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Comparative study on the biochemical factors and antioxidant enzymes of rainbow trout eggs and larvae in a recirculating and flow-through system

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ABSTRACT

Recirculating aquaculture systems (RASs) may cause stressful conditions, by which the biochemical compositions and immune system of the fishes can be influenced. Thus, in this research, effects of water reusing on the biochemical compositions and antioxidant enzyme activities of rainbow trout (*Oncorhynchus mykiss*) eggs and yolk-sac larvae in an airlift-based recirculating system and a flow-through system were investigated. Eggs and larvae were sampled weekly at 1, 7, 14, 21, 28, 35, and 42 days after fertilization (DAF) in triplicate for analyses of both biochemical and antioxidant enzyme activities. The results indicated that there were no significant differences between the two systems in terms of the major biochemical composition of the eggs and larvae, except for the total lipid at 42 DAF, which was higher in the flow-through system. In both systems, the moisture values of samples increased progressively during the experimental period. The total lipid values in the embryonic stage (at 1, 7, 14, and 21 DAF) were statistically constant, whereas in the larval stage (at 28, 35, and 42 DAF), increased significantly in both systems. There were no significant differences between the systems and amongst the sampling times during the study period in terms of ΣSFA, ΣMUFA, and ΣPUFA. The values of TAC, SOD, CAT, and GPX in both systems were statistically constant during the embryonic stage, whereas there were significant differences between the embryonic and larval stages. In addition, the antioxidant enzyme activities revealed significant differences between the experimental systems, with an exception of the SOD activity, on which effects of treatment were not significant. In conclusion, there were no significant differences between the flow-through and airlift-based recirculating systems in terms of biochemical compositions, fatty acid profiles, and antioxidant enzyme activities during the embryonic stage. Thus, the designed recirculating system was practically efficient in this stage. While, the lipid content and antioxidant enzyme activities of the yolk-sac larvae reared under the recirculating system were respectively lower and higher than of the larvae reared under the flow-through system, which was more likely due to the accumulation of ammonia in the recirculating system.

1. Introduction

Cultured fishes are subject to the surrounding water possessing several physiochemical factors that might be influenced by the management strategies and the environmental conditions. Fluctuating of the physiochemical factors like temperature, dissolved oxygen, pH, light, ammonia, solids, and salinity behind the normal range, can be stressful and affect the health and immune system of fish (Kolayli and Keha, 1999; Magnadottir, 2010; Uribe et al., 2011; Sinha et al., 2014).

An imbalance between oxidative and reductive processes makes oxidative stress in fish. In this situation, the immune system cannot counteract the increased reactive oxygen species (ROS) levels, and oxidative stress takes place. Consequently, levels of lipid hydroperoxides increase that can be estimated via the measuring of malondialdehyde (MDA). MDA is produced from the peroxidization of polyunsaturated fatty acids (PUFA), mainly arachidonic acid. Therefore, the end-products of the lipid peroxidation is malondialdehyde. In recent studies, the relationships amongst the reactive oxygen species, MDA content, and free radical damage were well demonstrated (Sinha et al., 2014).

Fish possesses a wide range of antioxidant defense systems to protect themselves from the harmful effect of reactive oxygen species (Tort, 2011). This system consist of non-enzymatic antioxidants such as vitamin C and E, and enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione-s-transferase (GST), and catalase (CAT) (Sinha et al., 2014; Zengin et al., 2015, 2016).

The stress response applies to a wide range of physiological

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mechanisms, including gene and protein changes, metabolism, energetic, immune, endocrine, neural and even behavioral changes that will first try to overcome that situation and then compensate for the imbalances produced by either the stressor or the consequences generated by the first array of responses (Tort, 2011).

Recirculating aquaculture systems offer several advantages such as reduction in water consumption, land requirement, and fuller control on water quality and pathogens (Irani and Agh, 2019). However, rearing of fish in continuously recycled water might cause stressful conditions such as low or high levels of physicochemical factors and accumulation of the substances that are released by the fish into the water. The body composition and antioxidant enzyme activities of fishes that are subject to these stressful conditions can be affected. Thus, in this study, we constructed an airlift-based recirculating hatchery system and investigated the biochemical factors, fatty acid composition, and antioxidant enzyme activities of Rainbow trout (*Oncorhynchus mykiss*) eggs and larvae compared with a flow-through system.

2. Material and methods

2.1. Experimental conditions

This research was conducted in the Artemia & Aquaculture Research Institute of Urmia University, Urmia, Iran. The study was performed at egg incubation and yolk-sac larvae stages of rainbow trout. Two egg incubation systems involved in an airlift-based recirculating system and a flow-through system with four trays were used (Irani and Agh, 2020). Each tray of flow-through and recirculating system received 5000 rainbow trout fertilized eggs, and they were maintained to yolk-sac depletion under the experimental conditions. During the experimental period, in the flow-through and recirculating systems, the temperature ranged from 11.36–13.51 °C and 10.83–12.59 °C, respectively, the pH values ranged from 7.84–8.07 and 8.24–8.59, respectively, and the dissolved oxygen values ranged from 7.77–8.92 mg/l and 6.86–9.10 mg/l, respectively. The values of total ammonia nitrogen (TAN) and nitrite nitrogen (NO_2-N) in the flow-through system, ranged from 0.032–0.074 mg/l and 0.004–0.0091 mg/l, respectively, and in the recirculating system, ranged from 0.189–1.676 mg/l and 0.04–0.289 mg/l, respectively.

