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# One step toward aquaculture sustainability of a carnivorous species: Fish meal replacement with barley protein concentrate plus wheat gluten meal in Caspian brown trout (*Salmo trutta caspius*)

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ABSTRACT

The present study evaluated the effect of replacing fish meal (FM) with barley protein concentrate (BPC) plus wheat gluten meal (WGM) on growth performance, carcass composition, digestibility, digestive enzyme activities, amino acid, and fatty acids profile of Caspian brown trout as a slow-growing and carnivorous fish species. Five experimental diets including Control (0 g/kg BPC), 25BPC (165 g/kg BPC), 50BPC (330 g/kg BPC), 75BPC (495 g/kg BPC), and 100BPC (660 g/kg BPC) were formulated. Also, WGM was added to diets to make them isonitrogenous. A total of 300 fish (13.53 ± 1.1 g) were farmed in three replicates per treatment for eight weeks. Results showed that FM could be replaced by BPC up to 50 % without any negative effect on growth performance (330 g/kg FM, 330 g/kg BPC). Fish was fed with dietary Control, 25BPC, and 50BPC digested protein and energy significantly better than those fed other diets. Accordingly, there were negative linear relations between BCP level in diets and trypsin, pepsin, and aminopeptidase activities ($P < 0.01$). There was no significant difference in the C20:5n3, C22:6n3, and long-chain polyunsaturated fatty acids (LC-PUFA) levels (g/kg) between individuals fed dietary Control (82.6, 148.5, 278, respectively) and 50BPC (67.0, 132.5, 244.7, respectively). Finally, pepsin and aminopeptidase had strong positive relations with weight gain, specific growth rate, the apparent digestibility coefficient of protein, lysine, and methionine values. Therefore, we suggest formulating diets with BPC up to 330 g/kg plus 40 g /kg WGM in Caspian brown trout diets based on unseen negative effects on investigated factors.

## 1. Introduction

Many projects have focused on finding different sustainable ways to provide food for humans in the coming decades. Among various agriculture sectors, aquaculture has attracted attention as fish can be farmed in marine water (marine water covers 72 % of our planet). Furthermore, aquaculture products are high-quality foods and beneficial for human health (FAO, 2020). As growth performance and feed efficiency can guaranty the profitability and sustainability of any aquaculture farm, every method for improving these parameters, such as dietary manipulations, can be a beneficial step toward achieving the sustainability of aquaculture (Asgari et al., 2020; Ghosi Mobaraki et al., 2020). Because of the balanced protein and amino acids, fish meal (FM) is considered the best natural raw ingredient in aquatic animal diets (Miles and Chapman, 2006). However, the limitation of FM resources in marine environments is a severe obstacle to developing aquaculture worldwide. Some carnivorous fishes like salmonids require a high amount of FM in their diets. Several studies in salmonids have focused on replacing FM with other protein sources, especially plant-based alternatives, to reduce the dependency on FM. For example, soybean meal (Booman et al., 2018; Kumar et al., 2020), rapeseed (Carter and Hauler, 2000; Slawski et al., 2012), potato protein concentrate (Refstie and Tiekstra, 2003; Tusche et al., 2013), safflower (Ustaoglu Tiril and Kerim, 2015), corn gluten meal (Stone et al., 2005; Yiğit et al., 2012; Doughty et al., 2019),

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rice (Palmegiano et al., 2006) camelina (Hixson et al., 2016), and chickweed leaf meal (Yılmaz and Ergün, 2013) were tested. Caspian brown trout (*Salmo trutta caspius*) requires a high amount of FM (65–75 % of total protein in diets should be provided by FM) for optimum growth compared with other salmonids (30–50 % of total protein in diets should be provided by FM). However, only one study investigated FM replacing with spirulina (*Spirulina platensis*) in Caspian brown trout (Roohani et al., 2019). By production up to 150 million tonnes, barley is among the top four produced grains worldwide. Nowadays, barley is gaining attention as a functional food because of its medicinal value and high-quality protein (Narwal et al., 2020). Barley protein concentrate (BPC), as a new alternative ingredient in aquaculture, has rarely been tested in fish (Morken et al., 2011; Burr et al., 2013; Rossi et al., 2013; Bell et al., 2016).

Caspian brown trout is sensitive to environmental stressors and hardly accepts formulated diets under farming conditions, and grows slowly compared to other salmonids. However, this species is one of the most expensive salmonids (12 times more expensive than rainbow trout (*Oncorhynchus mykiss*; Roohani et al., 2019). As part of enhancement programs in 2017, more than 500,000 juveniles of Caspian brown trout were released in rivers around the Caspian Sea (Roohani et al., 2019). In recent years, farmers have started to farm this species. As this fish requires a high amount of FM in diet and grows slowly, replacing FM with other alternative sources is necessary for Caspian brown trout aquaculture sustainability. Despite the importance as a premium species, there is no study about the potential use of BPC to spare FM during early growth stages. Also, most of the investigated parameters in this study are the first reports related to the impact of BPC on fish physiology. Therefore, the goal of this study was to examine replacing FM with BPC plus wheat gluten meal (WGM) on growth performance, body composition, amino acids profile, digestibility, fatty acids profile, and digestive enzyme activities of Caspian brown trout.

## 2. Materials and methods

### 2.1. Ethics statement

All procedures involving animals were conducted according to the Tarbiat Modares University protocols, which seek to optimize handling and minimize animal stress (Asadi et al., 2020; Ramezanzadeh et al., 2020a; Zeilab Sendijani et al., 2020).

### 2.2. Preparation of BPC and experimental diets

Barley seed (hull-less) was purchased from a local market and then powdered to prepare protein concentrate by alkaline method (Wang et al., 2010). According to this method, barley powder was mixed with deionized water (1:10 ratio), the mixture pH reached 11.5 using NaOH (0.5 mol/L) and stirred at room temperature for 25 min. Then, the resulting product was centrifuged at 8000 *g* for 15 min, then the supernatant was removed, and its pH reached 5 with 0.5 M HCl. After centrifugation, the precipitated protein was isolated and washed with deionized distilled water, followed by pH adjustment to 7. The samples were then freeze-dried, and the final product as BPC was used in this trial. For making experimental diets, required raw ingredients were purchased from the Mazandaran Animal & Aquatic Feed Company (Sari, Mazandaran, Iran). Lindo software was used to formulate five isonitrogenous (440 *g* crude protein/kg feed) and isoenergetic (22 kJ/g) diets. They were Control (0 *g*/kg BPC), 25BPC (25 % FM replacement: 165 *g*/kg BPC), 50BPC (50 % FM replacement: 330 *g*/kg BPC), 75BPC (75 % FM replacement: 495 *g*/kg BPC), and 100BPC (100 % FM replacement: 660 *g*/kg BPC). Also, wheat gluten was added to diets to make them isonitrogenous. We designed the treatments according to previous studies in this fish species and personal communication with a farmer. We first dried and mixed ingredients completely to make them a homogeneous mixture. In the next step, kilka fish oil and lecithin were weighed and added gradually to the mixture. The resulting mixture was ground by a meat grinder (Electrokar EC-1, Tehran, Iran) to form pellets with a 2 mm diameter. Then, pelleted granules were spread out on a tray and dried in an oven to ≥ 90 % dry matter at 60 °C for 24–48 hr. Finally, we stored the pellets in the fridge at 4 °C. Table 1 presented the experimental diets and their chemical compositions. Amino acids and fatty acids composition of five experimental diets, FM and BCP, were reported in Table 2 and Table 3, respectively.

**Table 1**
Formulation and proximate analyses of the experimental diets containing different levels of barley protein concentrate (BPC) (g/kg in diet).

| | g/kg, as-fed basis | | | | |
|---|---|---|---|---|---|
| | Control | 25BPC | 50BPC | 75BPC | 100BPC |
| Fish meal [1] | 660 | 495.0 | 330.0 | 165.0 | 0 |
| Barley protein concentrate [2] | 0 | 165.0 | 330.0 | 495.0 | 660.0 |
| Wheat flour [3] | 182.3 | 162.3 | 142.3 | 122.3 | 102.3 |
| Wheat gluten meal [3] | 0 | 20 | 40 | 60 | 80 |
| Fish oil [1] | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Mineral supplement [4] | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| Vitamin supplement [5] | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| Chrome oxide | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Other ingredients | 22.7 | 22.7 | 22.7 | 22.7 | 22.7 |
| **Proximate composition (g/kg dry matter)** | | | | | |
| Crude protein | 446.3 | 439.6 | 442.6 | 441.2 | 439.7 |
| Crude fat | 175.9 | 176.4 | 172.1 | 178.5 | 175.0 |
| Ash | 104.0 | 103.8 | 99.7 | 105.0 | 101.0 |
| Carbohydrate [6] | 160.0 | 172.8 | 172.3 | 160.7 | 173.5 |
| Moisture | 113.8 | 107.4 | 113.3 | 114.6 | 110.8 |
| Gross energy (kJ/g) [7] | 21.74 | 22.16 | 22.40 | 22.19 | 22.18 |

*__Other ingredients__ include lecithin 5 g, declaim phosphate 5 g, antifungal agent 2.5 g (Toxiban premix (Component: Alomino silicate, zeolite, bentonate, propionate ammonium)), antioxidant 0.2 g (Butylated hydroxytoluene (BHT)), molasses 10 g.

