THE EFFECT OF THE SYNBIOTIC ON REDUCING BODY WEIGHT, FEED CONSUMPTION, ACTIVITY AND HEALTH STATUS OF EXPERIMENTAL ANIMALS

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ABSTRACT

The current study aims to demonstrate the effectiveness of the synbiotic of eukaryotic and prokaryotic probiotics on the average weights of experimental animals (male rats) per week, the weights of the fed they consume, their activity and their health status daily by dosing each group with a probiotics and synbiotic except for the two control groups (positive and negative) were used. The results showed a decrease in the weights of experimental animals that were dosed with probiotics *L.GG, S.boul., L.GG+ S.boul*. and the synbiotic (*L.GG+ S.bou.l*+Psy) even though they were fed a standard diet with adding 10%Kcal, the synbiotic group *L.GG+S.boul.* +Psy was the most efficient and capable in reducing the rats weight by 11.7% as compared to the positive control group (cont2). The synbiotic group was closest to the negative control group (cont1), at a rate of 4.06%, since the latter feeds on a regular standard diet only, and the decrease in the body weights of the experimental animals of the dosed groups is accompanied by a decrease in the amount feed of consumed for the group of rats that were dosed with synbiotic, reaching a decrease of 8.34% when compared with the cont2 group, while the percentage of feed consumption was 0.73% when compared with the cont1 group. All dosed groups showed an inmprovment in activity and health status compared to the both control groups.

***Keywords*: obesity, *S. boulardii*, *L. rhamnosus* GG, psyllium, gut microbiota**
***Part of Ph.D. Dissertation of the 1st author.**

تأثير التآزر الحيوي في تخفيض الوزن والعليقة المستهلكة والنشاط والحالة الصحية لحيوانات التجارب

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المستخلص

هدفت الدراسة الحالية الى بيان فاعلية التآزر الحيوي للمعززات الحيوية حقيقية وبدائية النواة في معدل أوزان حيوانات التجارب (ذكورالجرذان) اسبوعياً واوزان العليقة التي تستهلكها ونشاطها وحالتها الصحية يومياً عن طريق تجريع كل مجموعة بالمعززات الحيوية والتآزر الحيوي باستثناء مجموعتي السيطرة (السالبة والموجبة) . أظهرت النتائج انخفاض اوزان حيوانات التجارب التي تم تجريعها بالمعززات الحيوية *L.GG* و *S.boul.* و *L.GG+ S.boul* والتآزر الحيوي *L.GG+ S.boul+Psy* رغم تغذيتها على عليقة قياسية زيادة 10% سعرات حرارية وكانت مجموعة التآزر الحيوي *L.GG+S.b+*Psy الأكفأ والأقدر في تخفيض تلك الأوزان ما نسبته 11.7% عند مقارنتها بمجموعة السيطرة الموجبة (Cont2) ، وكذلك تعد الأقرب من مجموعة السيطرة السالبة (Cont1) ما نسبته 4.06% كون الأخيرة تتغذى على عليقة قياسية اعتيادية فقط، ويرافق انخفاض اوزان جسم حيوانات التجارب للمجاميع المجرعة انخفاض في كمية العليقة المستهلكة لمجموعة الجرذان التي تم تجريعها بالمعززات الحيوية لا سيما المجموعة التي جرعت بالتآزر الحيوي وكان الانخفاض بواقع 8.34% عند مقارنتها بالمجموعة Cont2 في حين بلغت نسبة ما تستهلكه من العليقة 0.73% عند مقارنتها بالمجموعة Cont1، كما أظهرت جميع المجاميع التي تم تجريعها زيادة في النشاط والحالة الصحية مقارنة بمجموعتي السيطرة.

