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PROGRAMA DE POSGRADO EN BIOPROCESOS

EFECTOS DE LA INGESTIÓN DE *SPIRULINA* EN EL METABOLISMO LIPÍDICO Y MARCADORES DE INFLAMACIÓN EN UN MODELO MURINO DE OBESIDAD

OPCIÓN DE TITULACIÓN: ARTÍCULO DE INVESTIGACIÓN PARA OBTENER EL GRADO DE
MAESTRO EN CIENCIAS EN BIOPROCESOS

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“- Si te ayudo o no, no importa. Igual reprobarás.

- No lo haré. No puedo.

- ¿Por qué lo deseas tanto?

- Porque me dijeron que no lo lograría.”

Men of Honor (2000)

ÍNDICE

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1. RESUMEN

Los efectos de la suplementación de *Spirulina* en ratas sometidas a una dieta alta en grasas en términos de aumento de peso, la ingesta de la dieta, el perfil lipídico, así como la expresión de genes IL17, IL6, DGAT1 y ATGL. El análisis de la composición de la biomasa *Spirulina* reveló un 60% en el contenido de proteína, 9% de hidratos de carbono y 4,45% de lípidos totales. El perfil de lípidos de la muestra indica ácido palmítico como el ácido graso predominante que representa el 45% (16:00), seguido de ácido linoleico en 22% (18: 2 n6) y ácido linolénico en 22% (18: 3 n6). Los animales alimentados con una dieta alta en grasa (HFD) y la dieta alta en grasa suplementada con 2% *Spirulina maxima* (SMAX) tuvieron mayor ganancia de peso en comparación con el grupo de control; sin diferencia significativa entre estos grupos experimentales. No se observaron diferencias significativas entre los grupos de prueba en términos de consumo de la dieta a lo largo de la evaluación. El grupo SMAX presento una concentración sérica de colesterol total mayor e igual C-HDL que los del grupo HFD. Los resultados mostraron que la expresión de los genes IL17, IL6, DGAT-1 y ATGL no es estadísticamente diferente entre los grupos experimentales. Por lo que se propone realizar más estudios para evaluar las implicaciones que tendría la suplementación de *Spirulina* sobre estados de obesidad inducidos por dietas altas en grasa,

2. ABSTRACT

In the present study the effects of *Spirulina* supplementation in rats subjected to a high fat diet was studies in terms of weight gain, diet intake, lipid profile, as well as the expression of IL-17, IL-6, DGAT1 and ATGL genes. The analysis on *Spirulina* biomass composition revealed a 60% in protein content, 9% of carbohydrate and 4.45% of total lipids. The lipid profile of the sample indicates palmitic acid as the predominant fatty acid representing the 45% (16:00), followed by linoleic acid at 22% (18:2 n6), and linolenic acid at 22% (18:3 n6). Animals fed with a high fat diet (HFD) and high-fat diet supplemented with 2% spirulina maxima (SMAX) had a greater weight gain compared to the control group; with no significant statistical difference between these experimental groups. No significant differences between the test groups were observed in terms of diet intake throughout the evaluation. SMAX group had a higher total cholesterol and lower C-HDL than those of the HFD group. The results showed that expression of IL-17, IL-6, DGAT-1 and ATGL genes is not statistically different among the experimental groups. The implications of these results in the current literature on murine obesity models are discussed. More studies varying the Spirulina dose are suggested to determine whether or not *Spirulina* has the potential to attenuate obesity and the associated chronic inflammation.

3. INTRODUCCIÓN

Spirulina es una cianobacteria multicelular filamentosa perteneciente a la familia *Oscillatoraceae* (Eykelenburg 1979, Ciferri 1986). La *spirulina* puede crecer en agua dulce, agua salada y los cuerpos de agua salobre. Recientemente, muchos grupos de investigación se han centrado en el papel de los alimentos funcionales para reducir el riesgo de desarrollar enfermedades crónico-degenerativas (Fernández-Rojas et. Al 2014). En este contexto *Spirulina* tienen un gran potencial nutracéutico debido a la diversidad y concentración de nutrientes que producen (Capelli & Cysewski 2010). Esta cianobacteria es bien reconocida debido a sus altos niveles de proteínas vitaminas y minerales. Contiene cerca de un 60% de proteínas de alto valor biológico, debido a que en su estructura contiene todos los aminoácidos esenciales; y cantidades considerables de β-caroteno, otra parte, *Spirulina* es capaz de sintetizar ácidos grasos poliinsaturados tales como el ácido γ-linolénico (GLA; C18: 3 $^{\Delta 9,12,6}$), que comprenden 30% de los ácidos grasos totales (Capelli & Cysewski 2010; Hoseini et al. 2013). S*pirulina* es un elemento nutricional histórico utilizado desde la cultura azteca. El primer registro en el uso de la *spirulina* como alimento para los seres humanos data de 1521, en el que se establece que se extrae de las aguas del lago de Texcoco; siendo la especie *A*. *maxima* nativa de Texcoco y otros lagos de América Latina (Ramírez-Moreno et al. 2006)

Los estudios han demostrado que la composición de la dieta también afecta al metabolismo de los ácidos grasos en varios tejidos (Hoseini et al. 2012; Stinkens et al. 2015). El papel de los ácidos grasos no sólo es servir como fuente de energía, sino que también participan en varios procesos fisiológicos, incluyendo la absorción intestinal de grasa de la dieta, el almacenamiento intracelular de excedente de energía, la lactancia, la atenuación de la lipotoxicidad, el transporte de lípidicos y la transducción de señales (Shih et al. 2009).

Por otra parte, la obesidad induce la infiltración de macrófagos del tejido adiposo, dando por resultado una inflamación crónica de bajo grado, así como una mayor producción de moléculas pro-inflamatorias, tales como IL-6 o TNF-α (Mushref et al. 2012). El tejido adiposo es una de las principales fuentes de citoquinas inflamatorias y la grasa perirrenal mostró una fuente eficiente de ARNm en el modelo de inflamación crónica (Starr et al. 2009; Ma et al. 2015).

