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Cyanobacterium Spirulina platensis LUQS1: Effects on serum lipids and kidney in domestic cats, Felis catus

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ABSTRACT

Aims: Researchers found a wide range of therapeutic properties in *Spirulina* sp. including as anti-cholesterol or antihyperlipidemic agent. In this study, the lipid levels of domestic *F. catus* were induced in order to scrutinize the antihyperlipidemic effects of local *S. platensis* LUQS1 strain, specifically at concentrations of 0.5 g/day and 1.0 g/day.

Methodology and results: Elevation of serum lipid levels viz. total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG) as well as the status of kidney [creatinine (Cr) and blood urea nitrogen (BUN)] were observed in four groups of *F. catus* for 45 days. The highest levels for TC, LDL and TG (P<0.05) were recorded in high cholesterol diet group (CD) at day 45 with 291.67±2.87 mg·dL⁻¹, 111.60±9.73 mg·dL⁻¹ and 146.33±10.44 mg·dL⁻¹, respectively. HDL levels in *Spirulina*-treated groups (CA and CAA) were better than normolipidemic group (control, SD group), of which the maximum levels were displayed at day 30 specifically 72.87±6.08 mg·dL⁻¹ by cats-fed with high cholesterol diet treated with 0.5 g/day *S. platensis* LUQS1 (CA group). There were insignificant differences (P>0.05) in the BUN levels; however, the Cr levels in CAA group (day 30 and 45) were slightly out of normal range but did not classify under chronic condition.

Conclusion, significance and impact of the study: Alternative treatments on hyperlipidemic cats were rarely reported by researchers and medicinal practitioners. Thus, the findings of this present study provided a genuine knowledge concerning the lipid-lowering effect of *S. platensis* LUQS1 on the hyperlipidemic cats.

Keywords: Spirulina sp., serum lipids, creatinine, blood urea nitrogen, cat

INTRODUCTION

Awareness on hyperlipidemia, obesity and hypertension in human are greatly given by researchers around the world. Indeed, they have overlooked a few of isolated reports on animals owing to the fact that it is infrequent for animals to develop heart disease related to hyperlipidemia or hypolipidemia like human. As a matter of fact, animals are commonly linked to serious or even fatal ailments such as obesity, vision, pancreatitis and neurologic problems (Kline, 2005; Remillard, 2005; Steiner and Williams, 2005; Tucci-Prosěk, 2005).

Spirulina sp. is formerly huddled under blue-green algae, however, due to its prokaryotic structure, it now belongs to cyanobacteria. It is enriched with proteins and vital nutrients, of which 18 essential amino acids (out of 22) are available within *Spirulina*. This cyanobacterium is also completed with β -carotene, making it a perfect

antioxidant (El-Sabagh *et al.*, 2014), anti-inflammatory (Muhammad Nazrul *et al.*, 2014), anti-cholesterol (Kim and Kim, 2005), anti-pyretic (Muhammad Nazrul *et al.*, 2014), antibacterial (Ozdemir *et al.*, 2004) and also anticancer (Konícková *et al.*, 2014) agent.

The first clinical trial of *Spirulina* sp. as an antihyperlipidemic agent was done on albino rats (Devi and Venkataranam, 1983). Since then, bigger models have constructively been used for lipid-related studies including on human (Nakaya *et al.*, 1988), rabbits (Colla *et al.*, 2008; Cheong *et al.*, 2010) and pigs (Saeid *et al.*, 2013). Information on cats is restricted due to their resistance capabilities towards cardiovascular diseases (CVDs) mostly *atherosclerosis* (Xiangdong *et al.*, 2011). Cats are among animals with a null or low level of plasma cholesteryl ester transfer protein (CETP) and have naturally higher HDL particles in their bodies (Guyard-Dangremont *et al.*, 1998). Nonetheless, cats are the great

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choice for primary and secondary hyperlipidemic disorders study (Kenneth, 2011). These animals have been accounted for several manifestations of lipid-related disorders such as diabetes mellitus, idiopathic hyperlipidemia, pancreatitis. nephrotic disorder. cholesterol ester stockpiling malady (Mosallanejad et al., 2016) and lipid aqueous (Kluger et al., 2009; Xenoulis and Steiner, 2010). To date, no single animal models could utterly represent human atherosclerosis; albeit, there are two common cats' features that relate to human, particularly the mechanical endothelial injury and cholesterol feeding behaviour (Xiangdong et al., 2011).

