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RESEARCH ARTICLE

Evaluate the Effectiveness of Oral Dosage with Probiotic Bacteria Lactobacillus *rhamnosus GG*, Lactobacillus *Planetarium* and *Lactobacillus reuteri* on Some Immune Indicators in Rabbits

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Abstract

This study was designed to evaluate some immune Indicators for rabbits oral dosage with three types of probiotics bacteria culture Lactobacillus *rhamnosus GG, Lactobacillus planetarium* and *Lactobacillus reuteri.* Rabbits were divided into four treatments, each subjected as the followings: treatment A, used as a negative control. treatment B, sub divided into four groups: group1 used as a positive control and other three groups were oral dosage with 1.0 µl\100gm weight\day with *Lactobacillus rhamnosus GG, Lactobacillus planetarium* and *Lactobacillus reuteri* . Treatments C and D, the dose used was 200and 300µl\100gm weight\day from same. The results showed increasing in white blood cells (WBCs), red blood cells (RBCs) and hemoglobin (HB) and other parameters like immune origins weight (liver and spline) according to type of oral dosage bacteria and amount of dose, the dosage with *Lb. rhamnosus* GG bacteria was the beast compared with *Lactobacillus planetarium* which were better than *Lactobacillus reuteri* dosage and control. The study showed that dosage with 300µl\100gm weight\day was best comparing with other treatments and control respectively.

Keywords: *Oral dosage, Probiotics bacteria, Probiotics dose, Immune Indicators*.

Introduction

Probiotics have proven to be effective in enhancing the host's self-defence mechanisms by stabilizing intestinal flora as their presence in the intestine stimulates the specialized and non-specialized immune responses to different locations of their intestinal effects, resulting in different responses such as enhancing the effectiveness of natural killer cells.

For ingestion and others as well as promoting the secretion of cytokines to possess anti-inflammatory properties and play an important role in balancing the functioning of the immune system such as promoting the production of IL-10 [1].

Probiotics have the advantage of improving the host health when taken in sufficient numbers daily and a wide impact effect on the immune response, enhancing innate immunity and adaptive immunity [2].

Probiotics are a means of protecting human health and enhancing its immune system.

These organisms have health effects in the host, especially when consumed in accordance with the correct scientific rules. It reduces gastrointestinal infections and has anti-microbial properties, as well as improving lactose metabolism and lowering blood cholesterol reducing the incidence of cancer, and successful use in controlling the various cases of diarrhea [3].

Over the past decades, scientific research has shown that probiotics are useful for the prevention and treatment of diseases. The importance of using biopharmaceuticals in improving metabolism, enhancing immune system function [4].

Probiotics has many therapeutic qualities, most important of which is the enhancement of the immune system [5].

Therefore, the aim of this study was to evaluate the oral dose of probiotic bacteria Lactobacillus *rhamnosus GG*, *Lactobacillus planetarium* and *Lactobacillus reuteri* on some Indicators of rabbits immune.

Materials and Methods

Probiotic Bacteria Isolates

Pure cultures of Iraqi isolates *Lactobacillus rhamnosus GG*, *Lactobacillus planetarium* and *Lactobacillus reuteri* obtained kindly from Food Science and Biotechnology Department, College of Agriculture, Baghdad University, the collected isolates used in the present study were grown and kept in MRS medium (Himedia, India).

Probiotic isolates were activated in skim milk 12%, incubated at 37 °C for 24-48 h. or until crude production and probiotics bacteria were estimated using the Pour Plate Count method [6].

Experiment Design

Male rabbits were brought from local markets (Baghdad) with age 6-8 months and weights ranging from 500-1100 g, randomly divided into four treatments, in animal house with light period 12h.±3, 25°C ±2 temperature, and relative humidity ranged between 55-60% with the care, provision of feed and water, each group and sub-group containing 5 rabbits, treatments were subjected to the following: The first treatment: used as a negative control group, fed a local animal feed for 30 days. The second treatment: subdivided into four sub-groups:

- The first sub-group was oral dosage with 100µl skim milk (12%)\100gm weight\day as positive control.
- The second sub-group was oral dosage with 100µl *Lb. rhamnosus GG viable* cells suspended (1×10^9 cfu\ml) \100gm weight\day.
- The third sub-group was oral dosage with 100µl *Lb. planetarium viable* cells suspended (1×10^9 cfu\ml) \100gm weight\day.
- The fourth sub-group was oral dosage with 100µl *Lb. reuteri* viable cells suspended (1×10^9 cfu\ml) \100gm weight\day.

