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Histopathological indicators: a useful fish health monitoring tool in common carp (*Cyprinus carpio* Linnaeus, 1758) culture

Research Article

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Abstract: In order to evaluate the relationship between water quality in ponds and indices of histopathological changes occurring in the vital organs of the common carp (*Cyprinus carpio* L., 1758), two six-month field experiments were carried out using two different water supplies: from the nearby stream and a tube well. The fish were fed supplemental feed: raw cereals, pelleted and extruded compound feed. Histopathological analysis, alteration frequencies, and semi-quantitative scoring of the changes were used to assess the health status of the fish. Ponds supplied by stream water were characterized by higher water hardness, dissolved oxygen and pH values, while those supplied by the tube well had higher electroconductivity, total ammonium and orthophosphates content. Fish survival rate and habitat suitability index were lower in ponds supplied by stream water, while the weight gain did not differ between the two water supplies. The use of stream water resulted in a higher level of histopathological changes in gills and liver. Among the water quality parameters, pH level had the strongest influence on fish. Differences in water supply produced greater influence on the level of histopathological changes than the type of feed applied. Gills were the most sensitive organ, while the kidney was the least responsive.

Keywords: *Histopathology • Semi-quantitative scoring • Water quality • Pond • Common carp*

1. Introduction

The use of histopathological (HP) indicators is a frequent practice in fish health research, as they are able to provide information on chronic and sub-lethal effects of xenobiotics on organs [1,2] and for the assessment of fish stress [3,4]. They are usually applied in research of polluted aquatic ecosystems, since they are good indicators of altered or polluted environments [5-9]. Gills, liver, kidney, and skin are the most frequently used HP indicators in assessing health status of fish [10].

A variety of stressors are present in the fish pond. Stress can be initiated by inadequate water quality, stocking density, diet or feeding technique, infestation by parasites or a disease [11]. The level of stress in farmed fish is usually assessed using physiological parameters [12], while HP parameters are rarely used, especially in common carp (*Cyprinus carpio*) reared in earthen ponds [13-16]. Contrary to natural freshwater ecosystems, the water quality in fish ponds is monitored and maintained within certain limits. However, the interaction between different factors can affect the environment, making it less suitable and consequently induce stress in fish [12]. Environmental stress in fish triggers the hypothalamic-pituitary-inter-renal axis, which helps fish to adapt to environmental change [17]. This mechanism causes an increase in ACTH and cortisol, which alter fish metabolism and physiology, and if the stressor is chronic, changes in organ morphology occur [18]. Such changes can be detected by means of histopathology. In addition, by using a scoring system, it is possible to quantify HP changes and correlate them

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with the level of environmental stressors. The aim of the present study was to evaluate the relationship between water quality in ponds and different supplemental feeds with indices of HP changes occurring in vital organs of common carp.

2. Experimental Procedures

2.1 Field study design

Two six-month field experiments were carried out in 2008 and 2009 (from 29/04/2008 to 26/10/2008 and from 20/04/2009 to 18/10/2009) at the Center for Fishery and Applied Hydrobiology (CEFAH) at the Faculty of Agriculture, University of Belgrade. At the beginning of the growing season, in March, carp yearlings were transported to the CEFAH from the fish farm "St. Nicholas" (Neuzina, Serbia). After a one-month period of adaptation to new ponds, fish were placed in three earthen ponds, each measuring (LxW) 36x25 m, with average water depth of 0.61 m and each stocked with 400 individuals with an average body mass of 103.6±26.4 g (Mean ± SD) in the first and 114.9±20.7 g (Mean ± SD) in the second year of study. A different water supply was used each year: in 2008, water supply was from the nearby stream (SW), while in 2009 water from a tube well (TW), 125 m deep, was used. The stream is recipient of discharge from surrounding households, which are interspaced throughout the school estate land. In each of the three ponds, fish were fed with feed supplements: raw cereals (CF), pelleted (PF) and extruded (EF) compound feed. Cereals consisted of 1:1:1 ratio mixture of wheat, corn and barley grains. The other two feed types were made of the same components, but prepared using different treatments: one by steam pelleting and the other by an extruding process. Feed chemical composition is presented in Table 1. Feed was produced by the company "Veterinarski Zavod" (Subotica, Serbia). Once every 15 days, a sample of 50 fish from each pond was measured and their body mass was used as a correction to feeding rates. The daily feeding rate was up to 3% of total fish body mass, although the percentage was lowered if environmental conditions were unfavourable. Fish were not fed on sampling days, or when uneaten feed was found in the ponds. Water temperature, pH level, dissolved oxygen (DO), transparency and electroconductivity (EC) were monitored daily at 12 noon, using a Multi 340i water field kit (WTW, Germany). Water hardness, chemical oxygen demand through redox titrometry ($KMnO_4$) (COD), total ammonia-nitrogen (TAN), nitrates, total nitrogen, orthophosphates, and total phosphates were measured once each 15 days, at the time when fish were sampled. The latter parameters were analysed by the Institute for Public Health "Milan Jovanović-Batut" (Belgrade, Serbia). Concentrations of unionized ammonia-nitrogen (UAN) were determined using table values [19].

