J. Agric. Sci. Mansoura Univ., 28(8): 6389 - 6398, 2003

EFFECT OF *SPIRULINA* ON RUMEN BACTERIA AND ITS RELATION TO THE PROMOTION OF RAHAMANY CHEEP SEXUAL PUBERTY

Aly, M. S. * and M. A. Omar**

* Agricultural Microbiology Department*, Animal Reproduction Department**

** National Research Centre, Dokki, Cairo, Egypt

ABSTRACT

Spirulina was isolated from El-Khadra lake Wadi El-Natroun, Beheira, Egypt. The alga was identified as *Spirulina platensis*. The isolated algae were cultivated on a large-scale in a basin constructed near the lake. The dry biomass analysis showed that the algae biomass contains as high as 60 % crude protein, 5 % fats, 20 % carbohydrates, 7 % minerals and 5 % moisture. Unwashed biomass was found to be toxic to mice, while mice injected with washed biomass suspension still healthy survived. *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. The treatment also reduced the period to puberty and sexual maturity to 69 % of that of control animals. Addition of *Spirulina* to the diet significantly increased semen volume, semen mass activity and number of sperms in unite volume. The effect of *Spirulina* biomass and/or crude extract on dominant rumen bacteria (13 isolates) was studied.

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

INTRODUCTION

Cyanobacteria known as *Spirulina* is protein-rich aqueous organism. As marine algae, *Spirulina* grown in different climates and used as a human diet in some poor African countries. *Spirulina* is protein-rich alga (Carbonera *et al.*, 1982). 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 selling 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 animal (Omar, 1998). Improving their growth and fertility through optimizing serum and semen parameters can positively affect puberty and sexual maturity.

The microbial population in the rumen is enormous amounting up to 10^{10} cells/ml (Stanier, 1986). The full details of their biochemical activities are not yet totally understood. These microbes play role in the fast digestion of the plant materials in the diet. The net effect is clear: the cellulose and other complex carbohydrates are broken down to fatty and organic acids which is

absorbed in blood stream to various animal tissues and used in respiration to generate energy. These bacteria also take important part in the synthesis of essential amino acids and other valuable organic compounds (Stanier, 1986).

The present work objected to isolate *Spirulina* from El-Khadra Lake and study its characteristics to investigate whether its dry material can be used as diet promoter to shorten the periods to puberty and accelerate sexual maturity of Rahmany sheep.

MATERIALS AND METHODS

Algal isolation:

Samples were taken from different locations and different depths of El-Khadra Lake, 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 collection was grown on liquid media according to Aiba and Ogawa, 1977 for about 12 days. About 20 ml of each culture was then centrifuged at 1500 rpm for 10 minutes. The slurry was washed with distilled water examined and re-inoculated under microscope on algal peptone and/or yeast extract solid media. This process was repeated till obtaining an axenic culture.

Organism and growth condition:

The strain was cultivated in batch under sterile conditions in Zarouuk's medium pH 9.00 (Van shak1986). The sodium content of the medium was 250 mM, most of it as sodium bicarbonate. The flasks were kept on a ratory shaker at 30 C° and continuously illuminated with cool white fluorescent lambs providing 80 Umole photons m^2 at the surface of the flasks.

Mass cultivation:

A basin of 1000 Liter volume was constructed near El-Kadra lake for alga mass production. A plastic pipe was connected from the lake to the basin ended by a filter to remove large objects. The basin water was inoculated with the pure isolated algae prepared in the laboratory. Ten days later, the floated algae were harvested and sun dried.

Algal constituents:

- 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, total minerals content was determined according to Dubois *et al.* (1956).
- Moisture content was calculated on the base of difference between weight of the sun-dried biomass that oven dried at 105° C.

Toxicity tests:

Toxicity testes on mice were done:

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

Male-sheep growth and fertility promoter experiment:

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

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

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

-Age of puberty was determined depending on the complete appearance of the interior portion of penis.

-Semen was collected by artificial vagina parameters were determined (Omar and Younis (1995)

-Age of sexual maturity was determined after spermatozoa 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 Varley (1976).

Sampling of ruminant bacteria

Rahmany sheep feces samples were collected from around the place when the sheep were ranching using sterilized plastic bags.

