

## EFFECTS OF DIFFERENT ORGANIC MANURES ON BODY SIZE AND PRODUCTION OF *DAPHNIA MAGNA*

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### EFEKTI RAZLIČITIH VRSTA ORGANSKOG ĐUBRIVA NA VELIČINU TELA I PRODUKCIJU *DAPHNIA MAGNA*

#### Apstrakt

U akvakulturi se već decenijama masovno proizvodi zooplankton kao živa hrana za gajenje mlađi riba, rakova i mekušaca. Za slatkovodne ribe se osim naupliusa artemije i rotatorija može koristiti i veliki broj drugih organizama, naročito iz grupe Cladocera (*Daphnia magna*, *D. carinata* i dr.). Korišćenje organskog đubriva (stajnjaka) za proizvodnju žive hrane, može biti ekološki (uklanjanje nutrijenata) i ekonomski isplativo. Stajnjak je bogat izvor azota, fosfora i ugljenika kao i organskih materija i kao takav se može koristiti kao alternativni izvor nutrijenata za gajenje žive hrane za potrebe akvakulture.

Cilj ovog istraživanja je bio ispitivanje efekata 4 različite vrste stajnjaka (goveđi, ovčiji, koziji i riblji) na produkciju i veličinu tela *D. magna*. Eksperiment je realizovan u laboratorijskim uslovima, u 1L čašama, u dozi od 1g/L stajnjaka. Pored praćenja hemijskih parametara (kiseonik, elektroprovodljivost, pH, ukupan fosfor, nejonizovani amonijak), određivana je i ukupna brojnost dafnija kao i njihova veličina iz celih uzoraka. Tri veličinske klase dafnija (mala, srednja i velika) su ispitivane da bi se dobio uvid u populacionu strukturu i reproduktivni potencijal dafnija za čije su gajenje korišćeni različiti stajnjaci. Značajno veća brojnost dafnija ( $P < 0.05$ ) dobijena je u tretmanu sa goveđim stajnjakom, u odnosu na tretmane sa ovčijim i kozijim stajnjakom. Između ostalih nije bilo značajnih razlika. Kada se razmatraju veličinske klase, statistički značajno najveću brojnost su imale male *D. magna* u svim tretmanima u odnosu na velike. Na osnovu dobijenih rezultata, može se reći da je tretman sa goveđim stajnjakom pokazao najbolje rezultate i kada je u pitanju ukupna brojnost, ali i brojnost po veličinskim klasama (prosečna brojnost malih, srednjih i velikih dafnija je bila najveća). Osim toga ovaj tretman se izdvajao od ostalih i po svim hemijskim parametrima. U njemu je izmerna najveća prosečna koncentracija kiseonika, elektroprovodljivosti i pH, a najmanja

koncentracija ukupnog fosfora i nejonizovanog amonijaka. U zaključku se može reći da je goveđi stajnjak obezbedio najbolje uslove za rast i reprodukciju *D. magna*.

*Ključne reči: Daphnia magna, stajnjak, živa hrana, slatkovodne ribe*  
*Key words: Daphnia magna, manure, live food, freshwater fish*

## INTRODUCTION

Mass production of zooplankton as live food feeding of fish larvae, fry and adults, crustaceans and mollusks has been used in the aquaculture industry for decades (Hertrampf and Piedad-Pascual, 2000; Southgate, 2003).

For freshwater fish larvae except *Artemia salina* nauplii and rotifers a wider range of zooplankton can be used as live food especially cladocerans. *Daphnia magna* has been extensively used as live food for a range freshwater fish species due to their easy maintenance and adaptability to survive in low water quality conditions (Haisy and Porter, 1977; Dinges, 1982). Using animal manures for live food production can be ecologically (removing nutrients) and economically feasible. Animal manures are a rich source of nitrogen, phosphorus and carbon as well as organic mater and have been used as an alternative to other sources of nutrients for live food production (Wohlfarth and Schroeder, 1979).