A potential role of *Spirulina* sp. in lowering the cholesterol levels either in animal or human is totally lacking and remains unclear. Generally, *Spirulina* sp. will bind with cholesterol metabolites bile acids in the liver and later, reduce the cholesterol solubility (Deng and Chow, 2010). In this investigation, priority was given to the beneficial effect of *S. platensis* LUQS1 with highlights on hyperlipidemic *F. catus* and the profile of Cr and BUN in signifying the cats' kidney status.

MATERIALS AND METHODS

Source of S. platensis and maintenance

The strain of *S. platensis* LUQS1 was isolated in Tasik Dayang Bunting, Langkawi, Kedah, Malaysia and maintained in modified Zarrouk medium (Zarrouk, 1966) at ambient temperature of 30 ± 2 °C, alkaline pH (8.5 to 9.0) and under cool white fluorescent source (30 µmol photon m⁻²s⁻²).

Animals, cholesterol and Spirulina diets

Twelve healthy male of F. catus cats (size range: 2800-3161 g) were assigned into four experimental groups; standard commercial diet (SD), high cholesterol diet (CD), cholesterol with 0.5 g/day S. platensis LUQS1 diet (CA) and cholesterol with 1.0 g/ day S. platensis LUQS1 diet (CAA). A total of 9% cholesterol per body weight was added into the daily diet of CD, CA and CAA groups. The cholesterol preparation was done by adding the chloroform and dried in a fume hood, prior to mixing process in 100 g intact pellet. These 3 groups were fed with high cholesterol diet for 2 weeks and after that continued with normal commercial diet. The total treatment duration was 45 days and free water access ad libitum was provided throughout the experimental period. Concentrated S. platensis LUQS1 was given to the cats daily via oral-gavage procedure.

Protein and lipid composition in animal Feed

Protein and lipid contents were determined accordingly to the method of AOAC (1997).

Biochemical analysis

One millilitre (mL) of blood was drawn from a cephalic vein using a needle with the size of 23 G (Terumo, Japan). Blood was collected at day 0 (pre-treatment), 15, 30 and 45 (post-treatment). Samples were centrifuged at 5000 rpm for 10 min, prior to serum lipids evaluation (TC, LDL, TG and HDL) plus basic test of kidney-function (Cr and BUN). All samples were analyzed via automatic analyzer Roche Cobas Mira (Thermo Fisher Scientific, United State of America) and Hitachi 902 Machine (Hitachi, Japan).

Statistical analysis

Statistical differences between treatment and control groups were analyzed via ANOVA SPSS version 22 (SPSS Statistics, *IBM* Corporation) and significance was accepted at P<0.05. All data were expressed as mean±standard deviation and levels of significance were represented by superscript alphabet.

Animal ethics

The guidelines on handling the experimental animals were in compliance with the standard operational procedure of animal ethics which were verified by Animal Ethics Committee (Faculty of Veterinary Medicine) in Universiti Malaysia Kelantan.