2.2. Sampling and measurements

Ten eggs and larvae were sampled weekly at 1, 7, 14, 21, 28, 35, and 42 DAF for analyses of the biochemical composition and antioxidant activities. The samples were frozen immediately in the liquid nitrogen and stored at −80 until analysis. Fatty acid profiles, MDA levels, and antioxidant enzyme activities involving total antioxidant enzyme capacity, superoxide dismutase, glutathione peroxidase, and catalase were analyzed.

The analyses of biochemical parameters, including moisture, ash, crude protein, and total lipid of eggs and larvae, were carried out based on standard methods of AOAC (AOAC, 1995). Body moisture was calculated via drying the samples for 16 h at 105 °C in the oven (AMB50; ADAM, Milton Keynes, UK). Ash content was measured by incineration in a muffle furnace at 600 °C for 6 h. Crude protein was determined by the Kjeldahl procedure ($N \times 6.25$) using an automatic Kjeldahl system (Gerhardt, VAR 300; Germany), and total lipid by ether extraction method using a Soxhlet (Barnstead/Electrothermal, UK).

For analysis of fatty acid compositions, total lipids from the samples of eggs and larvae were extracted by homogenization in chloroform/methanol (2:1, *v*/v). Then, methyl esters were prepared by transmethylation using methanolic KOH and n-heptane. The fatty acid compositions were determined using an auto sampler gas chromatography (GC, Agilent technologies 7890 A, USA), equipped with a flame ionization detector (FID) and a cyanopropyl-phenyl capillary column (DB-225MS, 30 m × 0.250 mmID × 0.25 μm Film thickness, USA). Identification of the fatty acids was done by comparing their retention time with those of an external commercial standard mixture (Agh et al., 2014).

For analysis of antioxidant enzyme activities, malondialdehyde content, and soluble protein levels, the samples of eggs and larvae were homogenized in ice-cold lysing buffer (1:10). The homogenates were centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatants were collected and stored at −80 °C for further analysis. All assays were carried out using a microplate reader (BioTek, Synergy HT, USA) and commercial kits (Navand Lab Kit, Navand Salamat Co., Iran) in triplicate.

2.3. Statistical analysis

Statistical analyses were carried out using SPSS 22. Homogeneity of variances and normality of distribution were tested using the Levene's test and Shapiro-Wilk test, respectively. The mean values of the sampling times (from first to 42 DAF) and treatments (the flow-through and recirculating systems) were analyzed using two-way repeated measures ANOVA. Once the effects of time, treatment, and time*treatment interactions were significant, one-way ANOVA and Tukey's *post-hoc* test ($\alpha = 0.05$) was used to separate the significantly different values of the sampling times, and independent-samples *t*-test ($\alpha = 0.05$) was used to distinguish the significantly different values of the two systems at the same sampling time (Irani and Agh, 2020).

3. Results

3.1. Biochemical factors

Results of repeated measures ANOVA testing (Table 1) for the biochemical factors (moisture, ash, crude protein, and total lipid) indicated that time*treatments interactions were not significantly different ($p < .05$), except for the total lipid. The effects of the time were significant ($p < .05$) for the biochemical factors, except for the ash. However, the effects of treatment were not significant for these factors, except for the total lipid.

Analysis of independent-samples *t*-test ($p < .05$) for the biochemical factors exhibited that there were no significant differences between two systems, except for the total lipid at 42 DAF that was higher in the flow-through system. One-way ANOVA testing ($p < .05$) showed that

Table 1
Repeated measures ANOVA testing for the biochemical factors during the study period.

Source		df	Mean square	*F*-value	*P*-value	Partial eta squared
Moisture	Time	2.39	319.40	76.36	0.000	0.95
	Error (time)	39.94	9.55			
	Treatment	1.00	7.21	0.14	0.73	0.03
	Error (treatment)	4.00	52.54			
	Time * treatment	2.39	1.30	0.31	0.78	0.07
Ash	Time	2.49	0.97	3	0.088	0.43
	Error (time)	9.98	0.32			
	Treatment	1.00	0.86	5.76	0.074	0.59
	Error (treatment)	4.00	0.08			
	Time*treatment	6.00	0.10	0.76	0.60	0.16
Total lipid	Time	6.00	14.32	58.01	0.000	0.94
	Error (time)	24.00	0.25			
	Treatment	1.00	0.39	11.39	0.028	0.74
	Error (treatment)	4.00	0.04			
	Time * treatment	6.00	0.75	3.04	0.023	0.43
Crude protein	Time	2.38	10.19	3.12	0.085	0.44
	Error (time)	9.53	3.27			
	Treatment	1.00	1.69	0.53	0.51	0.12
	Error (treatment)	4.00	3.21			
	Time * treatment	2.38	7.18	2.20	0.16	0.35

