

Growth Performance Parameters of Experimental Rats Fed Diets Containing Different Lipid Sources with or without Probiotics

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Abstract

Our study aimed to investigate the effect of different lipid sources (animal and plant) on body weight and the relative weight of organs (heart, liver, kidney, and spleen) in male laboratory rats. It also aimed to evaluate the role of the probiotics *Bifidobacterium bifidum* and *Bacillus coagulans* in suppressing the potentially harmful metabolic effects of certain lipids. The experiment involved 65 male Albanian rats (average age: 2–3 months; average weight: 200–230 g). The rats were randomly assigned to 13 experimental groups, including a control group and groups fed diets supplemented with different lipid sources (cholesterol, fish oil, olive oil, and animal fats) alone or in combination with a continuous daily oral dose of the probiotics 1.5×10^8 colony-forming units (CFU) twice daily for 28 days. The results showed significant differences ($P \leq 0.05$) in final weight and weight gain. The animal fat group, without the probiotic (Af), recorded the highest weight gain (2.48 ± 0.36 g), accompanied by a marked increase in the relative weights of organs, with the highest values recorded for the heart (2.30 ± 0.02 g/100 g), liver (18.20 ± 3.30 g/100 g), and kidneys (3.80 ± 0.05 g/100 g). Conversely, the cholesterol group (Cho) had the highest spleen relative weight (2.26 ± 0.05 g/100 g). Furthermore, the intervention combining unsaturated oils with probiotics effectively curbed weight gain, resulting in a significant decrease in weight gain for the olive oil treatment with *Bifi. bifidum* (Oo+Bb) and the fish oil treatment with *B. coagulans* (Fo+Bc), amounting to 4.0 ± 0.12 g and 3.6 ± 0.02 g, respectively. This coincided with a significant reduction in liver and other organ hypertrophy. Specifically, the relative liver weight of the group treated with *Bifi. bifidum*-enriched fat (Af+Bb) decreased to 15.31 ± 2.58 g/100 g. These results demonstrate that the interaction between unsaturated oils and probiotics helps reduce visceral and tissue fat accumulation. It also helps mitigate the metabolic burden and pathological hypertrophy of major organs caused by high-fat diets.

Keywords: *Bifidobacterium bifidum*, *Bacillus coagulans*, lipid profile, probiotics.

Introduction

Recent studies indicate a strong link between high-fat diets and dyslipidemia. Increased intake of high amounts of (saturated) fat and cholesterol is a key feature of diets associated with dyslipidemia [11]. Dietary patterns directly or indirectly affect the lipid profile. Dyslipidemia is a major risk factor for cardiovascular disease, and a healthy lifestyle is the first line of defense for prevention and treatment [1]. There are well-established associations between traditional and Western dietary patterns

and elevated total cholesterol and LDL cholesterol levels. In this context, many studies recommend avoiding excessive or frequent consumption of either traditional or Western diets [16]. The development of oxidative stress can damage various organs, including the heart, pancreas, lungs, and kidneys [18]. Changes in organ weight are a sensitive indicator of organ damage resulting from metabolic influences due to external factors [15].

Probiotics are defined as live microorganisms that provide health benefits when consumed in sufficient and

balanced amounts [23]. They have numerous uses in human, animal, and plant health. Probiotics have been used to enhance resistance to fungal infections in plants and to improve livestock productivity [14][10]. They also offer significant health benefits to humans, possessing numerous antimicrobial and anticancer properties [2]. *Bifidobacterium* and *Bacillus* are among the most common probiotics. Other microorganisms that can be used as probiotics include *Saccharomyces*, *Lactobacillus*, *Enterococcus*, and *Streptococcus*. Probiotics play important roles in promoting overall health and treating various conditions, such as diabetes, infections, obesity, and cardiovascular diseases. Several studies indicate that probiotics can help regulate weight and blood sugar levels by modulating metabolism and improving insulin sensitivity [21].

