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The Advantageous Application of Spirulina in Mitigating Lipid Profile and Glycemic Alterations induced by a High Calorie Diet in Wistar Rat

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ABSTRACT

An unbalanced diet inevitably leads to weight and metabolic dysfunction. Spirulina (*Arthrospira platensis*) can be a useful supplement to remedy this situation. This work aims to study the effect of spirulina at 2% in the diet of unbalanced Wistar rat on weight and some biochemical parameters related to metabolism. The Wistar rats were being altered by a prolonged introduction of a high calorie diet. They receive the following experimental diets: a standard diet (S), a high calorie diet (H) and a diet enriched with Spirulina (SP). Rats had ad libitum access to their daily ration of 15 grams, which was provided each morning. The weight of the rats and serological parameters (glycemia, cholesterol and triglycerides) were measured and compared according to the three diets administered. The conducted measurements showed that the weight of the rats increased significantly (20.95 g) under H diet in comparison to those with diet S. The SP diet allowed a return to normal weight change (19.25 g) after 67 days. Glycemia, total cholesterol, triglycerides and LDL- C (low-density lipoprotein cholesterol), which increased very significantly in diet H (0.53 g/L, 0.25 g/L, 0.2 g/L respectively), have decreased in SP diet to be on the same line as the values recorded in diet S (0.35 g/L, 0.62 g/L, 0.46 g/L respectively). However, HDL-C (high-density lipoprotein cholesterol) rate is less influenced by diet change. All the results obtained are in favor of a beneficial effect of Spirulina on metabolic sphere of Wistar rats, hence the interest of its incorporation in the human diet.
Keywords: Spirulina, diet, Wistar rat, weight gain, metabolic dysfunction.

INTRODUCTION

Spirulina (*Arthrospira platensis*) is blue green algae originally from Chad [1]. This is a microorganism belonging to the class of cyanobacteria. It possesses exceptional nutritional qualities [2]. This species is the most famous and most used in the researches or during the planting of new crops [3]. Several studies have investigated the composition and therapeutic properties of Spirulina [4], [5], [6]. One of the first published studies carried out by [7]; has noticed that the alcoholic extract of spirulina more significantly prevented lipid peroxidation compared to chemical antioxidants. Furthermore, several studies on Spirulina platensis have already demonstrated anti-radical and microsomal lipid peroxidation-inhibiting properties [8]. This microalgae has various biological activities, namely antioxidant, anti-inflammatory, antiallergic, anticancer, anti-obesity, antidiabetic, antiviral

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and antibacterial [9],[10], [11]. Additionaly, the influence of Spirulina on bone growth in young male rats has been investigated. The research indicates that Spirulina can positively impact bone development by regulating hormones involved in growth processes [12].

Recently, several studies have allowed a better understanding of the spirulina beneficial nutritional effects [13], [14], [15]. Thus, the abnormally high protein content of Spirulina and its richness in vitamins, minerals, carotenoids and essential fatty acids are now well-recognized [13]. Overweight and obesity are on the rise, largely driven by contemporary lifestyles characterized by reduced physical activity and increased consumption of fast food and sugar-rich beverages[16],[17]. Saidi and his collaborators investigated the impact of Spirulina on diet-induced obesity in *Psammomys obesus*. Their study revealed that Spirulina can reduce obesity by downregulating genes involved in fat production [18]. This finding encouraged us to evaluate the potential of a specific spirulina-enriched diet (2%) in mitigating metabolic and morphological changes, including weight fluctuations, in *Wistar rats*, with the perspective of incorporating it into a regular diet.

MATERIALS AND METHODS

Spirulina sample preparation

The collection of spirulina *(Arthrospira platensis)* parented strain was carried out from the culture tanks in Chad. The collected quantity, was dried at room temperature 25 °C, and kept out from light until a crunchy texture is obtained. This is for the purpose of easing its consumption by experimental animals.

The experimental study and its conditions

The study was conducted on 45 male Wistar rats (*Rattus norvegicus*) weighing 134 ± 8g and aged about two months. The rats were provided by the Pasteur Institute of Algeria and treated in accordance with protection guidance and use of laboratory rats [17].The rats are kept in favourable conditions at a temperature of 26-28°C in a 12 hours a day lighted room [18].

