

Original Research

**CONCENTRATIONS OF METALS AND METALLOIDS IN MEAT OF THE
COMMON CARP (*Cyprinus carpio*) FARMED USING FEED BASED ON
INVERTEBRATE ANIMAL PROTEIN ORIGIN**

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Abstract

*The global shortage of fish meal (FM) presents a significant challenge to the sustainability of aquaculture and the aquafeed industry. Consequently, numerous studies have investigated alternative ingredients to replace FM in fish feed. However, the use of novel feed ingredients may pose risks not only to farmed fish but also to human consumers, particularly due to the potential accumulation of metals and metalloids in fish tissues. In this study, four experimental diets for common carp (*Cyprinus carpio*) were formulated using different animal protein sources: fish meal, earthworm meal, mealworm meal, and zooplankton meal. A 12-week feeding trial was conducted with juvenile (1+) carp under two rearing systems: (1) intensive (laboratory tanks), and (2) semi-intensive (earthen pond). The aim of the study was to determine the concentrations of harmful elements (Pb, As, Cr, Cu, Fe, Li, Zn, Ni, Mn, Cd, Co, Al, Sr) in carp fillets and to assess potential risks for consumers. Additionally, the study compares the effects of different protein sources and rearing systems on element accumulation.*

Keywords: aquaculture, fish meal, mealworm meal, earthworm meal, zooplankton meal.

INTRODUCTION

Aquaculture and captive fisheries have been the fastest growing food production sector in the past thirty years [1]. However, this growth has come at a cost, with the most significant negative impact being the reduction in the number of pelagic fish in the oceans due to systematic overfishing. This has jeopardised the sustainability of feed production for aquatic organisms because of the limitation of two key resources: fish meal (FM) and fish oil [2]. In

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recent decades, scientists have sought alternative ingredients to create balanced diets that meet the nutritional requirements and support optimal growth of farmed fish, without relying on FM. Alternative ingredients can be of plant or animal origin, with most studies focusing on plant sources for economic reasons. However, plants contain several anti-nutritional factors that reduce the growth and overall health of farmed fish [3]. In contrast, proteins of animal origin are better suited for inclusion in fish feed, and the most promising animals are insects [4]. Among these is the insect species at the centre of the present study – the yellow mealworm, *Tenebrio molitor* L., 1758 (TM). Two other invertebrate species, which are more common and frequently used as natural food for fish, are the earthworm *Eisenia fetida* (Savigny, 1826) (EF) and zooplankton (ZO), a mixture of different organisms from various taxa, mostly copepods, cladocerans and rotifers. Zooplankton from fish ponds has been used as a preferred food source, especially for nursing a fish larvae (and to a lesser extent for older fish), due to its high protein and lipid content [5].

Two nutritional assays were conducted using diets in which fish meal was replaced with the aforementioned invertebrate species. The focus species was the common carp (*Cyprinus carpio* L., 1758), one of the most important species in global aquaculture [6]. This fish is reared using all available systems, but in the present study, we used intensive (laboratory trial) and semi-intensive (pond trial) rearing systems.

The aim of this study was to determine the concentrations of 13 potentially toxic metals and metalloids (Pb, As, Cr, Cu, Fe, Li, Zn, Ni, Mn, Cd, Co, Al, and Sr) in fish meal (FM), earthworms (EF), mealworms (TM), and zooplankton (ZO), as well as in fish feeds containing these ingredients (CFM, CEF, CTM, and CZO), and in the fillets of common carp reared in intensive and semi-intensive systems. The main goal of the study was to determine whether some animal proteins pose a higher risk for fish nutrition and human consumption.

