RESEARCH ARTICLE

Effect of different diets on body mineral content, growth, and survival of barbel, *Barbus barbus* (L.), larvae under controlled conditions

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Abstract. The experiment tested three formulated dry diets at 25°C to determine their effects on larval *Barbus barbus* (L.) body mineral composition, growth, and survival. Live *Artemia* nauplii were fed to all the larvae for the first 5 days of the experiment. From day 6 to day 25 inclusive, nauplii were the reference diet in one group, whereas the three other groups were fed dry diets exclusively. On D1 the fish body comprised 6.71% ash, 1.20% P, 0.35% Ca, and 0.09% Mg (dry matter), but initial feeding with nauplii resulted in increased values of all these components. The highest statistically significant (P ≤ 0.05) final body ash and mineral content was recorded for larvae fed nauplii (ash 13.22%; P 2.04%; Ca 2.95% and Mg 0.15%; d.m.). Fish fed nauplii grew faster than those fed dry diets (final mean BW 214.5 mg and 84.3-118.9 mg, respectively; all differences significant), and their final survival rate was also significantly the highest (99.9% and 96.5-99.4%, respectively). As evidenced by the current results, even short-term (20 days) feeding exclusively dry formulated diets can lead to considerable deficiencies in essential minerals in the larval body.

Keywords: *Barbus barbus*, body ash, diets, growth, minerals, survival

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Introduction

Widely distributed in Europe, the barbel, *Barbus barbus* (L.), is a large cyprinid fish species inhabiting submontane and lowland reaches of clean, fast running rivers with gravel bottoms. During recent decades, its numbers have declined considerably locally as a result of river regulation, dam construction, spawning ground destruction, water pollution, and sometimes overfishing (Penczak and Kruk 2000, Poncin and Philippart 2002, Kottelat and Freyhof 2007, Britton and Pegg 2011). In some Central European countries, the ecological status of this species is relatively high and classified as at least near threatened (Witkowski et al. 1999, Lusk et al. 2004, Kottelat and Freyhof 2007, Britton and Pegg 2011). This has made *B. barbus* one of the most desirable cyprinid species for stocking programs in running waters, and, thus, it is playing an increasingly important role in European aquaculture.

Knowledge regarding *B. barbus* reproduction and rearing methods under controlled conditions for the production of stocking material has been increasing since the 1990s thanks to many experimental studies (e.g., Poncin 1992, Wolnicki and Górny 1995, Wolnicki 1997, Fiala and Spurný 2001, Poncin and Philippart 2002, Policar et al. 2007,

Kamiński et al. 2010). At present, it is known that the larvae of this species are better able, in comparison to most cyprinids, to attain satisfactory growth and survival when fed solely dry formulated diets from the first feeding, although results are much better with natural diets (Wolnicki 2005, Kamler and Wolnicki 2006).

Most young cyprinids fed a dry formulated diet characteristically exhibit a gradual increase in total body lipids and a decrease in body ash, thus mineral content (Chavez-Sanchez et al. 2000, Wolnicki et al. 2006). This can result in external body deformities, which become apparent after merely a few weeks of intensive feeding in most reared individuals (e.g. Wolnicki et al. 2000, 2009, Myszkowski et al. 2002, Kamler et al. 2006, 2008).

Phosphorus (P) and calcium (Ca) play important roles in the fish organism. The former is necessary for growth, bone mineralization, and lipid and carbohydrate metabolism, whereas the latter is important for bone formation, muscle contraction, nerve impulse transmission, and enzyme activation (Lovell 1993, Lall 2002). Due to the low P content in water, its primary source for fish is the diet (Lall 2002, Sales and Janssens 2003). P deficit in fish diets and low efficiency of its absorption from the diet can decrease P mineral content in bones and delay bone mineralization (Roy et al. 2002), which can lead to skeletal malformations (Sugiura et al. 2004). Fish are able to uptake Ca from both the aquatic environment and the diet, and Ca content in the latter affects the available dietary P (Lall 2002). Another essential mineral cyprinid fish get from dietary sources is magnesium (Mg), which is involved not only in skeletal tissue metabolism but also in osmoregulation and neuromuscular transmission (Lall 2002).

