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Original Research Article

Effect of *Lactobacillus acidophilus* and *Lactobacillus plantarum* on Weight Reduction in Obese Rats

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ABSTRACT

Obesity is a risk factor for diseases such as high blood pressure and type 2 diabetes. Over the years, alternatives to the use of drugs and surgery being used to treat obesity are being sought to improve health. This research aimed to determine the effect of two *Lactobacillus spp* on weight reduction in obese rats. Fifty 6-8weeks old female albino Wistar rats were grouped into two major groups; High fat diet (HFD) groups and Normal Diet (ND) groups. A concentration of 1 x 10^9 cfu/ml of the *Lactobacillus spp* was suspended daily in normal saline and orally given to rats for two weeks. The weights of both obese and non-obese rats were recorded. Results were analysed using the one-way analysis of variance. Results indicated that bodyweight increased more in the HFD groups compared to the ND groups. Analysis after probiotic treatments showed that *L. plantarum* (P1), and *L. acidophilus* + *L.plantarum* (PMIX) were effective in reducing the weight of the obese rats ($P<0.05$) while *L. acidophilus* (P2) was ineffective at reducing weight gain in the obese groups. The use of probiotics for weight loss can limit the adverse side effect associated with using conventionally produced anti-obesity drugs.

***Keywords*:** Obesity, Probiotics, Weight, High Fat Diet, *Lactobacillus.*

Introduction

Obesity is characterized by a combination interplay of environmental and genetic factors, which often arise from a long-term imbalance between energy intake and energy expended. Diets high in carbohydrates and fat accumulates lots of calories which when combined with a sedentary lifestyle are some of the factors responsible for the obesity pandemic. In addition, excessive consumption of Fatty foods can lead to an increase in adipose tissue which is also a factor leading to the development of metabolic and cardiovascular diseases such as fatty liver disease, insulin resistance, cancers and type 2 diabetes, and this is most common in individuals who are genetically susceptible to these diseases.[1] Any individual whose body mass index is above 25.0 kg/m^2 is considered obese and it has been predicted that by the year 2030, 3.3 billion people whose body mass index is equal or above 25.0 kg/m^2 will be affected by obesity.[2] The core method for treating obesity currently include regular and effective exercise, drug modulation, surgical therapy and dietary intervention. However, medical intervention in treating obesity differs greatly in medication effectiveness and the side-effect patterns, thus the use is limited to a few patients due to feasibility, safety and health insurance issues.[3] Living microorganisms consumed to provide a health benefit to the host thus improving microbial balance within the gut are known as probiotics.[4.] Using probiotics as additives in feed or directly feeding them to experimental animals has been shown to reduce intestinal pathogen colonization, improve productivity and facilitate the production of antibacterials.[5] Among the Lactic Acid Bacteria (LAB), *Lactobacillus* is the most distinguished and commonly used in health care, animal husbandry and food industries.[6] In order for the probiotic organism to be effective, it must reach the innermost part of the gut in sufficient amount to exert its benefits. A systematic review of various regulated trials confirmed that probiotic when administered substantially decreased fat percentage, body weight and body mass index when compared to placebo.[7] The effectiveness of probiotics is dependent on the type of strain used and may rely on the various metabolites developed by the probiotic organism.[8] The present study is aimed at investigating the ability of *Lactobacillus spp* isolated from fermented foods in the reduction of weight gain in rats fed a high-fat diet.

