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Original Article

Quantitative analysis of histopathological lesions in rainbow trout (*Oncorhynchus mykiss*) from northern Iran farms

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Abstract: This study was conducted to evaluate the health of rainbow trout on three farms in Mazandaran Province using a quantitative analysis of the histopathological alteration method. The fish were sampled within three classes: <100, 300-700, and >1500 g. Ten fish were sampled per size class from each farm. The fish gill, liver, kidney, and intestine pieces were collected, fixed, sectioned, mounted on slides, and stained for histopathological examinations. A quantitative approach was followed to examine the tissues alternation and fish. The results showed that the organ index of the gill in 300-700 and >1500 g fish was significantly higher than <100 g ones. The organ index of the liver in >1500 g fish was significantly higher than in the other two groups. There was no significant difference in the organ index of the kidney and intestine. Organ index within each fish size was as follow: gill> liver> kidney> intestine. Moreover, the total index was calculated based on the organ index in a fish and, accordingly, <100 g fish had the lowest total index than 300-700 and >1500 g fish. In conclusion, the fish gill exhibited more pathological changes due to direct contact with the ambient environment. Moreover, the fish intestines were generally healthy, suggesting suitable diet quality, although vacuolization and hypertrophy of the hepatocytes show that the dietary fat and feeding rate should be controlled.

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Introduction

The limitation of marine fish stocks and water resources and the increasing global population have forced humans to cultivate a variety of aquatic animals in artificial environments to provide healthy protein with high nutritional value (Paray et al., 2020). Aquaculture accounts for almost half of the global fish production (FAO, 2020). Aquaculture in Iran began importing non-native farmed fish species and has continued improving and promoting various aquaculture methods. Under intensive rearing conditions, fish are exposed to stresses threatening their health. Prolonged exposure to such adverse conditions makes the fish vulnerable to pathogens. Therefore, monitoring the health status of farmed fish is crucial (Hoseini et al., 2016a), as it provides valuable data for improving the rearing practices. Fish should be raised under conditions with minimum nutritional, environmental and infectious problems that guarantee the highest growth rate, feed efficiency, and survival (Hoseini et al., 2016b, c).

There are several methods for assessing the health status of fish. One of these methods is histological examinations. Hence, describing tissue changes in different organs can be an effective tool for assessing the health status of fish (Peyghan and Takamy, 2002; Al-Bairuty et al., 2013; Zepeda-Velázquez et al., 2015; Altun et al., 2017; Hoseini et al., 2019; Ghelichpour et al., 2020; Hoseini et al., 2021). Histopathological observation has been used as a diagnostic tool to assess fish health about environmental pollution, diet quality, and pathogen

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Table 1. Importance factors of the fish organs (Bernet et al., 1999).

Gill Lesions	Importance factor
Lamellar/filament hyperplasia	2
Lamellar/filament hypertrophy	1
Lamellar epithelial lifting/edema	1
Lamellar fusion	2
Aneurysm	1
Curling lamella	1
Branching filament	1
Liver Lesions	
Hepatocyte degeneration	3
Sinusoidal dilation	1
Hemorrhage/Congestion	1
Melanomacrophage aggregation	1
Hepatocyte vacuolization	1
Pyknotic nucleus	2
Hepatocyte hypertrophy	1
Exudate	1
Kidney Lesions	
Bowman's capsule dilation and glomerulus shrinkage	3
Hemorrhage/Congestion	1
Macrophage aggregation	1
Degeneration of hematopoietic tissue and tubular tissue	3
Intestine Lesions	
Hemorrhage	1
Submucosae and mucosae hyperplasia	2
Epithelium degeneration	3

exposure (Elahee and Bhagwant, 2007; Hedayati et al., 2014; Ghelichpour et al., 2016; Hoseini et al., 2016d; Dawood et al., 2021; Mangang and Pandey, 2021). In this regard, histopathological examinations can be used for fish of different sizes and ages, as it is believed lesion severity increases with age (Ahmadivand et al., 2014; Alavian Petroody et al., 2017).