Triacilgliceroles son el mayor tipo de moléculas en las que se almacenan los ácidos grasos; éstos consisten en una molécula de glicerol y tres ácidos grasos unidos por

enlaces éster. En la obesidad, existe una acumulación excesiva de triglicéridos en el tejido adiposo, que se asocia a una función anormal de varios órganos (Yen et al. 2008). La biogénesis de triacilgliceroles se produce por dos vías: la vía glicerolfosfato monoacilglicerol (Coleman et al. 2004; Riahi et al. 2015). Y la vía glicerolfosfato que se encuentra en la mayoría de las células, incluidos los adipocitos. Sin embargo, las dos vías tienen un paso en común: la unión de diacilglicerol con un acil-CoA graso para producir un triacilglicerol; que es catalizada por la diacilglicerol aciltransferasa (DGAT) (Coleman et al. 2004; Yen et al. 2008; Shih et al. 2009), la DGAT-1 es un miembro de familia de genes acil-CoA: colesterol aciltransferasa (ACAT) presente en los mamíferos (Shih et al. 2009). Siendo la expresión de ARNm de DGAT-1 en los seres humanos más alta en el tejido adiposo y el intestino delgado. Se ha demostrado que ratón (- / -) DGAT-1 resiste el aumento de peso bajo una dieta rica en grasas (21%) y presenta almohadillas de grasa más pequeñas en comparación con los ratones w.t. (Coleman et al. 2004). Además, en 2004 se descubrió una enzima capaz de hidrolizar triacilglicerol: lipasa adiposa de triglicéridos (ATGL), que se expresa en muchos tejidos, incluyendo el tejido adiposo blanco y marrón (Radovic et al. 2012). La alteración en la actividad de estas enzimas implicadas en las rutas metabólicas de lípidos ha sido estudiada por varios grupos. Sin embargo, el perfil de expresión de DGAT-1 y ATGL no se han analizado en detalle en los modelos de obesidad inducida por una dieta alta en grasa. A pesar de los muchos beneficios que la *spirulina* tiene, hay información limitada acerca de los efectos que podrían tener como nutracéutico en el tratamiento de la obesidad, así como las repercusiones en las alteraciones del metabolismo lipídico que se producen en esta condición.

4. OBJETIVO GENERAL

Identificar si la suplementación con Spirulina maxima en animales alimentados con una dieta alta en grasa, modifica la expresión génica de marcadores de inflamación y metabolismo lipídico en un modelo murino de obesidad exógena.

4.1 Objetivos Específicos

1. Cultivo, escalamiento y caracterización de *Spirulina maxima.*
2. Establecer un modelo de obesidad inducida por una dieta alta en grasa reportada en la literatura que permita evaluar el efecto de la suplementación con *Spirulina maxima* al 2%.
3. Determinar si esta suplementación podría modificar el peso corporal, la ingesta de alimento y el perfil lipídico de las ratas en presencia de una dieta alta en grasa.
4. Determinar si podría modificar los niveles de expresión génica de los marcadores moléculares de inflamación.
5. Determinar si modifica la expresión de genes que participan en el metabolismo lipídico.

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5. RESULTADOS

5.1. Caracterización bioquímica de biomasa Spirulina maxima

El análisis de la biomasa liofilizada de *Spirulina maxima* reveló un 60% en el contenido de proteína, 9% de hidratos de carbono y 4,45% de lípidos totales (Figura 1). El perfil de lípidos de la muestra indica ácido palmítico como el ácido graso predominante que representa el 45% (16:00), seguido de ácido linoleico en 22% (18: 2 n6) y ácido linolénico en 22% (18: 3 n6) (Figura 2).

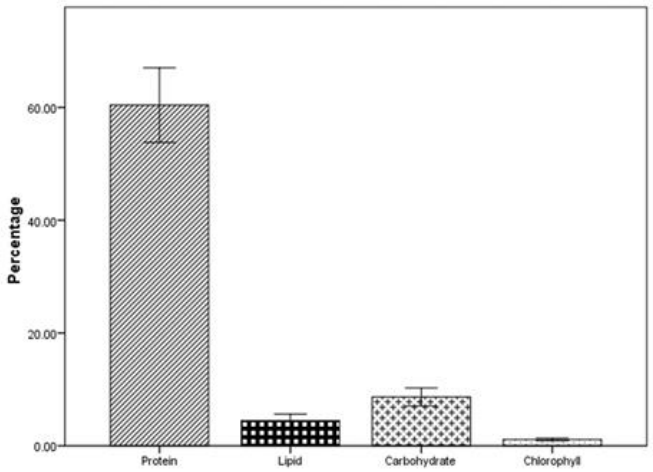

Figura 1. Composición de biomasa liofilizada de *Spirulina maxima.* La composición de la biomasa muestra el porcentaje de proteínas en un 60%, lo que concuerda con lo reportado en la literatura. Así mismo muestra los porcentajes de lípidos, hidratos de carbono y clorofila α.

