Nat.Volatiles&Essent.Oils,2021;8(5):8884-8892

NVEO
Natural Volatiles &
Essential Oils

Efficacy of fermented and unfermented cow's milk on the immune status and microbial balance of the gut in laboratory rats

***Rami Ahmed Abbas*[1] *Karkaz M. Thalij*[2]**

1,2Department of Food Sciences, College of Agriculture, Tikrit University, Tikrit, Iraq

Absract

The study was conducted in the laboratories of the Department of Food Science at the College of Agriculture, as well as in the animal house of the College of Veterinary Medicine at Tikrit University, for the period from the 1st of October 2019 until the end of February 2020. The aim of the study is determine the effect of some species of lactic acid bacteria in two groups: the first as (B1) *Streptococcus thermophillus*+ *Lactobacillus bulgaricus*+ *Lb.acidophilus* + *Bifidobacterium animalis ssp* and the second group of (B2) *S.thermophillus* + *Lb.bulgaricus* used in the fermentation of cow's milk and estimating the growth rate parameters, hematological and biological parameters, In addition the balance of the normal microbial flora in the intestine after feeding laboratory rats induced with immune reduction with Sandimmune and raising them for 28 days.Laboratory rats were separated into 8 groups, each of which included 4 animals. T1: negative control, T2: positive control: fed on the main ration and 0.3 ml of Sandimmune, T3: fed on the main ration supplemented with unfermented cow's milk. , T4: fed on the main ration supplemented with unfermented cow's milk and 0.3 of Sandimmune, T5: fed on the main ration of fermented cow's milk from starter B1, T6: fed on the main ration of fermented cow's milk from the starter B1 and 0.3 of Sandimmune, T7: fed on the basic diet supplemented with fermented cow milk from starter B2, T8 fed on the basic diet supplemented with fermented cow milk from starter B2 and 0.3 of Sandimmune.

Oral administration of the immunosuppressant caused a significant decrease in the values of IgG and IgA immunoglobulins in laboratory rats, which were at 1002 and 1986 mg/dL, respectively, compared to their values in the control group, which were 1461 and 3101 mg/dL, respectively. Oral fermented and unfermented milk improved their values to be similar with their values in the control group. Also, the microbial balance of the natural flora in the intestines was disturbed in the case of oral administration of the immunosuppressant, and the number of lactic acid bacteria was at 154 mcg/g. And feeding on fermented milk or feeding on unfermented milk contributed to a positive microbial balance to the flora. normal in the intestine.

Key words: fermented cow's milk, immunity, gut microbial balance, laboratory rats.

introduction

Recent studies have proven that fermented food contributes effectively to enhance human health, but the interactions caused by the components of those foods when consumed or the organisms used to ferment them in the intestine have not been clearly defined so far. And as stated in the old saying, “A person knows what you eat,” “You are what you eat,” and the accuracy of this saying has been demonstrated through the availability of accurate evidence on the effect of the state of microbial balance or not on the health status of humans (Bokulich et al., 2016).

One of the most microbiologically active ecosystems is the gastrointestinal tract (GT), which contains a mass of bacteria necessary for the activation of immune cells. A large number of bacteria coexist in the alimentary canal that reaches the intestine through ingestion of food. It has been shown that the interaction between microbes and their effect on improving the state of the immune system includes complex and multifaceted interactions to reach their effects on a person's health status. The state of immune activation is not effected by the types of intestinal microbes through their direct interaction with epithelial cells, but rather through the effect of their metabolites (Hooper et al., 2012).

The quality of the microbial balance in the intestine is determined by some factors, including the genetic factor (Turnbaugh et al., 2009), and the environmental factor, which represents the most influential food in it. Recent studies have shown a clear relationship between the types of some microbes and the state of balance between them in the intestine and the occurrence of a number of diseases in the human body, especially obesity, diabetes and infections related to the intestine (Laatikainen et al., 2016 Nagata et al., 2016).