The morphology and gram stain of isolates

The serial dilutions plate count method according to Allen (1953) the obtained isolates were kept on nutrient slants agar. The morphology and gram stain tests were done on 5 days cultures grown an either slants or nutrients broth.

Preparing *Spirulina* crude extract

The crude extract of *Spirulina* was obtained by extracting 50g of *Spirulina* powder for 3 hours in 80% of ethanol. The extraction was repeated three times using the same ethanol concentration. The filtrate extract was then concentrated under reduced pressure 0.1 ml.

Preparing *Spirulina* suspension

The *Spirulina* suspension was done by adding 60 mg of dry *Spirulina* biomass powder to 1000 ml of sterile distilled water. To test the stimulation or inhibitory effect of both *Spirulina* crude extract and 60 mg of dry *Spirulina* biomass. 0.1 ml of each was loaded on sterile filter paper after putting it on the surface of agar plates with nutrient agar medium.

RESULTS AND DISCUSSIONS

Morphology and Taxonomy:

Spirulina is a multicellular, filamentous cyanobacteria under the microscope, spirulina appears as blue-green filaments composed of cylindrical cells arranged in unbranched, helicoidally trichomas. 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.

Algae identification and characteristics:

The alga isolated from El-Kadra lake was identified as *Spirulina platensis* Fig.1). according to the taxonomic shewa of Rippka *et al* (1979) . Spriulina cell type and reproductive is a filamitovs trachomue containts vegetative cell, 'n dud uoled in G + OC rang III (40 – 678) kb and its G+ C chromosomal Content genera is 44 – 54 % Kb *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 supplementary diet for domestic animals.

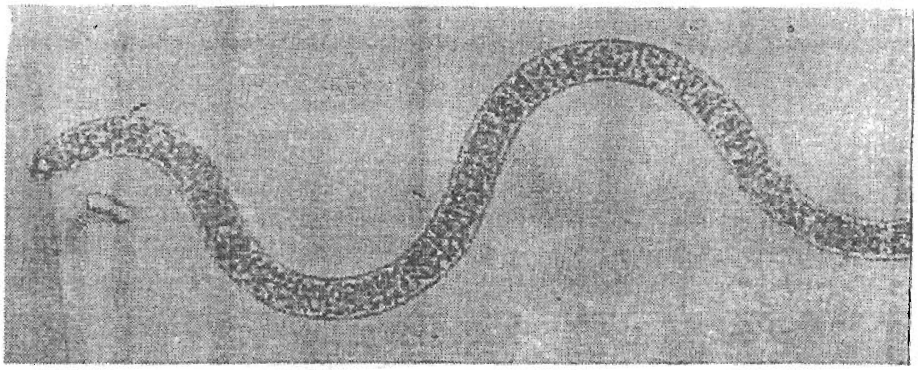

Fig 1: Morphology of the isolated *Spirulina platensis* 1000 X

Table 1: 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		

Physical and chemical composition of the Isolated Spirulina:

Major chemical composition of the isolated *Spirulina platensis* is shown in Table 1. 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.

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.

Toxicity:

The toxicity test declared that mice intra pretonally injected with *Spirulina* suspension before washing of the biomass were died after very short time (seconds). Meanwhile, others injected with the suspension of the washed biomass still healthy survived. It was found 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 used as diet.

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 *Spirulina* fed group as compared to control.

Spirulina was reported to convert nitrogen and nitrogenous material to protein in the rumen (Paterson Ct *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 Venkaram (1985).

Hot air dried Spirulina platensis, was found to be better than the other commercial promoters. It is interest than 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 for 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).

Interaction between *Spirulina* and ruminant bacteria

It is expected that the very high density of microbes in the rumen of the animal may have positive or negative interaction between each other while they are competing for nutrients. Among these interactions the inhibititory and stimulatory effects on beneficial and pathogenic microflora can take place: This can enhance the production of microbial products of significance to animal growth and reproduction. The inhibition of certain ruminant microbes can also play important role in the killing the pathogens in the rumen. The 13 isolates from the sheep feces (4 of gram negative and 9 gram positive bacteria) were found to be stimulated or inhibited by the addition of the *Spirulina* to the animal feed.

