Kirkuk University Journal for Agricultural Sciences
ISSN:2958-6585
https://kujas.uokirkuk.edu.iq

RESEARCH ARTICLE https://doi.org. 10.58928/ku24.15303

The effectiveness of fermented juices on weight gain and balance of microflora of the intestines in male rats.

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Received: 15/05/2024 **Revised:13/07/2024** **Accepted: 25/07/2024** **Published: 01/09/2024**

ABSTRACT

The current study aimed to evaluate the effectiveness of orange and pomegranate fermented juice with probiotics *Lactobacillus plantaram, Lactobacillus rhamnosus* and *Lactobacillus acidophilus* in weight gain and the balance of the natural intestinal flora in experimental rats. 21 male rats (Albino Sprague- Dawley) were used and were divided into seven groups with three replicates. The duration of the e experiment was 28 days. The results of the statistical analysis showed, with probability ($P<0.05$), that oral administration of various types of fermented juices led to a decrease in the weights animals. As for the fermented orange juice, the treatment supplemented with the probiotic L .*plantarum* was superior. The amount of weight gain was 65 grams, and the initial and final weight were (173 and 238) grams, respectively. As for pomegranate juice, the group of rats the fermented with the probiotic *L .acidophilus*, outperformed , the amount of weight gain was 54 grams, and the initial and final weight were (184 and 238.5) grams, respectively.As for the balance of microflora of the intestine, the results of the statistical analysis showed, with probability ($P<0.05$), the effect of fermented juices on the microflora balance of the intestines, as it caused a significant decrease in the total of coliform, Salmonella and Shigella, and a significant increase in the total count of Lactic acid bacteria (LAB) compared to the control groups.

***Keywords*:** Probiotics, fermented orange juice, fermented pomegranate juice, weight gain, gain, the gut microbial balance.

INTRODUCTION

Nowadays, food manufacturers and consumers are increasingly interested in what is known as functional foods [1], [2]. This trend is based on consumer awareness, and consumers have begun to turn to foods that are free of chemical additives and have health benefits. Functional foods improve the health functions of the body, outweighing their nutritional effects [3]. Functional foods are defined as foods that contain bioactive compounds such as fiber, oligosaccharides, and probiotics, the consumption of which can lead to the prevention of the risks of food-borne diseases. Probiotics are microorganisms that enhance health benefits to the host when consumed in adequate amounts. They are found naturally within the human intestine and promote intestinal health [4], [5]. The simplest way to enhance the survival and vitality of probiotics in fruit juices could be fortification with prebiotics that can provide protection [6].

Most foodstuffs containing probiotics and prebiotics on the market are based on milk origin, which is known to cause allergies and lactose intolerance, cholesterol diseases, dyslipidemia, and veganism. Therefore, attention is directed to non-dairy probiotic products, including fruit juices, which are considered healthy due to their high content of biologically active compounds. All age groups also consume them. Fruit juices with probiotics added can be an option for can be a choice for persons who cannot consume dairy products for health-related reasons with milk products [7], [8], [9]. Fruit juices also contain components that promote the viability of probiotics such as ascorbic acid, which reduces the redox potential and organic acids and saccharides that can usable as a carbon source [7], [10]. Fruits are an essential source of nutrients like vitamins, dietary fiber, and minerals such as potassium, magnesium, and manganese. It also contains phenolic compounds, antioxidants and enzymes that resist the formation of cancerous tumors [11].

Fruit juice market is one of the fastest growing markets around the world fermented fruits are referred to as healthy foods eaten by children and adults due to their nutritional and functional properties in the presence of probiotics [12].

A healthy gut microbiome is essential for overall health. However, lifestyle and diet of people nowadays are the two main factors that lead to well leading damage to gut microbiome and cause other diseases such as obesity [13], Therefore, the current article aimed to evaluate the effectiveness of pomegranate and orange juices fortified with probiotics in weights of rats and balancing the normal flora of rats.

MATERIAL AND METHODS

Collecting fruits and manufacturing juice Good quality orange and pomegranate fruits were purchased from the local markets in the city of Al- Shargat, washed, unwanted parts removed, squeezed and filtered, and 4% inulin (BDH, England) was added to them.

