FISHERIES & AQUATIC LIFE (2023) 31: 15 - 23
Archives of Polish Fisheries
DOI 10.2478/aopf-2023-0002

RESEARCH ARTICLE

Different susceptibility to body deformities in juveniles of 13 European species (Cypriniformes: Pisces) intensively fed dry formulated diet under controlled conditions

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Received – 12 October 2022/Accepted – 17 March 2023. Published online: 31 March 2023; ©National Inland Fisheries Research Institute, Olsztyn, Poland

Citation: Sikorska, J., Wolnicki, J., Kamiński, R. (2023). Different susceptibility to body deformities in juveniles of 13 European species (Cypriniformes: Pisces) intensively fed dry formulated diet under controlled conditions. Fisheries & Aquatic Life 31, 15-23

Abstract. The aim of this study was to compare the susceptibility to body deformities in juveniles of 13 common European cypriniform fish, caused by intensive feeding with the same commercial dry diet administered at 25°C according to a similar feeding schedule. The final share of individuals with body malformations differed considerably among species and was 0.0-87.0%. Unaffected by the intensive feeding were only *Barbus barbus* (L.), *Chondrostoma nasus* (L.), *Cyprinus carpio* L. and *Leuciscus aspius* (L.). They can be safely fed with the highest rations of dry diet constituting 2,5-3% of fish biomass daily. Most of the species exhibited body malformations in a short time of only 20-30 days of feeding. *Abramis brama* (L.), *Leuciscus idus* (L.), *Leuciscus leuciscus* (L.), *Scardinius erythrophthalmus* (L.), *Carassius carassius* (L.), *Rutilus rutilus* (L.) and *Squalius cephalus* (L.) proved to be highly prone to body deformities – daily ration of feed 2.5% of fish biomass resulted in high incidence of malformed individuals after 60 days of feeding (50-87%). For these species safe daily ration of a dry diet should not exceed 2% of total fish biomass. *Tinca tinca* (L.) and *Vimba vimba* (L.) showed medium susceptibility to body deformities (11 and 24%, respectively). They can be fed with the daily ration of a dry diet about 2,3% of fish biomass. Some regularities connected with different susceptibility to fish body deformities are discussed in the paper.

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Keywords: cypriniform fish, dry diet, intensive feeding, body malformations

Introduction

Body deformities are serious economical and health problem in aquaculture, that concerns especially fast growing young individuals of many cultured fish species representing different families (e.g. Vågsholm and Djupvik 1998, Rennert et al. 2003, Fjeldall and Hansen 2010, Myszkowski et al. 2010, Boglione et al. 2013a, 2013b, Kamiński et al. 2017, Kasprzak et al. 2018). Deformities can occur in different fish body parts and organs, but the most common in the spine (Rennert et al. 2003, Kamler and Wolnicki 2006, Kowalska et al. 2006, Baeverfjord et al. 2008, Kamler et al. 2008, Fjelldal et al. 2012, Kamiński et al. 2017). Moreover, many authors described cultured individuals with multiple deformities involving spine, jaws, gill covers and/or fins (e.g. Dabrowski et al. 1988, Madsen and Dalsgaard 1999, Afonso et al. 2000, Al-Harbi 2001, Lall and Lewis-McCrea 2007). Deformities can affect physiological processes in fish including growth, swimming and reproduction, susceptibility to predation and resistance to diseases

(Al-Harbi 2001, Eissa et al. 2021). They also contribute to poor economic value of fish and reduced their marketability (Eissa et al. 2021).

Fish sensitivity to factors inducing body malformations may change during the ontogeny (Mazurais et al. 2009) – young, fast-growing stages are considered as particularly prone to body deformities (Vågsholm and Djupvik 1998). Factors that might cause body deformities in fish can be variable, but in cultured fish they have been linked mainly to poor rearing conditions (Boglione et al. 2001), with the unsuitable diet and excessive feeding as the most frequent reasons (e.g. Wolnicki 2005, Kamler et al. 2006, Kamiński et al. 2017). Moreover, the likelihood of inducing body deformities during fish rearing increases at higher temperatures (Kamler et al. 2012, Kamiński et al. 2017).