From the above and the importance of reaching to clarify the positive effect of the types of lactic ferments from cow's milk and the types of fermented bacteria they contain or the products resulting from them and their relationship with the immune status and microbial balance in the intestine. In the intestinal parts of animals treated with fermented and non-fermented lactic ferments

Materials and methods

Preparation of laboratory animals:

Healthy and disease-free laboratory animals were obtained from the College of Veterinary Medicine / University of Tikrit in numbers of 32 adult Albino rats of 8-9 weeks of age, their weights ranged between 620-210 g, randomly distributed into eight groups of close weights, each group included four Animals: The animals were placed in cages made of plastic, after their floors were covered with sawdust, which was replaced four times a week. The animals were fed regularly using a special ration prepared as in (NRC, 2010). The staple food contained (gm/kg) pure protein, 158.5gm, 100gm oil, 5gm mixed vitamins, 50gm mixed mineral salts, 50gm of cellulose, 100gm of glucose and 536gm starch. The animals were raised under the supervision of a specialized veterinary staff, taking into account the aspect of hygiene

Sandimmune was given through oral administration at a concentration of 0.3 µl/g of animal weight for 7 consecutive days, depending on what was mentioned in (Abdel-Hamid and Thalij., 2020).

Experience design:

The experiment was designed to include totals of laboratory animal treatments according to the type of food given, which included T1: negative control: animals fed the basic ration only, T2: positive control: animals fed the basic ration and a dose of 0.3 ml of Sandimmune, T3: animals fed the main ration added. It has unfermented cow's milk, T4: animals are fed on the basic ration supplemented with unfermented cow's milk and a dose of 0.3 Sandimmune, T5: animals are fed on the primary ration to which fermented cow's milk has been added from the starter B1, T6: animals are fed on the primary ration to which milk has been added. Bovine fermented from starter B1 and a dose of 0.3 of Sandimmune, T7: Animals are fed on the basic ration supplemented with fermented cow milk from the starter B2, T8: Animals are fed on the basic ration supplemented with fermented cow milk from the starter B2 and a dose of 0.3 of Sandimmune.

Blood sample collection:

After the end of the specified period of the experiment, the animals were anesthetized by chloroform, then blood samples were drawn directly from the heart using the Cardiac Puncture method. A quantity between 5-8 ml of blood was withdrawn, which was placed in test tubes free of anticoagulant and left for a quarter of an hour. Approximately at room temperature, then separating the serum using centrifugation at a speed of 3000 rpm for 15 minutes and keeping the serum at (-20)ºC in clean plastic tubes until biochemical tests are performed on them, as in (Tietz.,2005). Examinations in laboratories specialized in blood tests.

Immunoglobulin concentration determination:

The method of Radial Immuno Diffusion (RID) or the so-called (Mancini method) was used. The principle of action was to show a quantitative relationship between the antigen in acarose and the antibodies present in the serum. The test was performed according to the manufacturer's instructions if calibrated dishes were prepared for both IgA and IgG from the Spanish company BioSystems, then the dishes were opened, and left at laboratory temperature for 5 minutes, then 5 microliters of serum were added to the samples under study in digging each of the dishes containing IgA and IgG antibodies into the gel using a micro pipette. The dishes were left for 15 minutes at laboratory temperature without stirring. Then the dishes were placed in the refrigerator at 4 C for 48 hours for the dishes used. The diameter of the sedimentation rings formed around the pits as a result of the reaction of the specific antigen, which is rat serum, with its antiserum in the gel, was measured using a special scope for measuring immunoassays called (Immunoviewer). Finally, the concentration value was extracted by comparing the diameter of the sedimentation ring with the values attached to the table attached to the dishes prepared by the manufacturer, and the concentration reading was in mg/dL.

Estimation of the total number of microorganisms:

Samples were collected from the intestines of the animals and their contents were emptied from the remains of the digested food and washed with sterile distilled water to be ready for the process of estimating the bacterial species in them.

