic per 100 mL of milk into the sterilized 1000 mL Erlenmeyer flask then it was incubated in 37°C for 48 h. Simultaneously, certain amount of *Lactobacillus casei* 01 stock were purified and kept under the anaerobic and sterilized condition, for further use. After this period, the milk in the Erlenmeyer flask was clotted and transformed to the yogurt shape in feature. The bacterial counting experiments by using the decreasing dilution providing method and investigation of anaerobic cultivation was conducted into a glass jar in MRS agar culture medium in order to make sure that there are no secondly factors and impurity.

***In vivo* feeding trial:** Thirty two male rat of race albino wistar, 180±30 g obtained from Pasteur Institute of Iran, were distributed among 4 groups of A, B, C and D, each of which containing 8 rats. Firstly, they passed the Adaptation period within a week under the cycle of 12 h light and 12 h darkness, under the base diet. On the 0th day, the weight of each rat was measured and recorded. Every day for 45 days, the B, C and D groups were fed respectively 32, 64 and 96 mL of the homogenated cultivated solution of probiotic containing 10^7 cfu mL^{-1} using the oral method. To the group A, only 1.5% fat milk without any probiotic was given. During the period the amount of the required food and water was monitored and at the end of period, the amount of the required food and water was scaled.

Blood analysis: Blood samples were collected from rats by carotid artery cannulation into potassium EDTA bottles. Cell counter (model: systmex KX21 Japon) was used to analyze the following blood parameters: Haemoglobin (HGB), Red Blood Cell (RBC), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Platelets (PLT), Mean Corpuscular 1 Haemoglobin (MCH), White Blood Cell (WBC), Hematocrit (Hct), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Granulocyte (Gran). Albumin and Total protein in serum were measured by common kits (Zist chim) and by spectrometer (Bio Wave S1 2000).

Statistics: Data gathered from haemotological parameters were analyzed using one way analysis of variance. Means were compared using TUKEY HSD test, all statistical analyses were performed with SPSS soft ware version 15.

RESULTS

The full blood count of rats is presented in Table 1. The albumin in group B was highest and there was significantly different ($p<0.01$) between B and C, moreover the albumin amount of rats in control group (A) was the same with group D. The amount of the total protein in group which had received the least amount of *Lactobacillus casei* 01 dose (B) in comparison with other groups was reported highest also the minimum of Total protein like albumin belonged to group C and there were meaningful difference between the group C and groups

Table 1: Full blood count of rats orogastrically dosed with different concentrations of *Lactobacillus casei* 01

Full blood count	A	B	C	D
ALB (G/DL)	2.77±0.03^{ab}	2.95±0.04^{b}	2.51±0.14^{a}	2.77±0.11^{ab}
T PRO (G/DL)	5.97±0.09^{b}	6.23±0.08^{b}	5.26±0.22^{a}	5.91±0.08^{b}
WBC×10^{3}/uL	7.08±0.93^{b}	7.25±0.77^{b}	5.47±0.78^{ab}	2.61±0.40^{a}
Lymph%	68.52±3.98^{b}	39.14±8.92^{a}	56.43±7.71^{ab}	35.22±8.05^{a}
Gran%	24.11±3.42^{a}	35.58±2.88^{ab}	34.62±6.61^{a}	55.67±7.20^{b}
HGB (G/DL)	12.62±0.85^{b}	12.12±0.19^{b}	9.81±0.57^{a}	15.12±0.39^{c}
RBC×10^{6} uL^{-1}	7.01±0.47^{b}	6.84±0.15^{b}	4.94±0.76^{a}	8.53±0.20^{b}
HCT%	35.70±2.27^{b}	34.82±0.49^{b}	24.92±3.79^{a}	41.46±1.15^{b}
RDW-CV%	12.78±0.79^{a}	14.31±0.18^{ab}	15.12±0.44^{b}	14.86±0.27^{b}
PMV (fL)	7.02±0.19^{b}	6.78±0.08^{ab}	6.51±0.20^{ab}	6.37±0.07^{a}
PDW	15.63±0.22^{b}	15.18±0.23^{ab}	15.30±0.27^{ab}	14.60±0.03^{a}

