



ORIGINAL RESEARCH PAPER

SPINAL DEFORMITIES IN THE GOLDFISH (*CARASSIUS AURATUS*) (LINNAEUS, 1758) AS SEEN BY MULTISLICE COMPUTED TOMOGRAPHY

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SYNOPSIS

The method of multislice computed tomography (MSCT) has been used to describe skeletal anomalies in two specimens of goldfish *Carassius auratus* obtained from the aquarium of the Novosibirsk Zoo (Western Siberia, Novosibirsk region, Russia). The deformities observed and described here pertain to kyphosis (dorsal curvature), lordosis (ventral curvature), ankylosis (fusion of vertebrae) and other minor anomalies. Deformities were mainly observed in the vertebrae, with slight abnormalities in the bones supporting the caudal fin. Both abdominal and caudal parts of the vertebral column were affected by the deformities, which were not as lethal as in full-grown specimens. This study discusses the conceivable reasons for these abnormalities, which related to the artificial diet such as, (1) the result of weakened bone structure; (2) the lack in skeletal mineralization was possibly the chief cause for the spinal anomalies in fish fed commercial foods; and (3) the imbalance of the three vital macroelements in skeletal metabolism are Ca, P and Mg.

SINOPSIS

SPINALNI DEFORMITET KOD ZLATNE RIBICE (*CARASSIUS AURATUS*) (LINNAEUS, 1758) POSMATRANI MULTISLAJSNOM KOMPJUTERSKOM TOMOGRAFIJOM

Metod multislajsne kompjuterske tomografije korišćen je za opis anomalija skeleta kod dva primjerka zlatne ribice *Carassius auratus* iz akvarijuma Novosibirskog zoološkog vrta (Zapadni Sibir, region Novosibirsk, Rusija). Deformiteti koji su uočeni i opisani odnose se na kifožu (dorzalna zakrivljenost), lordozu (ventralna zakrivljenost), ankiložu (srastanje pršljenova) i druge manje anomalije. Deformiteti su uglavnom primijećeni u pršljenima, sa blagim abnormalnostima u kostima koje podržavaju kaudalno peraje. Deformitetima su zahvaćeni i trbušni i kaudalni djelovi kičmenog stuba, s tim da nisu bili smrtonosni kao kod odraslih jedinki. Ovaj rad razmatra moguće razloge za ove abnormalnosti, koji se odnose na vještačku ishranu, kao što su: (1) rezultat oslabljene strukture kostiju; (2) nedostatak mineralizacije skeleta je vjerovatno bio glavni uzrok anomalija kičme u komercijalnoj hrani kojom se hrani riba; i (3) neravnoteža tri vitalna makroelementa u metabolizmu skeleta, Ca, P i Mg.

INTRODUCTION

Vertebral anomalies in members of the family Cyprinidae are common and reported on fish species from around the world (Hasanpour et al., 2016; Jawad et al., 2014; Jawad et al., 2016a, b; Jawad & Sudasinghe, 2017; Akmal et al., 2020; Golubtsov et al., 2021). Skeletal deformities reported from individuals of *C. auratus* deformities are infrequent and the information available on this species about the issue of anomalies is regarded to the developmental stages of the crucian carp (Wiegand et al., 1989; Kasprzak et al., 2019). Gubin et al. (2017) have used (MSCT) in their quantitative study on the carp species *C. auratus* in order to evaluate the use of (MSCT) in a case of skeletal deformity in this species. Recently, Jawad et al. (2020) reported six morphological deformities obtained from six specimens of *C. auratus* collected from the southern marshes of Iraq, Basrah. These anomalies include: eye abnormalities (exophthalmos and enophthalmos), mouth deformity (dextral twisted mouth), ankylosis (fusion of vertebrae), lordosis (ventral curvature), pudgheadness (deformation of the front head bones) and scoliosis (lateral curvature). All cases were not fatal as they occurred in adult individuals.

Scientists are usually kept an eye on the incidences of fish anomalies in both aquaculture facilities and the wild. The importance of knowing the case of abnormality of the fish will assist in the management of the fish population as such abnormalities has serious effects on health of fish, which lead to complication that is more commercial. On the other hand, reporting any case of fish anomaly can indicate the health status of the environment that the fish living in. In the present study a description of the cases of kyphosis, lordosis and other minor deformities observed in the two specimens of *C. auratus* is given. This report will be a significant addition to the archive of incidences of skeletal anomalies observed in the goldfish, which showing a severe lack of such information.