Weight gain and survival rate were calculated using the following equations: Weight gain (WG) = final body mass (g) - initial body mass (g); Survival rate (SR)=(N_t/N_o)×100; N_t - number of fish in the pond at the end of the field study, N_o - number of fish in the pond at the start of the field study. Habitat suitability index (HSI) was calculated using the following equation: HSI=$[2\times(V_7)^{1/2}+2V_{12}+V_{14}]/6$; V_7 - maximum midsummer water temperature in the pond; V_{12} - minimum dissolved oxygen levels during midsummer; V_{14} - pH levels during the year. This represents a modified equation for the water quality assessment of a lacustrine common carp habitat, established by Edwards and Twomey [20].

2.2 Histological analysis

From each of the ponds, three fish were sampled each 2.2 month and five at the end of experiment: a total of 20 fish per pond. Fish were anaesthetized with benzocaine and sacrificed with a quick blow to the head. Liver, kidney and gill samples were transferred to 4% formaldehyde (Lach-Ner, Czech Republic). Following the fixation in formaldehyde, tissues were placed in an automatic tissue processor Leica TP 1020 (Leica, Austria) and dehydrated in ethanol series, treated with xylene and embedded in paraffin. Paraffin blocks were serially sectioned at 5 µm thickness on a microtome Leica SM 2000R (Leica, Austria); sections were de-waxed and stained with haematoxylin and eosin (HE) or Periodic acid-Schiff (PAS) [21]. Microphotographs were

Component	Feed		
	EF	PF	CF
Crude proteins	26.5	28.5	11.3
Lipids	7.8	8.0	3.3
Crude fibers	3.5	3.3	7.4
Moisture	9.4	12.0	9.8
Ash	4.7	4.7	1.9
[1]Nitrogen-Free Extract (NFE)	57.5	55.5	76.1
[2]Gross energy (kJ /g)	17.5	18.0	15.7
[3]P/E ratio	15.1	15.8	7.2

Table 1. Chemical composition of feed used in the field study (%).

[1]Nitrogen free extract + fibre, (NFE) = 100 - (% protein + % lipid + % ash + % crude fibers);
[2]Calculated by: Crude protein=23.9 kJ/g, Crude lipids=39.8 kJ/g, NFE=17.6 kJ/g;
[3]P/E = Protein to energy ratio in g protein (kJ)/gross energy.

taken by a Leica DM LS microscope (Leica, Austria) equipped with a Leica DC 300 camera.

2.3 Semi-quantitative scoring system

A scoring system proposed by Bernet *et al*. [10] was applied for the assessment of pathological changes in fish that were caused by different degrees of pollution. According to this method, pathological changes are classified into five reaction patterns, namely: circulatory, regressive, progressive, inflammatory and neoplastic. An importance factor ranging from 1 (minimal alteration) to 3 (marked importance) is assigned to each alteration, determining the relevance of a lesion and its pathological importance. Depending on the degree and extent of lesions, a score value ranging from 0 (unchanged) to 6 (severe occurrence) is determined. By using the importance factor and the score value, an organ index is obtained. The method was used for estimating HP index in three organs: gills (IG), liver (IL) and kidney (IK) The total index (IT) was calculated as a sum of all three organ indices.