The problem of body deformities occurring in young fish reared on dry formulated diets under controlled conditions is important from biological and economic points of view. This explains why so many attempts are being made to identify the principle causes of fish body malformations and also to identify precautionary measures that can be taken to prevent this. The present study was designed to quantify the effects of three dry formulated diets (and live food as the reference diet), on larval *B. barbus* body mineral content, growth, and survival.

Material and methods

Fish and experimental conditions

The fish used in the 25-day experiment were 8-day-old first-feeding *B. barbus* larvae of an initial size of 12.5 ± 0.4 mm TL and 10.9 ± 1.0 mg BW (mean ± SD). They were pooled progeny of three female and five male spawners, cultured in tanks under indoor conditions. Ovulation was stimulated with Ovopel, a GnRH analogue (Horváth et al. 1997).

Actively swimming larvae were stocked at a density of 800 individuals per aquarium in eight 20-l glass flow-through aquaria. The aquaria, which were part of a recirculation system, were supplied continuously with filtered, aerated water heated to 25.0°C (range ± 0.5°C). For the first 5 days of the experiment (D1-D5), the larvae were fed exclusively live freshly-hatched *Artemia* nauplii ad libitum (commercial strain of 9.3% protein and 1.5% lipid content in wet matter; EG grade, INVE Aquaculture B.V., Belgium). From D6, in the control group ART, the fish were continuously fed only *Artemia* nauplii, which was the reference diet. The three remaining groups of larvae received, at an approximate level of satiation, the following dry formulated diets exclusively: Aller Futura Larvae, Denmark – commercial starter for fish larvae (64% protein, 12% lipid in wet matter – group AFL); Ewos AgloNorse, Norway – commercial starter for marine fish larvae (59% protein, 21% lipid – group EAN); Start, Belorussian Scientific-Research Institute of Fish Industry, National Academy of Sciences of Belarus – non-commercial starter for carp larvae (45% protein, 10% lipid – group STA). All data regarding feed composition are from the manufacturers. All treatment groups were in duplicate. Feeding was performed manually at 08:00, 11:00, 14:00, 17:00 and 20:00 h. The aquaria were lit with artificial fluorescent

illumination from 07:45 to 21:00 h at intensity of about 700 lx at the water surface.

Larval feces and leftovers were cleaned from the aquaria bottom every day in the morning and in the evening. Dead fish were removed from the aquaria and counted. During the experiment, dissolved oxygen saturation in the aquaria was maintained at 60-90%. Total ammonia and nitrites were kept below 0.1 and 0.06 mg dm^{-3}, respectively, whereas pH was close to 7.7.

Sampling and measurements

The initial sample of 500 freely swimming *B. barbus* larvae with remnants of yolk sac was taken just before the first feeding (at the beginning of D1) to determine the whole body ash and mineral (P, Ca and Mg) content. An intermediate sample of 320 (8 x 40 per aquarium) individuals with empty alimentary tracts was taken at the end of D5, immediately before weaning to dry diets. In each treatment group, the final samples for ash and body minerals consisted of 100 (2 x 50 per aquarium) fish with empty alimentary tracts. All samples were divided into 3 sub samples and frozen at -19°C for later analyses.

Chopped fish bodies were dried at 60°C to a constant dry matter (accuracy 0.01 mg), after which they were homogenized into a fine powder with an agate mortar. Samples of 100 mg of fish powder were burned in a combustion furnace at 450°C to determine body ash. For minerals, samples of 500-600 mg of fish powder were digested in an MDS 2000 microwave apparatus (CEM, USA) using nitric acid and hydrogen peroxide. P, Ca, and Mg contents in the fish bodies were analyzed with plasma mass spectrometry and an INTEGRA XL spectrometer (GBC, Australia), with the ICP technique and the analytic curve method. The total ash and mineral content of the diets were analyzed using the same methods.

The initial individual larval total length (TL, measured to the nearest 0.01 mm) and wet body weight (BW, 0.1 mg) were determined directly before the first feeding (n = 100); at the end of D5 and D25, using samples of 50 (2 x 25 per aquarium) individuals

from each treatment group. The final survival rates and the final share of individuals exhibiting typical features of macromineral deficiency (i.e., spinal curvature and malformations of opercula; Tacon 1992) were determined. All the surviving fish were anesthetized in a 2-phenoxyethanol water solution (0.4 ml dm^{-3}), and then counted and examined visually for external abnormalities.