The probiotic bacterium *Bifidobacterium bifidum* is an important strain used in the production of therapeutic food products. It has demonstrated numerous biological and functional effects and health benefits, placing it in the category of multi-probiotics. It helps enhance the integrity of the intestinal barrier and alleviate symptoms of gastrointestinal disorders and diseases [22]. Studies indicate that *Bifidobacterium bifidum* improves blood lipid levels, thereby aiding weight loss, and enhances insulin sensitivity and glucose metabolism without affecting the amount of food a person consumes [20].

Numerous studies have shown that consuming dietary supplements containing *Bacillus coagulans* strains offers several health benefits. Many studies have revealed therapeutic benefits for gastrointestinal diseases, as well as improved quality of life for individuals with overweight, obesity, or diabetes by modifying the gut microbiota and supporting the immune system [8][25].

Research has demonstrated the effectiveness of *B. coagulans* in weight management and improving liver tissue parameters effectively without side effects by regulating the gut microbiota [12]. Some studies suggest that *B. coagulans* may help prevent cardiovascular disease by reducing visceral fat and overall fat mass significantly in obese individuals. Therefore, *B. coagulans* can help manage weight without changes in diet or physical activity and without serious side effects [4].

Given the high fat content of modern diets and their detrimental health effects, our current study examines the impact of fats from plant and animal sources on cardiac function, blood lipid profile, and the potential health effects of the probiotics *Bifidobacterium bifidum* and *Bacillus coagulans*.

Material and Methods

Experimental Animals:

The laboratory animals were obtained from the animal house of the College of Veterinary Medicine, Tikrit University, and the biological experiment was conducted there. Sixty-five male albino rats, aged 2-3 months and weighing an average of 200-230 grams, were used. A specialist veterinarian performed the necessary examinations to ensure their health and freedom from disease. The animals were allowed 28 days to acclimatize and undergo the required metabolic changes, during which time they were fed a specially formulated diet. Following this period, the groups were administered oral probiotics in addition to their individual diets.

The experimental rats were divided into thirteen groups and placed in individual cages. The rats were randomly assigned to groups of five. They were fed different diets and provided with individual bottles of filtered water for each cage. The experimental groups were designed as follows: (Con): Control group, which

received the standard diet. (Cho): Rats in this group were fed a diet fortified with cholesterol only. (Cho+Bb): Group fed a diet fortified with cholesterol and the probiotic *Bifidobacterium bifidum*. (Cho+Bc): Group fed a diet fortified with cholesterol and the probiotic *B. coagulans*. (Fo): Group fed a diet fortified with fish oil only. (Fo+Bb): Group fed a diet fortified with fish oil and the probiotic *Bifidobacterium bifidum*. (Fo+Bc): The group fed a diet fortified with fish oil and the probiotic *B. coagulans*. (Oo)The group fed a diet fortified with olive oil only. (Oo+Bb)The group fed a diet fortified with olive oil and the probiotic *Bifidobacterium bifidum*. (Oo+Bc)The group fed a diet fortified with olive oil and the probiotic *B. coagulans*. (Af)The group was fed a diet fortified only with animal fat. (Af+Bb): The rats in this group were fed a diet fortified with animal fat and the probiotic *Bifidobacterium bifidum*. (Af+Bc) The group fed a diet fortified with animal fat and the probiotic *B. coagulans*.

Diets Preparation:

The basic diet for the laboratory animals was prepared as described in the NRC (1995). The diet was formulated to contain (g/kg): Casein 85 g, Protein 158.5 g, Sunflower Oil 100 g, Vitamin Mixture 5 g, Mineral Mixture 50 g, Cellulose 50 g, Glucose 100 g, and Starch 451.5 g [17].

For the other diets, a fat source was added to the standard diet according to the treatment specific to each group. The fat sources included: Cholesterol prepared from egg yolk 100 g, Fish Oil 100 g, Animal Fat 100 g, and Olive Oil 100 g.