2.4 Statistical analysis

Comparisons of water quality parameters and histopathology between ponds were performed by the non-parametric Kruskal-Wallis test, since variables did not meet the normality assumption. Two different water supplies were compared using the Mann-Whitney U test. Spearman`s non-parametric correlation test was used at a 95% confidence limit to assess the relationship between water quality parameters and HP indices. To assess the differentiation among the fish fed three different feeds and two different water supplies, based on the level of histopathological changes in each of the three assessed tissues, groups were compared by means of Canonical Discriminant Analysis. The scores for each specific pathological change in each of the tissues were used as the input variables.

3. Results

3.1 Differences among the three feed types

In terms of water quality, there were minor differences among the three ponds in both study years. In 2008 (SW), two parameters differed significantly ($P<0.05$) among lakes: electroconductivity (CF: 959.2±99.2, PF: 1044.9±75.0, EF: 949.7±77.1) and transparency (CF: 14.14±3.05, PF: 16.33±3.22, EF: 14.14±3.52). The only parameter that differed significantly in 2009 (TW) was the pH level (CF: 9.07±0.16, PF: 8.96±0.13, EF: 8.93±0.12). Histological alterations showed no differences among ponds in either of the tissue indices (Figure 1).

3.2 Differences between the two water supplies

Survival rate and HSI of fish reared in ponds supplied by stream water were both lower than in those supplied by water from a tube well, while fish weight gain did not differ significantly between the two groups (Table 2). Fish survival rate in ponds supplied by stream water was low (59%), compared to ponds supplied by tube water (88%). HSI values for all ponds were low (the maximum possible HSI value is 1), especially in ponds with stream water (0.040).

Ponds supplied by stream water had significantly higher water hardness, DO and pH level, while those supplied by water from a tube well had a significantly higher electroconductivity, total ammonium and orthophosphates. There were no differences between the two groups in other water quality parameters (Table 3). Variation in the majority of parameters was

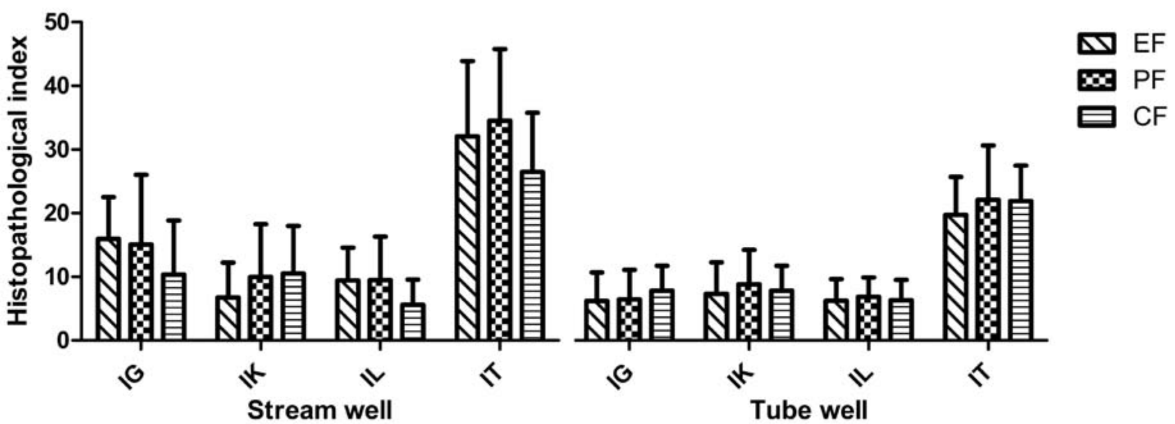

Figure 1. Histopathological index per fish group; data are presented as mean values ± SD; EF - extruded feed, PF - pelleted feed, CF - cereals, IG - gill histopathology index, IK - kidney histopathology index, IL - liver histopathology index, IT - total index.

Parameter	Water supply		P-value
	Stream water	Tube well	
Weight gain (g)	683.2±68.0	505.4±169.3	0.167
Survival rate (%)	59.16±4.69*	87.76±2.80*	0.000
Habitat suitability index	0.040±0.013*	0.206±0.053*	0.006

Table 2. Weight gain, survival rate and habitat suitability index of the common carp in ponds with two different water supplies.