Among cultured freshwater fish, many European cypriniform species are particularly susceptible to body deformities under the influence of feeding with dry formulated diets. This is because they do not possess functional stomach throughout their ontogeny. In effect no acid and pepsine secretion in their gut occurs, what makes the digestion considerable less effective as compared to fish species with functional stomach (Kaushik 1995, Hertrampf and Piedad-Pascual 2003, Barton 2007). Some dispersed and sparse data, that cannot be directly compared due to methodological differences, seem to indicate that the response of young stages of Cypriniformes fish to feeding with the same dry diet may differ significantly among species (Myszkowski et al. 2002, Rennert et al. 2003, Sikorska 2009, Kamiński et al. 2010, 2017). Such a feature would obviously be an important biotechnical obstacle in the development of widely understood cyprinid fish aquaculture.

Therefore, the aim of the present paper was to compare in juveniles of common European Cypriniformes fish the susceptibility to body deformities when fed intensively with the same dry diet according to very similar feeding schedule. Some regularities connected with the lower or higher susceptibility to body deformities in cypriniform species reared under controlled conditions will be discussed.

Material and Methods

Juvenile fish of 13 species belonging to three families in order Cypriniformes were used in the experiment: barbel *Barbus barbus* (L.), crucian carp *Carassius carassius* (L.), common carp *Cyprinus carpio* L., (all Cyprinidae), bream *Abramis brama* (L.), common nase *Chondrostoma nasus* (L.), asp *Leuciscus aspius* (L.), ide *Leuciscus idus* (L.), common dace *Leuciscus leuciscus* (L.), roach *Rutilus rutilus* (L.), rudd *Scardinius erythrophthalmus* (L.), chub *Squalius cephalus* (L.), vimba bream *Vimba vimba* (L.) (Leuciscidae), tench *Tinca tinca* (L.) (Tincidae). Fish were prepared to the experiment over 2-6 months to attain wet body weight about 0.5 g (Table 1). In the preparation period fish larvae were fed live freshly hatched Artemia nauplii that were substituted with commercial frozen Chironomidae larvae after metamorphosis (35-45 days post-hatch). Natural food was administered until satiation at 23-25°C.

The experiment was carried out in the Recirculation Aquaculture System (RAS) and lasted 60 days. Each species (n = 99-102) was investigated in triplicate in the aquaria stocked with 33-34 individuals. The experiments were carried out at different times in few series covering 2-4 species. Each stock was prepared in the way ensuring the same size distribution of the fish. Glass 20 dm^3 flow-through aquaria were fed with water at about 0.3 dm^3 min^{-1} and water temperature was kept at 25.0 ± 0.5°C. Photoperiod was set at 13L:11D (daily ~700 lx at the water surface). Dissolved oxygen in the aquaria was maintained at 70-95% of air saturation. Total ammonia and nitrites maximum concentrations reached 0.3 mg dm^{-3} and 0.04 mg dm^{-3}, respectively. Water reaction ranged 8.0-8.5 pH.

Fish diet used in the experiment was commercial dry feed Aller Futura GR 2 (0.9-1.6 mm, Aller Aqua, Denmark) containing 62.1% crude protein, 11.9% crude fat, 8.5% carbohydrates, 10.5% ash and 7.0% moisture in raw matter, with a caloric value of 22 kJ g^{-1} dry matter (manufacturer's data). Daily food rations (Table 1) were equally distributed among 5 feedings at 08:00, 11:00, 14:00, 17:00 and 20:00.

Table 1
Age, size and daily feeding rations in juveniles of 13 European cypriniform species intensively fed with a formulated dry diet for 60 days at 25.0°C (alphabetical order within fish families)

Species	Initial age of fish (months)	Initial wet body weight[1] (g)	Daily ration of dry feed[2] (% fish wet biomass d^{-1})
Barbus barbus	2	0.46 ± 0.05	2.6 (3.5→1.7)
Carassius carassius	6	0.81 ± 0.20	2.5 (3.4→1.6)
Cyprinus carpio	3	0.51 ± 0.08	2.6 (3.5→1.8)
Abramis brama	2	0.48 ± 0.06	2.5 (3.3→1.7)
Chondrostoma nasus	2	0.44 ± 0.04	2.6 (3.5→1.7)
Leuciscus aspius	2	0.44 ± 0.03	2.5 (3.4→1.7)
Leuciscus idus	2	0.50 ± 0.08	2.6 (3.4→1.7)
Leuciscus leuciscus	2	0.51 ± 0.08	2.7 (3.4→1.9)
Rutilus rutilus	2	0.53 ± 0.05	2.7 (3.3→2.0)
Scardinius erythrophthalmus	6	0.82 ± 0.06	2.5 (3.6→1.9)
Squalius cephalus	3	0.53 ± 0.08	2.6 (3.4→1.7)
Vimba vimba	2	0.47 ± 0.05	2.6 (3.4→1.8)
Tinca tinca	6	0.62 ± 0.10	2.6 (3.5→1.8)