Dietary treatments with different levels of BPC including Control (0 g/kg BPC), 25BPC (25 % fish meal replacement: 165 g/kg BPC), 50BPC (50 % fish meal replacement: 330 g/kg BPC), 75BPC (75 % fish meal replacement: 495 g/kg BPC), and 100BPC (100 % fish meal replacement: 660 g/kg BPC).

[1] . Kilka fish meal and fish oil (*Clupeonella delicatula*), Mazandaran Animal & Aquatic Feed Company (Mazandaran, Sari, Iran).

[2] . Barley protein concentrate contained 55.45 % protein, 10.6 % fat and 5.79 % ash.

[3] . Mazandaran Animal & Aquatic Feed Company (Mazandaran, Sari, Iran). Wheat gluten meal was added to diets to make them isonitrogenous.

[4] . **Every 100 g of premix mineral contain:** 3000 mg iron, 50,000 mg of zinc, 10 mg of selenium, 50 mg of cobalt, 300 mg copper, 250 mg of manganese, and 300 mg of choline chloride.

[5] . **5 kg Vitamin Supplementation** 0.5 % **contained:** vitamin A 80,000 IU/kg;vitamin D3 2000 IU/kg; vitamin k 20 mg/kg; thiamin 60 mg/kg; riboflavin 60 mg/ kg; pyridoxine 100 mg/kg; pantothenic acid 150 mg/kg; niacin 300 mg/kg; biothin 2 mg/kg; folic acid 20 mg/kg; vitamin B12 0.1 mg/kg; inositol 300 mg/kg; ascorbic acid 600 mg/kg; choline chloride 3000 mg/kg.

[6] . Carbohydrate = 100 - (crude protein + crude fat + ash + moisture).

[7] . Estimated gross energy was calculated based on 1 g crude protein being 23.6 kj, 1 g crude fat being 39.5 kj, and 1 g carbohydrate being 17.2 kj. NRC (2011).

### 2.3. Caspian brown trout farming and experimental conditions

For this experiment, a total of 300 juveniles Caspian brown trout (initial weight: 13.53 ± 1·10 g) was purchased from a reproduction center in Tonkabon (Mazandaran, Iran) and adapted three weeks with the experimental condition in the Aquarium Facility at Sari Agricultural Sciences and Natural Resources University. Fifteen 300- L fiberglass tanks within a semi-recirculating system were adjusted for five treatments (20 fish per tank). The static water system with daily exchange at a rate of 30%–40% for each tank was used in this study. Water quality parameters were regularly checked and kept in the standard levels for the culture of Caspian brown trout throughout the 8-week trial

**Table 2**
Amino acids profile in the experimental diets, barley protein concentrate (BPC), wheat gluten meal (WGM), and fish meal (g/kg protein).

| Amino acid | Control | 25BPC | 50BPC | 75BPC | 100BPC | Fish meal | WGM | BPC | SDM |
|---|---|---|---|---|---|---|---|---|---|
| Arginine | 59.8 | 49.9 | 46.7 | 51.7 | 42.7 | 55.5 | 35.3 | 34.0 | 6.5 |
| Histidine | 10.2 | 8.4 | 11.9 | 8.6 | 10.6 | 9.2 | 12.4 | 31.6 | 1.4 |
| Isoleucine | 31.5 | 39.1 | 35.7 | 34.7 | 36.6 | 34.2 | 32.0 | 28.7 | 5.6 |
| Leucine | 68.5 | 63.5 | 64.4 | 72.6 | 69.7 | 71.1 | 69.2 | 42.6 | 8.7 |
| Lysine | 47.7 | 44.3 | 41.2 | 37.2 | 30.3 | 67.4 | 14.4 | 18.9 | 7.9 |
| Phenylalanine | 32.7 | 32.6 | 32.4 | 35.5 | 44.1 | 32.4 | 47.5 | 39.7 | 5.5 |
| Threonine | 29.5 | 24.8 | 25.9 | 21.1 | 21.9 | 31.9 | 29.1 | 25.3 | 6.1 |
| Valine | 31.8 | 35.0 | 43.6 | 45.1 | 44.7 | 43.0 | 30.7 | 44.8 | 6.8 |
| Methionine | 20.3 | 21.6 | 16.7 | 14.5 | 10.5 | 23.7 | 11.0 | 8.7 | 4.5 |
| Total essential amino acids | 332 | 319.2 | 318.5 | 321 | 311.1 | 368.4 | 281.6 | 274.3 | 36.8 |
| Glutamic acid | 126.5 | 136.6 | 153.8 | 181.2 | 197.8 | 116.2 | 360.4 | 156.6 | 17.3 |
| Serine | 47.8 | 44.8 | 52.7 | 48.0 | 51.3 | 40.9 | 51.3 | 37.7 | 10.0 |
| Aspartic acid | 62.0 | 60.7 | 56.2 | 56.9 | 45.7 | 76.3 | 30.1 | 34.0 | 9.5 |
| Glycine | 60.8 | 67.0 | 69.7 | 70.0 | 52.1 | 70.1 | 34.6 | 32.1 | 10.2 |
| Alanine | 43.3 | 37.8 | 39.7 | 31.7 | 27.2 | 52.8 | 23.1 | 28.2 | 7.5 |
| Tyrosine | 15.3 | 23.2 | 29.2 | 19.5 | 21.5 | 18.2 | 20.5 | 22.7 | 8.2 |
| Total non-essential amino acids | 355.7 | 370.1 | 401.3 | 407.3 | 395.6 | 374.5 | 520.0 | 311.3 | 27.0 |

**Table 3**
Fatty acids profile (g/kg Fatty Acid Methyl Esters (FAMEs)) of the experimental diets, barley protein concentrate (BPC), and fish meal.

| Fatty acid | Control | 25BPC | 50BPC | 75BPC | 100BPC | Fish meal | BCP | SD (average) |
|---|---|---|---|---|---|---|---|---|
| C14:00 | 33.2 | 35.1 | 26.2 | 21.0 | 18.6 | 41.2 | 1.2 | 4.9 |
| C16:00 | 150.6 | 169.5 | 181.5 | 203 | 229.5 | 107.8 | 235.4 | 21.2 |
| C18:00 | 31.4 | 38.1 | 44.9 | 43.0 | 35.5 | 12.5 | 26.5 | 5.6 |
| SFA † | 220.5 | 248.6 | 260.0 | 275.9 | 281.4 | 161.6 | 269.7 | 27.4 |
| C16:1n1 | 35.4 | 30.8 | 27.8 | 19.0 | 17.6 | 27.0 | 2.4 | 6.3 |
| C18:1n-7 | 21.4 | 20.5 | 24.3 | 26.4 | 23.9 | 10.3 | 23.5 | 5.8 |
| C18:1n9 | 135.6 | 140.5 | 157.6 | 167.3 | 171.2 | 65.4 | 104.5 | 23.7 |
| C20: n-1 | 30.4 | 29.8 | 29.7 | 20.5 | 21.2 | 38.7 | 1.4 | 6.2 |
| C22: n-1 | 104.8 | 89.7 | 74.2 | 68.6 | 58.9 | 194.5 | 0.8 | 20.12 |
| MUFA ‡ | 334.1 | 321.5 | 328.6 | 310.5 | 298.5 | 340.0 | 13.87 | 34.5 |
| C18:2n6 | 89.5 | 131.1 | 153.9 | 183.2 | 202.8 | 14.9 | 514.0 | 25.9 |
| C18:3n3 | 26.8 | 27.5 | 29.4 | 31.5 | 23.12 | 11.1 | 34.5 | 3.0 |
| C20:3n-6 | 10.6 | 10.2 | 9.8 | 10.1 | 8.7 | 14.3 | 1.2 | 2.4 |
| C20:4n6 | 10.1 | 14.5 | 13.7 | 12.8 | 12.1 | 3.7 | – | 2.3 |
| C20:5n3 | 81.9 | 62.0 | 56.8 | 41.6 | 35.9 | 156.8 | – | 7.9 |
| C22:6n3 | 168.3 | 143.2 | 122.1 | 103.4 | 96.5 | 286.5 | 9.6 | 17.0 |
| PUFA § | 394.2 | 400.2 | 389.7 | 386.6 | 385.0 | 491.1 | 56.9 | 43.5 |
| LC-PUFA §§ | 270.9 | 229.9 | 202.4 | 167.9 | 153.2 | 461.3 | 67.7 | 19.8 |
| n-3 ¶ | 277.0 | 232.7 | 208.3 | 176.5 | 155.52 | 454.4 | 44.1 | 32.7 |
| n-6 ¶¶ | 110.2 | 155.8 | 177.4 | 206.1 | 223.6 | 16.1 | 515.2 | 17.6 |
| n3/n6 | 2.51 | 1.49 | 1.17 | 0.86 | 0.70 | 28.22 | 0.08 | 0.3 |