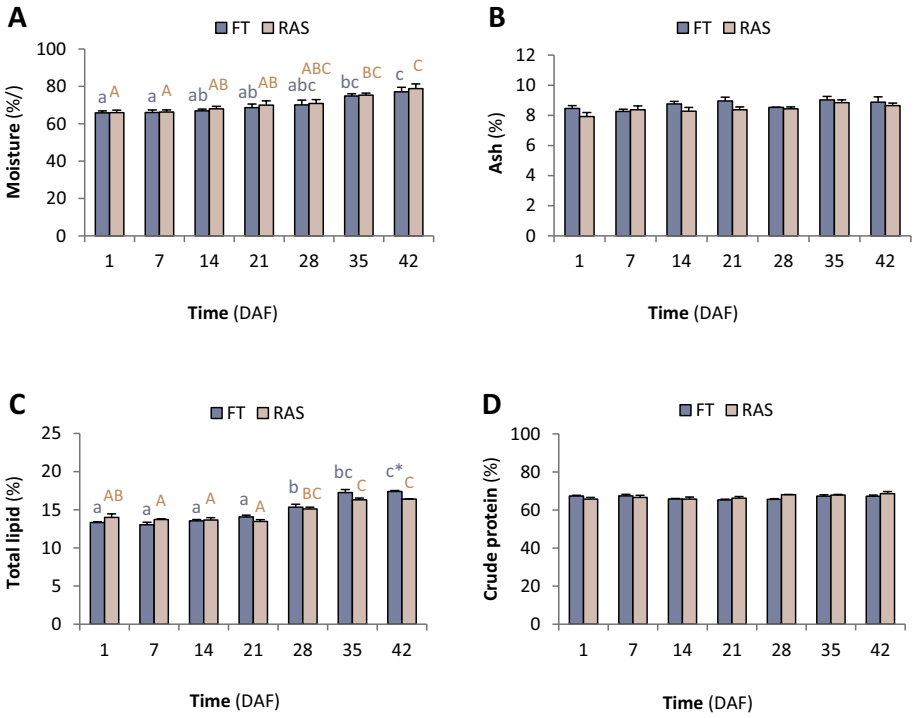

Fig. 1. The values of moisture (A), ash (B), total lipid (C), and crude protein (D) of rainbow trout eggs and larvae in the flow-through (FT) and recirculating (RAS) systems. Different letters (small letters for the FT and capital letters for the RAS) indicate significant differences amongst the sampling times (one-way ANOVA, $\alpha = 0.05$) in each system. * indicates significant differences between the treatments (independent-samples *t*-test, $\alpha = 0.05$) at the same sampling time.

the values of the moisture (Fig. 1A) and the total lipid (Fig. 1C) during the experimental period had significant differences in both systems, whereas there were no significant differences amongst the values of both ash (Fig. 1B) and total protein (Fig. 1D).

The values of moisture during the embryonic stage (i.e., at 1, 7, 14, and 21 DAF) ranged from 65.83–68.60% in the flow-through system, and 65.97–69.93% in the recirculating system, whereas the moisture levels increased to 77.10% and 78.83% at the end of yolk-sac stage, respectively. During the experimental period, the ash values ranged from 8.26–9.03% and 7.92–8.85% in the flow-through and recirculating systems, respectively. The total lipid values in the yolk-sac larvae stage were significantly more than in the embryonic stage at both systems. The values ranged from 13.03–17.40% in the flow-through system and 13.46–16.39% in the recirculating system. The crude protein values in the flow-through and recirculating systems ranged from 65.32–67.46% and 65.71–68.61%, respectively.

3.2. Fatty acid profiles

The values of total saturated fatty acids (ΣSFA) of embryos and larvae ranged from 18.68–19.60% and 18.62–19.91% in the flow-through and recirculating systems, respectively (Table 2). There were no significant differences between the two systems and amongst the sampling times in terms of ΣSFA. No significant differences were observed between the experimental systems and during the study period in terms of total monounsaturated fatty acids (ΣMUFA). The values in the flow-through and recirculating systems ranged from 45.93–48.24% and 46.56–47.89%, respectively.

The values of C18:2n-6, dominant PUFA-n-6, from the hatching time onwards (i.e., at 28, 35, 42 DAF) were significantly more than the first two weeks in the flow-through system. Conversely, the values of C20:2n-6 at the first two weeks was significantly more than at the last two weeks in this system.

There were no significant differences amongst the sampling times in terms of n-3 series PUFA in the flow-through system, whereas in the recirculating system, the values of C20:3n-3 and C20:5n-3 in the whole yolk-sac larvae stage were significantly lower than at 1 DAF. The values exhibited no differences between the experimental systems.

The values of total poly unsaturated fatty acids (ΣPUFA) ranged from 42.51–44.68% in the flow-through system, and from 42.30–44.72% in the recirculating system. There were no significant differences between the systems and amongst the sampling times.

The values of Σn-3/Σn-6 ratio at the hatching time (i.e., 28 DAF) were significantly lower than at both first and last two weeks in the flow-through system, whereas there were no significant differences in the recirculating system during the study period (Table 2).

