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Original Article

Which is a better chelating agent in beluga, *Huso huso* (L.), coriander, *Coriandrum sativum*, or active charcoal?

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Abstract: To compare the chelating effect of coriander, *Coriandrum sativum*, and active charcoal on beluga, *Huso huso*, a total of 270 beluga (260±2 g) were initially fed with a diet containing 0, 5, 10, 15 and 20 g of coriander and charcoal per kg of diet for 60 days. The fish were then subjected to 1 mg/L of heavy metals (lead, cadmium, and copper) for seven days. The best growth and feeding performances before and after challenging with heavy metals were observed in fish fed with 15 g/kg active charcoal. Also, the best growth performance before challenging with heavy metals was observed in fish fed with 5 and 10 g/kg coriander followed by fish fed with 10 and 15 g/kg coriander after the challenging stage. The growth and nutritional efficiency were better in coriander than the charcoal treatments, especially in the pre-challenge stage. No mortalities were observed in charcoal treatments at all. While mortality occurred only in fish fed with 20 g/kg coriander in the first stage, a 100% survival rate was observed only in the second stage of fish fed with 10 g/kg coriander. In both stages of the experiment, the highest percentages of carcass protein and lipid were obtained in fish fed with 10 g/kg coriander and 15 g/kg of charcoal. Also, the lowest ash and the highest moisture were related to the control group. The amounts of protein and ash were higher in carbon treatments while the amounts of lipid and moisture were higher in coriander treatments. Minimum amounts of cadmium, copper, and lead were obtained in fish fed with 15 and 20 g/kg charcoal and coriander. The concentration of the heavy metals was significantly lower in fish fed with carbon ($P<0.05$). Therefore, 5-10 g/kg coriander is a better additive in low heavy metal concentration. But in an environment with a higher concentration of heavy metals, adding 15 g/kg active charcoal to beluga diet is recommended.

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Introduction

Due to the effect of heavy metal pollutions on the health of fishes and consequently humans, using dietary supplements called chelating agents, which can absorb heavy metals and accelerate their excretion from the body, is of great importance. Chelation is a process by which a chemical or natural compound binds a metal ion and holds it firmly. Substances with such property have been used in the treatment of metal poisoning (Aaseth et al., 2016). Various chelating agents identified so far such as ethylene diamine tetraacetic acid (EDTA), methyl sulfonylmethane (MSM), active charcoal (AC), coriander, garlic, and *Chlorella* algae (Mirzavand et al., 2015; Abdel-Tawwab et al., 2017a). Active charcoal refers to a group of charcoalaceous substances with high porosity and high internal surface area, which are of paramount importance due to their significant internal area, powdery and porous structure, high absorbability, surface reactivation ability, and low cost compared to other adsorbents (Bradley et al., 1996; Yoo et al., 2005).

It should be noted that negative side effects in the use of chemical chelating agents have also been reported. Even EDTA, with much lower toxicity than MSM, can excrete some metal elements like zinc. Therefore, using natural substances with lower side effects and costs have attracted researchers (Aaseth et al., 2016). Coriander, *Coriandrum sativum*, or Chinese parsley, from the family of Umbelliferae/ Apiaceae, is a glabrous aromatic, herbaceous annual plant, with effective compounds of metallothionein and

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glutathione compounds, for removing metals such as mercury, cadmium, lead, copper, and aluminum (Abidhusen et al., 2012). Since coriander leaves can attach to heavy metal and excrete these particles from the body. Coriander can also excrete heavy metals from the bloodstream (Abidhusen et al., 2012).

Various studies have been reported the detoxification of heavy metals by AC in fish. Also, they indicated AC positive effects on the growth efficiency, and the body chemical composition, including Nile tilapia, *Oreochromis niloticus*, (Abdel-Tawwab et al., 2017a), Red tilapia, *O. mossambicus × O. niloticus*, (Michael et al., 2017) and Japanese flounder, *Paralichthys olivaceus*, (Thu et al., 2010). Moreover, the chelating effect of coriander, itself or in combination with other substances, has been studied in fish and other animals. For example, coriander has led to the non-accumulation of aluminum and lead in mice (Aga et al., 2001; Kansal et al., 2011). The chelating effect of coriander in iron excrement is much higher than parsley (Mirzaei and Khatami, 2013). Also, the use of coriander, garlic, and *chlorella* algae in *Carassius gibelio* has shown a positive effect (2% individually) on kidney protection against 10 mg/L of cadmium (Nicula et al., 2016). Besides, using 2% coriander powder in rainbow trout, *Oncorhynchus mykiss*, diet reduced cadmium amount by 20-30% in the liver and kidney of fish (Ren et al., 2006).

Beluga is one of the most important sturgeon species of the Caspian Sea with high economic value for producing meat and caviar (Chebanov and Galich, 2013; Hoseini et al., 2013; Gisbertet al 2014; Asgari et al., 2014). Since the Caspian Sea is polluted with heavy metals due to wastewater influx from farms and industries (Parizanganeh and Lakhan, 2007; Saeedi Saravi and Shokrzadeh, 2013; Saghali et al., 2014 Abadi et al., 2018), bioaccumulation of heavy metals in the tissues and muscles of sturgeons with a lengthy culture is highly important. Hence, the present study aims to investigate and compare the effects of two available and cheap chelating agents, coriander as a natural substance and active charcoal as a chemical substance, on growth efficiency, fish nutritional value, and chelating of heavy metals in beluga.