The aim of the present investigation was to study the effect of four different organic manures (cow, sheep, goat and fish) on the production and body size of *Daphnia magna*. The three size classes of investigated organisms will give insight into the population structure and reproduction capability of daphnia fed different diets. The organic manure that provides the best conditions for growth of *D. magna* in terms of abundance and body size can be used either as live food for fish larvae and fry in hatcheries or as exogenously added zooplankton for juveniles or adult freshwater fish especially in ornamental fish production.

## MATERIAL AND METHODS

The experiment was conducted by using a laboratory clone of *Daphnia magna* obtained from RECETOX (Masaryk University) initially grown on a combination of *Scenedesmus quadricauda* and bakers yeast. *D. magna* was reared in 1L beakers with 4 different organic manures (cow, sheep, goat and fish) during 30 days. Before the beginning of the experiment manures were left to dissolve in dechlorinated water for 12 days at a dosage of 1g/L, in glass beakers. The beakers were kept in a water bath at 24 °C and were thoroughly mixed every day. Every type of manure was applied in triplicates. On the 13th day each beaker was inoculated with 5 medium size adult *D. magna* individuals. The light regime was 12 hr day and 12 hr night.

Water quality parameters such as temperature, dissolved oxygen (DO), pH and conductivity (COND) were measured every other day using WTW MULTI 340i/SET (WTW, Germany). Water samples for laboratory analysis of total ammonia nitrogen and total phosphorus (TP) were taken once a week from all three replicates per treatment in equal amounts (50 ml) as an integral sample (150 ml). Total ammonia nitrogen and total phosphorus were measured following APHA (1998). Un-ionized ammonia (UIA) was calculated from total ammonia nitrogen taking into account pH and temperature (Alabaster and Lloyd 1980).

At the end of the experiment, water from every beaker was filtered through a 25  $\mu\text{m}$  sieve and fixed with 4% formalin. All individuals of *Daphnia magna* from samples were counted and body length measured from the tip of the head to the base of the tail spine in Sedgwick Rafter chamber using a microscope ocular micrometer. According to the measurements animals were grouped into three size classes: small (700 - 1400  $\mu\text{m}$ ), medium (1400 - 2000  $\mu\text{m}$ ) and large (2000 - 3200  $\mu\text{m}$ ).

#### Statistical analysis

All data are presented as mean  $\pm$  SE. Two-way ANOVA was used to test differences ( $P < 0.05$ ) in *Daphnia magna* total densities and densities between size classes and type of manure. One-way ANOVA was used to test differences ( $P < 0.05$ ) in water chemistry parameters depending on type of manure used for rearing. In the cases where water chemistry parameters showed linear change over time paired t-test was used to test for statistical differences ( $P < 0.05$ ). All statistical tests were performed with Sigma Plot version 9 software (Systat Software, Inc.).

## RESULTS

Water temperature was constant during the experiment in all treatments, 22°C. One way ANOVA showed significant differences between all measured chemical parameters except for un-ionized ammonia. Mean values of all parameters are show in Table 1. In all treatments, excluding beakers with goat manure, dissolved oxygen had an increasing trend from the beginning to the end of the experiment (Figure 1.a). A striking increase of this parameter was evident after day 18 in fish and goat manure and ranged from 3.54 and 4.24 mg/L up to 9.12 and 9.35 mg/L, respectively (Table 1.). On average, the highest level of dissolved oxygen was obtained in the treatment with cow manure (Table 1.).