3.3. Antioxidant enzymes and AMD

Results of repeated measures ANOVA testing (Table 3) for the antioxidant enzyme activities (i.e., total antioxidant capacity, superoxide dismutase, glutathione peroxidase, and catalase) indicated that the activity of these enzymes influenced significantly by time ($p < .05$). The effects of treatment and time*treatment interactions on the antioxidant enzyme activities and MDA levels were significantly different, except for the SOD, on which effects of treatment were not significant (Table 3).

One-way ANOVA testing ($p < .05$) showed that there were significant differences amongst the values of total antioxidant capacity (Fig. 2A), superoxide dismutase (Fig. 2B), glutathione peroxidase (Fig. 2C), and catalase (Fig. 2D) in terms of the sampling time. The peak level of TAC in both flow-through (41.71 nmol/mg protein) and recirculating (64.64 nmol/mg protein) systems was observed at 42 DAF, having a significant difference in compare to other sampling times. The TAC values under the recirculating system at 35 and 42 DAF were significantly more than under the flow-through system at the same sampling time (independent sample *t*-test, $p < .05$). Like TAC, the peak levels of the SOD and CAT in both flow-through (73.38 nmol/mg protein and 179.26 nmol/min/mg protein, respectively) and recirculating (94.37 nmol/mg protein and 307.81 nmol/min/mg protein, respectively) systems occurred at 42 DAF, having significant differences compared with other sampling times. The SOD and CAT values increased from the hatching time onwards, and the values at 42 DAF in the recirculating system were significantly more than in the flow-through system. The peak level of GPX in the flow-through system (30.80 nmol/min/mg protein) was observed at 42 DAF that showed

Table 2
One-way ANOVA and independent samples *t*-test analysis for the fatty acids of rainbow trout eggs and larvae during the study period.