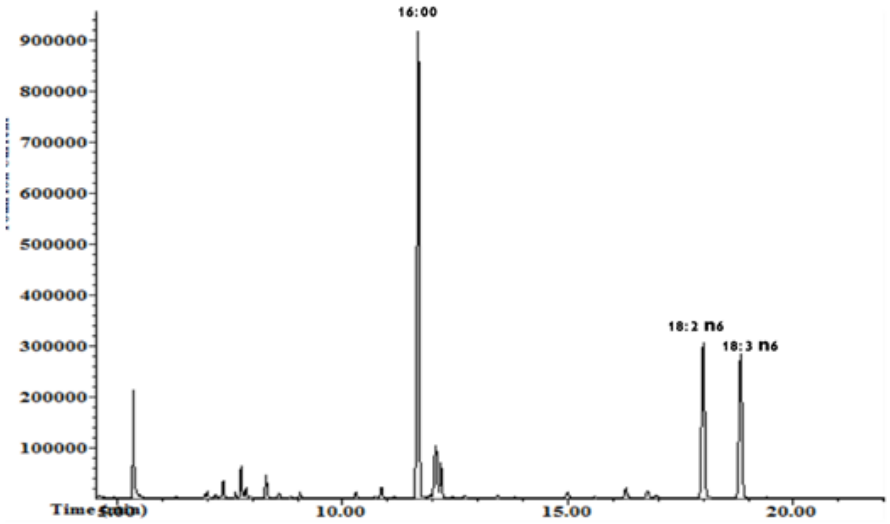

Figura 2. Perfil lipídico de *Spirulina maxima*. El cromatograma muestra el perfil lipídico de la biomasa de *Spirulina maxima*, el cual muestra los principales ácidos

grasos presentes; siendo el ácido palmítico predomínate; así mismo muestra los ácidos linoleico y linolénico.

5.2. Ganacia de peso y control de Ingesta

Tanto la ingesta en la dieta como el peso corporal se registraron en animales de prueba durante un período de 8 semanas. Los resultados mostraron que los animales alimentados con una dieta alta en grasa (HFD) y la dieta alta en grasa suplementada con 2% *Spirulina maxima* (SMAX) tuvieron una ganancia de peso mayor en comparación con el grupo de control; sin embargo, no muestran diferencia estadística significativa entre estos grupos (Fig. 3). Así mismo, no se observaron diferencias significativas entre los grupos de prueba en términos de consumo de la dieta a lo largo de la evaluación (Figura 4).

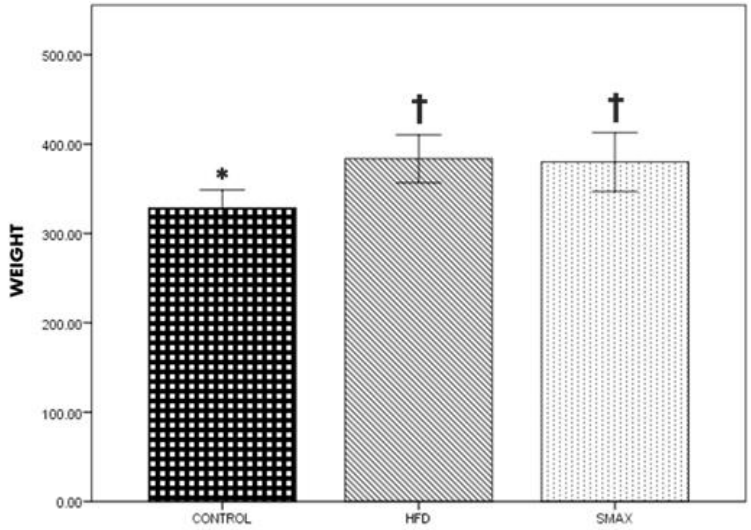

Figura 3. Ganacia de peso. Se muestra la ganancia de peso en cada uno de los grupos al termino de 8 semanas del proyecto, se observa una diferencia significativa entre los grupos alimentados con una dieta alta en grasa y una dieta alta en grasa suplementada con 2% de *Spirulina maxima* comparados con la dieta control. Así mismo no existe diferencia entre los grupos experimentales (* $p<0.05$).

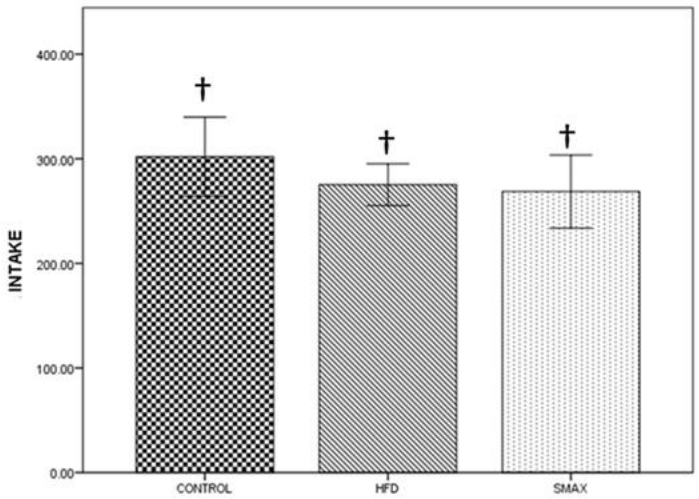

Figura 4. Ingesta La ingesta de los grupos experimentales no muestra diferencias significativas entre ellos († p<0.05)

5.3. Perfil bioquímico

El perfil de bioquímico en suero que se manifestó en los animales alimentados con HFD y SMAX muestra diferencias significativas en comparación con el grupo control. El perfil lipídico mostró una disminución en el C-HDL. Por otro lado, aumento en el colesterol total y urea no solo en comparación con el control, sino también en relación de HFD y Smax, siendo este último tratamiento el que presenta valores más elevados El resto de los valores analizados no muestra diferencias significativas entre los tratamientos. (tabla 1)

	Glucose	Cholesterol T.	Triglycerides	C-HDL	Urea	Creatinine	Uric Acid
Control	125.4 ±21.96	64.6 ±2.41	43.4 ±6.66	25.2 ±1.50	34.4 ±4.03	0.72 ±0.07	2.46 ±0.43
HFD	81.60[a] ±13.56	170.00[a] ±33.08	32.8 ±12.72	13.84[a] ±5.52	20.08[a] ±3.17	0.7 ±0.07	1.83 ±0.28
Smax	105.20[a] ±14.96	294.60[b] ±54.05	45.4 ±12.72	18.70[a] ±3.50	26.72[b] ±2.87	0.75 ±0.06	1.92 ±0.63

a *P<0.05* vs Control group; b *P<0.05* vs HFD group

Tabla 1. Tabla de análisis bioquímico en suero Los grupos experimentales muestran diferencias significativas en relación al grupo control en los parámetros de glucosa, colesterol total, C-HDL y Urea. Los grupos alimentados con dieta alta en grasa (HDF) y dieta alta en grasa suplementada con 2% *Spirulina maxima* (SMAX) muestran una diferencia significativa entre ellos, mostrando una mayor con concentración sérica de Colesterol Total y Urea en el grupo SMAX.