The third and fourth treatments used the same as second treatment except the size of dosage which was increased to 200 and 300 µl / 100 g weight / day, respectively. All groups were fed local feed allotted daily for 30 days. Then, rabbits were weighed, blood samples were collected by cardiac puncture, blood was drawn from the heart by a medial syringe.

Blood samples were collected from each group of experimental animals in tubes. The following readings were taken using Hemolyzer (Analytic on- Germany)Number of white blood cells (WBC) 10^9 /L, number of red blood cells (RBC) 10^{12} /L, Hemoglobin (HB) g / L. then sacrificed rabbits , dissected and weighed immune organs that included liver and spleen.

Results and Discussion

White Blood Cells (WBC)

The results show in Figure (1) the rate of white blood cell count (WBC) 10^9 / L of the treatments, which reached 5.91×10^9 / L blood in the treatment of 300 µl *Lb. rhamnosus* GG / 100 g / day compared with other treatments of the two types *Lb. planetarium* and *Lb. reuteri* and control treatments. The increase of white blood cells number is due to the immune response of the host to resist bacterial invasion as an initial defensive line.

The number of white blood cells is rapidly affected by inflammatory processes and leads to the rapid migration of white blood cells to the location of foreign bodies by some attractant substances. To reduce the number of bacteria in the area of infection and the effectiveness of white cells in the killing and destruction of foreign bodies and pathogenesis produced molecules that can stimulate T cells and stimulate acquired immunity [7].

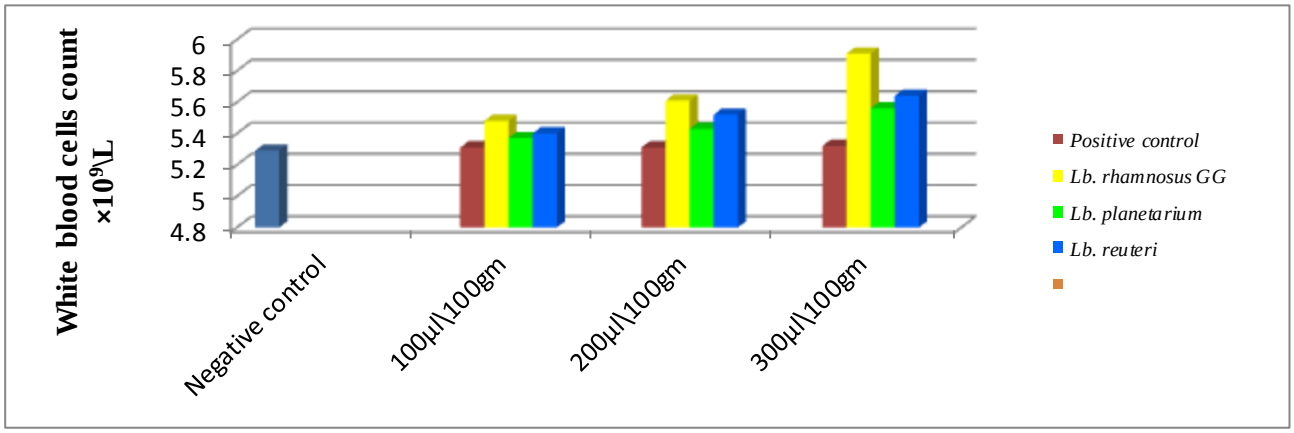

Fig. 1: White blood cell count (WBC) 10^9 / L blood for the treatments

Neutrophils play an important role in the phagocytosis process and express for receptors associated with specialization on the lining of the infected area. These cells swallow and digest the foreign bodies with the enzymes [8].

Macrophage cells are stimulated to secrete a number of cytokines such as TNF-α (Tumer Necrosis Factor-α) and interlukine, which in turn stimulate the response of T-cells. Macrophage cells have antibody receptors on their surface and receptor receptors are very important to complement the process of inhalation of antibodies, the role of integrators is that they cover the surface of the cells and activates the complement Cascade, which promotes and increasing the effectiveness of photosite [9].

