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ORIGINAL PAPER

The influence of *Opisthorchis felineus* on the nutritional quality and processing factors of fish meat

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Abstract

The aim of this study was to assess the influence of *Opisthorchis felineus* infection on the nutritional quality and processing factors of fish meat, and to determine the resistance of *O. felineus* metacercariae colonizing fish muscles to various meat processing methods. The prevalence and severity of *O. felineus* infection were evaluated in three economically important fish species: *Abramis brama* (Linneus, 1758), *Carassius auratus* (Linneus, 1758), and *Leuciscus idus* (Linneus 1758). The fish came from the Birtaban--Shalkar lakes in the Akmola region of Kazakhstan. The microbiological quality of fish meat was evaluated using conventional methods based on the results of sensory analysis (n=34), analysis of proximate chemical composition, counts of mesophilic aerobic and facultative anaerobic microorganisms (CMAFAnM), coliforms, *Salmonella* spp., *Listeria monocytogenes*, and heavy metal concentrations (Pb, Hg, Cd, Cu, Fe, Zn, Mn, Ni, and Co) in the meat of infected and non-infected fish. Metacercariae were resistant to various meat processing methods. *Opisthorchis felineus* metacercariae were identified in all analyzed fish species. The meat of infected and control fish differed in proximate chemical composition. The content of heavy metals did not exceed maximum permissible limits. In comparison with non-infected fish, the meat of infected fish was characterized by a higher content of Fe (by 1.7%) and Zn (by 12.9%) and a lower content of Ni (by 5.5%), Cu (12.5%), Co (by 43.8%), Mn (by 59%), and Pb (by 37.3%). The content of Ca and Hg in fish meat was similar in both groups. Freezing, thermal processing, and salting destroyed 100% of *O. felineus* metacercariae in fish. Drying without pre-freezing was the only processing method that did not eliminate liver fluke larvae from fish meat.

Keywords: *Opisthorchis felineus,* metacercariae, fish meat, infection, technological processing

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INTRODUCTION

Fish are regarded to be a valuable source of animal protein and fat in human diets. Fish meat is known to be a source of protein rich in essential amino acids (isoleucyne, lysine, methionine, cysteine, threonine, and tryptophan). Omega-3 and omega-6 fatty acids (FAs), which play an important role in human nutrition and health promotion, are essential compounds in fish lipids. The amounts of amino acids and fatty acids in fish can be changed by diet. Fish are widely consumed by humans, and the presence of contamination in the form of pesticides or parasite infections may cause health problems (Paritova et al. 2014, Kaczyński et al. 2017).

Invasions by liver flukes of the genera *Opisthorchis* (*O. felineus* and *O. viverrini*) and *Clonorchis* (*C. sinensis*) pose a significant health hazard in many countries around the world. These parasitic flukes cause a disease known as opisthorchiasis. According to estimates, more than 40 million people are currently infected with liver flukes and around 680 million are at risk of infection. Liver flukes also decrease the quality of fish meat and cause significant economic losses. Meat processing plays an important role in disease prevention because most human infections occur after the consumption of contaminated raw meat (Pozio et al. 2013) and inadequate preparation of fish meat for human consumption (Choi et al. 2004, Salo et al. 2020).

Opisthorchis felineus (Rivolta 1884, Blanchard 1895) is a parasitic fluke that causes infections in predators and humans. This parasite has a wide geographic distribution, ranging from Western Siberia (Kazakhstan) to Eastern Europe, and it poses a significant epidemiological and economic problem (Syskova et al. 2001, Ilyinskikh 2002, Chemych et al. 2014, Yurlova et al. 2017). *Opisthorchis felineus*, pathogenic microorganisms, toxins, and heavy metals can be carried by fish (Safaeifan, Khanzadi 2017). Flukes of the family Opisthorchiidae are dangerous parasites that can also be transmitted to humans (Saijuntha et al. 2021).

Freshwater snails of the genus *Bithynia* are the first intermediate hosts of *O. felineus* (Mordvinov et al. 2012). The second intermediate hosts include fish of the family Cyprinidae, metacercariae, which are encysted fluke larvae, colonize the muscles of susceptible fish (Brusentsov et al. 2013). When raw fish is consumed by the definitive hosts, metacercariae leave the cyst and migrate through the common bile duct to the gallbladder, where they develop into adult flukes.