First:

Estimation of the total number of colon bacteria in the intestine: Weigh 10 g of each part of the animal intestine and add it to 90 ml of physiological saline. The necessary dilutions were made up to the sixth dilution and 0.1 ml of the fifth and sixth dilution was withdrawn and spread on MacConkey Agar medium. The plates were incubated upside down at 37 °C for 24 hours after which the colonies were counted on dish for each sample (Feng et al., 2002)

Second:

Estimation of the total number of lactic acid bacteria: 0.1 mL of the dilution prepared in the physiological solution in the previous step was withdrawn and spread on the surface of MRS-$CaCO_3$

solid medium and incubated anaerobic at 35 ºC for 24-48 hours. Then the numbers of cream-shaped colonies with clear areas around them were calculated (Hove et al., 1999.

Statistical analysis:

The data was statistically analyzed through the experimental system within the ready statistical program (SAS, 2012) and through the use of the complete random design system (CRD), as the averages were chosen according to the (Dancan, 1955) multi-range test to determine the significance of the differences between the averages of the factors affecting the studied traits in If it is at the level (0.05).

Results and discussion:

Efficacy of oral administration of fermented milk on the level of immunoglobulins in experimentally immunosuppressed laboratory rats.

Table (1) shows the effect of oral administration of fermented and unfermented cow's milk on the level of immunoglobulins in experimentally immunocompromised laboratory rats fed for 28 days. The results showed that the effectiveness of oral administration of fermented and unfermented cow's milk caused a significant ($p<0.05$) increase in the average values of IgA and IgG immunoglobulins in laboratory rats that had experimentally reduced immunity compared with the group that used Sandimmune alone.

From the results it was found that the values of the immunoglobulin parameters in the above had significantly decreased their levels ($p<0.05$) in the serum of the experimentally reduced immune laboratory rats, and their values were at 1002 and 1986 mg/dL, respectively, compared to their values in the control group, which were at 1461 and 3101 mg/dL, respectively.

Table 1. Efficacy of oral administration of fermented milk on the level of immunoglobulins in experimentally reduced immunized laboratory rats fed for 28 days.

		(IgG) mg/dl	(IgA) mg/dl
	T1	3101 a±13.86	1461a±13.6
	T2	1986 f±18.55	1002f±7.82
	T3	2731 d±17.70	1187d±11.34
	T4	2935 d±16.37	1201d±9.74
	T5	2694 e±14.54	1172e±10.59
	T6	2974 e±20.66	1385e±14.69
	T7	2780 e±18.22	1176d±12.53
	T8	3111 b±13.75	1415b±12.27

Different letters in the same column mean that there are significant differences at the 0.05 probability level.

T1:Negative control (without additives), T2: positive control (SN dose only), T3: (non-fermented cow's milk only), T4: (unfermented cow's milk +SN), T5: (fermented cow's milk B1 only), T6: (fermented cow's milk B1 only) Fermented bovine B1+SN), T7:(B2 fermented cow's milk only), T8(fermented bovine milk B2+SN)... SN= Sandimmune, B1=Bacterial Initiator (S.thermophillus+ Lb.bulgaricus+ Lb.acidophilus + Bifidobacterium animalis ssp), B2=Bacterial Initiator (S.thermophillus + Lb.bulgaricus).

The condition of treatment using both fermented or non-fermented milk with both types of starters caused a significant increase in IgA and IgG values, bringing its ranges between 1172 to 1417 mg/dL and between 2694 to 3111 mg/dL.

The results agreed with previous studies in the occurrence of differences in the levels of immune globulins from the normal limit (Franco et al., 2007: Douer et al., 1981) by comparing the levels of immune globulins in patients with the control group, as it showed that IgA and IgM from the animal group Experimentally lowered immunocompromised patients had their values lower than their normal range. As mentioned, (Oborilová et al., 2004) that low values of IgA, IgG mostly indicate cases of immunotoxicity to cells in the body.