[1] mean ± SD, n = 99-102; [2] mean values, in brackets initial (max.) and final (min.) values.

Feeding intensity was close to the level of satiation determined on the basis of the previous experience (Kamler et al. 2006, Sikorska 2009).

All fish were individually measured (total length; accuracy to 0.1 mm) and weighed (wet body weight; to 0.01 g) just before start of the experiment. During the experiment the fish were weighed every 10 days and then daily food rations were adjusted to the actual fish biomass according to the accepted feeding protocol with taking into account also their appetite. On these days and at the end of the experiment (D60) the fish were inspected for the presence of any visible external deformities of their body. The manipulations were done after a food deprivation over the night and under a short-term mild anesthesia induced by 0.4 g dm^{-3} (for *C. carpio* and *C. carassius* – 0.5 g dm^{-3}) water solution of 2-phenoxyethanol.

Relative growth rate (% day^{-1}) was calculated as $RGR = 100 (e^G - 1)$; $G = \ln(BW_{t0}/BW_t)/(t - t_0)$, where BW_{t0} and BW_t are wet body weights at the beginning and the end of growing period (at the moments of time t_0 and t), respectively. Final percentages of fish with visible body deformities were normalized using angular transformation (Sokal and Rohlf 1969) and the differences were considered significant at $P \leq 0.05$. All growth data were tested for normality of distribution. No significant differences between means (TL, BW) from three aquaria within each group were found, therefore the data from triplicate aquaria were pooled. The significance of differences among experimental groups was tested with one-way ANOVA followed by Duncan's multiple range test, $P \leq 0.05$ (Statistica for Windows).

Results

In the course of the experiment no losses occurred in any of the investigated fish species. Fish growth differed considerably – juveniles of each species attained the final average body weight from 1.18 g (*R. rutilus*) to the maximum of 3.78 g (*C. carassius*) (Table 2). The relative growth rates for body weight (RGR) ranged from 1.34 % d^{-1} in *R. rutilus* to 3.00 % d^{-1} in *C. carpio*.

At the termination of the experiment external body deformities were found in juveniles of 11 species (Table 2). Two exceptions were *B. barbus* and *Ch. nasus*. In *C. carpio* and *L. aspius* the final share of individuals with deformities equalled to 2.0% and

Table 2
Final incidence of external body deformities and growth in juvenile fish of different cypriniform species intensively fed in 25.0°C with Aller Futura dry diet (mean daily rations about 2.5% of fish biomass) for 60 days under controlled conditions

Species	DD*	Share of fish with particular types of deformities (%)			Total share of individuals with body deformities (%)	Final body weight (g)	RGR (% d^{-1})
		spinal curvature	jaw deformities	cranium deformities			
Barbus barbus	-	0.0^{g}	0.0^{c}	0.0^{d}	0.0^{g}	1.95 ± 0.38	2.44
Chondrostoma nasus	-	0.0^{g}	0.0^{c}	0.0^{d}	0.0^{g}	1.75 ± 0.33	2.33
Cyprinus carpio	40	2.0^{f}	0.0^{c}	0.0^{d}	2.0^{f}	3.01 ± 0.73	3.00
Leuciscus aspius	50	3.0^{f}	0.0^{c}	0.0^{d}	3.0^{f}	1.86 ± 0.15	2.43
Vimba vimba	30	10.0^{e}	0.0^{c}	2.0^{c}	11.0^{e}	1.77 ± 0.21	2.23
Tinca tinca	40	24.0^{d}	0.0^{c}	0.0^{d}	24.0^{d}	2.90 ± 1.39	2.60
Squalius cephalus	30	39.0^{c}	14.0^{b}	24.0^{b}	50.0^{c}	2.60 ± 0.46	2.69
Rutilus rutilus	30	36.0^{cd}	44.0^{a}	0.0^{d}	58.0^{bc}	1.18 ± 0.17	1.34
Carassius carassius	20	64.0^{b}	0.0^{c}	0.0^{d}	64.0^{b}	3.78 ± 1.09	2.60
Scardinius erythrophthalmus	30	78.0^{a}	0.0^{c}	0.0^{d}	78.0^{a}	2.28 ± 0.35	1.72
Leuciscus leuciscus	30	60.0^{b}	47.0^{a}	39.0^{a}	80.0^{a}	1.34 ± 0.23	1.62
Leuciscus idus	30	84.0^{a}	14.0^{b}	41.0^{a}	86.0^{a}	2.38 ± 0.62	2.63
Abramis brama	20	87.0^{a}	0.0^{c}	0.0^{d}	87.0^{a}	1.91 ± 0.43	2.33