Fatty acid		1	7	14	21	28	35	42
C14:0	FT	0.34 ± 0.02*	0.34 ± 0.01	0.34 ± 0.01	0.36 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.34 ± 0.02
	RAS	0.31 ± 0.04	0.36 ± 0.01	0.32 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.35 ± 0.01	0.33 ± 0.01
C16:0	FT	12.75 ± 0.10	12.37 ± 0.19	12.25 ± 0.19	12.73 ± 0.10	12.11 ± 0.06	12.82 ± 0.20	12.93 ± 0.72
	RAS	11.87 ± 0.47^{a}	12.12 ± 0.12^{ab}	12.56 ± 0.10^{ab}	12.95 ± 0.01^{ab}	12.80 ± 0.26^{ab}	13.33 ± 0.50^{b}	12.63 ± 0.15^{ab}
C18:0	FT	6.51 ± 0.05^{b}	6.49 ± 0.11^{b}	6.10 ± 0.13^{ab}	6.34 ± 0.05^{ab}	6.27 ± 0.29^{ab}	5.77 ± 0.07^{ab}	5.63 ± 0.31^{a}
	RAS	6.47 ± 0.08	6.17 ± 0.04	5.74 ± 0.48	5.61 ± 0.50	6.34 ± 0.12	6.23 ± 0.10*	5.74 ± 0.01
ΣSFA	FT	19.60 ± 0.06	19.20 ± 0.11	18.68 ± 0.17	19.43 ± 0.15	18.69 ± 0.22	18.92 ± 0.28	18.90 ± 0.28
	RAS	18.65 ± 0.42	18.65 ± 0.09	18.62 ± 0.57	18.92 ± 0.50	19.49 ± 0.35	19.91 ± 0.60	18.70 ± 0.14
C16:1n-7	FT	2.33 ± 0.06	2.40 ± 0.03	2.43 ± 0.03	2.52 ± 0.01	2.30 ± 0.09	2.45 ± 0.01	2.50 ± 0.05
	RAS	2.02 ± 0.27	2.41 ± 0.05	2.28 ± 0.15	2.52 ± 0.02	2.62 ± 0.06	2.59 ± 0.05	2.46 ± 0.04
C18:1n-9	FT	21.17 ± 0.34	22.10 ± 0.21	22.62 ± 0.42	22.95 ± 0.20	23.27 ± 0.34	22.03 ± 0.43	21.14 ± 2.21
	RAS	22.27 ± 0.54	22.76 ± 0.54	22.92 ± 0.36	22.98 ± 0.27	22.02 ± 0.83	21.01 ± 0.26	22.82 ± 0.07
C18:1n-7	FT	2.44 ± 0.06	2.37 ± 0.03	2.47 ± 0.07	2.44 ± 0.02	2.49 ± 0.03	2.54 ± 0.06	2.67 ± 0.17
	RAS	2.46 ± 0.06	2.66 ± 0.06	2.68 ± 0.14	2.48 ± 0.01	2.61 ± 0.11	2.46 ± 0.13	2.31 ± 0.23
C20:1n-9	FT	1.04 ± 0.01^{cd}	1.10 ± 0.03^{d}	0.96 ± 0.01^{bc}	0.91 ± 0.02^{b}	0.86 ± 0.05^{b}	0.73 ± 0.01^{a}	0.72 ± 0.01^{a}
	RAS	1.16 ± 0.06^{d}	1.09 ± 0.01^{cd}	1.05 ± 0.05^{cd}	1.01 ± 0.01^{bc}	0.87 ± 0.02^{ab}	0.81 ± 0.02^{a}	0.75 ± 0.02^{a}
ΣMUFA	FT	46.58 ± 0.41	47.21 ± 0.28	47.15 ± 0.45	48.24 ± 0.02	47.63 ± 0.21	46.67 ± 0.66	45.93 ± 3.13
	RAS	46.56 ± 0.37	47.57 ± 0.86	47.55 ± 0.24	47.89 ± 0.21	47.62 ± 0.48	46.78 ± 0.61	47.05 ± 0.45
C18:2n-6	FT	15.29 ± 0.02^{a}	15.26 ± 0.26^{a}	16.01 ± 0.10^{ab}	15.67 ± 0.08^{ab}	16.28 ± 0.04^{b}	16.15 ± 0.04^{b}	16.44 ± 0.30^{b}
	RAS	15.51 ± 0.20	16.05 ± 0.41	16.71 ± 0.27	15.53 ± 0.24	15.56 ± 0.13	16.26 ± 0.53	16.59 ± 0.18
C20:2n-6	FT	1.86 ± 0.04^{cd}	1.94 ± 0.02^{d}	1.79 ± 0.07^{bcd}	1.72 ± 0.03^{abc}	1.67 ± 0.01^{abc}	1.59 ± 0.06^{ab}	1.52 ± 0.01^{a}
	RAS	2.06 ± 0.12^{b}	1.92 ± 0.08^{ab}	1.82 ± 0.10^{ab}	1.77 ± 0.01^{ab}	1.71 ± 0.04^{a}	1.63 ± 0.03^{a}	1.63 ± 0.03^{a}*
C20:4n-6	FT	8.73 ± 0.25	8.56 ± 0.15	8.28 ± 0.13	8.20 ± 0.05	8.59 ± 0.26	8.68 ± 0.18	8.46 ± 0.18
	RAS	9.06 ± 0.44	8.52 ± 0.10	8.78 ± 0.26	8.39 ± 0.07	8.48 ± 0.29	8.83 ± 0.05	8.42 ± 0.08
Σn-6 PUFA	FT	25.88 ± 0.20	25.76 ± 0.09	26.07 ± 0.26	25.56 ± 0.07	26.54 ± 0.23	26.27 ± 0.32	26.41 ± 0.49
	RAS	26.64 ± 0.74	26.50 ± 0.31	27.31 ± 0.44	25.69 ± 0.32	25.74 ± 0.20	26.71 ± 0.51	26.64 ± 0.28
C18:3n-3	FT	1.29 ± 0.06	1.30 ± 0.06	1.30 ± 0.01	1.29 ± 0.01	130 ± 0.02	1.30 ± 0.01	1.38 ± 0.04
	RAS	1.28 ± 0.03	1.32 ± 0.04	1.34 ± 0.02	1.27 ± 0.03	1.28 ± 0.01	1.34 ± 0.05	1.34 ± 0.02
C20:3n-3	FT	0.09 ± 0.004	0.10 ± 0.003	0.09 ± 0.013	0.09 ± 0.014	0.08 ± 0.004	0.08 ± 0.009	0.08 ± 0.004*
	RAS	0.10 ± 0.011^{b}	0.10 ± 0.008^{b}	0.08 ± 0.010^{ab}	0.08 ± 0.010^{ab}	0.05 ± 0.015^{ab}	0.06 ± 0.003^{ab}	0.05 ± 0.003^{a}
C20:5n-3	FT	1.56 ± 0.02	1.48 ± 0.02	1.49 ± 0.01	1.43 ± 0.01	1.50 ± 0.05	1.46 ± 0.02	1.46 ± 0.07
	RAS	1.60 ± 0.08^{b}	1.49 ± 0.01^{ab}	1.46 ± 0.01^{ab}	1.44 ± 0.01^{ab}	1.45 ± 0.04^{ab}	1.39 ± 0.01^{a}	1.38 ± 0.03^{a}
C22:6n-3	FT	15.14 ± 0.20	14.73 ± 0.22	14.35 ± 0.03	14.14 ± 0.15	14.11 ± 0.07	15.30 ± 0.42	15.34 ± 0.45
	RAS	15.10 ± 0.85	14.22 ± 0.77	14.11 ± 0.05	13.81 ± 0.32	14.29 ± 0.49	14.82 ± 0.44	14.74 ± 0.12
Σn-3 PUFA	FT	18.09 ± 0.16	17.61 ± 0.18	17.23 ± 0.03	16.96 ± 0.17	16.98 ± 0.11	18.11 ± 0.44	18.27 ± 0.55
	RAS	18.08 ± 0.91	17.13 ± 0.73	16.98 ± 0.07	16.61 ± 0.28	17.08 ± 0.53	17.63 ± 0.41	17.52 ± 0.17
ΣPUFA	FT	43.97 ± 0.35	43.37 ± 0.09	43.31 ± 0.29	42.51 ± 0.10	43.52 ± 0.34	44.38 ± 0.76	44.68 ± 1.04
	RAS	44.72 ± 1.64	43.63 ± 0.44	44.29 ± 0.37	42.30 ± 0.04	42.83 ± 0.73	44.34 ± 0.11	44.15 ± 0.45
Σn-3/Σn-6	FT	0.70 ± 0.001^{b}	0.68 ± 0.009^{bc}	0.66 ± 0.008^{ab}	0.66 ± 0.006^{ab}	0.64 ± 0.002^{a}	0.69 ± 0.009^{bc}	0.69 ± 0.008^{bc}*
	RAS	0.68 ± 0.016	0.65 ± 0.035	0.62 ± 0.013	0.65 ± 0.019	0.66 ± 0.016	0.66 ± 0.028	0.65 ± 0.001
ΣSAT/ΣUNSAT	FT	0.22 ± 0.001	0.21 ± 0.001	0.21 ± 0.002	0.21 ± 0.002	0.21 ± 0.002	0.21 ± 0.003	0.21 ± 0.002
	RAS	0.21 ± 0.008	0.20 ± 0.001	0.20 ± 0.005	0.21 ± 0.005	0.22 ± 0.003	0.22 ± 0.005	0.21 ± 0.002

