## Chlorella vulgaris IFR 111 influence on structural features of rabbits eyes

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#### Abstract

Chlorella vulgaris (green algae) is used in the field of human and animal nutrition. The aim of the performed study was to investigate the effects of Chlorella vulgaris strain IFR 111 on eyes structural elements, to compare the amount of water soluble lens proteins in control and experimental groups and to evaluate distribution of these proteins between fractions of different molecular mass. A-mode ultrasonography was used to determine globe axial length, anterior chamber depth, axial lens thickness and axial vitreous length in control and experimental groups. Globe axial length, anterior chamber depth and axial vitreous length were not statistically different between the groups, apart from the lens thickness. The lens thickness of experimental group rabbits was reduced by 12.49 %.

Keywords: chlorella vulgaris IFR 111, eye, ultrasonic biometry.

### Introduction

Chlorella likely appeared on Earth 1.5 to 2 billion years ago. It is a fresh-water, one-celled green algae that is widely found in lakes and marshes all over the world. The name chlorella is a compound of Greek (chloros, meaning green) and Latin (ella, meaning small thing), and it was discovered and named by M.W.Beyerinck of Holland in 1890.

Chlorella is 2 to 10 microns in size (slightly smaller than a red blood cell) and with an optical microscope youcan see its green and almost spherical shape. Its classified botanically in the division of chlorophyta, the class chlorophyceae order chlorococcales, family of oocystaceae, and genus of chlorella. Its an ancestor of such vegetables as spinach and pumpkins.

Compared to other plants, chlorella has a high concentration of chlorophyll, so its capability for photosynthesis is many times higher than that of other plants. Photosynthesis is the process by which green plants synthesize nutrients such as starch out of water and carbon dioxide by absorbing energy from the sun. Chlorella also has a compound called chlorella extract that is capable of multiplying cells into four parts every 20 hours. This impressive cellmultiplication capability is unique to chlorella and is rarely seen in other living beings.

Chlorella helps maintain human health and prevent and treat discase because of its high-quality, plant-based protein, vitamins, minerals, dietary fiber, antioxidant compounds and chlorella extract.

*Chlorella vulgaris,* a unicellular green algae, has been studied in a variety of practical approaches. In the last years, several reports demonstrated the protective effects of this algae agains bacterial and virus infection, tumours and peptic ulcers (Morimoto et al., 1995). Recently, the use of *C.vulgaris* for the removal of toxic metals from natural waters or waste waters has been reported by several authors (Wilde et al., 1993). This microalgae has been used for analytical purpose to quantitatively recover ions, such as lead, cadmium, chromium and nickel, from natural water samples. These experiments demonstrated the presence of very high affinity metal ion binding sites in these organisms (Matsunaga et al., 1999). This metalbinding property seems to be related to the presence of chloroplast in the cellular wall, an organelle rich in sulphur, potassium, calcium and phosphorus. The ability of sulphydryl-containing compounds to chelate metals is well established in the literature and this could be the main factor involved in the in vitro removal by *C.vulgaris* of heavy metal contaminants.

Chelation therapy, first developed as a method for treating heavy metal poisoning, has recently been demonstrated to be effective against atherosclerosis, coronary heart disease and peripheral vascular disease. Its supposed benefits include increased collateral blood circulation, decreased blood viscosity, improved cell membrane function, improved intracellular organelle function, decreased arterial vasospasm, decreased free radical formation, inhibition of the aging process, reversal of atherosclerosis; decrease in angina, reversal of gangrene, improvement of skin color and healing of diabetic ulcers (Ernest, 2000).

Concerns about the safety of chelation have focused on experimental evidence in animals and on clinical experience. The adverse effects of chelators include hypersensitivity reactions, hyperpyrexia, tachycardia, hypertension, transient elevations of hepatic transaminases, conjunctivitis, lacrimation, salivation, renal failure, leucopenia, thrombocytopenia, hematuria, proteinuria, eosinophilia and anorexia (Quan et al., 2001).