RESULTS AND DISCUSSION

Growth performance

Table 1.0 presents the diet compositions for all investigated groups and Table 2.0 displays the body weight differences for F. catus throughout 45 experimental days. According to the results of the final weights, it can be concluded that all diets managed to increase the cats' body weights. Yet, the highest daily weight gain (P<0.05) was recorded by CAA group (14.60±0.22 g/day) followed by CA (9.98±0.84 g/day), SD (9.78±1.20 g/day) and CD (5.77±0.45 g/day) group. The total protein (33.10±0.07%) and lipid (4.20±0.05%) contents in CAA group diet have significantly assisted the cats in increasing their body platensis Besides, the weights. S. LUQS1 supplementation which is generally known for its high protein, lipid and carbohydrate, has also contributed to the weight gain. As observed, the daily weight gain in CAA group (1.0 g/day) group has denoted 46% higher than the CA group (0.5 g/day). Comparing to the standard diet or normal group (SD), the CAA group increased to about 49% while the CD group stated a 41% reduction. Only the CD group demonstrated lower daily weight gain compared to others (CA and CAA groups) despite being supplied with the same cholesterol percentage (9% per body weight) in the diets. The daily weight gain between SD and CA groups displayed insignificant difference albeit their diet compositions (protein and lipid) were significantly different.

Protein, carbohydrate and fat notoriously contribute in weight gaining either in human or animal. These elements consist of the distinguishable value of calories, approximately 4 calories per gram deposited by protein and carbohydrate and about 9 calories per gram by fat.

 Table 1.0:
 The proximate composition of the experimental diets.

Compositions (%)	SD	CD	CA	CAA
Crude protein	19.80±	21.07±	26.53±	33.10±
	0.07ª	0.04 ^{ab}	3.55°	0.07°
Crude lipid	3.75±	4.12±	4.18±	4.20±
-	0.10ª	0.06 ^b	0.03 ^b	0.05 ^b

*SD: standard commercial diet group; CD: cholesterol diet group; CA: cholesterol with *S. platensis* LUQS1 (0.5 g/day) diet group; CAA: cholesterol with *S. platensis* LUQS1 (1 g/day) diet group; Values (mean ±standard deviation) with different superscripts in the same row were significantly different at the 5% level

Table 2.0: Initial and final weight of animals during the treatment.

Treatment group	Initial weight (g)	Final weight (g)	Daily weight gain (g/day)
SD	2870.67±47.11	3310.67±7.11	9.78±1.20 ^b
CD	2988.33±5.56	3248.00±26.00	5.77±0.45 ^a
CA	3139.33±14.44	3588.33±23.56	9.98±0.84 ^b
CAA	2967.00±4.67	3624.00±11.33	14.60±0.22 ^c
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*Values (mean \pm SD) with different superscripts in the same column were significantly different at the 5% level.

The supplementation of S. platensis LUQS1 resulted in a greater live weight and also daily weight gain of F.catus. These outcomes were in agreement with Nagaoka et al. (2005), Colla et al. (2008), Heidarpour et al. (2011) and El-Sabagh et al. (2014) who have tested the effects of Spirulina sp. on rats, rabbits, calves and lambs, respectively. A complete set of nutrients in Spirulina sp. such as amino acids, vitamins, essential fatty acid, minerals and other nutrients, has possibly stimulated the gut microflora to exude extracellular enzymes that indirectly induced the growth of animals (Tovar-Ramírez et al., 2002; Gershwin and Belay, 2008). It is also worth to mention a report by Azabji-Kenfack et al. (2011) that the supplementation of S. platensis towards a group of emaciated HIV-infected adults in Africa has successfully gained 4.8 kg of their average weight. In human, essential amino acids are pivotal for anabolism and muscle mass reconstitution. According to Deng and Swanson (2015), the gut of canine and feline are similar to human's gut especially their faecal microbial phylogeny and functional capacity. These similarities include the functional groups related to protein, metabolism of vitamins, carbohydrate, DNA and other major capacities.

Status of kidney

The nitrogenous end products of metabolism in human and animal are urea and creatinine. Thus far both elements are the most appropriate markers for renal (kidney) damage detection. Table 3.0 denotes the average levels of BUN and Cr in *F. catus* throughout the study period. The normal ranges of BUN and Cr levels obtained from the SD group were 44.33-65.33 mg·dL⁻¹ and 1.60-1.83 mg·dL⁻¹, respectively. By referring to the BUN, all levels in the tested groups were within the normal ranges. As for Cr, at day 30 and 45, the levels in CAA group were slightly out of normal ranges albeit not categorized under chronic condition. The Cr levels for both days were 1.93±0.11 mg·dL⁻¹ (day 30) and 2.13±0.04 mg·dL⁻¹ (day 45).