The decrease in the values of immunity of IgA and IgG types in the case of oral administration of Sandimmune, which consists of cyclosporine produced from the fungus Tolypocladium, and its effectiveness in binding with the external receptors of lymphocytes called cyclophilin in cells, causing inhibition of the production of cytokines, which causes a decrease in the formation of Interleukins 2 and 4 as well as tumour necrosis factor alpha interferon and interferon gamma all cause a decrease in lymphocyte activity (Advani et al., 2007).

The results showed that the use of fermented and non-fermented milk with both types of probiotics led to an increase in the significant improvement in the immune parameters of IgA and IgG types, and it reached similarity with the immunological values in the serum of laboratory rats from the control group, and this indicates the effect of these factors in improving and raising the immunity of the reduced animals. experimentally immunocompromised.

Efficacy of oral administration of fermented milk on the balance of the natural flora in the intestines of experimentally reduced immune lab rats:

Table (2) shows the effectiveness of oral administration of fermented milk on the balance of gut bacteria for each of the groups of experimental animals given from SN and immunocompromised for a period of 28 days. The results of the statistical analysis at (P<0.05) show that the balance of species of coliform bacteria and types of lactic bacteria present in the intestines of rats differed significantly in the groups used in the research, where lactic acid bacteria decreased and increased significantly (P<0.05) in contrast, the number of coliform bacteria In laboratory rats whose immunity was reduced as a result of using SN, the number of lactic bacteria at 154 µg/gm and the number of coliform bacteria at 499 µg/gm compared with their numbers in the intestines of rats in the control group which were at 221 for lactic bacteria and 332.5 for coliform bacteria. There was an increase in the number of lactic bacteria in the given groups of the probiotic bacteria, as the numbers ranged between 195 and 507 CFU/gm, and in contrast there was a decrease in the number of intestinal bacteria, where the numbers ranged between 163 and 419 CFU/gm.

Table 1. Efficacy of oral administration of fermented milk on the level of immunoglobulins in experimentally reduced immunized laboratory rats fed for 28 days.

Different letters in the same column mean that there are significant differences at the 0.05 probability level.

T1: Negative control (without additives), T2: positive control (SN dose only), T3: (non-fermented cow's milk only), T4: (unfermented cow's milk +SN), T5: (fermented cow's milk B1 only), T6: (fermented cow's milk B1 only) Fermented bovine B1+SN), T7:(B2 fermented cow's milk only), T8(fermented bovine milk B2+SN)... SN= Sandimmune, B1=Bacterial Initiator (S.thermophillus+ Lb.bulgaricus+ Lb.acidophilus + Bifidobacterium animalis ssp), B2=Bacterial Initiator (S.thermophillus + Lb.bulgaricus).

	lactic bacteria numbers	coli bacteria numbers
	(CFU/gm)	
T1	12.5±221.5 e	10.5±332.5 d
T2	18.0±154 g	12.0±499 a
T3	14.0±218 e	9.0±351 c
T4	15.0±195 f	12.5±419 b
T5	5.0±363 c	8.5±180 e
T6	13.0±324.0 d	22.5±164 f
T7	21.0±507.0 a	14.5±163 f
T8	12.5±417.5 b	12.5±175 e

The results of the study agreed with what was found by (Lin et al., 2014) in his study about the ability of lactic acid bacteria to influence the level of the normal flora of the intestine and stimulate antioxidants in the host's body, which reduces the possibility of oxidative stress processes, as they agreed with (Si et al. , 2019) which was found to increase the antioxidant capabilities of black garlic by removing hydroxyl radicals and free radicals.

The results also agreed with (Alvaro et al., 2007), who found that oral administration of lactic bacteria helped reduce the number of colon bacteria in diabetic patients who suffer from an imbalance in the intestinal microflora and improve insulin levels.

The reason for the decrease in the level of the total numbers of intestinal bacteria compared to the increase in the total numbers of lactic bacteria can be due to their ability to secrete lactic acid and other organic acids resulting from fermentation processes in the intestine, in addition to their ability to produce hydrogen peroxide and their ability to stick to the inner walls of the intestine, which enables them to Competition and displacement of other microbes from adhesion with gut epithelial cells (Li et al., 2014).

