MORPHOMETRICAL STUDY OF INTESTINAL FOLDS OF CARP FED DIFFERENT ADDED FEED IN SEMIINTENSIVE SYSTEM

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MORFOMETRIJSKO ISPITIVANJE NABORA CREVNE SLUZOKOŽE ŠARANA HRANJENOG RAZLIČITOM DODATNOM HRANOM U POLUINTENZIVNOM SISTEMU

Apstrakt

U poluintenzivnom sistemu gajenja šarana u poslednjoj deceniji dolazi do promena u vrsti dodatne hrane koje povećavaju održivost proizvodnje. Sa žitarica koje su se godinama koristile kao dodata hrana, sada se prelazi na peletiranu i ekstrudiranu hranu. Ova zamena uzrokuje i promene u fiziologiji varenja riba, a kao posledica se dešavaju morfološke promene na crevima. Najčešće se na organima za varenje riba gajenih u akvakulturi ne nalaze teže histopatološke promene, pa se pribegava kvantifikaciji i morfometriji histoloških parametara koje mogu da ukažu na odstupanja od normalne građe/funkcije. Metod evaluacije dužine crevnih nabora je korišćen u velikom broju studija koje su se bavile efektima različitih tipova hrane i/ili aditiva na crevo riba. Cilj rada je da se utvrdi efekat različitih tipova hrane (žitarice, peletirana, ekstrudirana hrana) na crevo šarana merenjem dužine crevnih nabora kod riba koje su gajene poluintenzivno. Dužina crevnih nabora je korelisana sa prirastom ribe.

Kretanje dužine crevnih nabora po jezerima/vrsti dodate hrane je imalo pravilan tok (Fig. 1). Najkraći crevni nabori su izmereni kod riba hranjenih žitaricama. U sva tri jezera primetan je pad dužine nabora u junu. U ovom mesecu su vrednosti najmanje, manje čak i od inicijalnih na početku eksperimenta (u aprilu), kao i vrednosti dobijenih u maju. Od juna dužina nabora creva konstantno raste kod riba u svim jezerima, bez obzira na tip dodate hrane.

Rezultati su pokazali visok stepen statistički značajne korelacije između mase tela ribe i dužine crevnih nabora u svim jezerima (Tab. 2). Ukoliko se posmatraju pojedinačna jezera, vrednosti se kreću između 0.82 kod riba hranjenih peletiranom hranom, preko

0.83 kod riba hranjenih žitaricama do 0.86 kod riba hranjenih ekstrudiranom hranom. Dužina crevnih nabora je koristan parametar u eksperimentima ishrane riba i često se primenjuje u proceni efekta određene hrane na crevo riba. Smanjenje dužine nabora može da ukaže na pojavu enteritisa, na nedovoljnu ishranu ili gladovanje. Naši rezultati pokazuju pozitivnu korelaciju između mase tela ribe i dužine crevnih nabora. Oni su u skladu i sa sličnim istraživanjima koja su sprovedena na drugim životinjskim vrstama, mahom na domaćim životinjama. Crevni nabori na početku eksperimenta ne rastu, čak pokazuju trend pada sve do juna meseca i to iako ribe pokazuju konstantan prirast u svim jezerima, što se objašnjava količinom prirodne hrane koja je u to doba najveća u jezerima. Naime, na osnovu analize prirodne hrane tokom ovog perioda možemo videti da postoji velika biomasa zooplanktona u toku aprila, maja i juna dok je zoobentos na niskom nivou u svim jezerima u toku celog eksperimenta i ne prelazi 10 g/m^2. Ovo uzrokuje promene u načinu ishrane ribe od juna meseca. Do tada se šaran hranio uglavnom zooplanktonom, koga je bilo u izobilju, a nakon toga, početkom juna, zbog nedostatka prirodne hrane,riba počinje da sve više konzumira dodatu, što uzrokuje i povećanje dužine crevnih nabora, s obzirom da su pelete, kojom se ribe hrane voluminoznije i većih dimenzija od zooplanktona. Hranjenjem, ribe pune creva i samim tim ih i šire, što omogućava povećanje apsorpcione površine povećanjem dužine crevnih nabora. Ovaj fenomen u stvari predstavlja adaptaciju creva na različite tipove hrane kod riba.