MATERIALS AND METHODS

Experimental diets

Four isoproteinaceous experimental diets were formulated to replace fish meal (FM) with different animal protein sources: earthworm meal (EM), mealworm meal (MM), and zooplankton meal (ZM) (Table 1). The origins of the alternative protein sources were as follows: (1) EM was produced from earthworms reared at the earthworm farm “Ranč Jablan” (Surdulica, Serbia) on a mixture of manure collected from local farmers and fruit and vegetable waste; (2) MM was produced from microwave-dried whole mealworms, purchased online from the “RANZ natural pet food” company (China); (3) ZO was collected from earthen ponds at two fish farms (“Kapetanski rit”, Kanjiža, Serbia, and the “Centre for Fishery and Applied Hydrobiology” (CEFAH), Faculty of Agriculture, University of Belgrade), from water abundant in zooplankton species, using a plankton net with a mesh size of 55 µm and a ring diameter of 55 cm.

Table 1 List of ingredients (%) in experimental feeds for common carp

Ingredients	Experimental diet			
	CFM	CEF	CTM	CZO
Fish meal	18.1	-	-	-
Earthworm meal	-	17.1	-	-
Mealworm meal	-	-	21.2	-
Zooplankton meal	-	-	-	24.1
Soybean seed, whole	25.0	25.0	25.0	25.0
Sunflower seed	20.0	20.0	20.0	20.0
Wheat	14.7	15.0	19.8	10.2
Maize	11.0	10.0	5.0	8.0
Corn starch	6.0	6.0	5.0	6.0
Soybean oil	2.9	2.9	-	2.6
Monocalcium phosphate	0.4	0.9	0.9	1.0
NaCl	0.2	0.2	0.2	0.2
CaCO ₃	0.5	1.7	1.7	1.7
Lysine	0.2	0.2	0.0	0.2
Mineral and vitamin premix	1.0	1.0	1.0	1.0

Feeding trials

Two feeding trials were conducted with one- year-old juvenile common carp. The fish were hatched at CEFAH, reared in earthen ponds, and fed commercial compound feed (FM) prior to the start of the feeding trials. The fish were acclimatised to tanks/cages and new environmental conditions for 14 days. Both trials lasted 12 weeks; this duration was chosen to distinguish between the transfer of elements from feed to muscle under laboratory conditions and in earthen ponds. In earthen ponds, common carp also feed on benthic macroinvertebrates and zooplankton species, and are exposed to other sources of metals originating from the environment (water and sediment). In both studies, fish were provided with feed amounting to 2.5% of their total body mass. The calculated daily feed ration was distributed manually in two meals per day.

First experiment – laboratory trial

The first experiment was conducted in the Laboratory of Fish Nutrition (Faculty of Agriculture, University of Belgrade). A total of 120 common carp juveniles (12.4 ± 2.7 g) were transferred from CEFAH and randomly distributed into 12 indoor conical-bottomed tanks – T (four experimental groups in triplicate, 10 fish per replicate, totalling 30 fish per group), each with a volume of 120 L. During the acclimation period, the fish were fed FM feed *ad libitum*. Dechlorinated municipal tap water was used, and each tank had a separate flow-through system with an exchange rate of 0.36 L/min. A natural photoperiod was maintained (March–June).

Second experiment – field study in earthen ponds

Cage system K was established at CEFAH in one of the ponds. After an acclimation period, fish were measured and stocked into 12 cages (0.22 m^3 ; $0.6 \times 0.6 \times 0.6 \text{ m}$), with 10 individuals per cage (10.1 ± 1.0 g). The cages were arranged in two rows with a 40 cm walkway between them to allow monitoring, feeding, and handling. The distance between cages was approximately 30 cm, and they were suspended on a support cable with their upper

parts above the water surface for easier access. To prevent oxygen deficiency, each cage was connected to an air compressor via diffusers and tubing to ensure adequate aeration. The cages were covered with protective mesh to prevent fish escaping.