Materials and Methods

Fish and experimental design: This research was carried out at Qaraboron Sturgeon Culture Center (Sari, Iran). A total of 270 beluga (with the average weight and length of 260±2 g and 40±1 cm, respectively, was randomly selected and stocked in 27 concrete ponds (1.70×1.50 m) with a depth of 0.6 m and a watering depth of 0.3 m. This trial consisted of a control group (no coriander powder and active charcoal addition), four experimental treatments received coriander powder (Qaemshahr, Iran), and four treatments received active charcoal (Tina Chem., Iran) orally at 5, 10, 15 and 20 g/kg of diet (Abdel-Tawwab et al., 2017a, b). Each treatment had three replications. The first stage of the experiment lasted for 60 days and 12 fish were sampled from each treatment for carcass and heavy metal analysis. The rest fish were challenged with heavy metals for seven days as the second stage of the experiment (Abdel-Tawwab et al., 2017a, b).

Table 1 represents the Caspian Sea water analysis in the Qaraboron Center regarding the concentration of heavy metals in 12 months, conducted by the laboratory of Alborz Health Test, Iran. Accordingly, the highest amount of the above three heavy metals was related to lead (0.9 mg/L). Therefore, heavy metals as lead nitrate, copper sulfate, and cadmium chloride (Merch, Germany) were each challenged at the concentration of 1 mg/L. It should be noted that in the pre-challenging stage, the fish rearing was carried out in ponds with continuous water exchange, while in the second stage, flowing water was not used to maintain the concentration of the above heavy metals at a constant concentration (1 mg/ L). Therefore, the ponds were first watering and in addition to using oxygen, one-third of the ponds water was siphoned from the bottom especially after feeding and freshwater was added to the ponds together with the same doses of heavy metals (Abdel- Tawwab et al., 2017a, b).

Water quality parameters: During the experimental process, the Qaraboron Center water was supplied from a well that flowed into the system after aeration. The water flow rate was 15 L/s for all ponds in the

Table 1. Physico-chemical parameters and heavy metals concentrations of the Caspian Sea and well waters used in this study.

Parameter	Parameters	
	Caspian Sea water	Well water
temperature (ºC)	13 (winter) - 25 (summer)	17.1±0.2
Dissolved oxygen (mg/L)	9-14	8±0.11
Salinity (ppt)	10-13	0.3±0.05
pH	8.3-8.9	7.51±0.03
Total alkalinity (mg/L)	260	370±2.01
Total hardness (mg/L)	5100	500 ±3.00
NH_4(mg/L)	0.08- 0.096	0.576±0.032
No_3 (mg/L)	3.5-11.1	23.79±2.21
No_2 (mg/L)	0.011-0.11	0.01±0001
Copper (mg/L)	<0.1	<0.002
Cadmium (mg/L)	<0.01	<0.001
Lead (mg/L)	0.013-0.9	<0.0005

Center (Chebanov and Galich, 2013). The chemical properties of water are given in Table 1. During the experiment, factors such as temperature, oxygen, and pH were measured daily using a multimeter (HI 9828, Multiparameter Meter, HANNA Instruments, United States).

Diet preparation: The basal diet used in this study was a pellet (2 mm) made by Qaraboron Company, Iran (Table 2). For coriander diets preparation, the coriander was washed and dried in a cool environment and circulating air which was away from direct sunlight. The leaves were then powdered by grinding machine, dissolved in distilled water (5 g of coriander in 70 ml of water), and finally, added to per kg of diet. About active charcoal, the diets were prepared manually too, by dissolving the active charcoal powder in distilled water (5 g of charcoal in 70 ml of water) and adding to per kg of diet. The diets were then grounded in a meat grinder. After re-pelleting, the diets were dried in an oven for 48 hrs and stored at 4 °C. The diets were prepared once a week and fed to fish 5 times a day at 24, 5, 10, 14, and 20 hrs until its satiation (Chebanove and Galich, 2013).

Growth and feeding parameters: The growth and nutritional performances were calculated based on the following formulae (Abdel- Tawwab et al., 2017a).

Body weight gain (BWG, %) = Final weight (g) – Initial weight (g)/ Initial weight (g) × 100

Specific growth rate (SGR, %/day) = $[(\text{Ln } W_2 - \text{Ln } W_1)/T_2 - T_1] \times 100$

Where, LnW_1= Natural logarithm of initial weight, LnW_2= Natural logarithm of final weight and $T_2 - T_1$= Experimental period.

Survival rate (SR, %) = $(N_t/N_0) \times 100$

Where N_t = Number of fish at the end of experimental period and N_0 = Number of fish at the beginning of experimental period.