The study is conducted on duration of 67 days and consists of 2 parts: the adaptation period (D1 to D7) and the experimental period (D8 to D67). Upon receipt, the rats are divided into two separate batches of 15 rats and 30, respectively for the control group and the experimental group. All rats received a standard diet (S) as usually recommended [21] with some modifications in at a rate of 15g per rat per day throughout the whole period of adaptation. The diet S was also distributed to the control group during the experimental period. Thirty rats of the experimental batch received a high calorie diet (H) during a period of 30 days (D8-D37), considered as the first experimental period. The diet (H) is distributed in order to cause a metabolic dysfunction and destabilize some serological components (glycemia, cholesterol and triglycerides). At D38, three rats from each batch are sacrificed for serological measurements. During the second experimental period (D38-D67), the rats in the experimental batch were randomly divided into two groups. One group received the high calorie diet (H) and the other received the 2% Spirulina enriched diet (SP).

At D68, three rats taken from each group are sacrificed for serological measurements. Similar measurements were performed on the control group for a comparison. The experimental procedure is well illustrated in figure 1.

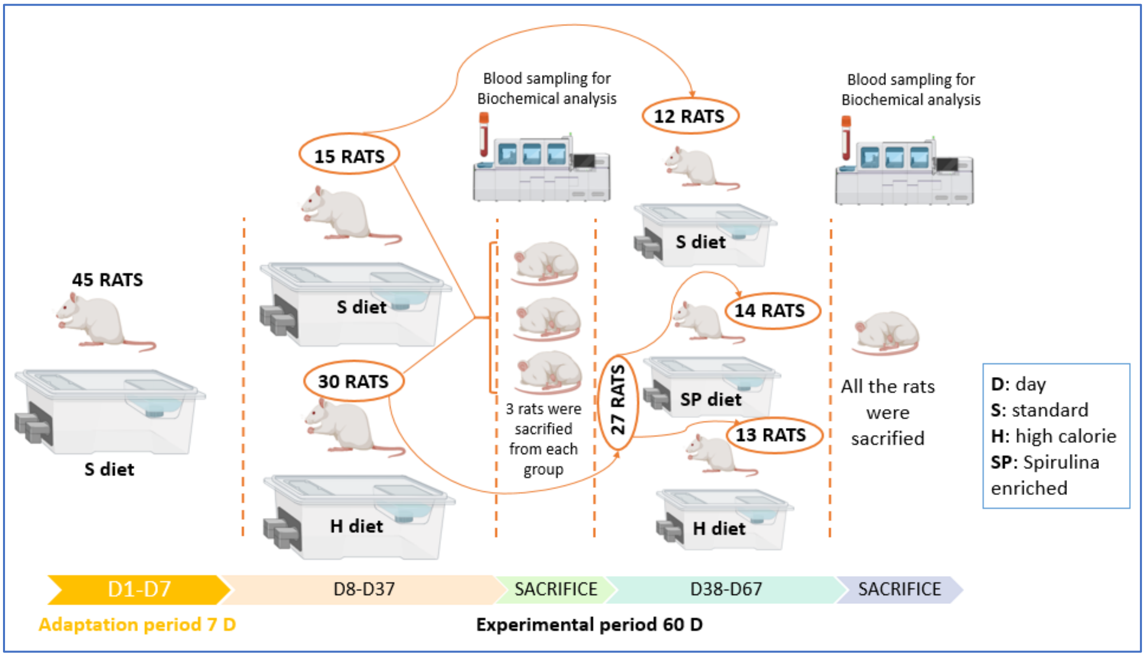

Figure 1. The experimental design to study the impact of different diets on lipid and glycemic parameters

The diets used in the study were produced locally in the laboratory (Table 1).