**Table 1.** Average values (mean  $\pm$  standard error) of chemical parameters, number of *Daphnia magna* individuals in three size classes and their total number in all treatments

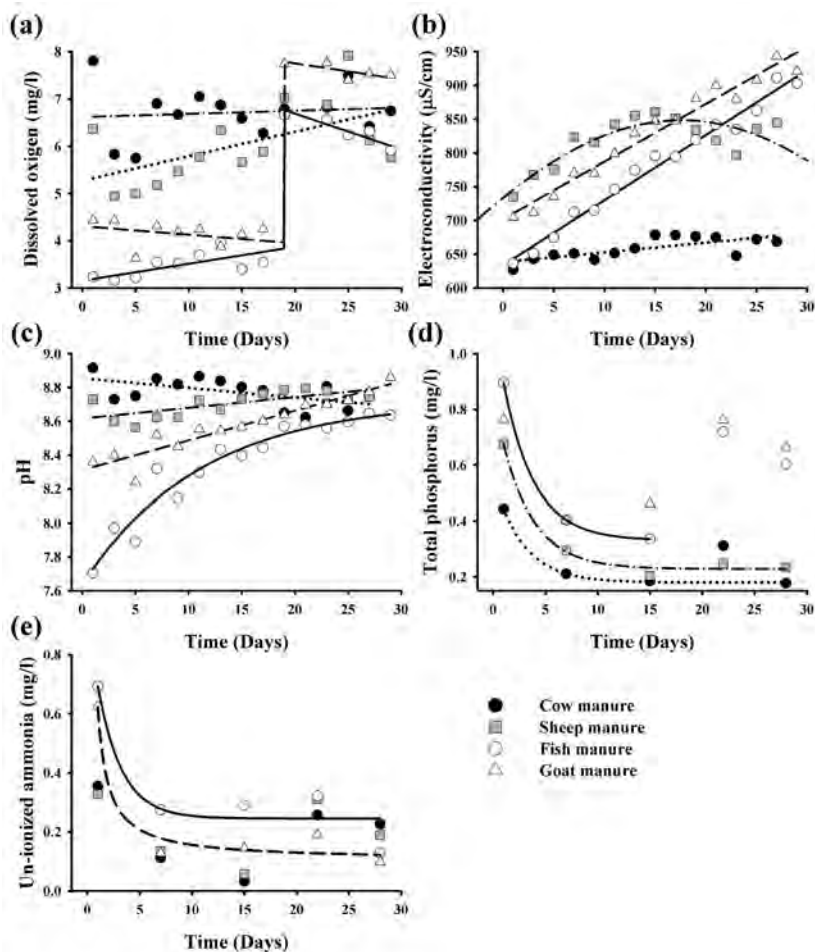
Types of manure	DO	COND	pH	TP	UIA	Small size	Medium size	Large size	Total number
Cow	6.7 $\pm$ 0.2 <sup>a</sup>	659 $\pm$ 4 <sup>a</sup>	8.78 $\pm$ 0.02 <sup>a</sup>	0.27 $\pm$ 0.05 <sup>a</sup>	0.20 $\pm$ 0.06	155 $\pm$ 71	88 $\pm$ 26	41 $\pm$ 19	95 $\pm$ 28 <sup>a</sup>
Sheep	6.0 $\pm$ 0.2 <sup>a</sup>	818 $\pm$ 10 <sup>a</sup>	8.70 $\pm$ 0.02 <sup>b</sup>	0.33 $\pm$ 0.09	0.20 $\pm$ 0.05	14 $\pm$ 5	62 $\pm$ 8	7 $\pm$ 3	28 $\pm$ 9 <sup>a</sup>
Fish	4.5 $\pm$ 0.4 <sup>a</sup>	778 $\pm$ 22 <sup>a</sup>	8.35 $\pm$ 0.08 <sup>a,b</sup>	0.59 $\pm$ 0.10 <sup>a</sup>	0.34 $\pm$ 0.09	93 $\pm$ 34	42 $\pm$ 17	10 $\pm$ 2	48 $\pm$ 16
Goat	5.4 $\pm$ 0.5 <sup>a</sup>	830 $\pm$ 20 <sup>a</sup>	8.57 $\pm$ 0.04 <sup>a</sup>	0.61 $\pm$ 0.08 <sup>a</sup>	0.24 $\pm$ 0.10	22 $\pm$ 15	14 $\pm$ 10	11 $\pm$ 4	16 $\pm$ 6 <sup>a</sup>

\* data in the same column sharing the letter (<sup>a, b, c</sup>) are statistically different ( $p < 0.05$ ).