After Chinese carp, Rainbow trout, *Oncorhynchus mykiss* is the main farmed fish (FAO, 2019). Because of the importance of rainbow trout as an aquaculture species in Iran (Hoseini et al., 2020), the present study aimed to assess histopathological alternations in vital organs of the farmed trout during their growth based on their size increase in Mazandaran Province, as one of the main regions of trout culture in Iran.

Materials and methods

Sampling was done in three farms in Tonekabon, Mazandaran Province, north of Iran). Sampling was performed in three weight groups, including <100, 300-700, and >1500 g. From each farm, 30 fish were randomly caught (10 per size class). A sharp blow on the head euthanized fish according to the protocol approved by the Council of Europe. Then, each fish was examined for appearance deformity. Then, the abdominal area of the fish was examined for appearance, including shape and color, and the presence or absence of visible lesions.

Preparation of the tissue sections: After the euthanasia, a gill arch (second arch of the left side) was cut by scissors, washed with distilled water, and fixed in 10% buffered formalin. Then, the scissors opened the abdominal cavity, and a piece of the liver, middle kidney, and posterior part of the intestine was removed, washed with distilled water, and fixed in the buffered formalin. The fixative was renewed after 24 h. A standard protocol was used to prepare the tissues, parafinized blocks, tissue sections (5µ), and slides. Then, the slides were stained using hematoxylin and eosin (H&E) (Eagderi et al., 2013). The sections were analyzed by light microscopy (Di Giulio and Hinton, 2008).

