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Spirulina platensis AS NUTRITIONAL PROMOTER FOR SEXUAL PUBERTY IN RAHMANY SHEEP

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ABSTRACT

Seven cyanobacteria groups recorded in Wadi-EL-Natrun lakes included five genera (two *Spirulina*, two *Oscillatoria* , one *Nostoc*, one *Chlorella* and one *Naviculs*) .

Spirulina was isolated from El-Khadra lake , Beheira, Egypt. The alga was identified as *Spirulina platensis*, and purified as axenic strain. Effect of antibiotic treatment on the *Spirulina platensis* was studied. Bacterioplankton were different in their antibiotic sensitivity, ampicillin was more effective. Axenic *Spirulina platensis* strain (bacteria free) was cultivated on a large-scale. The dry biomass algal yield contains as high as 60 % crude protein, *Spirulina* dry biomass added to the diet of Rahmany male sheep in the rate of 50g/Kg significantly increased Ca, P, Mg and Cu concentrations in the blood serum of the animals. This treatment reduced the period to puberty and sexual maturity to 69 % of that in control animals. Moreover, addition of *Spirulina* to the diet significantly increased semen volume, semen activity and number of sperms in unit volume. As *Spirulina* could optimize the blood picture and accelerates growth of male sheep, it could be recommended as growth promoter in the diet of sheep and similar animals.

Keywords: *Spirulina*, isolation, identification, toxicity, sheep promoter

INTRODUCTION

Phylogentically algae, especially micro algae, belong to the oldest and ecologically most variable group of organisms on the earth.

The health promoting effect of marine algae has been early described in Chinese references from 2700 BC, this case for micro algae. In Asia, cyanobacteria extracts prepared by boiling are used as beauty for skin and hair care .

Use of micro algae as food is not widespread. Human consumption of cyanobacteria of the genus Nostoc is reported from Mongolia, China, Japan, and Peru and seems to be valid for other Asian countries like Myanmar . Legendary are the descriptions of the natural mass cultures of Mexican and African *Spirulina* and their use by local populations as health promoting food (Piccardi *et al*,1999).

Nobora (1991) announced the efficient role of *Spirulina platensis* in prevention and treatment of heratites and cirrhosis. He mentioned that *Spirulina* is the way to save and clean the "medicine pickled" liver of the Japanese people.

Cyanobacteria known as *Spirulina* is protein-rich aqueous organism. As marine algae, *Spirulina* grown under different climatic conditions and used as a human diet in some poor African countries as protein-rich alga (Carbonera *et al.*, 1980). In addition *Spirulina* powder contains valuable ratios of carbohydrates, fats, and minerals (Hendrickson, 1989). A recent attention is given to *Spirulina* as a human health food. Its products are sold as powder, flakes or tablets as natural source of energy, thiamin, riboflavin and vitamin B_{12} (Hendrickson, 1989).Many studies were done to test *Spirulina* as animal

feed. Dehydrated *Spirulina* was used for protein replacement in swine starting diet (Hugh *et al.*, 1985), poultry diet (Mokadey *et al.*, 1979) silkworms (Hou and Chen, 1981) and fish (Granoth and Porath, 1984).

Sheep are important source for meat, milk, wool and manure. Rahmany sheep is wide spread as short breeding cycle sheep (Omar, 1998). Improving their growth and fertility through optimizing serum and semen parameters can positively affect puberty and sexual maturity.

The present work objected :

- Isolation, purification and Identification of the collected strains of cyanobacterial is dates from the different locations under study .
- Cultivation of the highly bioactive cyanobacteria to select the best isolates of these bacteria using synthetic media.
- Study the effect of antibiotic treatment on the bacterial load.
- Purification the isolated *Spirulina* as bacteria free strain (axenic strain).
- Determination of the physical and chemical composition of the isolated *Spirulina*:
- Use of cyanobacteria as food for Rahmany male-sheep and study its effect on puberty and sexual maturity.