Preparation of juices fortified with probiotics

The manufactured juices were distributed in glass bottles and were pasteurized at a temperature of 85°C in a water bath for 10 minutes. They were then left to cool for room temperature and were inoculated with broth cultures of the types of probiotics used in the study: *L. plantarum*, *L. acidophilus*, and *L. rhamnosus* at a rate of 3.75%, in two replicates for each type of juice, it was incubated anaerobically at a temp erature of 37°C for 24 and 48 hours and was stored in the refrigerator at 4°C until use [14]. Colonies of the specific probiotic bacterial strains employed in the juice formulations were enumerated on solid MRS (de Man, Rogosa, and Sharpe) medium, maintaining the viability and numbers of viable cells in the juice, which should not be less than 10^6 CFU /ml.

Bacteria used in the study

The types of probiotics used in the study were obtained *L. plantarum*, *L. acidophilus* and *L. rhamnosus* from the laboratories of the College of Agriculture and the College of Science at Tikrit University, Using the Vitek2 Compact system device, the diagnostic and purification procedures for these probiotic species were also verified.

Vital Experiment

Preparing the bacterial suspension:

The bacterial suspension of the bacterial species was prepared by growing them on nutrient agar, and then the tryptone broth was inoculated with all the bacteria. Next, 1 ml of the bacterial suspension and orange and pomegranate juice were transferred and compared with McFarland's solution to obtain a cell count of 1.5×10^8 cells/ml. Then the animals were dosed with 2 ml of the bacterial suspension and therapeutic juices using a dosing machine, specially prepared for this purpose, to deliver it to the animal's stomach.

Preparing feed for laboratory rats

The special feed for the animal was prepared based on [15] and included the following ingredients (g/kg). Distilled water was added to the ingredients to obtain a suitable dough, then it was placed in stainless steel utensils and dried at 50° using an electric oven.

Material	Weight / gm
Casein	158.5
Cellulose	50
Starch	536.5
Sunflower oil	100
Vitamin mixture	5
Mixture of mineral elements	50
Glucose	100

Experiment design

The experiment was designed using 24 male rats (Dawley-Albino Sprague), with an average age between 8 and 10 weeks and average weights between 145 and 147 grams. Appropriate conditions were provided for their upbringing and care, including appropriate nutrition, temperature, lighting, humidity, and appropriate humidity, as mentioned in [16]. After that, the rats were divided into eight groups with three replicates of the group, and the treatments were as follows:

C1: control group.

T1: A group of rats given orange juice fermented with *L.acidophilus*.

T2: A group of rats given orange juice fermented with *L. plantaram*.

T3: A group of rats given orange juice fermented with *L. rhamnosus*.

G1: A group of rats given pomegranate juice fermented with *L. acidophilus*.

G2: A group of rats given pomegranate juice fermented with *L. plantarum*.

G3: A group of rats given pomegranate juice fermented with *L.rhamnosus.*

The experiment continued for 28 days. The daily oral dose was 2 ml of juices fermented with probiotics for the infected groups after comparison with McFarland solution at numbers of 1.5×10^8 CFU. After the search experience ended, the rats were starved for 20 h, then anesthetized, and blood was drawn to conduct the tests specified in the experiment. Weight gain was calculated based on the difference between the initial weight and the final weight. As for the balance of the normal flora was estimated according to the method [17] by 10 grams of intestine were weighed and transferred to a flask containing 90 ml of physiological solution. A number of decimal dilutions were performed up to the fifth dilution. 0.1 ml of the fifth dilution was taken and spread on a set of differentiation media specific to the bacterial species, which included MRS-CaCO3, SS Agar and MacConkey Agar and incubated at 37°C for 24 h, then counting was carried.