Histopathological analyses: Tissue lesions and

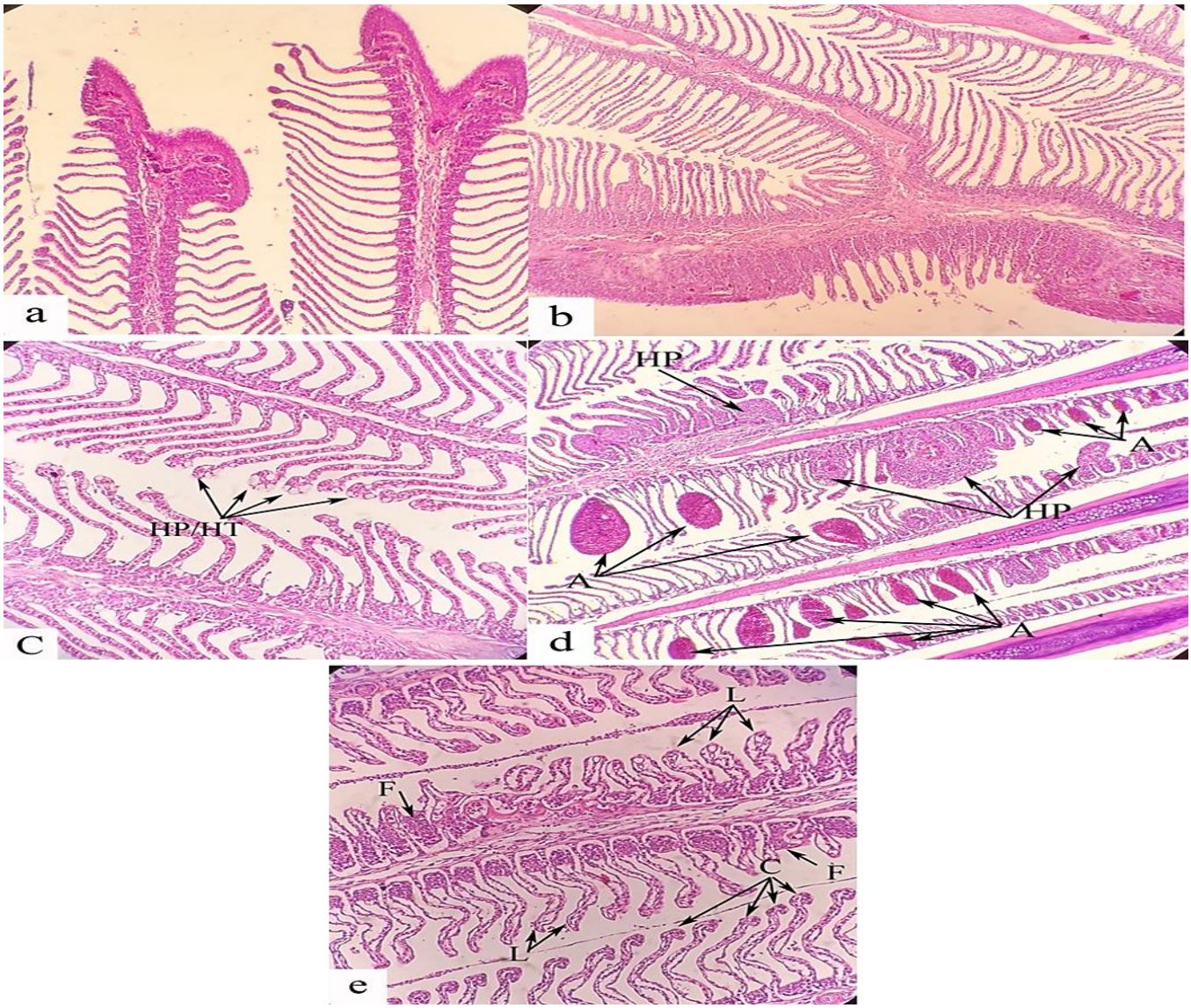

Figure 1. The gill histopathological lesions of three rainbow trout size classes (<100, 300-700, and >1500 g) in north of Iran. a-b) filament branching; c) HP: hyperplasia, HT: hypertrophy; d) HP: hyperplasia, A: aneurysm; e) F: fusion, C: curling lamella, L: lifting epithelial.

alternations were quantitatively analyzed based on Bernet et al. (1999). First, the importance of each lesion was pathologically evaluated from 1 to 3 as importance factor (Table 1). Importance factor 1 implies a low pathological significance: it can be readily reversed after the causative agent is eliminated. Importance factor 2 indicates a moderate pathological significance: tissue damage may often reverse when the agent is eliminated. Importance factor 3 is of great pathological importance. In this condition, the tissue damage is irreversible, and the organ function is generally affected. Also, the tissue changes were ranked into four groups based on the degree and extent of observation (Score value). Number 0 means no change, number 2 means low severity of tissue lesion, number 4 indicates moderate severity, and number 6 indicates severe tissue lesion (Bernet et al., 1999). Therefore, importance factors and score values were determined for each tissue. Then, the organ index was calculated as a sum of each lesion's importance factor × score value. The organ index was used to represent the organ's pathological status. The sum of different organ indexes within a fish provides a total index, which indicates the general health status.

Statistical analyses: All statistical analyses were performed in SPSS v.22 software. The data was analyzed with non-parametric tests. Mann-Whitney U