الكلمات المفتاحية: السمنة، السيليوم، النبيت المعوي
***البحث مستل من أطروحة دكتوراه للباحث الاول**

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INTRODUCTION

The World Health Organization (WHO) showed in 2020 that obesity is an epidemic syndrome spread throughout the world and is characterized by the accumulation of a mass of fatty tissue in the body, and it has almost tripled worldwide between 1975 and 2016. In 2016 there was more than 1.9 billion adults are overweight (39%), more than 650 million are obese (13%), and more than 340 million children and adolescents between the ages of 5 and 19 are overweight or obese. In 2019, there were about 38 million children under the age of five suffering from the same problem. In Iraq, the percentage of those suffering from obesity and overweight for adults reached 59.8%, and the percentage of those suffering from obesity for ages between 5-15 years is 10-15%. The percentage of children who are overweight between the ages of 2-4 years is 28.79% (23). Rebalancing the gut microbiota is an effective treatment for obesity and other chronic diseases and is of great importance. Diet plays a profound role in shaping the formation of these organisms. Probiotics, prebiotics, and synbiotics receive a lot of attention in this regard. In addition, previous experiments showed that the effects of the use of probiotics on body weight and body mass index (BMI) vary according to the strain and dose used (20). According to the FAO and WHO probiotics are defined as live microorganisms that, when ingested in appropriate quantities, give health benefits to the host (13). Prebiotics have also been defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as carbohydrates selectively fermented by microorganisms present in the host to confer a number of health benefits (11). Gibson and Roberfroid, in 1995, introduced the term Synbiotic to describe a synthesis of the synergistic action of a mixture of probiotics and prebiotic (10) , accordingly, synbiotic is defined as the joint relationship that combines the probiotics and the prebiotic that take place either by adding them together in the food, or by adding the probiotic alone in the event that the prebiotic is naturally available in some foods, in order to achieve the maximum therapeutic benefit, through what the prebiotic provide from support and stimulation of vital probiotics, including increased growth and survival rates (12). The introduction of these components specifically in the digestive system leads to a beneficial effect on the health of the host through selective stimulation of the growth and/or metabolic activation of beneficial microorganisms in the gut microbiota (22). Probiotic strains can be isolated from different sources, especially gut microbiota and oral, or from dairy products (19). Probiotic and prebiotics are added to foods to give health benefits to the body and prolong the shelf life of those foods, especially when added to fermented dairy products (15).

In view of the increasing rates of obesity in Iraq, the Arab world and the entire world at an increasing rate, and its health risks of obesity on human health, and the fact that probiotic, prebiotic and synbiotic products are among the effective solutions for this phenomenon, and for the purpose of delivering these factors to the consumer through a food products, this became the aim of this study.

MATERIALS AND METHODS

Activation of eukaryotic and prokaryotic probiotics and synbiotic Preparation

The lyophilized capsule (US-Sarrow Fomalas Company) content of the *S.boulardii* probiotic was emptied into sterile liquid YPD medium (5). The lyophilized capsule probiotic *L. rhamnosus* (GG) (US- Valio Company) was activated using sterile liquid MRS medium (21) and incubated at 37°C for 24 hours and repeated three times individually for both reinforcers individually. The activation process was also repeated three times, but using 20 ml of skimmed milk medium (French Regala) 12% (w/v) + 2% sucrose for the yeast and without adding sugar to the bacteria, to prepare an active liquid culture for both of them (2). In addition, the logarithm of the live numbers of each of them was calculated according to the method described by (4). To prepare Psyllium seed powder prebiotic and the synbiotic cultuer, 5-1% (yeast - bacteria) was mixed and then the prebiotic (psyllium) was added to them at a concentration of 0.125%, and the live numbers of each probiotic were calculated separately as stated in the method (1)

Experimental animals

Forty-two male albino rat aged 4-6 weeks with an average weight of 100-120g were divided into six groups. All rats were Individually housed in plastic cages under standard conditions (such as heat, humidity, light, and ventilation) in a room at a controlled temperature (22 ± 2C) with a relative humidity of 55 ± 5% under a 12 h light–dark cycle. After 1 week of acclimation, the rats were equally divided into six groups with 7 individuals per group. The groups included a: 1- The negative control group fed a standard diet (Cont1) (A)

2- The positive control group (Cont2) fed a standard diet plus 10% Kcal (B)

3- A group fed diet (B) with the probiotic *L. rhamnosus GG* (*L.GG*)

4- A group fed diet (B) with the probiotic *S.boulardii* (*S.b*).