5.4. Expresión Génica

Los resultados mostraron que, en la expresión de genes relacionados con la inflamación, tales como IL-6 e IL-17 no existe diferencia estadísticamente significativa entre los grupos experimentales. (Fig. 5) Del mismo modo, los genes implicados en el metabolismo de los triglicéridos (DGAT-1 y ATGL) no mostraron diferencias estadísticamente significativas entre los grupos tratados con una dieta alta en grasa o con dieta alta en grasa suplementada con 2% de *Spirulina maxima* (Fig. 6)

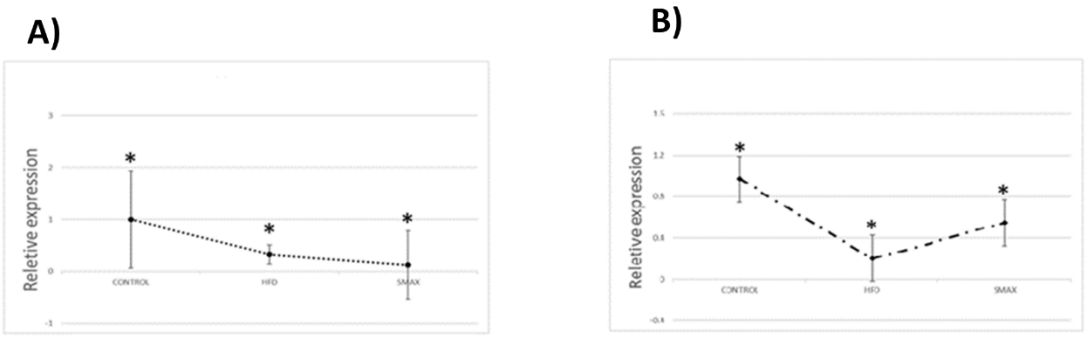

Figura 5. Expresión relativa de los genes relacionados a inflamación A) Expresión relativa de IL-6 en tejido adiposo perirenal de los animales alimentados con los diferentes tratamientos B) Expresión relativa de IL-17 de tejido adiposo perirenal de los animales alimentados con los diferentes tratamientos (* $p<0.05$).

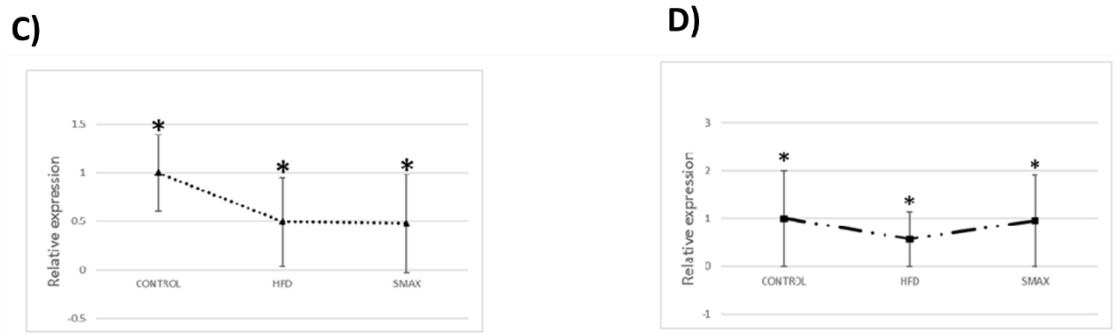

Figura 6. Expresión relativa de los genes relacionados al metabolismo lipídico. C) Expresión relativa del gen DGAT-1 en tejido adiposo perirenal en animales alimentados con los distintos tratamientos. D) Expresión relativa del gen ATGL en tejido adiposo perirenal en animales alimentados con los distintos tratamientos ($p<0.05$).

6. CONCLUSIONES

La *Spirulina maxima* contiene la cantidad de proteínas, lípidos y carbohidratos que se han reportado presentes en las cepas más estudiadas de este género.

La dieta alta en grasa reportada es capaz de generar una obesidad exógena en los animales que son expuestos a ella al termino de 8 semanas, por lo que permite establecer un modelo de estudio más económico para estudios posteriores sobre obesidad y sus posibles interacciones biológicas.

La ganancia de peso, así como los cambios observados en los animales alimentados con dieta alta en grasa y dieta alta en grasa suplementada con 2% de *Spirulina maxima* no muestran diferencias entre ellos, pese a que *Spirulina* ha mostrado resultados diferentes en la literatura, existe muy poca evidencia de la interacción con una dieta alta en grasa, por lo que la presencia de dicha dieta podría afectar de forma negativa los efectos benéficos que podría tener el consumo de *Spirulina*; de la misma forma, el porcentaje suplementado de *Spirulina maxima*, está por debajo de los niveles reportados en la literatura con los que se han observado efectos benéficos, por lo que son necesarios más estudios en los cuales la suplementación sea más elevada.