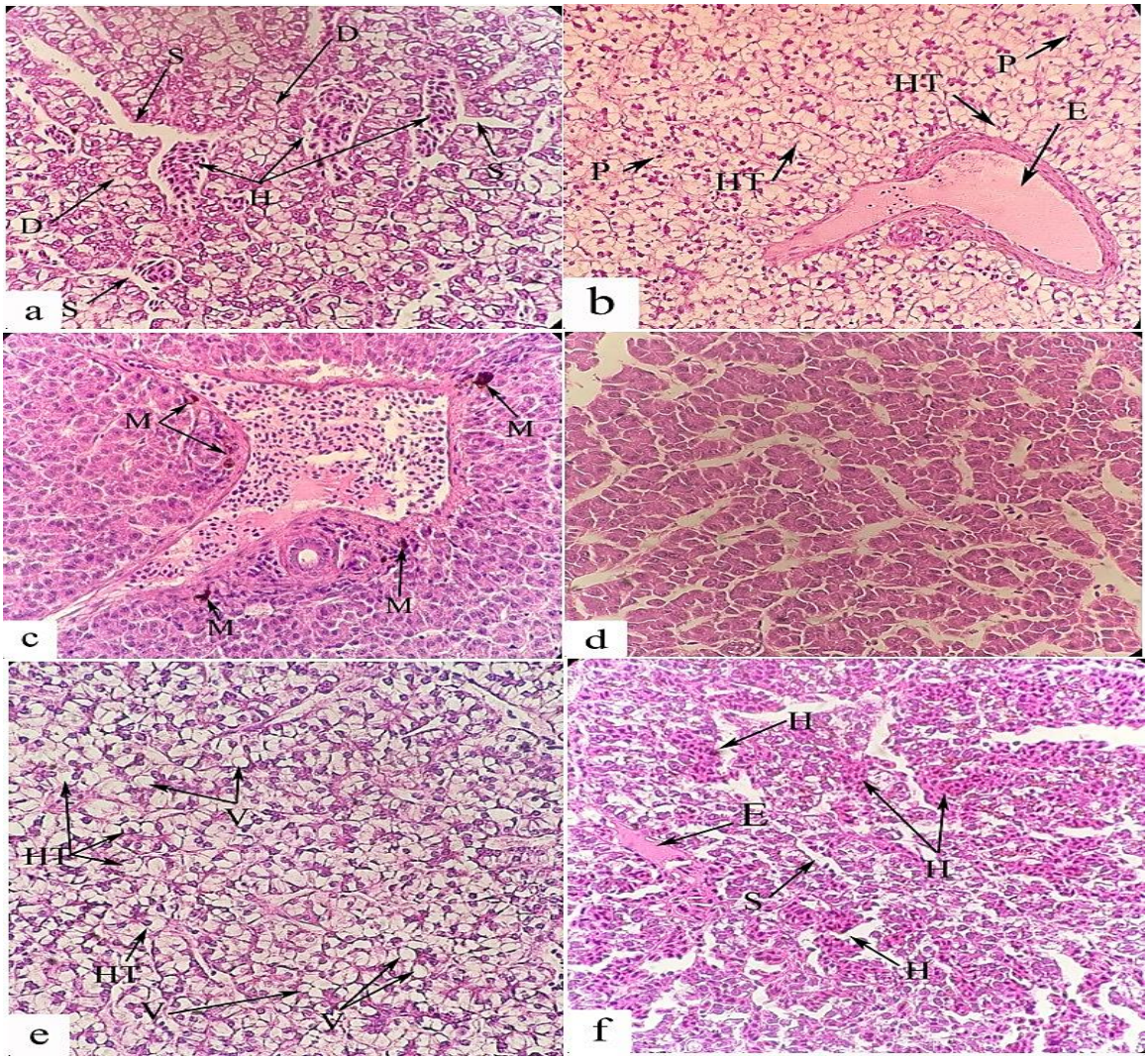

Figure 2. The hepatic histopathological lesions of three rainbow trout size classes (<100, 300-700, and >1500 g) in north of Iran. a) S: sinusoidal dilation, H: hemorrhage, D: hepatocyte degeneration; b) HT: hepatocyte hypertrophy, P: pyknotic nucleus, E: Exudate; c) M: melanomacrophage aggregation; d) sinusoidal dilation; e) HT: hepatocyte hypertrophy, V: hepatocyte vacuolization; f) H: hemorrhage/congestion, E: exudate, S: sinusoidal dilation.

and Kruskal–Wallis tests determined the significant differences among groups.

Results

The lamellar / filament hyperplasia, lamellar / filament hypertrophy, lamellar epithelial lifting / edema, lamellar fusion, aneurysm, curling lamella, and branching filament were alternations observed in the fish gills (Fig. 1). The results indicated that hyperplasia and edema of the epithelial layer were the most common gill lesions, whereas the aneurysm was the second most common lesion. The gill structural deformities, e.g. filament branching, were observed in the larger fish.

The hepatocyte degeneration, sinusoidal dilation, hemorrhage/congestion, melanomacrophage aggregation, hepatocyte vacuolization, pyknotic nucleus, hepatocyte hypertrophy, and exudate were among the histopathological lesions observed in the fish liver (Fig. 2). The hyperemia, sinusoidal dilatation, and hepatocyte vacuolization were the main injuries seen in the hepatic sections; however, fat accumulation was mainly observed in some hepatocytes' cytoplasm. In rare cases, fat vacuoles were occasionally observed in the hepatocytes.

