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Red yeast (*Phaffia rhodozyma*) and its effect on growth, antioxidant activity and color pigmentation of rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Efficacy of orally used of red yeast (*Phaffia rhodozyma*) (1.6 mg astaxanthin/g product) at 15.5 g (P1), 37.3 g (P2) and 47 (P3) g per kg diet were studied on performance, carcass composition, antioxidant activity and fillet pigmentation in rainbow trout (*Oncorhynchus mykiss*) weighing 208–212 g for eight weeks at 11 °C. Synthetic astaxanthin (AX) (0.5 g/kg diet) and basal diet were used as controls. With an increase in *P. rhodozyma* concentration in diet, weight gain and specific growth rate exhibited an increase compared to basal diet ($P < 0.05\%$). A significant difference was seen among the treatments but only P3 diet significantly demonstrated a better growth than AX diet ($P < 0.05$). Activity of antioxidant enzymes; superoxidase dismutase, glutathione peroxidase, total antioxidant activity, glutathione reductase, and catalase exhibited an enhancement in serum or liver samples of fish fed *P. rhodozyma* compared to basal diet ($P < 0.05$). Protein content in fillet of fish fed P3 diet was higher than both basal and AX diets ($P < 0.05$), but no significant difference was seen in contents of lipid, moisture, ash and pH values either among the treatments or between treatments and basal diet ($P > 0.05$). Water holding capacity and lipid loss in fillets of treatments were lower than fish fed basal diet ($P < 0.05$). By increasing *P. rhodozyma* concentration in fish diets, redness value exhibited a progressive increase, and the highest value was seen in P3 diet compared to other treatments ($P < 0.05$). These data show application of *P. rhodozyma* at 47 g/kg diet in trout could provide a better performance, antioxidant activities, and fillet pigmentation.

1. Introduction

The color of fish muscle especially salmonid species is one of critical factors of fish fillet quality. Typical red to pink fillet color of aquaculture fish is due to astaxanthin (3,3′-dihydroxy-β, β-carotene 4,4′-dione) that is a xanthophyll carotenoid available in various microorganisms and marine animals (Higuera-Ciapara et al., 2006; Ranga, 2011) including *Haematococcus pluvialis*, a green microalga, and red-yeast, *Phaffia* (Sarada et al., 2002; Ranga, 2011; Sarada et al., 2002; Higuera-Ciapara et al., 2006; Pashkow et al., 2008). Except the natural astaxanthin commercially obtained from *Phaffia* yeast and *Haematococcus*, some commercial astaxanthin are obtained through chemical synthesis (Ranga et al., 2009; Ranga et al., 2010; Lorenz, 1999), but more concerns associated with the level of safety are required on such synthetic products. However, due to its prevention or reduction of various disorders risk in humans and animals (Guerin et al., 2003; Kidd, 2011) astaxanthin application as a nutritional supplement has been rapidly growing in foods, feeds, nutraceuticals and pharmaceuticals.

Phaffia rhodozyma is a basidiomycetous pink yeast that until recently was found exclusively in slime fluxes of certain broad-leafed trees in the

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northern hemisphere. The slime fluxes are rich in sugars and other carbohydrates and takes advantages of its fermentative abilities to use these substrates. *P. rhodozyma* is a member of *Cryptococcaceae* and based on the available data it contains 22% protein, 23% lipid, 3% ash, 5% moisture and astaxanthin of 3 mg/g product (Sanderson and Jolly, 1994; Nakano et al., 1999). Although, there are some works exhibited the effect of *Phaffia* yeast on fish fillet pigmentation or growth such data are limited especially associated with the red yeast dosage optimization (Nakano et al., 1999; Amar et al., 2004). This study aimed to evaluate different dosages of *P. rhodozyma* on growth and nutritional performances, antioxidant activity, chemical composition of flesh fish and pigmentation of fillet in rainbow trout in compared with basal diet and synthetic astaxanthin recommended at current dosage.

2. Materials and methods

2.1. Diet preparation

Five isonitrogenous and isocaloric diets including basal diet (without astaxanthin), diet contains 500 mg/kg diet synthetic astaxanthin (AX) (10% purity, Lucantin® Pink CWD, Australia), and diets containing 15.5 (P1), 37.3 (P) and 47 (P3) g *P. rhodozyma* powder (BPAX-AL, Bioproton Pty Ltd, Australia) per kg diet were used. *P. rhodozyma* ingredients were agar 1.5%, yeast extract 0.3%, malt extract 0.3% and bacto-peptone 0.5%, and 1.6 mg astaxanthin/g product. Grow-out pellets produced by Faradaneh Aquatic Animals Feed Producer (Table 1) were obtained from dryer section before adding the oil. The weighed pellets were dissolved in water before being minced with a mincer. The diets were mixed thoroughly with AX and *P. rhodozyma* and were minced again. The prepared diets were then analyzed for chemical composition (Table 2).

2.2. Fish and feeding regime

Healthy rainbow trout obtained from a trout farm were accommodated in 300 L tanks (15 tanks) each 20 fish and were adapted to new conditions for two weeks before the experiment. Afterwards, fish were weighed individually after being anesthetized with clove oil. Fish (208–212 g) were randomly divided in five groups each in three replicates (20 fish/replicate) and were fed the experimental diets for 56 days at 1.3% body weight/day and the rate of feeding was corrected each week interval after weighing of fish in each tank. Water quality including temperature, dissolved oxygen, nitrite, un-ionized ammonia and pH were 11 °C, > 8 mg/L, < 0.1 mg/L, < 0.01 mg/L and 7.8, respectively using well water flow with aeration. Any mortality was collected daily and recorded.

Table 1
Diet ingredients (g/kg diet) used in this study. P1, P2 and P3 are experimental diets contain 15.5 g, 37.3 g and 47 g *Phaffia rhodozym* per kg diet, respectively. AX = 0.5 g synthetic astaxanthin per kg diet.

	Diets				
Ingredient	AX	P3	P2	P1	Control
Sardine fishmeal	150	178	167.53	154.4	150
Canned tuna by-products meal	150	75	95	130	150
Soybean meal	250	250	250	250	250
Wheat fluor	235	235	235	235	235
Wheat gluten	50	50	50	50	50
Phaffia rhodozyma	0	47	37.3	15.5	0
Soybean oil	100	100	100	100	100
Binder (gelatin)	50	50	50	50	50
Vitamin premix	3	3	3	3	3
Mineral premix	3	3	3	3	3
Choline	3	3	3	3	3
Salt	3	3	3	3	3
Acidifier	3	3	3	3	3
Synthetic astaxanthin (10%)	0.5	0	0	0	0

Table 2
Chemical composition of the ingredients used for diet preparation. P1, P2 and P3 are experimental diets contain 15.5 g, 37.3 g and 47 g *Phaffia rhodozym* per kg diet, respectively.

Characters	Proximate analysis (% in dry basis)				Astaxanthin (mg/kg diet)
	Moisture	Lipid	Protein	Ash	
Raw ingredients:					
Sardine fishmeal	6.53	6.55	67.1	20.1	0.0
Canned tuna by-products meal	9.51	15.93	55.39	15.90	0.0
Soybean meal	10.8	1.02	43.49	6.21	0.0
Wheat flour	13.5	1.5	9.93	0.78	0.0
Wheat gluten	4.64	1.04	76.13	0.83	0.0
Yeast pigment	7.65	1.49	46.46	6.34	0.0
Fish oil	0.5	99.5	0	0	0.0
Soybean oil	0.5	99.5	0	0	0.0
Binder (gelatin)	9.52	0.49	81.81	19.4	0.0
Experimental diets:					
Control	9.03	14.01	39.48	8.15	0.0
P1	9.1	14.2	39.44	8.14	24.80
P2	8.99	13.74	39.39	8.02	59.68
P3	8.91	13.3	39.35	7.87	75.2
Synthetic astaxanthin	8.86	13.06	39.38	7.82	500

2.3. Sampling and processing of samples

At the end of experiment, fish were subjected to a mild sedation of clove oil and the weight and length of all fish of each treatment were individually weighed. Six fish of each replicate (18 fish per treatment) were randomly chosen for blood collection from caudal vein after being anesthetized with clove oil. These fish were used for liver and fillet collection. Blood samples were kept at 4 °C overnight and the sera samples were separated and kept in liquid nitrogen tank (−196°C) until sued. Liver and fillet samples were obtained immediately after dissection and were kept in liquid nitrogen and 4 °C, respectively. The color of each flesh fillet sample was immediately measured using a colorimetric assay. The fillets were then minced aseptically by a mincer, and each was divided in several equal samples for the following assays.