MATERIAL AND METHODS

Two samples of teleost fishes, *C. auratus* (150 and 170 mm TL; age 1⁺ year), family Cyprinidae exhibited spinal deformities. The specimens were obtained from the aquarium of the Novosibirsk Zoo (Western Siberia, Novosibirsk region, Russia) in 2017. One normal specimen was also obtained from the same aquarium for comparison (fig. 1). Firstly, the body and fins were thoroughly inspected for the presence of parasites, malformations, amputations or any other morphological deformities. Then, the specimens were examined using the multislice computed tomography (MSCT) technique, for which Toshiba Aquilion-64 (Japan) and GE Light Speed VCT systems (USA) were used. The technical conditions for the spiral scans were 120 kV, 80 mA on a 24-cm study field (Fov), 3-mm slice thickness, and post processing of slices with a thickness of 0.5 mm (Gubin et al., 2017). The abdominal part of the vertebral column is the region of the vertebral column that contains abdominal vertebrae, whereas the caudal part contains the caudal vertebrae (28–30). Abdominal vertebrae are located directly posterior to the skull and lack haemal processes, while caudal vertebrae have haemal processes fused together creating haemal spine ventrally. The length of a vertebra is designated as parallel to the cranial- caudal axis, whereas the width is perpendicular. Fishes at the aquarium set up aquarium of the Novosibirsk Zoo (Western Siberia, Novosibirsk region, Russia) kept in a 6 cubic meter pool with aeration in a warm pavilion at a temperature of 18-20 degrees and pH -7-7.5. The food consisted of pellets contain wheat germ, high quality ingredients, vitamins, essential fatty acids-Omega-3, beta-glucan.

RESULTS

An account of the cases of kyphosis, lordosis and other minor deformities observed in the two specimens of *C. auratus* is given below. A normal specimen of this species, which was

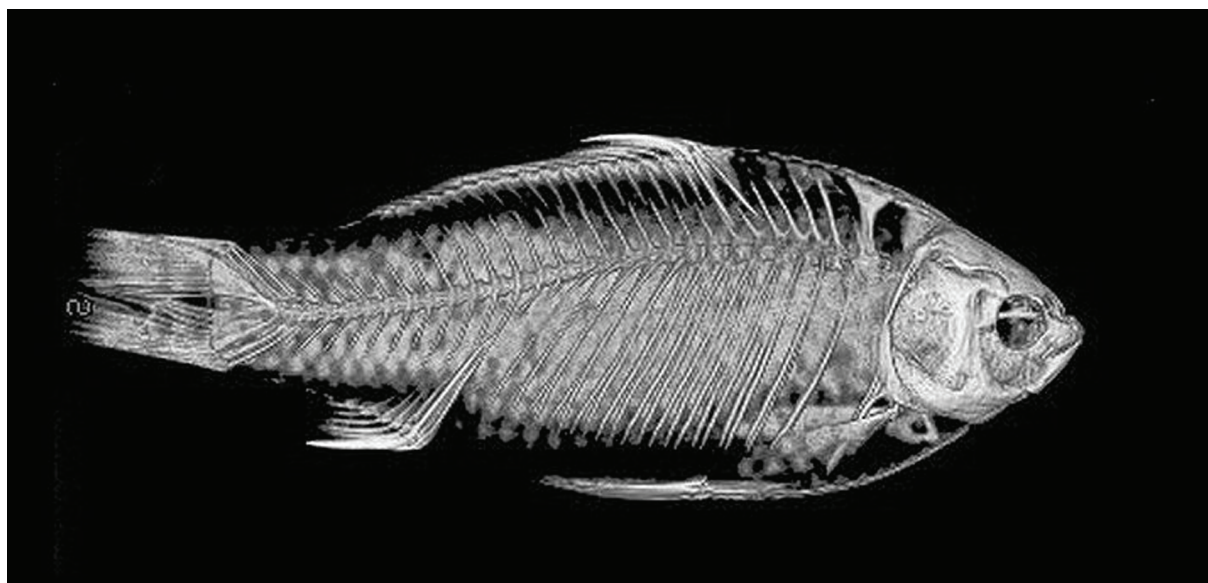


Figure 1. Normal skeleton of *Carassius auratus*. TL 170 mm.

obtained from the same source as the abnormal specimens, was used for comparison (fig. 1).