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Materials and Methods

Isolation of Lactobacillus: Two species of *Lactobacillus* previously isolated from locally fermented Ogi (Pap) and yoghurt and characterized for some probiotic features were used for this study. The species were identified as *Lactobacillus plantarum* and *Lactobacillus acidophilus*

Prepararion of the probiotic treatment
Before oral administration of the probiotic treatment, the probiotic to be administered was prepared daily by inoculating sterile normal saline with the *Lactobacillus* spp and incubating anaerobically at 37^{o}C for 24 hours. The concentration of the bacterial in the mixture was 1 x 10^9 cfu/mL

Experimental design
Covenant University Health and Research Ethical Committee (CUHREC) approved this study with an approved assigned protocol number CHREC/53/2020. A 60% diet high in fat was used to model obesity. Fifty 6-8weeks old female albino Wistar rats were purchased. The rats were kept in cages under controlled temperature (21-23^{o}C) and 12hours light and dark cycle. The rats were grouped as follows: (i)

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High Fat Diet group (HFD) which was further sub divided into four groups; group HfD+P1 fed on HFD thoughout the experimental period and were treated with L. plantarum, Group HFD + P2 were treated with L. acidophilus while group HFD + PMIX were treated with a mixture of both *L. acidophilus* and *L. plantarum*. HFD alone groups were fed with HFD throughout the exeperiment (ii) Normal Diet group (ND) which was further sub divided into four groups; group ND+P1 fed on ND thoughout the experimental period and were treated with L. plantarum, Group ND + P2 were treated with L. acidophilus while group ND + PMIX were treated with a mixture of both *L. acidophilus* and *L. plantarum*. ND alone groups were fed with ND throughout the exeperiment. The probiotic treatment was carried out daily for two weeks. The mean weights of the rats were recorded weekly both before and after treatment to check for the effect of the probiotic treatment on the weight of the obese rats.

Determination of obese rats
Some parameters were used to classify the rats as obese and non-obese and these parameters include:

Body Weight
The bodyweight of the experimental animals was one of the ways by which the rats were grouped as obese or non-obese. During the feeding period (10 weeks), the mean weight of rats was determined weekly using an electronic weighing balance.

Body Mass Index
The BMI of the animals was carried out to determine which of the group is obese. The animals were pinned on a flat table and their length measured from the nose to the anal region with a tape. The weights were recorded using a weighing balance. BMI of the animals was calculated using the formula:

$$\text{BMI} = \frac{\text{Weight (g)}}{\text{Length}(\text{cm}^2)}$$

Results were recorded as g/cm^2.

Lee index
Analysis of the Lee index was carried out using the method of Malafaia *et al*.[9] Lee index was thus calculated using the formula:

$$\text{Lee index} = \frac{\text{cube root of body weight (g)}}{\text{Nose} - \text{to} - \text{anus length(cm)}} \times 1000$$

Results were recorded as g/cm.

Effect of probiotic treatment on body weight
after two weeks of treatment, the mean weight of rats in the treated and untreated group were analyzed to check if the probiotic organisms had any effect on the body weight of the obese rats. This was done before the rats were sacrificed for further experiments.

Statistical analysis
Graphpad prism software version 8.0 was used for the statistical analysis. Results were recorded as mean with their standard error of mean. One-way analysis of variance followed by Tukey's post hoc comparison test was used to check for significant difference among groups. Differences between groups were defined as significant if $P < 0.05$.