† Saturated fatty acid—the sum of all fatty acids without double bonds; includes C18:0, C20:0, and C22:0 in addition to individually reported SFAs.
‡ Monounsaturated fatty acids—the sum of all fatty acids with a single, double bond; includes 18:1n-7, 22:1n-11, and 22:1n-9 in addition to individually reported MUFAs.
§ Polyunsaturated fatty acids—the sum of all fatty acids with ≥ 2 double bonds; includes 16:2n-4 in addition to individually reported PUFAs.
§§ Long-chain PUFAs: the sum of all fatty acids with chain length ≥ 20 carbon atoms and ≥ 3 double bonds.
¶ Sum of all n-6 fatty acids; includes 20:3n-6 in addition to individually reported n-6 fatty acids.
¶¶ Sum of all n-3 fatty acids.

(temperature 14 ± 1 °C; DO 8.5 ± 1 mg /L; pH 7.5 ± 0.4; total ammonia nitrogen< 0.05 mg /L). Photoperiod was maintained at 12D:12 L, and fish were hand-fed three times daily to apparent satiation. The temperature was measured by a thermometer (Zomorodazma Company, Iran) and dissolved oxygen and pH by Electrochemistry Meters (AQUALYTIC, AL15, Germany).

### 2.4. Evaluation of growth indices

At the end of the experimental period, all fish were fasted for 24 h and were then anesthetized with the clove oil stock solution (50−70 ppm) (Esmaeili et al., 2017a; Hosseinpour Aghaei et al., 2018). The survival rate and growth indices were determined using standard formulas and reported in the footnote of Table 4. Three fish per tank was randomly selected, and then their respective liver was sampled and weighed. We used carcass for performing body composition analysis.

### 2.5. Chemical analysis of diets and fish carcass

The proximate composition of diets and carcass samples were analyzed using AOAC methods (AOAC, 2000). Briefly, crude protein was determined by the Kjeldahl method, using an automatic Kjeldahl system (Kjeltec Analyser unit 2300, Sweden). Crude fat was analyzed with the Soxhlet extraction method (Soxtec 2050 FOSS Model, Switzerland). Moisture was determined by drying samples in an oven at 105 °C for 12 h. A Nabertherm muffle furnace (Model K, Germany) was used for the determination of ash (550 °C for 4 h) (Safavi et al., 2019). Nitrogen- free extract plus fiber, representing carbohydrate, was calculated using the formula: carbohydrate = 100 − (protein + fat + ash + moisture) (Aksnes and Opstvedt, 1998). Gross energy of the diet and feces was calculated according to the National Research Council (NRC, 2011):

Energy (MJ/kg) = (protein ×23.6 kJ/g) + (fat ×39.5 kJ/g) + (carbohydrate ×17.2 kJ/g).

**Table 4**
Growth performance of Caspian brown trout fed experimental diets containing different levels of barley protein concentrate (BPC) for eight weeks.

| | Control | 25BPC | 50BPC | 75BPC | 100BPC | ANOVA | Liner | Quadratic |
|---|---|---|---|---|---|---|---|---|
| Initial weight (g) | 13.84 ± 2.11 | 13.21 ± 1.55 | 13.50 ± 0.56 | 13.79 ± 0.74 | 13.30 ± 0.8 | | | |
| Final weight (g) | 34.37 ± 2.68 [a] | 35.94 ± 1.22 [a] | 34.41 ± 3.00 [a] | 27.07 ± 2.61 [b] | 25.92 ± 1.08 [b] | 0.01 | 0.00 | 0.05 |
| Weight gain (%) | 248.3 ± 30.3 [a] | 272.1 ± 34.8 [a] | 254.9 ± 32.5 [a] | 196.3 ± 34.6 [b] | 194.9 ± 10.5 [b] | 0.02 | 0.00 | 0.16 |
| DGR (g) | 0.62 ± 0.08 [a] | 0.64 ± 0.02 [a] | 0.61 ± 0.05 [a] | 0.48 ± 0.06 [b] | 0.46 ± 0.02 [b] | 0.00 | 0.00 | 0.11 |
| SGR | 2.27 ± 0.32 | 2.35 ± 0.14 | 2.30 ± 0.08 | 1.93 ± 0.12 | 1.92 ± 0.02 | 0.07 | 0.01 | 0.31 |
| FCR | 2.11 ± 0.14 [b] | 2.16 ± 0.09 [b] | 2.79 ± 0.12 [ab] | 3.40 ± 0.12 [a] | 3.41 ± 0.02 [a] | 0.00 | 0.00 | 0.75 |
| HSI | 1.36 ± 0.07 | 1.26 ± 0.08 | 1. 27 ± 0.05 | 1.34 ± 0.11 | 1.36 ± 0.14 | 0.28 | 0.48 | 0.09 |
| Survival rate (%) | 100 | 100 | 100 | 100 | 100 | | | |

Values are represented means ± SDM of triplicate tanks; means without letter labels are not significantly different. According to Duncan's multiple range tests, the letters a and b indicate significant differences in the treatments ($P < 0.05$).
Weight gain (%) = (weight gain (g) / Initial weight (g)) × 100.
Daily growth rate (DGR) = (Final body weight – Initial body weight/number of rearing days.
SGR = [(Ln $W_2$ - Ln $W_1$) / number of rearing days] × 100.
FCR = Amount of feed taken / body weight gain (g).
Hepatosomatic index (HSI) = [Weight of liver (g) / body weight (g)] × 100.
Survival rate (%) = (Initial number − number of mortalities / initial number) × 100.

### 2.6. Apparent digestibility coefficient (ADC)

For analyzing ADC, chrome oxide (5 g) was supplemented to the diets as a marker for measuring the apparent digestibility of protein, fat, dry matter, ash, and energy. Feces were collected 3–4 h after feeding for 14 days (Esmaeili et al., 2017b) (from day 36th to 50th of the experimental period). Feces were carefully collected from the tank floor by siphoning, and extra attention was intended to this procedure to avoid contamination or misleading samples. Additionally, extra and/or uneaten feed pellets were collected 30 min after feeding. Once feces samples were completely collected, they were freeze-dried and kept at -20° for further analysis (Esmaeili et al., 2017b). The level of chrome oxide in diet and feces was analyzed by atomic absorption spectrophotometry (Williams et al., 1962). The ADC was calculated by the below formula (Maynard and Loosli, 1969):

ADC (%) = 100 − [100(%chrome in feed/%chrome in feces) × [% nutrient in feces / %nutrient in feed)].

### 2.7. Amino acid analyses

For the detection of amino acids in carcass and diets, the method of o-phthaldialdehyde (OPA) was used (Lindroth and Mopper, 1979). Briefly, after hydrolysis of samples in 6 N HCl for 24 h at 110 °C, samples were derivatized with OPA. The total amino acids were analyzed by HPLC (Wissenschaftliche Gerätebau Dr. Ing. Herbert Knauer GmbH / Hegauer Weg 38 / D-14,163

Berlin, Germany) at the flow rate of 1 mL/min with a fluorescence detector (excitation 330 nm, emission 450 nm).

### 2.8. Fatty acids analyses

We analyzed fatty acids in diets and carcasses by extracting total lipids by chloroform/methanol method (Folch et al., 1957). Following adding methanol to the sample, lipid was methanolized with boron trifluoride (BF3). After observing the two phases, the upper layer was separated and kept at −20 °C until infusion to gas chromatography (GC) mass instrument. From GC (Varian Analytical Instrument, CP 3800, Walnut Creek, CA, Netherland) equipped with a flame ionization detector fitted with a permanently bonded polyethylene glycol, fused silica capillary column (PBX70 SGE Analytical Science; 120 m x0.25 mm internal diameter, film thickness 0.25 μm, Melbourne, Australia) was used for separating methyl esters (FAMEs) fatty acids. The injection volume was 1.0 μL, and the carrier gas was helium. The injector and detector temperature were respectively 230 °C and 260 °C. A split injection approach of 20:1 was used, and the temperature was programmed to increase from 160 °C to 180 °C at a rate of 2 °C min-1 and held at 180 °C for 85 min. Individual FAMEs were identified using the external standard as a reference (Sigma-Aldrich, Steinheim, Germany) (Tazikeh et al., 2020).