Feed conversion ratio (FCR) = Weight gain (g)/feed eaten (g)

Protein Efficiency Ratio (PER) = Protein eaten (g)/weight gain (g)

Nutritional value of fish flesh: Before and after challenging with heavy metals, six fish per treatment were randomly selected for carcass composition analysis. The skin of the fish was isolated and crushed. After removing viscera, they were placed in a meat grinder (Pars Khazar, MG 1400, Iran). This was performed separately for all treatments. Afterward, they placed into a plate and the total weight of plate and fish was measured by a digital scale. The specimens were then inserted into an oven of 55 °C for 24 hrs and re-weighed with the same scale to get moisture content. The important parameters calculated for approximate fish carcass analysis were crude protein, total lipid, and total ash which were estimated by Kjeldahl device (Kjeltec Auto Analyzer, 2300 Tecator, Sweden), Soxhlet (dissolving fat in ether), and standard method of AOAC (1990) (placing samples in an electric furnace (Herius-Germany) at 550°C for 4 hrs), respectively.

Heavy metals in fish flesh: At the end of both stages of the experiment, six fish per treatment were

Table 2. Ingredients and proximate analysis of the experimental diets used in this study (NRC, 1993).

Ingredients	Amount (%)
Fish Meal (57.35% Protein)	37.07
Wheat Gluten	10
Wheat Flour	10
Canola Oil	5.5
Soybean Meal	24
Meat Meal	10
*Mineral Premix	1.5
*Vitamin Premix	1.5
Anti-Fungi	0.1
Stable Vitamin C	0.13
Binder (Molasses)	0.2

Parameter (%)	0	5	10	15	20	0	5	10	15	20
Protein	34.5±0.3	34.4±1.2	34.4±1.00	34.2±0.08	34.0±0.01	34±0.6	34±1.4	33.9±0.003	33.7±0.09	33.5±0.04
Lipid	14.8±0.11	14.8±0.03	14.8±0.50	14.8±0.16	14.8±0.07	14.8±0.30	14.7±0.50	14.7±0.13	14.7±0.01	14.6±0.24
Ash	9.2±0.30	9.2±0.006	9.3±0.25	9.3±0.11	9.4±0.72	9.2±0.008	9.7±0.46	10.2±1.31	10.7±1.90	11.2±2.01
Moisture	14.5±0.92	14.5±1.08	14.5±1.7	14.5±1.36	14.5±1.00	14.8±0.80	14.5±1.91	14.5±1.98	14.4±2.05	14.0±0.66

*Mineral premix, provided following amounts per gram of premix: Mg: 70 mg, Fe: 50 mg, Cu: 3 mg, Co: 0.01 mg, Mn: 20 mg, Zn: 30 mg, I: 0.1 mg, Se: 0.1 mg. *Vitamin premix, provided following amounts per gram of premix: A: 1000 IU, D_3: 200 IU, E: 10 IU, K: 2 mg, B_1: 4 mg, B_2: 4 mg, B_{12}: 4 mg, Pantothenic Acid: 10 mg, Niacin: 20 mg, Pyridoxine: 4 mg, Biotin: 0.02 mg, Folic Acid: 1 mg, C: 44 mg, Choline Chloride: 98 mg.

randomly selected in each stage and kept at –20°C in the laboratory for five days for heavy metal analysis. To digest frozen flesh, all fish were placed in a dryer for 48 hrs until they completely dried. Thereafter, the dry tissues (0.2 g) were separately powdered and poured into test tubes. After adding concentrated nitric acid (5 ml), samples were placed in ambient temperature for 1 hr. and completely digested on a hot plate at 100 °C for 3 hours. After cooling down, the volume of samples reached 25 ml by adding distilled water (Okoye, 1991). In order to estimate the values of heavy metals (copper, lead and cadmium), the samples were filtered with acetate cellulose (0.2 µ) and the concentrations of heavy metals were measured in all fish by an atomic absorption device (Model ICP-OES) and the following formula:

$$C = G_s \times V/W$$

Where C = metal concentration in the solid sample (mg/ g dry weight), G_s = metal concentration in solution from digestion (ppm), V = dilution volume (ml) and W= the dry weight of the sample (g).

Statistical analysis: This research was conducted in a factorial design including one control group, four groups with coriander treatments, and four groups with active charcoal treatments. All data were tested for normal distribution by the Kolmogorov– Smirnov test. Moreover, data were analyzed by two-way analysis of variance (Factorial Two-Way ANOVA). Besides, mean values were compared by Tukey's test through SPSS software (version 23) at a 95% confidence level.

Results

Growth and nutritional performances before and after challenging with heavy metals: The results of pre and post- challenging with heavy metals showed that the highest BWG, SGR, and PER and the lowest FCR amounts were obtained in fish fed with 15 g/kg active charcoal without significant differences with fish fed 10 g/kg carbon in the first stage and the other treatments except for PER in the second stage ($P>0.05$). The best growth and feed utilization results were obtained in fish fed with 5 g/kg coriander without significant differences with fish fed 10 g/kg coriander before challenging with heavy metals, while in the next stage, the best growth and feeding parameters were obtained in fish fed with 10 g/kg coriander with no significant differences with the fish fed 15 g/kg coriander. In the first phase, SR was 100% in all carbon treatments which was the same for fish in all

Table 3. Growth and feeding parameters of beluga fed with different levels of coriander and active charcoal before challenging with heavy metals.