Table 1. Composition of experimental diets (g/100g of nutrients)

Diet composition	Types of diets		
	Standard Diet (S)	High calorie Diet (H)	Spirulina enriched diet (SP)
Cornstarch	55.82	25.82	53.82
Sucrose	10.00	20.00	10.00
Casein (>85% Protein)	22.00	22.00	22.00
DL-methionine	0.18	0.18	0.18
Saturated fat	0	20.00	0
Sunflower oil	5.00	5.00	5.00
Mineralized vitamin complex	4.00	4.00	4.00
Spirulina	0	0	2.00
Agar-agar	3.00	3.00	3.00
Metabolizable energy KJ/g	16.95	21.14	16.63

Gravimetric analysis

Throughout the duration of the experiment, the growth curve was established according to the current recommendations [19], from individual daily weighing rats.

Sacrifices and serological tests

Blood was sampled at retro orbital sinus. Plasma samples were decanted after centrifugation of the blood at 3000 rpm for 15 min [20]. Glycemia is measured according to GOD-PAP method [21]. Plasma cholesterol is determined by an enzymatic colorimetric test [22].

Plasma triglyceride levels are determined using the "Test-combination GPO-PAP" kit from Boehringer, according to an enzymatic method [23]. HDL cholesterol fraction, determined using cholesterol liquicolor kit and the concentration of LDL cholesterol is calculated according to the formula [24]:

LDL cholesterol = Total Cholesterol– HDL cholesterol – 1/5 (triglyceride)

Statistical analysis

Statistical treatment of the results was performed using the “Statistica software” (Version 6.1, 2004). The comparison of two means is performed by the Student's T test and comparison of several means are performed by variant analysis (ANOVA).

RESULTS

Weight in the adaptation period

The adaptation period (D1-D7) allowed to evaluate the impact of the standard diet (S) on the evolution of the rats weight (Figure 2). The general trend (Figure 2) is in favor of a linear increase in body weight of rats.

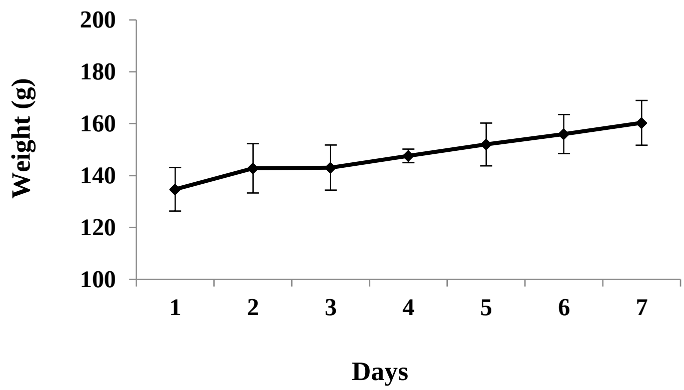

Figure 2. Evolution of the rats weight during the adaptation period D1 to D7 (1st Week) first experimental period

Weight in the first experimental period

During this period, the weight of the rats under H diet is significantly higher ($p<0.001$) compared to those under S diet (Figure 3). The H diets distinguished by an additional energy concentration of 4.19 kJ/g of nutrients.

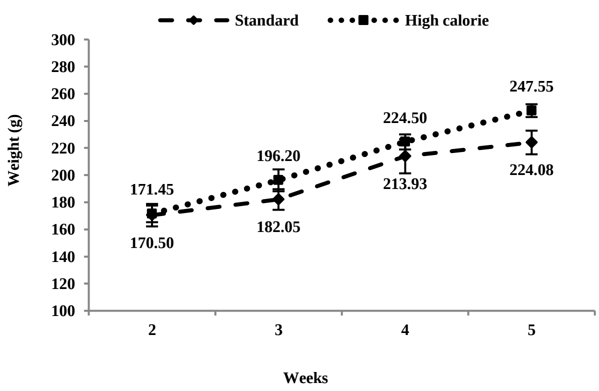

Figure 3. Evolution of the weight of rats during the first experimental period D8 to D37 (Weeks 2 to 5)

Weight in the second experimental period

Animals continue to receive the standard diet (S) distributed from the beginning of the experiment, and keep increasing their weight (Figure 4).