### 2.9. Digestive enzyme activities

The stomach and whole intestine of each fish were homogenized on ice in an electric homogenizer. Stomach enzyme activity (pepsin) and pancreatic enzyme (amylase, lipase, trypsin, chymotrypsin, and aminopeptidase) measurements were taken by dividing the collected digestive tracts from each fish into two sections. The intestine and pyloric caeca were examined to assay pancreatic enzymes, and the stomach section was used to analyze pepsin enzyme activity (Ramezanzadeh et al., 2020b). The complete assay procedure of digestive enzyme activities was reported in our previous research (Ramezanzadeh et al., 2020b).

### 2.10. Statistical analysis

This experiment was conducted in a completely randomized design with five treatments and three replications. Data were analyzed by one-way analysis of variance (ANOVA) after checking the normality of data and homogeneity of variance. Some researchers think the Mean Separation tests after ANOVA are not technically correct for quantitative data such as that generated by feeding graded levels of a nutrient. Therefore, for providing a comprehensive analysis, we additionally determined if the relation of adding BPC to diets with measured factors was linear and/or quadratic using orthogonal polynomial contrasts. We used the SPSS software (version 21.0 for Windows) and assessed the differences among five treatments in growth performance factors, body composition, digestibility, fatty acid profile, amino acid profile, and digestive enzymes by Duncan's multiple range tests ($P < 0.05$). The relationships between the investigated parameters and digestive enzymes were examined by Pearson correlations in SPSS.

## 3. Results and discussion

### 3.1. Growth performance

The present study is the first research in Caspian brown trout to test BPC as an alternative protein source for FM. The replacement of FM by other protein sources has been a hot topic in fish nutrition for many years. As the portion of WGM protein at the highest level (100BPC) was still lower than 14 % of total proteins, we did not focus on it in the results

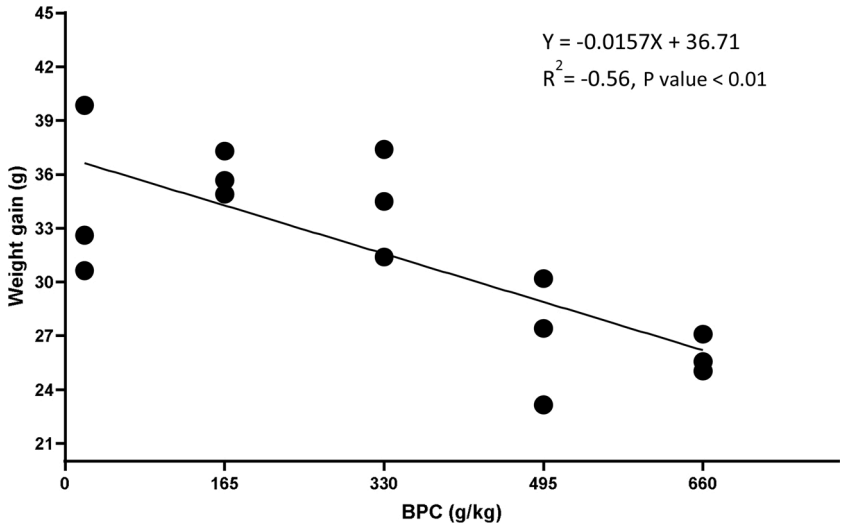


**Fig. 1.** Orthogonal polynomial (linear) relationship of weight gain (g) and the level of barley protein concentrate (BPC) in diets with a linear model in Caspian brown trout fed these diets for eight weeks.

and discussion section. In the 100BPC diet, more than 83 % of total protein contents were provided from BPC. According to Table 4, FM could be replaced with BPC up to 50 % without any adverse effect on growth performance. More precisely, weight gain (%) was higher in Control (248.3 %), 25BPC (272.1 %), and 50 BPC (254.9 %) treatment as compared to others ($P < 0.05$). Also, lower FCR values in fish were fed with dietary Control (2.11) and 25BPC (2.16) compared with those were fed with 75BPC (3.40) and 100BPC (3.41) diets were observed ($P < 0.05$). Also, there was a negative linear relationship between the weight gain (g) and BPC levels in diets (Fig. 1). There was no significant difference in HSI and survival rate, showing that the health status of fish was in an optimum condition. We can hypothesize that 330 g/kg FM is the threshold for this fish species to provide the same growth as Control (660 g/kg). The output of this study can be an outstanding achievement as farmers usually formulate diets with 550−650 g/kg FM for Caspian brown trout of this size. There is just one study available regarding FM replacement in this fish species (Roohani et al., 2019). Roohani et al. (2019) reported no growth impairment with decreasing FM in the fish diet up to 600 g/kg. However, they suggested higher levels of FM replacement should be tested as this level was the highest in their experimental design. It is worth highlighting that we added enough fish oil to the diets (100 g/kg) to prevent omega 3 deficiency. This species relies on fish oil for growth, and the level of FM replacement depends on fish oil contents in the diet. In only one fish oil replacement study in Caspian rainbow trout (Kenari et al., 2011), investigators formulated 670 g/kg FM in diets and could totally replace fish oil with vegetable resources. However, fish of similar size did not grow enough in their study and, after 60 days, just gained a 50−70% increase in weight gain. However, their work is one of the earliest researches in this fish species, and during these ten years, much progress has been achieved in farming Caspian brown trout. More research on this fish is needed to illustrate how much FM and fish oil can be replaced in their diets. Some fish studies revealed that BPC could be potentially formulated as a reliable alternative protein source for FM replacing. In the red drum (*Sciaenops ocellatus*), unlike our data, fish fed a control diet (580 g/kg FM) had significantly higher growth than those fed a diet contained 290:340 g/kg FM: BPC (Rossi et al., 2013). In Atlantic salmon, when 90 g juveniles fed a diet that contained FM (150 g/kg) had similar growth in comparison with those fed BPC and soybean protein concentrate (Bell et al., 2016). As we can see, there is no study in terms of FM replacement only by BPC in the literature available in aquatic studies. From this point, we can also say, the presented research is the preliminary report on aquaculture species.

In general, the present results suggest that FM can be replaced by BPC up to 330 g/kg plus 40 g/kg WGM in diets of Caspian brown trout. Exceeding this limit probably increases the level of antinutrients and, subsequently, reduces the absorption of nutrients and feed palatability, followed by adverse effects on fish growth. Digestibility and digestive enzyme activities also confirmed this issue.

The deficiency of lysine and methionine is another issue that probably impaired growth in 75BPC and 100PBC treatments in our study. The lysine and methionine contents of Control and 100BPC diets were 47.7 and 20.3 g/kg, 30.3 and 10.5 g/kg, respectively. Further, WGM was added to make diets isonitrogenous, and it was inevitable. This issue also worsened lysine and methionine deficiency as WGM is a poor source of these amino acids (14.1, 11.0 g/kg protein). We did not add any amino acid supplementation to diets as we wanted first to observe the fish performance under this condition. However, this research is a preliminary study, and in future works, supplementing lysine and methionine to diets is suggested.

### 3.2. Carcass composition

The chemical composition of fish is a significant determinant for customers. They prefer to buy and eat fish with fewer lipids but high levels of omega 3 fatty acids and more protein contents. A wide range of

**Table 5**
Carcass chemical composition (g/kg) of Caspian brown trout fed experimental diets containing different levels of barley protein concentrate (BPC) for eight weeks.

| | Control | 25BPC | 50BPC | 75BPC | 100BPC | ANOVA | Liner | Quadratic |
|---|---|---|---|---|---|---|---|---|
| Protein | 656.2 ± 17.4 | 655.2 ± 12.1 | 639.5 ± 14.9 | 641.2 ± 21.1 | 633.9 ± 19.5 | 0.34 | 0.05 | 0.91 |
| Fat | 232.3 ± 10.0 | 231.2 ± 8.9 | 231.0 ± 9.8 | 238.8 ± 10.9 | 240.4 ± 8.7 | 0.75 | 0.30 | 0.55 |
| Ash | 100.4 ± 5.7 | 97.2 ± 6.4 | 93.2 ± 4.9 | 93.9 ± 4.3 | 94.6 ± 6.6 | 0.47 | 0.14 | 0.29 |
| Moisture | 744.1 ± 20.1 | 748.4 ± 15.6 | 740.1 ± 18.4 | 740.8 ± 22.2 | 740.1 ± 20.0 | 0.98 | 0.67 | 0.98 |

Values are represented by means ± SDM of triplicate tanks; means without letter labels are not significantly different. According to Duncan's multiple range tests, the letters a and b indicate significant differences in the treatments ($P < 0.05$).