Parameter	Concentration (g/kg) Chelating agent	0	5	10	15	20
Body weight gain (%)	Coriander	88.33 ± 3.25^{a}	127.88 ± 3.03^{bA}	121.09 ± 13.42^{bA}	100.44 ± 4.84^{abA}	96.07 ± 7.14^{aA}
	Active charcoal	88.33 ± 3.25^{a}	88.92 ± 3.13^{aB}	91.89 ± 2.96^{aB}	100.74 ± 0.01^{bA}	75.66 ± 2.91^{cB}
Specific growth rate (%/day)	Coriander	0.453 ± 0.02^{a}	0.597 ± 0.01^{bA}	0.573 ± 0.04^{bA}	0.503 ± 0.02^{cA}	0.487 ± 0.03^{acA}
	Active charcoal	0.453 ± 0.02^{ac}	0.462 ± 0.01^{acB}	0.470 ± 0.01^{abB}	0.500 ± 0.00^{bA}	0.410 ± 0.01^{cB}
Survival rate (%)	Coriander	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	93.33 ± 0.00^{bA}
	Active charcoal	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{aB}
Feed conversion ratio	Coriander	2.13 ± 0.044^{a}	1.39 ± 0.02^{bA}	1.49 ± 0.16^{bA}	1.77 ± 0.06^{cA}	1.97 ± 0.11^{aA}
	Active charcoal	2.13 ± 0.044^{a}	2.12 ± 0.05^{aB}	2.01 ± 0.05^{abB}	1.85 ± 0.01^{bA}	2.34 ± 0.07^{aB}
Protein efficiency ratio	Coriander	1.71 ± 0.048^{a}	2.10 ± 0.04^{bA}	1.98 ± 0.21^{bA}	1.65 ± 0.05^{acA}	1.49 ± 0.09^{cA}
	Active charcoal	1.71 ± 0.048^{ad}	2.06 ± 0.05^{bdA}	2.18 ± 0.06^{bcB}	2.36 ± 0.01^{cB}	1.87 ± 0.05^{adB}

Data are presented as means ± standard deviation. Different lowercase letters show significant difference of each parameter at different levels of coriander and active charcoal treatments with the control group and each other; whereas, different uppercase letters show significant difference of each parameter in each level of coriander and active charcoal (Factorial two- way ANOVA, *P*<0.05).

Table 4. Growth and feeding parameters of beluga fed with different levels of coriander and active charcoal after challenging with heavy metals.

Parameter	Concentration (g/kg) Chelating agent	0	5	10	15	20
Body weight gain (%)	Coriander	3.75 ± 0.33^{a}	4.47 ± 0.74^{aA}	6.03 ± 1.01^{bA}	4.95 ± 1.02^{abA}	4.42 ± 0.59^{abA}
	Active charcoal	3.75 ± 0.33^{a}	3.88 ± 0.10^{aA}	3.91 ± 0.14^{aB}	4.27 ± 0.18^{aA}	3.50 ± 0.31^{aA}
Specific growth rate (%/day)	Coriander	0.227 ± 0.03^{a}	0.270 ± 0.04^{aA}	0.363 ± 0.06^{bA}	0.300 ± 0.06^{abA}	0.267 ± 0.03^{aA}
	Active charcoal	0.227 ± 0.03^{a}	0.232 ± 0.01^{aB}	0.237 ± 0.01^{aB}	0.260 ± 0.01^{aA}	0.213 ± 0.02^{aA}
Survival rate (%)	Coriander	93.33 ± 0.00^{a}	93.33 ± 0.00^{aA}	100.00 ± 0.00^{bA}	97.67 ± 0.00^{cA}	83.33 ± 0.00^{dA}
	Active charcoal	93.33 ± 0.00^{a}	100.00 ± 0.00^{bB}	100.00 ± 0.00^{bA}	100.00 ± 0.00^{bB}	100.00 ± 0.00^{bB}
Feed conversion ratio	Coriander	2.42 ± 0.12^{a}	1.79 ± 0.27^{bcA}	1.42 ± 0.15^{bA}	1.86 ± 0.38^{bcA}	2.21 ± 0.27^{bcA}
	Active charcoal	2.42 ± 0.12^{ab}	2.26 ± 0.07^{abA}	2.13 ± 0.06^{abB}	1.94 ± 0.08^{aA}	2.62 ± 0.25^{abA}
Protein efficiency ratio	Coriander	1.23 ± 0.097^{a}	1.65 ± 0.26^{bA}	2.07 ± 0.23^{cA}	1.61 ± 0.32^{bdA}	1.33 ± 0.18^{adA}
	Active charcoal	1.23 ± 0.097^{a}	1.29 ± 0.04^{aA}	1.37 ± 0.04^{aB}	1.50 ± 0.06^{bA}	1.12 ± 0.10^{aA}

Data are presented as means±standard deviation. Different lowercase letters show significant difference of each parameter at different levels of coriander and active charcoal treatments with the control group and each other; whereas, different uppercase letters show significant difference of each parameter in each level of coriander and active charcoal (Factorial two- way ANOVA, *P*<0.05).

coriander treatments except fish fed with 20 g/kg coriander. In the second phase, the 100% survival rate was also observed in all carbon treatments but it was seen only in fish fed with 10 g/kg coriander. The comparison of carbon and coriander treatments showed that except SR and PER (only in the pre-challenging stage), coriander yielded better results than carbon in growth and nutritional parameters. Except for PER, no significant difference was obtained in fish fed with 15 g/kg carbon or coriander, in the first stage (*P*>0.05; Table 3). In the next stage, a significant difference was only obtained in fish fed with 10 g/kg of carbon and coriander (*P*<0.05; Table 4).