Statistical analysis

Statistical analysis was conducted using the statistical program [18] and the CRD system. The averages were selected according to Duncan test [19] to find the significance of the differences between the means for the factors impacting the tested traits at (0.05)

RESULTS AND DISCUSSION

The effect of fruit juices fortified with various types of probiotics on the average weight of experimental rats:

Table (1-1) and Figure (1-1) shows the effect of the different treatments C, T1, T2 and T3 on weight gain for groups of rats with orange juice supplemented with probiotic *L.plantarum, L.acidophilus,* and *L.rhamnosus* during the experiment period, which was 28 days, The results showed a significant increase at (P<0.05) that the rate of final weight gain for the C group of rats amounted to (18) gm. The final weight was (283.5) gm, while the initial weight was (265.5) gm. The table also indicates that the highest final weight gain appeared in male rats T1, as it reached 76.5 gm, and the initial and final weight were (177 and 253.5) gm, respectively, while the lowest final weight gain was in group T2, which was 65 grams, and the initial and final weight was 173 and 238 gm, respectively. As for group T3, the weight gains at the end of the experiment period reached 76 gm, while the initial and final weight was (164 and 240) grams for the aforementioned groups, respectively. Different letters in the same column indicate significant differences at the probability threshold of 0.05.

Table (1-1) The effect of orange fermented juice with probiotics on weight gain in rats.

Treatments	Initial weight gm	Final weight	Initial weight
C	165.5 a	283.5 a	118 a
T1	177 b	253.5 b	76.5 b
T2	173 b	238 c	65 c
T3	164 c	240 c	76 b

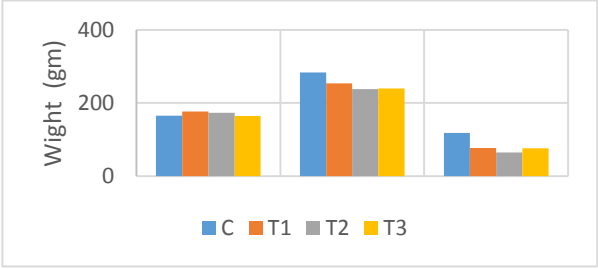

Figure (1-1) The effect of orange fermented juice with probiotics on weight gain in rats.

As for Table (2-1) and Figure (2-1), it shows the effect of the different treatments C, G1, G2, and G3 on the rate of weight gain for groups of rats and treated with pomegranate juice supplemented with the aforementioned types of probiotic during the duration of the experiment. The results showed Statistical analysis: At probability (P<0.05), the final weight gain rate for the replicates of group **C** rats reached (15) gm, and the final weight was (277) gm, while the initial weight was 262 gm. The table also shows that the highest final weight gain appeared in male rats G2, as it reached 81.5 gm, and the initial and final weight were (159 and 241.5) gm, respectively, while the lowest final weight gain was in the G1 group, which was 54 gm, and the initial and final weight was (184 and 238.5) gm, respectively. As for group G3, the weight gain at the end of the experiment period reached 72 gm, respectively, while the initial and final weight was (169 and 273) gm for the aforementioned groups, respectively. Different letters in the same column indicate significant differences at the probability level 0.05.

Table (2-1) The effect of pomegranate fermented juice with probiotics on weight gain in rats.

Treatments	Initial weight gm	Final weight	Initial weight
C	165.5 a	277 a	111.5 a
G1	184 b	238.5 c	54.5 d
G2	159.5 c	241 c	81.5 b
G3	169.5 c	273 a	72 c

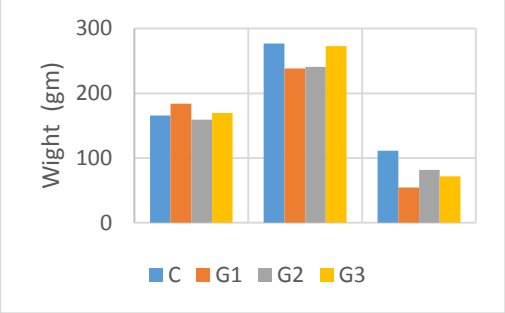

Figure (2-1) The effect of pomegranate fermented juice with probiotics on weight gain in rats

The reason for the average weight gain in rats is the accumulation of fat due to high concentrations of cholesterol and triglycerides and high low-density fats [20].