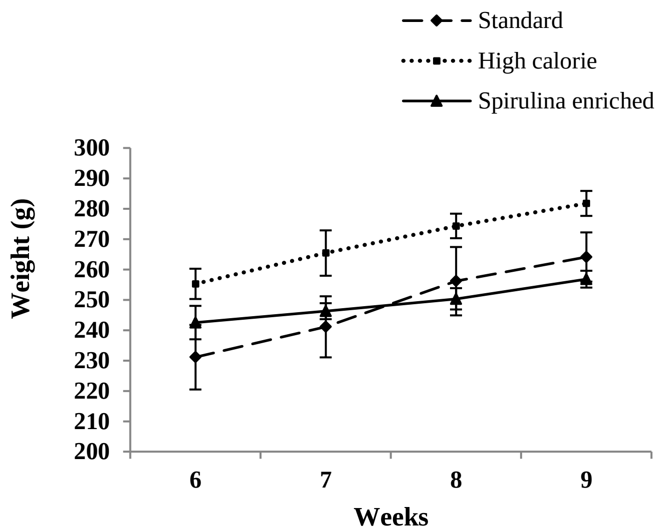

Figure 4. Evolution of the weight of rats during the second experimental period D38 to D67 (Weeks 6 to 9)

Variation of serological parameters depending upon diet

First experimental period

The assessment of some metabolic parameters from serum samples of rats was recorded in Table 2.

Table 2. Serological parameters of rats at the end of the first experimental period (D38)

Serological parameters (g/L)	Norms [17]	Type of diet		*p*
		Standard (S)	High calorie (H)	
Glycemia	1.15 - 2.59	1.17 ± 0.04	1.70 ±0.01	***
Triglycerides	0.53 - 2.20	1.09 ±0.01	1.14 ±0.01	***
Total Cholesterol	0.69 - 1.39	0.72 ±0.01	0.97 ±0.02	***
HDL-C	0.09 - 0.19	0.11 ±0.01	0.14 ±0.01	*
LDL-C	0.47 – 0.77	0.40 ±0.01	0.60 ±0.01	***

p: statistical probability of the Student's test on the serological parameters of the rats during the first experimental period (Weeks 2 to 5); ***: $p < 0.001$; *: $p < 0.05$.

3.3.2. Second experimental period

In the second experimental period, the measure of serological parameters at D68, for rats receiving different diets showed the results listed in Table 3.

Table 3. Serological parameters of rats at the end of the second experimental period (D68)

Serological parameters (g/L)	Norms [17]	Types of diet			*p*
		Standard (S)	High calorie (H)	Spirulina enriched (SP)	
Glycemia	1.15 - 2.59	1.18 ± 0.01	1.94 ± 0.04	1.59 ± 0.01	***
Triglycerides	0.53 - 2.20	0.68 ± 0.01	0.95 ± 0.01	0.59 ± 0.04	**
Total Cholesterol	0.69 - 1.39	0.79 ± 0.01	1.28 ± 0.05	0.66 ± 0.01	***
HDL-C	0.09 - 0.19	0.13 ± 0.0	0.23 ± 0.01	0.15 ± 0.05	NS
LDL-C	0.47 - 0.77	0.53 ± 0.05	0.86 ± 0.04	0.40 ± 0.04	**

p: statistical probability of ANOVA test of one serological parameters factor at the end of the second experimental period (D68). [NS]: No significant difference ($p > 0.05$); *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

DISCUSSION

During the adaptation period (D1-D7), the impact of the standard diet (S) on the weight fluctuation of rats was evaluated and the outcomes are demonstrated in the graph of Figure 2. The linear increase in body weight of rats indicated that the standard diet resulted in a classical weight gain of young animals.

During the first experimental period (D8-D37), the weight of the rats receiving H diet was significantly higher (p<0.001) compared to those receiving the S diet (Figure 3). The H diets, characterized by an additional energy concentration of 4.19 kJ/g of nutrients, successfully achieved the objective of promoting weight gain.