Carcass composition before and after challenging with heavy metals: The highest percentages of carcass protein was measured in fish fed with 10 g/kg coriander which was not significantly different from other treatments (except fish fed with 20 g/kg coriander in the pre-challenging stage and fish fed with 15 and 20 g/kg coriander in the post-challenging stage). The highest amount of lipid was obtained in fish fed with 10 g/kg coriander which was not significantly different from other coriander treatments in both stages (*P*>0.05; Table 5). In charcoal treatments, however, the highest percentages of carcass protein and lipids were measured in fish fed with 15 g/kg carbon in both stages. In the first stage, carcass ash percentage did not differ significantly between all treatments while in the next stage, it increased significantly in 10, 15, and 20 g/kg coriander compare to other treatments. In carbon treatments, the percentage of carcass ash increased significantly with increasing carbon content in both

Table 5. Carcass composition of beluga fed with different levels of coriander and active charcoal before challenging with heavy metals.

Parameter	Concentration (g/kg) Chelating agent	0	5	10	15	20
Total protein (%)	Coriander	15.65±0.250^{a}	15.77±0.252^{aA}	16.37±0.153^{aA}	15.53±0.153^{abA}	14.93±0.115^{bA}
	Active charcoal	15.65±0.250^{a}	16.57±0.378^{bcA}	17.00±0.200^{bA}	17.23±0.305^{bB}	15.93±0.902^{acA}
Total lipid (%)	Coriander	8.58±0.265^{a}	8.833±0.153^{bA}	9.008±0.115^{bA}	9.000±0.000^{bA}	8.900±0.100^{bA}
	Active charcoal	8.58±0.265^{a}	8.400±0.100^{aB}	8.667±0.208^{bcB}	8.800±0.1000^{cB}	8.500±0.100^{aB}
Total ash (%)	Coriander	1.88±0.08^{a}	1.67±0.15^{aA}	1.70±0.10^{aA}	1.77±0.15^{aA}	1.67±0.06^{aA}
	Active charcoal	1.88±0.08^{a}	2.01±0.02^{bB}	2.39±0.03^{cB}	2.43±0.03^{cB}	2.54±0.11^{cB}
Moisture (%)	Coriander	74.30±0.100^{a}	72.97±0.235^{bA}	72.77±0.030^{bA}	71.91±0.080^{bA}	71.75±0.089^{bA}
	Active charcoal	74.30±0.100^{a}	70.02±0.965^{bB}	71.50±0.500^{cB}	71.83±0.289^{cA}	68.93±0.950^{bB}

Data are presented as means±standard deviation. Different lowercase letters show significant difference of each parameter at different levels of coriander and active charcoal treatments with the control group and each other; whereas, different uppercase letters show significant difference of each parameter in each level of coriander and active charcoal (Factorial two- way ANOVA, $P<0.05$).

Table 6. Carcass composition of beluga fed with different levels of coriander and active charcoal after challenging with heavy metals.

Parameter	Concentration (g/kg) Chelating agent	0	5	10	15	20
Total protein (%)	Coriander	15.29±0.115^{ab}	15.38±0.202^{abA}	15.80±0.346^{aA}	15.07±0.115^{bcA}	14.60±0.529^{cA}
	Active charcoal	15.29±0.115^{a}	16.50±0.200^{bB}	16.66±0.210^{bB}	17.15±0.150^{bB}	14.63±0.077^{cA}
Total lipid (%)	Coriander	8.167±0.153^{a}	8.800±0.000^{bA}	8.900±0.173^{bA}	8.833±0.153^{bA}	8.767±0.252^{bA}
	Active charcoal	8.167±0.153^{a}	8.600±0.100^{bA}	8.067±0.115^{aB}	8.800±0.100^{bA}	8.667±1.53^{bA}
Total ash (%)	Coriander	1.80±0.10^{a}	1.80±0.00^{aA}	2.04±0.00^{bA}	2.12±0.00^{bA}	2.10±0.10^{bA}
	Active charcoal	1.80±0.10^{a}	2.13±0.15^{bB}	2.39±0.00^{cB}	2.47±0.15^{cB}	2.67±0.15^{cB}
Moisture (%)	Coriander	76.48±0.040^{a}	74.47±0.351^{bA}	73.74±0.151^{bA}	72.50±0.500^{bA}	72.00±0.500^{bA}
	Active charcoal	76.48±0.040^{a}	73.23±0.586^{bB}	73.00±1.000^{bA}	72.16±0.110^{cA}	70.12±0.080^{dB}