A normal cat has about 0.2-0.5 g·L⁻¹ (20-50 mg·dL⁻¹) serum urea and 6-19 mg·L⁻¹ (0.6-1.9 mg·dL⁻¹) serum Cr (Chetboul *et al.* 2003). According to our recent results, only the serum Cr was in agreement with the aforementioned outcomes. Deguchi and Akuzawa (1997) have reported that the average levels of BUN and Cr in cats with chronic renal damage were 136.7 mg/100 mL and 5.09 mg/100 mL, respectively. To obtain a more precise result, it is recommended to measure the quantity of glomerular filtration, the quantity of urine excreted and also the tubular re-absorption rate (Deguchi and Akuzawa, 1997).

Serum lipids of *F. catus*

Table 4.0 discloses the overall results for serum lipids in *F. catus.* For TC, significant differences (P<0.05) between the control group (SD) and CD, CA and CAA groups were detected at day 15, 30 and 45. At the end of the experimental period, CD group gave the highest TC levels compared to others. Meanwhile, the CAA group displayed the lowest TC level. Comparing to the control group (SD) at day 45, the increase of TC levels in CD group was by 94% while the TC levels in *Spirulina* supplemented groups have reduced by 60% (CA group) and 64% (CAA group).

In regard to LDL levels, there was a drastic increase recorded at day 30 in CD group of which the level hit up from 24.37 \pm 3.58 to 71.87 \pm 2.58 mg·dL⁻¹. The level was gradually increased at day 45 with 111.60 \pm 9.73 mg·dL⁻¹ (P<0.05) that representing the highest LDL levels in this experiment. Almost the same scenario was also observed in TG levels. The cholesterol intake has forced the CD group to attain the highest TG levels at day 30 with 190.97 \pm 2.64 mg·dL⁻¹ (P<0.05). The LDL and TG levels for both treated groups (CA and CAA) were slightly similar to the control group (SD).

Table 3.0: Values of blood urea nitrogen (BUN) and creatinine (Cr) in the cats on day 0, 15, 30 and 45 of experimental period.

Kidney's enzymes (mg⋅dL ⁻¹)/ Treatment group	Day 0	Time (Day) Day 15	Day 30	Day 45
BUN				
SD	58.27±2.18 ^a	65.33±3.78 ^a	53.73±3.16 ^{ab}	44.33±2.22 ^a
CD	62.73±2.49 ^a	57.33±5.56ª	65.53±5.02 ^b	52.67±4.22 ^a
CA	64.00±2.00 ^a	64.00±4.67 ^a	56.87±6.09 ^b	43.00±3.33 ^a
CAA	62.33±3.11ª	64.83±1.22 ^a	39.00±1.33 ^a	44.67±7.56 ^a
Creatinine				
SD	1.60±0.13 ^a	1.67±0.04 ^b	1.83±0.44 ^{bc}	1.8±0.07ª
CD	1.70±0.07 ^a	1.60±0.07 ^b	1.47±0.04 ^a	1.83±0.04ª
CA	1.70±0.07 ^a	1.13±0.04 ^a	1.60±0.07 ^{ab}	1.83±0.04ª
CAA	1.60±0.07 ^a	1.80±0.07 ^b	1.93±0.11°	2.13±0.04 ^b

*Values (mean ±SD) with different superscripts in the same column were significantly different at the 5% level.

Table 4.0: Values of total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG) and high-density lipoprotein (HDL) in the cats throughout the experimental period.