During the second experimental period, the rats were different from the others by a significant higher weight (p <0.001, 268.63 ± 11.36 g) the difference with the weight of rats receiving S and SP diets being respectively 20.95 g and 19.25 g. Such a diet is associated with significant overweight observed in humans [25]. We did not observe the state of obesity that we have set at the beginning of the experiment. This state is usually achieved with high-energy diets (21.14 KJ/g) and a rodent species predisposed to becoming obese and/or diabetic (*Psammomys obesus*) [26]. The SP diet increases the weight of the rats at a lower level compared to that of subjects receiving H diet and, whatever the considered week during this experimental period (Figure 4). The increase in weight is more pronounced with the H diet than it is with the S and SP diets.

Regarding, the variation of some metabolic serological parameters depending upon diet, we stated that at D38, the H diet has allowed a significant increase (p <0.001) of rats glycemia compared to the control group (S). It is of the order of 1.70 ± 0.01 g /L. However, this increase maintained the glucose value in the normal range [18]. The observed increase in glycemia of rats receiving H diet is an expected result since the glycemic response depends on the nature of ingested nutrients [26]. Compared to rats receiving the S diet, those receiving H diet, show a significant increase (p <0.001, Table 2) of TG (1.09 ± 0.01 to 1.14 ± 0.01 g/L). The impact of plasmatic triglycerides in the incidence of coronary heart disease has long been the subject of intense debate. Other studies have shown a significant relationship between triglycerides and coronary heart disease independently of the concentration of LDL cholesterol/HDL cholesterol ratio [27], [28], [29]. The H diet also induced a significant increase (p <0.001) of the total cholesterol level, compared to the control group (Table 2). The H diets characterized by a relatively high caloric content (21.14 KJ/g) [30]. The H diet caused a significant increase (p <0.001) of the lipoprotein fraction LDL cholesterol compared to that observed with the diet S (Table 2). An increase in total cholesterol and LDL cholesterol is associated with an increased coronary heart disease risk [31].HDL-C concentration was significantly increased (p <0.05) with the diet H (Table 2). It is now accepted that the HDL-C presents a preventive factor for cardiovascular disease risks [32], [33]. In the second experimental period, the measure of serological parameters at D68, for rats receiving different diets showed the following results (Table 3). Animals under H diet showed a significant increase (p <0.001) of the Glycemia compared to those under S diet. This difference maintains glycemia values within a range of normal values (Table 3). Triglyceride rate induced by the diet H increased significantly (p<0.01) compared to the diet S, without exceeding the upper critical value [18]. As for total cholesterol, under diet H, it increased significantly (p <0.001) compared to that induced by diet S. Cholesterol and triglyceride are implicated in increasing vascular disease risk [33]. We observed a significant increase (p <0.01) of the lipoprotein LDL fraction in the diet H compared to that induced by the diet S atD68, however exceeding the normalized values [18]. HDL-C has not changed significantly (p> 0.05, Table 3). The fraction HDL-C plays a major role in maintaining the homeostasis of total cholesterol [34], [35]. Furthermore, the spirulina intake on SP diet induced at D68, a significant decrease (p<0.001) of the glycemia (Table 3); the same for triglycerides, total cholesterol and LDL-C. The decrease in glycemia, triglycerides, total cholesterol and LDL-C is related to the reduction (about 19%) of the energy rate of the ingested diet [36]. On the contrary, this diet caused no change in the level of HDL-C (p> 0.05, Table 3).

This result is supported by those in the scientific literature which showed that the administration of methanolic extract of *Arthrospira platensis* (at 15 and 10 mg/Kg of body weight) caused an anti-hyperglycemic activity by reducing high blood sugar levels [37]. Spirulina, a single-cell protein, is abundant in essential nutrients and vitamins, making it an excellent ingredient for creating functional foods. A significant issue in the food industry is the reliance on synthetic additives, which are linked to an increased cancer risk. Spirulina's chemical composition features 55-70% protein (all essential amino acids in complete balance), 25% carbohydrates, 18% essential fatty acids, vitamins (notably vitamin B12, pro-vitamin A (β-carotne)), minerals (like iron, calcium, chromium, copper, magnesium, manganese, phosphorus, potassium, sodium, zinc), pigments like carotene, chlorophyll A, and phycocyanin, it is also one of the rare sources of dietary γ-linolenic acid and phenolic compounds.