Data are presented as means±standard deviation. Different lowercase letters show significant difference of each parameter at different levels of coriander and active charcoal treatments with the control group and each other; whereas, different uppercase letters show significant difference of each parameter in each level of coriander and active charcoal (Factorial two- way ANOVA, $P<0.05$).

stages of the study. However, no significant differences were observed in fish fed with 10, 15, and 20 g/kg carbon ($P>0.05$; Tables 5, 6). Also, in both stages of the experiment, the highest moisture was related to the control group with significant differences with other treatments (Tables 5, 6). The comparison of fish nutritional value in both carbon and coriander treatments, the amounts of protein and ash were higher in carbon treatments while the amounts of lipid and moisture were higher in coriander treatments (Tables 5, 6). In the first stage, significant differences in protein, lipid and ash contents were obtained only in fish fed with 15 g/kg carbon and coriander ($P<0.05$). This result was obtained in fish fed with 10 g/kg carbon and coriander after challenging with heavy metals (Table 6).

Heavy metal amounts in beluga before and after challenging with heavy metals: For both stages of the experiment, the amounts of carcass heavy metals significantly decreased compared to the control group by adding both chelating agents to the diet, ($P<0.05$; Tables 7, 8). The lowest levels of the above metals were obtained in fish fed with 15 and 20 g/kg coriander with an only significant difference about the lead amount in fish fed with 10 g/kg coriander in the first stages. In the second stage, there was no significant difference in the amount of copper between the coriander treatments. The lowest amounts of the other two heavy metals were also obtained in fish fed with 15 and 20 g/kg coriander. In the case of carbon treatments, the lowest amounts of the heavy metals were observed in fish fed with 20 g/kg carbon, which was only significant in terms of copper content compared to fish fed with 15 g/kg carbon in both stages of the study ($P<0.05$; Tables 7, 8). A comparison of the results of the two chelating agents showed that the amounts of cadmium, copper, and lead were lower in the fish fed with activate carbon than the fish fed coriander. In the pre-challenging stage, no significant difference was only obtained in

Table 7. Heavy metal amounts of beluga flesh fed with different levels of coriander and active charcoal before challenging with heavy metals.

Parameter	Concentration (g/kg) Chelating agent	0	5	10	15	20
Cadmium (×10^{-3} mg/g)	Coriander	5.00±1.000^{a}	3.33±0.577^{abA}	2.33±0.577^{bA}	2.00±0.000^{bA}	2.00±0.000^{bA}
	Active charcoal	5.00±1.000^{a}	2.33±0.577^{bA}	2.00±0.000^{bcA}	1.33±0.577^{bcA}	0.33±0.577^{cA}
Copper (mg/g)	Coriander	1.517±0.058^{a}	1.433±0.058^{aA}	1.167±0.058^{bA}	1.200±0.100^{bA}	1.200±0.000^{bA}
	Active charcoal	1.517±0.058^{a}	1.300±0.000^{bB}	1.000±0.000^{cB}	0.500±0.000^{dB}	0.200±0.000^{eB}
Lead (×10^{-3} mg/g)	Coriander	260.80±0.72^{a}	207.33±6.43^{bA}	198.67±1.15^{cA}	150.30±0.26^{dA}	148.17±3.55^{dA}
	Active charcoal	260.80±0.72^{a}	191.53±0.50^{bB}	189.43±0.51^{bB}	135.00±1.00^{cB}	129.00±3.60^{cB}

Data are presented as means±standard deviation. Different lowercase letters show significant difference of each parameter at different levels of coriander and active charcoal treatments with the control group and each other; whereas, different uppercase letters show significant difference of each parameter in each level of coriander and active charcoal (Factorial two- way ANOVA, P<0.05).

Table 8. Heavy metal amounts of beluga flesh fed with different levels of coriander and active charcoal after challenging with heavy metals.

Parameter	Concentration (g/kg) Chelating agent	0	5	10	15	20
Cadmium (×10^{-3} mg/g)	Coriander	9.00±0.577^{a}	7.00±1.000^{abA}	5.67±0.577^{bcA}	4.33±0.577^{cdA}	3.67±1.15^{dA}
	Active charcoal	9.00±0.577^{a}	6.33±0.577^{bA}	4.67±0.577^{cA}	1.67±0.577^{dB}	1.00±0.000^{dB}
Copper (mg/g)	Coriander	6.167±0.045^{a}	5.200±0.436^{bA}	5.027±0.025^{bA}	5.017±0.015^{bA}	5.013±0.042^{bA}
	Active charcoal	6.167±0.153^{a}	4.500±0.100^{bB}	2.100±0.100^{cB}	0.600±0.000^{dB}	0.267±0.058^{eB}
Lead (×10^{-3} mg/g)	Coriander	930.33±15.04^{a}	834.00±15.09^{bA}	731.33±20.13^{cA}	680.00±10.0^{dA}	660.33±26.65^{dA}
	Active charcoal	930.33±13.61^{a}	795.67±5.86^{bA}	763.67±14.84^{bA}	232.00±22.00^{cB}	223.67±16.20^{cB}

Data are presented as means±standard deviation. Different lowercase letters show significant difference of each parameter at different levels of coriander and active charcoal treatments with the control group and each other; whereas, different uppercase letters show significant difference of each parameter in each level of coriander and active charcoal (Factorial two- way ANOVA, P<0.05).

cadmium amounts in the fish fed coriander and carbon (P>0.05; Table 7). In the post-challenging stage, significant differences were obtained in the amounts of all three metals in fish fed with 15 and 20 g/kg coriander and carbon (P<0.05; Table 8).