Red Blood Cells (RBC)

The results showed in Figure (2) the number of red blood cells (RBC) 10^{12} / L blood for the treatments, showing the treatment of the dosage with 300 µl of *Lb. rhamnosus* GG / 100 g / day increased in the red blood cell count to 133.17 × 10^{12} / L blood compared with the treatments of the two types *Lb. planetarium* and *Lb. reuteri* and control.

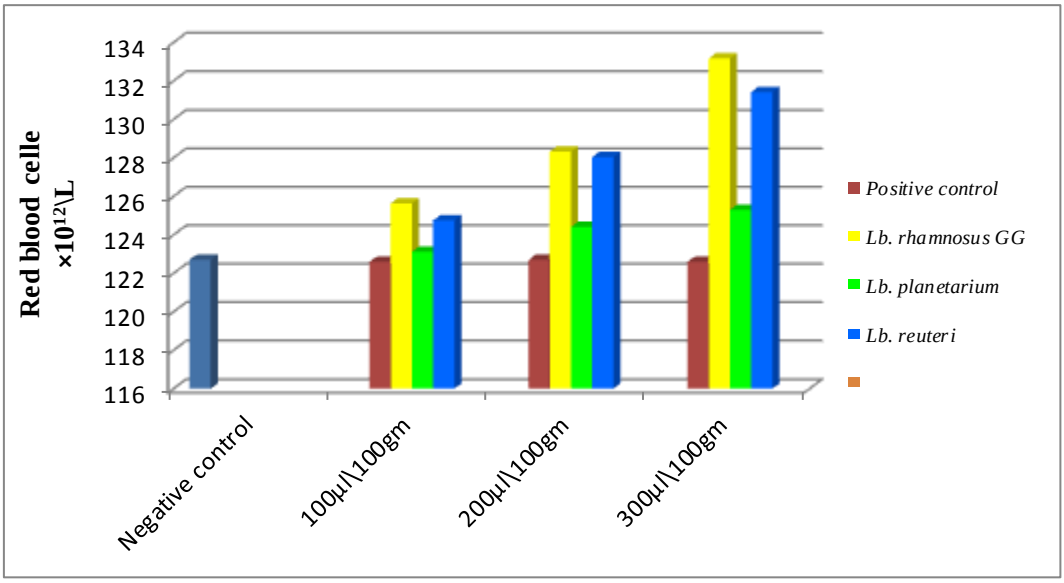

Fig. 2: Red blood cells (RBC) 10^{12} / L blood for the treatments

Hemoglobin (HB)

Figure (3) shows the average amount of hemoglobin (HB) g / L of blood for the treatments, the results showed that the treatment of the dosage 300 µl of *Lb. rhamnosus* GG / 100 g / day the highest increase in the mean amount of hemoglobin was 122.6 g / L blood compared with the treatments of the two types *Lb. planetarium* and *Lb. reuteri* and control.