**Table 6**
Digestibility of nutrients (g/kg) in Caspian brown trout fed experimental diets containing different levels of barley protein concentrate (BPC) for eight weeks.

| | Control | 25BPC | 50BPC | 75BPC | 100BPC | ANOVA | Liner | Quadratic |
|---|---|---|---|---|---|---|---|---|
| Dry matter | 848.5 ± 17.7 | 850.1 ± 10.3 | 841.4 ± 20.4 | 843.7 ± 19.5 | 840.9 ± 19.0 | 0.94 | 0.50 | 0.96 |
| Fat | 939.0 ± 21.1 | 937.6 ± 24.3 | 937.8 ± 16.0 | 921.5 ± 21.4 | 935.3 ± 17.1 | 0.77 | 0.51 | 0.73 |
| Protein | 881.7 ± 22.5 [a] | 889.9 ± 10.9 [a] | 877.5 ± 20.2 [a] | 846.5 ± 14.7 [b] | 841.8 ± 15.2 [b] | 0.01 | 0.00 | 0.23 |
| Ash | 786.4 ± 19.7 | 780.9 ± 19.5 | 785.4 ± 18.0 | 786.8 ± 17.4 | 787.5 ± 19.9 | 0.98 | 0.76 | 0.77 |
| Energy | 701.5 ± 18.9 [a] | 686.4 ± 18.4 [a] | 683.2 ± 19.0 [a] | 652.1 ± 19.2 [b] | 650.0 ± 19.5 [b] | 0.01 | 0.00 | 0.95 |

Values are represented by means ± SDM of triplicate tanks; means without letter labels are not significantly different. According to Duncan's multiple range tests, the letters a and b indicate significant differences in the treatments ($P < 0.05$).

internal and external factors such as age, gender, size, water quality, season, and geographical differentiate the chemical composition of fish. Still, the main reason usually comes from the diet (Shearer, 1994). According to Table 5, there were no significant differences in levels of crude protein, fat, and ash among treatments. However, protein contents in fish had a negative linear relationship with the BPC contents in diets ($P < 0.05$). There are few studies available regarding the effect of BPC on the chemical composition of fish. While the difference was not significant, an increase in fat and protein contents in Atlantic salmon was observed (Burr et al., 2013). These data, in terms of protein contents, were compatible with our study. Increasing moisture and decreasing fat and ash contents in the body of Atlantic salmon were fed with dietary BPC were observed (Rossi et al., 2013). As we can see, the response of proximate composition varied between studies. The experimental design, size of fish, BPC, and FM levels in the diet, species, etc., can be reasons for these differences.

### 3.3. Apparent digestibility of nutrients

Nutrients digestibility is one of the most important factors for evaluating whether a protein source can be a reliable alternative for FM or not. Proteins with low digestibility decrease feed efficiency and cause consuming more protein by fish. Consuming more feeds and proteins eventually causes more excretion of ammonia products to the water. Reducing digestibility, availability, and uptake of nutrients are common problems when FM has been replaced by other protein resources (Gasco et al., 2018). Table 6 showed no significant difference in ADC (g/kg) of fat, dry matter, and ash among treatments. Fish fed dietary Control, 25BPC, and 50BPC digested protein and energy contents significantly better than those fed other diets ($P < 0.05$). The results of ADC of nutrients showed that up to 50 % FM replacing fish can digest nutrients well, but upper than this threshold, ADC of fat, protein, and energy were decreased ($P < 0.05$). Morken et al. (2011) examined the effects of partial FM replacement by BPC in the diet of rainbow trout and observed no adverse impact on ADC of nutrients. They did not test the high BPC level, and thus, the results in treatments with lower than 330 g/kg BPC in our study are compatible with rainbow trout. Among seven investigated ingredients (spirulina, corn concentrate, canola concentrate, barley concentrate, yeast protein, fish processing by-product, and FM), BPC, along with FM, had higher digestibility of nutrients in Florida Pompano (*Trachinotus carolinus*) (Riche et al., 2017). This study is a further line of evidence that BPC is a high-quality protein for formulation in fish diets. However, more research is needed to illustrate the response of each species to BPC level in diets. In general, the decreasing growth in 75BPC and 100 PBC treatments can be due to lower ADC of protein, fat, and energy. As discussed in the growth performance section, high antinutritional contents in 75BPC and 100 BPC diets can decrease protein, fat, and energy digestibility. Similarly, many studies in salmonids reported high antinutritional factors from plant-based proteins reduced the ADC of nutrients (Krogdahl et al., 2010; Kokou and Fountoulaki, 2018).

### 3.4. Profile of amino acids

Protein is the most critical and expensive nutrients present in the fish diet, performing a wide range of vital physiological activities. A balanced amino acid profile can guarantee growth, feed efficiency, and fish health (Liu et al., 2019). Any action to reduce FM content in the fish diet can be considered an essential step toward aquaculture sustainability. The main obstacle to achieve this goal is the unbalanced amino acids of plant-based diets. The indispensable amino acids (IAA) of the fish carcass can be a reliable indicator for estimating the fish response to different experimental diets (Conceição et al., 2003). According to Table 2, BPC and WGM had lower contents of six amino acids, including alanine, arginine, aspartic acid, glycine, lysine, and methionine, compared with FM. The result of amino acids changes in carcass

**Table 7**
Carcass amino acid profile (g/kg protein) of Caspian brown trout fed experimental diets containing different levels of barley protein concentrate (BPC) for eight weeks.

| | Control | 25BPC | 50BPC | 75BPC | 100BPC | ANOVA | Liner | Quadratic |
|---|---|---|---|---|---|---|---|---|
| Arginine | 57.0 ± 8.1 | 60.2 ± 6.3 | 49.3 ± 5.4 | 47.9 ± 4.3 | 45.3 ± 4.4 | 0.07 | 0.01 | 0.85 |
| Histidine | 10.3 ± 2.1 | 9.8 ± 1.2 | 9.7 ± 0.8 | 10.2 ± 0.7 | 10.8 ± 0.6 | 0.86 | 0.56 | 0.37 |
| Isoleucine | 44.6 ± 8.1 | 46.0 ± 6.1 | 48.9 ± 5.2 | 45.8 ± 6.1 | 45.0 ± 7.1 | 0.93 | 0.97 | 0.49 |
| Leucine | 64.1 ± 5.5 | 64.9 ± 7.5 | 66.1 ± 8.6 | 63.8 ± 9.5 | 65.3 ± 8.6 | 0.99 | 0.91 | 0.89 |
| Lysine | 71.2 ± 10.4 [a] | 59.0 ± 8.6 [a] | 66.0 ± 7.2 [a] | 52.8 ± 6.7 [b] | 46.3 ± 8.6 [b] | 0.02 | 0.00 | 0.67 |
| Phenylalanine | 29.5 ± 8.1 | 29.8 ± 6.2 | 30.7 ± 4.1 | 30.0 ± 5.1 | 30.5 ± 6.4 | 0.99 | 0.80 | 0.79 |
| Threonine | 51.1 ± 7.5 | 50.7 ± 7.4 | 49.4 ± 4.0 | 51.2 ± 6.6 | 50.9 ± 5.2 | 0.98 | 0.99 | 0.81 |
| Valine | 47.5 ± 6.1 | 57.3 ± 7.3 | 48.5 ± 8.4 | 56.8 ± 9.0 | 46.0 ± 8.5 | 0.21 | 0.79 | 0.14 |
| Methionine | 21.7 ± 4.3 [a] | 21.2 ± 2.7 [a] | 20.1 ± 5.3 [a] | 12.7 ± 3.0 [b] | 10.2 ± 2.1 [b] | 0.01 | 0.00 | 0.21 |
| Total EAA | 397 ± 45.6 | 398.9 ± 51.0 | 388.7 ± 39.6 | 371.2 ± 30.5 | 350.3 ± 49.1 | 0.64 | 0.16 | 0.59 |
| Glutamic acid | 130.0 ± 14.1 | 131.7 ± 15.5 | 132.3 ± 16.2 | 140.2 ± 17.2 | 138.5 ± 20.3 | 0.87 | 0.35 | 0.98 |
| Serine | 54.2 ± 6.7 | 50.5 ± 5.6 | 51.7 ± 4.5 | 55.7 ± 7.4 | 53.0 ± 6.9 | 0.83 | 0.79 | 0.71 |
| Aspartic acid | 95.5 ± 8.3 | 106.5 ± 8.8 | 107.2 ± 7.9 | 96.9 ± 10.3 | 98.3 ± 9.6 | 0.28 | 0.78 | 0.10 |
| Glycine | 101.1 ± 10.5 | 109.5 ± 11.0 | 105.6 ± 7.8 | 111.2 ± 15.4 | 104.6 ± 10.7 | 0.72 | 0.63 | 0.34 |
| Alanine | 74.3 ± 7.3 | 70.2 ± 8.4 | 70.3 ± 6.2 | 68.2 ± 5.5 | 69.0 ± 7.3 | 0.82 | 0.33 | 0.60 |
| Tyrosine | 27.1 ± 3.1 | 21.8 ± 6.1 | 28.0 ± 4.2 | 25.8 ± 3.1 | 26.5 ± 2.0 | 0.56 | 0.75 | 0.73 |
| Total NEAA | 482.2 ± 62.6 | 490.2 ± 55.2 | 495.1 ± 37.7 | 498 ± 37.6 | 489.9 ± 44.4 | 0.95 | 0.80 | 0.75 |

Values are represented by means ± SDM of triplicate tanks; means without letter labels are not significantly different. According to Duncan's multiple range tests, the letters a and b indicate significant differences in the treatments ($P < 0.05$).