Bowman's capsule dilation and glomerulus shrinkage, hemorrhage/congestion, macrophage aggregation in the form of melanomacrophage or accumulation of macrophages, hemosiderin, degeneration of hematopoietic tissue, and tubular tissue were observed in the kidney sections (Fig. 3).

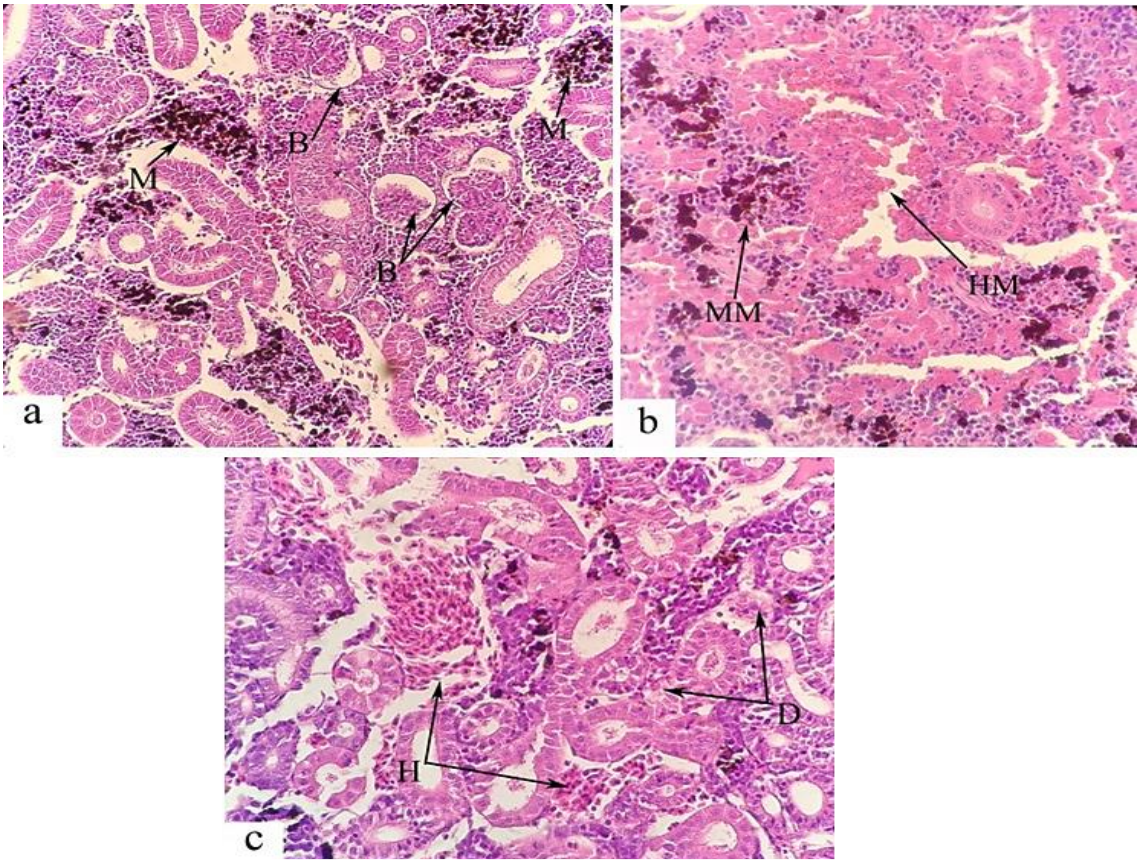

Figure 3. The kidney histopathological lesions of three rainbow trout size classes (<100, 300-700, and >1500 g) in north of Iran. a) M: melanomacrophage aggregation, B: bowman's capsule dilation and glomerulus shrinkage; b) MM: melanomacrophage aggregation, HM: hemosiderin aggregation; c) H: hemorrhage/congestion, D: degeneration.