Results and Discussion

The study aimed to investigate the effects of probiotics, *L. acidophilus* and *L. plantarum* on weight reduction in diet-induced obese rats using a high-fat diet. The components of the High Fat diet used is shown in Table 1. In this study, the high-fat diet was used to mimic an unhealthy diet pattern while the normal diet was used to represent a healthy diet. Several studies have been carried out on the potential of probiotics being used as a therapeutic agent in the management of obesity.[10,11] Using probiotics in the management of obesity has been confirmed to increase metabolic rate and energy consumption, decrease a fat deposit in the body and maintain nutrient balance which is altered as a result of high-calorie foods.[12] Body weights, Body mass index and Lee index were the parameters used to classify the experimental animals as obese and non-obese. Body weight was measured weekly using an electronic weighing balance. The mean weights of rats per group (Table 2) showed that rats in the High-fat diet groups gained more weight than rats fed regular diets. The rate of weight gain was highest in the HFD alone group throughout the experiment.
The mean Body mass index of rats is presented in Figure 1. Even though the BMI of the HFD was higher than that of the ND group ($P<0.05$), there was only a little difference in the BMI of both groups when compared.
The analysis of the Lee index is presented in Figure 2 and the results also showed that the mean Lee index of the rats in the high-fat diet group was higher than that of the normal groups ($P<0.05$).
To check for the effect of the two *Lactobacillus spp* on weight reduction in both obese and non-obese rats, a concentration of 1×10^9 cfu/ml of the *Lactobacillus spp* was suspended daily in normal saline and orally given to rats for two weeks. From the analysis, *L. acidophilus* was unable to reduce weight gain in the high-fat diet-fed rat ($P>0.05$) in both the first and second week of treatment, this finding agrees with previous studies which reported that *L. acidophilus* was unable to reduce weight gain in mice fed high-fat diet [1,13]. However an effect was noticed when combined with *L. plantarum* (Figure 3). The inability of *L. acidophilus* to reduce weight gain could be as a result of the inability of the organism to adhere to the intestinal wall of the rats or it could be that the duration of the treatment is not sufficient for the organism to exert its effect on the weight of the rats. However, when L. acidophilus was used as a combined dose with L. plantarum, it was able to exert an effect on the weights of the rats, this can indicate that *L. acidophilus* as a single dose might not have an effect on weight reduction singly unless it is used as a combined dose. In the normal diet group, however, while no significant observation was noted in the first week of treatment (Figure 4), a reduction in weight gain was noted in all groups treated with probiotics ($P<0.05$) in the second week of treatment. This could be as a result of the organisms being able to attach adequately to the intestinal wall of the animals thereby preventing excessive fat storage and thus preventing the animals from being obese. Finally, it is not enough to be consuming probiotics only, adequate physical activities and reducing the consumption of calories will help to sustain healthy intestinal homeostasis, decrease obesity and promote wellness.

Table 1: components of the High fat diet

Ingredients	Composition (kg)
Maize	4.4
Wheat offal	2.0
Rice brown	2.0
Groundnut cake	2.0
Lard	14.0
Bone	0.1
Vitamin Mix	0.05
Lysine	0.05
Methionine	0.05
Salt	0.05

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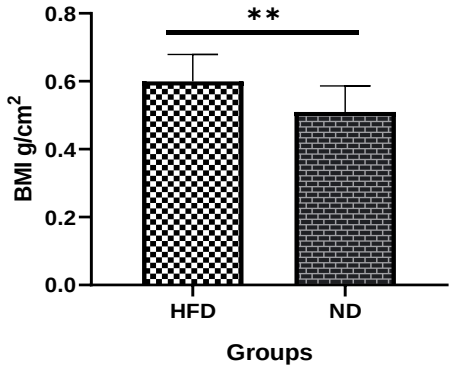


Figure 1: Body mass index of experimental animals (line with symbols on top indicates there is a significant difference between the groups)

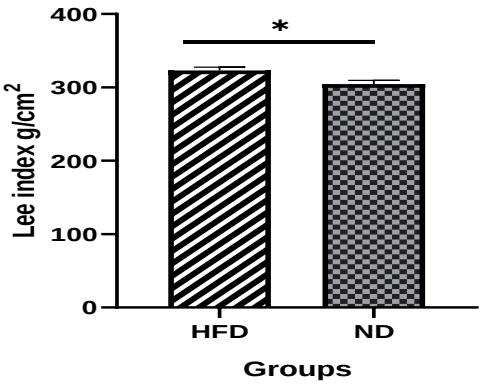
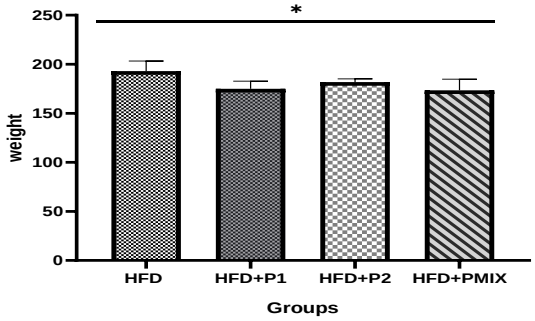