Naše istraživanje je pokazalo da praćenje histologije šarana može da ukaže kako se odvija proces varenja zavisno od dela sezone/razvoja zajednice zooplanktona, ali i vrste dodate hrane u poluintenzivnom sistemu.

Ključne reči: *morfometrija, histologija, crevni nabori, šaran, poluintenzivno gajenje*

INTRODUCTION

In order to enhance sustainability of the semiintensive carp production system in the first decade of the 21st century, replacement of traditionally used cereals occurs in Serbia and the region (Marković et al., 2010). The shift from cereals to pelleted and extruded supplemental feed affect fish nutritional physiology and therefore results in morphological changes in the intestine (Segner et al., 1987, Epler et al., 2009). Our research of intestinal histology has demonstrated that no major pathological changes occur on carp intestine in feeding experiments. The alteration observed couldn't seriously impair the function of digestion. A number of studies use morphometry for evaluation of effects of different feeds on fish intestinal histology (Sanden et al., 2005; Zhou et al., 2007; Liu et al., 2009; Qiyou et al., 2011, Rašković et al., 2011).

The aim of the present study was to evaluate effects of different added feed on intestinal folds (mixture of cereals, pelleted, and extruded feed) using morphometry. Intestinal fold length of carp grown in a semiintensive production system was correlated with carp growth rate.

MATERIAL AND METHODS

The experiment was performed in three earthen ponds, surface of 650 m^2 each, placed side by side at the experimental fish farm of the Center for Fishery and Applied Hyd-

robiology, Faculty of Agriculture, University of Belgrade, Serbia. 400 common carps (*Cyprinus carpio* L.) were placed in each pond. They were 11 months old with average weight of 150 ± 18 g. The diseases free fish was provided from the commercial fish farm "Neuzina", Serbia. After a period of adaptation of three weeks, fish were grown for one production season (29th April - 26th October 2008) using feed of 3 different treatment level: mixture of grains (wheat, corn and barley mixture in 1:1:1 relation; GF), pelleted feed (PF) and extruded feed (EF). PF and EF were made of the same components (Table 1), but were treated differently – by pelletting and extruding process, respectively. Fish were fed on daily basis, with 3% of their body mass.

Table 1. Chemical composition of the feed.

Feed	GF	EF	PF
Proteins	11.3±0.8	28.5±0.9	26.5±0.8
Lipids	3.3±0.2	7.8±0.1	8.0±0.1
Ash	1.9±0.3	4.7±0.2	4.7±0.3
Fibers	7.4±0.6	3.5±0.1	3.3±0.4
Moisture	9.8±0.7	9.4±0.3	12.0±0.5
Carbohydrates	66.2±2.0	46.1±1.3	45.5±1.7

At the beginning of experiment (29th April 2008) 6 fish were sacrificed and formed the initial group for histology examination. Subsequently, two fishes from each pond were taken every 30 days until 26th October 2008. Specimens were sacrificed with a quick blow to the head, and distal intestine was quickly removed, fixed in 4% formaldehyde, and processed using a standard histological technique: dehydration in an ethanol series, embedding in paraffin, and serially sectioning at 5 μm. Sections were stained with hematoxylin and eosin (H/E) (Humason, 1979). Microphotographs were taken with a Leica DML microscope with the Leica DC 300 camera. Lengths of intestinal folds were measured from the base to the tip of the fold at average of 60 folds per fish. STATISTICA 7.0 (StatSoft, USA) was used in statistical analysis of values. LSD test was used for determination of significant differences between samples. For correlation a Pearson r correlation was used.

RESULTS

Intestinal fold length had a rather regular growth in the three ponds studied (Fig. 1). Fish fed cereals as added feed had the shortest folds. In all three ponds a fall in intestinal fold length occurred in June. In June fold length was even shorter than at experiment start in April and in May. Intestinal folds length constantly increased from June onward in all ponds, regardless of feed used.