7. ARTICULO

Effects of Spirulina ingestion on lipid metabolism and inflammation markers in an obesity rat model

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Effects of *Spirulina* ingestion on lipid metabolism and inflammation markers in an obesity rat model

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Abstract

In the present study the effects of Spirulina supplementation in rats subjected to a high fat diet was studies weight gain, diet intake, lipid profile, as well as the expression of IL17, IL6, DGAT1 and ATGL genes. The analysis on Spirulina biomass composition revealed a 60% in protein content, 9% of carbohydrate and 4.45% of total lipids. The lipid profile of the sample indicates palmitic acid as the predominant fatty acid representing the 45% (16:00), followed by linoleic acid at 22% (18:2 n6), and linolenic acid at 22% (18:3 n6). Animals fed with a high fat diet (HFD) and high-fat diet supplemented with 2% spirulina maxima (SMAX) had a greater weight gain compared to the control group; with no significant statistical difference between these experimental groups. SMAX group had a higher total cholesterol and lower C-HDL than those of the HFD group. The expression of Il17, Il6, DGAT-1 and ATGL genes is not statistically different among the experimental groups. The implications of these results in the current literature on murine obesity models are discussed. More studies varying the Spirulina dose are suggested to determine whether or not Spirulina has the potential to attenuate obesity and the associated chronic inflammation.

Keywords: Obesity, Inflammation, Gene expression, Lipid profile, High fat diet.

1. Introduction

Spirulina is a multicellular unbranched filamentous cyanobacterium belonging to the Oscillatoraceae family [1] [2]. Spirulina can grow in freshwater, saltwater and brackish water bodies. Recently many research groups have focused on the role of functional foods and other products in reducing the risk to develop chronic degenerative diseases or as support treatment [3]. In this context, cyanobacteria of the genus *Arthrospira* (*Spirulina*), which grows in fresh water, salt water or brackish water bodies, have nutraceutical potential due to the diversity and concentration of nutrients they produce [4]. *Spirulina* is a historic nutritional element used since the Aztecs culture. The first record on the use of *Spirulina* as food for humans is from 1521, which states that it was harvested from the waters of Lake Texcoco. *A. maxima* species is native to the Texcoco and other lakes in Latin America [5] this *cyanobacterium* is well recognized as a potential food supplement for humans because of its high levels of protein vitamins and minerals. Spirulina is about 60% protein of high biological value, as it contains all the essential amino acids; and contain considerable amounts of β-carotene and Moreover, Spirulina is able to synthesize polyunsaturated fatty acids such as glycerolipid γ-linolenic acid (GLA; C18:3 $^{\Delta 9,12,6}$), which comprise 30% of the total fatty acids [4][6].

Studies have shown that diet composition also affects the fatty acids metabolism in several tissues [7][8]. The role of fatty acids is not only to serve as source of energy, they are also involved in several physiological processes, including dietary fat intestinal absorption, intracellular storage of surplus energy, lactation, attenuation of lipotoxicity, lipid transportation, and signal transduction [9].

Adipose tissue is one of the major sources of chronically produced inflammatory cytokines and perirenal fat showed an efficient source of RNAm in inflammation model [10][11]. Moreover, obesity induces macrophage infiltration of adipose tissue,

resulting in a low-grade chronic inflammation as well as increased production of pro-inflammatory molecules, such as IL-6 or TNF-α [12].

Triacylglycerols are the largest type of molecules in which fatty acids are stored; these consist of a glycerol molecule and three fatty acids linked by ester bonds. In abesity, an excessive accumulation of triacylglycerols in adipose tissue, which is associated to an abnormal function of several organs [13]. Triacylglycerols biogenesis occur by two routes: glycerolphosphate pathway or route monoacylglycerol [13][14]. Although the glycerolphosphate pathway is found in most cell types, the monoacylglycerol route occur in certain cell types, including adipocytes. However, the two pathways have the joining of of diacylglycerol with fatty acyl-CoA to yield a triacylglycerol as a common reaction; which is catalyzed by the diacylglycerol acyltransferase (DGAT) [9][13][14] DGAT-1 is a member of the mammalian acyl-CoA:cholesterol acyltransferase (ACAT) gene family [9]. DGAT-1 mRNA expression in humans is highest in adipose tissue and small intestine. It has been demonstrated that DGAT-1 - / - mouse resists weight gain under a 21% fat diet and presents smaller fat pads when compared to the wild type mice [14]. Furthermore, in 2004 an enzyme capable of hydrolyzing triacylglycerol was discovered: adipose triglyceride lipase (ATGL), which is expressed in many tissues, including white and brown adipose tissue [7]. The alteration in the activity of these enzymes involved in the lipid metabolic pathways has been studied by several groups. However, the expression profile of DGAT-1 and ATGL have not been analyzed in detail in models of obesity induced by a high fat diet. Despite the many benefits that *Spirulina* had, there is limited information about the effects that could have as nutraceutical in the treatment of obesity as well as the repercussions on lipid metabolism alterations that occur in this condition.

The aim of this study was to determine the effects of the diet supplementation with *Spirulina maxima* in rats subjected to a high fat intake, in terms of weight gain, lipid profile, and triacylglycerides metabolism.

2. Materials and methods

2.1 Animals

Wistar male Rats (n=15) were purchased from Universidad Autónoma de San Luis Potosí, Mex. The rats groups (n=5) were maintained at 22°C to 24°C with 50% to 60% relative humidity and a 12-hour dark/light cycle. All experiments were performed according to the ethical guidelines of the Ethics Committee of the Facultad de Ciencias Químicas, UASLP.

2.2 Spirulina maxima

Spirulina biomass was produced by establishing cultures in a 90 L column. The Schlosser´s medium was used and culture conditions were: 25°C; cycles of 12 hours with light-dark at 560 lux; and constant aeration. *Spirulina maxima* was harvested by sieving using a 20 and 30 µm strainer. The biomass was freeze-dried and subsequently characterized by determining lipid profile, determined by gas chromatography-mass spectrometry using a GCD-1800B equipment; as well as the content of chlorophyll, proteins, carbohydrates and phycobiliproteins by spectrophotometry methods using a DR 5000 ™ Laboratory Spectrophotometer UV-Vis equipment.