5-A group fed diet (B) with a mixture of *L. rhamnosus GG + S.boulardii* (*L.GG+S.b*)

6- A group that took diet (B) with the Synbiotic *L. rhamnosus GG + S.boulardii +* Psyllium seed powder (*L.GG+S.b*+Psy)

***All groups consumed a standard diet, a 10% increase in calories, excluding the first group (Cont1).**

Preparing the standard feed for experimental animals

The standard diet for feeding experimental animals was prepared according to what was mentioned (6) and the same standard diet was modified with an increase of 10% calories for all groups except for the control group (Cont1). As shown in Table 1, the ingredients of the two feeds for feeding experimental animals.

Table 1. Ingredients of the two diets for feeding experimental animals (g/100g)

Ingredients	Standard Diet	standard diet plus 10% Kcal
Casein	22.5	22.5
Sunflower oil	7.0	14.0
Cellulose	5.0	3.9
Starch	50.5	50.0
Sucrose	10.0	4.6
Vitamins and minerals	5.0	5.0
Total calories	395 Kcal	434.5 Kcal

Measuring some nutritional and health indicators:

Measuring the weights of experimental animals

The weight of the experimental animals was recorded regularly every week using a sensitive electronic scale, Then, the covariance ratios for these weights were calculated.

Measuring the weight of the feed consumed

The weights of the feed consumed by the experimental animals were estimated daily, taking into account the weights of the feed scattered in the cage according to the following equation:

Amount of feed consumed = Quantity of feed given to the animal - (amount of feed remaining + feed scattered in the cage)

Monitoring the health status of experimental animals

Monitoring the motor activity and health status of the experimental animals daily, recording observations and abnormal cases, following up on the color of the eyes, tail, hair and cases of diarrhea to ensure the health and safety of these animals.

RESULTS AND DISCUSSION

The effect of Synbiotic on the weights of experimental animals

The results in Figure 1 show the average weights of experimental animals (rats) per week for the duration of the experiment (8 weeks), and table 2 shows the percentages of variance for the average of those weights.

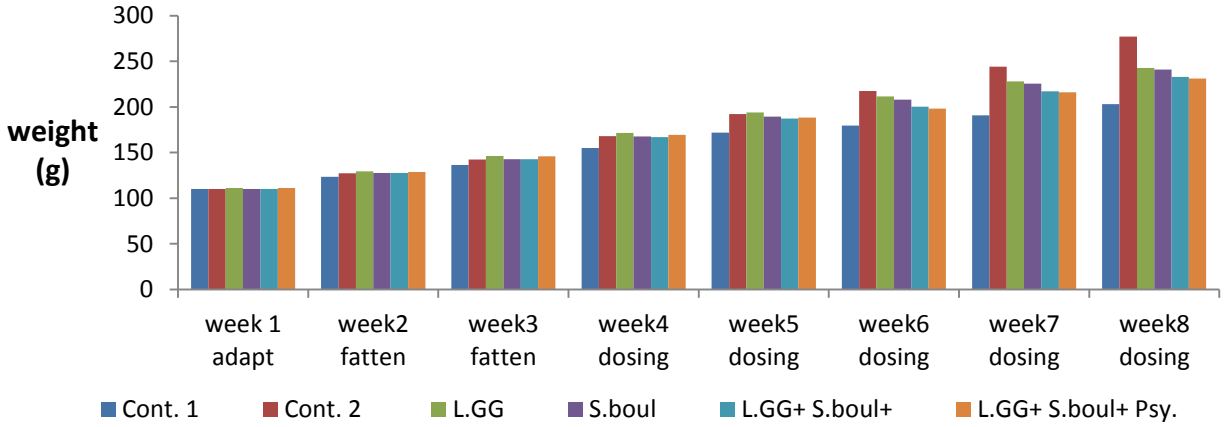

Fig 1. Effect of probiotic and Synbiotic on the weights of experimental animals (g)

The negative control group (Cont1) showed an increase in the average of those weights from 110g after a week of acclimatization to 203g after eight weeks of feeding on the standard diet

Table 2. Percentage variance of weights of experimental animals and the difference between the dosed and control groups at the end of the experiment