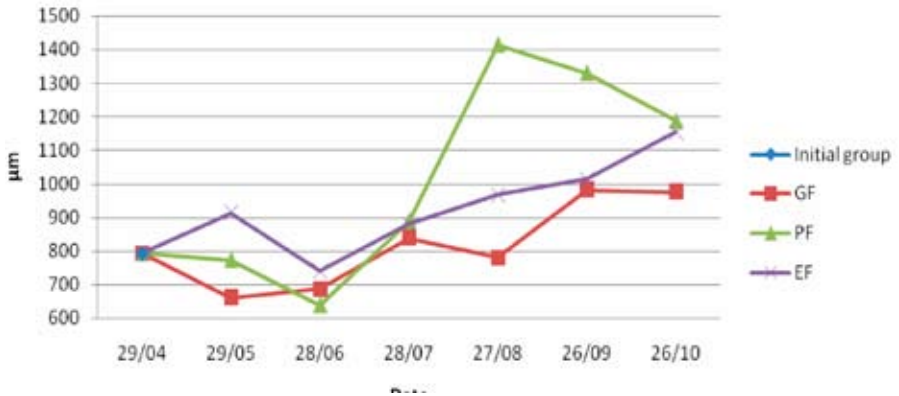

Figure 1. Intestinal fold length (in µm) over a period of experiment duration (29th April-26th October).

The results have shown high correlation level between carp body mass and intestinal fold length in all ponds (Tab. 2). Correlation between fold length and body mass in each pond is given in Tab.2: it was 0.82 in the pond PF, 0.83 in GF, and 0.86 in EF. As it can be seen from the table all correlations obtained were statistically significant.

Table 2. Relationship between length of intestinal folds and body mass of fish in each pond.

Feed	GF	PF	EF
Correlation coefficient	0.83	0.82	0.86
Statistical significance	p=0.02	p=0.02	p=0.01

DISCUSSION

Measurement of the length of intestinal folds is a useful parameter in feeding experiments. It is used in evaluating effects of feed on fish intestine (Ostazevska et al., 2005; Zakes et al., 2008). When intestinal fold length decreases, it can indicate enteritis (Baeverfjord and Krogdahl, 1996) or suboptimal feeding or starvation (Mc Leese and Moon, 1989). Fish were fed 3% of their body mass in our experiment, thus the added feed quantity increased with body mass, and therefore suboptimal feeding couldn't be a reason for fold length decrease.

Our results have shown a positive correlation between body mass and intestinal fold length. They are in accordance with similar findings on other domestic animals such as: pigs (Li et al. 1991), calves (Brooks et al. 1998) broilers (Loh et al., 2010) and turkeys (Ritz et al., 1995).

In our study intestinal folds length has a decreasing trend in carp from all studied ponds until the month of June, although the growth rate increased (Table 3; Dulić et al., 2009). This can be explained by the quantity of natural food gradually reaching its maximum in the pond in this period of the season (Paterson, 1993). According to the analysis of natural food the highest zooplankton biomass is found in months of April, May and June, especially in ponds with PF and EF. Additionally, in this part of the season, the

main Cladoceran species was *Bosmina logirostris*, and Copepod Cyclops. sp. However zoobenthos in all ponds investigated during the whole experiment never exceeded 10 g/m^2 (Dulić et al., 2009). Obviously carp consumed natural food during April and even in May, while in June, most probably was a period of adaptation to added feed. After this period of maximum development of natural food, added feed started to be increasingly used. This affected intestinal folds length which increased in size. The reason for this could be explained by a larger volume pellets occupy compared to zooplankton dimensions (for instance, size of *Bosmina longirostris* is 200-400 μm, pellet size is ø3 x 10 mm) and possibly by the high digestibility of natural food, providing additional enzymes (Kibria et al., 1997). The increased absorption area by increased fold length could represent an adaptation to different food type in fish as stressed by Refstie et al. (1997).

Our study has shown that monitoring carp histology during the production season can give insight into the digestion process, its efficiency in the semiintensive system depending on the part of the season /development of natural food, and of the type of added feed.

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