Figure 2: Lee index of experimental animals



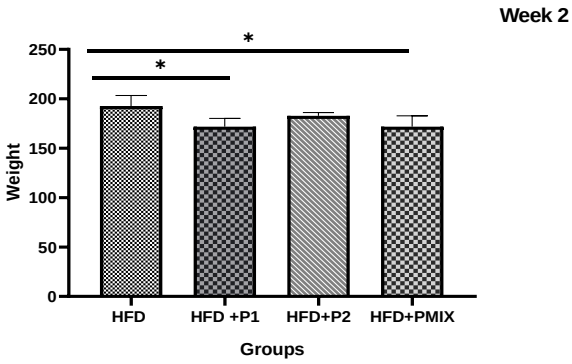


Figure 3: Effect of probiotic treatment on the weight of rats placed on probiotic treatments (P1= *L.acidophilus*, P2 = *L. plantarum*, PMIX = *L. acidophilus* + *L. plantarum*. Line with symbol on top indicate there is a significant difference between the groups).

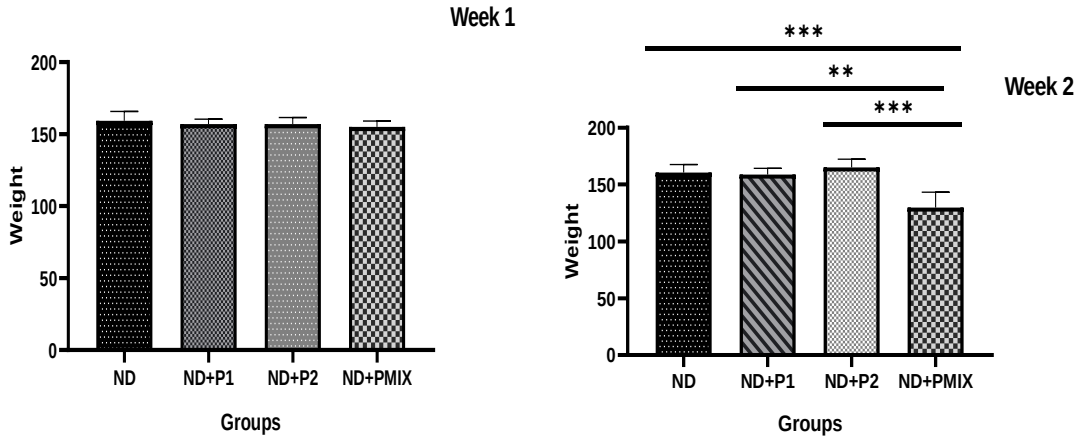


Figure 4: Effect of probiotic treatment on the weight of rats placed on probiotic treatments in the normal diet groups (P1= *L.acidophilus*, P2 = *L.plantarum*, PMIX = *L.acidophilus* + *L. plantarum*. Line with symbol on top indicate there is a significant difference between the groups).

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Table 2: Mean weight of rats with their standard error of mean

	HFD		ND group	
	Mean	**S.E**	**Mean**	**S.E**
Week 1	127.5	5.0	119.7	2.5
Week 2	130.2	1.7	130.4	5.0
Week 3	137.0	1.6	133.5	4.9
Week 4	143.6	1.7	136.1	5.0
Week 5	149.4	1.8	138.9	4.9
Week 6	154.2	1.8	142.3	4.8
Week 7	160.1	1.7	145.4	4.8
Week 8	165.5	1.8	148.8	4.8
Week 9	171.1	1.7	151.7	4.8
Week 10	177.0	1.9	155.4	4.9

Conclusion

In this study, Rats fed with a high-fat diet have a higher level of weight increase compared to those fed with normal compositions. This study has shown that adequate physical activities, reducing calorie consumption and probiotic administration can help to sustain healthy intestinal homeostasis, decrease obesity and promote wellness. .

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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