Probiotic bacteria

The hermetically sealed, freeze-dried probiotic capsules were obtained from Danisco for *Bifidobacterium bifidum* and from Wren Laboratories Limited for *Bacillus coagulans*. The bacteria were activated as described by Yassin et al. (2020). The bacteria were transferred to MRS broth twice, each for 48 hours, and

incubated at 37°C. The third transfer involved inoculating pasteurized skimmed milk at 121°C for 5 minutes, followed by 24 hours of incubation at 37°C. The activated colony was then refrigerated until use [24]. The groups were then dosed with the indicated probiotic bacteria until the end of the experiment. Rats in the designated groups were dosed with a bacterial suspension at a concentration of 1.5×10^8 colony-forming units (CFU) twice daily.

Measuring body weight, relative organ weight, and collecting and analyzing blood.

Initial and final body weight were measured at the beginning and end of the experiment, and the average weight difference was calculated according to the following equation:

$$\begin{aligned} \text{Weight difference} \\ &= \text{Final weight} \\ &\quad - \text{Initial weight} \end{aligned}$$

The relative weight of the heart, liver, kidneys, and spleen was calculated according to the following equation:

$$\begin{aligned} \text{relative organ weight} \\ &= \frac{\text{organ weight (g)}}{\text{body weight (g)}} \times 100 \end{aligned}$$

Three milliliters of blood were collected using the cardiac pricking method. Serum was isolated by centrifugation (3000 rpm) for 15 minutes, and the blood lipid profile was measured.

Statistical analysis

The statistical analysis of the results was carried out according to the principles of Analysis of Variance (ANOVA) at a statistical significance level ($P \leq 0.05$) to test for statistically significant differences between the groups.

Results and Discussion

Body weight

Table 1 shows significant differences ($P \leq 0.05$) in the relative weights of organs

in laboratory rats that were orally administered various combinations of lipid sources, with or without probiotics, for 28 days. The response varied depending on the type of lipid source and the probiotic used. Treatment with animal fats without the probiotic Af recorded the highest values in most of the organs studied. The relative weight of the heart was 2.30 ± 0.02 g/100 g (a), while the relative weight of the liver was 18.20 ± 3.30 g/100 g (a). The relative weight of the kidneys was 3.80 ± 0.05 g/100 g, indicating a significant increase compared to the other treatments. The cholesterol treatment without the Cho booster also showed similarly high values, with the relative weight of the liver reaching 18.02 ± 2.41 g/100 g and that of the spleen 2.26 ± 0.05 g/100 g, the highest value for the spleen among the treatments. In contrast, the treatments with unsaturated oils, particularly olive oil and fish oil,

showed a relative decrease in organ weights, with (Oo) and (Oo+Bc) registering the lowest values for the heart and liver, respectively. Meanwhile, the fish oil treatments Fo, Fo+Bb, and Fo+Bc exhibited relatively stable weights compared to the animal fat and cholesterol treatments.

These results partially agree with those of Puttarat et al. (2023) [19], who found that administering probiotic strains to rats on a high-cholesterol diet reduced body weight gain and improved lipid profiles and the gut microbiota. Their study also showed that the effect was more pronounced when multiple probiotic strains were used, supporting the idea that probiotics may modulate the effects of high-fat, high-cholesterol diets on body weight and lipid metabolism.

Table 1. Average weights of Rats orally administered combinations of different lipid sources with or without probiotics.

