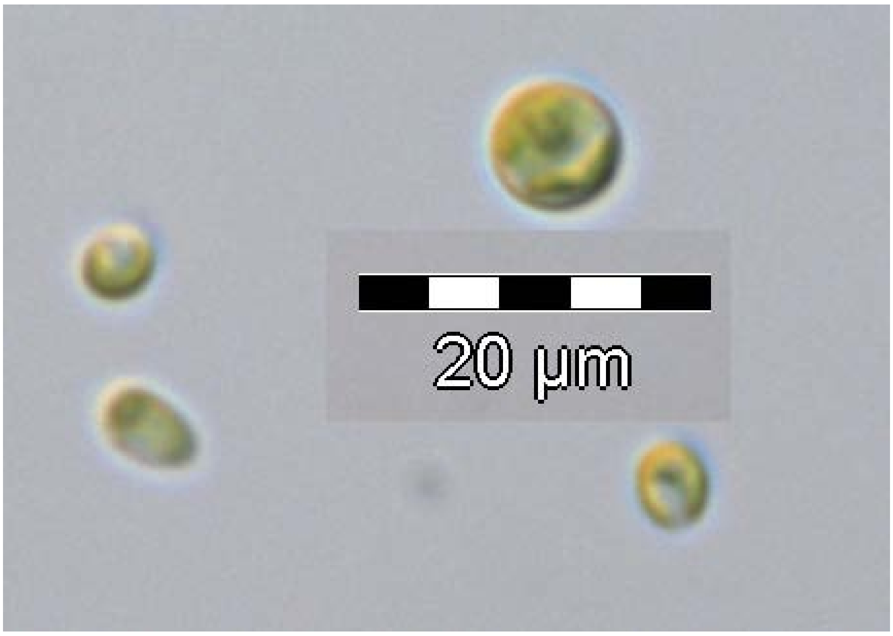
1. Introduction

The green micro-algae, or *Chlorophyceae*, of which *Chlorella vulgaris* Beijerinck is an example, have existed since the very beginning of the life on Earth. Fossil evidence shows that the algae have existed since the Precambrian époque, 2.5 Billion years ago. The micro-algae assimilated CO_2 from the atmosphere and produced oxygen, and so let other forms of life to develop. With time, the algae colonized the whole planet and today they can be found everywhere: primarily in marine environments such as lakes and water tanks but also in geysers, ice, snow, high mountains, soil and in waste water. The term “micro-algae” describes different unicellular eukaryotic organisms belonging to several classes like *Cyanophyceae* (cyanobacteria), *Chlorophyceae*, *Euglenophyceae*, *Xanthophyceae*, *Bacillariophyceae*, and *Rhodophyceae*, having different photosynthetic assimilatory pigments. There are more then 40,000 species known to date and, thanks to the modern molecular techniques, new species are still being discovered.

Figure 1. *Chlorella vulgaris* (microscope Olympus IX70, differential interference contrast, camera SIS Color View 12, Software AnalySIS Pro 1.0)