**Table 8**
Carcass fatty acid profile (g/kg Fatty Acid Methyl Esters (FAMEs)) of Caspian brown trout fed experimental diets containing different levels of barley protein concentrate (BPC) for eight weeks.

| | Control | 25BPC | 50BPC | 75BPC | 100BPC | ANOVA | Liner | Quadratic |
|---|---|---|---|---|---|---|---|---|
| C14:00 | 32.9 ± 4.5 | 33.2 ± 5.2 | 27.8 ± 3.8 | 31.0 ± 4.0 | 31.6 ± 5.1 | 0.68 | 0.60 | 0.40 |
| C16:00 | 157.6 ± 18.9 | 152.2 ± 20.1 | 164.4 ± 19.4 | 170.0 ± 25.6 | 176.5 ± 30.3 | 0.50 | 0.11 | 0.66 |
| C18:00 | 52.4 ± 5.4 | 57.8 ± 4.3 | 54.9 ± 5.5 | 56.0 ± 4.4 | 54.7 ± 2.9 | 0.80 | 0.78 | 0.44 |
| SFA | 247.2 ± 27.3 | 250.7 ± 29.2 | 252.1 ± 35.4 | 262.4 ± 26.0 | 270.4 ± 29.2 | 0.88 | 0.33 | 0.79 |
| C16:1n1 | 32.8 ± 4.5 | 41.2 ± 5.2 | 46.6 ± 5.0 | 38.9 ± 4.1 | 39.9 ± 4.8 | 0.10 | 0.25 | 0.04 |
| C18:1n-7 | 35.7 ± 3.8 | 37.3 ± 4.8 | 36.5 ± 5.5 | 40.3 ± 4.1 | 35.5 ± 3.6 | 0.63 | 0.74 | 0.38 |
| C18:1n9 | 145.0 ± 20.0 | 142.8 ± 15.4 | 145.4 ± 15.5 | 147.4 ± 16.3 | 151.5 ± 28.1 | 0.98 | 0.60 | 0.76 |
| C20: n-1 | 28.5 ± 1.9 | 28.4 ± 2.1 | 29.0 ± 2.0 | 30.0 ± 1.8 | 31.3 ± 3.1 | 0.54 | 0.12 | 0.54 |
| C22: n-1 | 70.4 ± 8.6 [a] | 69.8 ± 7.7 [a] | 67.5 ± 10.9 [a] | 59.7 ± 5.3 [b] | 59.2 ± 11.3 [b] | 0.02 | 0.00 | 0.58 |
| MUFA | 316.2 ± 20.4 | 323.5 ± 19.9 | 328.4 ± 21.3 | 321.7 ± 15.0 | 325.4 ± 17.2 | 0.93 | 0.62 | 0.64 |
| C18:2n6 | 114.0 ± 14.3 | 120.6 ± 15.7 | 125.5 ± 14.6 | 133.7 ± 17.9 | 133.9 ± 11.2 | 0.50 | 0.09 | 0.78 |
| C18:3n3 | 30.2 ± 2.5 [a] | 28.5 ± 2.4 [a] | 29.1 ± 1.9 [a] | 32.4 ± 2.8 [a] | 24.7 ± 3.3 [b] | 0.02 | 0.12 | 0.09 |
| C20:3n-6 | 21.6 ± 1.7 [a,b] | 24.9 ± 2.0 [a] | 21.6 ± 1.6 [a,b] | 17.9 ± 3.5 [b] | 17.0 ± 2.9 [b] | 0.00 | 0.00 | 0.06 |
| C20:4n6 | 25.5 ± 2.0 | 26.1 ± 1.9 | 23.5 ± 3.1 | 25.6 ± 2.6 | 24.0 ± 1.8 | 0.59 | 0.43 | 0.94 |
| C20:5n3 | 82.6 ± 14.3 | 73.3 ± 19.6 | 67.0 ± 20.2 | 61.1 ± 18.6 | 58.2 ± 15.5 | 0.31 | 0.04 | 0.68 |
| C22:6n3 | 148.5 ± 22.8 | 139.6 ± 24.3 | 132.5 ± 35.0 | 122.2 ± 19.2 | 120.4 ± 23.5 | 0.61 | 0.13 | 0.84 |
| PUFA | 427.4 ± 35.6 | 419.3 ± 39.0 | 404.5 ± 35.5 | 399.6 ± 42.1 | 382.5 ± 40.0 | 0.63 | 0.14 | 0.92 |
| LC-PUFA | 278.0 ± 30.2 | 263.9 ± 25.6 | 244.7 ± 20.4 | 226.8 ± 41.2 | 217.6 ± 18.5 | 0.14 | 0.01 | 0.86 |
| n-3 | 263.1 ± 20.4 [a] | 241.4 ± 19.7 [a] | 228.6 ± 16.9 [b] | 215.6 ± 15.5 [b] | 203.3 ± 22.3 [b] | 0.05 | 0.00 | 0.71 |
| n-6 | 161.1 ± 17.2 | 171.6 ± 18.5 | 170.6 ± 17.0 | 177.2 ± 18.9 | 172.9 ± 18.8 | 0.84 | 0.38 | 0.57 |
| n3/n6 | 1.62 ± 0.2 | 1.41 ± 0.1 | 1.34 ± 0.2 | 1.22 ± 0.2 | 1.17 ± 0.3 | 0.15 | 0.02 | 0.58 |

Values are represented means ± SDM of triplicate tanks; means without letter labels are not significantly different. The letters a, and b indicate significant differences in the treatments according to Duncan's multiple range tests ($P < 0.05$).
[c]Sum of all n-6 fatty acids; includes 20:3n-6 in addition to individually reported n-6 fatty acids.
[d]Sum of all n-3 fatty acids.
[e]Polyunsaturated fatty acids—the sum of all fatty acids with ≥ 2 double bonds; includes 16:2n-4 in addition to individually reported PUFAs.
[f]Long-chain PUFAs: the sum of all fatty acids with chain length ≥ 20 carbon atoms and ≥ 3 double bonds.
[a] Saturated fatty acid—the sum of all fatty acids without double bonds; includes C18:0, C20:0, and C22:0 in addition to individually reported SFAs.
[b] Monounsaturated fatty acids—the sum of all fatty acids with a single, double bond; includes 18:1n-7, 22:1n-11, and 22:1n-9 in addition to individually reported MUFAs.

followed the trend in diets (Table 7) so that individuals fed Control (7.12, 2.17), 25BPC (5.90, 2.12), and 50 BPC (6.61, 2.01) diets had a higher value of lysine and methionine, respectively as compared with other groups. With matching amnio acid profile of diets and carcass, we can conclude that lysine and methionine supplementations are essential when replacing FM with BPC and WGM. Many studies in salmonids reported that a diet containing a high level of plant-based proteins had a lower lysine and methionine (Cheng et al., 2003; Luo et al., 2006; Espe et al., 2007; Kumar et al., 2020). Therefore, they should be supplemented to the diets. We similarly suggest adding these amino acids in future studies to replace FM with BPC and WGM. The present study is the first one reporting the effect of supplementing BPC to diets on the amino acids profile of aquaculture species. In general, it can be hypothesized that unbalanced lysing and methionine could cause a lower growth rate in individuals fed diets containing less than 330 g/kg FM.