Specimen 1: 150 TL mm

Case of ankylosis

Tomograms revealed that vertebrae in the anterior abdominal region of the vertebral column show different grades and types of vertebral compression and ankylosis (fig. 2). The anteriormost 9 abdominal vertebrae form three groups of ankylosed vertebrae: group I contains V1-V3, group II comprises V4-V6 and group III encloses V7-V9. In group I, the centra of the first and second abdominal vertebrae were compressed, with a slight deformity. The centrum of the third vertebra was less compressed, but showed severe anomalies in both the neural and haemal spines. The neural spines of the first and second abdominal vertebrae looked normal, while their haemal spines looked deformed, as they were grown together. In vertebrae of group II, the body of the vertebra looked normal, but amalgamated. Slight deformities were observed in their neural and haemal spines and arches were not alignment or twisted. In group III, the centra of the 3 vertebrae were severely deformed as the seventh and ninth vertebrae had lost their posterior and anterior parts respectively, while the 8th vertebra had a compacted centrum. The

neural spines of the seven vertebrae were missing, the eight vertebra was normal and those of the ninth vertebra were non-aligned.

The haemal spines and arches of the 3 vertebrae in this region were displaced and deformed slightly.

Case of kyphosis

The Tomogram showed that ten thoracic vertebrae (V10-V19) were intricate in that the vertebral column curved upward (kyphosis) (fig. 2). The rising arm of the arch is formed of the vertebrae 10-14 and the downward arm of the vertebrae 15-19. The vertebrae 14-16 were on the top of the arch with bent centra. The neural and haemal spines of the vertebrae were normal except for a slight distortion due to the curving of the centra of the vertebrae.

The five calculated ratios showed a disturbance caused by the location of the vertebra and the curving of the vertebral column. Vertebrae 10-14 exhibited an augmented height on the ventral side (0.020-0.026) and a decreased height on the dorsal side (1.220-1.250). Vertebrae 15-19 were wedged (1.334-1.355) (had a diminished ventral length relative to their dorsal length) and reduced midline widths (0.024-0.030). The 18th and 19th vertebrae were wedged such that the dorsal length was reduced relatively to the ventral length and the amphicoelous centra