Sample code	Initial weight (g)	Final weight (g)	Weight gained (g)
Con	214.7 ± 11.67 a	238.3 ± 21.67 c	23.6 ± 1.14 c
Cho	215 ± 15 a	247.5 ± 23.5 a	32.2 ± 3.47 b
Cho+Bb	214.3 ± 21.67 a	221.7 ± 22.33 e	7.4 ± 0.96 e
Cho+Bc	214.7 ± 29.91 a	220.3 ± 21.67	5.6 ± 1.01 f
Fo	215 ± 13 a	216 ± 23 f	1.0 ± 0.04 g
Fo+Bb	215 ± 24.89 a	212.3 ± 24.33 g	-2.7 ± 0.03 m
Fo+Bc	215.3 ± 19.45 a	211.7 ± 19.67 g	-3.6 ± 0.02 n
Oo	213.8 ± 20.5 a	215 ± 20 f	1.2 ± 0.05 g
Oo+Bb	215 ± 23.06 a	211 ± 21.08 g	-4 ± 0.12 n
Oo+Bc	216.7 ± 16.67 a	215.3 ± 23.91 f	-1.4 ± 0.04 h
Af	214 ± 22.5 a	250 ± 21 a	36 ± 2.48 a
Af+Bb	214.3 ± 17.67 a	226 ± 13.79 d	11.7 ± 1.06 d
Af+Bc	214 ± 23.06 a	219.3 ± 20.33 e	5.3 ± 0.98 f

Numbers represent mean $\pm$ standard error. Numbers shown represent the average of five replicates for each sample. Different lowercase letters within the same column indicate statistically significant differences at a probability level of ($P \leq 0.05$). Con: Control, Cho: Cholesterol without booster, Cho+Bb: Cholesterol + *Bifi.bifidum*, Cho+Bc: Cholesterol + *B.*

coagulans, Fo: Fish oil without booster, Fo+Bb: Fish oil + *Bifi.bifidum*, Fo+Bc: Fish oil + *B. coagulans*, Oo: Olive oil without booster, Oo+Bb: Olive oil + *Bifi.bifidum*, Oo+Bc: Olive oil + *B. coagulans*, Af: Animal fat without booster, Af+Bb: Animal fat + *Bifi.bifidum*, Af+Bc: Animal fat + *B. coagulans*.

The results of the fish oil treatments were also consistent with those reported by Ciesielska et al. (2025), whose study showed that a high-fat diet supplemented with fish oil exhibited a trend toward reduced weight gain compared to other fats, such as animal fat or olive oil, and showed better effects on fat tissue. This is consistent with the reduced weight gain observed in the fish oil treatments in the current table, especially when combined with probiotics [5]. Furthermore, while the source of the fat may cause limited changes in weight gain, it does not affect other lipid and tissue parameters. This suggests that the weight response may depend not solely on the type of fat, but also on interactions among the type of fat, the dose, the trial duration, and the probiotic used [26].

Therefore, the results obtained can be interpreted as follows: animal fats and cholesterol without a probiotic led to a significant increase in weight, possibly due to their high energy density and ease of fat storage. The weight loss observed in the fish oil and olive oil treatments, when combined with certain probiotics, may be related to the role of unsaturated fatty acids in enhancing fat oxidation. Additionally, probiotics may help modify the gut microbiota and reduce fat or cholesterol absorption. Thus, the current findings support the modern trend that the biological effects of fats depend not only on their role as an energy source but also on their chemical composition and the presence of accompanying probiotics.

Organs relative weights

Table 4 shows significant differences ($P \leq 0.05$) in the relative weights of organs in laboratory rats orally administered various combinations of lipid sources, with or without probiotics, for 28 days. The

response varied depending on the type of lipid source and the probiotic used. Treatment with animal fats without the probiotic Af recorded the highest values in most of the organs studied. The relative weight of the heart was 2.30 ± 0.02 g/100 g (a), while the relative weight of the liver was 18.20 ± 3.30 g/100 g (a). The relative weight of the kidneys was 3.80 ± 0.05 g/100 g, indicating a significant increase compared to the other treatments. The cholesterol treatment without the Cho booster also showed similarly high values, with the relative weight of the liver reaching 18.02 ± 2.41 g/100 g and the spleen reaching 2.26 ± 0.05 g/100 g, the highest value for the spleen among the treatments. In contrast, the treatments with unsaturated

Oils, particularly olive oil and fish oil, showed a relative decrease in organ weights, with (Oo) and (Oo+Bc) registering the lowest values for the heart and liver, respectively. Meanwhile, the fish oil treatments (Fo, Fo+Bb, and Fo+Bc) exhibited relatively stable weights compared to the animal fat and cholesterol treatments.