2.4. Growth and nutritional parameters

Growth and nutritional factors including final weight (FW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), hepatosomatic index (HSI), and visceral somatic index (VSI) were determined using following equations (Bransden et al., 2003; Karalazos et al., 2007; Montero et al., 2008).

Weight gain (WG) = final weight – initial weigh

Specific growth rate (SGR %) = (Ln final weight of the fish-Ln initial weight of fish) × 100/days of feeding trial

Feed conversion ratio (FCR) = food intake (g)/living weight gain (g)

Hepatosomatic index (HSI %) = 100 x (liver weight [g] /whole fish weight [g])

Visceral somatic index (VSI %) = 100 x (viscera weight [g]/whole fish weight [g])

2.5. Carcass chemical composition

Chemical compositions including protein, lipid, moisture and ash of each fillet sample were measured according to standard methods (AOAC, 1995; Latimer, 2012). Crude protein content was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl system (Autoanalyzer, PDU500SI, PecoFood, Iran). Crude lipid was measured by the ether extraction method using a Soxtec extraction system (Peco,

Iran). Moisture was measured by oven drying at 105 °C until a constant weight was obtained. Ash content was determined after samples were placed in an oven at 550 °C for 4 h. A 5 g of each minced fillet sample was mixed with 50 ml of 4ºC-sterile distilled water (pH 7) and was homogenized thoroughly before the pH being measured by a calibrated pH meter.

2.6. Antioxidant activity

Liver samples were first homogenized before being used for antioxidant assay. Antioxidant activities of both sera and liver samples were determined for glutathione peroxidase (GPx), glutathione reductase (GR) and superoxidase dismutase (SOD) all measured by the Randox kit (The UK); and total antioxidant activity (TAC), catalase activity (CA) and myeloperoxidase activity (MPO) were measured by Noxifer™, Nactaz™ and Nampox™ kits (Navand Lab Kit, Iran), respectively. The activity of malondialdehyde (MDA) was determined using Nalondi™ kit (Nalondi™ Lipid peroxidation (MDA) assay kit, Navand Lab Kit, Iran). The assay for each sample was repeated three times and the average was used for further analysis.

2.7. Water holding capacity

Water holding capacity of the fillet samples kept at − 18 °C for 45 days was measured with the press filter press method according to Gómez-Guillén et al. (2000) and Komolka et al. (2020) with slight modifications. The expressible moisture/total liquid loss was determined as the volume of liquid squeezed from fillets upon compression. Briefly, 2 g of the minced fillet sample was transferred into a sterile tube before being covered with a weighed-Whatman filter paper. The sample was centrifuged at 4000 g for 10 min, and the filter paper was dried at 50ºC overnight and reweighed again. This was carried out to minimize the weighing error because of filter paper absorption of fat from the sample. The expressible moisture, based on the fluid absorbed by the filter paper divided by the sample weight was calculated using following equation. The aqueous plus fatty fraction (AF) which was retained in the filter was dried to constant weight. Fat release was calculated as dry weight of AF × 100/initial weight of sample. All determinations were carried out in triplicate.

Expressible moisture (total liquid loss) (%) = [(weight of filter paper after absorbing the water sample (g) – initial weight of filter paper (g))/weight of fillet sample (g)] × 100

Water holding capacity (%) = [(weight of filter paper after absorbing water sample (g) – weight of dried filter paper (g))/ weight of fillet sample (g)] × 100

Lipid loss (%) = [(weight of dried filter paper (g) – initial weight of filter paper (g))/weight of fillet sample (g)] × 100

2.8. Fillet colorimetric assay

Individual fillets from each fish were used for tristimuli color measurement according to the L*a*b* CIELAB system using a Minolta Chroma Meter (TES 135 Color Meter, Taiwan) and methods described by Komolka et al. (2020). The L* value describes the lightness between 0 (black) and 100 (white). On the a* axis, green and red are opposite to each other, while the b* axis runs between blue and yellow. Lightness, redness, and yellowness values were gained as the mean from three measure points each of the dorsal and ventral muscles along the horizontal septum (Komolka et al., 2020).

2.9. Statistical analysis

The normality of the data was investigated by Kolmogorov–Smirnov test. Then, the mean ± standard deviation (SD) of the data were analyzed by one-way analysis of variance (ANOVA) and the significant differences among the means were detected using Dunkan's post-hoc test at $P < 0.05$. All the statistical tests were conducted by the SPSS software for windows (Version 22, Chicago, USA).

3. Results

3.1. Growth performance

Results of growth performance and nutritional efficiency are shown in Figs. 1.1–1.6. With an increase in *P. rhodozyma* in the diet, FW, WG and SGR exhibited higher increase in fish fed the experimental diets than basal diet ($P < 0.05$) (Fig. 1.1–1.3). Also, the growth factors were significantly different among fish fed different levels of *P. rhodozyma* ($P < 0.05\%$), and fish fed 47 g *P. rhodozyma* significantly demonstrated a better growth than fish fed AX diet ($P < 0.05$) (Fig. 1.1–1.3). FCR was reduced by an increasing in level of *P. rhodozyma* in the diets and the lowest FCR was seen in fish fed 47 g/kg diet (Fig. 1.4). The lowest and the highest percentages of HSI and VSI were obtained in fish fed 47 g *P. rhodozyma* /kg deit and both basal and AX diets, respectively (Fig. 1.5–1.6). No mortality was seen in the experimental diets and the controls, except one fish died in fish fed basal diet.

3.2. Antioxidant activity

Antioxidant activities of serum and liver samples are presented in Fig. 2.1–2.7. A progressive increase in SOD was measured in both serum and liver samples of fish fed *P. rhodozyma* and the highest activity was seen in 47 g/kg diet ($P < 0.05$) (Fig. 2.1). No significant difference was obtained in SOD in liver samples between AX group and 15.5 or 37.3 g/kg diets ($P > 0.05$), but these were all significantly higher than basal diet ($P < 0.05$). SOD activity in sera samples of fish fed diets of AX, basal diet, 15.5 *g*/kg, and 37.3 *g*/kg was insignificant ($P > 0.05$), but all were significantly lower than 47 g *P. rhodozyma* diet ($P < 0.05$). GPx activity in liver samples of fish fed *P. rhodozyma* exhibited a progressive increase and showed a significant difference compared to basal diet ($P < 0.05$), but it was insignificant between15.5 or 37.3 g *P. rhodozyma* and AX diet ($P > 0.05$). No significant difference was seen in GPx among the sera samples of experimental diets and between experimental diets and AX diet ($P > 0.05$), but all these were higher that fish received basal diet ($P < 0.05$). TAC activity in both serum and liver samples of experimental diets exhibited a progressive increase (Fig. 2.3) and the highest TAC activity was seen in 47 g/kg diet followed by 37.3 g and AX diets. TAC activity in fish fed 15.5 g *P. rhodozyma* were higher than basal diet but was insignificant in liver samples ($P > 0.05$). GR activity was higher in fish of experimental diets than fish fed basal diet ($P < 0.05$) (Fig. 2.4), and a higher activity was seen in fish fed 47 g *P. rhodozyma*/kg diet compared to other treatments and both basal and AX diets ($P < 0.05$). No significant difference was observed in GR activity between fish fed AX and fish fed 15.5 or 37.3 g *P. rhodozyma* ($P > 0.05$). CAT in both serum and liver samples of fish fed *P. rhodozyma* exhibited a progressive enhancement and the highest activity was seen in 47 g/kg diet, while the lowest activity was obtained in basal diet ($P < 0.05$) (Fig. 2.5). Fish fed AX, 15.5 g and 37.3 g *P. rhodozyma* demonstrated identical activity in CAT ($P > 0.05$). No significant differences were observed in MDA (Fig. 2.6) and MPO among the experimental diets and among experimental diets, AX and basal diets ($P > 0.05$) (Fig. 2.7).