Table 1 shows the morphology and gram staining of 13 isolates from the feces of Rahmany sheep. Among the isolates the cocci bacteria were dominating (11 isolates) and the other two isolates were short and long rods. Bacteria of the genus *Ruminococcus* were described in Bergey's (1994) to inhabit the rumen, large bowel, and cecum of mammals. The effect of

Spirulina on the dominant ruminant bacteria from Rahmany sheep is presented in Fig (2). The results show that four isolates were stimulated by the *Spirulina* nonliving cell suspension whereas; the other nine isolates were inhibited by the same treatment with the algae nonliving cell suspension. The intensity of the stimulation or inhibition was dependent on the incubation period, being higher with the extended duration of incubation (6 days). The inhibitory effect was much higher with using *Spirulina* crude extract (Fig 3) as compared with *Spirulina* non-living cell. One isolate (number 15) was stimulated with the crude extract, whereas the rest of the isolates were inhibited. The degree of inhibition by the crude extract was much higher with several isolates (4, 6, 7, 8, 10 and 11). These data show that the biomass of *Spirulina* and its crude extract exert effect on the bacteria densely populating the digestive system of the sheep. The stimulation or inhibition of these bacteria may have general effect on the animal productivity and reproduction through the transformations of feed in the rumen and enriching it with necessary amino acids, vitamins and other compounds; and or through controlling pathogenic bacteria which many adversely affect the animal growth. Several authors found that the biological agents including ruminant bacteria and protozoa play role in modification of nutritional status of feed (Anna and Ung, 1984; Ushida *et al.*, 1984; Veira *et al.*, 1984).

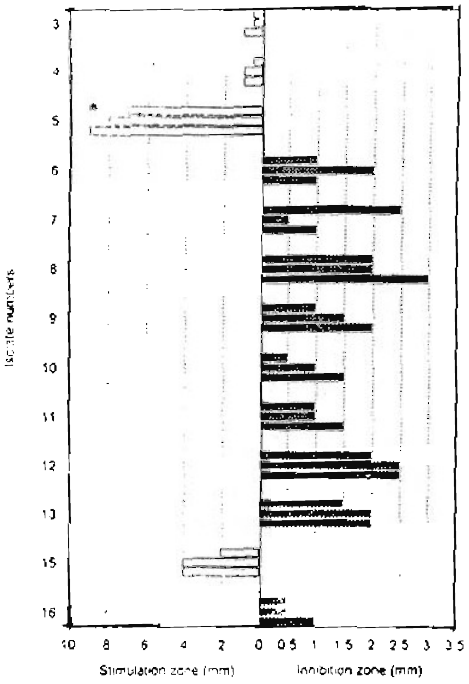

Fig.2: The stimulatory and inhibitory effects of *Spirulina platensis* on 13 ruminant bacterial isolates from Rahmany sheep

(* Each cluster of three histograms means three sampling dates (2, 4, 6 days up to down))

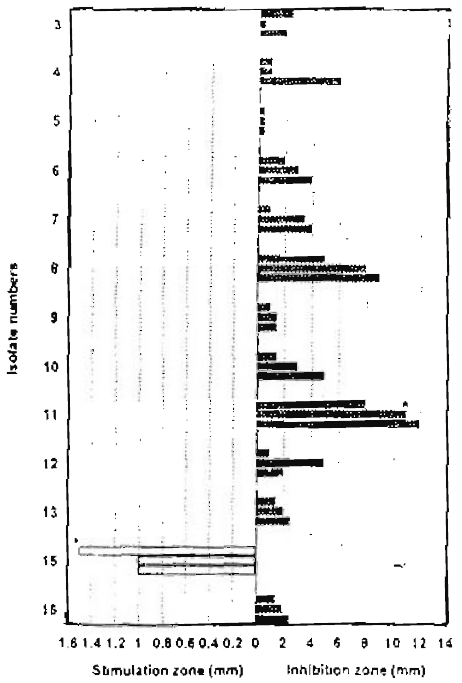

Fig.3. The stimulatory and inhibitory effect of *Spirulina platensis* crude extract on 13 ruminant bacterial isolates from Rahmany sheep

(* Each cluster of three histograms means three sampling dates (2, 4, 6 days up to down))

This clearly shows that the presence of the *Spirulina* in the feed can exert noticeable effect in composition of ruminant bacteria through simulative and/or inhibition effect on the bacteria. The change in ruminant microflora composition may change the growth and reproduction of the animal through the secretion of growth promoting substances and release of minerals from the complex organic.