*DD – day when first individuals with body deformities were recorded.
In columns data with different superscripts differ significantly at $P \leq 0.05$; n = 99-102. In some individuals, different types of deformities occurred simultaneously, therefore the total share of individuals with body deformities does not have to be the sum of the shares of their individual types.

2.9%, respectively. The highest share of fish with deformities, ranged 77.8-86.9%, was recorded in *S. erythrophthalmus*, *L. leuciscus*, *L. idus* and *A. brama*. In all these species the first deformities became noticeable as early as on D20 or D30 of the experiment.

The most common type of body deformity was spinal curvature, particularly scoliosis (Table 2, Fig. 1). It occurred in all of 11 species showing any deformities. In six species – *C. carpio*, *L. aspius*, *V. vimba*, *T. tinca*, *C. carassius* and *S. erythrophthalmus* – only this type of body abnormality was recorded. Other types of abnormalities in fish body were deformed jaws (found in 14-47% juveniles of *R. rutilus, S. cephalus*, *L. idus* and *L. leuciscus*) and cranium deformities (24-41% in *S. cephalus* and *L. idus* and *L. leuciscus*).

Figure 1. Juvenile crucian carp with spinal curvature in the caudal region.

Discussion

The present work is the first ever attempt to compare the susceptibility to body deformities induced by intensive feeding with a commercial dry diet in different species of fishes belonging to three Cypriniformes families – Cyprinidae, Leuciscidae and Tincidae. Most of them are particularly difficult in rearing exclusively on dry formulated diets because of lack of functional stomach and ineffective digestion of such diets (Smith 1980, Hertrampf and Piedad-Pascual 2003). Commonly observed effects of intensive use of commercial dry diets on cypriniform fishes are high growth rates and high survival rates, but also excessive body fat deposits and deficiency in macromineral content what results in high incidence of body deformities (Kamler et al. 2012). One of the most important observations made in this work was the finding of considerable differences in susceptibility of different cypriniforms to body deformities when fed the same dry diet with the same intensity. Among 13 species studied, the final share of individuals with body malformations was highly diversified and ranged from 0.0% up to 87.0%. Only very few species remained unaffected by intensive feeding with the dry diet (*B. barbus* and *Ch. nasus*). Most of the species exhibited body malformations in a short time of only 20-30 days of feeding. Fact of the existence of such big differences certainly has a considerable practical aspect.

The fish used in our studies can be divided into groups of species of low, medium and high susceptibility to body deformities. The least prone to body deformities proved *B. barbus*, *Ch. nasus*, *C. carpio* and *L. aspius*. In this group of fish, the share of juveniles with deformities did not exceed 3.0% what can be considered as a satisfactory result from the practical point of view. These species can be fed safely with exclusively dry diet at the mean daily level of 2.5-3.0% of fish biomass. To group of medium susceptibility can be included *V. vimba* and *T. tinca* (11.0% and 24.0% of deformed individuals, respectively). They can be fed safely at slightly lower rates of about 2.3% of fish biomass. As many as 7 of the 13 species should be included to the group of high susceptibility to body deformities with their final share of 50.0% or higher. All of them require the mean daily rates of a dry diet not exceeding about 2% of their total biomass. To this group belong *S. cephalus*, *L. leuciscus*, *R. rutilus*, *C. carassius* and *S. erythrophthalmus*, and two species turned out to be the most prone – *L. idus* with 86% of malformed fish and *A. brama* with 87.0%. Additional interesting finding is that only in this group were recorded fish with multiple deformities.