Opisthorchiasis is prevalent in the northern and north-eastern regions of Kazakhstan, in the drainage basins of the Irtysh River, its tributaries (Yesil and Tobol), and the Nura River, and in the north-western regions of Kazakhstan, in the basin of the Ural River, which separates Europe and Asia (Proskurina et al. 2020). *Opisthorchis felineus* colonizes 14 freshwater fish species of the family Cyprinidae (including the ide, carp, common bream,

common roach, common dace, common rudd, and tench). The ide (*Leuciscus idus*) is most susceptible to infections caused by *O. felineus* metacercariae. In Kazakhstan, *O. felineus* infections sometimes reach epidemic proportions (Beysenbiyeva et al. 2016). The sale of fish colonized by parasitic flukes contributes to the spread of infection outside fishing grounds (Sultanov et al. 2018).

A study of sheep infected with common liver flukes demonstrated that some heavy metals are accumulated in the organs of definitive hosts. Infections caused by *O. felineus* flukes lead to the deposition of Al in the host's liver, and adult flukes accumulate Fe and Al in their tissues. Fluke infections can also lead to Mn, Zn, and Cu deficiencies in the host organism (Khatemeh, Imani Baran 2022).

The aim of this study was to assess the influence of *Opisthorchis felineus* infection on the nutritional quality and processing factors of fish meat.

MATERIALS AND METHODS

The study has been approved by the Bioethics Committee of the Faculty of Veterinary Medicine, Kazakh National Agrarian Research University. The research methodology is consistent with the Code of Veterinary Medical Ethics and the provisions of the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (permission No 19/2023). Fish were analyzed in the Food and Environmental Safety Laboratory of the Kazakh-Japanese Center for Innovation, in December 2021.

The study was conducted in 2021-2022. Fish were harvested from the Birtaban-Shalkar lake system in the drainage basin of River Nura in the Korgalzhyn Nature Reserve in Akmola Region in central Kazakhstan. Fish were harvested according to the official guidelines for sampling biological material from commercial fishing grounds in winter. The applied sampling techniques, harvest time, and the conditions and temperature of fish storage were always identical. The harvested fish were identified to species level based on the identification keys for fish and invertebrates (Bogutskaya et al. 2013). A total of 71 fish belonging to three species were examined, including 27 common bream (*Abramis brama,* Linneus, 1758), 18 crucian carp (*Carassius auratus*, Linneus, 1758), and 26 ides (*Leuciscus idus*, Linneus, 1758).

Opisthorchis felineus metacercariae were identified by visual inspection and a microscopic analysis of the internal organs, eyes, and edible fish parts, according to the methodological guidelines for parasitological examinations of fish (Bogutskaya et al. 2013). The life stage of liver flukes was determined by examining fish muscles. Samples of fish muscles were analyzed for the

presence of larvae. The average larval counts per sample were determined by dividing the number of counted larvae by the number of samples. The results were used to assess the severity of *O. felineus* infection. The skin was separated from subcutaneous tissue. Thin layers of fish muscle (with a thickness of up to 2-3 mm) were scraped off with a scalpel or incised, and placed between a microscope slide and a coverslip, and the specimens were examined under a microscope. The severity of *O. felineus* infection was assessed based on the number of metacercariae in 10 g samples of dorsal muscles.

The sensory analysis involved 34 samples of fish muscles collected from crucian carp (two samples from mildly infected fish and three samples from non-infected fish), common bream, and ide (three samples each from mildly, moderately, and severely infected fish and non-infected fish). Body length, body fat content, body mass, skin condition, skin color, presence or absence of external damage, mucus, scales, eyes, ventral region, rectum, gill color, aroma, muscle consistency, and cooking parameters were determined in all samples.