MATERIALS AND METHODS

Morphology a nd Taxonomy :

Water samples were taken from different depths in four lakes (El-Khadra, El-hamra, El-Baida and El-Gaar Lake) at Wadi El-Natrone, Beheira, Egypt to determine their monthly mass production. At the heaviest growth period, algae floating on the water surface were collected in transparent bottles.

A part of the collected bio mass was grown on liquid media according to Aiba and Ogawa, 1977 for 12 days. About 20 ml of each culture was then centrifuged at 1500 rpm for 10 minutes. The slurry was washed with distilled water and examined under microscope.

The different types of Micro algae found in Wadi – EL- Natron lakes were identified microscopically according to Holmes and Whitton (1981) described by Salwa and Bader (1980)

The scheme of idintification was based on the colour of the living cells and their dimensions under microscope . Diameters and length of 100 vegetative cells , 100 hetero-cysts and a kinetes were measured. In the case of flamentous algae, the diameters and length of basel, median , and asppical pant of the vegetative flamentous were also measured .

Growth condition:

Spirulina was cultivated in batch culture under sterile conditions in Zarouk's medium at pH 9.00 (Van shak1986). The sodium content of the medium was 250 mM, most of it as sodium bicarbonate (13 grams per liter). The flasks were kept on a rotary shaker at 30 C° and continuously illuminated with cool white fluorescent lamps providing 80 Umole photons /m^2 at the surface of the flasks.

Purification of *Spirulina platensis* as axenic strain :

Cultures of S. *platensis* grown were filtered through sterilized Whattman 41 filter paper and Washed three times with modified Zarouk medium. The washed filaments were suspended in Zarouk medium covered with aluminum foil and kept in the dark at 35° C for 2.5 h. To this suspension, the following ingredients (final concentration) were added : glucose (1 %). peptone (0.5 %) . yeast extract (0.3 %), Na cl (0.5 %). In previous studies (Nobaru 1998) the optimeen antibiotics consantration which did not barn *Spirulina* alga was 100 Mg/ ml. so in the present study we used this concentration to control the bacterio-plankton Antibiotics i.e. ampicillin (100Mg / ml), Cefoxitin (100Mg/ml) and imipenen (100Mg / ml) were added separately to *Spirulina cultures* . These cultures were incubated at 35 C° for 48 h. in the dark , filtered and washed six times and suspended in zarouk media . Ferris and Hirsch.(1991) Algal filament and heterotrophic bacteria counts of this suspension were obtained. .

Mass cultivation:

A basin of 1000 Liter volume was constructed beside El-Kadra lake for alga mass production. A plastic pipe was connected from the lake to the basin ended by a filter to remove impurities. The basin water was inoculated with the purified isolate (axenic strain) prepared in the laboratory. Ten days later, the floated algae was harvested and air dried for further studies.

Determinations:

- Crude protein content of the algae was determined as total N x 6.25
- Lipid content was determined as ether extract according to A.O.A.C. (1965)
- Total carbohydrate, and total minerals contents were determined according to Dubois *et al.* (1965).
- Moisture content was calculated on the base of difference between weight of the sun-dried biomass and that oven dried at 105° C.

Toxicity tests:

Toxicity testes were carried out using mice tolerant to different doses of treated algae as follows:

- Fresh mass of the isolated algae was homogenized and centrifuged. Different doses of the suspension were injected interpretoneal to mice.
- Other part of the slury was washed several times with distilled water. Then the slury was homogenized and centrifuged. Different doses of the suspension were inter pretoneally injected to experimental mice.

Male-sheep growth and fertility promoter experiment:

Two Rahmany male-sheep groups were used. Each group consists of 5 animals in the same age (one month) was treated as follows:

Group 1(control): fed daily with 3.0 Kg Bovine-milk without any additions

Group 2: fed daily with 3.0 kg Bovine - milk + 150 g algae powder

- Age of puberty was determined depending on the complete appearance of the exterior portion of penis.
- Semen was collected by artificial vagina (Omar (1998))
- Age of sexual maturity was determined after spermatozoa become distict inside the seminiferous tubules.