The results agreed with [21], as they showed that probiotics have a very significant effect in reducing body weight if they are taken daily and within acceptable limits. Moreover, probiotics can reduce the average size of fat cells in the adipose tissue of experimental animals, as [22] indicated that probiotics can stimulate the secretion of many enzymes such as proteolytic and lipolytic, and this helps in digesting nutrients in the intestine, Thus, it improves metabolism within the intestines and helps in losing body weight. The study by [23] also showed that probiotics have an effect in reducing weight and regulating blood glucose, which is related to the feeling of fullness. In another study conducted on experimental animals to determine the effect of the probiotic, it was found that taking the probiotic for a long period led to a reduction in body weight and belly fat.

The effect of fermented juices on the balance of intestinal flora:

The effect of fermented juices on the microflora balance of the intestines of rats is shown in Tables **(3-1)** and **(4-1) ,** Figure **(3-1)** and **(4-1).** The results of the statistical analysis showed, at probability ($P<0.05$), the presence of a significant increase in the total count of coliform, Salmonella and Shigella, and a significant decrease in the total count of LAB in the group control. At the same time, oral dosing with fermented juices caused a significant increase in the numbers of probiotic bacteria and a decrease in the numbers of pathogenic coliform, Salmonella and Shigella bacteria. Different letters in the same column indicate significant differences at the probability level 0.05 Different letters in the same column indicate significant differences at the probability level 0.05.

Table (3-1) The effect of orange fermented juice with probiotics on the gut microbial balance in rats.

Treatments	Total count of LAB	Total count of *Coliform*	Total count of *Salmonella*& *Shigella*
C	17 d	298 b	0.0 a
T1	305 a	145 bc	0.0 a
T2	181 c	389 a	0.0 a
T3	214.5 b	195 c	0.0 a

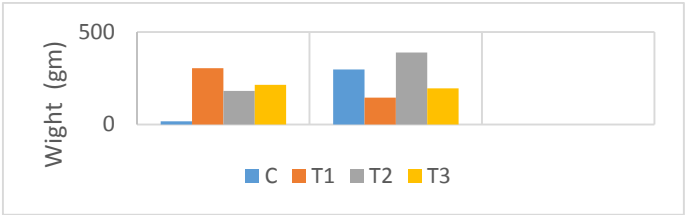

Figure (3-1) The effect of orang fermented juice with probiotics on on the gut microbial balance in rats.

Table (4-1) The effect of pomegranate fermented juice with probiotics on on the gut microbial balance in rats.

Treatments	Total count of LAB	Total count of *Coliform*	Total count of *Salmonella*& *Shigella*
C	18 d	295 a	18.0 a
G1	651 a	253 b	0.0 b
G2	297 b	182.5 c	0.0 b
G3	234 c	269 b	0.0 b

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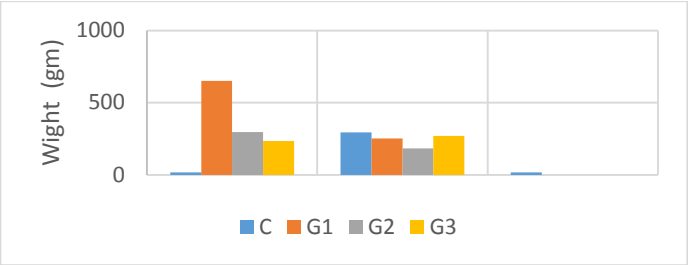

Figure (4-1) The effect of pomegranate fermented juice with probiotics on the gut microbial balance in rats.