Statistical analysis

The data of the initial and intermediate body ash and mineral content as well as dietary ash and minerals were not analyzed statistically because of single samples. The final TL, BW, and survival data obtained from the duplicate aquaria were compared for each experimental group. Since no significant differences were found, the pooled data from the duplicate aquaria were used to calculate the final values of these parameters for each experimental group. The final TL and BW data and the data of the whole fish body ash and mineral content, Ca:P, Ca:Mg, P:ash and Ca:ash ratios were analyzed using Duncan's multiple range test (Statistica for Windows). Survival percentages were normalized using angular transformation (Sokal and Rohlf 1969). The level of significance was set at P ≤ 0.05.

Results

Chemical composition of diets

Substantially higher ash, P, and Ca content in dry matter was determined for two commercial starters, Aller Futura Larvae and Ewos AgloNorse, in comparison with the two other diets (Table 1). The same was found for Ca:P and Ca:Mg ratios. Mg content was similar in all diets (0.19-0.25%). *Artemia* nauplii contained considerably less Ca than the dry formulated diets did; therefore, the lowest Ca:P and Ca:Mg ratios of 0.09 and 0.47, respectively, were recorded for this diet.

Table 1
Mineral composition of diets used in the experiment with *B. barbus* larvae

| Parameter | Diet* | | | |
	Aller Futura Larvae (AFL)	Ewos AgloNorse (EAN)	Start (STA)	Artemia nauplii (ART)
Ash (% d.m.)	11.54	13.52	6.74	5.66
P (% d.m.)	1.46	1.46	0.88	0.98
Ca (% d.m.)	2.34	2.17	0.59	0.09
Mg (% d.m.)	0.20	0.25	0.19	0.19
Ca:P ratio	1.60	1.49	0.67	0.09
Ca:Mg ratio	11.94	8.79	3.12	0.47

*in brackets denotations of the experimental groups

Chemical composition of the fish body

At the beginning of D1, immediately before the first feeding, the larval body comprised 6.71% ash and 1.20% P in d.m., whereas Ca:P and Ca:Mg ratios were 0.29 and 3.72, respectively (Table 2). The initial five days of the feeding with live *Artemia* nauplii resulted in a substantial increase in all of the chemical parameters studied, especially ash (to 10.82%) and Ca (to 1.51%). A moderate rise in P to 1.53% was also recorded. Consequently, Ca:P and Ca:Mg ratios increased to values of 0.99 and 10.94, respectively.

Table 2
Mineral body composition of *B. barbus* larvae before the first-feeding (D1) and after 5 days of feeding exclusively with live Artemia nauplii (D5)

| Parameter | Experimental days | |
	D1	D5
Ash (% d.m.)	6.71	10.82
P (% d.m.)	1.20	1.53
Ca (% d.m.)	0.35	1.51
Mg (% d.m.)	0.09	0.14
Ca:P ratio	0.29	0.99
Ca:Mg ratio	3.72	10.94

In comparison to D5 (Table 2), the 20-day period of feeding with commercial dry diets and live *Artemia* nauplii increased fish body Ca content substantially, whereas ash content increased distinctly in nauplii-fed individuals from group ART and in those from group AFL (Table 3). Increased P content was noted only in the fish from the former group. The highest statistically significant ($P \leq 0.05$) ash content in the final fish body composition was 13.22% in the fish from group ART. The values of the other chemical parameters measured were also significantly the highest in this group, except for the P:ash ratio which was the same in all treatment groups. Excluding this one, all the remaining parameters were significantly the lowest for the fish fed non-commercial starter in group STA. In all groups, the final content of body Ca exceeded that of P content.

Fish growth, survival and malformations

By the end of D5 of the experiment, the fish had reached 16.0 ± 0.4 mm TL and 30.1 ± 2.9 mg BW (mean ± SD). All differences in final fish size were significant (Table 3). The longest and heaviest were fish in group ART (29.3 mm TL and 214.5 mg BW), where most of them became juveniles shortly before the end of the experiment. The smallest were larvae from group STA (21.3 mm TL and 84.3 mg BW). The differences between final survival rates were significant in all cases, with the maximum value of 99.9% recorded for group ART and the minimum of 96.5% for group AFL. No external fish body malformations were recorded in any treatment group.