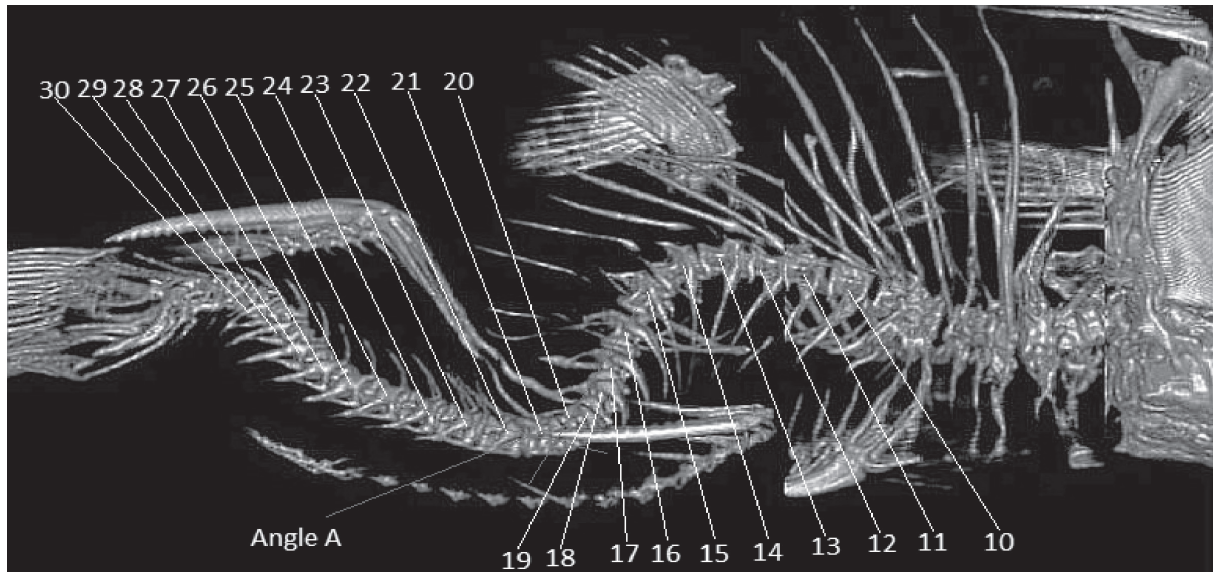


Figure 2. Tomograph of *Carassius auratus* specimen, TL 150 mm.

of these vertebrae were distorted such that the height was upsurged on the dorsal side (0.002–0.003). Slenderness and thickness were less in vertebrae 10–14 (0.002–0.004).

Cases of lordosis

The Tomogram showed that the fish body was expanded ventrally in the area below the anal fin starting from the anus and continuing to the posterior edge of the anal fin. The skeleton of the deformed specimen was similar to that of the normal one. One flexion of the vertebral column at the caudal region was present. Ten caudal vertebrae (V10–V20) were intricate due to the lordosis observed. Vertebrae located toward the posterior end of the vertebral column showed a distortion in their centra in both neural and haemal spines, especially for V29 and V30.

The ratio of the vertebral column to the total fish length of the deformed individual was 0.6, against 0.8 for the normal specimen. The haemal spines of the caudal vertebrae V20 and V21 appeared undulating and bent backwards. The amphicoelous centra of vertebrae 23–26 were distorted so that the height was increased on the dorsal side and decreased on the ventral side (0.023–0.026). Vertebrae 19 and 20 were wedged so that the dorsal length passed through the sides of the vertebral column.

Deformities of the bones of the skeleton of the caudal fin

A severe anomaly occurred in the shape of the bones supporting the caudal fin of *C. auratus* (fig. 3). All hypural bones were narrow and curved rather than being broad and straight



Figure 3. Tomograph showing deformed caudal skeleton of *Carassius auratus* specimen, TL 150 mm.

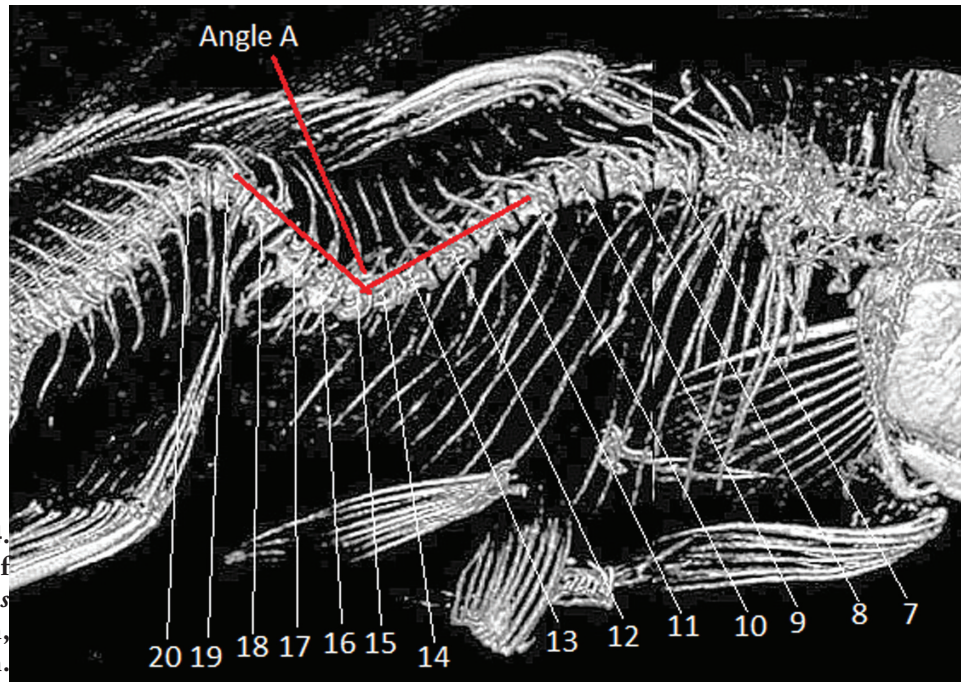


Figure 4. Tomograph of *Carassius auratus* specimen, TL 170 mm.

as seen in the normal specimen (fig. 1). In addition, the ural vertebra appeared distorted and deformed severely.