Element analysis

At the end of the experiment, muscle tissue was sampled from two individuals per cage (left dorsal side below the dorsal fin), and the samples from each cage/tank were pooled into a single bag. All samples were stored at $-20\text{ }^{\circ}\text{C}$ prior to analysis. The concentrations of 13 elements ($\mu\text{g g}^{-1}$ dry weight, dw) were determined using inductively coupled plasma optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer). Wavelength lines (λ , nm) of the measured elements were as follows: Pb (220.353), As (193.696), Cr (267.716), Cu (327.393), Fe (238.204), Li (670.784), Zn (206.200), Mn (257.610), Ni (231.604), Cd (228.802), Co (228.616), Al (396.153), and Sr (407.771). To evaluate potential contamination from the reagents used in this study, four analytical blanks (without tissue) were processed following the same procedure as the samples. Quality control of the analytical process was assessed using certified reference materials, including IAEA-336 Lichen (AQCS, International Atomic Energy Agency) and BCR-185R Bovine Liver (European Commission Joint Research Centre). For all analysed elements, the measured concentrations ranged between 90% and 115% of the certified values.

Element concentrations in muscle tissue were evaluated against the maximum allowed concentrations (MACs) established for fish meat under the national legislation of the Republic of Serbia [7] and the regulation of the European Commission [8]. These regulatory frameworks are aligned with regard to the MACs for Cd ($0.05\text{ }\mu\text{g/g}$) and Pb ($0.3\text{ }\mu\text{g/g}$). Furthermore, Serbian national legislation defines additional MACs for Cu ($30\text{ }\mu\text{g/g}$), Zn ($100\text{ }\mu\text{g/g}$), and As ($2\text{ }\mu\text{g/g}$).

Statistical analysis

All values are presented as mean $\pm$ standard deviation (SD). Normality was assessed using the Shapiro–Wilk test, and homogeneity of variances (homoscedasticity) was evaluated using Levene’s test. As the data did not meet the assumption of normality, the non-parametric Kruskal–Wallis H test was applied, followed by the Mann–Whitney U test as a post hoc analysis. Statistical analyses were performed using Statistica 12.0 software (StatSoft Inc., USA). The level of statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

A number of studies have been published recently in which novel foods derived from invertebrate species are screened for the presence of metals and other elements, and the risk to humans is assessed [9–11]. Concentrations of elements usually do not exceed maximum allowable concentrations, but there are exceptions, and some elements have been found in elevated concentrations [9–11]. The concentration of elements depends on the environment in which invertebrates are reared and the food they consume, and these data are usually not known when these products are acquired. In contrast, fish are well known for their bioaccumulation properties for certain elements [12].

The concentrations of Pb, As, Cd, and Co were below the detection limits in all cases studied, and Ni ($0.005 \pm 0.001 \mu\text{g/g}$) was detected only in CZO. Although variations in mean values were observed, no statistically significant differences were identified among the groups for the analysed elements, which is most likely attributable to the limited sample size included in the study (Table 2).

Table 2 Element concentrations ($\mu\text{g/g}$) in fish feed and fillets of common carp reared in laboratory tanks and earthen ponds in a semi-intensive system. All values are presented as mean $\pm$ standard deviation (SD). ND indicates values below the detection limit: $0.005 \mu\text{g/g}$ for Cr and Cu, $0.002 \mu\text{g/g}$ for Li, $0.01 \mu\text{g/g}$ for Al, and $0.001 \mu\text{g/g}$ for Sr