** parameter differed significantly between the two groups (Mann-Whitney U test, P<0.05)*

Parameter	Water supply				P-value
	Stream water		Tube well		
	Mean ± SD	Interval	Mean ± SD	Interval	
Temperature (°C)	23.9 ± 4.6	12.4 - 34.4	22.8 ± 2.3	16.3 - 30.3	0.394
Transparency (m)	0.15 ± 0.02	0.02 - 0.27	0.16 ± 0.03	0.06 - 0.20	0.187
EC (µS/cm)	987.0 ± 71.6*	728.0 - 1414.0	2051.1 ± 107.9*	1801.0 - 2260.0	0.000
DO (mg/L)	11.00 ± 2.97*	0.70 - 33.70	3.99 ± 0.91*	1.00 - 13.00	0.000
pH	9.63 ± 0.54*	7.57 - 10.93	9.00 ± 0.11*	8.75 - 9.47	0.000
Nitrates (mg/L)	3.42 ± 1.89	0.60 - 11.00	4.97 ± 2.47	2.10 - 10.50	0.082
COD (mg/L)	17.97 ± 8.44	1.80 - 30.50	18.28 ± 2.98	5.25 - 28.85	0.858
Water hardness (dH)	21.13 ± 2.14*	15.10 - 21.00	5.07 ± 2.14*	2.46 - 14.04	0.000
Orthophosphates (mg/L)	0.043 ± 0.046*	0.001 - 0.309	0.224 ± 0.091*	0.093 - 0.487	0.000
Total phosphates (mg/L)	0.387 ± 0.118	0.019 - 0.970	0.619 ± 0.637	0.086 - 1.700	0.141
TAN (mg/L)	0.13 ± 0.07*	0.01 - 0.47	0.34 ± 0.24*	0.01 - 0.90	0.001
UAN (mg/L)	0.11 ± 0.08	0.01 - 0.32	0.11 ± 0.08	0.01 - 0.27	0.916
Total nitrogen (mg/L)	3.38 ± 1.20	1.10 - 5.76	2.55 ± 1.34	0.50 - 4.31	0.182

Table 3. Values of water quality parameters (mean values ± SD and ranges) in ponds with two different water supply; TAN - total ammonia, UAN - unionized ammonia, EC - electroconductivity, DO - dissolved oxygen, COD - chemical oxygen demand.

** parameter differed significantly between the two groups (Mann-Whitney U test, P<0.05)*

higher in ponds with stream water, reaching extreme values in certain periods, such as those for water temperature (34.4°C), pH level (10.93) and DO value (0.7 mg/L).

HP indices differed significantly between the ponds with different water supplies (Figure 2). Fish from ponds supplied with stream water had a higher total (P<0.001), gills (P<0.001) and liver (P<0.01) HP index, while kidney HP index did not differ between the two groups (P=0.860).

The most frequent gill alterations observed during this study were hyperaemia, hyperplasia (Figure 3A) and the lifting of epithelium (Table 4). In fish from ponds with stream water, a higher frequency of hyperplasia (P<0.05) and the lifting of epithelium (P<0.05) were observed. Irreversible alterations: complete lamellar

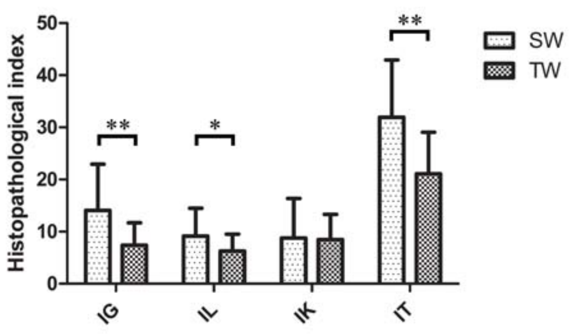

Figure 2. Histopathological index between the ponds with two different water supply; data are presented as mean values ± SD; IG - gill histopathology index, IK - kidney histopathology index, IL - liver histopathology index, IT - total index, SW - stream water, TW - tube well; Statistical significance level: *P<0.01, **P<0.001.