Furthermore, the addition of probiotics reduced the relative weight of certain organs compared with the unboosted treatments. The relative weight of the liver in the animal fat treatment fortified with *Bifidobacterium bifidum* (Af+Bb) decreased to 15.31 ± 2.58 g/100 g compared to 18.20 ± 3.30 g/100 g in (Af), and the heart weight decreased from $2.30 \pm 0.02a$ to $1.85 \pm 0.02c$. Similarly, the addition of the probiotic to the cholesterol treatments (Cho+Bb and Cho+Bc) reduced the relative weight of the liver and kidneys compared to the unfortified cholesterol treatment (Cho), indicating a modulatory

effect of the probiotics in mitigating the effects associated with high dietary fat.

The results are consistent with those of Chen et al. (2024) [3], whose study showed that the use of multi-strain probiotics in rats fed a high-fat diet led to a reduction in relative liver weight and improved metabolic indicators related to lipid metabolism, while also reducing pathological changes resulting from fat accumulation in tissues. They also indicated that the probiotics improved gut microbial balance and mitigated the adverse effects of high-fat diets, which is consistent with the significant reduction in liver and kidney weights observed in the probiotic-enriched treatments in the current study.

The results also align with those of Huang et al. (2023), who found that administering

Bacillus coagulans to mice fed a high-fat, high-sugar diet led to reduced liver weight, improved histological markers, and decreased inflammatory responses and oxidative stress compared with untreated animals. The researchers attributed these effects to probiotics' ability to regulate lipid metabolism and reduce lipid accumulation within organs, a pattern similar to the relative decrease in organ weight observed with *Bacillus coagulans* in the current study [13]. Furthermore, the type of fat source can directly influence physiological markers and organ function. Unsaturated fats, such as fish and olive oils, have a lesser effect on organ hypertrophy than animal fats or cholesterol [7].

Table 2. The relative weight of the organs of laboratory rats was determined after they were given combinations of different lipid sources orally, with or without probiotics.

Sample code	Relative weight g/100 g			
	Heart	Liver	Spleen	Kidney
Con	1.59 ± 0.01 d	12.88 ± 2.13 e	1.40 ± 0.03 e	2.63 ± 0.05 d
Cho	2.08 ± 0.02 a	18.02 ± 2.41 a	2.26 ± 0.05 a	3.45 ± 0.06 a
Cho+Bb	1.91 ± 0.01 b	16.01 ± 3.26 b	1.86 ± 0.04 b	2.04 ± 0.04 e
Cho+Bc	1.88 ± 0.03 c	15.89 ± 2.86 b	1.90 ± 0.05 b	3.14 ± 0.06 b
Fo	1.97 ± 0.01 b	14.00 ± 4.17 d	1.61 ± 0.02 d	2.38 ± 0.04 c
Fo+Bb	1.72 ± 0.03 c	13.21 ± 3.01 e	1.56 ± 0.02 d	2.75 ± 0.03 d
Fo+Bc	1.77 ± 0.04 c	13.30 ± 2.73 e	1.69 ± 0.03 c	2.80 ± 0.05 c
Oo	1.54 ± 0.03 d	13.00 ± 2.22 e	1.36 ± 0.03 d	2.69 ± 0.05 d
Oo+Bb	1.65 ± 0.01 d	13.09 ± 2.11 e	1.55 ± 0.03 d	2.81 ± 0.03 c
Oo+Bc	1.58 ± 0.02 d	12.96 ± 2.47 e	1.52 ± 0.05 d	2.65 ± 0.02 d
Af	2.30 ± 0.02 a	18.20 ± 3.30 a	2.03 ± 0.04 a	3.80 ± 0.05 a
Af+Bb	1.85 ± 0.02 c	15.31 ± 2.58 c	1.73 ± 0.03 c	2.95 ± 0.06 b
Af+Bc	1.99 ± 0.03 b	15.80 ± 3.09 b	1.81 ± 0.05 b	2.98 ± 0.04 b