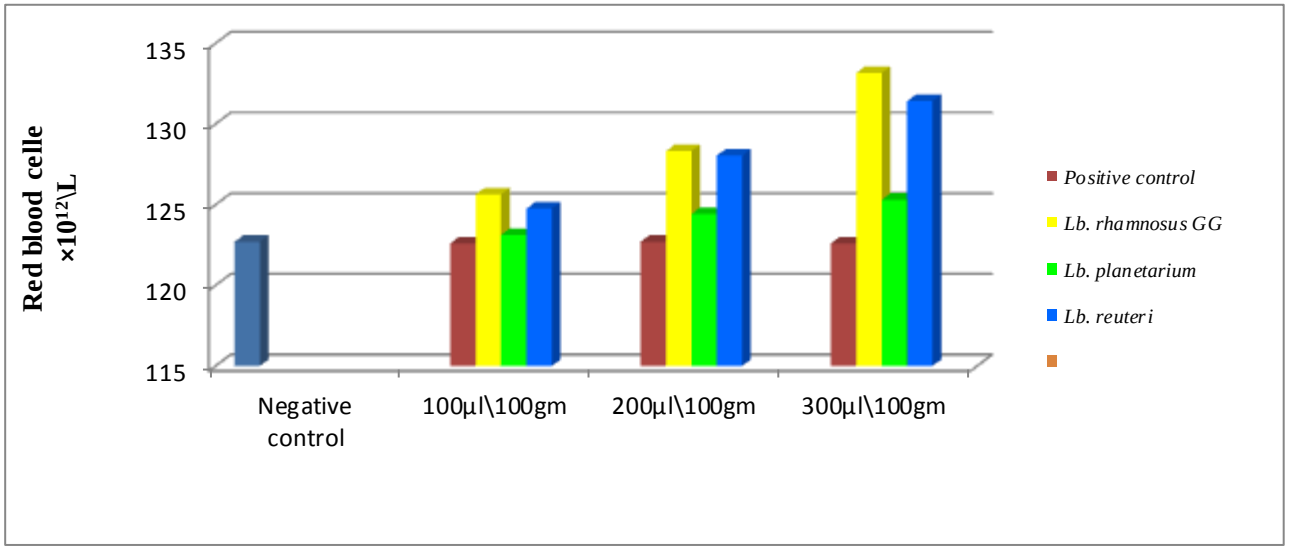

Fig. 3: Hemoglobin (HB) / g for the treatments

Studies have shown that probiotics can affect both red cell and white cell levels as well as their role in influencing hemoglobin ratio. Cetine et al [10] in a study using a combination of probiotics and indicating significant increase hemoglobin in the ratio, AL-Saiad [11] found the same results when using *Lb.acidophilus* and *Lb.plantarum*, and indicated an increase in hemoglobin and red blood cells numbers.

Mercenier et al [12] described the role of probiotics in promoting and enhancing immune response in vitro and in vivo.

And he explained the difference in the mechanisms effect whichdepending on the species and strain. This difference is due to several factors, including the sources of isolation and genetic pattern as well as the type of inhibitory substances produced by the bacterial and cellular wall components, also the difference in the type of immune cells targeted by probiotics may lead to different effects.

The Weight of Liver and Spleen

Results in Figures (4, 5) showed the weight of immune organs which were included liver and spleen in the treatments, it appears clearly increased in liver weight for the treatment with probiotics bacteria compared to the positive control group. the weight of the liver was 4.89, 3.93 and 4.27 g and the weight of the spleen was 0.049, 0.038 and 0.042 g in the treatment of 300 µl / 100 g / day of *Lb. rhamnosus* GG, *Lb. planetarium* and *Lb. reuteri* respectively compared to positive control group and other treatments, the dosage of 300 µl / 100g / day was also shown to be the best compared to other dosage treatments.

The results certified of increasing weight to the ability of the selected isolates as probiotics in improving the environment intestinal balance of experimental animals through competition with pathogenic bacteria on nutrients in the gastrointestinal tract as well as their role in improving the absorption of nutrients in the small intestine [13].

Probiotics also producing some biological compounds that remove the toxic effects and producing short-chain fatty acids, especially butyric acid which is the major energy source for epithelial cells and its activity in producing certain growth factors that affect the activation of epithelial cells which leading to an increase in weight of the treatments compared to the control group [14].

The utilization of *Lb.plantarum* inhibits the transfer process It reveals this inhibition by measuring some liver enzymes such as Aspartate trans aminase (AST) and alanine trans aminas (ALT) in the blood, Probiotics have a role in promoting liver cells functions by suppressing the effect of pathogens and their effect on the general health status [13]. Spleen functions produce red and white blood cells in the fetus but the spleen production stops for red cells after birth.