Oral administration of fermented milk to each of the experimental animals with reduced immunity and containing high numbers of lactic bacteria and its metabolite probiotic products caused modification of the numbers of bacterial species present in the intestine, as the number of coliform bacteria decreased and in contrast the numbers of lactic bacteria increased

The human gut plays the role of host to trillions of bacteria collectively known as the gut flora. Changes in the composition of the normal flora of the digestive system, as well as alterations in the host's diet and immune system, have a clear impact on the balance of the normal gut flora allowing for Changes in the bacterial population (Zheng et al., 2020), the balance of gut microbiota within the host, and the abundance and diversity of bacterial species present within the GI tract are affected by various factors including pH, transit time, nutrient availability, age and health status of the host. If effects on health occur, it leads to an imbalance of the natural flora within the intestine and an increase in harmful colon bacteria.

The action of lactic acid bacteria is to attach to the intestinal epithelial cells of the host, causing immunostimulation through their production of immunosuppressive and inhibitory substances for other microbial species (Galdeano et al., 2019). It also appeared that higher doses of probiotics given in short courses were more effective than lower doses in chronic or immune diseases. Effects also depend on the interaction with the gut immune system and the duration of treatment (Minelli and Benini., 2008). The dose and the time of administration are also very important in the treatment of inflammatory immunodeficiency diseases. Different product combinations of lactic acid bacteria stimulate intestinal lymphocytes in different ways, providing further support for the immune system by stimulating specific parts of the immune system differently, for example L. delbrueckii subsp. bulgaricus stimulates peripheral blood mononuclear cells (PBMNCs) when used at a concentration higher than 104 cfu/ml, while Bifidobacteria can stimulate anti-inflammatory cytokines more than lactobacilli, but the pattern of stimulation is different with the highest concentration of bacteria (107 cu ft/ml). that stimulates PBMNCs to produce anti-inflammatory cytokines (Helwig et al., 2006).

In addition to what was mentioned, gut microbes perform many important physiological functions that include regulating energy levels and metabolism, neutralizing drugs and carcinogens, modifying gut motility, immune regulation and protection against pathogens, as well as affecting behavior and cognitive functions such as learning, memory, and even determining the mood of the host (Adikari et al. al.,2020), this diverse ecosystem is closely linked to physiological processes that affect many organ systems including cardiovascular, nervous, immune and metabolic. On the other hand, the high number of harmful colon bacteria can cause many problems, including the production of inflammatory substances, which may cause diabetes, heart disease and obesity (Jin and Flavell., 2013).

References

Abdel-Hamid Z.D. and Thalij K.M. (2020). The Effects of Oral Dosage of Glutathione and some Biological Agents on Immunity and Oxidative Stress Parameters in Male Rats Induced with Immunosuppression. Eurasian Journal of Biosciences. Volume 14 Issue 2: 2933-2939.

Adikari, A. M. G. C. P., Appukutty, M., & Kuan, G. (2020). Effects of Daily Probiotics Supplementation on Anxiety Induced Physiological Parameters among Competitive Football Players. *Nutrients*, *12*(7), 1920.

Advani, R., Horwitz, S., Zelenetz, A., & Horning, S. J. (2007). Angioimmunoblastic T cell lymphoma: treatment experience with cyclosporine. *Leukemia & lymphoma*, *48*(3), 521-525.

Bokulich, N. A., Chung, J., Battaglia, T., Henderson, N., Jay, M., Li, H., ... & Blaser, M. J. (2016). Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Science translational medicine*, *8*(343), 343ra82-343ra82.

Douer, D., Levin, A. M., Sparkes, R. S., Fabian, I., Sparkes, M., Cline, M. J., & Koeffler, H. P. (1981). Chronic myelocytic leukaemia: a pluripotent haemopoietic cell is involved in the malignant clone. *British journal of haematology*, *49*(4), 615-619.