3.3. Chemical composition of fillets

A higher crude protein content was obtained in the fillets of fish fed *P. rhodozyma* than basal diet (Fig. 3.1) ($P < 0.05$), but it was insignificant among fish treated with different levels of *P. rhodozyma* ($P > 0.05$). Also, protein level in fillets of fish fed *P. rhodozyma* at 47 g/kg diet was higher than fish fed AX diet ($P < 0.05$). Contents of lipid, moisture and

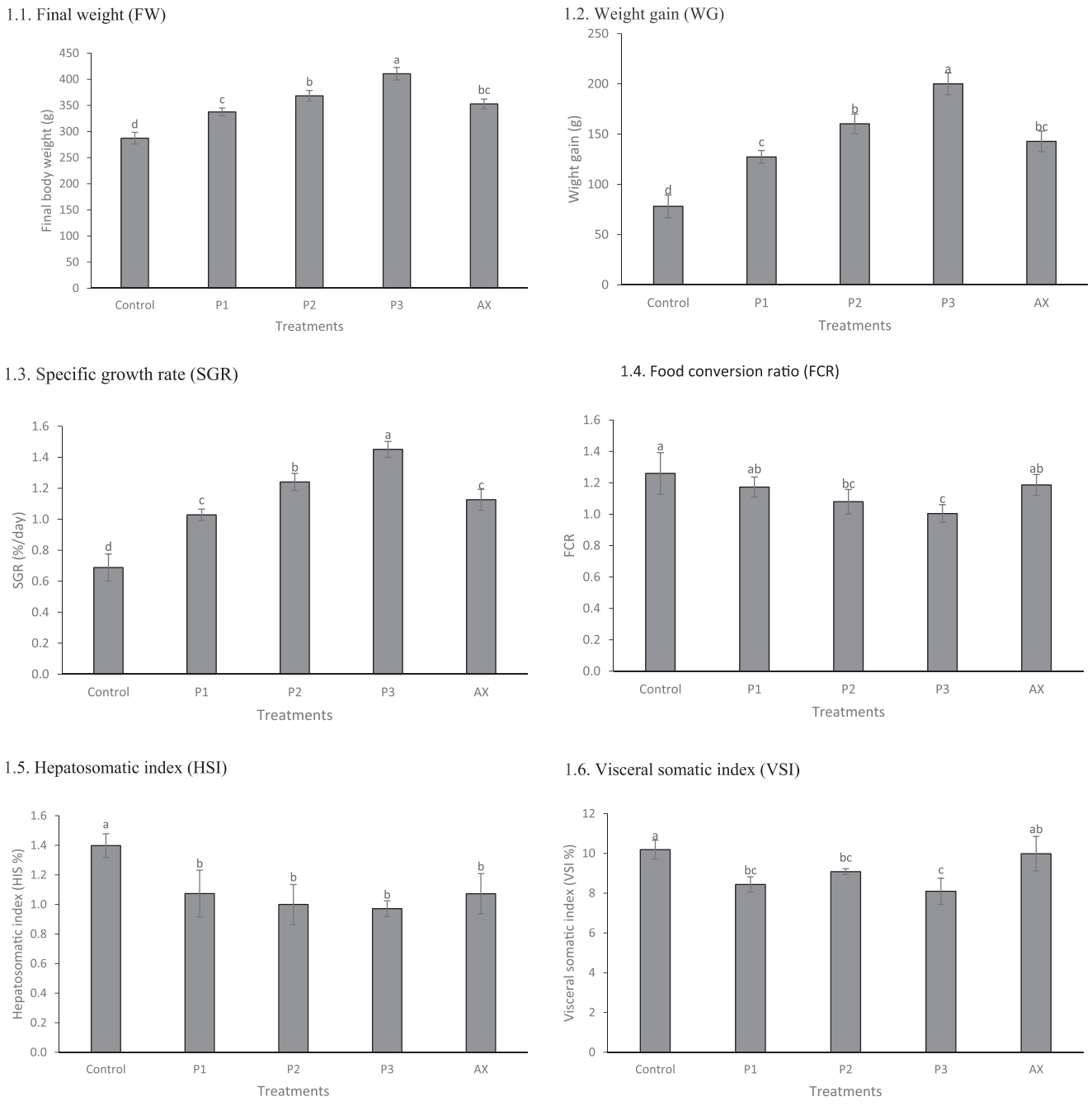


Fig. 1. Growth and nutritional parameters of rainbow trout fed red yeast (*Phaffia rhodozyma*) at different levels for 56 days at 11ºC. P1, P2 and P3 are diets contain 15.5 g, 37.3 g and 47 g *P. rhodozyma* /kg diet, respectively. Control = Basal diet, AX = 0.5 g synthetic astaxanthin /kg diet. Data are mean ± SD, n = 60, except control that n is 59. Means on the same line not sharing the same letter are significantly different (P < 0.05).

ash ae well as pH value were insignificant among the treatments and between treatments and controls (P > 0.05) (Fig. 3.2–3.5).

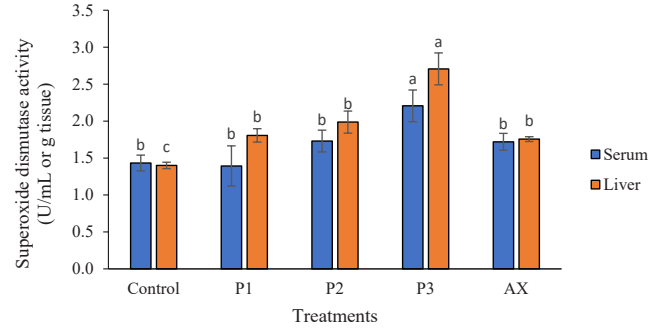
3.4. Water holding capacity

Results of water holding capacity of the fillet samples kept at − 18 C for 45 days post-sampling are given in Fig. 4.1 to 4.3. Expressive moisture (water loss) in fillets of experimental diets was lower than basal diet (P < 0.05) (Fig. 4.1). Also, water loss in fillets of fish fed *P. rhodozyma* was different with a higher loss seen in 15.5 g treatment *(*P < 0.05*)*. The fish fillets of 37.3 g deit revealed the lowest water loss, while those of 47 g and AX treatments were insignificant (P > 0.05). In addition, with an increase in level of *P. rhodozyma* in fish diets the content of total liquid loss was reduced and the lowest level was obtained in fillets of fish fed 47 g *P. rhodozyma* (P < 0.05) (Fig. 4.2). Further, fillets of fish fed *P. rhodozyma* demonstrated a lower total liquid loss than AX and basal diets (P < 0.05) (Fig. 4.2). With an increase in level of *P. rhodozyma* in the diets, the content of lipid loss in fillets samples was reduced, and it was significantly lower than AX and basal diets (P > 0.05) (Fig. 4.3). No significant difference was seen in lipid loss between AX and 37.3 g treatments (P > 0.05).