In the analysis of proximate chemical composition, the moisture content and the content of fat, protein, and ash (on a mass basis) were determined in selected samples of dorsal muscles from nine infected and three non-infected fish. The results were used to calculate the energy value of meat from fish with varied severity of *O. felineus* infection. Moisture content was determined in a thermogravimetric analysis in drying cabinets set at a temperature of 103±2°C. Fat content was determined by Soxhlet extraction (Choi et al. 2004). Protein content was determined by the Kjeldahl method by multiplying the nitrogen content by a conversion factor of 6.25. Ash content was determined by thermal demineralization of dehydrated and defatted samples at a temp. of 500-650°C (Nogala-Kałucka 2016).

The microbiological quality of fish meat was evaluated in 12 samples of dorsal muscles from *Leuciscus idus* infected with *O. felineus* and in three samples of dorsal muscles from non-infected *Leuciscus idus*. Mesophilic aerobic and facultative anaerobic microorganisms (CMAFAnM) were enumerated by culturing meat samples on meat peptone agar. Coliforms were enumerated by culturing meat samples on Kesseler's nutrient medium and transferring positive samples to Endo agar. *Staphylococcus aureus* was enumerated by culturing meat samples on fish peptone broth with salt and transferring positive samples to egg yolk agar with salt. *Salmonella* spp. were enumerated by culturing meat samples on selenite-enriched broth and transferring the emerged colonies to selective bismuth sulfite agar. *Listeria monocytogenes* were enumerated by culturing meat samples on Fraser broth base and transferring the emerged colonies to nutrient agar (Kačániová et al. 2007).

The heavy metal content of fish muscles was determined in five ides severely infected with *O. felineus* (more than 50 metacercariae in 10 g of fish meat) and in five non-infected ides. The fish were freeze-stored at -18°C

before the analysis. Dorsal muscles without skin or bone were sampled from the left side of each fish, in the amount of 2 g (10 samples in total). The samples were rinsed with diluted nitric acid. Digested meat samples were analyzed for the content of Fe, Mn, Zn, Cu, Co, Ni, Hg, Pb, and Cd with the use of an atomic absorption spectrophotometer (model AA100831106, Analytik Jena, Germany). Heavy metal concentrations were expressed in mg kg^{-1} of raw muscles and compared with Kazakh food quality standards.

In the last stage of the study, the effectiveness of various meat processing methods in eradicating *O. felineus* was evaluated in 33 meat samples from moderately and severely infected ides (more than 21 larvae in 10 g of fish meat) in a total of 11 experiments. Fish meat samples were subjected to freezing, cooking, salting, and drying.

The survival of metacercariae was determined under a microscope based on the presence of at least one motile larva in 1% HCl solution in the field of view.

The results were processed statistically using Statistica ver. 13 software. The arithmetic mean and standard deviation were calculated. The significance of differences was determined by the Tukey's test (StatSoft 2018).

RESULTS

Infection with *O. felineus* was detected in all analyzed species of freshwater fish, including in 24 of the 26 examined ides (92%), 7 of the 27 examined common bream (26%), and 2 of the 18 examined crucian carp (11%) – Figure 1.

The visual inspection and the sensory analysis revealed that the quality parameters of fish that were mildly, moderately, and severely infected with *O. felineus* were within the norm and did not differ from the quality parameters of non-infected fish, i.e. the mucus was abundant, transparent, without

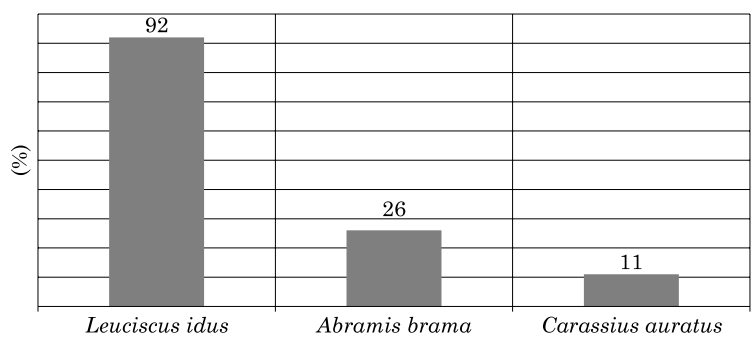

Fig. 1. Fish infected with *Opisthorchis felineus* (%)

* Source: own elaboration

any foreign odor, the scales were smooth, shiny, difficult to remove, the eyes were convex, transparent, the cornea was transparent, the mouth was closed, the gills were bright red, the mucus was sticky and transparent, the abdomen was not very swollen. The internal organs were visible, the muscle tissue was flexible and difficult to separate from the bones, and the entire body, immersed in water, sank to the bottom of the tank.