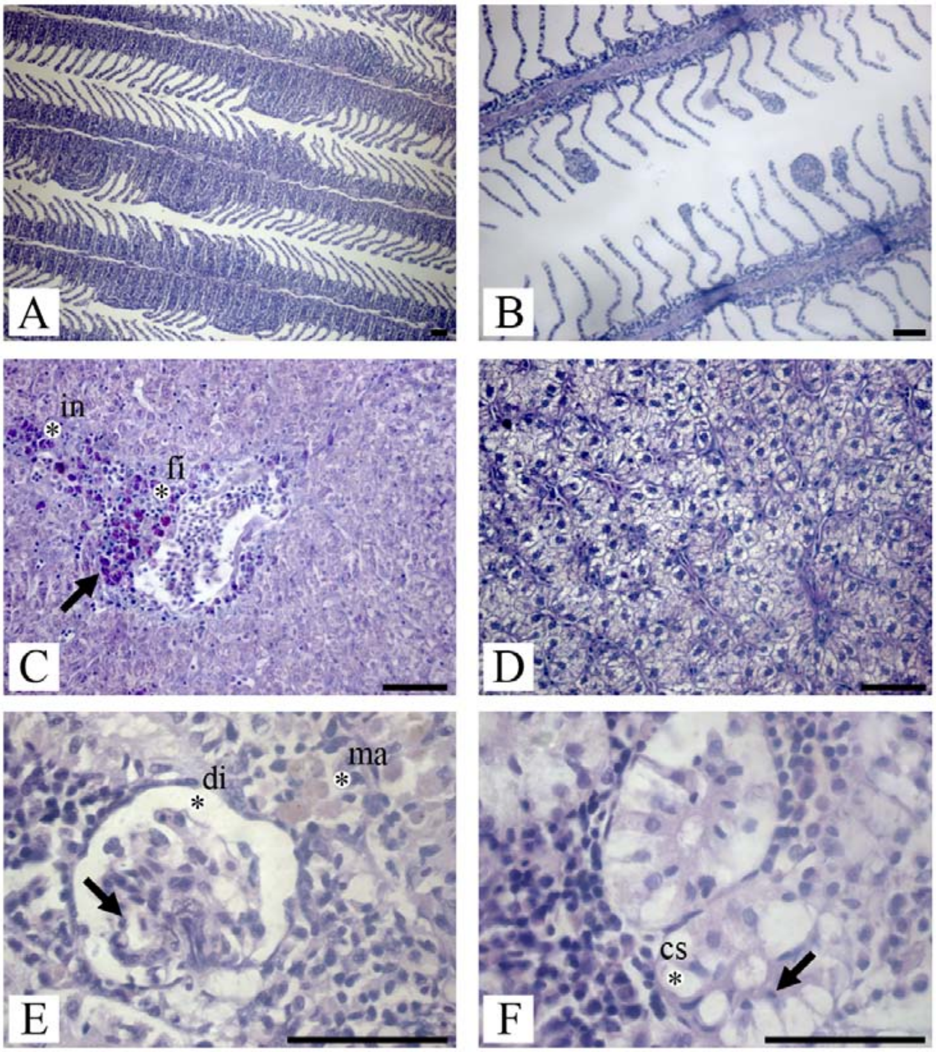

Figure 3. Histopathological alterations found in the study: A. Gills: hyperplasia of the branchial epithelium (HE x10); B. Gills: aneurism of terminal capillaries (HE x20); C. Liver: arrow = presence of EGC, fi = fibrosis, in = leukocyte infiltration (PAS x40); D. Liver: sinusoidal capillaries congestion (HE x40); E. Kidney: arrow = dilation of glomerular capillary, di = dilation of the Bowman's space, ma = macrophage aggregates (HE x100); F. Kidney: note decrease of tubular lumen, arrow = pyknotic nucleus, cs = cloudy swelling (HE x100); bar = 50 μm.

fusions and necrosis ($P<0.05$) of the branchial epithelium were present in fish from ponds with stream water, but not in those from ponds with water from the tube well. However, a higher presence of circulatory changes, such as hyperaemia ($P<0.05$), aneurism (Figure 3B; $P<0.05$) and stasis, was recorded in fish from ponds with tube well water.

Tissue pathologies in blood vessels and circulatory changes in liver were higher than those in gills. The most common alterations included fibrosis (Figure 3C) and the congestion of blood vessels (Figure 3D), as well as the presence of eosinophilic granular cells (EGC). Fibrosis and the congestion of blood vessels were both more frequent in fish from ponds with stream water ($P<0.05$). Other alterations were minor and there were no differences between the groups, with the exception of leukocyte infiltration, which was more frequent in fish from ponds with tube well water. Alterations in the kidney did not differ between the two groups. The dilation of glomerular capillaries was the only lesion that differed between the two groups (Figure 3E; $P<0.05$), and the occurrence of this alteration was high, particularly in ponds with stream water. Two lesions had a high HP index in kidneys as well: dilation of Bowman's space (Figure 3E) and tubular cloudy swelling (Figure 3F). Regenerating nephrons were also noticed in experimental fish, but their occurrence was not different between years.