Group	Weeks							Difference in End of experiment	
	Fatten %			Dosing%					
	2	3	4	5	6	7	8	with Cont1	with Cont2
Cont. 1	10.93	19.31	12.13	20.65	24.12	28.57	32.84	0	-15.76
Cont. 2	13.59	22.74	15.17	25.85	34.51	41.62	48.60	15.76	0
L.GG	14.29	24.10	14.65	24.56	30.80	35.81	39.69	6.85	-8.91
S.boul	13.73	22.89	14.94	24.68	31.36	36.74	40.76	7.92	-7.84
L.GG+ S.boul	13.73	22.95	14.51	23.73	28.65	34.21	38.73	5.89	-9.87
L.GG+ S.boul+ Psy.	13.75	23.84	13.93	22.60	26.39	32.56	36.90	4.06	-11.7

As for the Cont2 group, their average weight increased from 110g to 277g after eight weeks of feeding. When comparing it with the Cont1 group, we notice an increase of 15.76% in weights. This is due to the nature of the diet containing a 10% increase in calories, and it showed significant differences at the level of ($P<0.05$) starting from the fourth week of the experiment and it was increasing weekly, this is consistent with what was mentioned by Mazloom et al., (17) that consuming more calories results in an increase in body weight and the accumulation of fatty tissues, in addition to this, it leads to disrupt the balance of the gut microbiota, which in turn increases the extraction of energy from food components. It was also noticed that there was an increase in the average weights of the *L.GG* group, starting from 111g to 242g, after eight weeks of feeding, compared to the Cont1 group, and this increase in the average weights was less compared to what happened with the Cont2 group, even though they consumed the same diet (10% increase in calories). It showed a significant decrease in weight at the level ($P<0.05$) starting from the seventh week of the experiment (the fourth week of dosing), and the difference in the percentage of variation at the end of the experiment was 8.91% less compared to the Cont2 group, while the difference reached 6.85% when compared with Group Cont1. The decrease in the weight of the *L.GG* group compared to the Cont1 group is due to the mechanisms possessed by these bacteria in showing its effect in reducing weight. Cheng and Liu, (7) revealed that the administration of probiotic *L.GG* to the mice led to a decrease in their weight despite being fed a HFD diet. The results also showed that the *S.boulardii* group had an increase in their average weights starting from 110 g to 240 g compared to the Cont1 group, and this increase was less compared to the Cont2 group, despite the fact that they were fed the same diet (10% increase in calories) to show a significant difference at the level ($P< 0.05$) starting from the seventh week of the experiment (the fourth week of dosing) and the difference in

the percentage of heterogeneity at the end of the experiment was 7.84% less compared to the Cont2 group, while the difference was 7.92% when compared with the Cont1 group, and it is noted that *S.boulardii* yeast is less efficient in Weight reduction in comparison with *L.GG* bacteria. The results are consistent with that reported by Everard et al., (8) That the ability of *S.boulardii* yeast to reduce the weight of mice fed a high-calorie diet by modifying the gut microbiota, reducing inflammation and reducing fat mass in mice body. The results also showed an increase in the average weights of the *L.GG* + *S.b* group during the trial period, starting from 110g to 233g compared to the Cont1 group. This increase is due to the natural growth of experimental animals and their consumption of a standard diet, an increase of 10% of calories. However, this increase in the average weights is not at the same level as the weights of the Cont2 group fed the same standard diet, due to dosing rats with the co-culture eukaryotic and prokaryotic probiotics (*L.GG+Sb*), which was more effective in reducing weight and in a shorter period compared to using it alone despite being fed a standard diet with an increase of 10% of calories, this group showed a significant difference at the level ($P<0.05$) starting from the sixth week of the experiment (the third week of dosing), with a difference in the percentage of variance amounted to 9.87% less compared to group Cont2, while the difference was 5.89% when compared with group Cont1.