2.3 Study design

Animals were randomized into 3 groups receiving one of the following dietary regimens: group 1 or control, fed with standard diet (lab diet 300); group 2, fed with High Fat Diet (diet containing standard pellet diet supplemented with 0.5% (w/w) cholic acid, 20% (w/w) lard and 2% (w/w) cholesterol) [15]; group 3, fed with High

Fat Diet supplemented with 2% of *Spirulina maxima* biomass. All animals were fed during eight weeks *ad libitum* (water/diet) and daily food intake registered once every three days. The animals were bled at baseline and then once every fifteen days to determine serum levels of glucose, triglycerides, total cholesterol, C-HDL, urea, creatinine, uric acid. After the eight weeks of treatment the animals were subsequently sacrificed by decapitation after overnight fasting. The truncal blood was collected and samples were centrifuged at 3500 rpm for 15 min to recover serum, which was stored at -80°C until further use. Samples of perirenal fat were resected, frozen with liquid nitrogen and subsequently stored at -80°C until further analysis.

2.4 Gene expression

Total RNA extraction was performed with the Trizol® reagent method using a FastPrep®-24 Instrument. The RNA quality extracted was checked by Experion™ Automated Electrophoresis System and analysis kits StdSens Experion™ RNA. cDNA synthesis was performed using a commercial kit for Superscript® III First-Strand Synthesis System for RT-PCR. And the relative transcript expression was measured by qRT-PCR technique with StepOne™ Real-Time PCR System using the following pairs oligonucleotide: IL-17A 5'- CTCATCCCTCAAAGTTCAGTGT -3´, 5´- GCTAAGGGAGTTGAGGACTTTC -3; IL-6 5'-GCCCTTCAGGAACAGCTATGA-3', 5'-TGTCAACAACATCAGTCCCAAGA-3'; DGAT1 5´- CGGGTTCTTGAGATGCTCTTT-3´, 5´-GGCTTCATGGAGTTCTGGATAG-3´; ATGL 5´-GAGTTTCGGATGGAGAGAATGT-3´, 5´- GCCACAGTACACAGGGATAAA-3´

2.5 Statistical analysis

One way ANOVA was performed using the IBM SPSS 20 software, where P values <0.05 were considered as statistically significant.

Results

3.1 *Spirulina maxima* analysis

The analysis on Spirulina biomass composition revealed a 60% in protein content, 9% of carbohydrate and 4.45% of total lipids (Figure 1). The lipid profile of the sample indicate palmitic acid as the predominant fatty acid representing the 45% (16:00), followed by linoleic acid at 22% (18:2 n6), and linolenic acid at 22% (18:3 n6) (Figure 2).

3.2 Weight gain

Diet intake and body weight were registered in test animals over an 8-week period. The results showed that the animals fed with a high fat diet (HFD) and high-fat diet supplemented with 2% spirulina maxima (SMAX) had a greater weight gain compared to the control group; with no significant statistical difference between these experimental (Fig. 3). No significant differences between the test groups were observed in terms of diet intake throughout the evaluation (Fig 4).

3.3 Biochemical serum profile

The serum lipid profile revealed that animals fed HFD and SMAX have significant differences when compared to the control group. For instance, SMAX group had a higher total cholesterol and lower C-HDL than those of the HFD group. The rest of the analyzed values shows no significant difference between treatments. (table 1)

3.4 Gene expression

The results showed that expression of inflammation-related genes like IL-6 and IL-17 is not statistically different among the experimental groups. (Fig. 5) Similarly, genes involved in the metabolism of triglycerides (DGAT-1 and ATGL) showed no statistically significant differences between the groups treated with high fat diet alone or high fat diet supplemented with 2% of *Spirulina maxima* (Fig. 6)

4. Discussion

The use of *Spirulina spp*. has been popularized and became one of the most important functional foods due to its nutrients content, e.g. contains 60% of protein with a biological value since it contains all the essential amino acids, being *S.* peaceful and *S.* platensis the most characterized species [4]. Our study revealed that *Spirulina maxima*, native of Latin America, falls in the described range of nutrient composition. For instance, the reported fatty acids profile for *S. platensis indicate a content of* 10-17% of unsaturated fatty acids, such as linoleic and linolenic [16]. The biomass generated for this study contained 22% of polyunsaturated fatty acids, comprising linolenic and linoleic acids. This higher content respect to other reports could be attributed to the growth conditions. In fact previous studies have shown that the growth media composition exerts an influence on the biomass composition [17]. Therefore, our data suggests that the use of the Jourdan medium is suitable for the production of biomass to be used for nutraceuticals production since an increase in the content of bioactive metabolites is achieved.

According to the literature, high fat consumption is associated to a higher prevalence of cancer. For instance, some studies have found that the offspring of animals exposed to a high-fat diet during pregnancy showed an increased susceptibility to breast cancer in adulthood [16] [17]. There is also evidence, derived from studies performed with children and their parents, on the transgenerational amplification of obesity, metabolic syndrome and decreased reproductive capacity [18].

In the present study although a non-constant pattern has been established for high-fat diet, especially in terms of composition of saturated fatty acids [19], the diet used in the present study induced a significantly higher weight gain respect to the control group, which is consistent with previous reports [15]. Therefore, our conditions allowed to set a model of obesity caused by a high fat diet. Previous reports showed

that Spirulina diet supplementation induced a weight gain [20]. Interestingly, the weight gain in the group treated with high fat diet supplemented with Spirulina was equal to that of the group treated with high fat unsupplemented diet, despite that no significant difference on the intake of these groups was observed; next explored the effects of Spirulina supplementation on the biochemical profile. The high fat diet induced an increase in total cholesterol levels [21] as expected. Such effect was significantly higher in the group treated with *Spirulina maxima*, however no significant difference in terms of triglycerides levels was observed among these groups. Some reports have showed that *Spirulina platensis* consumption decreases triglycerides levels depending on the dose administered [22], however the evaluation was conducted in animals subjected to a normal diet. In contrast, when the effect was studied on a high-fat diet model, a poor change in serum triglyceride levels was observed [23] using a 57 mg/kg body weight Spirulina supplementation. Since our study comprised a 2% Spirulina supplementation, one can speculate that these levels are not sufficient to achieve hypolipemiant effects.