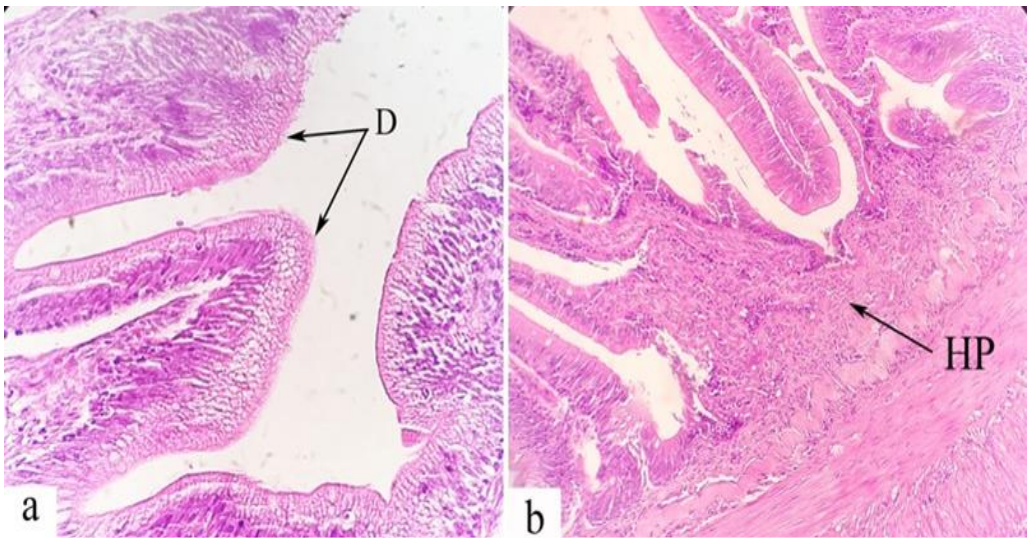

Figure 4. The intestinal histopathological lesions of three rainbow trout size classes (<100, 300-700, and >1500 g) in north of Iran. a) D: epithelium degeneration; b) HP: hyperplasia.

Examination of intestinal tissue sections did not show severe lesions. Histopathological lesions observed in the intestine sections were some alternations such as hemorrhage, sub-mucosae hyperplasia, mucosae hyperplasia, and epithelium degeneration (Fig. 4).

Comparisons between the fish size classes revealed that the gill lesions were more severe in 300-700 g and >1500 g fish than <100 g (Fig. 5). Moreover, >1500 g fish exhibited more severe hepatic lesions than the others. However, there were no size effects on the