The reasons for such a big diversity in susceptibility to deformities in cypriniform fish fed dry diet are not clear and obvious. Wild fish consume prey with high moisture contents, whereas cultured fish are generally fed with commercial dry diets of low water content what makes them less digestible. Moreover, increased feeding frequency with dry diets can result in faster intestine evacuation, which has been found to diminish the efficiency of digestion and absorption of ingested food (Le et al. 2019). However, this does not explain the great differences in the incidence of body deformities among different species of cypriniform fishes fed the same dry diet. Susceptibility to body deformities in these fishes seems to be diet- and species-related. In contrast to dry formulated diets, natural food does not induce external abnormalities in cypriniform fishes, regardless of the species and feeding intensity (Wolnicki 2005, Kamler et al. 2006, Kasprzak et al. 2018). However, the cost of any natural food is high and its availability on the market may be strongly limited.

In our study we recognized the particular fish species as those of low, medium and high susceptibility to deformities. However, there is no clear relation between susceptibility and their classification in families, life strategies or habitats. Among the largest group of fish highly susceptible to external abnormalities, we can find both rheophilic species (*L. leuciscus*) and preferring stagnant waters *C. carassius*. They may be herbivorous species as *S. erythrophthalmus* or *Ch. nasus*, or omnivores feeding on both plant material and various aquatic animals, including larvae of insects and molluscs (e.g., *R. rutilus* or species from genus Leuciscus) (Kottelat and Freyhof 2007). Similar diversity characterizes species of low susceptibility to deformities. It can be found there rheophilic *B. barbus* and *V. vimb*a as well as *C. carpio* preferring rather still waters. The latter is omnivorous, whereas *L. aspius* is predatory species though not in early juvenile period (Kottelat and Freyhof 2007).

Beside of different natural food preferences, from herbivorousness to piscivorousness, the gut structure in different species belonging to Cypriniformes is quite similar – differs mainly in the longitude – and characterizes with lack of stomach and pylorus (Smith 1980), and therefore lack of acid and pepsin secretion in the gut (Hofer 1991). Most of the discussed species have relatively short gut with only a single loop – e.g. piscivorous *L. aspius* and many euryphagous species with more or less carnivorous tendencies, like *L. leuciscus, S. cephalus, R. rutilus, S. erythrophthalmus*, *T. tinca* and *V. vimba* (Junger et al. 1989). But some species with herbivorous or omnivorous feeding tendencies have relatively long gut showing a more complex pattern of loops and coils (e.g. *B. barbus, C. carpio* and *Ch. nasus*) and these species in our experiment turn out to be much more resistant to deformities or at least less susceptible.

Histological analyses of the alimentary tracts in fish fed with commercial dry diets revealed significant pathologies including lowered hepatocyte and enterocyte proliferation, as well as shortened intestinal folds (Kasprzak et al. 2019). Another typical effect is excessive body fat deposits and lower content of macrominerals essential for proper skeleton mineralization, i.e. P, Ca, Mg (Kamler et al. 2012, Kamiński et al. 2017, Kasprzak et al. 2018). It seems that the content of fat in dry diets may, in general, exceed requirements of young cypriniforms, although for most of the species the optimum lipid content in their diets was not determined. High incidence of deformities in fish in our experiment may also be due to the fact that juvenile cypriniform fish have a physiological problem with enzymatic adaptation to composition of dry feeds. It is known fact that the content of dietary lipid can stimulate the activity of lipase in the gut, but only to the certain level (Buchet et al. 2000). Another key enzymes seem to be phosphatases secreted by the intestinal brushborder. They are responsible for hydrolysis of inorganic phosphates, modification of amino acid chains, stimulation of Ca ions absorption and transport of nutrients through the brushborder membrane into the enterocytes (López-Ramirez et al. 2011). The activity of phosphatases could be a good marker in evaluation of food digestion efficiency and nutrient absorption in juvenile Cypriniformes (Kasprzak et al. 2019). Regarding amylase activity, the omnivorous species demonstrate higher activity than the carnivores, although digestive enzyme activities of fish are affected by the diet composition and feeding habit (Al-Saraji and Nasir 2013).