Specimen 2: 170 TL mm

Vertebral coalescence

This specimen showed a case of coalescence of the anteriormost vertebrae, lordosis in the posterior abdominal and anterior caudal vertebrae, kyphosis in the caudal vertebrae and deformity in the skeleton supporting the caudal fin (fig. 4). The vertebrae 1-6 were compacted, with a severe deformity. The first and second vertebra had their centra compacted, the third vertebra had no centrum and vertebrae 4th, 5th and 6th had deformed centra. All the anterior six vertebrae had an abnormal neural arch and spines (fig. 5).

Case of lordosis

The skeleton of the abnormal specimen was compared with that of the normal one. The Tomogram showed that the vertebral column of the aberrant specimen had one flexion at the abdominal region including 8 vertebrae (V7-V14). Vertebrae V12-V14 showed distorted centra together with their neural and haemal spines. Vertebrae V7 - V13 had slightly deformed centra V12 and V13.

The ratio of the vertebral column to the total fish length of the abnormal specimen was 0.5 against 0.7 for the normal specimen. The amphicoelous centra of vertebrae 12 - 14 were distorted so that the dorsal side was higher,

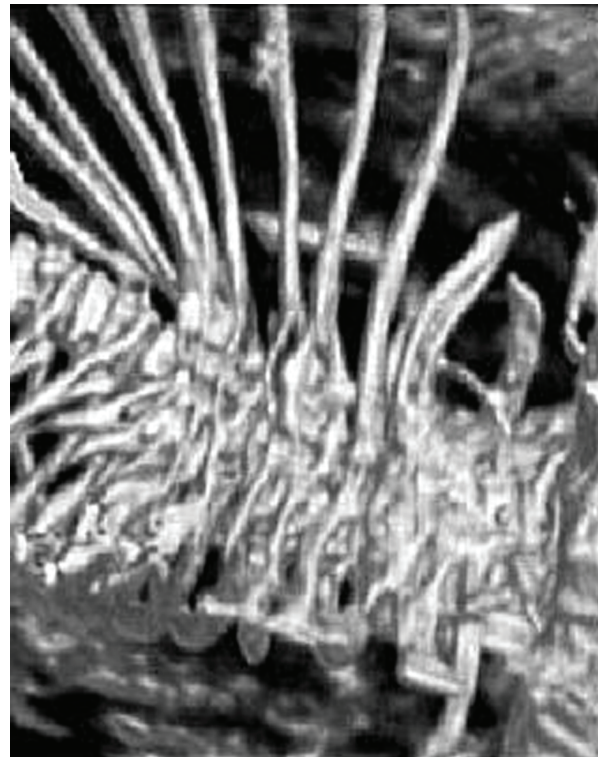


Figure 5. Tomograph showing coalescence of anterior most abdominal vertebrae of *Carassius auratus* specimen, TL 170 mm.

but the ventral side was lower (0.022 - 0.024). Vertebra 13 and 14 were wedged so that the dorsal length passed through the sides of the vertebral column and the curvature was 95°.

Case of kyphosis

The Tomogram showed that six caudal vertebrae (V14-V20) were intricate in that the vertebral column was bent upward (kyphosis) (fig. 4). The rising part of the bow was composed of the vertebrae 14 - 20 and the downward part of the vertebrae 21-24. The vertebrae 18-20 were on the top of the bow with curved centra, with the neural spines curved strongly backward, while the haemal spines were non-aligned (V18, 19, 20) due to the curving centra.

Deformities of the preural vertebrae and the skeleton reinforcing the caudal fin

The urostyle and the six-preural vertebrae were compressed and severely distorted, with their neural and haemal spines wavy and curved posteriorly. The bones of the skeleton supporting the caudal fin were also deformed, the hypural bones were distorted and the completely caudal skeleton structure was directed ventrally. The rays of the caudal fin were wavy instead of straight (fig. 6).