### 3.5. Fatty acid analysis

Fatty acids in fish are known as essential energy sources, vital for growth, survival, and several physiological mechanisms (Tocher et al., 2019). In the present study, we reported the fatty acid (g/kg) changes in fish was fed with BPC diets compared with Control. This ingredient had around 100 g/kg fat, accompanied by a commercial product previously formulated in the fish diet (Rossi et al., 2013). Unsurprisingly, with increasing the BPC level in experimental diets, saturated fatty acids and long-chain polyunsaturated fatty acids (LC-PUFA) in diets increased and decreased, respectively (Table 3). The profile of fatty acid in fish tissues is broadly a reflection of the dietary fatty acid composition (Matani Bour et al., 2018; Montazeri Parchikolaei et al., 2020). In the present research, the profile of fatty acid from carcass followed the trend in experimental diets. According to Table 8, there was a negative linear relationship between C20:5n3, LC-PUFA, and n3/n6 contents and BPC levels in the diets($P < 0.05$). Also, there was no significant difference between individuals fed dietary Control (82.6, 148.5, 278) and 50BPC (67.0, 132.5, 244.7) in terms of C20:5n3, C22:6n3, and LC-PUFA values. However, as observed, 330 g/kg BPC in diets seems to be a threshold and upper than this level; replacing FM with BPC and WGM negatively affected the omega 3 content. There is no study about the effect of BPC on fatty acids in aquatic species to compare our results with them. When FM was replaced with other protein sources in salmonids, a negative impact on fatty acids profile was observed (Esmaeili et al., 2017b).

### 3.6. Digestive enzyme activities

Digestive enzyme activities have been reliable indicators of the growth performance and digestibility of nutrients (Rungruangsak Torrissen and Male, 2000). Many studies connected the increase of digestive enzyme activities to improve growth in salmonids (Esmaeili et al., 2017b; Bilen et al., 2020; Ramezanzadeh et al., 2020a; Wang et al., 2020). We already reported few digestive enzyme data in a paper in Persian language related to the effect of BPC on fish (Zaretabar et al., 2019). As we wanted to focus on correlation analysis, the complete digestive enzymes data in the presented research were reported. Higher activities of trypsin (mU/mg protein) and pepsin (U/mg protein) were observed in Caspian brown trout fed Control (0.64, 1.61), 25BPC (0.63, 1.54), and 50BPC (0.62, 1.59) diets, respectively, in comparison with other groups ($P < 0.05$) (Fig. 2). Amylase showed a different trend, so that individuals fed dietary 50BPC (105.0), 75BPC (101.5), 100PBC (102.9) had a higher value of this enzyme as compared with others ($P < 0.05$). Also, aminopeptidase in the Control group was significantly higher than the BPC groups ($P < 0.05$). Finally, there was no significant difference in chymotrypsin and lipase among treatments. Enzymes responsible for protein digestion had the same direction with growth, unlike those are responsible for digestion fat (lipase) and carbohydrates (amylase). Caspian brown trout is extremely carnivorous, and these results were expectable. Many studies similarly reported decreasing FM in diets suppressed digestive enzyme activities of fish (Santigosa et al., 2008; Gai et al., 2012; Esmaeili et al., 2017b). However, in our study,

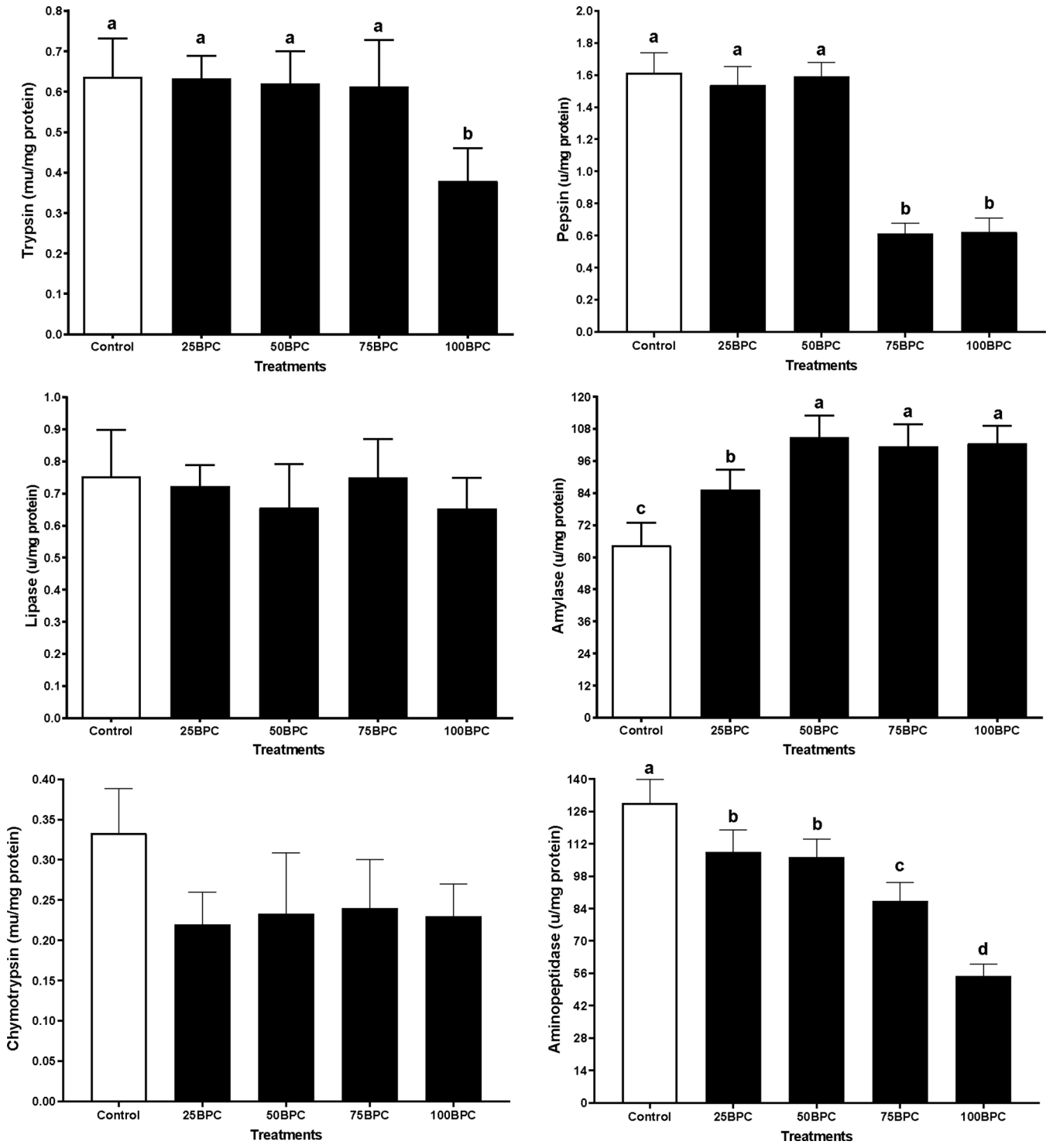


**Fig. 2.** Digestive enzyme activities of Caspian brown trout fed experimental diets containing different levels of barley protein concentrate (BPC) for eight weeks. Values are represented by means ± SDM of triplicate tanks; means without letter labels are not significantly different. According to Duncan's multiple range tests, the letters a and b indicate significant differences in the treatments ($P < 0.05$). There were negative linear relations between BPC levels and trypsin, pepsin, and aminopeptidase activities.

amylase was higher in BPC treatments, incompatible with the above-mentioned works. BPC is the richest source of β-glucan among cereals. Some fish studies revealed that this polysaccharide could stimulate digestive enzymes and potentially increase growth rate (Mohammadian et al., 2019). On the other hand, the antinutritional factors such as phytic acid, tannin, and antitrypsin in plant-based proteins can suppress digestive enzyme activities in fish (Francis et al., 2001; Bowyer et al., 2012; Chikwati et al., 2013). It seems that fish in the presented study have experienced distinct responses for each family of digestive enzymes depending on the antinutrient and β-glucan contents in diets. To our knowledge, fish response to different levels of antinutritional factors and β-glucan is mostly unexplored, and further investigations to determine a threshold for them are warranted. By matching the digestive enzymes and growth performance data, we can hypothesize that proteases play a

**Table 9**
Correlation between digestive enzymes with investigated factors in Caspian brown trout fed experimental diets containing different levels of barley protein concentrate (BPC) for eight weeks.