Since 1919, when Otto Warburg chose *Chlorella* (unicellular micro organism of 5 – 10 µm cell diameter – Fig.1) for his experiments on photosynthesis, the green micro-algae became more and more popular. At the beginning, researchers’ interest was focused on the micro-algae themselves – their nutritional and cultivation requirements were defined. After the protein, lipid and other content of the organisms were established, investigations developed towards the possibility of industrial production of micro-algae for aquaculture and agriculture. Historical documents show that several species of micro-algae were cultivated and used as human food, or food supplements since the 18th century in Japan. In other Asiatic countries, as well as in South America, the micro-algae have been used in human nutrition for centuries. All this made the micro-algae attractive for researchers from different scientific fields. In the 1940s researchers from western countries discovered the huge potential of the green micro-algae as a protein source for human and animal nutrition. In the face of the continuously increasing demand for meat in fast developing western countries, there is always a problem in getting enough protein sources for animal nutrition. Intensive animal production needs good protein sources: cheap and of good quality. Higher plants cannot meet all animal requirements; some of them contain anti-nutritive components, (for example soy) that make them unsuitable for the feed industry. The micro-algae could be therefore a solution. Even though the principle of cultivation of *Chlorella* is relative simple, high production costs did not allow for the industrial production for large scale and wide use in animal or human nutrition in northern countries, where closed cultivation system have to be built.Since photosynthesis, and thus plant growth, requires sunlight micro-algae can only be grown economically using natural sunshine. Only in countries like Japan or Taiwan do the climatic conditions allow for economically viable cultivation in open tanks,, this is one of the reasons that micro-algae have been used in these countries for centuries. Looking for more economic cultivation systems, efficient closed photo bioreactors have been developed – one such systems has been developed and patented in Germany, in Kloetze (Pulz, 2000) – and the production costs decreased, but they still remain at 20 – 25 per kilogram of dried algae biomass. But the simplicity of the cultivation systems has allowed the producers to commercialize the micro-algae, and today the worldwide yearly micro-algae production value reaches 500 Million US $. *Chlorella vulgaris* belongs to one of about 30 cultivated algae species and is being sold as a nutritional supplement in powder or tablets form, or as a component in cookies, noodles and other foodstuff, nowadays. Another commercial use of the green micro-algae is in the cosmetics industry (Ecke, 2004).

As already mentioned, one of the early ideas for the use of micro-algae was its introduction into human or animal food as a protein source. The protein content of micro-algae is very high – similar or even higher than in higher plants – so theoretically the algae could become a good protein source. But first feeding results showed the digestibility of the protein of raw algal biomass was quite low. The reason for that was that the very robust cellulose cell wall did not allow digestive enzymes to penetrate the algal cell and to digest the cell contents. In order to overcome this, biotechnologists tried out and developed different methods such as boiling, autoclaving, homogenizing, high pressure homogenizing, and several drying procedures. The protein digestibility was not always increased and production costs did not always decrease in parallel to the introduction of the novel processing techniques (Komaki et al., 1998; Bock & Wuensche, 1967; Cook et al., 1963; Cook, 1962). When applying different technological treatments to algal biomass, one has to also remember that such treatment could lead to destruction of algal cell constituents, thus reducing the nutritional value of the algae. The Institute for Cereal Processing in Bergholz – Rehbruecke, Germany, is one of the companies working to develop alternative, cost-effective ways of increasing the bioavailability of algal nutrient. Technologists from this institute have developed two new techniques: electroporation and ultrasonication prior to spray drying of the algal biomass, which could lead to improvement of cell wall destruction and to a decrease in the market prices of the green micro-algae. In order to prove the efficacy of these methods, nitrogen balance study was conducted on rats and the results of this experiment are a part of this dissertation.

One way of decreasing of production costs, and at the same time offering the possibility of decreasing greenhouse gas production, is locating the photo bioreactors in fossil fuel power stations. The CO_2 produced in power stations, which is one of the gases causing the greenhouse effect, can be successfully used for micro-algae cultivation, thus reducing the energy costs needed for the cultivation and the global CO_2 emission (Kremer et al., 2004). Also waste water treatment with micro-algae allows double use of this microorganism. The algae adsorb phosphates, nitrites and other substances from the sewage, cleaning the water. They can then be collected and used as a source of food for humans and animals (Oswald, 1993).

There are more and more “civilization diseases” like some kinds of cancer, hyperlipidosis, or systemic high pressure. There are more and more pharmaceuticals developed to overcome these diseases, but new chemical substances are not always without side effects. Public opinion and industry are interested in looking for novel applications of natural products. *Chlorella vulgaris* (as well as other micro-algae) is such a potential product. The knowledge about micro-algal activity in animals and humans is rising all the time. Today it is know that *Chlorella* possesses immunomodulating activity and can act as a preventive against several tumors, can decrease systemic high pressure or high serum cholesterol levels, and can also help to clear animal's organism from some harmful compounds (like dioxins). There are also suggestions that micro-algae can influence the reproductive performance of swine or birds, for example causing an increase in the number and quality of laid eggs. This effect is not fully proven; therefore one of aims in these investigations was to evaluate the influence of the *C. vulgaris* on reproductive parameters in mice.