DISCUSSION

Spinal deformities are commonly observed in both wild and farmed fish, due to a multifactorial etiology, including a variety of injuries which can be classified as physical, environmental, nutritional, infectious, and genetic/heritable (Divanach et al., 1997; Gorman & Breden, 2007; Arbuatti et al., 2013; Jawad et al., 2013; Jawad & Liu, 2015). Investigators have discovered several reasons for such deformities including genetic (Ishikawa, 1990) and epigenetic influences (Bogliione et al., 1995), in addition to environmental issues such as temperature, light, salinity, pH, low oxygen concentrations, insufficient hydrodynamic settings and parasites (Gavaia et al., 2009; Chatain, 1994). Even though the exact cause of the spinal deformities observed in the present study was

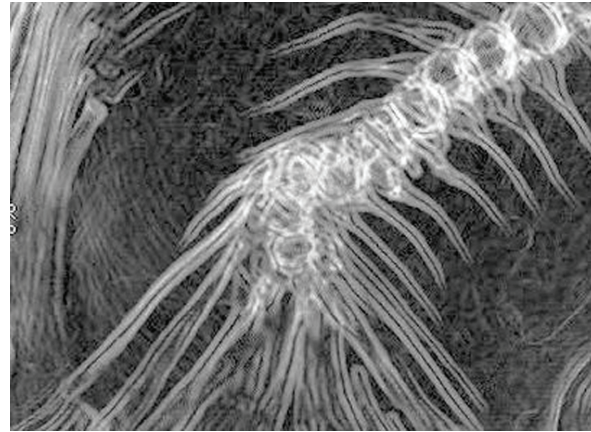


Figure 6. Tomograph showing deformed caudal skeleton of *Carassius auratus* specimen, TL 170 mm.

not determined and since they were found in only two specimens of the many specimens held at the aquarium of Novosibirsk Zoo (Western Siberia, Novosibirsk region, Russia), they still provided useful information on the optimal maintenance of *C. auratus* in captivity, since an increased incidence of skeletal deformities may be a signal of environmental deterioration that needs timely corrective actions (Eissa et al., 2009).

The current mode to study the osteological characters is using computed tomography. However, such usage is limited due to the high cost of using the Ct-Scan machine (Bakal et al., 1998; Kacem et al., 1998; Gorman et al., 2010; Govett et al., 2004).

Gubin et al. (2017) studied the quantitative aspects of the deformities found in *C. auratus*, no reference was made to a description of the abnormalities, and the type and number of vertebrae involved in these anomalies. Therefore, the study at hand aimed to describe cases of deformities found in two specimens of *C. auratus*, a species that Gubin et al. (2017) examined, and to add them to the available information about the abnormalities in this species. The description will show a clear link between these aberrations and environmental issues, such as contaminants.

Jawad et al. (2020) reported one case of lordosis in one specimen collected from the southern marshes of Iraq. The present study describes more complicated and ever cases

of lordosis in goldfish specimens, where the vertebral column was bent more than once and in acute to obtuse angles. Such changes in the vertebral column will certainly exert a malfunction to the vertebral column and the body organs associated with it. Therefore, further incidences are added to the already known data on abnormalities on this fish species.

The external deviations in the instances of lordosis and kyphosis in the two individuals of *C. auratus* are associated with an anterior-posterior (i.e. cranial-caudal) compression along the spine. The tomography showed the presence of structural changes in the amphicoelous-shaped vertebrae of the two specimens examined. Gorman et al. (2010) noticed similar changes or deviations from the normal vertebral column shape in *Poecilia reticulata*. They reckoned that the detected variations in vertebral bone structure might be produced by either (1) a distortion of the normal vertebral shape or (2) the active remodelling of the vertebral osteoid bone as a result of external forces. Consequently, additional investigations on the vertebral wedging in *C. auratus* caused by lordosis and kyphosis should test cellular action at the intervertebral area, the assumed growth area of guppy vertebrae, to evaluate a possible variation of growing in curved individuals.