The synbiotic group (*L.GG+S.b*+Psy) showed an increase in the body weights from 111g to 231g compared to the Cont1 group. This height is lower compared to the Cont2 group as it was given in that significant difference at the level ($P<0.05$) starting from the sixth week of the experiment (the third week of dosing), with the highest difference in the heterogeneity rate reaching 11.70% less. It is noteworthy that the co-cultuer of eukaryotic and prokaryotic probiotics or its prebiotic additive psyllium was used for the first time in achieving weight reduction in a way that exceeds each of them individually. This study findings are in agreement with that of Ferrarese et al., (9) who reported that probiotics and synbiotic modulate the gut microbiota through competition, adhesion to epithelial mucosal membranes and the ability to absorb lipids through cleavage of chelated salts and inhibition of the enzyme LPL that controls triglyceride deposition in adipocytes and a decrease in LPS compound, which leads to the regulation of the work of hormones, including those that control appetite, the most important of which are leptin as well as insulin and improve their sensitivity, as well as improve the function of the intestinal barrier, which leads to control body weight.

Effect of probiotics and synbiotic on the amount of feed consumed

Figure 2 shows the average weights of feed consumed weekly throughout the experiment period (8 weeks). Table 3 shows the percentages of variance for the average of those weights.

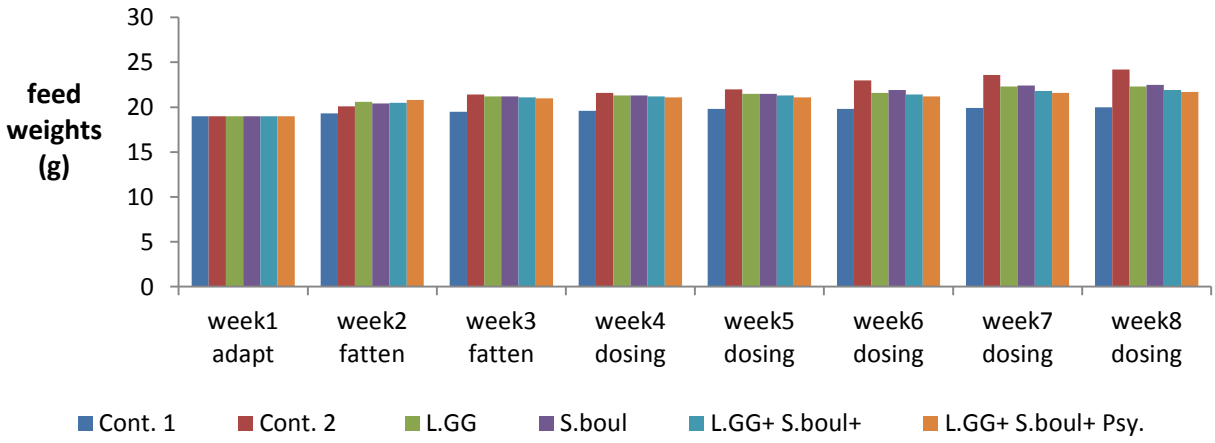

Fig 2. Effect of the synbiotic of eukaryotic and prokaryotic probiotics on feed weights (g)

It is noted that the weights of the feed consumed in the Cont1 group increased after a week of adaptation from 19g to 20g, and this is due to the nature of growth .

Table 3. Percentage variance of feed weights consumed by experimental animals and the difference between the dosed and control groups at the end of the experiment

Group	Weeks							Difference end of experiment	
	Fatten%			Dosing%					
	2	3	4	5	6	7	8	with Cont1	with Cont2
Cont. 1	1.55	2.56	0.51	1.52	1.52	2.01	2.5	0	-9.07
Cont. 2	5.47	11.22	0.93	2.73	6.96	9.32	11.57	9.07	0
L.GG	7.77	10.38	0.47	1.4	1.85	4.93	4.93	2.43	-6.64
S.boul	6.86	10.38	0.47	1.4	3.2	5.36	5.78	3.28	-5.79
L.GG+ S.boul	7.32	9.95	0.47	0.94	1.4	3.21	3.65	1.15	-7.92
L.GG+ S.boul+Psy	8.65	9.52	0.47	0.47	0.94	2.78	3.23	0.73	-8.34