- Serum testosterone was assayed using radioactive kits according(Yallow and Berson, 1971).
- Serum calcium was colorimetrically determined according to Tietz (1970)
- Serum inorganic phosphorus was determined.
- Serum magnesium, iron, copper and zinc were determined by using atomic absorption spectrophotometer according to(Omar (1998)).

RESULTS AND DISCUSSIONS

Algae identification and characteristics:

The seven cyanobacteria groups recorded in Wadi-EL-Natrun lakes were included five genera (two *Spirulina*, two *Oscillatoria* , one *Nostoc*, one *Chlorella* and one *Naviculs*) .

Spirulina is a multicellular , filamentous Cyanobacteria, under the microscope, it appears as blue-green filaments composed of cylindrical cells arranged in unbranched ,helicoidally trichomas, The diameter of the cells ranges from 4 to 6 um in *Spirulina platensis*. The most external or other membrane layer is *composed* of material arranged linearly in parallel with the trichome axis and is considered analogous to that present in the cell wall of gram-negative bacteria.

The diameter of the cells ranges from 1 to 3 Mm in the *smaller species (S. Caldaria) and from* 3 to 12 Mm in the larger one (*S. platensis*) diameter 6 to 8 mm. *S. platensis* have a granular cytoplasm containing gas vacules and easily visible septa – Under certain conditions trichomes of *S. platensis* is as long as 20mm have been observed .

The taxonomy of cyanobacteria is extremely difficult due to the paucity of morphological characteristics. Thus, names of genera have changed frequently and a number of subspecies are known.

Only unicellular and filamentous cyanobacteria are known, the latter being dominant, either branched or unbranched. None of these forms are flag elated , but some species are able to glide, e. g. Oscillatoria .

Many species are imbedded in a thick layer of extra celluar poly saccharides .

Cyanobacteia are gram negative with a typical peptidoglucan cell wall. (Tsuzuki and Miyachi, 1991).

Spirulina platensis and *spirulina caldaria* were dominant in EL Khadra lake fig (1 & 2) *Chlorella vulgaris* and *Naviculs canalis* were dominant in EL GAAR lake shown in fig (3 & 4). *Oscillatoria okeni* and *Oscillatoria. willei* were dominant in El Beida Lake shown in fig (5 & 6) *Nostoc* sp. was *dominant in El Hamra Lake shown in fig.* 7.

At present *spirulina* is given clinically to patients recuperating from surgery and patients with damaged livers. It is unfortunate we are still in the observation stage so that the statistics have not yet been completely gathered, but insorfa as we have been able to observe it appears that there has been great improvement (Gerwick, 1994; Sirenko *et al.* 1999).

Because of its exceptional nutrition properties, *Spirulina* is particularly interesting. Currently available *spirulina* isolated from natural lake (El-Khartoum) as well as from culture collection centers are not free of bacteria. The cohabiting bacteria are well adapted to the *spirulina* culture conditions.