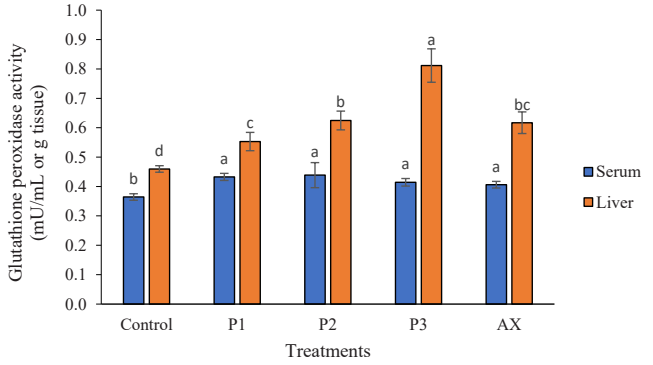
3.5. Fillet pigmentation

Results of color pigmentation of the fish fillet are shown in Fig. 5.1–5.3. Fillets of fish fed *P. rhodozyma* at 37.3 g/kg and 47 g/kg

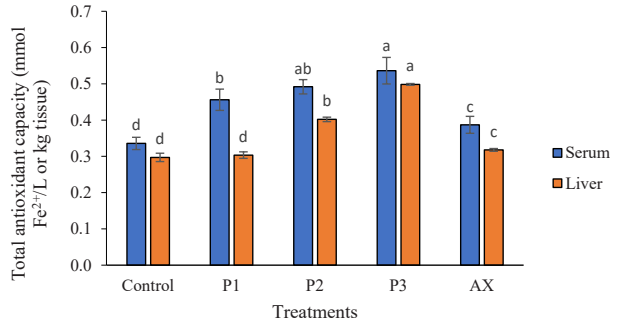
2.1. Superoxidase dismutase (SOD)



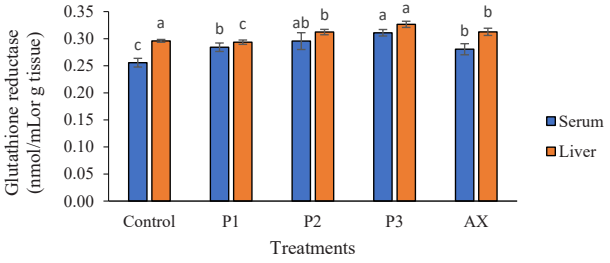
2.2. Glutathione peroxidase (GPx)



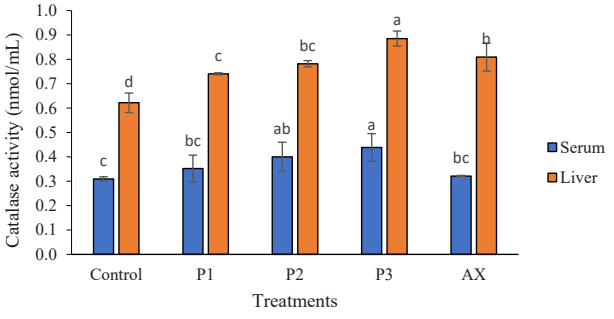
2.3. Total antioxidant activity (TAC)



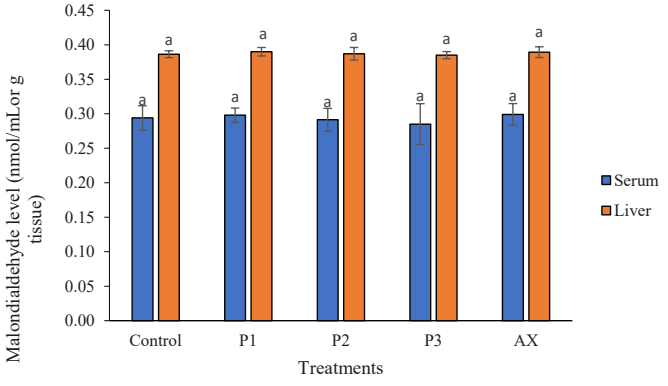
2.4. Glutathione reductase (GR)



2.5. Catalase (CA)



2.6. Malondialdehyde (MDA)



2.7. Myeloperoxidase activity (MPO)

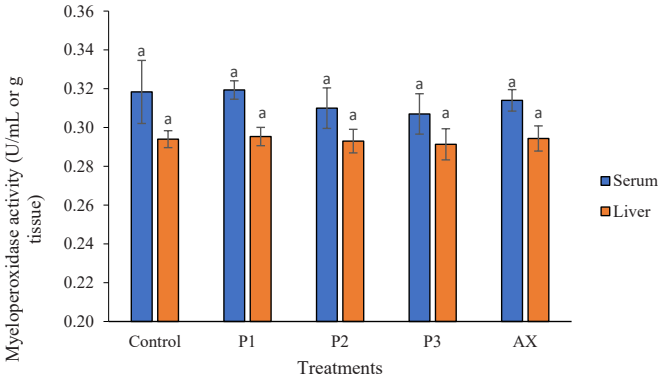


Fig. 2. Antioxidant activity in serum and liver samples of rainbow trout fed red yeast (*Phaffia rhodozyma*) at different levels for 56 days at 11ºC. P1, P2 and P3 are diets contain 15.5 g, 37.3 g and 47 g *P. rhodozyma* /kg diet, respectively. Control = Basal diet, AX = 0.5 g synthetic astaxanthin /kg diet. Data are mean ± SD, n = 18. Means on the same line not sharing the same letter are significantly different ($P < 0.05$).