Values are means±SE for 8 rats per group. Means along the row with different superscript are significantly different ($p<0.05$)

Table 2: Hematological parameters did not have significant differences in four groups

Full blood count	A	B	C	D
Lymphocyte≠10^{3} uL	15.31±9.81^{a}	3.53±0.32^{a}	2.44±0.62^{a}	0.96±0.44^{a}
Gran≠10^{3}	1.61±0.09^{a}	2.56±0.37^{a}	2.02±0.64^{a}	1.10±0.31^{a}
Mid%	7.60±1.45^{a}	7.35±2.43^{a}	8.87±2.49^{a}	10.54±3.03^{a}
MCV	51.08±0.86^{a}	50.98±0.76^{a}	50.55±0.74^{a}	49.01±0.74^{a}
MCH	17.61±0.24^{a}	17.67±0.22^{a}	17.54±0.24^{a}	17.65±0.12^{a}
MCHC	30.43±2.87^{a}	34.77±0.16^{a}	34.82±0.34^{a}	34.87±1.13^{a}
RDW-SD	27.57±1.18^{a}	26.80±0.28^{a}	27.44±0.63^{a}	26.91±0.39^{a}
Plt×10^{3}	464.12±53.27^{a}	531.5±37.4^{a}	427.87±68.73^{a}	483.87±25.74^{a}
PCT	0.32±0.03^{a}	0.36±0.02^{a}	0.28±0.04^{a}	0.3±0.01^{a}

Values are means±S.E. for 8 rats per group. Differences among the means of the mentioned parameters in four groups were not statistically significant

(A, B, D), with ($p<0.01$) C was lower than groups A, D and with ($p<0.001$) was lower than group B too. WBC count's group B liked the two parameters above and group D which had received the highest dose of *Lactobacillus casei* 01 was lowest, the amount of group D's WBC with ($p<0.01$) was significantly lower than group A and B. The Mid×10^{3} amount was observed like WBC as well but there was only a significantly difference between group A and B with ($p<0.01$). The Lymphocyte% was highest in control (A) and significantly different from the other treatments with ($p<0.01$) except C. The MPV and PDW were highest in control and significantly different from the D group rats both with ($p<0.01$). Granulocytes% in group D was significantly highest when compression with control and all of treatment groups also there was a significantly different between D and A with ($p<0.01$).The amount of HGB in group C and D, respectively, were the highest and the lowest amount. The amount of HGB in group control with ($p<0.01$) is lower than group D, moreover with ($p<0.01$) higher than group C. In counting, the amount of RBC×10^{3} and HCT group D was highest and group C was lowest when compression with control and treatment groups and there was significantly different between D and C with ($p<0.01$). Investigating RDW-CV% the maximum percentage was observed in group C and minimum in group A. The average percentage of this parameter was significantly ($p<0.01$) lower in the control than treatment groups. It should be noted that studying the parameters, Lymphocyte. Gran, Mid%, MCV, MCH, MCHC, RDW-SD, PT, PCT, a meaningful difference ($p<0.05$) was not found among the four groups (A, B, C and D) while referring to the Table 2 as in which the numbers are mentioned.

DISCUSSION

There has been a number of studies that reveal the probiotic potential of Lactobacillus as health promoting bacteria in man and human (FAO/WHO, 2001). In the last two decades the potential of probiotic lactobacilli to restore gastrointestinal health has received interest. *Lactobacillus casei* 01 is one of the bacteria associated with the fermented milk productions. To assess the safety of these probiotic bacteria *L. case* 01, it was fed to wistar albino rats. The hematological parameters of rats was observed. The result of hematological parameters shows that rats dosed with *L. casei* 01, show signs of better health based on their hematological status. In a previous study (Shu *et al*., 1999), reported that 4 weeks consumption of *L. rhamnosus* HN001 (DR20), *L. acidophilus* HN017 and *Bifidobacterium lactics* HN019 (DR10) strains had no adverse effects on mice general health status, hematology, blood biochemistry, gut mucosa histology or the incidence of bacterial translocation. There were general increase in the count of HCT, RBC, HGB, GRAN% especially in rats dosed high with *Lactobacillus casei* (D) when compared to the control (A) (Table 1). A high level of Hb, PCV and RBC is an indication that the rats are not anemic while a lower level is a sign of anemia (Aboderin and Oyetayo, 2006;