Different letters in each row indicate significant differences (one-way ANOVA, $\alpha = 0.05$).
* Indicates significant differences between the treatments (independent-samples *t*-test, $\alpha = 0.05$) at the same sampling time.

significant differences compared with the other sampling times, except 35 DAF. In the recirculating system, the peak level occurred at 14 DAF (16.18 nmol/min/mg protein), the values decreased afterwards and then increased slightly again at 42 DAF.

The values of MDA in the recirculating system exhibited no significant differences during the experimental period, whereas in the flow-through system, the differences of values were significant. The peak level in this system, occurred at 7 DAF (38.01 nmol/mg protein), showing significant differences compared with 1, 35, and 42 DAF. In addition, there were significant differences between the systems in terms of the MDA values at 7, 14, and 21 DAF (Fig. 2F).

4. Discussion

Recirculating aquaculture systems in spite of many advantages (Irani and Agh, 2019) may cause stressful conditions such as fluctuations in the levels of dissolved oxygen, pH, and nitrogen compounds, by which the immune system of cultured fish can be influenced. (Kolayli and Keha, 1999) reported that the antioxidant capacity in rainbow trout might be related to the physical and chemical characteristics of environment. In their study, the antioxidant activities and MDA levels of rainbow trout were influence by salinity. Dissolved oxygen level in the rearing water plays an important role in adjusting the immune response (Bowden, 2008). Fluctuations in pH levels can make different results in immune response, such as levels of lysozyme and IgM (Uribe et al., 2011). Ammonia ranks second after oxygen in importance in water quality assessment. This is so because ammonia is toxic to fish. If it accumulates in rearing water, it hinders ammonia excretion in fish and net uptake of ammonia from the environment occurs. Moreover, increased blood and tissue ammonia levels can damage tissues and, by affecting various physiological processes, increases the generation of reactive oxygen species and activation of the antioxidant enzymes (Sinha et al., 2014). These conditions can occur in the recirculating systems because of insufficient water purification. Thus, in this research, effects of water reusing on the biochemical compositions and antioxidant enzyme activities of rainbow trout eggs and yolk-sac larvae in an airlift-based recirculating system were investigated and compared to a flow-through system.

The moisture values the yolk-sac larvae were significantly more than of the embryos, this agrees with the findings of Dayal et al. (2003). They reported that the dry matter of Asian seabass (*Lates calcarifer*) decreased from the fertilized eggs to the beginning of exogenous feeding. The increasing values of moisture during the early life stages is a normal phenomenon in the freshwater fish, having massive yolk sac, as during the vitellogenesis process, nutrients are deposited into the oocyte in the compressing manner to occupy little space. However,

Table 3
Repeated measures ANOVA testing for the antioxidant enzyme activities and ADM contents during the study period.

Source		df	Mean square	*F*-value	*P*-value	Partial eta squared
TAC	Time	3.24	2895	377	0.000	0.99
	Error (time)	12.96	7.67			
	Treatment	1	256	466	0.000	0.99
	Error (treatment)	4	0.55			
	Time * treatment	3.24	182	23.77	0.008	0.86
SOD	Time	6	3808	3188	0.000	0.99
	Error (time)	24	1.20			
	Treatment	1	113	5.04	0.088	0.56
	Error (treatment)	4	22.51			
	Time * treatment	6	93.94	78.63	0.000	0.95
GPX	Time	6	71.55	5.73	0.001	0.59
	Error (time)	24	12.48			
	Treatment	1	437	151	0.000	0.97
	Error (treatment)	4	2.90			
	Time * treatment	6	97.59	7.82	0.000	0.66
CAT	Time	2.40	107,146	417	0.000	0.99
	Error (time)	9.60	257			
	Treatment	1	4414	31.47	0.005	0.89
	Error (treatment)	4	140			
	Time * treatment	2.40	8530	33.18	0.000	0.89
MDA	Time	6	95.83	1.50	0.22	0.27
	Error (time)	24	63.88			
	Treatment	1	1071	36	0.004	0.90
	Error (treatment)	4	29.74			
	Time * treatment	6	208	3.26	0.017	0.45

during the development and growth of the embryos and larvae, based on the endogenous feeding, the yolk sac is depleted gradually, and the body moisture increased to support the routine and healthy activities of several metabolic and catabolic processes. According to this phenomenon, levels of body moisture progressively increased during the study period, as the values in the larvae at 35 DAF were significantly more than in the green eggs (i.e., 1 and 7 DAF), and the values at the end of yolk-soc stage (i.e., 42 DAF) were higher than at both green and eyed eggs.