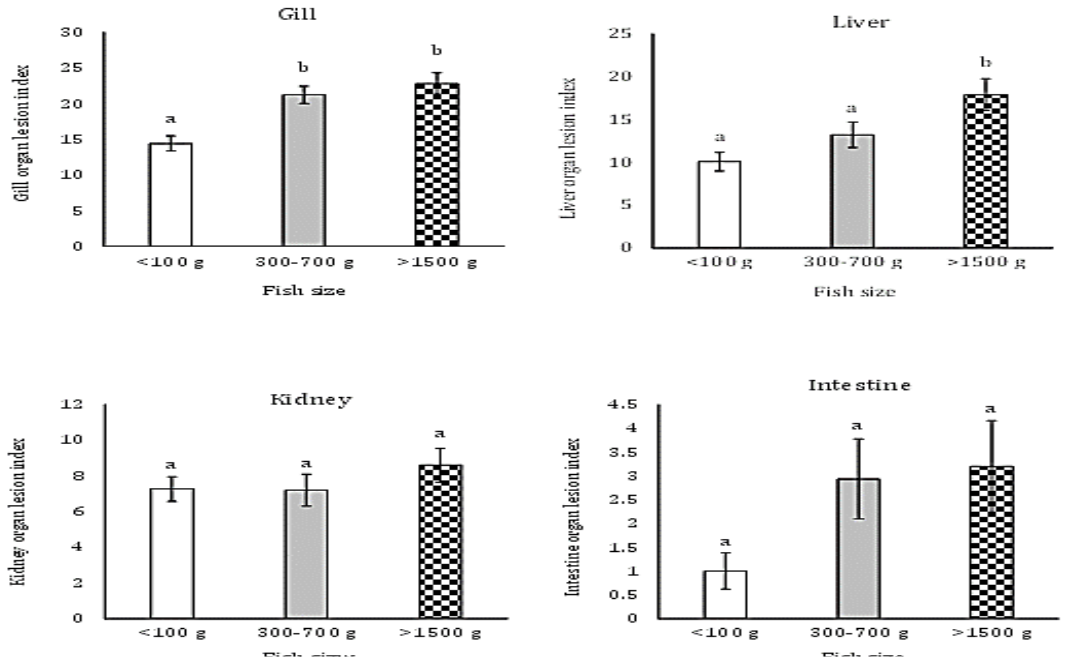

Figure 5. Comparison of the average organ index (mean ± SE) in the gill, liver, kidney, and intestine tissues among three different fish size classes. Different letters above the bars indicate significant differences among the fish size classes (P<0.05; n= 10).

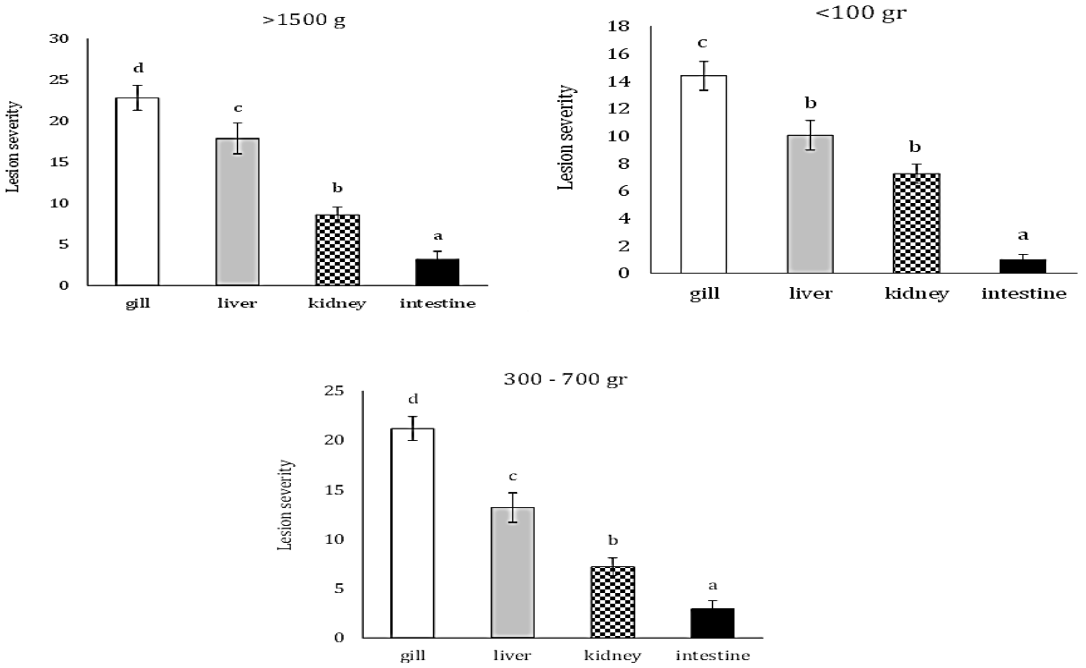

Figure 6. Lesion severity (mean ± SE) in different organs (gill, liver, kidney, and intestine) of fish with different size classes. Different letters above the bars indicate significant differences among different organs (P<0.05; n= 10).

kidney and intestine lesions (Fig. 5). Comparison of organ index within each size class showed that the most severe lesions in <100 g fish were related to the fish gill, followed by liver and kidney. The lowest lesion severity was related to the intestine in <100 g fish. The severity of organs' lesion in 300-700 g and >1500 g fish was as follows: gill> liver> kidney> intestine (Fig. 6). The total index (Fig. 7) indicates that the small fish (<100 g) had less pathological lesions than 300-700 and >1500 g fish.