Feng, P., Weagant, S. D., Grant, M. A., Burkhardt, W., Shellfish, M., & Water, B. (2002). BAM: Enumeration of Escherichia coli and the Coliform Bacteria. *Bacteriological analytical manual*, *13*.

Franco, S., Basu, U., Zarrin, A., Yan, C., Chaudhuri, J., Perlot, T., ... & Alt, F. W. (2007). Evolution of the immunoglobulin heavy chain class switch recombination mechanism. *Advances in immunology*, *94*, 157-214.

Galdeano, C. M., Cazorla, S. I., Dumit, J. M. L., Vélez, E., & Perdigón, G. (2019). Beneficial effects of probiotic consumption on the immune system. *Annals of Nutrition and Metabolism*, *74*(2), 115-124.

Helwig, U., Lammers, K. M., Rizzello, F., Brigidi, P., Rohleder, V., Caramelli, E., ... & Campieri, M. (2006). Lactobacilli, bifidobacteria and E. Coli nissle induce pro-and anti-inflammatory cytokines in peripheral blood mononuclear cells. *World journal of gastroenterology: WJG*, *12*(37), 5978.

Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *science*, *336*(6086), 1268-1273.

Hove, H., Norgaard, H., & Mortensen, P. B. (1999). Lactic acid bacteria and the human gastrointestinal tract. *European Journal of Clinical Nutrition*, *53*(5), 339-350.

Jin, C., & Flavell, R. A. (2013). Innate sensors of pathogen and stress: linking inflammation to obesity. *Journal of Allergy and Clinical Immunology*, *132*(2), 287-294.

Laatikainen, R., Koskenpato, J., Hongisto, S. M., Loponen, J., Poussa, T., Hillilä, M., & Korpela, R. (2016). Randomised clinical trial: low-FODMAP rye bread vs. regular rye bread to relieve the symptoms of irritable bowel syndrome. *Alimentary pharmacology & therapeutics*, *44*(5), 460-470.

Li, Y. B., Xu, Q. Q., Yang, C. J., Yang, X., Lv, L., Yin, C. H., Liu, X. L., & Yan, H. (2014). Effects of probiotics on the growth performance and intestinal micro flora of broiler chickens. *Pakistan journal of pharmaceutical sciences*, *27*(3 Suppl), 713–717.

Lin, C. S., Chang, C. J., Lu, C. C., Martel, J., Ojcius, D., Ko, Y. F., ... & Lai, H. C. (2014). Impact of the gut microbiota, prebiotics, and probiotics on human health and disease. *Biomedical journal*, *37*(5).

Minelli, E. B., & Benini, A. (2008). Relationship between number of bacteria and their probiotic effects. *Microbial Ecology in Health and Disease*, *20*(4), 180-183).

Nagata, S., Asahara, T., Wang, C., Suyama, Y., Chonan, O., Takano, K., ... & Yamashiro, Y. (2016). The effectiveness of Lactobacillus beverages in controlling infections among the residents of an aged care facility: a randomized placebo-controlled double-blind trial. *Annals of Nutrition and Metabolism*, *68*(1), 51-59.

National Research Council. (2010). Guide for the care and use of laboratory animals.

Oborilová, A., Mayer, J., Korístek, Z., Hofmanová, Z., Vásová, I., Navrátil, M., ... & Matuska, M. (2004). Evaluation of the clinical effectivity and toxicity of the FDN regimen (fludarabin, mitoxantron, dexamethason) in patients with follicular lymphoma. *Casopis lekaru ceskych*, *143*(10), 685-690.

Si, L., Lin, R., Jia, Y., Jian, W., Yu, Q., Wang, M., & Yang, S. (2019). Lactobacillus bulgaricus improves antioxidant capacity of black garlic in the prevention of gestational diabetes mellitus: a randomized control trial. *Bioscience reports*, *39*(8), BSR20182254.

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., et al. (2009). A core gut microbiome in obese and lean twins. Nature 457, 480–484. doi: 10.1038/nature07540.

Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell research*, *30*(6), 492-506.