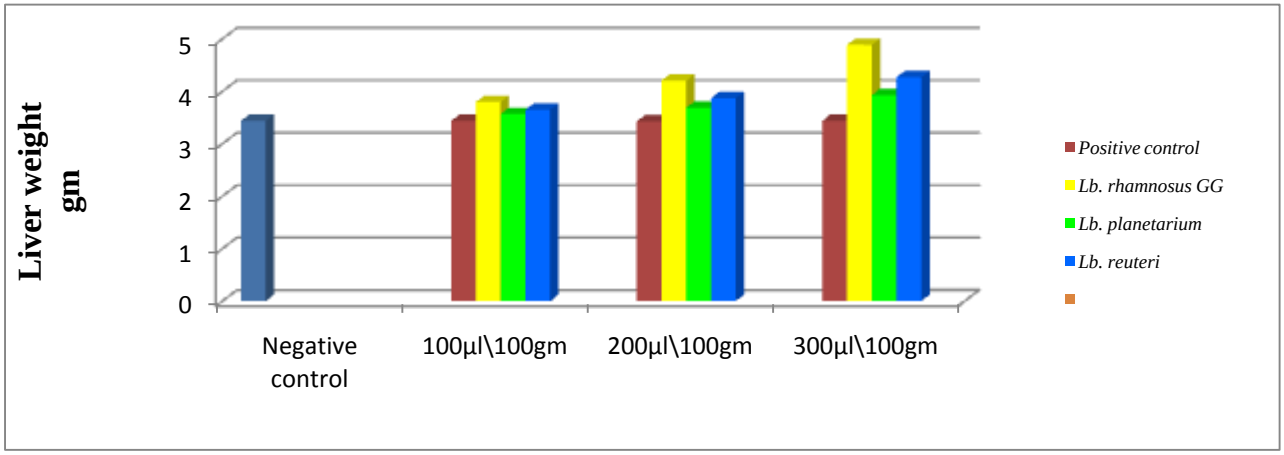

Fig. 4: The rate of liver weights for the treatments

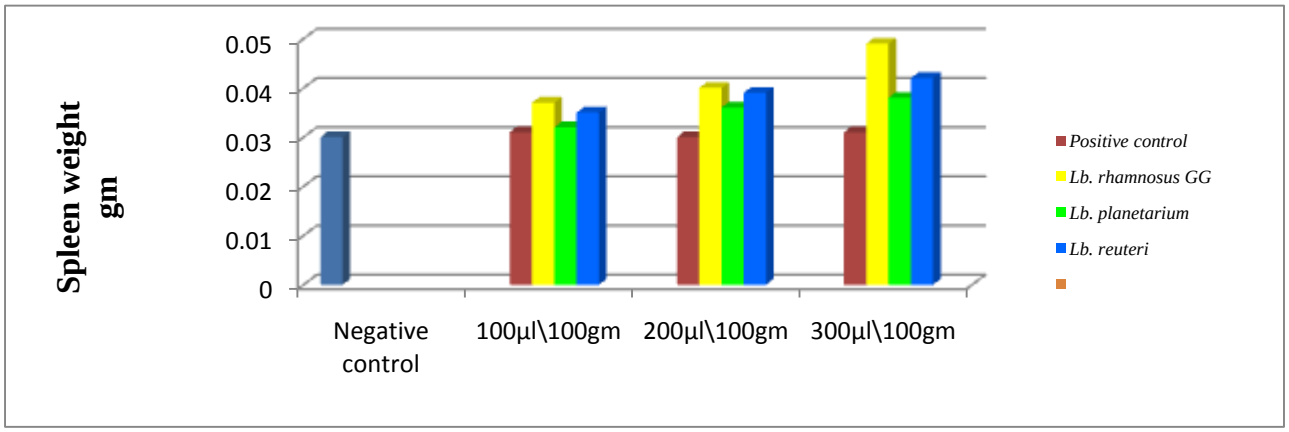

Fig. 5: The rate of spleen weights in terms of inflation for transactions

Large spleen cells destroy the old red blood cells that have to be replaced by new red blood cells Also, The spleen manufactures lymphocytes for the lymphatic cycle and the spleen stores a blood amount of about 5% of the human blood, for stress, pregnancy, bleeding and poisoning, or when the blood oxygen content of the decreases, the spleen push its content from blood to circulation, spleen divides into two sites, a part of the red pulp store and a rich part of the white pulp cells that produce antibodies which contribute effectively role during humoral immune response [15]. Probiotics have an important role in promoting spleen and peyer's patches and immune response,In a study, mice fed a combination of probiotics

for 28 days the level of IL-2 and IFN-ϒ increased in both the spleen and the peyer's patches, Probiotics adhere to mast cells and respond to the Tool-Like Reseptor system, which is produced from Th1 cells by IFN-ϒ and IL-2, as a result probiotics activate ,cericulation and differentiation of B cell subunits under Th1 and cytokine control, these effectes increase the weight of the spleen , also, these activities lead to increased the spleen weight when using these doses in rat feeding [16].

Chen et al [17] studied increased the weight of spleen which is the result of its development of immune activity and enhancing the production of cytokines as a result of probiotics effect.

Conclusions

The study concluded that the dosage of probiotics gives an effective effect in enhancing the function of the immune system and increasing the effectiveness of immune markers, that's depend on the type of bacteria used and the amount of dosage Rabbits in this study gain useful health indicators and seemed to be in excellent to the control group.

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