In terms of glucose levels, the group treated with standard diet showed a significantly higher levels when compared with the other experimental groups. However this unexpected results seems of low relevance since the levels are not considered hyperglycemia in rats [24]. According to the literature, spirulina consumption at 50/100 mg per kg body weigth under a standard diet induces a protective effect on renal injury due to a decrease in the concentration of urea [22]; however under a high-fat diet, this effect was not observed. This evidence suggests that the exposure to a high fat diet may affect the response to supplementation *Spirulina.*

Regarding to the inflammatory response associated to obesity, it should be considered that the stromal vascular fraction of the adipose tissue approximately

comprises 10% of macrophages (CD14 + and CD31), which produce the TNF-α present in the tissue and are responsible for the production of about 50% of the IL-6 present in the microenvironment [25] [26]. Furthermore, recent studies have shown that IL-17 levels are increased in obese mice, which is associated with the induction of insulin resistance [27]. Although the mechanisms by which Il-17 exerts such effects are still unknown, it has been reported that IL-17 induced expression of inflammatory chemokines / cytokines through a GSK3B-dependent mechanism [28] [29]. Many studies have focused in the effects of a high fat diet on the serum cytokine levels, however in tissues only the expression of the IL-17 receptor has been explored reporting no significant changes [30]. Interestingly, exposure to a high fat diet has been associated with elevated mRNA expression of IL-17 in the skin in a model of dermatitis [31]. In the present study, no significant difference in the expression of IL-17 and IL-6 genes was found, which is coincident with a previous study where no significant changes in the levels of these citokynes were found in serum upon a high fat diet [32]. Although the expression of some genes related to lipid metabolism has been assessed in perirenal fat tissue [33], no reports on the expression of cytokine genes were found in the literature. Thus, our protocol will be a useful tool to study the inflammatory response in perirenal tissue upon several conditions.

Considering that the balance between synthesis and degradation of lipids is crucial for the prevention of metabolic diseases, we evaluated for the first time the expression of enzymes involved in triglycerides metabolism (DGAT-1 and ATGL) [34]. Our results showed that *S. maxima* supplementation does not induce changes in the expression of DGAT-1 and ATGL genes. Interestingly a study in sheep was focused on estimating the expression of genes related to lipid metabolism in subcutaneous adipose tissue upon high fat diet supplemented with *S. platensis* [35],

observing that a high dose of *S. platensis* (20% w/v) induces a decrease in the expression of ADRB3 FASD, and BTG2 genes, which are related to fat deposit in peripheral tissues. We speculate that the elected dose for our study (2% w/w) is not sufficient to exert effects in the parameters of interest. Thus, further studies evaluating higher supplementation are proposed.

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Figure 1 Spirulina biomass composition
Percentage main components Spirulina maxima
Figure 2 Lipid profile
Fatty acid components in Spirulina maxima
Figure 3 Weight gain
Weight gain among different treatments
* Significant difference p<0.05, † No difference between treatment p<0.05
Figure 4 Intake
This graphic shows the intake in grams between different treatments in 8 weeks
† No difference between treatment p<0.05
Figure 5 Relative expression of inflammation genes
The graphic shows the relative expression of perirenal fat of genes a) IL-17 and b) IL-6 of different treatment
*No difference between group p<0.05
Figure 6 Relative expression of lipid metabolism genes
The graphic shows the relative expression of perirenal fat of genes a) DGAT-1 and b) ACTL of different treatment
*No difference between group p<0.05

Table 1. Serum Analysis

	Glucose	Cholesterol T.	Triglycerides	C-HDL	Urea	Creatinine	Uric Acid
Control	125.4 ±21.96	64.6 ±2.41	43.4 ±6.66	25.2 ±1.50	34.4 ±4.03	0.72 ±0.07	2.46 ±0.43
HFD	81.60[a] ±13.56	170.00[a] ±33.08	32.8 ±12.72	13.84[a] ±5.52	20.08[a] ±3.17	0.7 ±0.07	1.83 ±0.28
Smax	105.20[a] ±14.96	294.60[b] ±54.05	45.4 ±12.72	18.70[a] ±3.50	26.72[b] ±2.87	0.75 ±0.06	1.92 ±0.63