The proximate chemical composition of fish meat not infected with *O. felineus* was also determined. In a study by Blazhekovikj-Dimovska and Ahmed (2022), the proximate chemical composition of the edible portion of lake carp (g 100 g^{-1}) was determined at: moisture content – 73.223±0.274, protein – 15.333±0.047, fat – 3.483±0.190, ash – 1.053±0.004, and energy value – 95.41 kcal 100 g^{-1} on average.

In the meat of fish that were mildly, moderately, and severely infected with *O. felineus*, moisture content was 2.6%, 3.6%, and 4.3% higher ($p \leq 0.05$), respectively, whereas protein content was 1.7%, 2.3%, and 3% lower, respectively, than in non-infected fish. Fat and ash content was also lower in all fish infected with metacercariae than in non-infected fish. The analyzed meat quality parameters decreased with a rise in infection severity. The energy value of fish meat was also lower in fish that were more severely infected with *O. felineus* (Table 1).

Table 1

Proximate chemical composition of *Leuciscus idus* muscles

Parameter	Proximate chemical composition of *Leuciscus idus* meat (g 100 g^{-1})			
	non-infected (control) *n*=3	infected with *O. felineus,* infection severity (number of metacercariae in 10 g of dorsal muscles)		
		<20, *n*=3	21-50, *n*=3	>51, *n*=3
Moisture	74.2±0.39^a	76.8±0.1	77.8±0.52^b	78.5±0.39^c
Protein	21.7±0.007^a	20±0.12	19.4±0.25	18.7±0.19^b
Fat	2.5±0.17^a	2.2±0.22	2±0.17	1.9±0.29^b
Ash	1.1±0.069	1±0.027	0.8±0.062	0.9±0.049
Energy value of 100 g of meat (kcal)	109.3^a	99.8	95.6^b	91.9^c

* Source: developed by the authors

The microbiological analysis revealed that ide muscles were not contaminated with pathogenic microflora and that the counts of conditionally pathogenic microorganisms did not exceed maximum permissible limits. The only exception were severely infected fish (more than 51 metacercariae), where the number of CMAFAnM colony-forming units (CFU) exceeded the statutory limit. In addition, CMAFAnM counts were 1.6, 2.7, and 4.3 higher in fish

that were mildly, moderately, and severely infected with *O. felineus* compared with non-infected fish.

The content of the tested metals did not exceed permissible limits in the meat of *Leuciscus idus* (Table 2). In the group of micronutrients, the meat of severely infected fish was characterized by a somewhat higher content of Fe (by 1.7%), lower content of Ni (by 5.5%) and Cu (12.5%), and a significantly lower content of Co (by 43.8%) and Mn (by 59%). In the meat of severely infected fish, the content of Zn was 12.8% higher, whereas the content of Pb was 37.3% lower than in non-infected fish. The levels of Cd and Hg were similar in infected and non-infected fish. These results suggest that infection with *O. felineus* metacercariae contributes to the accumulation of Fe and Zn in fish muscles and decreases the content of Cu, Ni, Mn, and Co within permissible limits.

Table 2

Content of metals in *Leuciscus idus*

Metals	Metal content (mg kg^{-1}) of fish muscles		
	infected with *Opisthorchis felineus,* infection severity >50 metacercariae in 10 g of muscles, *n*=5	non-infected, *n*=5	average content in fish of the family Cyprinidae (Łuczyńska 2014, Sultanov et al. 2014, Szkoda et al. 2016, Różycki, Podolska 2019).
Micronutrients			
Cu	2.4±0.03	2.7±0.057	0.269 - 0.685
Fe	12±0.075	11.8±0.041	1.59 - 2.95
Mn	2.2±0.01	3.5±0.021	0.108 - 0.221
Ni	9.0±0.079	9.5±0.069	1.32 - 39
Co	5.0±0.025	8.9±0.051	3.9-10
Zn	8.6±0.05	7.5±0.051	3.40 - 6.15
Toxic elements			(Mojoudi 2013)
Pb	0.69±0.03	1.1±0.06	0.2/0.5
Cd	0.1±0.04	0.1±0.005	0.05/0.5
Hg	0.004±0.0001	0.004±0.0001	0.5/ 0.5