The results agree with what was indicated in [24] that the *Lb. acidophilus* and other probiotic can inhibit the growth of *E. coli* through the production and accumulation of secondary metabolites of the bacteria, hydrogen peroxide, bacteriocins, and organic acids, which have an inhibitory effect for bacterial growth and reproduction [25], [26], [27], also the results are agreed with what was indicated in [28], [29]. A healthy gut microflora is essential for the general health of the body. However, an unhealthy diet and lifestyle of people are the two main factors causes that damage intestinal health, which negatively affects the natural flora of the intestine and contributes to the development of many diseases such as intestinal inflammation, rectal and colon cancer, obesity, and insulin resistance [30], [31]. Functional foods are considered an important Suggestions to overcome this problem, represented by fermented fruit juices, as they provide the body with a group of health benefits compared to other probiotic products [32], In addition, fruits are an essential source of nutrients such as fiber, vitamins, minerals such as potassium, magnesium, manganese, and antioxidants that resist the formation of cancer cells [11] These nutrients and the acidic nature of fruits and vegetables provide a suitable medium for fermentation by probiotic, which as an addition to food has health benefits for the intestine [33]. Such as enhancing the immune system [34], improving gastrointestinal system functions [35], and reducing cholesterol [36] and lowering the possibility of colon cancer [37], [13]. [13]also indicated that juices had an inhibitory effect on some pathogenic microbes by providing a high acidity environment, and that fermenting juices increases the shelf life of vegetables and fruits and enhances many beneficial properties, such as nutritional value and flavor, and reduces toxicity and fermented foods they are foods that contain many biologically active compounds, such as dietary fiber, sugars, and vitamins, as prebiotics, so they maintain the balance of intestinal bacteria [38], [39], [40]. Fortifying probiotics with prebiotics can also protect probiotics during cold storage [6]. Fiber contributes to influencing the sensory properties of juices. Adding brewer's yeast to beetroot and carrot juices, along with the addition of L. acidophilus, led to an increase in probiotics during fermentation as well as a reduction in fermentation time [41], [42]. Adding inulin to fig juice fermented with *L. delbrueckii* increased the number of probiotic bacteria compared to the control sample, as well as its content of antioxidants and polyphenols and improved sensory characteristics [43]. The study conducted by [44] recommended adding probiotics to many foods, nutritional supplements, and animal feeds, and for many therapeutic and preventive uses.

Conclusions

The current study's results lead us to the conclusion study that adding probiotic bacteria to fruit juices is a good alternative to probiotic dairy products and a healthy nutritional supplement because fruits contain many nutrients, antioxidants and dietary fiber, which makes them have a preventive and therapeutic effect for reducing weight, treating digestive tract problems, and increasing the number of beneficial bacteria. In the digestive tract environment, which is free of allergens, unlike dairy products, juices are also acceptable to all age groups.

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العصائر المتخمرة في الوزن المكتسب وتوازن النبيت الطبيعي في ذكور الجرذان.

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[3]كلية الطب البيطري، جامعة كركرك، كركوك، العراق.

الخلاصة
هدفت الدراسة الحالية تقييم فاعلية عصائر البرتقال والرمان المدعمة بانواع البكتيريا العلاجية *Lactobacillus Lactobacillus plantarum* و *Lactobacillus rhamnosus* , *acidophilus* في الزيادة الوزنية وتوازن النبيت الطبيعي للامعاء في حيوانات التجربة تم استخدام *21* جرذ ذكر نوع *(Albino Sprague – Dawley)* قسمت إلى *7* مجموعة بواقع ثلاث مكررات وكانت مدة التجربة 28 يوما.اظهرت نتائج التحليل الاحصائي وعند الاحتمالية (*P<0.05*) ان التجريع الفموي من انواع العصائر المتخمرة ادت الى انخفاض اوزان الحيوانات، بالنسبة لعصير البرتقال المتخمر تفوقت المعاملة المدعمة بالمعزز الحيوي *L.plantarum* وكان مقدار الزيادة الوزنية *65* غم وكان الوزن الابتدائي والنهائي (*173*و *238*) غم على التوالي ، اما بالنسبة لعصير الرمان تفوقت مجموعة الجرذان المعطاة عصير الرمان المتخمر بالمعزز الحيوي *L.acidophilus* وكان مقدار الزيادة الوزنية *54* غم وكان الوزن الابتدائي والنهائي (*184* و *238.5*) غم على التوالي. اما فيما يخص توزان النبيت الطبيعي للامعاء يبينت نتائج التحليل الاحصائي وعند الاحتمالية (*P<0.05*) تأثير العصائر المتخمرة على التوازن الميكروبي لامعاء الجرذان وتبين حصول انخفاض معنوي في الاعداد الكلية لبكتريا القولون وبكتريا السالمونيلا والشكيلا وارتفاع معنوي في العدد الكلي لبكتريا حامض اللاكتيك مقارنة بمجاميع السيطرة .

الكلمات المفتاحية: المعززات الحيوية ، عصير البرتقال المتخمر، عصير الرمان المتخمر ، الزيادة الوزنية ، النبيت المعوي.