| | FM contents | Trypsin | Pepsin | Chymotrypsin | Amylase | Lipase | Aminopeptidase | Weight gain | SGR | FCR | Protein | Fat | ADCs of protein | Lysine | Methionine | EAA | NEAA | LC-PUFA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FM contents | **1.00** | 0.62* | 0.88** | 0.43 | −0.79** | 0.24 | 0.93** | 0.81** | 0.73** | −0.76** | 0.54* | −0.32 | 0.19 | 0.73** | 0.75** | −0.43 | 0.08 | 0.68* |
| Trypsin | 0.62* | **1.00** | 0.53* | 0.11 | −0.34 | 0.37 | 0.66** | 0.55* | 0.52* | −0.52* | 0.45 | −0.32 | 0.42 | 0.30 | 0.50 | −0.31 | 0.17 | 0.40 |
| Pepsin | 0.88** | 0.53* | **1.00** | 0.22 | −0.52* | 0.10 | 0.81** | 0.86** | 0.78** | −0.79** | 0.38 | −0.37 | 0.78** | 0.70** | 0.89** | −0.32 | 0.11 | 0.57* |
| Chymotrypsin | 0.43 | 0.11 | 0.22 | **1.00** | −0.24 | 0.39 | 0.58* | 0.09 | 0.05 | −0.17 | 0.76** | 0.60* | 0.55* | 0.68** | 0.01 | −.03 | 0.39 | 0.53* |
| Amylase | −0.79** | −0.34 | −0.52* | −0.24 | **1.00** | −0.08 | −0.55* | −0.50 | −0.46 | 0.58* | −0.18 | 0.48 | −0.24 | −0.34 | −0.53* | 0.50 | 0.20 | −0.42 |
| Lipase | 0.24 | 0.37 | 0.10 | 0.39 | −0.08 | **1.00** | 0.35 | −0.07 | −0.13 | −0.15 | 0.59* | 0.52* | 0.30 | 0.25 | 0.21 | 0.04 | 0.35 | 0.34 |
| Aminopeptidase | 0.93** | 0.66** | 0.81** | 0.58* | −0.55* | 0.35 | **1.00** | 0.74** | 0.65** | −0.66** | 0.66** | −0.09 | 0.82** | 0.86** | 0.68** | −0.29 | 0.23 | 0.70** |
| Weight gain | 0.81** | 0.55* | 0.86** | 0.09 | −0.50 | −0.07 | 0.74** | **1.00** | 0.98** | −0.87** | 0.33 | −0.48 | 0.71** | 0.56* | 0.68** | −0.48 | −.12 | 0.38 |
| SGR | 0.73** | 0.52* | 0.78** | 0.05 | −0.46 | −0.13 | 0.65** | 0.98** | **1.00** | −0.87** | 0.28 | −0.49 | 0.63* | 0.46 | 0.59* | −0.48 | −0.15 | 0.31 |
| FCR | −0.76** | −0.52* | −0.79** | −0.17 | 0.58* | −0.15 | −0.66** | −0.87** | −0.87** | **1.00** | −0.29 | 0.38 | −0.58* | −0.51 | −0.70** | 0.51 | 0.24 | −0.27 |
| Protein | 0.54* | 0.45 | 0.38 | 0.76** | −0.18 | 0.59* | 0.66** | 0.33 | 0.28 | −0.29 | **1.00** | 0.53* | 0.80** | 0.54* | 0.18 | −0.1 | 0.55* | 0.63* |
| Fat | −0.32 | −0.32 | −0.37 | 0.60* | 0.48 | 0.52* | −0.09 | −0.48 | −0.49 | 0.38 | 0.53* | **1.00** | 0.19 | 0.12 | −0.45 | 0.39 | 0.48 | 0.1 |
| ADCs of protein | 0.19 | 0.42 | 0.78** | 0.55* | −0.24 | 0.30 | 0.82** | 0.71** | 0.63* | −0.58* | 0.80** | 0.19 | **1.00** | 0.76** | 0.57* | −0.16 | 0.39 | 0.66** |
| Lysine | 0.73** | 0.3 | 0.70** | 0.68** | −0.34 | 0.25 | 0.86** | 0.56* | 0.46 | −0.51 | 0.54* | 0.12 | 0.76** | **1.00** | 0.51 | −0.04 | 0.15 | 0.63* |
| Methionine | 0.75** | 0.50 | 0.89** | 0.01 | −0.53* | 0.21 | 0.68** | 0.68** | 0.59* | −0.70** | 0.18 | −0.45 | 0.57* | 0.51 | **1.00** | −0.23 | 0.00 | 0.47 |
| EAA | −0.43 | −0.31 | −0.32 | −0.03 | 0.50 | 0.04 | −0.29 | −0.48 | −0.48 | 0.51 | −0.1 | 0.39 | −0.16 | −0.04 | −0.23 | **1.00** | 0.37 | 0.17 |
| NEAA | 0.08 | 0.17 | 0.11 | 0.39 | 0.20 | 0.35 | 0.23 | −0.12 | −0.15 | 0.24 | 0.55* | 0.48 | 0.39 | 0.15 | 0.00 | 0.37 | **1.00** | 0.67** |
| LC-PUFA | 0.68** | 0.4 | 0.57* | 0.53* | −0.42 | 0.34 | 0.70** | 0.38 | 0.31 | −0.27 | 0.63* | 0.1 | 0.66** | 0.47 | 0.47 | 0.17 | 0.67** | **1.00** |

SGR: specific growth rate; FCR: feed conversion ratio; EAA: essential amino acids; NEAA: non-essential amino acids; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; LC-PUFA: long-chain polyunsaturated fatty acid.

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

crucial role in the digestion of BPC and, eventually, the growth of Caspian brown trout.

### 3.7. Correlation between digestive enzyme activities and other measured parameters

In the present study, a positive correlation between digestive enzyme activities with growth performance, lysine, methionine, and LC-PUFA was observed. Pepsin had a strong negative relationship with BPC contents in diet (0.88), weight gain (0.86), specific growth rate (SGR) (0.78), ADCs of protein (0.78), lysine (0.70), and methionine (0.89) ($P < 0.01$) (Table 9). Aminopeptidase also had strong negative relationships with BPC contents in diet (0.93), weight gain (0.74), SGR (0.65), protein content in body (0.66), ADCs of protein (0.82), lysine (0.86), methionine (0.68), and L-C PUFA (0.70) ($P < 0.01$) (Table 9). These results were surprising for us as we hypothesized that trypsin and chymotrypsin would have a strong relationship with growth and other investigated parameters. Similarly, some research observed a positive correlation between growth rate and digestive enzyme activities (Johnston et al., 2004; Esmaeili et al., 2017b; Magouz et al., 2020; Zemheri-Navruz et al., 2020). Conversely, other studies have reported no direct relationship between growth rate and digestive enzyme activities (Lemieux et al., 1999; Rungruangsak-Torrissen, 2012; Ramezanzadeh et al., 2020b). This issue is still under question, and we do not know that elevated growth increases digestive enzyme activities or vice versa.

It has been well known that cholecystokinin (CCK)/ gastrin-like peptides play a critical role in controlling digestive physiology in vertebrates. Enzymes and nutrients regulate CCK secretion in the body (Navarro-Guillén et al., 2017; Sridhar, 2020). Therefore, it is reasonable to speculate that endogenous enzyme activities were probably the cause of CCK secretion changes, directly affecting feed intake and growth performance. However, further endocrinology and gene expression analysis are necessary to illustrate this issue.

There were strong negative correlations between weight gain and BPC contents in the diet (0.81), ADCs of protein (0.71), and methionine (0.68). These results show that how FM is crucial for fish growth and ADCs of protein. We can conclude that for an extreme carnivorous fish like Caspian brown trout, ADCs of protein can be an indicator of protein quality. Finally, we can highlight the importance of methionine in fish growth and its relationship with digestive enzymes. Only one study investigated the effect of supplementing of methionine on fish digestive enzyme activities (Xiao et al., 2011). They reported trypsin, amylase, chymotrypsin, and lipase activities significantly increased with elevated methionine contents in diets of Jian carp (*Cyprinus carpio* var. *Jian*). However, Xia et al. (2011) did not measure pepsin and aminopeptidase in their studies to see how methionine could affect the activities of these enzymes. More studies are needed to illustrate how amino acids can directly affect digestive enzymes and, eventually, growth performance.

## 4. Conclusion

In general, the current results indicated that Caspian brown trout fed the 50BPC diets (330 g/kg FM) grew similar to those that ate the dietary Control (660 g/kg FM). We hypothesized that the high digestibility of nutrients, balanced amino acid and fatty acid, and increased trypsin, pepsin, and lipase activities in the 50BPC group caused this treatment to grow as same as Control. Considering all factors, we suggest the potential benefits of using BPC plus WGM as alternative sources for FM up to a certain level in fish diets. Too much inclusion of these ingredients caused lysine and methionine deficiency, and adding amino acid supplementation to diets is suggested for future research. BPC as a high-quality protein represented a significant potential benefit for formulating in the diet of an extreme carnivorous fish like Caspian brown trout. Formulating new protein resources in fish diets is an ongoing step toward developing new aquafeeds to ensure aquaculture food security of this species in the 21st century.

## Data availability statement

Data available on request due to privacy/ethical restrictions (The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions).

## Declaration of Competing Interest

There is no conflict of interest to declare by the authors.

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