Concentration of serum lipids (mg·dL ⁻¹)/Treatment group	0	Time (Day) 15	30	45
Total cholesterol (TC)				
SD	153.93±0.76 ^a	150.20±2.27 ^a	164.07±2.76 ^a	150.03±6.04 ^a
CD	153.73±2.31ª	188.37±1.82 ^b	271.33±1.56 ^b	291.67±2.87 ^b
CA	149.33±4.22 ^a	91.37±1.58°	63.57±1.71°	59.43±3.91°
CAA	144.67±2.44 ^a	86.17±2.11°	48.90±4.60 ^d	53.63±3.76°
Low density lipoprotein (LDL)				
SD	15.20±0.80 ^a	39.93±1.91 ^a	20.40±3.60 ^a	28.70±6.27ª
CD	15.60±1.73ª	24.37±3.58 ^b	71.87±2.58 ^b	111.60±9.73 ^b
CA	22.57±2.38 ^b	22.40±3.07 ^b	18.53±3.69ª	44.3±5.13ª
CAA	16.13±2.09 ^{ab}	24.50±4.33 ^b	22.30±2.87 ^a	35.60±4.40 ^a
Triglyceride (TG)				
SD	41.60±2.13 ^a	64.90±4.60 ^a	52.27±5.51ª	62.33±8.89 ^a
CD	62.57±2.38°	72.37±4.24ª	190.97±2.64 ^b	146.33±10.44 ^b
CA	42.53±2.36 ^a	60.30±3.53 ^a	62.53±3.38ª	67.33±3.56ª
CAA	51.70±2.20 ^b	60.83±5.22 ^a	64.60±5.73 ^a	65.40±6.93ª
High density lipoprotein (HDL)				
SD	43.80±1.87 ^a	45.27±4.84 ^a	43.40±6.93 ^{ab}	38.37±6.911 ^{ab}
CD	46.67±1.78 ^a	37.10±5.40 ^a	28.53±6.36 ^a	25.60±4.40 ^a
CA	46.67±2.89 ^a	47.73±5.82ª	72.87±6.08°	61.63±3.76°
CAA	41.20±1.47 ^a	44.22±4.22 ^a	60.67±5.56 ^{bc}	57.67±7.78 ^{bc}

*Values (mean ±SD) with different superscripts in the same column were significantly different at the 5% level.

Table 5.0:	Effect	of	S.	platensis	on	weight	performance,	serum	lipids	and	kidney	in	different	animal	models
supplement	ed with	S. J	olate	ensis.											

Animal	Dose of S. platensis	Duration	Effect of <i>S. platensis</i> on weight performance, serum lipids and kidney	References
Lambs	1 g/10 kg body weightday	35 days	Weights increased 9.7% higher than control group TG and TC levels were significantly reduced at day 17 (P<0.05) HDL levels were not reported Urea was not significantly changed at day 35	El-Sabagh <i>et al</i> ., 2014
Rabbits	0.5 g/day	60 days	Weights increased 3.6% higher than control group Only TC levels denoted significant decreases (P<0.05) HDL levels showed significant increases with P<0.05 Kidney status was not reported	Colla <i>et al</i> ., 2008
Rats	48.03 g/100 g	10 days	8.5% body weight increase recorded TC and LDL+VLDL levels were significantly decreased (P<0.05) HDL levels were significantly increased (P<0.05) Kidney status was not reported	Nagaoka <i>et al</i> ., 2005
Calves	25 g/day	57 days	104% weights gain higher than control group TC and LDL levels were significantly decreased (P<0.05) HDL levels induced (P<0.05) BUN levels slightly similar to the control levels	Heidarpour <i>et al</i> ., 2011

Both single and double doses of *S. platensis* LUQS1 (CA and CAA groups) demonstrated the better enhancement in HDL levels compared to SD and CD groups. The highest level for HDL was recorded at day 30 in the CA group with about 68% higher than the SD group. A comparison of the effect of *S. platensis* on different animals was summarized in Table 5.0. Based on the results, *S. platensis* provided several beneficial advantages on animals mostly in their weight performance and also serum lipids elevation. Besides, the kidney of a few of the tested animals remained healthy and unaffected. The pre-clinical tests have proven that the positive effects of this cyanobacterium occur at an extensive range of animals' species.