It was also noted that the Cont2 group consumed an increase of 10% calories at an increasing rate after a week of anaphylaxis, from 19 g to 24 g. An increase of 10% of calories led to an imbalance in the gut microbiota and an increase in the number of *Firmicutes* bacteria that work to harvest calories extracted from food, which leads to obesity and thus a decrease in the brain's response to the hormone leptin, which is responsible for satiety. The results also recorded an increase in the average weights of the feed consumed daily during the week in the *L.GG* group, which consumed the same standard feed in the Cont2 group, but this increase was not at the same rates, as the average weights of the fed consumed by it, decreased with a significant difference at the level ($P<0.05$) starting from the seventh week of the experiment (the fourth week of dosing) and continued in this decline until the end of the experiment to give a difference in the percentage of heterogeneity amounted to 6.64% decrease compared to the Cont2 group and an increase of 2.43% compared to the Cont1 group. This is consistent with what Cheng and Liu, (7) stated that feeding mice fed an HFD diet with the probiotic *L.GG* leads to an increase in the feeling of satiety as a result of raising the sensitivity of the leptin hormone responsible for this function. The results also showed the effect of *S.boulardii* yeast on the weights of the feed consumed in the group of experimental animals that were dosed with this eukaryotic probiotic. The average weight of what they consumed daily during the week increased from 19g to 22.5g, and the rate of this increase in the feed was significantly less than the increase in the Cont2 group at level ($P<0.05$) in the seventh week of the experiment (4th week of dosing). At the end of the experiment, the difference in the heterogeneity rate were 5.79% less as compared to the Cont2 group, while the increase was 3.28% compared to the Cont1 group. The results showed that the *L.GG+Sb* group had an increase in the weights of the feed consumed from 19 g to 21 g, but this increase was not by the same amount in the Cont2 group despite eating the same feed (10% increase in calories). The combined eukaryotic and prokaryotic probiotics, which showed a superior effect in reducing the weights of the feed consumed in a shorter period, and gave a significant difference at ($P<0.05$) starting from the sixth week of the experiment (the third week of dosing), and at the end of the experiment a difference in the variance ratio was 7.92% less compared to the Cont2 group, while the increase was 1.15% compared to the Cont1 group. It is evident from the results presented in the group *L.GG+S.b+Psy* that the average weight of the consumed feed increased from 19g to 21.7g, which is lower than the amount consumed in the Cont2 group despite consumed the same feed (10% increase in calories). An additional factor and a synergistic effect for co-culture, which is the prebiotic (psyllium) in the treatment *L.GG+S.b+Psy*, to give a more efficient effect in reducing the consumed diet compared to the rest of the groups that were dosed with probiotics, as the difference in the percentage of variation reached 8.34% less compared to the Cont2 group, the increase was 0.73% compared to the Cont1 group.

Effect of probiotics and synbiotic on the health status of experimental animals

Table 4 shows what was monitored for the health status of the experimental animals, their

activity and their general appearance throughout the experiment period. It was noted that the Cont1 group that was fed a standard diet only gave better health indicators compared to the Cont2 group that was fed a standard diet, with an increase of 10% in calories. A little roughness was observed with little hair loss, and a slight spotting, however this group was moderately active and diarrhea was not observed. The emergence of some undesirable minor signs during the experiment period maybe due to a change and the beginning of an imbalance in the gut microbiota with its progression in age. Despite all of this, it is healthier than the Cont2 group. These findings were in accordance with that stated by Kim and Benayoun, (16) that advancing age leads to an imbalance in the gut microbiota

Table 4. Effect of probiotics and synbiotic on the health status of experimental animals

Groups	Hair	Eyes	Tail	Movement and activity	Cases of diarrhea
Cont1	**Slight roughness with little hair loss**	**normal**	**slightly keratinized, with little spotting**	++	**none**
Cont2	**High coarseness with visible hair loss**	**Abnormal dull color (sleepy)**	**corny with spots and abnormal color**	+	**2**
L.GG	**Smooth** +++	**natural and shiny**	**normal**	+++	**none**
S.boul.	**Smooth** +++	**natural and shiny**	**normal**	+++	**none**
L.GG + Sb.	**Smooth** ++++	**natural and shiny**	**normal**	++++	**none**
***L.GG+ Sb* +psy**	**Smooth** ++++	**natural and shiny**	**normal**	++++	**none**