Cheesborough, 1991). The WBC (leucocytes) counts was higher in the low probiotic dose group (B) when compared to the control and other treatments dosed with *Lactobacillus casei* 01 however, there is not more different between (A and B). White Blood Cell (WBC) is important in defending our body against infection (Aboderin and Oyetayo, 2006). The leucocytes counts however cannot give specific information and this necessitated the differential leucocytes counts. In the differential leucocytes count, the Gran% were lower in the rats of the control (A) group when compared to groups B, C and D. Neutrophil is majorly responsible for phagocytosis of pathogenic microorganisms during the first few hours after their entry into tissues. The lymphocyte % of rats dosed with *Lactobacillus casei* 01 was lower and significantly different ($p<0.01$) in groups B and D when compared with the control (A). The primary role of lymphocytes is in humoral antibody formation and cellular immunity (Aboderin and Oyetayo, 2006). In essence, the increase in the lymphocyte count observed for rats in group control shows sign of immuno-mostimulatory effect. had earlier reported that oral ingestion of lactic acid bacteria by rats increases lymphocyte proliferation and interferon a production (Aattouri *et al*., 2002). The mean lymphocytes counts of the probiotics (*Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14) fed rats did not change significantly, indicating that these two strains did not invoke any peripheral lymphocytosis in healthy animals. A human study with these strains, results shows that oral ingestion of *L. rhamnosus* GR-1 and *L. fermentum* RC-14 did not lead to immunoglobulin or cytokine changes beyond normal values.

This finding indicates that in healthy individuals, consumption of probiotics is safe In previous study which was designed to assess the effect of probiotic Yeasture on growth, composition of gastrointestinal microflora and blood parameters in newborn calves, results showed that probiotic Yeasture in experimental group significantly on 43 g (8.9 %) increased the average daily weight gains compared to control group ($p<0.05$). There were no significant differences in the hematological indicators of blood between two groups (Oberauskas *et al*., 2006). Total protein and albumin which investigated in the serum, both were increased at the control and lowest probiotical groups so they were same. Diet supplemented with probiotics (*Streptococcus thermophilus* and *Lactobacillus helveticuson*) the wistar rats showed Total serum proteins and globulin were higher in probiotic animals (Dock-Nascimento *et al*., 2007). The results obtained from these study showed that milk fermented by *Lactobacillus casei* 01 is safe and it has immunostimulatory and haematological parameters effects which were dose dependent.

REFERENCES

Aattouri, N., M. Bouras, D. Tome, A. Marcos and D. Lemonnier, 2002. Oral ingestion of lactic acid bacteria by rats increases lymphocyte proliferation and interferon-gamma production. Br. J. Nutr., 87 (4): 367-373.

Aboderin, F.I. and V.O. Oyetayo, 2006. Haematological studies of rats fed different doses of probiotic, *Lactobacillus plantarum*, isolated from fermenting corn slurry. P. J. Nutr., 5 (2): 102-105.

Berg, R.D., 1999. Bacterial translocation from the gastrointestinal tract. Adv. Exp. Med. Biol., 473: 11-30.

Bertazzoni, M.E., A, Benini, M, Marzotto, H. Hendriks, A. Sbarbati and F. Dellaglio, 2001. Preliminary screening of health-promoting properties of new *Lactobacillus strain*: *In vitro* and *in vivo*. HEALFO abstracts, Italy.