* Source: developed by the authors

The meat of *Leuciscus idus* was subjected to several disinfection treatments to eliminate *O. felineus* metacercariae. The applied treatments are presented in Table 3.

The analysis revealed that freezing, cooking, and salting treatments destroyed 100% of *O. felineus* metacercariae in fish meat, and that these treatments effectively neutralized all pathogens in the examined samples, rendering them safe for human consumption.

Table 3

Disinfection treatments applied in fish infected with *Opisthorchis felineus* (an example of small specimens of *Leuciscus idus*)

N° n/n	Number of samples	Physical and chemical disinfection treatments	Duration (exposure)
1	3	freezing at -18°c	7 days
2	3	freezing at -20°c	2 days
3	3	freezing at -28°c	32 hours
4	3	thermostat treatment at 60°c±10°c	35 min
5	3	boiling in water (until the temp. inside the specimen reached min. 80°c)	10 min (from the moment of boiling)
6	3	flattened pieces (100 g) fried in fat on open baking sheets at a temp. of 150°c	15 min
7	3	NaCl salting, 50 g L^{-1} (5%)	30 days
8	3	NaCl salting, 100 g L^{-1} (10%)	21 days
9	3	NaCl salting, 140 g L^{-1} (14%)	15 days
10	3	NaCl salting, 150 g L^{-1} (15%)	10 days
11	3	drying without pre-freezing	21 days

* Source: developed by the authors

DISCUSSION

Most studies on opisthorchiasis have examined infections in humans, and the effectiveness of freezing in neutralizing pathogens in fish meat has been rarely investigated. According to estimates, 70% of the Thai population and 27% of the Laotian population are infected with *O. viverrini* (Zhan et al. 2017, Saijuntha et al. 2022).

Approximately 1300 to 1500 cases of opisthorchiasis are reported each year in Kazakhstan, mainly in the regions of Akmola, East Kazakhstan, Kostanay, Pavlodar, Karaganda, West Kazakhstan, and North Kazakhstan. In the past decade, the average long-term incidence of opisthorchiasis was determined at 13.6 cases per 100,000 population. According to public health statistics, the relevant epidemiological risk is particularly high in the regions of Akmola and West Kazakhstan. Based on the data published by the Department of Sanitary and Epidemiological Control of the Medical and Pharmaceutical Control Committee of the Ministry of Health of the Republic of Kazakhstan, in 2021-2022, the incidence of opisthorchiasis reached 196-212 cases per 100,000 population in Akmola Region, which accounts for 20.4-22.1% of the total number of opisthorchiasis cases registered in Kazakhstan (Koszerowa et al. 2009). A high rate of infection in commercially important fish species of the family Cyprinidae (ide), as well as socioeconomic

factors, including a thriving fisheries sector (including an increase in the number of fishermen), high consumption of fish and fish products (including undercooked fish), uncontrolled export of fish and fish products from areas affected by opisthorchiasis, and illegal trade in fish, indicate that the epidemic risk of opisthorchiasis is highest in Akmola Region. These factors contribute to a high and persistent incidence rate of opisthorchiasis in Akmola Region, where 14 fish species of the family Cyprinidae are infected with *O. felineus* metacercariae (Nurgalieva 2010; http://zhuldyz.kz).

According to a local veterinary laboratory, water bodies in Korgalzhyn District, in particular the Birtaban-Shalkar lake system, are regarded as hotspots of opisthorchiasis. The present study demonstrated that 92% of ides, 25.9% of common bream, and 11.1% of crucian carp from freshwater fish populations were infected with *O. felineus*, which validates the general observation that ides play the most important role in the epidemiology of opisthorchiasis. These findings confirm the presence of natural foci of opisthorchiasis in the analyzed lake system (Beysenbiyeva et al. 2016).