Discussion

In the natural habitat, all the dietary requirements of fish are usually met by natural food sources that contain all the essential minerals in easily assimilable forms. Therefore, under controlled conditions, larval and juvenile cyprinids fed natural food grow quickly

Table 3

Final mineral body composition, growth performance and survival of *B. barbus* larvae fed different diets for 25 days

Parameter	Experimental diet*			
	AFL	EAN	STA	ART
Body composition				
Ash (% d.m.)	11.61 ± 0.58^b	10.54 ± 0.17^c	7.48 ± 0.26^d	13.22 ± 0.53^a
P (% d.m.)	1.65 ± 0.06^b	1.58 ± 0.06^b	1.11 ± 0.03^c	2.04 ± 0.05^a
Ca (% d.m.)	2.15 ± 0.05^b	2.09 ± 0.03^b	1.21 ± 0.02^c	2.95 ± 0.13^a
Mg (% d.m.)	0.13 ± 0.00^b	0.13 ± 0.00^b	0.10 ± 0.00^c	0.15 ± 0.00^a
Ca:P ratio	1.31 ± 0.02^b	1.32 ± 0.06^b	1.09 ± 0.02^c	1.45 ± 0.03^a
Ca:Mg ratio	16.48 ± 0.07^b	16.42 ± 0.54^b	12.48 ± 0.12^c	19.56 ± 0.79^a
P:ash ratio	0.14 ± 0.01^a	0.15 ± 0.00^a	0.15 ± 0.01^a	0.15 ± 0.01^a
Ca:ash ratio	0.19 ± 0.01^b	0.20 ± 0.00^b	0.16 ± 0.01^c	0.22 ± 0.02^a
Growth and survival				
TL (mm)	22.9 ± 1.9^c	23.9 ± 1.7^b	21.3 ± 1.4^d	29.3 ± 2.3^a
BW (mg)	100.9 ± 31.9^c	118.7 ± 30.6^b	84.3 ± 20.6^d	214.5 ± 46.4^a
Survival rate (%)	96.5^d	98.5^c	99.4^b	99.9^a

*see Table 1

Data for body composition: n = 3; for TL and BW: n = 50; for survival: n = 1600

Significant differences were found between groups with different superscripts in the same row (P ≤ 0.05)

and do not develop external body malformations resulting from nutritional deficiencies (review: Wolnicki 2005). In contrast, young cyprinids are highly prone to body deformities when intensively fed dry formulated diets exclusively (e.g. Kamler et al. 2006, 2008, Wolnicki et al. 2006). This includes *B. barbus* (Wolnicki et al. 2000).

The chemical body composition of *B. barbus* larvae, recorded at the beginning of the present experiment, seems to be typical for this species at this moment of its ontogeny. The very close similarity between the current results and those obtained by Çalta (1998) permit drawing this conclusion. Later in the experiment, considerable chemical changes occurred in the fish body within just five days of initial feeding with the live diet (D1-D5). During this period, larval body ash content increased almost twofold and Mg did so more than fourfold despite the fact that *Artemia* nauplii contained relatively few minerals in dry matter, especially of ash and Ca in comparison to the dry diets. Further feeding with the live diet consistently raised the ash and mineral contents in the fish in group ART to reach the maximum values of all the treatment groups on D25. The 20-day feeding with dry diets had a much less pronounced effect on the chemical composition of the

fish body and this effect was clearly diet-influenced. For example, ash remained at more (group AFL) or less (EAN) a similar level, whereas in group STA it decreased considerably.

All of these findings indicate that the organic complexes of essential macrominerals (e.g., phosphate complexes) with proteins, lipids and/or carbohydrates, that live *Artemia* nauplii contained, were substantially more readily available to fish than the inorganic forms included in dry formulated diets. This appears to explain why feeding with Ewos AgloNorse or Aller Futura Larvae dry feed resulted in slower larval growth and lower body ash content in comparison to the fish fed live food, even though the mineral content of both diets was considerably higher than that of the nauplii.