Eye structural elements, especially lens, are very sensitive to various influence – dietary deficiency, accumulation of metabolites, radiation and others. These influences can cause abnormalities of eye parameters.

#### Materials and methods

The aim of the experiment was to study the effects of *Chlorella vulgaris* strain IFR 111 on the rabbits eyes parameters. Seventeen adult New Zealand white rabbits

were used in the experiment. The rabbits were divided into two groups – control (n=11) and experimental (n=6). Control group rabbits were fed a standart diet and got drinking water, rabbits of experimental group were fed a standart diet and got drinking water with *Chlorella vulgaris* IFR 111 extract 50 ml every 30 days.

Using A-mode ultrasonography, we investigated structural parameters of eyes after eyeball enucleation (Fig. 1, 2). The intraocular dimensions were measured as follows: the distance between the anterior cornea to the surface of the anterior lens, the thickness of the lens, the distance between the surface of the posterior lens to surface of the retina, the distance from the anterior cornea to the retina, which represents the total axial length.

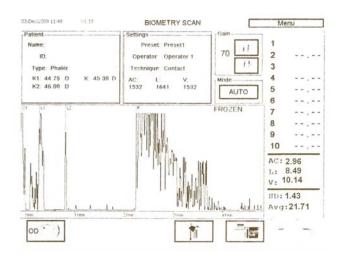
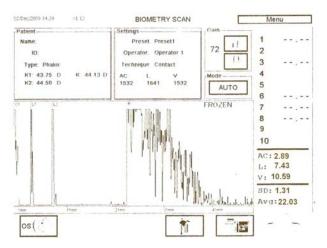


Fig.1. Ultrasonic measurements of control group rabbits eye parameters



# Fig.2. Ultrasonic measurements of experimental group rabbits eye parameters

We have realized this work in cooperation with the Laboratoty of ophtalmology of Kaunas Medical University.

### Results

Using the A-mode ultrassonography, we investigated structural parameters of rabbits eyes.

Data about measurements of the ocular dimensions are presented in Table 1. A comparison of the mean eyes

structural elements measurements of the control group rabbits and experimental group rabbits, which got drinking water with *chlorella vulgaris* IFR 111 extract was done. There were no significant differences of the eye axial length, the depth of the anterior chamber and the axial length of the vitreous between the control and the experimental rabbits groups (p>0,05). Only one significant difference of the lens thickness was found (p<0,05) – the lens of experimental group rabbits was thinner.

	0	Depoth of the anterior chamber	Thickness of the lens	Axial length of the vitreus
Control group	21,71±1,43	2,96±0,39	8,49±0,72	10,14±1,04
Experimental group	22,03±1,31	2,89±0,36	7,43±0,69	10,59±1,11
р	>0,05	>0,05	<0,05	>0,05

Table 1. Measurements of the rabbits eyes structural elements (mm)

Percentage relation between the total axial length and other ocular dimensions among rabbits groups is presented in Table 2. There were no significant differences of the percentage relation of the depth anterior chamber. The percentage relation between the total axial length and the thickness of the lens and the axial length of the vitreous show that lens occupy less and vitreous much more of the total axial length experimental group rabbits.

 $Table \ 2. \ {\bf Percentage \ relation \ between \ total \ axial \ length \ and \ other \ ocular \ dimensions$ 

	Depoth of the anterior chamber	Thickness of the lens	Axial length of the vitreus
Control group	13,08	37,38	44,65
Experimental group	13,12	33,73	48,07

#### Discussion

*Chlorella vulgaris*, a unicellular green algae, has been studied in a variety of practical approaches. In the last years several reports demonstrated the protective effects of this algae against bacterial and virus infections, tumours and peptic ulcers. The use of *Chlorella vulgaris* for the removal of toxic metals from natural waters or waste waters has been reported. *Chlorella vulgaris* IFR 111 is generally used in the field of human and animal nutrition.