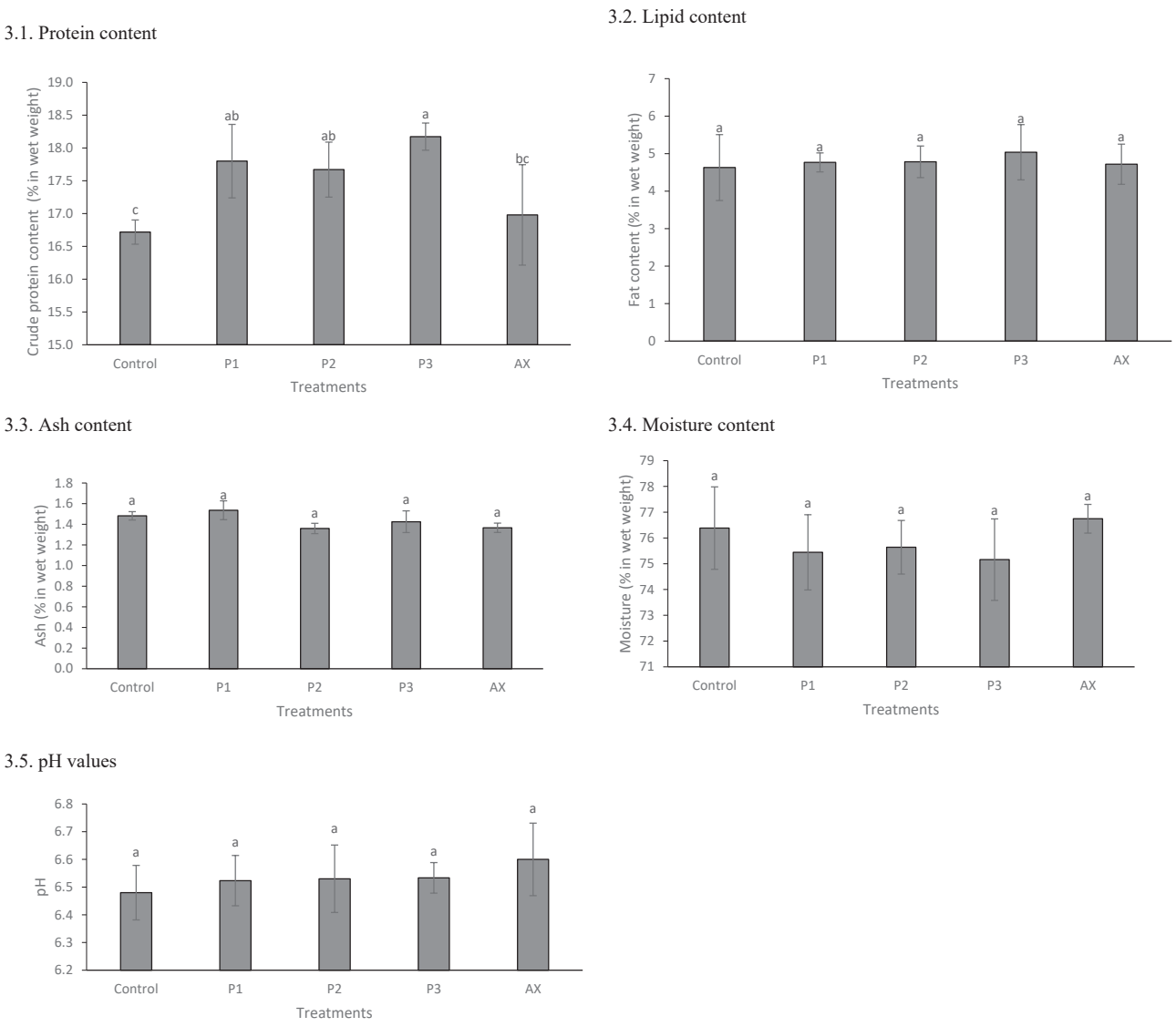


Fig. 3. Chemical composition and pH value of rainbow trout fed red yeast (*Phaffia rhodozyma*) at different levels for 56 days at 11ºC. P1, P2 and P3 are diets contain 15.5 g, 37.3 g and 47 g *P. rhodozyma* /kg diet, respectively. Control = Basal diet, AX = 0.5 g synthetic astaxanthin /kg diet. Data are mean ± SD, n = 18. Means on the same line not sharing the same letter are significantly different ($P < 0.05$).

diet exhibited the lowest luminosity (L*) compared to other treatments, while the highest luminosity was seen in fish fed basal diet (Fig. 5.1). The level of redness (a*) exhibited that with an increase in use of *P. rhodozyma* in fish diets, the level of a* indicator was enhanced, and the highest level was seen in fish fed 47 g red yeast compared to other treatments and AX deit, while the lowest indicator was observed in fish fed basal diet (Fig. 5.2). Also, with an increase in *P. rhodozyma* in diets the yellowness (b*) level exhibited an enhancement, and the highest level was seen in fillets of fish fed 47 g/kg diet ($P < 0.05$) (Fig. 5.3). This indicator was the lowest in basal diet and was insignificant between 15.5 g and AX diets ($P > 0.05$).

4. Discussion

Carotenoids are reported to enhance fish health status probably in part due to a positive effect on intermediary metabolism in aquatic organisms (Segner et al., 1989; Rashidian et al., 2020), that can improve nutrient utilization resulting in increasing in the animal growth (Amar et al., 2001). A balancing in the intestinal flora breaking down indigestible feed components to extract more nutrients and to stimulate the production of enzymes transporting fats for growth instead of storage is also another possible mechanism induced by the carotenoids on fish growth (Kalinowski et al., 2011).

In the present study, an increase was seen in trout growth after feeding with *P. rhodozyma* and diet contains higher *P. rhodozyma* exhibited a better growth compared to fish fed AX diet. This was supported by the data where the crude protein content in the fillets of fish fed *P. rhodozyma* were significantly higher than basal diet, and fish fed 47 g red yeast exhibited a higher protein content than AX diet. The red yeast contains an appreciable amount of protein and fat, thereby can affect the nutritional profile of the diets. This can contribute to variable growth performance, thus, providing the nutritional profile for the diets may result in a significant deviation from the basal diet and nutrient requirements for the fish. However, the analysis of amino acid and lipid profiles of each experimental diet would be helpful to support such differences in the growth parameters, particularly since the sardine fishmeal and canned tuna byproduct meal components were different across the experimental diets to provide for increased inclusion of the red yeast. Also, Astaxanthin may increase lipid utilization in whole fish and liver, giving a more energy and consequently improving animal growth (Kalinowski et al., 2011). A 150-day oral use of *P. rhodozyma* (Red Star BioProducts) at 75 mg astaxanthin/kg feed in forms of

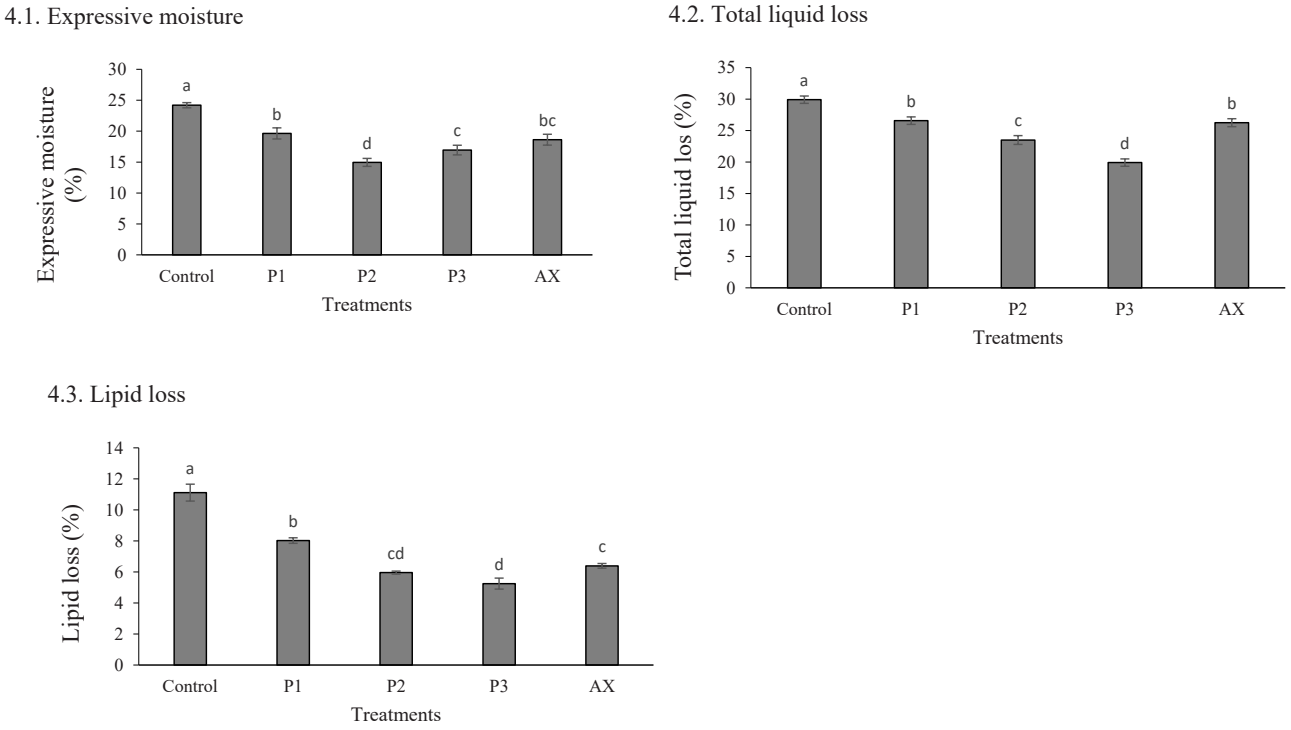


Fig. 4. Expressive moisture of rainbow trout filet fed red yeast (*Phaffia rhodozyma*) at different levels for 56 days at 11°C and analyzed after 45 days post-holding at −18 °C. P1, P2 and P3 are diets contain 15.5 g, 37.3 g and 47 g *P. rhodozyma* /kg diet, respectively. Control = Basal diet, AX = 0.5 g synthetic astaxanthin /kg diet. Data are mean ± SD, n = 18. Means on the same line not sharing the same letter are significantly different ($P < 0.05$).