Conductivity showed an increasing trend during the investigation period in all four treatments (Figure 1b). Overall, significantly lower ( $P < 0.05$ ) average values of COND were noted in cow manure. When paired t-test was applied conductivity of fish manure was significantly lower ( $P < 0.05$ ) than goat manure. pH values increased during the experiment in all beakers apart from those with cow manure where a slight decrease of this parameter was noted (Figure 1c). On average, treatment with cow manure had significantly higher ( $P < 0.05$ ) values of pH than treatments with fish and goat manures. In paired t-test pH of fish manure was significantly higher than goat manure. A notable decrease of total phosphorus was evident in all treatments until day 15, while thereafter in-

crease in fish and goat treatments was observed (Figure 1d). Significantly higher average values ( $P < 0.05$ ) of TP were evidenced in fish and goat treatments compared to the cow manure treatment. Un-ionized ammonia was the only parameter that showed no significant differences between treatments. A decreasing trend of this parameter was observed in all treatments with a slight increase in the second half of the study (Figure 1e).

Two-way ANOVA showed that there was significant difference in total number of *D. magna* between treatments ( $P < 0.05$ ). However, treatments and size classes of daphnia were statistically independent. The total number of daphnia in treatment with cow manure was significantly higher ( $P < 0.05$ ) than in sheep and goat manure. However, no significant difference was found between other treatments. Among the three size classes of daphnia, significantly higher numbers were obtained in the small class compared to the large size class in all treatments.



**Figure 1 a–e.** Measured chemical parameters (dissolved oxygen, electroconductivity, pH, total phosphorus and un-ionized ammonia) in four treatments during the 30 day experiment

Between other size classes no significant differences was observed. Overall, total number of *D. magna* in all size classes were on average higher in treatment with cow manure than in other three treatments.

## DISCUSSION

Dissolved oxygen in all four treatments had a general decreasing trend from the beginning towards the end of the experiment. This was expected due to higher consumption rates on bacteria and ciliates present in manures by increased number of daphnias over time. Similar results were obtained by other authors (Jana and Chakrabarti, 1993, Srivastava et al. 2006). Srivastava et al. (2006) investigated effect of 4 different dosages of a mixture of cattle manure and poultry droppings on the production of *Ceriodaphnia carinata*, where irrespective of manure dosage a subsequent increase of DO towards the end of the experiment was observed.

*Daphnia magna* is often found in wastewater treatment ponds loaded with organic matter (Kibria et. al. 1999, Cripp and Kumar, 2003). This species is known for its high utilization of nitrogen ammonia and total phosphorus. Therefore *D. magna* can significantly improve the water quality due to their high filtering capabilities while grazing on pathogenic and fecal bacteria as *E. coli* (Kima et al., 2003, Shiny et al., 2005, Jung et al., 2009.). The results of our study are in line with above mentioned investigations. *D. magna* reduced the amount of total phosphorus in all types of manures regardless of their initial levels. This holds for un-ionized ammonia as well, a clear decrease in all treatments was evident throughout the present study. While being a more toxic form of ammonia for most aquatic animals, zooplankton, especially cladoceran can sustain high concentrations of UIA.

Considering the effect of treatments on the production and body size of daphnia, it seems that cow manure treatment provided the ideal conditions for growth and reproduction of *D. magna*. The overall highest number of individuals was obtained in cow manure. Additionally, on average, all size classes of daphnia were more abundant in this treatment than in the remaining ones.

In conclusion, cow manure was distinctively different from the rest of the manure treatments in all chemical parameters showing highest dissolved oxygen, conductivity and pH and the lowest total phosphorus and un-ionized ammonia. These findings select cow manure as the best growth media for culturing *Daphnia magna*.

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