Due to its valuable properties, Spirulina can serve as an excellent substitute for many antimicrobial compounds and synthetic antioxidants, offering a safer alternative that can enhance consumer health. Effectively, this includes immunological applications (suppresses pro-inflammatory cytokines release (interferon-γ (IFN-γ), and TNF- α) and enhances the levels of anti-inflammatory cytokines (IL-10). Besides, it improves anti-allergic effects by inhibiting the release of histamine), anti-cancer effects, probiotic benefits, neuroprotective qualities, hypoglycemic and hypolipidemic properties, as well as anti-nephrotoxicity, anti-genotoxicity, antiviral activity, anti-inflammatory effects, and anti-obesity benefits. Additionally, Spirulina offers technological advantages in various applications. [38], [39],[40].

The possible mechanism is due to the enhancement of pancreatic insulin secretion by islets of β cells or an increased transport of blood glucose to peripheral tissues in diabetic rats treated with spirulina [41]. Foods rich terms of flavonoids and polyphenols possess the ability to prevent type 2 diabetes and obesity. Several substances are included such as apigenin, rutin, luteolin, p-coumaric acid, gallic acid, vanilic acid, quercetin, cinnamic acid. These molecules play an inhibitory effect on the activity of intestinal and pancreatic lipase. They also, demonstrate protective effects against hyperglycemia and atherosclerosis by inhibiting α-amylase increasing insulin secretion, preventing insulin resistance, and decreasing body and adipose tissue weights[45], [46],[47], [48],[49]. Overall, the evidence in the literature suggests that spirulina improves several well-established cardiovascular disease risk factors including hyperlipidemia and seems to provide benefits around weight loss [42],[43]. As a whole, supplementing spirulina at 2–8 g/day may improve lipid profiles, particularly by reducing triglycerides, LDL-C and improving HDL-C; apolipoprotein A1 and reducing apolipoprotein B, aiding weight loss and reducing body mass index.

Spirulina also seems to improve insulin resistance, antioxidant / anti-inflammatory properties, blood glucose and blood pressure [44]. Spirulina has recently risen to the forefront of nutritional research due to its impressive nutritional composition, which has been shown to be effective in treating several conditions including blood sugar and kidney failure in diabetic rats [45], [46].

Spirulina boasts a promising composition, including carotenoids, phenolic compounds, phycocyanin, and chlorophylls. It has been widely utilized as a nutritional supplement in various forms, such as powder, flakes, or capsules. Additionally, it has been incorporated into easily accepted food products like cookies, pasta, sauces, ice cream, and snacks [43]. Spirulina-based food supplements are nutritionally rich but may currently be inaccessible to economically disadvantaged populations due to affordability constraints. To enhance nutrition and health outcomes, their use could be expanded by integrating spirulina into national nutrition programs, ensuring broader accessibility and coverage. While there are food safety risks associated with spirulina consumption, effective mechanisms to mitigate these hazards are well-established. Growing consumer interest in spirulina has also drawn the attention of food regulatory authorities worldwide, further supporting its potential for widespread and safe use [55]. Our study can be considered a foundational step in addressing the limitations of spirulina's application as a food ingredient or supplement, particularly concerning cost and safety risks. Incorporating just 2% spirulina into food products helps minimize costs and potential risks while still offering significant health benefits. Further studies are necessary to validate the safety and efficacy of this spirulina percentage (2%) in humans.

CONCLUSIONS

In this study, it was shown that the high calorie diet (H) produced metabolic disorders characterized by weight gain and a significant increase of serological parameters. However, the SP diet increased the weight of rats to a lower level compared to that of subjects receiving the H diet, regardless of the week considered during the experimental period. Weight gain is more pronounced with the H diet than with the S and SP diets. Moreover, animals under H diet showed a significant increase of the Glycemia, Triglyceride rate, total cholesterol and the lipoprotein LDL fraction compared to that induced by diet S. Cholesterol and triglyceride are implicated in increasing vascular disease risk. HDL-C has not changed significantly. The fraction HDL-C plays a major role in maintaining the homeostasis of total cholesterol.