Discussions

Based on the results, the best growth and feeding performances before challenging with heavy metals were observed in fish fed with 5 and 10 g/kg coriander followed by fish fed with 10 and 15 g/kg coriander after the challenging stage. In carbon treatments, the best results were obtained in fish fed 10 and 15 g/kg carbon in both stages of the study. The positive effect of carbon and coriander on growth and nutrition efficiency was not significant in the second stage due to the short period of rearing at this stage. But in the first phase, in particular, the results indicated a higher efficacy of coriander than carbon. Considerable effect of coriander and carbon on growth and feeding parameters is related to their ability in enhancing the status of the digestive system and food digestion, along with absorbing gases and food toxicants (Pish Jang, 2011; Abdel-Tawwab et al., 2017a). Active charcoal enhances the activity of intestinal function by increasing the length of intestinal villi and enterocytes, thereby raising food intake associated with increased growth rate (Michael et al., 2017). But coriander with more nutritional value can be more effective on growth rate than carbon. The aromatic oil in coriander is a digestive stimulant. The oil contains linalool and other important compounds include flavonoids, phenolic acids and mucilage (a soluble fiber) (Bhat et al., 2014). Ramakrishna et al. (2003) reported that the effectiveness of pancreatic lipase and amylase were increased through the supplementation of essential oils by coriander. Coriander also contains some substances with mild anti-bacterial activity (Kubo et al., 2004). Although, in the post-challenging phase, due to the better effect of carbon on the removal of heavy metals, the difference in growth rate in carbon and coriander treatments was not significant.

The effect of coriander on animals such as Vanaraja chicken (Kumari et al., 2014), and mice (Aga et al., 2001; Ren et al., 2013) has been widely investigated with a proven positive effect on growth. Moreover, 1% dietary coriander led to the highest growth rate of broilers (Ali Taneh et al., 2016). According to

Hosseinzadeh et al. (2014), a treatment containing 2% coriander reduced growth rate and raised gut content viscosity due to increasing crude fiber in the diet along with changes in the diet taste and palatability. This might end to a decrease in growth rate in beluga fed with 20 g/kg coriander. There are limited reports on the effect of coriander on fish growth rate. Jia et al. (2009) for example, showed that the addition of coriander to rainbow trout diet did not effect on fish growth which was attributed to the types of fish diet and digestive enzymes. In this study, although 5 to 15 g/kg dietary coriander powder increased the growth of beluga, more coriander amounts might be toxic.

Abdel-Tawwab et al. (2017a) have reported an optimal active charcoal amount of 7 g/kg diet for Nile tilapia. On the other hand, Pirarat et al. (2015) reported a better growth rate in fish fed with 20 g/kg carbon in the same species. No significant difference in the growth performance of Nile tilapia was reported by using the same amount of active charcoal (Boonanuntanasarn et al, 2014). There are contrasting results about other species, too. Michael et al. (2017), for instance, introduced 30 g/kg carbon as the optimum amount in Red tilapia while Thu et al. (2010) reported 5 g/kg carbon as an appropriate amount for Japanese flounder. The differences in optimum concentrations could be attributed to differences in the charcoal production source, and fish feeding habits. Also, the absorption capacity of active charcoal depends on the challenging duration so that slow-feeding fish needs less active charcoal for better growth, while in those that feed rapidly, active charcoal has a lower time to expose to toxic compounds and toxic gases, resulting in their increased in charcoal demands (Thu et al., 2010).

Although there is no report about the effects of coriander on nutritional parameters in fish, an improvement in FCR was reported by using 1% coriander powder in broilers (Ghazanfari and Mohammadi, 2015; Ali Taneh et al., 2016). The best FCR and PER values in Red tilapia were obtained in fish fed with 30 and 40 g active charcoal (Michael et al., 2017) while in Nile tilapia, FCR amounts were not significantly different by increasing charcoal (Abdel-Tawwab et al., 2017a).

Here, no mortalities were recorded in charcoal treatments. Likewise, Abdel-Tawwab et al. (2017a) have noticed a survival rate of 96.7-100% in Nile tilapia, so that no toxic effects were observed in dietary active charcoal. This result was not obtained in using coriander especially at high concentration in this study. Coriander can be added to the diet at 10-15 g/kg without mortality. It should be noted that there was no mortality in the fish fed active carbon even during the heavy metal challenge. The result was only obtained in fish fed with 10 g/kg coriander. It can relate to interactions between the two chelating agents and the heavy metals, which made the active carbon a better chelating agent.