	Cr	Cu	Fe	Li	Zn	Mn	Al	Sr
FM	ND	0.005 ± 0.002	0.182 ± 0.006	ND	0.095 ± 0.003	0.009 ± 0.001	0.090 ± 0.006	0.203 ± 0.029
EF	ND	0.013 ± 0.002	1.185 ± 0.145	ND	0.123 ± 0.007	0.071 ± 0.013	1.371 ± 0.220	0.016 ± 0.002
TM	ND	0.023 ± 0.006	0.049 ± 0.023	ND	0.105 ± 0.030	0.011 ± 0.004	0.015 ± 0.017	0.006 ± 0.002
ZO	0.010 ± 0.006	0.013 ± 0.010	2.793 ± 2.390	0.010 ± 0.008	0.075 ± 0.011	0.238 ± 0.203	0.044 ± 0.013	0.005 ± 0.009
CFM	ND	0.041 ± 0.043	0.175 ± 0.037	ND	0.099 ± 0.013	0.059 ± 0.001	0.058 ± 0.004	0.045 ± 0.004
CEF	ND	0.015 ± 0.002	0.366 ± 0.043	ND	0.076 ± 0.018	0.059 ± 0.008	0.240 ± 0.034	0.010 ± 0.001
CTM	ND	0.021 ± 0.002	0.189 ± 0.044	ND	0.088 ± 0.003	0.059 ± 0.005	0.072 ± 0.009	0.010 ± 0.001
CZO	0.006 ± 0.001	0.034 ± 0.008	1.701 ± 0.065	0.005 ± 0.001	0.090 ± 0.005	0.144 ± 0.008	3.085 ± 0.090	0.162 ± 0.005
K _{CFM}	ND	ND	0.014 ± 0.016	ND	0.050 ± 0.030	0.001 ± 0.001	0.011 ± 0.011	0.007 ± 0.004
K _{CEF}	ND	ND	0.015 ± 0.018	ND	0.036 ± 0.029	0.001 ± 0.002	0.011 ± 0.011	0.005 ± 0.007
K _{CTM}	ND	0.003 ± 0.002	0.083 ± 0.090	ND	0.072 ± 0.016	0.002 ± 0.001	0.021 ± 0.001	0.008 ± 0.008
K _{CZOO}	ND	0.004 ± 0.002	0.020 ± 0.021	ND	0.058 ± 0.036	0.002 ± 0.001	0.013 ± 0.014	0.005 ± 0.003
T _{CFM}	0.008 ± 0.009	0.011 ± 0.015	0.026 ± 0.029	0.008 ± 0.011	0.053 ± 0.056	0.003 ± 0.002	0.009 ± 0.003	0.001 ± 0.001
T _{CEF}	ND	ND	0.007 ± 0.003	ND	0.020 ± 0.008	0.001 ± 0.001	0.011 ± 0.010	0.002 ± 0.001
T _{CTM}	ND	ND	0.002 ± 0.001	ND	0.021 ± 0.005	0.001 ± 0.001	ND	ND
T _{CZOO}	ND	ND	0.019 ± 0.004	ND	0.026 ± 0.001	0.001 ± 0.001	0.023 ± 0.003	0.004 ± 0.004

In the present study, most metals and metalloids were found at lower concentrations in muscle tissue compared to raw ingredients or fish feed. In cyprinids, the bioaccumulation pattern in tissues is typical for most metals: the highest concentrations are found in the liver, the lowest in muscles, with other organs (such as gills) positioned in between [13]. The concentrations of six elements in muscle tissue were below the maximum allowed concentrations (MACs) established by both national legislation and European Commission regulations. Therefore, common carp meat can be considered safe for human consumption.

In a recent study, concentrations of three metals (Pb, Cd, and Ni) with adverse effects on animals were detected in various insect-based products available for purchase via e-commerce in the European Union [9]. Average concentrations ranged from 0.02 to $2.23 \mu\text{g/g}$, which contrasts with the present study, as two of these three elements were below the detection limit. The highest detected concentration in feed was Al, found in zooplankton feed. This is expected, as invertebrates are known to contain high concentrations of Al [14-15]; however, it is questionable why Al concentrations in zooplankton were lower than those found in the

feed. A probable reason is the addition of other plant-based ingredients, which lowered the concentrations in zooplankton.

CONCLUSION

The significance of this preliminary study lies in assessing whether alternative feed sources for common carp (yellow mealworm, earthworm, and zooplankton) are safe for fish consumption and whether they influence fish meat quality in terms of the accumulation of potentially toxic elements. Although the observed differences were not statistically significant due to the small sample size, it was determined that common carp meat is safe for human consumption.

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