The analysis of fish quality parameters did not reveal differences in the sensory attributes of dorsal muscles between ides infected and not infected with *O. felineus*. However, infected fish were characterized by a less desirable proximate chemical composition. Moisture content increased, whereas protein, fat, and ash content, and the energy value of fish muscles decreased with a rise in infection severity.

The microbiological parameters of meat from infected fish did not exceed the permissible limits indicated in the Customs Union Technical Regulations on the safety of fresh fish. The only exception were CMAFAnM counts (CFU), which were higher in severely infected fish. The above parameter was 1.6-4.3 times higher in infected than in non-infected fish. At the same time, a direct correlation was observed between infection severity and microbial contamination of fish. Samples of infected meat were colonized by conditionally pathogenic microorganisms that were transported across the skin by fluke larvae and weakened the hosts' resistance to infection (Kačániová et al. 2007).

The Birtaban-Shalkar lake system is situated in the drainage basin of the Nura River, which receives wastewater from a nearby metallurgical plant. The above implies that the drainage basin could be contaminated with heavy metals. Significant quantities of heavy metals and other pollutants reach water bodies with wastewater evacuated from industrial plants. These pollutants tend to accumulate in the biocenosis, in particular at the second and third trophic level of the food chain, including in fish. These toxins are ingested by humans with the consumed fish.

Metals play an important role in nutrient cycling in various ecosystems. Many metals, including Fe, Cu, Zn, and Mo, participate in biological processes and are essential for plant, animal, and human health. Iron is one of the most common elements on the Earth, and it is crucial for all living organisms (Bury, Grosell 2003). Manganese occurs naturally in surface waters, but high Mn concentrations are toxic for organisms (Krauskopf, Bird 1995). Zinc

is both an essential nutrient and an environmental contaminant for aquatic organisms (Glover, Hogstrand 2003). Excessive Zn levels are toxic for fish and humans because this element is deposited in tissues and can cause numerous diseases. In aquatic environments, high Cu levels combined with low water pH are lethal for fish (Cogun, Kargin 2004). Nickel inhibits the activity of various enzymes and binds to proteins, including albumins (Dallas and Day 1993). Freshwater fish are exposed directly to Ni in water and indirectly through contaminated feed and sediments (Dallinger, Kautzky 1985). In aquatic biocenoses, the greatest threat is posed by Hg, Pb and Cd. The toxic effects of Pb on fauna and flora can be modified by various factors. In general, Pb is only toxic for the ecosystem when it forms free ions (van Aardt, Venter 2004). Cadmium is a heavy metal with an unknown biological function in animals (Xie and Klerks 2004). Mercury is a natural component of the Earth's crust, but Hg levels are generally very low in the natural environment. It is also an indicator of pollution in water bodies and aquatic organisms (Al-Sulaiti et al. 2022).

CONCLUSIONS

The severity of infection with *O. felineus* metacercaria was highest in the ides (92%) and lowest in the crucian carp (11.1%). The tested fish infected with flukes had similar visual and organoleptic indicators. Analysis of the chemical composition of fish meat showed that with the increase in infection, the moisture content increased, while the content of protein, fat and ash, as well as the energy value of fish meat, decreased. Microbiological analysis showed that *Leuciscus idus* meat was not contaminated with pathogenic microflora, except for the conditionally pathogenic CMAFanM. The number of CMAFanM (CFU) increased with increasing infection severity from 2.9×10^4 (low infection) to 5.1×10^4 (severe infection). Average heavy metal concentrations in the muscles of severely infected ides did not exceed the norm for fresh fish products. Freezing, cooking and salting destroyed 100% of *O. felineus* metacercariae in fish meat, while they were destroyed in all fish meat samples dried without pre-freezing.

Author contribution

G.T. – data collection, writing – review and editing, A.Z., A.A., A.U. – conceptualization, data analysis and interpretation, writing – original draft preparation, editing, J.M. – conceptualization, A.U. – writing – original draft preparation, proofreading, M.W-D. – writing – proofreading, S.W. – editing.

All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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