However, the incorporation of spirulina with a rate of 2% has allowed to correct weight gain caused by the H diet of Wistar rats. In addition, Spirulina has allowed reducing glycemia, cholesterol and triglyceride levels. This confirms its positive effect on glucose and lipid metabolism. Therefore, spirulina enriched diet should be considered desirable, even if all the mechanisms of action of these micro-algae are not yet well elucidated.

However, a larger sample size per group would have enhanced the statistical power of the study. Additionally, this study is only preliminary and should be followed up with a longer-term study (with extended experimental periods) to induce more pronounced metabolic disorders and to provide stronger validation of the findings.

Ethical approval: In this study, rats were treated in accordance with the guidelines for the protection and use of laboratory rats (Council of the European Communities, 1986).

Supplementary Materials: Not applicable.

Author Contributions: "Conceptualization, K.A.; methodology, K.A. and M.A.; validation, S.A., D.D. S.F. and M.M.; formal analysis, K.A. and M.A.; investigation, K.A. and M.A.; data curation,, K.A. M.A. and L.K.; writing—original draft preparation, K.A. and M.A.; writing—review and editing, L.K.; visualization, M.A..; project administration, **S.F**; validation, visualization, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: The data presented in this study are available for a limited time upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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التطبيق المفيد للسبيرولينا في التخفيف من اضطرابات الملامح الدهنية والتغيرات السكرية الناتجة عن نظام غذائي عالي السعرات الحرارية لدى جرذان ويستار

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ملخص

إن النظام الغذائي غير المتوازن يؤدي حتمًا إلى زيادة الوزن وحدوث اختلالات أيضية. ويمكن أن تكون السبيرولينا (*Arthrospira platensis*) مكملًا مفيدًا لمعالجة هذه الحالة. يهدف هذا العمل إلى دراسة تأثير إضافة السبيرولينا بنسبة 2% في غذاء جرذان ويستار غير المتوازن على الوزن وبعض المعايير البيوكيميائية المرتبطة بالأيض. تم إحداث التغيرات لدى جرذان ويستار عبر إدخال مطوّل لنظام غذائي عالي السعرات الحرارية. وتلقت الحيوانات الأنظمة الغذائية التجريبية التالية: نظام غذائي قياسي (S)، نظام غذائي عالي السعرات الحرارية (H)، ونظام غذائي مُدعّم بالسبيرولينا (SP).أُتيح للجرذان الوصول الحر (ad libitum)إلى حصتها اليومية البالغة 15 غرامًا، والتي قُدمت كل صباح. تم قياس وزن الجرذان والمعايير المصلية (سكر الدم، الكوليسترول، والدهون الثلاثية) ومقارنتها تبعًا للأنظمة الغذائية الثلاثة المطبقة. أظهرت القياسات المنجزة أن وزن الجرذان ازداد بشكل ملحوظ (20.95 غ) تحت النظام الغذائي Hمقارنةً بالنظام S.وقد سمح النظام SPبالعودة إلى تغير وزني طبيعي (19.25 غ) بعد 67 يومًا. كما أن سكر الدم، والكوليسترول الكلي، والدهون الثلاثية، وكوليسترول البروتينات الدهنية منخفضة الكثافة LDL-C، التي ارتفعت بشكل كبير جدًا في النظام H (0.53 غ/ل، 0.25 غ/ل، 0.2 غ/ل على التوالي)، قد انخفضت في النظام SPلتصبح على نفس مستوى القيم المسجلة في النظام S(0.35 غ/ل، 0.62 غ/ل، 0.46 غ/ل على التوالي). في المقابل، كان معدل كوليسترول البروتينات الدهنية عالية الكثافة HDL-Cأقل تأثرًا بتغير النظام الغذائي. جميع النتائج المتحصل عليها تصب في صالح وجود تأثير مفيد للسبيرولينا على المجال الأيضي لدى جرذان ويستار، مما يبرز أهمية إدماجها في النظام الغذائي للإنسان.

الكلمات الدالة: السبيرولينا، النظام الغذائي، جرذ ويستار، زيادة الوزن، الاختلال الأيضي.

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