6. Summary

Green micro-algae have been used as nutritional supplement or food source in Asiatic countries for hundreds of years. Use of the green micro-algae in human and animal nutrition has been studied intensively since the mid-50s of last century. Since then many groups have investigated different micro-algae cultivated under different conditions. Beneficial immunomodulatory properties of the green micro-algae (mainly *Chlorella vulgaris*) were discovered and its potential for use in human medicine was reported. In my dissertation five experiments are described, in which we investigated the nutritional value of micro-algal protein as well as assessing the safety of feeding the micro-algae on animal health. We also investigated if feeding micro-algae can influence the reproductive efficiency and growth parameters of laboratory animals.

In order to make the constituents of micro-algae accessible to mammals the cell wall must be destroyed. Several processes for making micro-algae more digestible have been developed but their efficacy and economic utility were poor. New efficient and cheap cell-opening methods are needed if the micro-algae are to fulfill their potential as novel, widely-available, natural, modern feed supplements. Treatment of algal cells with electrical field (electroporation) or ultrasound (ultrasonication) prior to spray-drying have shown promise as ways of releasing algal nutrients. We aimed to investigate if these new methods are indeed efficient at enhancing the bioavailability of algal protein, using rodents as models. This experiment was part of project "Entwicklung und Produktionseinführung von speziellem Kraftfutter mit Zusatz von aufgewerteten Mikroalgen" ("Development and introduction into production of special concentrate with supplement of valuated micro-algae") of the Frankerförder Forschungsgeselschaft Ltd. Three processed products derived from the green micro-algae *Chlorella vulgaris* (C1) were therefore investigated:spray dried only,; electroporated and spray dried and, finally, ultrasonicated and spray dried. A nitrogen-balance study was undertaken. Male Wistar rats with a mean weight of 135-145 grams were housed separately in metabolism cages and were fed the three algal products as the sole protein source at 150 mg N per 100 g of body weight. A control group of rats was fed with casein at the same amount as mentioned above. Apparent crude protein digestibility for spray dried *C. vulgaris* was 46.9 ± 12.7% (mean ± SD), for electroporated *Ch. vulgaris* 44.3 ± 7.5%, for ultrasonicated micro-algae 56.7 ± 13.7%. The protein efficiency ratio was 1.4 ± 0.3; 1.0 ± 0.5; 2.1 ± 0.3, respectively. N-balance was 41.86 ± 32.8 mg; 31.3 ± 17.3 mg; and 66.7 ± 30.1 mg,

respectively. The biological value was 93 ± 9.5 %; 93.6 ± 10 %; and 101 ± 5%, respectively. The differences between nutritional parameters (except for biological value) for ultrasonized and electroporated *C. vulgaris* were statistically significant ($p<0.05$ in HSD-Tukey test). The digestibility and biological value of *C. vulgaris* was enhanced by ultrasonic treatment and reduced by electroporation, thus ultrasonication may be a helpful technological process in processing of the green micro-algae in food industry.

Micro-algae contain high amounts of nucleic acids, which are source of purine bases that after absorption are transformed to uric acid (in humans) and allantoin (most animals). Thus feeding microalgae could lead to an increase of uric acid formation with subsequent development or enhancement of pathological conditions associated with this metabolite(i.e. gout, kideny stones). To investigate this and general algal influence on animal, we fed adult and growing rats with a diet containing 20% of micro-algae (supplemental or as sole protein source) for one month. Uric acid and allantoin were measured periodically in serum and urine. Urine production was also recorded. Multiple serum biochemistry and haematological parameters were measured. Internal organs were weighed and histological examinations were performed for intestines, liver and kidneys. No negative influence of micro-algal feeding could be observed. Uric acid level did not change after algal feeding. Allantoin excretion increased in the first two weeks of algal feeding and thereafter returned to the initial level. Serum biochemistry and haematology showed a decrease of full name (ALP) activity in algal group, no other changes were observed. Intestines were heavier and longer in rats fed micro-algae. No histological abnormalities were observed in organ slices. Feeding rats with micro-algae at 20% in feed led to no adverse effects and this amount can be considered as safe.

The effect of feed supplementation with 1.0 % spray-dried green alga *Chlorella vulgaris* powder on mouse reproduction and growth was investigated. The study was continued over three generations of mice, strain Fzt:DU. Females (F_0) were fed the control diet (commercial chow) or the algae-supplemented diet starting from 21st day of life (weaning). They were mated randomly on the 63rd day of life and all gave birth to pups. Litters were weighed, counted and then standardized (4 males and 5 females per litter). Pups were weighed and counted on day 10 and 21. After weaning 2 females and 2 males (F_1) from each litter were kept further. Females were mated on 63rd day of life. On the 18th day of pregnancy 57 and 59 (control and algae group) were sacrificed, 51 and 53 (respectively) gave birth to pups. Live and dead pups, absorbed fetuses and *corpora lutei* were counted, live fetuses were weighed. Live-born pups were counted and weighed and kept by dams without standardization, their number and weight were recorded on days 10 and 21. 2 females and 2

males (F_2) were weaned and kept further, the procedure as above was repeated. No difference in number of fetuses, *corpora lutei* or live-born pups was noted between groups or between generations. Litters from the algae group were slightly heavier at the weaning, the females and males from algae group also developed slightly better than the ones from control group, and this tendency was seen in all generations.

Technological processing of microalgae by ultrasonication can be efficiently used in practice, but still the industry must find a way to reduce the processing costs. There are still more experiments needed to establish the amount of micro-algae in feed that will be efficient and beneficial for farm animals without increasing animal production costs.