Phosphorus occurs in the fish body in combination with calcium, and these two minerals are usually considered together. In contrast to P, Ca can be absorbed from water to meet some of the nutritional requirements of the fish. However, Ca absorbed from water cannot be deposited in mineralized tissue if there is a dietary P shortage (Baeverfjord and Åsgård 1998). Excessive quantities of dietary Ca are known to have a negative effect, for example, on scale ash and mineral deposition as well as on vertebrae

deposition of zinc (Ye et al. 2006). This might suggest that for *B. barbus* Ca content should not exceed the content of P in the formulated diets, as was noted in the reference diet, *Artemia* nauplii, in the current experiment.

Chavez-Sanchez et al. (2000) reported that phosphorus might not be absorbed by the intestine, when there is an excess of Ca over P in the diet since they can form tricalcium phosphate, which is of very low availability to cyprinid fish (Paul et al. 2004). In the current experiment, only the live *Artemia* nauplii and the Start dry diet had P contents exceeding that of Ca. According to many authors, P available from the diet significantly affects not only its content in the fish body, but also body ash, lipids, and proteins (Helland et al. 2005, Mai et al. 2006, Yang et al. 2006, Ye et al. 2006, Zhang et al. 2006, Uyan et al. 2007). In the present experiment the final share of P in body ash was the same in all treatment groups despite significantly differentiated P and ash content in the fish body.

Dry diet ingredients vary in phosphorus and calcium content as the availability of these minerals to fish does. For example, fish meal, which is used as an essential ingredient of dry feeds for fish, is rich both in P and Ca. However, as stomachless fish, with no acid secretion in the alimentary tract, cyprinids have a low capability for utilizing the bone phosphate present in fish meal. According to Ogino et al. (1979), only 10–30% of P from fish meal is available to common carp, *Cyprinus carpio* L. Likewise, the P present in plant phytate is poorly absorbed by fish (Robinson et al. 2001), especially those without functional stomachs. Phosphorus deficiency results in the acceleration of fatty acids synthesis from amino acids via citrate (Rodeshutscord 1996), which can affect body lipid content greatly. This phenomenon is well known in the rearing of fish on dry formulated diets.

In the present experiment, the Ca:Mg ratio of 3.72 was noted in first-feeding *B. barbus*, which is similar to the value of 3.0 recorded under comparable conditions by Çalta (1998). The current data concerning the final chemical body composition of the fish fed nauplii seem to suggest that the proper level of Mg in reared *B. barbus* larvae may be close to 0.15% d.m. with a Ca:Mg ratio of about 19.6. This value is much lower in comparison to that of adult common carp (Ca:Mg = 50, Ogino and Chiou 1976) or parr of Atlantic salmon, *Salmo salar* L. (Ca:Mg = 40, El-Mowafi and Maage 1998). In the current experiment, the fish fed dry diets, especially those in group STA, seemed to exhibit Mg deficiency. This was probably due to the impaired absorption from dry diets of not only Mg but also P. It is known from other studies that fish fed diets deficient in P exhibit losses of whole-body Ca and Mg despite sufficient dietary provision of these minerals (Sugiura et al. 2004). It seems obvious then that if the diet is rich in P but in a form with limited availability to the fish, they may also exhibit losses of body Ca and Mg.

In summary, none of the dry formulated diets used in the present study produced final results comparable to those obtained with live food, except for satisfactorily high survival rates. The poorest were the results attained with the non-commercial Start diet. Feeding this diet, with its low mineral content and availability, resulted in considerable decrease in body ash, P, Ca, and Mg, and the slowest fish growth. It is probable that prolonged use of this diet would result in fish body deformities, which become visible usually after 30-40 days of feeding (Wolnicki 2005, Wolnicki et al. 2000, 2009). In contrast, the commercial dry diets, Ewos AgloNorse and Aller Futura Larvae, have similar chemical composition and mineral availability. However, the former seems to be superior for larval cyprinid feeding since it produces larger fish (present paper and Wolnicki et al. 2009). Ewos AgloNorse also appears to be safer for fish with regard to the subsequent risk of the appearance of body deformities (Wolnicki et al. 2009).

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