Bomba, A., R. Nemcova, S. Gancarcikova, R. Herich, P. Guba and D. Mudronova, 2002. Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. Br. J. Nutr., 88 (1): S95-S99.

Chang, H., J. Kim, H. Kim, W. Kim, Y. Kim and W. Park, 2001. Selection of a potential probiotic *Lactobacillus strain* and subsequent *in vivo* studies. Antonie Van Leeuwenhoek, 80: 193-199.

Cheesborough, M., 1991. Medical Laboratory Manual for Tropical Countries. 2nd Edn. Tropical Health Technology and Butterworth Scientific Limited, 1: 494-526.

De Waard, R., J. Garssen, J. Snel, G.C.A.M. Bokken, T. Sako, J.H.J. Huis, T. Inveld and J.G. Vos, 2001. Enhanced antigen-specific delayed-type hyper-sensitivity and immunoglobulin G2b responses after oral administration of viable *Lactobacillus casei* YIT9029 in Wistar and brown Norway rats. Clin. Diagn. Lab. Immunol., 8 (4): 762-767.

Dock-Nascimento, D.B., J. Kelly, E. Jose and de. Aguilar-Nascimento, 2007. Rapid restoration of colonic goblet cells induced by a hydrolyzed diet containing probiotics in experimental malnutrition. Acta. Cir. Bras., 22: 72-76.

FAO/WHO, 2001. Report of a joint FAO/WHO. Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria (LAB). American Cordoba Park Hotel, Cordoba, Argentina, pp: 1-4.

Fuller, R. and G.R. Gibson, 1997. Modification of the intestinal microflora using probiotics and prebiotics. Scand. J. Gastroenterol. Suppl., 222: 28-31.

Mirzaei, H., 2004. Probiotics and Introduction to Their Role in Human Health. 1st Edn. Islamic Azad University Press, pp: 1-2.

Mirzaei, H., S. Mahboob, K. Kazeman-Alanag, G. Karim, 2006. Interaction of *Bifidobacterium bifidum*, *B. angulatum* and *Lactobacillus casei* with *Salmonella typhimurium* during Co-culture. J. Ardabil University Med. Sci. Health Services: JAUMS, 6: 409-416. ISBN: 1735-2452.

Naidu, A.S., W.R. Bidlack and R.A. Clemens, 1999. Probiotic spectra of Lactic Acid Bacteria (LAB). Crit. Rev. Food. Sci. Nutr., 39 (1): 13-126.

Oberauskas, V., R. Sutkeviciene, J. Laugalis, R. Zelvyte, I. Monkeviciene, I. Kantautaite and A. Sederevicius, 2006. The effect of probiotic yeasture on the growth, microflora of the digestive system and health of neonate calves. Veterinarija Ir Zootechnika, 34: 29-35.

Ouwehand, A.C., P.V. Kirjavainen, M.M. Gronlund, E. Isolauri and S.J. Salminen, 1999. Adhesion of probiotic micro-organisms to intestinal mucus. Int. Dairy J., 9 : 623-630.

Oyetayo, V.O., F.C. Adetuyi and F.A. Akinyosoye, 2003. Safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent *in vivo*. Afr. J. Biotechnol., 2 (11): 448-452.

Salminen, M., S. Tynkkynen, H. Rautelin, M. Saxelin, M. Vaara, P. Ruutu, S. Sarna, V. Valtonen and A. Jarvinen, 2002. *Lactobacillus bacteremia* during a Rapid Increase in Probiotic Use of *Lactobacillus rhamnosus* GG in Finland. Clin. Infect. Dis., 35: 1155-1160.

Shu, Q., J.S. Zhou, K.J. Rutherfurd, M.J. Birtles, J. Prasad, P.K. Gopal and H.S. Gil., 1999. Probiotic lactic acid bacteria (*Lactobacillus acidophilus* HN017, *Lactobacillus rhamnosus* HN001 and *Bixdobacterium lactis* HN019) have no adverse effects on the health of mice. Int. Dairy J., 9: 831-836.