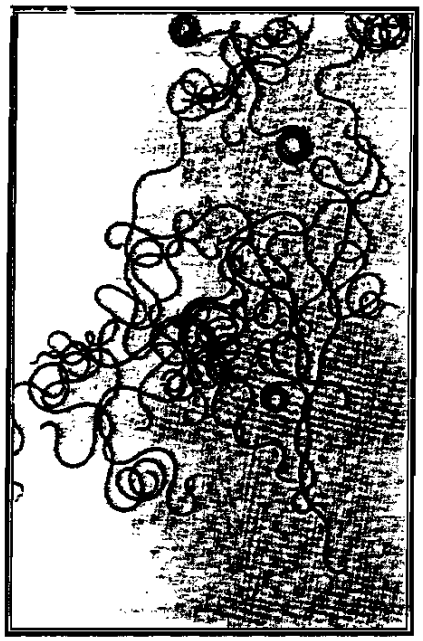

Fig (1) : *Spirulina platensis.*

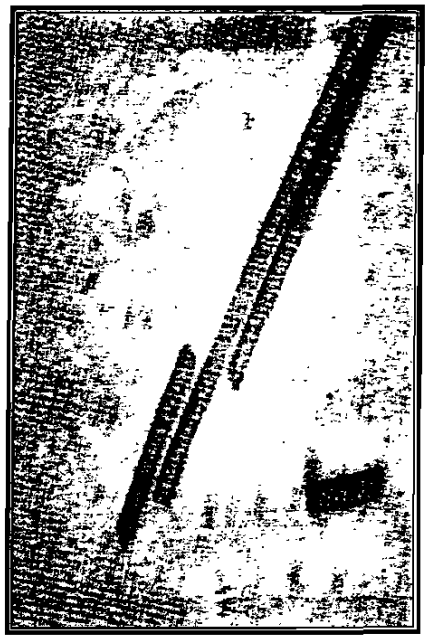

Fig (2) : *Spirulina caldaria.*

Fig (3) : *Chlorella vulgoris.*

Fig (4) : *Naviculs canalis.*

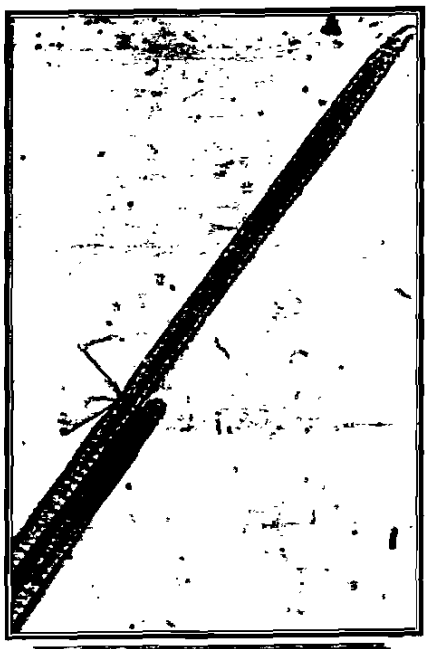

Fig (5) : *Oscillatoria okeni.*

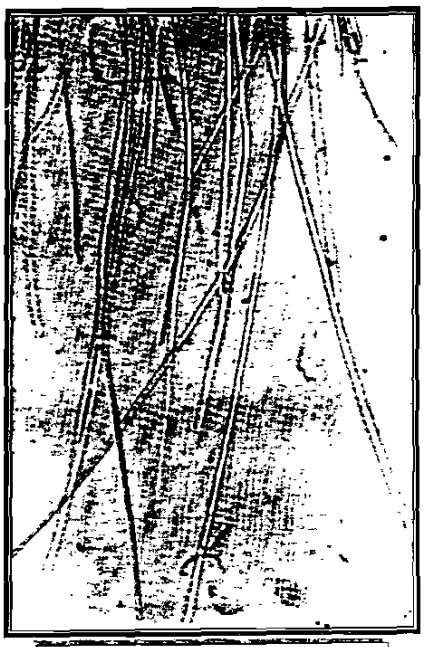

Fig (6) : *Oscillatoria willei.*

Fig (7) : *Nostoc sp.*

Where they grow embedded in the mucilaginous sheath secreted by *spirulina*, therefore, their physical separation from *spirulina* is very important while we have been succeeded to get axenic cultures of *spirulina* platensis by the method we applyied

Spirulina platensis isolated, have some naturally cohabiting bacteria. In our collection we observed that these bacteria are embedded in the mucilageinous sheath covering the spirulina filamentus (bacterioplanktons). Thus simple differential filtration was not sufficient to obtain axenic *spirulina* cultures.