Histopathological change	Water supply		P-value
	Stream water	Tube well	
Gills			
Epithelial lifting	1.74 ± 1.57*	0.33 ± 0.55*	0.000
Hyperplasia of epithelium	1.23 ± 1.58*	0.24 ± 0.48*	0.005
Hyperemia	1.15 ± 1.48*	1.63 ± 1.23*	0.045
Stasis	0.48 ± 1.15	0.72 ± 1.28	0.159
Aneurism	0.14 ± 0.38*	0.63 ± 1.23*	0.020
Complete lamellar fusions	0.03 ± 0.12	0.00 ± 0.00	0.070
Necrosis	0.09 ± 0.23*	0.00 ± 0.00*	0.008
Liver			
Fibrosis of blood vessels	1.93 ± 1.49*	1.12 ± 1.05*	0.013
Congestion of blood vessels	2.53 ± 1.31*	1.02 ± 1.05*	0.000
Presence of EGC	1.67 ± 1.21	1.33 ± 1.08	0.296
Macrophage aggregates	0.00 ± 0.00	0.02 ± 0.09	0.231
Leukocytes infiltration	0.22 ± 0.74*	0.32 ± 0.58*	0.034
Stasis	0.16 ± 0.82	0.11 ± 0.40	0.395
Cloudy swelling	0.09 ± 0.53	0.00 ± 0.00	0.253
Necrosis	0.18 ± 0.48	0.12 ± 0.40	0.296
Kidney			
Dilation of Bowman's space	1.94 ± 1.78	1.54 ± 1.64	0.264
Increased Bowman's capsule thickness	0.10 ± 0.34	0.03 ± 0.10	0.500
Dilation of glomerular capillaries	1.22 ± 1.38*	0.54 ± 0.97*	0.006
Tubular cloudy swelling	1.36 ± 1.32	1.93 ± 1.48	0.103
Decrease of the tubular lumen	0.33 ± 0.73	0.58 ± 1.03	0.234
Macrophage aggregates	0.53 ± 0.56	0.64 ± 0.63	0.496
Stasis	0.48 ± 1.12	0.63 ± 1.18	0.276
Fibrosis of blood vessels	0.17 ± 0.57	0.15 ± 0.47	0.844
Regenerating nephrons	0.25 ± 0.55	0.15 ± 0.23	0.776
Necrosis	0.51 ± 0.38	0.48 ± 0.57	0.208

Table 4. The frequency of the most important histopathological lesions found in the study; scores are presented as mean values ± SD; tissue alterations were scored as follows: 0 = none, 1 = mild, 3 = moderate and 5 = severe.

** parameter differed significantly between the two groups (Mann-Whitney U test, P<0.05)*

3.3 Canonical Discriminant Analysis

The Canonical Discriminant Analysis suggested a moderate differentiation among the six compared groups (Figure 4). As can be observed in the Figure, there was a stronger differentiation based on the type of water supply applied, than on the type of feed. Moreover, histopathological changes in fish from ponds with tube well water were on average more uniform than those in fish from ponds with stream water. Two canonical functions (CV) together accounted for 64.5% of the total heterogeneity (CV1 - 33.4% and CV2 - 31.1%). Fish from ponds with stream water were separated from those with tube well water along the first canonical function, mostly by congestions of blood vessels in liver, lifting of epithelium and local proliferations in gills, as well as by dilation of glomerular capillaries in kidneys and liver fibrosis. There was only a low differentiation of groups along the second canonical function, mostly by the fusion of the tips of primary lamellae and oedema in gills, as well as by glomerular necrosis in kidneys.

3.4 Correlations

Correlation between HP indices and water quality parameters are presented in Table 5. There was a positive correlation between the HP index of gills and the total index and EC, pH and COD levels (P<0.05). The liver histopathology index was positively correlated with pH level (P<0.05).