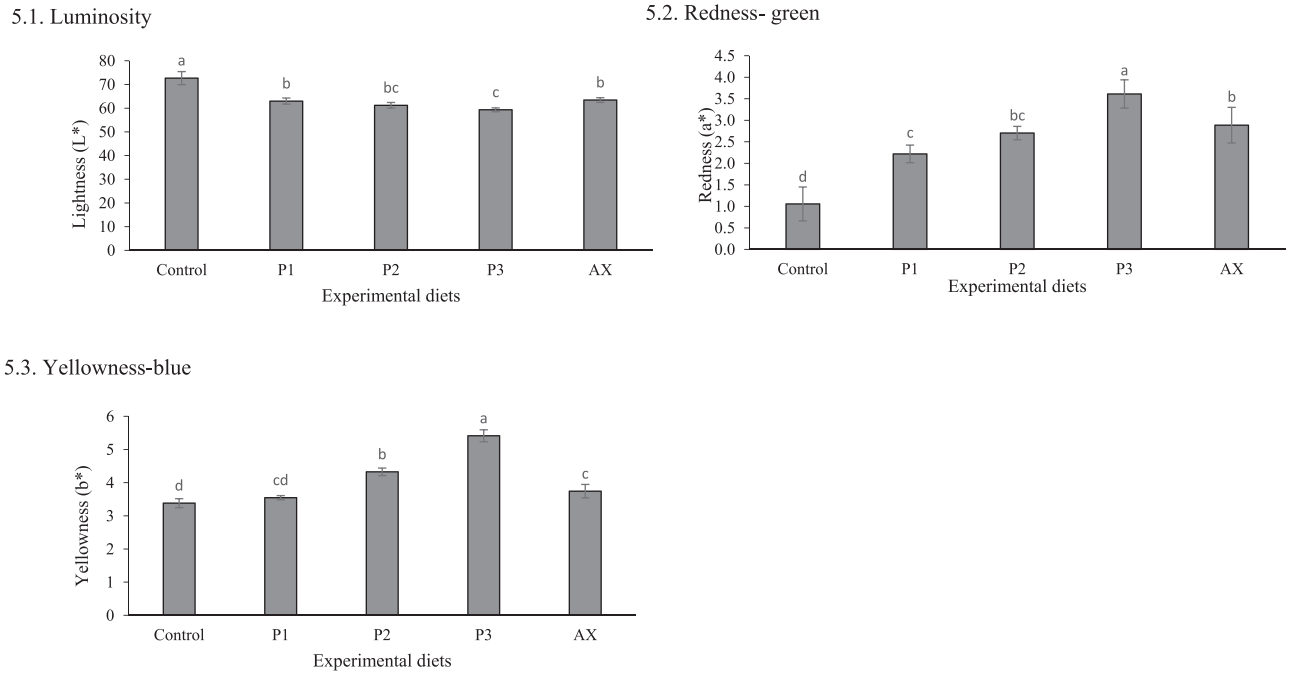


Fig. 5. Pigmentation of rainbow trout filet fed red yeast (*Phaffia rhodozyma*) at different levels for 56 days at 11°C. P1, P2 and P3 are diets contain 15.5 g, 37.3 g and 47 g *P. rhodozyma* /kg diet, respectively. Control = Basal diet, AX = 0.5 g synthetic astaxanthin /kg diet. L* = lightness with 100 = absolute white and 0 = absolute black, a* = "redness" coordinate; b* = "yellowness" coordinate. Data are mean ± SD, n = 18. Means on the same line not sharing the same letter are significantly different ($P < 0.05$).

spray-dried untreated *P. rhodozyma*, heat-treated and spray-dried *P. rhodozyma*, and heat-treated and chemically treated spray-dried *P. rhodozyma* in Atlantic salmon exhibited insignificantly an increase in fish growth in all experimental diets (Whyte and Sherry, 2001). A 9–10-week feeding rainbow trout *P. rhodozyma* at 50–200 mg/kg feed exhibited no difference in growth compared to control fish and proximate composition of fish muscle (Choi et al., 2016). Such difference may be in part due to, the period of administration, quality of feed and water

quality condition especially water temperature. However, pufferfish (*Takifugu obscurus*) fed astaxanthin at 80–320 mg/kg diet significantly increased WG and SGR compared to control fish (Cheng et al., 2018), and crucian carp (*Carassius auratus*) fed 200–800 mg astaxanthin/kg diet for two months revealed an increased in WG, FCR, intestinal digestive enzymes (protease, lipase and amylase), serum SOD, CAT, acid phosphatase, alkaline phosphatase, lysozyme, complement and interleukin (IL)− 10 and exhibited a significant resistance to *Aeromonas hydrophilia* infection. Theses authors suggested 400 mg/kg as an optimum dosage for use in this fish species (Wu and Xu, 2021).

Reactive oxygen species (ROS) are oxidative products, induced under normal aerobic cellular metabolism and respiratory burst (Ranga et al., 2014), that play a significant role in a various biological functions such as cell growth, inducing and maintaining of the transformed state, and cell death process (Finkel, 2003). However, ROS can damage healthy cells if they are not eliminated (Chew and Park, 2004). Under normal physiological conditions, the excessive ROS is removed by internal antioxidants and anti-oxidative systems (Chen et al., 2015; Burgos-Aceves et al., 2018) e.g., SOD, CAT, and GPx, large molecules (albumin, ferritin, and ceruloplasmin) and small molecules (ascorbic acid, α-tocopherol, β-carotene, and uric acid) (Martinez-Alvarez et al., 2005). Despite their lower consumption, carotenoids are one of the most widely used dietary antioxidants in animal diets (Chew and Park, 2004) because they can reach the highest plasma and tissue concentrations (Olmedilla et al., 2001). In our study, a progressive increase in SOD activity was measured in fish fed *P. rhodozyma* but it was not significant between fish fed AX diet and fish fed 15.5 or 37.3 g *P. rhodozyma.* Similar findings were seen in TAC, GPx, GR and CAT activities in fish fed experimental diets but only diet of 47 g red yeast gave a higher activity than AX diet. No significant differences were however, observed in the levels of MDA and MPO between the experimental diets and among experimental diets, AX and basal diets. Thus, application of red yeast at 15.5–47 g/kg diet can improve the antioxidant capacity resulting in improving fish health status as also reported by other researchers who reported a higher immune responses in non-salmonid fish fed red yeast than control fish (e.g., Cheng et al., 2018; Wu et al., 2021) or salmonid species such as rainbow trout (Choi et al., 2016; Amar et al., 2004). Also, detary *P. rhodozyma* may effectively suppress the of lipid peroxides of tissue and normalize liver function as well as improving muscle pigmentation of trout (Nakano et al., 1999), suggesting red yeast could have a reducing effect on oxidized oil-induced oxidative stress in fish.

Drip loss is a process involving the transfer of water from myofibrils to the extracellular space affected by structural features at different levels within muscle tissue (Bertram et al., 2002; Huff-Lonergan, 2009). Manipulation of the net charge of myofibrillar proteins and the structure of the muscle cell and its components i.e., myofibrils, cytoskeletal linkages and membrane permeability, and the amount of extracellular space within the muscle are some factors that can influence the retention of water holding capacity of fillet. Drip loss is an important factor associated with the palatability, quality and acceptability of meat (Forrest et al., 2000). Genomic research has shown that the genes involved in muscle structure integrity, glycolysis, and calcium signaling (Heidt et al., 2013; Ponsuksili et al., 2008) are responsible for the major variations in drip loss. Also, protein metabolism is correlated with drip loss in some animal meats such as beef cattle (Guo and Dalrymple, 2017). In the present study, total lipid in fillets of fish treated with *P. rhodozyma* was dose-dependent and the lowest water loss was seen in fish fed 47 g red yeast in diet. In addition, with an increase in the yeast concentration in fish diets the contents of expressive moisture and lipid loss were reduced. Further, fillets of fish fed *P. rhodozyma* demonstrated a lower lipid loss and total liquid loss than fish fed AX and basal diets.