Table (1):Effect of antibiotic treatment on *S. platensis* and its bacterio-plancton content.

Treatment	Bacteria (CFU/ml)	*Spirulina platensis* (filament / ml)
Ampicillin (100Mg/ml)	101×10^3	101×10^5
Cefoxitin (100Mg/ml)	2.8×10^4	3.9×10^4
Imipenen (100Mg/ml)	0.9×10^4	8.7×10^7

The bacterioplanktons did not have similar antibiotic sensitivity as shown in table 1. Moreover, even eliminate the sensitive bacteria. This is perhaps due to the poor permeability of the *spirulina* sheath to the antibiotics used or to an unknown mechanism of protection of the bacteria by the *spirulina* filaments. Hence, to eliminate the cohabiting bacteria load in *spirulina* cultures sufficiently so that upon dilution and distribution of the culture some filaments of *spirulina* free of bacteria could be isolated.

The alga isolated from El-Kadra lake was identified as axenic *Spirulina platensis* (Photo1). In addition to its nutritional value as food and feed, ability of *Spirulina* species to grow on a wide range of environments e.g. soil, marches, brackish water, fresh and seawater (Soong, 1980) raise the interest of using them as diet for domestic animals.

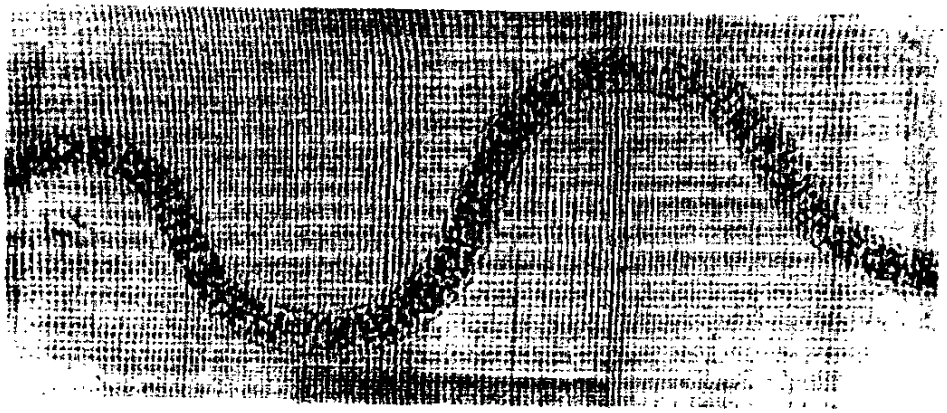

Photo 1: Morphology of axenic *Spirulina platensis*

Physical and chemical composition of the isolated *Spirulina*:

The major chemical composition of the isolated *Spirulina platensis* is shown in Table 2. It is obvious that the alga contains high protein percentage compared to other conventional protein sources. On the other hand, its low fat and relatively high mineral content put *Spirulina* biomass among the healthy diets.

Table 2: Major physical properties, minerals and chemical composition of the isolated *Spirulina platensis* dry biomass

Chemical composition		Physical properties	
Component	**Value (%)**	**Composition**	***100% Spirulina***
Crude protein	60.0	Appearance	fine powder
Total lipids	5.00	Color	dark blue-green
Total carbohydrates	20.0	Odor and taste	mild like seaweed
Minerals	7.00	Bulk density	0.35 to 55 kg/ litre
Moisture	5.00	Partial size	64 mesh trough
Minerals (per 10 grams)			
Calcium	75 mg	Copper	110 mg
iron	18 mg	Chloride	nil
phosphorus	78 mg	Potassium	130 mg
iodine	nil	sodium	90 mg
zinc	0.4 mg		

Moreover, protein of *Spirulina platensis* is rich in essential amino acids (Paoletti *et al.*, 1971), which renders its use as non-conventional protein source in the animal diet.

Biomass of *spirulina* as powder was produced from axenic culture of *spirulina platensis*, we managed to produce 750 grams through our study by cultivation of *spirulina platensis* axenic culture, harvesting cells or filaments from solution (10-20% d.w.) and dehydrating it.