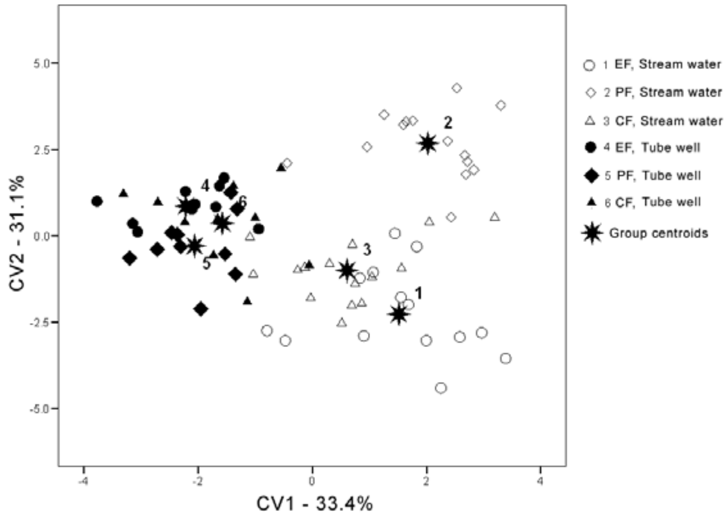

Figure 4. Canonical Discriminant Analysis applied on the histopathological changes in common carp with three different feeds and two different water supplies applied; the input variables are the scores for each specific pathological change in each of the three assessed tissues; CF - cereals; PF - pelleted feed; EF - extruded compound feed.

4. Discussion

Influence of the protein level in supplemented feed on water quality in fish ponds is documented in the literature [22]. It is estimated that 87.9% of the nitrogen input to ponds is accounted to feed [23], and supplemental feed can indirectly influence fish health [24]. Even though two commercial feeds in the present study had a higher protein level than cereals, there were no differences observed among the three ponds with regard to the water quality. The probable reason was that the amount of nitrogen in the water was proportional to the carp biomass [25,26].

Common carp has a broad ecological spectrum and can tolerate extreme environmental conditions. Nevertheless, fish loss is often recorded in semi-intensive aquaculture, and is estimated at 20-40% during rearing season [27]. Fish survival in ponds supplied by water from the tube well was higher than that in ponds with stream water. This loss was partly influenced by a reduced water quality, directly or indirectly by higher pH level, which is reported to reduce fish growth and survival rate at values close to 10.3 [28]. Moreover, other parameters, such as temperature, DO, COD, TAN and UAN, were also frequently outside optimal levels for carp production, as suggested by Boyd [29]. These factors affected vital organs, resulting in HP changes.

Salinity in ponds with tube well water (measured through EC) was well above values appropriate for freshwater, classifying it as sub-saline water. Common carp is moderately tolerant to salinity. Low levels of salinity, such as those measured in ponds with stream water, are usually well tolerated and in some trials even result in weight gain improvement [30]. Common carp is often reared in sub-saline conditions [31]. Higher values, however, such as those detected in ponds with tube well water, are able to affect fish metabolism [32], causing physiological changes and reduced growth [33], although increased mortality was not reported. Besides salinity, carp is also tolerant to low levels of DO and can survive for more than 7 days in the water with 0.5 mg/L of DO [34]. COD levels were high, as compared to natural freshwater, but these levels are

	IG	IL	IK	IT
TAN	-0.19	-0.02	-0.27	-0.30
UAN	0.03	-0.02	-0.09	-0.03
Nitrates	-0.03	0.08	-0.10	-0.06
EC	0.46*	0.24	0.17	0.53*
pH	0.51*	0.34*	0.15	0.60*
DO	0.28	0.15	0.04	0.22
COD	0.33*	0.03	0.10	0.33*

Table 5. Correlation matrix of histopathological indices and water quality parameters; TAN - total ammonia; UAN - unionized ammonia; EC - electroconductivity; DO - dissolved oxygen; COD - chemical oxygen demand; IG - gill histopathology index; IK - kidney histopathology index; IL - liver histopathology index; IT - total index.

**parameters were significantly correlated (Spearman`s correlation test, P<0.05)* tolerated in semi-intensive aquaculture and are related to the quantity of supplemented feed [27]. According to the available literature [35], COD levels are correlated with TAN values. Several studies have documented a relationship between water temperatures and the toxicity of different compounds in aquatic environments, including ammonium [19,36-38].