The animals employed in this study were adult and healthy domestic cats. Their diets were mixed with cholesterol in order to achieve a hyperlipidemic condition. Atherosclerosis is one of the hyperlipidemic events which could occur in human and animal. However, cats, rats, mice, pigs, cows, horses and dogs are among animals with atherosclerosis resistance owing to their low CETP level (Guyard-Dangremont et al., 1998). CETP is responsible to assist the transport of cholesterol and triglycerides between the lipoproteins. Lacking in CETP resulted in more cholesterol to be transported as HDL. Report by Manning and Clarkson (1970) was not proportional to the statement as there was atherosclerosis event detected in cats. Based on their study, atherosclerosis has successfully developed in the abdominal aorta and coronary artery. They have induced the serum cholesterol levels using a diet rich in lard at concentrations of 0.5% (for 8 months) and 2% (for 4 months). Thus, understanding the basic lipoprotein metabolism is imperative since many diseases could amend this process due to the elevation levels in plasma lipid (Johnson, 2005). In this experiment, the application of higher cholesterol percentage specifically (9% cholesterol per body weight) has effectively elevated the serum lipids in domestic F. catus after 45 days of the study period. These outcomes were aligned with Ginzinger et al. (1997) where the application of 30% fat and 3% cholesterol showed significant changes in serum lipids, providing the basis evaluation for susceptibility of New Zealand lipase-deficient to diet-induced lipoprotein cats atherosclerosis (Ginzinger et al., 1999).

Previous studies have reported the valuable therapeutic functions of Spirulina sp. as anti-cholesterol, anti-inflammatory, anti-pyretic, antibacterial and anticancer agent (Ozdemir et al., 2004; Kim and Kim, 2005; El-Sabagh et al., 2014; Konícková et al., 2014; Muhammad Nazrul et al., 2014). Referring to Deng and Chow (2010), any agents with antioxidant and antiinflammatory activities own a tremendous potential for combating CVDs. Commonly, antioxidant properties could lessen the oxidation of lipids and proteins that indirectly defend the body from arterial stiffening and atherosclerosis (Carty et al., 2000; Carpenter et al., 2003; Ellingsen et al., 2009). In Spirulina sp., phycocyanin, βcarotene and y-linolenic acid are among super antioxidants which have been recognized affecting the CDVs by numbers of researchers (Nagaoka *et al.*, 2005; Colla *et al.*, 2008; Deng and Chow, 2010). Scavenging free radicals, reducing nitrite production and inhibiting liver microsomal lipid peroxidation are among phycocyanin's abilities (Remirez *et al.*, 2002; Romay *et al.*, 2003; Khan *et al.*, 2006; Cherng *et al.*, 2007; Riss *et al.*, 2007). βcarotene involves in lipid peroxidation (Schafer *et al.*, 2002) while γ-linolenic acid, a precursor to body's prostaglandin, engages with cholesterol synthesis (Krone *et al.*, 1988).

A few accessible reports regarding cats were commonly on renal failure (Deguchi and Akuzawa, 1997), hypertension (Chetboul *et al.*, 2003), obesity (German, 2006) and lipid-related study (Ginzinger *et al.*, 1999; Datz *et al.*, 2009). Based on this recent data, a tremendous potential in treating lipid-related events in *F. catus* using natural cyanobacterium was discovered. It can be a new promising and cheaper source to be supplemented in the cats' daily diets.

CONCLUSION

Studies assessing the efficacy of *Spirulina* sp. and clinical practice in *F. catus* are constrained. In this experiment, *S. platensis* LUQS1 (CA and CAA groups) has effectively reduced the TC, TG and LDL levels in cats-fed high cholesterol which simultaneously induced the HDL levels without affecting the kidney condition. Thus, this cyanobacterium has proven its anti-hyperlipidemic function as previously reported by researchers in this study herein.

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