This is in agreement with Akin 1984 who stated that the population of rumen microorganisms is itself diverse, and variations exist in the manner in which microbial types attack the plant cell walls and convert the forages. Therefore, complexity in the utilization of forages is brought about by diversity among the forage plants, diversity in the microbial population, and interactions between the plants and microorganisms.

Using the isolated Spirullna as promoter for Rahmany male-sheep:

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). In addition, containing of *Spirulina* on a 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 by 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.

Table 2: 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 with the same letters are not significantly different, P<0.01*)

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

Table (3): Morphology and Gram staining of isolated ruminant bacteria

Isolate numbers	Cell shape	Gram staining
3	Cocci	+
4	Cocci	+
5	Short rod	–
6	Cocci	+
7	Cocci	+
8	Cocci	+
9	Cocci	+
10	Cocci	+
11	Cocci	–
12	Cocci	–
13	Cocci	+
15	Cocci	–
16	Long rod	+

CONCLUSION

From the present work, it can be concluded that:

Addition of *Spirulina* dry biomass to Rahmany male-sheep diet led for improving the blood characteristics, semen picture and reduction in the periods to puberty and sexual maturity compared to control animals. The *Spirulina* extract and the biomass were found to stimulate several isolates of ruminant bacteria. Other group of isolates was found to be inhibited by *Spirulina*. This may have effect on the bacterial composition of ruminant microflora and in turn on the nutritive value of feed amended with *Spirulina*.

REFERENCES

A.O.A.C. (1965). Official methods of analysis. 10th Ed. Association Official Agricultural Chemist, Washington D.C., pp. 780.

Akin, D. E. (1984). Chemical and biological structure in plants as related to microbial degradation of forage cell wasils , plant structurt and degradtion , 139 : 155 .

Alba, S. and Ogawa T. (1977). Assessment of growth yield of blue green alga, S. platensis, in axenic and continuous culture, J. gen. Microbiol., 102:179-182.

Allen, O. N. (1953). Experiments in soil Bacteriology. (1st Ed.), Burgess Publ. Co.,U.S.A

Anna, M. Denholm and J. R. Ung (1984). In vitro metabolism of bacterial cell wall by rumen Protozoa. Cam. J. Anim. sci. 64 (suppl.): 18-19.

Becker, E.W. and L. Verikaraman (1992) Biotechnology and Exploration of Algae,The Indian Approach, Germany agency for Technical cooperation Eschborn, Germany.

Bergey's Manual (1994). Bergey's Manual of Determinative Bacteriology.. John G. H.; Noel R. K: Peter H. A.; Smeath S.; James T. s.; Stanley T. Ninth Edition. Willians and Wilkins company.

Carbonera, D.; Garetta and S. Geitleri (982). Immuno electrophoretic investigation on *Spirulina platensis*. FEMS Microbiol. Lett., 14: 229-232.

Veira, D. M.; M. Ivan and P. Y. Jui T. (1984). The effect of ciliate Protozoa on the flow of amino acids from the stomach of sheep. Cam. J. Anim. sci. 64 (suppl.): 22-23.

Dubois, M.; K. A. Gilles; J. K. Hmilton; P.A. Rebers and F. Smith (1965). Colorimetric method for determination of sugars and related substances. Anal. Chem., 28(3): 350-365.

Garnoth, G. and D. Porth (1984). An attempt to optimize feed utilization by Tilapia in a flow through aquaculture. Proc. of Int. Symp. On Tilapia and Aquaculture. Nazareth, Israel. pp. 550-558.

Hendrickson, R. (1989). Earth food Spirulina. Ronore Enterprises. Laguna Beach, California. U.S.A.

Hou, R. H. and R. S. Chen (1981). The blue-green alga *Spirulina platensis* as a protein source for artificial feeding of *Bombyx mori*. Appl. Entomol. Zool., 16:169-171.