Mean UAN values in the present study were 0.11 mg/L. Such levels either did not cause histopathological changes in laboratory experiments [39,40], or the changes were only mild [41]. However, the problem is increased ammonia toxicity, when the water pH level is elevated [40]. Therefore, we assume that the pH level had the most important, but indirect effect on histopathology of the gills in this study. In alkaline waters, such as those in ponds supplied by stream water, fish have difficulties with ammonia excretion [42-44]. Even low UAN levels could endanger fish health when pH levels are elevated, due to high ammonia content in blood plasma [45]. This is supported by the fact that fish gill tissue was not affected during the exposure to extreme pH levels (10) when ammonium was not present in the water [40]. A positive correlation between TAN or UAN and HP changes in gills has been established in the literature [40,41,46], however this was not the case in the present study. Instead, a positive correlation was established between HP indices and pH, COD and EC. The potential interaction among these factors could produce a suitable environment for increased UAN toxicity [38], especially in the case of high temperature and pH levels, and low DO values [19,47], as was recorded in ponds with stream water. This was confirmed by HSI values being lower in all ponds with stream water. Such extreme conditions are able to cause histopathological changes and while the recovery is possible, it is often slow and will last only until water quality deteriorates again [48,49]. Other parameters, namely nitrate levels, orthophosphates and total phosphates, total nitrogen, water hardness and transparency were within optimal ranges for fish rearing [29].

Alterations observed in the assessed vital organs were mostly mild and reversible [10]. Necrosis and complete lamellar fusion found in the gills are characterized as severe changes [50]. Necrosis is an irreversible change, but its frequency in the present study was low and necrotic changes were focal. The exception was in the kidneys, with a higher frequency of necrosis than in the other tissues, although there was no difference between the two water supplies.

The most frequent HP alterations in all organs in the present study were circulatory changes. To cope with a low DO level in ponds with tube well water and extreme pH and temperature values in ponds with stream water, carp had to adapt the gill surface to pond conditions [51]. Hyperplasia and lifting of epithelium are the most common alterations found in gill histopathology studies [52]. These changes represent the fastest and easiest adaptations to low water quality, with the purpose of decreasing the respiratory surface and increasing diffusion distance [47]. On the other hand, blood flow in fish gills is much higher during hypoxia or reduced oxygen level, which might explain the higher value of gill circulatory changes in fish from ponds with tube well water [53].Unlike gills, the liver is protected from physical exposure to the external environment [18]. Moreover, since xenobiotics were not present in fish ponds, hepatocyte alterations were rare, with a low frequency of cloudy swelling and macrophage aggregates confirming these findings. However, in contrast to the gills, the most common liver lesions were vascular changes. These are frequently reported in fish exposed to organic contaminants [54], while Koponen *et al*. [55] concluded that the appearance of fibrosis in portal and periportal areas is due to seasonal changes. EGC are often found in connective tissue or in the blood vessels and their presence could indicate response to inflammation [56].

The histopathology of the kidneys did not show sensitivity to the water quality parameters. A number of alterations were observed on both glomerular and tubular tissues. A majority of alterations of nephrons and tubules observed are considered mild and commonly found on freshwater reference sites [57]. The only moderate change was a decrease of the tubular lumen [6], but this lesion had a low frequency in the present study. A presence of regenerative tubules and glomeruli is observed in healthy and young fish [58] and represents evidence of good fish health status.

5. Conclusions

To conclude, the results obtained in the present study have demonstrated that histopathology represents a useful indicator of the water quality in fish ponds where a semi-intensive culture system is practiced. Gills were the most sensitive organ, while the kidney was the least responsive. The majority of HP alterations were mild and reversible.

Despite this, the level of mortality that occurred in ponds with stream water was obviously a consequence of deteriorated water quality, which also induced histological changes. The results of the study indicate that, among the observed water parameters in the fish pond, a crucial role is played by the pH level. In the present study, water quality in ponds with tube well water was worse than in those with stream water, but the pH level was the limiting factor. Although tube well water used had a high salinity, ammonia and low DO levels, vital organ histopathology was not markedly affected. While the average values of water quality parameters were more favorable in ponds with stream water, occasional extreme values of certain parameters caused lower HP indices. Finally, the study confirmed that HP changes are not stressor-specific, they rather indicate all possible interactions among the factors (additive, synergistic, or/and antagonistic). In addition, scoring of HP changes offers an accurate measure of the level of environmental stress.

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