Numbers represent mean ± standard error. Numbers shown represent the average of five replicates for each sample. Different lowercase letters within the same column indicate

statistically significant differences at a probability level of ($P \leq 0.05$). Con: Control, Cho: Cholesterol without booster, Cho+Bb: Cholesterol + *Bifi.bifidum*, Cho+Bc: Cholesterol + *B. coagulans*, Fo: Fish oil without booster, Fo+Bb: Fish oil + *Bifi.bifidum*, Fo+Bc: Fish oil + *B. coagulans*, Oo: Olive oil without booster, Oo+Bb: Olive oil + *Bifi.bifidum*, Oo+Bc: Olive oil + *B. coagulans*, Af: Animal fat without booster, Af+Bb: Animal fat + *Bifi.bifidum*, Af+Bc: Animal fat + *B. coagulans*.

The results obtained can be interpreted as follows: the marked increase in the relative weight of the liver, kidneys, and heart in the animal fat and cholesterol treatments may be due to increased fat deposition in tissues and a higher metabolic burden resulting from the consumption of saturated fats and cholesterol. This leads to relative enlargement of some organs, particularly the liver, as the primary organ responsible for fat metabolism. The increased spleen weight in some treatments may also be linked to the activation of inflammatory or immune responses associated with high-fat diets.

The increased animal fat and cholesterol levels observed in certain treatments may be attributed to increased fat deposition in tissues and a higher metabolic burden resulting from the consumption of saturated fats and cholesterol. This leads to relative enlargement of some organs, particularly the liver, the primary organ responsible for fat metabolism. The increased spleen weight observed in some treatments may also be linked to the activation of inflammatory or immune responses associated with high-fat diets.

Conversely, the relative decrease in organ weight with the addition of probiotics may be attributed to their role in improving the balance of gut microbiota, reducing fat absorption, enhancing fatty acid oxidation, and mitigating inflammation and oxidative stress. These explanations are supported by recent studies examining the effect of probiotics in reducing metabolic disturbances associated with high-fat diets [9][6]. Therefore, the current findings suggest that the physiological responses of organs are not solely dependent on the type of fat source but are also influenced by the

presence of accompanying probiotics, which may play a protective role in mitigating the negative effects of high dietary fat.

Conclusion

The findings of this study revealed several conclusions:

There is a direct correlation between saturated fats and weight gain. Diets high in animal fats and cholesterol stimulate fat storage and rapid weight gain, accelerating the onset of obesity and its associated metabolic disorders.

The results also demonstrated the pathological effect of high-fat diets on internal organs. High consumption of fats and cholesterol leads to hypertrophy and an increase in the relative weight of the liver, kidneys, and heart. This is a significant physiological indicator of fat deposition within cellular tissues, thus increasing the metabolic burden on the liver and kidneys. Furthermore, it stimulates an immune and inflammatory response, as reflected by increased spleen weight.

The study also demonstrated the physiological superiority of unsaturated oils (fish oil and olive oil) over saturated fats. They maintained stable weights of internal organs and prevented their hypertrophy. This may be attributed to the role of unsaturated fatty acids (such as omega-3 fatty acids and oleic acid) in promoting fat oxidation rather than fat storage.

Both probiotic strains, *Bifidobacterium bifidum* and *Bacillus coagulans*, also demonstrated a positive modulatory effect. They successfully inhibited weight gain and reduced the relative weights of

enlarged organs. This effect may be attributed to their mechanisms of action, which include modifying the gut microbiota, inhibiting the absorption of fats and cholesterol in the intestine, and reducing levels of inflammatory cytokines and oxidative stress.

The results of our current study demonstrate that the weight and histological responses of body organs are not limited by the type of fat alone, but rather by the biological interaction between the lipid source and the accompanying probiotic dose. Therefore, this opens up practical applications for using these combinations as safe functional foods for weight management and the prevention of fibrosis and organ enlargement.

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