The aim of the performed study was to investigate the effects of Clorella vulgaris strain IFR-111 on eyes structural elements, because there is no information about green algae influence to eye ball structural parameters. The cornea and the lens are most powerful optical portion and refracting surface and most sensitive tissues in the eye. Differences between the lens and cornea are their sources of nutrients, the solubility of structural protein, the amount of mucopolysaccharide and the physical characteristics responsible for transparency. Various nutrition supplements, dietary deficiency, accumulation of metabolites, and radiation can cause cataracts. A-mode ultrasonography was used to determine the globe axial length, the anterior chamber depth, the axial lens thickness

and the axial vitreous length in the control group and the experimental group rabbits, which got drinking water with *Chlorella vulgaris* IFR 111 extract. According to our investigations, there were no significant differences of the eye total axial length, the depth of the anterior chamber and the axial length of the vitreous between control and experimental rabbits groups (p>0,05). Only one significant difference of the lens thickness was found (p<0,05), the lens of experimental group rabbits, which got drinking water with *Chlorella vulgaris* IFR 111 extract was thinner.

Immature cataracts demonstrated a trend towards reduced thickness, while the previous studies on cataract pathobiology have suggested a reduction in lens thickness in immature cataract through lens protein loss and an increase in thickness in mature cataracts (Williams, 2004). Transparency of the lens depends on the physicochemical state of its proteins. Changes in inorganic ion concentration, osmotic pressure, pH or enzyme activity alter the lens proteins and a cataract results.

The data analysis revealed that *Chlorella vulgaris* IFR 111 influences morphological and biochemical features of the lens, the lens becomes cataractous. Further total investigations and the lenticular protein examination are being continued.

#### References

 Morimto T., Nagatsu A., Murakami N., Sakakibara J., Tokuda H., Nishino H. et al. Anti-tumor-promoting glyceroglycolipids from the green alga, *Chlorella vulgaris*. Phytochemistry. 1995. Vol.40. P.1433-1437.

- 2. Wilde E. W., Benemann J. R. Bioremoval of heavy metals by the use of microalgae. Biotechnol. Adv. 1993. No. 11. P.781-812.
- Matsunaga T., Takeyama H., Nakao T., Yamazawa A. Screening of marine microalgae for bioremediation of cadmium-polluted seawater. J. Biotechnol. 1999. No. 70. P.33-38.
- Ernest E. Chelation therapy for. Coronary heart disease: an overview of all clinical investigations. Am Heart J. 2000. No.140. P.139-141.
- Quan H., Ghali W. A., Verhoef M. J., Norris C. M., Galbraith P. D., Knudtson M. L. Use of chelation therapy after coronary angiography. Am. J. Med. 2001. No.111. P. 729-730.
- Williams D. L. Lens morphometry determined by B-mode ultrasonography of the normal and cataroctous canine lens. Veterinary Ophthalmology. 2004. Vol. 2. P.91-95.

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# Žaliojo dumblio *Chlorella vulgaris* IFR 111 poveikis struktūrinėms triušių akių savybėms

#### Reziumė

Žaliasis dumblis *Chlorella vulgaris* naudojamas žmonių ir gyvūnų mityboje. Darbo tikslas – nustatyti *Chlorella vulgaris* IFR 111 poveikį struktūriniams akies elementams, palyginti tirpiųjų baltymų kiekį kontrolinių ir maisto papildą gavusių triušių akies lęšyje, taip pat įvertinti šių baltymų pasiskirstymą skirtingos molekulinės masės frakcijose. Taikant kontaktinį A ultragarsinį akies struktūrinių elementų matavimo būdą išmatuota sagitalinė akies ašis, priekinės kameros gylis, lęšio ir stiklakūnio storiai. Palyginus kontrolinės ir eksperimentinės grupių triušių akių matmenų duomenis nustatyta, kad akies ašies ilgio, priekinės kameros gylio ir stiklakūnio storio skirtumai labai menki, išskyrus lęšio storį – eksperimentinės grupės triušių lęšis yra 12,49 proc. plonesnis.

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