The L*a*b* measurement is an approved and well-documented method in meat science (Komolka et al., 2020). In our study fillets of fish fed *P. rhodozyma* at 37.3 g/kg and 47 g/kg diets exhibited the lowest luminosity (L*) compared to other treatments, while the highest luminosity was seen in fish fed basal diet. The level of redness (a*) exhibited an increase with an increase in concentration of *P. rhodozyma* in diets, and the highest redness level was seen in fish administered 47 g yeast per kg diet compared to other treatments and AX diet, while the lowest indicator was observed in fish fed basal diet. (Whyte and Sherry, 2001) exhibited that astaxanthin concentration was not significantly different between Atlantic salmon fed the basal diet or treated with *P. rhodozyma* at 75 mg astaxanthin/kg feed and fish fed AX diet within 150 days of feeding. Although total carotenoid and astaxanthin concentrations in muscle of rainbow trout showed no significant difference after fish being fed solid fermented soybean meal with *P. rhodozyma* at 50–100 ppm astaxanthin the redness values of muscle from all treated fish were significantly higher than control diet (Choi et al., 2016). It is however, notable to say that the deposition of *P. rhodozyma* astaxanthin in the trout flesh may be dependent on the proper preparation of the red yeast cells before their inclusion into the feed as rainbow trout fed intact red yeast exhibited no coloring, but fish fed the red yeast cells partially digested by enzymes demonstrated the most efficient deposition of astaxanthin in their muscles (Johnson et al., 1980).

5. Conclusion

Orally used of *P. rhodozyma* at 15.5–47 g/kg diet exhibited an improvement in trout growth and higher dosages were superior to lower dosages. Also, trout fed higher dosages of *P. rhodozyma* revealed a better growth than synthetic astaxanthin at 0.5 g/kg diet. Antioxidant activities in serum and liver samples of trout fed *P. rhodozyma* were dose-dependent and a higher dosage exhibited more antioxidant activity. Higher dosage of the red yeast also revealed a higher protein content in fillets than fish fed synthetic astaxanthin and basal diet, while water holding capacity and lipid loss in the fillets of fish fed the experimental diets were lower. The best pigmentation color was seen in fillets of fish fed higher red yeast concentration. Overall, application of *P. rhodozyma* at 47 g/kg diet in trout could provide a better performance, antioxidant activities, and fillet pigmentation.

Authors statement

This manuscript entitled "red yeast (*Phaffia rhodozyma*) and its effect on growth, antioxidant activity and color pigmentation of rainbow trout (*Oncorhynchus mykiss*)" written by Mehdi Soltani and the colleagues. The MS describes the efficacy of orally used of red yeast (*Phaffia rhodozyma*) at various levels on the performance, carcass composition, antioxidant activity, and fillet pigmentation of rainbow trout (*Oncorhynchus mykiss*) under a standard water quality condition and feeding quality. Synthetic astaxanthin and basal diet were also used as controls. The data show how the red yeast is positively effective on fish immune-physiological and growth criteria. I do clarify that the data are original and have not been published or submitted to other journals.

Declaration of Competing Interest

Authors have no conflict of interest on this work.

Data availability

Data availability is depended on the request from the authors.

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References

Amar, E.C., Kiron, V., Satoh, S., Watanabe, T., 2001. Influence of various dietary synthetic carotenoids on bio-defence mechanisms in rainbow trout, Oncorhynchus mykiss (Walbaum). Aquac. Res. 32, 162–173. https://doi.org/10.1046/j.1355-557x.2001.00051.x.

Amar, E.C., Kiron, V., Satoh, S., Watanabe, T., 2004. Enhancement of innate immunity in rainbow trout (*Oncorhynchus mykiss* Walbaum) associated with dietary intake of carotenoids from natural products. Fish. Shellfish Immunol. 16 (4), 527–537. https://doi.org/10.1016/j.fsi.2003.09.004.

AOAC, 1995. Official Methods of Analysis. Association of Official Analytical Chemists, Inc.,, Arlington, Virginia, USA.

Bertram, H.C., Purslow, P.P., Andersen, H.J., 2002. Relationship between meat structure, water mobility, and distribution: a low-field nuclear magnetic resonance study. J. Agric. Food Chem. 50 (4), 824–829. https://doi.org/10.1021/jf010738f.

Bransden, M.P., Carter, C.G., Nichols, P.D., 2003. Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): effect on growth performance, tissue fatty acid composition and disease resistance. Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 135 (4), 611–625. https://doi.org/10.1016/S1096-4959(03)00143-X.

Burgos-Aceves, M.A., Cohen, A., Smith, Y., Faggio, C., 2018. MicroRNAs and their role on fish oxidative stress during xenobiotic environmental exposures. Ecotoxicol. Environ. Saf. 148, 995–1000. https://doi.org/10.1016/j.ecoenv.2017.12.001.

Chen, Y.Y., Lee, P.C., Wu, Y.L., Liu, L.Y., 2015. In vivo effects of free form astaxanthin powder on anti-oxidation and lipid metabolism with high-cholesterol diet. PLoS One 10 (8), e0134733. https://doi.org/10.1371/journal.pone.0134733.

Cheng, C.-H., Guo, Z.-X., Ye, C.-X., Wang, A.-L., 2018. Effect of dietary astaxanthin on the growth performance, non-specific immunity, and antioxidant capacity of pufferfish (*Takifugu obscurus*) under high temperature stress. Fish Physiol. Biochem. 44 (1) https://doi.org/10.1007/s10695-017-0425-5.

Chew, B.P., Park, J.S., 2004. Carotenoid action on the immune response. J. Nutr. 134, 257–261. https://doi.org/10.1093/jn/134.1.257S.

Choi, J., Rahman, M.M., Lee, S.Y., Chang, K.H., Lee, S.M., 2016. Effects of dietary inclusion of fermented soybean meal with *Phaffia rhodozyma* on growth, muscle pigmentation, and antioxidant activity of juvenile rainbow trout (Oncorhynchus mykiss). Turk. J. Fish. Aquat. Sci. 16 (1), 091–101. https://doi.org/10.4194/1303-2712-v16_1_10.

Finkel, T., 2003. Oxidant signals and oxidative stress. Curr. Opin. Cell Biol. 15 (2), 247–254. https://doi.org/10.1016/S0955-0674(03)00002-4.

Forrest, J.C., Morgan, M.T., Borggaard, C., Rasmussen, A.J., Jespersen, B.L., Andersen, J. R., 2000. Development of technology for the early postmortem prediction of water holding capacity and drip loss in fresh pork. Meat Sci. 55 (1), 115–122. https://doi.org/10.1016/S0309-1740(99)00133-3.

Gómez-Guillén, M.C., Montero, P., Hurtado, O., Borderías, A.J., 2000. Biological characteristics affect the quality of farmed Atlantic salmon and smoked muscle. J. Food Sci. 65 (1), 53–60. https://doi.org/10.1111/j.1365-2621.2000.tb15955.x.

Guerin, M., Huntley, M.E., Olaizola, M., 2003. Haematococcus astaxanthin: applications for human health and nutrition. Trends Biotechnol. 21 (5), 210–216. https://doi.org/10.1016/S0167-7799(03)00078-7.