Vertebral coalescence often concurs with spinal curvature anomalies in cultivated fish (Boglione et al., 2013). Nevertheless, the analogy between species needs to be ascertained because of the diversity in diagnosis of this malformation (Witten et al., 2009). In this study, the occurrences of vertebral union were very possibly a result of weakened bone structure. Ytteborg et al. (2012) proposed that there are four stages, which may characterized vertebral coalescence. These stages are the stage of upsurge of disordered and multiplying cells at the growth zones protracted along the edges of combining vertebral bodies. Instances from numerous mammalian investigations exhibited that variations in the balance between cell death and cell proliferation may cause such defects (Miura et al., 2004). Ytteborg et al. (2012) found that amplified growth of osteoblasts at the

development areas is partially alleviated by the following factors, upsurged cell death, the phase of metaplastic move and its part in vertebral fusion, the phase of damage of notochordal sheath integrity, where this sheath was found in a reinstated shape after ephemeral abnormality (Yu et al., 2005). Therefore overflowing of this sheath may start the spinal anomalies. Ectopic bone formation and remodelling caused by a deficiency of osteoclasts in the bone development areas, displaying that normal endochondral ossification, may be controlled (Ytteborg et al., 2012).

Generally, the influence of vertebral coalescence on fish wellbeing is vague, but it is expected that such deformations may worsen with time (Witten et al., 2006). It is conceivable that the abnormal specimen of *C. auratus* was confronted with factors related to the artificial diet that its body was unable to tolerate that may have led to this type of vertebral anomalies. Since the specimen of *C. auratus* was an adult, the abnormalities were not fatal, but it definitely disturbed the movement of the fish in some way.

The lack in skeletal mineralization was possibly the chief cause for the spinal anomalies in fish fed commercial foods, since decreased mineral contents causes bones to become softer and, therefore, more vulnerable to winding and twisting (Lall & Lewis-McCrea, 2007). The three vital macroelements in skeletal metabolism are Ca, P and Mg (Lall, 2002). The pathological variations suggest that a lack of phosphorus maybe the main reason for the failure of bone mineralization. Analogous comments were made for phosphorus-deficient common carp (Ogino & Takeda, 1976), rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) (Deschamps et al., 2016; Fontagné et al., 2009; Poirier Stewart et al., 2014) and the Atlantic salmon (Fjelldal et al., 2012; Helland et al., 2005; Witten et al., 2016). Kasprzak et al. (2019) studied the effect of the type of food on the growth of skeletal deformities in goldfish reared in tanks. They showed that commercial diets do not fulfil the nourishing requirements of juvenile crucian carp. Fish nourished on such a diet suffered from a

multitude of developmental disarrays. They lost a certain level of minerals from their skeleton and developed several anomalies. Kasprzak et al. (2019) concluded that the plausible reason for many of these anomalies was a shortage of phosphorus, although additional nutritional aspects could not be omitted. On the other hand, they could not eliminate a conceivable coexistence of additional nourishment -linked methods as the growth of bone deformities is a composite phenomenon that can be produced by an unevenness of other constituents (Lall & Lewis-McCrea, 2007). Obviously, individuals with such deformities will have a lower chance of survival than the normal ones.

The economic consequences of vertebral malformations are visible in terms of decreased weight and a much diminished value per kg fish caught. Hence, additional attempts such as enhancements in administration of the fisheries and ornamental fish industries must be made to discover the different sources of such anomalies.

CONCLUSION

The method of multislice computed tomography (MSCT) appeared to be a good tool to show skeletal deformities in the fish *Carassius auratus*. The deformities observed and described here pertain to kyphosis (dorsal curvature), lordosis (ventral curvature), ankylosis (fusion of vertebrae) and other minor anomalies. The observed deformities were located in both the abdominal and caudal parts of the vertebral column. This study explains the conceivable reasons of these abnormalities.

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Conflict of interest

Authors of this work declare no conflict of interest.

Ethical Statement

The authors declare that ethical issues are not applicable in the present study as the fish specimens were dead at the time of the examination.

Author contributions

LAJ: conceptualization; formal analysis; investigation; methodology.

GVD: data duration; investigation; resources; visualization.

AVG: funding; acquisition; project administration.

KAD: data duration

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