a *P<0.05* vs Control group; b *P<0.05* vs HFD group

Figures

Figure 1

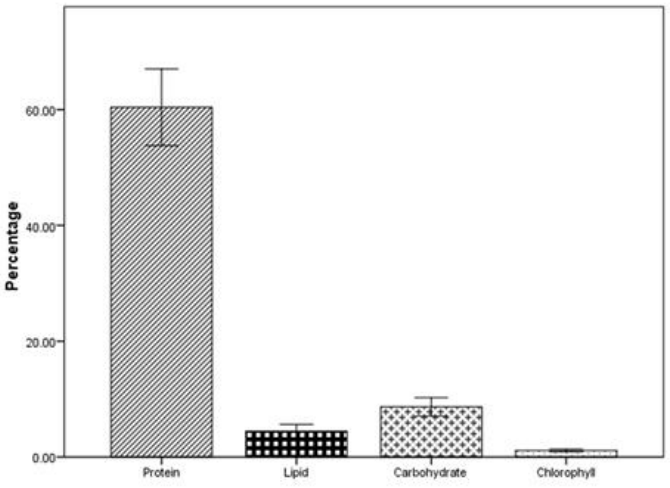


Figure 2

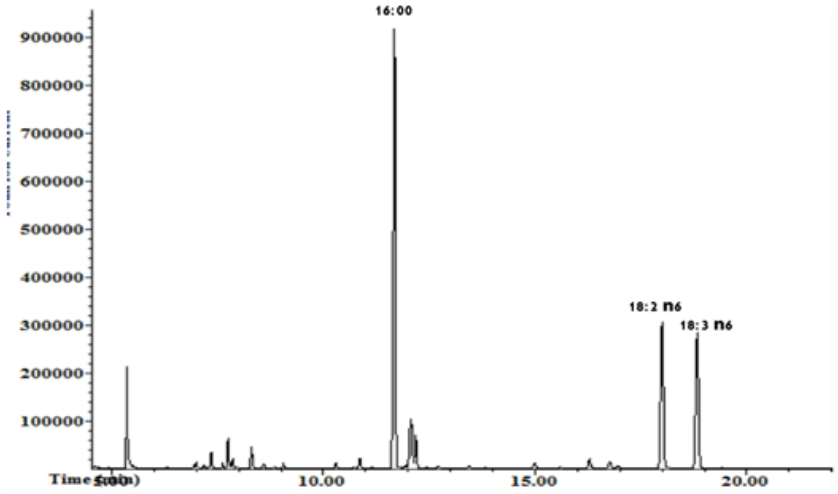


Figure 3

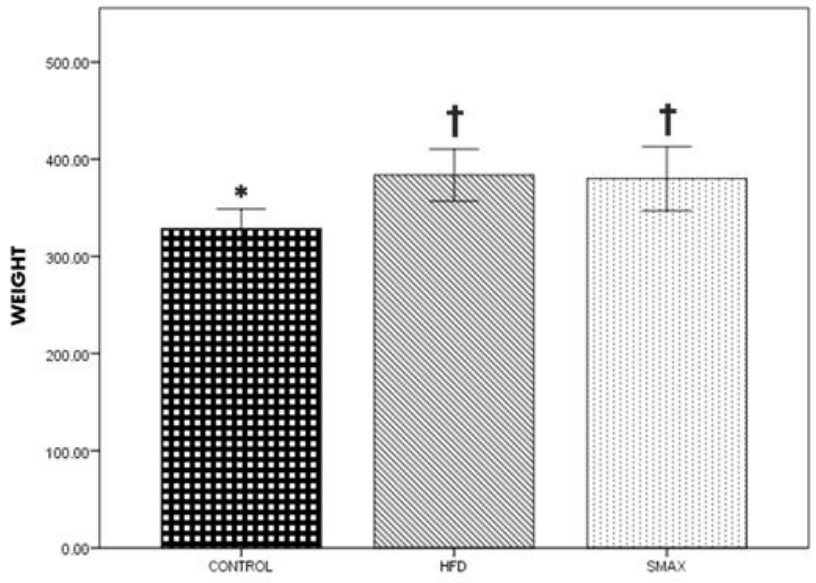


Figure 4

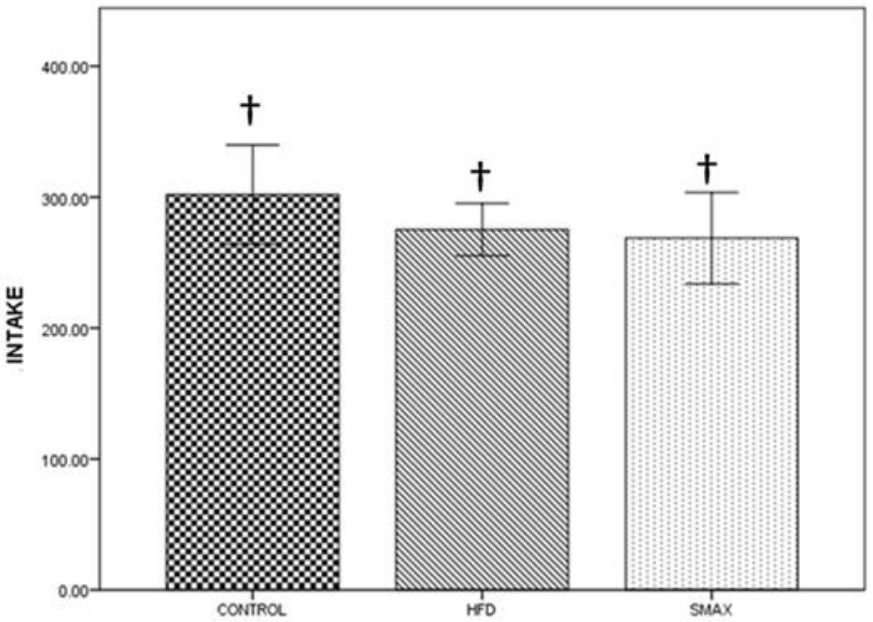
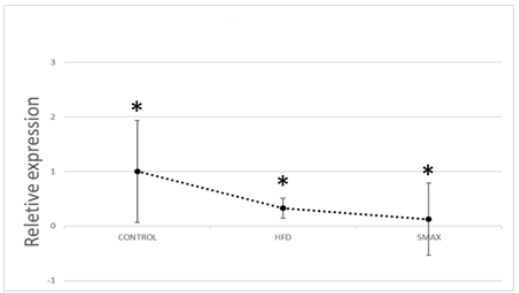


Figure 5



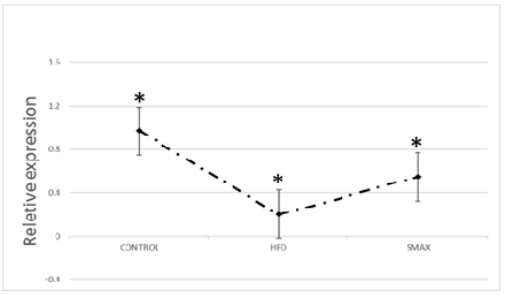


Figure 6