Toxicity:

Remakes of the toxicity test declared that mice intra pretonelly injected with *Spirulina* suspension before washing of the biomass were died after 1.58 seconds. Meanwhile, others injected with the suspension of the washed biomass still healthy survived. It was discovered that the biomass before washing contains a high concentration of H_2S, which is toxic to mice. Thus, it is recommended that *Spirulina platensis* biomass must be washed before drying and use as diet.

Using the isolated *Spirulina* as promoter for Rahmany male-sheep:

Highly significant shortening ($P<0.01$) of ages of puberty and sexual maturity which means early starting of the animal reproductive life or sheep breeding cycles were observed in treated group as compared to control.

Spirulina had a magic importance to convert nitrogen and nitrogenous material to protein in the rumen (Paterson at al, 1983) which improves the nutrients level in the low protein level ration. These results were in accordance to Hugh *et al*, (1985) and Becker and Van shak (1988)

Spirulina which discovered in National Research Centre were better than the other commercials promoters. It is of great interest that the most superior growth or fertility promoter or functional proboscis was the hot air dried *Spirulina* platensis, not only for shortening ($P<0.01$) both ages of puberty and sexual maturity; but also improving the levels of all semen characteristics, testosterone hormone levels as well as both minerals (calcium, inorganic phosphorus and magnesium) and trace elements (iron ; cupper and zinc) as shown in table (2)

Addition of *Spirulina* powder to the diet of Rahmany Male-sheep was found to significantly increase calcium, phosphorus magnesium and cupper in the blood serum of the animals (Table 3).

Table 3: Ages of puberty and sexual maturity, testosterone level, semen picture and nutrient concentrations in the blood serum of Rahmany male-sheep fed with *Spirulina* additives in the diet compared to control animals

Character	Control animals	Animals fed with dry *Spirulina* in the diet
Age of puberty (days)	185 b	150 a
Testosterone (ng/ml)	11.89 ± 0.7 a	14.79 ± 0.92 a
Age of sexual maturity (days)	285 b	197 a
Semen picture:		
Volume (ml)	0.58 ± 0.1 a	0.92 ± 0.08 b
PH	6.56 ± 0.2 a	6.60 ± 0.03 a
Mass activity	3.6 ± .08 a	4.52 ± 0.13 b
Motility	85.6 ± 7.52 a	94.85 ± 11.52 a
Sperm number (million/ml)	2362 ± 67 a	2994 ± 89 b
Abnormal sperms (%)	8.43 ± 1.01 a	5.82 ± 0.42 a
Dead sperms (%)	7.42 ± 0.9 a	5.82 ± 0.62 a
Serum nutrient concentrations:		
Ca (mg/100 ml)	6.82 ± 1.18 a	18.31 ± 1.61 b
P (mg/100 ml)	6.32 ± 0.52 a	8.11 ± 1.61 b
Mg (mg/100 ml)	1.39 ± 0.03 a	10.82 ± 0.05 b
Fe (mg/l)	1.49 ± 0.31 a	1.98 ± 0.23 a
Cu (mg/l)	1.79 ± 0.38 a	2.29 ± 0.31 b
Zn (mg/l)	1.32 ± 0.6 a	2.01 ± 0.22 a

Character with the same letters are not significantly different, $P<0.01$

In addition, containing of *Spirulina* on high protein percentage of high efficiency ratio (Mokady *et al.*, 1979) and rich in essential amino acids (Paoletti *et al.*, 1971, Ladygina and Gurevich, 2000), pigments and vitamins (Hendrickson, 1989) can accelerate the physiological processes lead to a faster growth. As a response to the optimization of blood characteristics occurred by *Spirulina* in male-sheep diet, age of puberty and age of sexual maturity were significantly shortened ($P < 0.01$). Both parameters were reduced compared to the control animals. It was found also that semen picture of this group was improved, where semen volume, mass activity and numbers of sperms were significantly increased, which ascertain fertility increase of the animals.