Discussion

This study investigated the histopathological lesions of rainbow trout's gill, liver, kidney, and intestine on

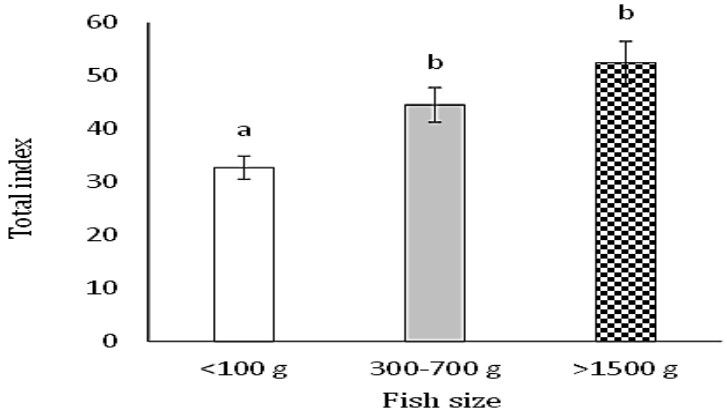

Figure 7. Total index (mean ± SE) of three fish size classes. Different letters above the bars indicate significant differences among different fish size classes (P<0.05; n =10).

three farms in north Iran. Since gill, liver, and kidney have high metabolic activity and important roles in biological transformation and excretion of wastes, they are generally analyzed in most fish health studies. Intestinal tissue is also directly exposed to the substances ingested by fish. Therefore, its health status is very important same as gill, liver and kidney (Wolf et al., 2014).

The histopathological investigations are performed to describe the effect of pollutants on fish in natural water resources and/or diagnose pathogens (Altun et al., 2017; Jovanović et al., 2018; Mokhbatly et al., 2020) and diseases of farmed fish (Hirazawa et al., 2016; Ortega Asencios et al., 2016; Chen et al., 2018). However, monitoring the fish health during the culture period may be important, as it provides a picture of general fish health. In this regard, Saraiva et al. (2015) found several histopathological lesions in different organs of farmed seabass (*Dicentrarchus labrax*); whereas, Saraiva et al. (2016) found no significant pathological lesions in farmed turbot (*Scophthalmus maximus*), suggesting context-dependency of the pathological lesions.

The present study used the histopathological method to analyze the health of rainbow trout as a valuable species in Mazandaran Province (north of Iran) fish farms. Most publications report histopathological lesions qualitatively (Al-Bairuty et al., 2013; Altun et al., 2017; Chen et al., 2018); however, quantification of the lesions provides a standard tool to analyze the fish health statues. Furthermore, as many lesions are organ-specific, quantitative approaches enable one to compare different organ health status (Bernet et al., 1999). The highest organ index was observed in the fish gills in this study. These results are in line with those obtained by Saraiva et al. (2015), who showed that the gill was the most damaged organ in farmed seabass. Since the gill is in direct contact with the aquatic environment, water condition has greater and faster effects than the other organs. Therefore, gills are remarkably vulnerable to disturbing, toxicological and infectious agents (Wolf et al., 2014). However, the regenerative capabilities of gill tissue because injured gills are capable of recovering the injuries somewhat (Wolf et al., 2014).

The liver was observed as the second organ in lesion severity due to its role as the center of metabolic activity, detoxification, and regulation of many physiological activities (Hoseini et al., 2014; Ghelichpour et al., 2020). The third lesion severity was related to the kidney, which may be associated with the main function of the fish kidney in osmoregulation (Bernet et al., 1999). Overall, gill, liver, and kidney have high metabolic activity and important roles in biotransformation and exclusion of harmful substances of endogenous and exogenous origin (Wolf et al., 2014). The present results demonstrated that the examined fish had generally normal intestines, in line with studies on farmed

seabass Saraiva et al. (2015) and turbot Saraiva et al. (2016).

The total index showed that histopathological lesions of the <100 g fish were significantly lower than 300-700 and >1500 g fish, which may be because younger fish have experienced un-favor conditions for a shorter period than older fish, and it is generally believed that histopathological lesions of an organism magnify over time. Moreover, small fish are more susceptible than the larger ones; therefore, feed suppliers provide more qualified feed for small fish, supporting them in a higher health condition. In conclusion, the present results demonstrated that farmed trout have some histopathological lesions, which increase the fish size. The main reasons for such lesions are not clear, and further studies are encouraged on monitoring the farm water quality, the fish diets and the presence of specific pathogens.

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