The micro-algae can also act as a carrier for additional dietary components, such as unsaturated fatty acids, glycoproteins or other substances, which may have a positive influence on humans or animals. Selection and focused cultivation can lead to the production of new strains rich in active components, and with decreased levels of anti-nutritive substances (such as the reduction of glucosinolate and erucic acid levels in "Double Zero" rapeseed during the search for new protein sources from higher plants). Development of "healthy food" is of great public interest and this perception may represent the future of micro-algae in human and animal nutrition.

But before any new plant component can be introduced for use in animal nutrition, it must be checked for its safety. The high nucleic acid content of green micro-algae could, for instance, lead to the development or enhancement of diseases caused by elevated uric acid production, like gout, even though the percentage of humans suffering from this disease is low. In most animals uric acid is further metabolized to allantoin and the problem does practically not exist but in humans uric acid is the end-product of purine metabolism. Measurement of excretion of both metabolites in laboratory rodents fed with high level of micro-algae can give an indication of whether nucleic acids consumed in food rich in micro-algae could be harmful for humans. This was therefore also part of these studies.

Summarizing, there is global interest in production of micro-algae, on account of their potential pharmaceutical and nutritional activities and properties. Cultivation processes for growing micro-algae may lead to a reduction in global pollution – reducing CO_2 emission and waste water production. However, there is still a high production cost that remains a big problem and novel production techniques must be established to produce a cheap product of good nutritional quality. *Chlorella vulgaris* alone, or in combination with other micro-algae, can become a novel healthy food or feed component, but, before introduction into animal nutrition, general "safety" studies must be undertaken to ensure that there are no major negative consequences.

The investigations presented here focused on three main components: the influence of novel processing techniques for destroying algal cell walls on algal nutritional parameters when fed to rats; the general influence of micro-algae on animals' health in experiments on rats and mice; and influence of algae on reproduction in experiments on mice. The experiments were accomplished on laboratory animals, as model animals in nutritional, health and reproduction studies.

1.1. Investigation of effects of *Chlorella vulgaris* in animal nutrition

Several procedures have been developed for disrupting algal cell walls, such as boiling, autoclaving, high pressure homogenization, spray drying or drum drying. The effect of these procedures is questionable. There is still the need for development of a cheap and efficient method of algal cell opening. Potential procedures have been developed at The Institute for Cereal Proceeding in Bergholz – Rehbruecke, Germany, these are electroporation and ultrasonication prior to spray drying. In order to prove if these methods were more or less efficient than spray drying alone, a nitrogen balance study was conducted to determine the nitrogen digestibility and nutritional value of micro algal protein. Rats were chosen as model laboratory animals for economical reasons.

1.2. Investigation of effects of *Chlorella vulgaris* on animal health

One concern connected with feeding animals or humans with micro-algae is the high nucleic acids content in algal cells. As the nucleic acids are transformed after absorption to uric acid, and in this form eliminated with urine (in most mammals and humans), it cannot be excluded, that feeding with micro-algae would lead to high uric acid production and thus to development or enhancement of diseases like gout or kidney-stones formation. Introducing a novel feed component requires undertaking of general safety studies. Therefore it was investigated, whether feeding of micro-algae in large amounts could negatively influence metabolism of animals in studies on model laboratory animals - rats.

Because of limited technical possibilities, nutritional physiology studies comprising general health control, blood chemistry parameters and histological examination of tissue samples were performed.

1.3. Investigation of effects of *Chlorella vulgaris* on animal reproduction

There is little data about the influence of micro-algae on reproduction, some indications can be found in literature but there have only been a few studies conducted, and the existing data is inconclusive as to whether there is an effect. Enhancement of reproductive rates of farm animals can lead to a reduction of the production costs associated with meat production, and so is important for farmers. As this would be an interesting action of micro-algae, it was decided to conduct an experiment on mice fed a diet supplemented in 1.0% of *Chlorella vulgaris*. The mice model was chosen because of the financial aspects (cost of the micro-algae) and the possibility to accomplish an experiment on large number of animals in relatively short time, on several generations, to get statistically significant results.