CONCLUSIONS

From the present work, it can be concluded that:

- The isolated alga is protein-rich and can be used as unconventional protein source for animal feeding. However, the harvested slurry must be washed several times before drying to avoid animal toxicity.
- Addition of *Spirulina* dry biomass to Rahmany male-sheep diet led to optimization of the blood characteristics, improvement of semen picture and reduction in the periods to puberty and sexual maturity compared to control animals.

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إستخدام طحلب " سبيرولينا بلاتنسيس" كمنشط لنمو أغنام الرحماني وسرعة نضجها الجنسي.

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**** قسم تكاثر الحيوان ، المركز القومي للبحوث – الدقي – القاهرة.**

لقد تم عزل وتصنيف سبعة مجاميع من السيانو بكتريا من ٤ بحيرات من بحيرات وادي النطرون المغلقة ، تضم هذه المجاميع الخمس الأجناس التالية :

١- جنس الاسبيريولينا وكان سائداً في بحيرة الخضراء.

٢- جنس الـــــــــــكوليرا وكان سائداً في بحيرة الجعـــار .

٣ - جنس النافاكوليـــس وكان سائداً في بحيرة الجعـــار.

٤ - جنس الاسولوتاريا وكان سائداً في بحيرة البيضاء.

٥ - جنس النوستـــــــوك وكان سائداً في بحيرة الحمراء .

كما تم تنقية سلالة من الإسبيريولينا بلاتنسيس من البكتريا النامية في الغلاف الجيلاتينـــي المحيط بها (بكتريو بلانكتون) و دراسة حساسيتها لثلاث مضادات حيوية هي :-

١ــ الأمبيسيلين بتركيز ٥ ملليجرام / مللى لتر

٢ــ سيفاكسين بتركيز ٥ ملليجرام / مللى لتر

٣ــ امبيسنين بتركيز ٥ ملليجرام / مللى لتر

و لقد ثبت فاعلية الأمبيسيلين بالمقارنة بالمضادات الأخرى في تنقية سلالة الاســبيريولينا بلاتنسيس من البكتريو بلانكتون

بعد الحصول على السلالة النقية تم تنميتها و جمع الكتلة الحيويـــــة للطحلـــب وتحليـــل محتواها من البروتين الخام الذي وصل إلى ٦٠% ، والدهـــون ٥ % ، وكربوهيـــدرات ٢٠ % ، معادن ٧ % ورطوبة ٥ % .

وأوضحت نتائج التقييم البيولوجي لهذا الطحلب ومدى سميته عن طريق حقـــن فـــئران التجارب بخلايا الطحلب الغير مغسولة فقد أدت إلى موت حيوانات التجارب ، في حين أن الفـئران التي حقنت بخلايا الطحلب المغسولة بقيت حية وذات حيوية عالية ، مما يدل على زوال الســموم " التكسينات " عن طريق غسل خلايا الطحلب .

كما أوضحت نتائج تجارب تغذية ذكور الأغنام " الرحماني " على مســـحوق الطحـــالب المغسولة تحسن حالتها الصحية وزيادة تركيز الفسفور والكالسيوم والمغنسيوم كعناصر غذائية فـــي سيرم دم الحيوان ، كما أدت إضافة الطحلب إلى عليقة الحيوان إلى سرعة البلوغ والنضج الجنســي لتلك الحيوانات بمعدل ٦٩ % عن تجربة المقارنة بدون تغذية بتلك العليقة المشتملة على مســـحوق الطحلب المغسول.

ولذا يوصى بإضافة مسحوق هذا الطحلب إلى عليقة تغذية تلك الأغنام حيث أنها تحتـوي على نشاط لزيادة نموها وارتفاع محتواها من البروتين الخام والكربوهيدرات والدهون.