Hugh, W. t.; I. Dominy and E. Duerr (1985). Evaluation of dehydrated *Spirulina platensis* as a protein replacement in swine started diets. Res. Ext. Ser., 1985: 3-10.

Ladygina, U. P. and Gurevich, I. (2000). Protist plankton from the Khnak lake. Izv Akad. Nauk ser Biol., 3: 361-376.

Mokadey, S.; S. Uannai; P. Einav and Z. Berk (1979). Algae grown on waste water as source of protein for young chickens and rats. Nutr. Rep. Int., 20: 31-39.

Omar, M. A. (1998). Reproductive patterns of sheep breeds under desert conditions. Proc. 5th Sci. Con., Fac. Vet. Med. Zagazig, Egypt, 1998.

Paoletti, C.; R. Materassi and E. Pelosi (1971). Variazione delle composicione di alcuni ceppi mutanti di *Spirulina platensis*. Ann. Micr., 21:65.

Paterson, J.A.; B.M. Anderson; D. Bowman; R. L. Morrison and J. E. William (1983). Effects of protein source and lasalocid on nitrogen digestibility growth by ruminants. J. Anim Sci 571537.

Roger, *et el.* (1979). Rippka R., Deruelles J., Waterbury J.B , Herdman M Stonier R.Y.Genetic assign memet , strain histories and properties of pure Cestuses cyamoladeria gem Microbial III, 1 – 61

Roger, Y. Stanier; John L. Ingraham; Mark L. Wheelis; Page R. Painter (1986). The Microbial World. Prentice Hall College Div.

Soong, P. (1980). Production and development of Chlorella and Spirulina in Taiwan, p. 97-113 in G. Shelef and C. J. Soeder (Ed.) Algae biomass. Biochemical Press, Elsevier/Holland

Teitz, (1970). Fundamentals of clinical chemistry W.B. sounders, philaderphia.

Ushida, K.; J. P. Jouany; Bernadette Lassalas and P. Thivend (1984). Protozoal contribution to nitrogen digestion in sheep. Cam. J. Anim. sci. 64 (suppl.): 20-21.

Varley, (1976). Practical clinical biochemistry. 4th edn. Ariold Hieinemanm, New Delhi.

Ward, G.M. and F.B. Johnson (1962). Chemical methods of plant analysis. Canada Dept. Agric., 10 (64), 1.

Yallow, and Person (1971). Principal of competitive protein bnding assayi. Eds. J.B. Lippencott, Co. Philadelphia 1 - 19.

تأثير طحلب اسبيرولينا على بكتريا الكرش فى أغنام الرحمانى وعلاقتة بالخصوبة والنضج الجنسي

محمد سعد على، محمد عبد العظيم عمر
قسم الميكروبيولوجيا ، قسم التكاثر الحيوانى، المركز القومى للبحوث

تم عزل طحلب اسبيرولينا من بحيرة الخضراء بوادي النطرون محافظة البحيرة وقد تم تعريف الطحلب على انه اسبيرولينا بلاتنسيس وتم تنميته الطحلب على مستوى كبير فى بحيرة مصنعة بجوار البحيرة الخضراء وكانت نتائج تحاليل الكتلة الحيوية تبين أن هذه الكتلة تحتوى على بروتين بنسبة ٦٠% بروتين خام، ٥% دهن، ٢٠% كربوهيدرات، ٧% معادن، ٥% رطوبة وقد وضح ان الكتلة الحيوية الغير مغسولة تحتوى على سمية للفئران ولكن الكتلة التى تم غسلها فلم تظهر هذه السمية بل على العكس عاشت الفئران بصحة.

والكتلة الجافة من سبيرولينا اذا أضيفت فى عليقة الخراف الرحمانى بمعدل ٥٠ جم/كجم أدت إلى زيادة تركيز الكالسيوم والفوسفور والمغنسيوم والنحاس فى سيرم الدم للحيوانات.وأدت إلى ضعفه فترة النضج الجنسى الى ٦٩% من حيوانات المقارنة وكذلك زيادة حجم السائل المنوي والكتلة المنوية والنشاط وعدد الحيوانات المنوية فى وحدة الحجم كما درس أيضا تأثير مستخلص الاسبيرولينا على ميكروبات الكرش.