Adding coriander to beluga diet, increased the amount of lipid and decreased the amount of carcass moisture. The highest percentage of carcass protein was obtained in fish fed with 10 g/kg coriander in both stages. The highest percentages of carcass protein and lipids were obtained in fish fed with 15 g/kg charcoal. Also, the lowest carcass ash and highest moisture content were related to the control group. The comparison of carcass composition in both carbon and coriander treatments, the amounts of protein and ash were higher in carbon treatments while the amounts of lipid and moisture were higher in coriander treatments. This result showed that charcoal was more suitable as an additive to beluga diet.

The decrease of carcass moisture is related to the increase of other carcass constituents such as protein, lipid, and ash as well as a high absorption of water by active charcoal (Abdel-Tawwab et al., 2017a). The result of this study is similar to other researchs about increasing carcass protein amount by a rising amount of dietary charcoal in Nile tilapia (Boonanuntanasarn et al., 2014; Abdel-Tawwab et al., 2017a), Red tilapia (Michael et al., 2017), and Japanese flounder (Thu et al., 2010). The above studies suggested that the digestibility of dietary protein increases dramatically in charcoal-contained diets, giving rise to the protein content of fish fed with active charcoal compared to the control group. Also, the stimulatory effects of coriander essential oils on gastrointestinal secretions result in more digestion and absorption of lipids in the digestive tract and hence, improving carcass lipid (Ali Taneh et al., 2016). Mandal and Mandal (2015) showed that fish fed with coriander had an increase in concentrations of Eicosapentaenoic acid and Docosahexaenoic acid. There are also different reports focused on other carcass components such as moisture, total lipid, and ash. For example, Boonanuntanasarn et al. (2014), Thu et al. (2010) and Michael et al. (2017) reported no significant differences in the above-mentioned components other than protein, while Abdel-Tawwab et al. (2017a) reported a significant difference in all parameters.

Based on the results, exposure to environmental heavy metals did not affect protein and lipid amounts considerably which may because of the protective role of coriander and carbon in reducing the toxicity of heavy metals. Furthermore, the decrease of lipid and protein contents is because of changes in their synthesis or degradation in the body after challenging with heavy metals since they provide energy against ensuing stress, a process observed in the control group. However, the effects of metals were largely neutralized by increasing coriander and charcoal concentrations, especially in fish fed with 10 and 15 g/kg carbon or coriander.

In this study the highest amounts of cadmium, copper, and lead were measured in the control group while the lowest amounts were obtained in fish fed with 15-20 g/kg of active charcoal and coriander, respectively. The amount of copper in fish carcass was higher compared to cadmium and lead in both stages of the experiment. Also, the amount of lead in beluga was higher than cadmium. Similar results have been reported in three fish species of the Caspian Sea (Saeedi Saravi and Shokrzadeh, 2013). Comparing two chelating agents in this study, the amounts of cadmium, copper, and lead were lower in fish fed with active carbon than the fish fed with coriander. At very low concentrations of metals, no considerable difference was found but in cadmium amounts in fish fed with coriander and carbon while at 1 mg/L concentration of each metal, differences were obtained in fish fed with 15 and 20 g/kg coriander and carbon. In fact, at higher concentrations, carbon was a better chelating agent than coriander.

Several researches have shown increasing accumulation of heavy metals, particularly in the gills, liver and muscle of fish by rising concentrations of metals in the environment or fish diet (Boonanuntanasarn et al., 2014; Al-Asgah et al., 2015). The present study also confirmed this result in fish muscles. Also, our results indicated that although both substances were able to reduce the accumulation of heavy metals in beluga, active charcoal was more effective. Active charcoal can reduce heavy metals bioaccumulation through excretion and /or absorption because of its micro-porous structure through surface adsorption mechanism. Similar results were reported in Nile tilapia in which heavy metal accumulation reduced by increasing the amount of charcoal (Abdel-Tawwab et al., 2017a). Metallothionein and glutathione compounds of coriander are combined with heavy metals; besides, accelerating the urinary excretion of these metals reduces the bulk accumulation of them (Kansal et al., 2011). Aga et al. (2001) suggested that coriander removes toxins from the cell and it was recommended to be mixed with other chelating agents for better disposal. Moreover, Nicula et al. (2016) reported that using 2% garlic powder with 2% coriander powder had the most protective role against cadmium toxicity in *C. gibelio*. Coriander was shown to combine with cadmium chloride, stabilize it, and remove the metal in rainbow trout (Ren et al., 2009). Ren et al. (2006) reported that inclusion of 2% dried coriander powder by freeze-drying reduced the 20-30% cadmium accumulation in the liver of rainbow trout; in fact, the accumulation of toxic form of cadmium was greater in the control group but its effect on preventing the kidney accumulation of cadmium was less. Coriander also protects the cells against free radicals produced by the lead (Wangensteen et al., 2004; Kansal et al., 2011). Lead can promote the generation of reactive oxygen, and affect antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase (Tellez-Lopez et al., 2017).

Conclusion

The heavy metals were chelated better in fish fed with 15 to 20 g/kg of active charcoal and coriander. But considering other parameters such as growth performance, survival rate, and carcass composition led to better results in fish fed with 10 g/kg coriander and 15g/kg active charcoal. However, coriander is a better additive in an environment with a low concentration of heavy metals. But in an environment with a higher concentration of heavy metals, adding 15 g/kg active charcoal to beluga diet is recommended.

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