In the present study, the values of lipid increased from hatching time onwords, while Dayal et al. (2003) reported the decreasing values of lipid in the eggs and early larval stage of Asian seabass that might be related to the species-specific differences. In addition, lipid content of larvae reared under the recirculating system was significantly lower than those reared under the flow-throw system, which is more likely due to the stressful conditions caused mainly by accumulation of ammonia during the last two weeks, when the ammonia level in the recirculating system was about 20–24 times more than in the flow-through system. Lipid content of eggs is vital for the successful embryonic development and larval survival, as lipids are the primary energy source used by fish from larval stage onwards (Sink et al., 2010). Besides, stress increases ATP requirements of fish via inducing a wide range of ATP-consuming processes (Tort, 2011).

Beside the main nutrition (i.e., carbohydrate, lipid, and protein) that is vital for the production of high-quality eggs and larvae, the optimal fatty acid compositions provide the high chance of success for developing embryos and larvae (Tocher, 2003). In this study, the palmitic acid (C16:0) was the major saturated fatty acid in the samples obtained from the flow-through system (the values ranged from 12.11–12.93%) and the recirculating system (the values ranged from 11.87–13.33%). Similar findings were reported for rainbow trout eggs and larvae (Agh et al., 2019; Zengin et al., 2015, 2016), marketable size rainbow trout (Trbović et al., 2012), and channel catfish eggs, *Ictalurus punctatus*, (Sink et al., 2010). The dynamics of SFA values remained insignificant during the present study, whereas progressively increased values were reported during the embryonic stage of rainbow trout in the previous studies (Agh et al., 2019; Zengin et al., 2015).

Although the values of ΣMUFA remained almost unaltered during the study period, the values of gondoic acid (C20:1n-9) in the yolk-sac larvae (i.e., final two weeks) were significantly lower than in the embryos developed under both flow-through and recirculating systems. Like several previous studies (e.g., Agh et al., 2019; Zengin et al., 2015), the dominant MUFA in both systems was oleic acid (C18:1n-9), having the values ranged from 21.14–23.27% and 21.01–22.98%, respectively.

The results of total PUFA-n-6 and total PUFA-n-3 in the flow-through and recirculating systems support the results obtained by Agh et al. (2019), while were different from the values reported by Zengin et al. (2015, 2016). The linoleic acid (C18:2n-6) and DHA (C22:6n-3) were the dominant fatty acid amongst the PUFA-n-6 and PUFA-n-3, respectively.

In the present study, there were no significant differences in terms of SFA, MUFA, PUFA-n-6, and PUFA-n-3 between the two experimental systems during the study period. Consequently, the designed airlift-based recirculating system had no significant adverse effects on the fatty acid composition of rainbow trout eggs and larvae.

The oxidative stress markers are essential indicators of the physiological state of fish. Superoxide dismutase, catalase, and glutathione peroxidase are considered to be the most important antioxidants (Sinha et al., 2014). The values of TAC, SOD, CAT, and GPX in both systems were statistically constant during the embryonic stage that could be a consequence of many reasons: First, rainbow trout may not be able to produce these antioxidant enzymes during the embryonic stage, and the low and constant levels of activities might related to the maternally-derived antioxidant enzymes that were transferred from broodstock to eggs before and during the spawning season. As previous studies demonstrated, in many teleosts, including salmonids, hormones and immune factors can be taken up by the oocyte during vitellogenesis, and they play critical role during the early developmental stages, when fish are unable to produce certain enzymes and hormones endogenously (Taylor et al., 2016).

Second, levels of reactive oxygen species resulted from lipid peroxidation have not been high enough to increase the activation of antioxidant enzymes. This situation, especially was met in the recirculating system that MDA levels were constant and low (ranged from 13.28–17.07 nmol/mg protein) during the embryonic stage compared to the previously reported MDA values in juveniles rainbow trout muscle that was more than 300 nmol/mg protein (Tkachenko et al., 2014, 2016) and in the different tissues of adults *Salmo trutta*, more