The Cont2 group showed abnormal symptoms and deterioration of its health condition, starting from the first week of consuming a standard diet, an increase of 10% in calories, and some clear signs of inactivity, lethargy, pale eyes, coarseness, and obvious hair loss in different parts of the body with spots of the tail. In addition to two cases of diarrhea, and this is one of the most important signs of a worsening of their health condition due to the imbalance in the gut microbiota. This is in accordance our previous finding which indicated that the consumption of the Cont2 group for a standard diet increased by 10% calories led to a decrease in the live numbers of *Lactobacillus* bacteria and an significant increase in the live numbers of bacteria of the *Firmicutes* phyla, which leads to the emergence of disease symptoms, and perhaps the most important indicator is diarrhea. These results agree with what Hussain et al., (14) stated that HFD diets lead to an imbalance in the gut microbiota and encourage pathogenic microorganisms to exercise their antagonistic effects on beneficial microorganisms, which leads to the emergence of disease symptoms, such as diarrhea (caused *Clostridium difficile*). Some strains of probiotics are effective in inhibiting aerobic and anaerobic pathogenic bacteria (3).

It became clear through the results and through what was monitored during the experiment, that dosing groups of experimental animals with probiotic and synbiotic led to an improvement in their health condition, even though they consumed a standard diet, an increase of 10% calories, after some unhealthy signs appeared during the two weeks of fattening (before dosing), including a little hair loss and inactivity. During the weeks of dosing, they gradually began to regain their health condition to give all groups at the end of the experiment a clear healthy effect, represented by some important signs, such as smooth hair, shiny natural eyes and natural tails. Considering, the movement and activity, all groups gave a high activity, and the two groups that were dosed were characterized by co-culture (*L.GG+Sb*) in addition to the prebiotics (*L.GG+S.b+Psy*), as noted through the results, that the kinetic status and activity of all groups that were dosed with the probiotics and the synergists were better compared to even the Cont1 group. In addition, no cases of diarrhea were observed, despite the consumption of a diet that had a higher calorie consumption, which resulted from the increase in the proportion of oil in it. This is explained by the fact that dosing experimental animals with probiotics and

synbiotics throughout the dosing days led to restoring the balance of the gut microbiota and increasing the antagonistic action of beneficial microorganisms against the diarrhea-causing bacteria. This is consistent with Pandey et al., (18) reporte that probiotics and synbiotic enhance anti-pathogenic activity, treat diarrhea and constipation, as well as nutrient synthesis and improve their bioavailability, Reducing the symptoms of allergies, cancer, AIDS, respiratory and urinary tract infections and their beneficial effects on aging, fatigue, autism, osteoporosis, type 2 diabetes and obesity, due to the mechanisms that they perform inside the body, including the production of inhibitory substances such as H2O2, bacteriocins and organic acids and blocking the adhesion sites of pathogenic bacteria and competition with them for Nutrients also degrade toxins, block their receptors, and modulate immune responses.

REFERENCES

1. Al-Ogaidi, A. A. A. and A. A. A. Daher, 2019. The study of some chemical properties of Plantago ovata seeds and its utilization as synbiotics in fermented milk, Plant Arch,. 19, (2): 1824–1829,.

2. Al-Shammary,H. A. A., A. A. A.-S. Daher, and S. M. Salih,. 2018. Effect of probiotic Saccharomyces boulardii in weight losing and cytokines levels in mice before and after Infection by Salmonella typhimurium. Al-Anbar Journal of Veterinary Sciences., 11, (1): 1999-6527

3. Alziadi R. E., A. A. A.-S. Thaher, and N. A. Al-Issawi, 2020. Utilization of isolated therapeutic bacteria in topical ointment for infection treatment, Iraqi J. Agric. Sci., 51, (6): 1661–1669,.

4. Bashar,M. A. A., and A. M. A. Thaher. 2015. "New synbiotic sweet produced from almond skin and some probiotic bacteria, pp. 2319–7064, Jan., doi: 10.21275/ART20175022.