It is known that microflora of fish digestive tract plays significant role in digestion and metabolism. In the intestinal bacteriocenoses of the digestive tract of freshwater fish inhabiting natural water bodies prevail the bacteria of the genera *Aeromonas, Lactobacillus, Pseudomonas* and *Flavobacterium-Cytophaga*. But in farmed fish fed on dry diets the structure of the bacteriocenosis is different with a dominant share of *Enterobacteriaceae*, which may make up to 50% of all bacteria (Ganguly and Prasad 2012). Gastrointestinal bacteria take part in the decomposition of nutrients and provide the host with enzymes, some amino acids and vitamins. Thus the microbial population of the gut represents an important and diversified enzymatic potential, which interfere with a host metabolism and dry food has an adverse effect on the intestinal microflora (Ganguly and Prasad 2012). These changes certainly have an impact on digestion efficiency and fish performance.

Interesting are also some regularities that can be found among fish from the groups of low and high susceptibility to body deformities. Comparison of the present results with some literature data concerning response of larval cypriniform fish on the feeding with dry starter feeds indicates some similarities in fish performance. Such species as *B. barbus*, *Ch. nasus*, *C. carpio* and *V. vimba*, in larval period of their life, are characterized by relatively high growth rates and high survival rates when fed exclusively dry diets just from the first feeding (Krupka 1985, Wolnicki and

Myszkowski 1998, Fiala and Spurný 2001, Wolnicki 2005). These four species in our classification were categorized as fish of low or – at worst – of medium susceptibility to deformities. By contrast, *L. idus*, *L. leuciscus*, *S. cephalus* and *T. tinca* in larval period were characterized by low ability to effective digestion of dry diets and should not be fed exclusively with them from the first feeding (Wolnicki and Górny 1995a, 1995b, Wolnicki and Myszkowski 1999, Lepičova et al. 2002, Shiri Harzevili et al. 2004, Wolnicki 2005). The same can be said about juveniles of the aforementioned species because of their high susceptibility to dry diet-induced deformities.

It is commonly observed in different fish species that during the first month of life their gut undergoes morphological changes that are related to the transition from endo- to exogenous feeding. But in juveniles the enzymatic contents in the gastrointestinal tract were in the same order of magnitude as those previously measured in adult individuals (Caruso et al. 2009). Despite this, the data presented above indicate that the ability of cypriniforms to use dry diets effectively appears to be only slightly better in juvenile fish than in larvae of the same species. It means that 2-6 months of the ontogeny, as in our experiment, may not be enough to guarantee much more effective utilization of dry feeds in juveniles as compared to larvae.

The fact of existing among cypriniform fish species considerable diversity in the response to intensive feeding with the same dry diet seems to indicate that there is a little chance for working out universal formula of starter feed that would be equally appropriate for all species belonging to that order. From the same reason it would be difficult to work out the general method of intensive rearing of juvenile cypriniforms with the use of exclusively dry diets. This fact is an explanation why so many attempts were made to date to improve the effects of fish feeding with dry diets by suplementing them with small amounts of natural food (Quirós and Alvariño 1998, Celada et al. 2009, Kamler et al. 2008 and many others) or with probiotics, prebiotics or symbiotics (Ganguly and Prasad 2012, Akhter et al. 2015, Van Doan et al. 2019), or else by treating them with organic acids (Sugiura et al. 1998, Kamiński et al. 2021). In fish farms the reduction in the number of skeletal deformities that affect commercial fish value may be also achieved by semi-intensive systems characterized by lower densities and larger rearing units (Boglione and Costa 2011). Otherwise, intensive use of any dry diet after some time can lead to body deformities in almost all species belonging to Cypriniformes (Wolnicki 2005).

Acknowledgments. The study was supported with funding from Project Z-005 of the National Inland Fisheries Research Institute, Olsztyn, Poland.

Author contributions. J.S. designed the work, investigation, wrote the original draft, reviewed, edited; J.W. investigated, wrote original draft, reviewed; R.K. investigated, reviewed. All authors approved the manuscript for publication.

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