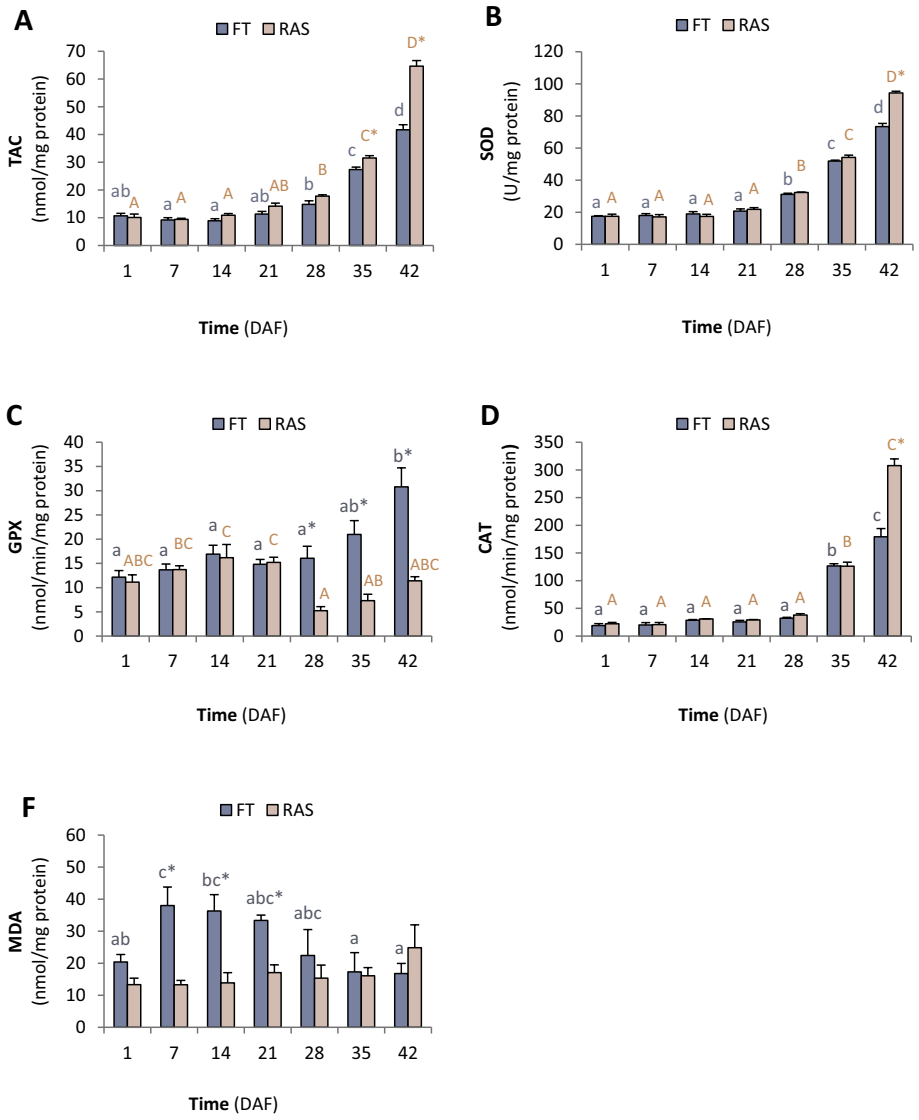

Fig. 2. The values of total antioxidant capacity (A), superoxide dismutase (B), glutathione peroxidase (C), catalase (D), and MDA of rainbow trout eggs and larvae in the flow-through (FT) and recirculating (RAS) systems. Different letters (small letters for the FT and capital letters for the RAS) indicate significant differences amongst the sampling times (one-way ANOVA, $\alpha = 0.05$) in each system. * indicates significant differences between the treatments (independent-samples *t*-test, $\alpha = 0.05$) at the same sampling time.

than 50 μmol/mg protein (Kurhalyuk et al., 2009).

Third, the considered antioxidant enzymes do not play significant roles during the embryonic stage of rainbow trout. Sinha et al. (2014) reported that in response to high environmental ammonia, SOD and CAT activities in the kidney of juveniles rainbow trout remained statistically unaltered and suggested SOD and CAT are not their first defense to counter ammonia-mediated ROS production.

Conversely, activities of all assessed antioxidant enzymes progressively increased from hatching time onwards, with the exception of GPX at the recirculating system that decreased suddenly at the hatching time and then increased. Previous studies revealed that the antioxidant enzyme activities are different in the various fish species (Kolayli et al., 1997; Trenzado et al., 2006), in the various tissues of fish (Kolayli and Keha, 1999; Trenzado et al., 2006), and in the different life stages, especially during the early life stages (Zengin et al., 2015, 2016).

The increased values of the antioxidant enzyme activities after the hatching time in both experimental systems indicate that the endogenous production of these enzymes in rainbow trout begins after the hatching time. The significant differences observed between the two systems during or at the end of the yolk-sac larvae stage might be a consequence of the elevated ammonia values in the recirculating system (maximum level: 1.676 mg/l) compared to the flow-through system (maximum level: 0.074 mg/l).

5. Conclusion

An airlift-based recirculating system was constructed for rainbow trout larvae production. In the previous paper (Irani and Agh, 2020) the efficacy of this system were evaluated, and in this study, the effects of water reusing on the biochemical compositions and antioxidant enzyme activities of rainbow trout eggs and yolk-sac larvae investigated. The results indicated that there were no significant differences between the flow-through and airlift-based recirculating systems in terms of biochemical compositions, fatty acid profiles, and antioxidant enzyme activities during the embryonic stage. However, the lipid content and antioxidant enzyme activities of the larvae reared under the recirculating system were significantly different from the larvae reared under the flow-through system, which is more likely due to accumulation of ammonia in the recirculating system. In addition, the constant values of antioxidant enzyme activities in the embryos and the

subsequent increased values in the larvae demonstrate that the endogenous production of these enzymes in rainbow trout begins after the hatching time.

CRediT authorship contribution statement

Abdoljabbar Irani: Conceptualization, Methodology, Software, Data curation, Writing - original draft, Investigation.**Farzaneh Noori:**Supervision, Validation, Investigation, Writing - review & editing, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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