5. Cardoso, V. M., B. M.Borelli, C. A. Lara, M. A.Soares, C. Pataro, E. C.Bodevan, and C. A.Rosa. 2015. The influence of seasons and ripening time on yeast communities of a traditional Brazilian cheese, Food Res. Int., (69): 331–340,.

6. Çelik, M. N. and M. Ü. Söğüt, 2019. Probiotics improve chemerin levels and metabolic syndrome parameters in obese rats, Balkan Med. J., 36, (5): 270,.

7. Cheng Y.-C. and J.-R. Liu,. 2020. Effect of *Lactobacillus rhamnosus GG* on energy metabolism, leptin resistance, and gut microbiota in mice with diet-induced obesity, Nutrients, 12, (9): 2557,.

8. Everard A., S. Matamoros, L. Geurts, N. M. Delzenne, and P. D. Cani,. 2014. *Saccharomyces boulardii* administration changes gut microbiota and reduces hepatic steatosis, low-grade inflammation, and fat mass in obese and type 2 diabetic db/db mice, MBio, 5, (3): e01011-14,.

9. Ferrarese,R., E. R. Ceresola, A. Preti, and F. Canducci,. 2018. Probiotics, prebiotics and synbiotics for weight loss and metabolic syndrome in the microbiome era, Eur Rev Med Pharmacol Sci, 22, (21): 7588–7605,.

10. Gibson G. R. and M. B. Roberfroid,. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics, J. Nutr., 125, (6): 1401–1412,.

11. Gibson, G. R., R. Hutkins, M. E. Sanders, S. L. Prescott, R. A. Reimer, , S. J.Salminen, and G. Reid. 2017. Expert consensus document: The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics, Nat. Rev. Gastroenterol. Hepatol, 14, (8): 491–502,.

12. Grimoud, J., H. Durand, C .Courtin, P.Monsan, F. Ouarné, V.Theodorou, , and C .Roques. 2010. In vitro screening of probiotic lactic acid bacteria and prebiotic glucooligosaccharides to select effective synbiotics, Anaerobe,. 16, (5): 493–500,.

13. Hotel A. C. P. and A. Cordoba. 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, Prevention, 5, (1): 1–10,.

14. Hussain, M., G. Bonilla-Rosso, C. K. K. Chung, L.Bäriswyl, M. P.Rodriguez, B. S.Kim, , ... and M.Noti,. 2019. High dietary fat intake induces a microbiota signature that promotes food allergy, J. Allergy Clin. Immunol., 144, (1): 157–170,.

15. Khaleel, M. M. and A. A. Thaer. 2017. Using probiotics and inulin to prolong ferminted dairy products shelef life, Iraqi J. Agric. Sci., 48, (2): 608–617,.

16. Kim, M., and B. A.Benayoun, 2020. The microbiome: An emerging key player in aging and longevity. Translational Medicine of Aging, (4): 103-116.
17. Mazloom,K., I. Siddiqi, and M. Covasa,. 2019. Probiotics: how effective are they in the fight against obesity?, Nutrients, 11, (2): 258,.
18. Pandey,K. R., S. R. Naik, and B. V Vakil,. 2015. Probiotics, prebiotics and synbiotics-a review, J. Food Sci. Technol., 52, (12): 7577–7587,.
19. Rasheed, H. T., K. J. K. Luti, and M. A. Alaubydi,. 2020. a probiotic application of *Lactobacillus acidophilus* ht1 for the treatment of some skin pathogens, Iraqi J. Agric. Sci., 51, (6): 1559–1571,.
20. Ríos-Covián D., P. Ruas-Madiedo, A. Margolles, M. Gueimonde, C. G. De Los Reyes-gavilán, and N. Salazar. 2016. Intestinal short chain fatty acids and their link with diet and human health, Front. Microbiol., 7, 185,.
21. Robinson, R. K. 2005. Dairy microbiology handbook: the microbiology of milk and milk products. John Wiley and Sons, (3): 551-554
22. Skalkam, M. L., M. Wiese, D. S.Nielsen, , and G.Van Zanten. 2016. In vitro screening and evaluation of synbiotics, Univ. Copenhagen Copenhagen, Denmark, 477–486,.