Guo, B., Dalrymple, B.P., 2017. Chapter 11 - transcriptomics of meat quality. In: Purslow, P.P. (Ed.), New Aspects of Meat Quality. Woodhead Publishing, pp. 259–320. https://doi.org/10.1016/B978-0-08-100593-4.00012-6.

Heidt, H., Cinar, M.U., Uddin, M.J., Looft, C., Jüngst, H., Tesfaye, D., Becker, A., Zimmer, A., Ponsuksili, S., Wimmers, K., Große-Brinkhaus, C., 2013. A genetical genomics approach reveals new candidates and confirms known candidate genes for drip loss in a porcine resource population. Mamm. Genome 24 (9), 416–426. https://doi.org/10.1007/s00335-013-9473-z.

Higuera-Ciapara, I., Felix-Valenzuela, L., Goycoolea, F.M., 2006. Astaxanthin: a review of its chemistry and applications. Crit. Rev. Food Sci. Nutr. 46 (2), 185–196. https://doi.org/10.1080/10408690590957188.

Huff-Lonergan, E., 2009. Fresh meat water-holding capacity. Improving the Sensory and Nutritional Quality of Fresh Meat, 147–160.

Johnson, E.A., Villa, T.G., Lewis, M.J., 1980. *Phaffia rhodozyma* as an astaxanthin source in salmonid diets. Aquaculture 20 (2), 123–134. https://doi.org/10.1016/0044-8486(80)90041-1.

Kalinowski, C.T., Robaina, L.E., Izquierdo, M.S., 2011. Effect of dietary astaxanthin on the growth performance, lipid composition and post-mortem skin colouration of red porgy Pagrus *pagrus*. Aquac. Int. 19 (5), 811–823. https://doi.org/10.1007/s10499-010-9401-0.

Karalazos, V., Bendiksen, E.Å., Dick, J.R., Bell, J.G., 2007. Effects of dietary protein, and fat level and rapeseed oil on growth and tissue fatty acid composition and metabolism in Atlantic salmon (*Salmo salar* L.) reared at low water temperatures. Aquac. Nutr. 13 (4), 256–265. https://doi.org/10.1111/j.1365-2095.2007.00471.x.

Kidd, P., 2011. Astaxanthin, cell membrane nutrient with diverse clinical benefits and anti-aging potential. Altern. Med. Rev. 16 (4), 355–364.

Komolka, K., Bochert, R., Franz, G.P., Kaya, Y., Pfuhl, R., Grunow, B., 2020. Determination and comparison of physical meat quality parameters of percidae and salmonidae in aquaculture. Foods 9 (4), 388. https://doi.org/10.3390/foods9040388.

Latimer, G.W., 2012. Official Methods of analysis of AOAC International, nineteenthth ed. AOAC International, Rockville, MD, USA.

Lorenz, R.T., 1999. A technical review of *Haematococcus* algae. Nat. Tech. Bull. 60, 1–12.

Martinez-Alvarez, R.M., Morales, A.E., Sanz, A., 2005. Antioxidant defenses in fish: biotic and abiotic factors. Rev. Fish Biol. Fish. 15 (1–2), 75–88. https://doi.org/10.1007/s11160-005-7846-4.

Montero, D., Grasso, V., Izquierdo, M.S., Ganga, R., Real, F., Tort, L., Caballero, M.J., Acosta, F., 2008. Total substitution of fish oil by vegetable oils in gilthead sea bream (*Sparus aurata*) diets: effects on hepatic Mx expression and some immune parameters. Fish. Shellfish Immunol. 24 (2), 147–155. https://doi.org/10.1016/j.fsi.2007.08.002.

Nakano, T., Kanmuri, T., Sato, M., Takeuchi, M., 1999. Effect of astaxanthin rich red yeast (*Phaffia rhodozyma*) on oxidative stress in rainbow trout. Biochim. Et. Biophys. Acta (BBA) Gen. Subj. 1426 (1), 119–125. https://doi.org/10.1016/S0304-4165(98)00145-7.

Olmedilla, B., Granado, F., Southon, S., Wright, A.J., Blanco, I., Gil-Martinez, E., van den Berg, H., Corridan, B., Roussel, A.M., Chopra, M., Thurnham, D.I., 2001. Serum concentrations of carotenoids and vitamins A, E, and C in control subjects from five European countries. Br. J. Nutr. 85 (2), 227–238. https://doi.org/10.1079/BJN2000248.

Pashkow, F.J., Watumull, D.G., Campbell, C.L., 2008. Astaxanthin: a novel potential treatment for oxidative stress and inflammation in cardiovascular disease. Am. J. Cardiol. 101 (10), S58–S68. https://doi.org/10.1016/j.amjcard.2008.02.010.

Ponsuksili, S., Jonas, E., Murani, E., Phatsara, C., Srikanchai, T., Walz, C., Schwerin, M., Schellander, K., Wimmers, K., 2008. Trait correlated expression combined with expression QTL analysis reveals biological pathways and candidate genes affecting water holding capacity of muscle. BMC Genom. 9 (1), 1–14. https://doi.org/10.1186/1471-2164-9-367.

Ranga, R., Sarada, A.R., Baskaran, V., Ravishankar, G.A., 2009. Identification of carotenoids from green alga *Haematococcus pluvialis* by HPLC and LC-MS (APCI) and their antioxidant properties. J. Microbiol. Biotechnol. 19 (11), 1333–1341. https://doi.org/10.4014/jmb.0905.03007.

Ranga A., R., 2011. Production of Astaxanthin from Cultured Green Alga *Haematococcus pluvialis* and its Biological Activities, (Ph.D. thesis). University of Mysore,, Mysore, India.

Ranga, R.A., Phang, S.M., Ravi, S., Aswathanarayana, R.G., 2014. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—a review. Mar. Drugs 12, 128–152. https://doi.org/10.3390/md12010128.

Ranga, R.A., Raghunath Reddy, R.L., Baskaran, V., Sarada, R., Ravishankar, G.A., 2010. Characterization of microalgal carotenoids by mass spectrometry and their bioavailability and antioxidant properties elucidated in rat model. J. Agric. Food Chem. 58 (15), 8553–8559. https://doi.org/10.1021/jf101187k.

Rashidian, G., Rainis, S., Prokic, M., Faggio, C., 2020. Effects of different levels of carotenoids and light sources on swordtail fish (*Xiphophorus helleri*) growth, survival rate and reproductive parameters. Nat. Prod. Res. 34 (4), 2413–2423. https://doi.org/10.1080/14786419.2020.1723091.

Sanderson, G.W., Jolly, S.O., 1994. The value of Phaffia yeast as a feed ingredient for salmonid fish. Aquaculture 124 (1–4), 193–200. https://doi.org/10.1016/0044-8486(94)90377-8.

Sarada, R., Tripathi, U., Ravishankar, G.A., 2002. Influence of stress on astaxanthin production in *Haematococcus pluvialis* grown under different culture conditions. Process Biochem. 37 (6), 623–627. https://doi.org/10.1016/S0032-9592(01)00246-1.

Segner, H., Arend, P., Von Poeppinghausen, K., Schmidt, H., 1989. The effect of feeding astaxanthin to Oreochromis niloticus and Colisa labiosa on the histology of the liver. Aquaculture 79 (1–4), 381–390. https://doi.org/10.1016/0044-8486(89)90480-8.

Whyte, J.N., Sherry, K.L., 2001. Pigmentation and composition of flesh of Atlantic salmon fed diets supplemented with the yeast *Phaffia rhodozyma*. North Am. J. Aquac. 63 (1), 52–57. https://doi.org/10.1577/1548-8454(2001)063<0052:PACOFO>2.0.CO;2.

Wu, S., Xu, B., 2021. Effect of dietary astaxanthin administration on the growth performance and innate immunity of juvenile crucian carp (*Carassius auratus*). Biotech 3, 151. https://doi.org/10.1007/s13205-021-02700-3.