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Full length article

Dietary phytoimmunostimulant Persian hogweed (*Heracleum persicum*) has more remarkable impacts on skin mucus than on serum in common carp (*Cyprinus carpio*)

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Abstract

Immunostimulation through the use of sustainable and eco-friendly dietary additives is one of the current prophylactic strategies in fish husbandry. Plant-based immunostimulants are highly considered for this intent, both for their scientific and practical advantages. Persian hogweed (*Heracleum persicum*) is a flower-bearing herb that possesses interesting pharmacological importance due to its bioactive compounds. It is commonly used as a spice, food additive, dietary supplement and traditional remedy. The present study evaluated the potential of *H. persicum* as a dietary phytoimmunostimulant in common carp (*Cyprinus carpio*). The powder form of *H. persicum* was incorporated in the basal diet at three different inclusion levels: 2.5, 5 and 10 g Kg^{-1}. The basal diet (0 g Kg^{-1} of *H. persicum*) served as control. Experimental diets were administered to the fish for a period of 8 weeks. At the termination of the feeding experiment, impacts on fish immunity and performance were evaluated.

Inclusion of *H. persicum* in the diet significantly elevated several immunological factors such as immunoglobulins, lysozyme, protease and alternative complement activities in carp. Interestingly, the changes were more pronounced in the skin mucus than in the serum. Performance was significantly improved in the fish groups that received the candidate phytoimmunostimulant. Specifically, final weight, weight gain, specific growth rate and feed conversion ratio were significantly improved in the fish that received dietary *H. persicum* at inclusion levels 5 g Kg^{-1} and higher. This study demonstrated the potential of Persian hogweed as a candidate dietary phytoimmunostimulant in carp, impacting

mainly the skin mucosal defenses. The study supports the current trend in the exploration of sustainable plant-based dietary supplements that are capable of boosting the immunological defenses of farmed fish.

Keywords: aquaculture; fish health; immunostimulant; mucosal immunity; Persian hogweed

1. Introduction

Immunomodulation is one of the major prophylactic strategies being advanced in aquaculture [1]. The approach lies on the fundamental concept that modulating the immunological defenses of fish potentiates the capability of the organism to respond more effectively in an event of danger (*i.e.* pathogen, stress insult), thereby conferring them resistance and protection [2]. Dietary supplements, particularly those with immunomodulatory properties, have been explored for this purpose.

An immunostimulant is a naturally occurring substance that has a modulatory effect upon the immune system particularly by increasing the resistance of the host against diseases that in most cases are caused by pathogens [2, 3]. In the last decade, several substances have been identified as potential immunostimulants in fish. However, many candidate immunostimulants cannot be used due to high production cost and limited effectiveness [4]. The interest has been diverted to the use of plant-based products, in which many have been customarily used in traditional human medicine. Phytoimmunostimulants have little side effects, are easily degradable, and are abundantly available, making them as cheap and sustainable alternatives to conventional microbial-based immunostimulants [5].

Heracleum persicum, commonly known as Golpar or Persian hogweed, is a plant belonging to the Apiaceae family and is considered native to Iran [6, 7]. This flower-bearing herb grows about 150 to 200 cm high with bristly haired stems of up to 50 cm in thickness. They are widely distributed in Iran, but grow best in moist and nutritious areas

70 especially in northern mountainous regions with an altitude ranging from 1500-2500 m.
71 Traditionally, they are being used as a spice, food additive and supplement. They are also
72 widely known by the locals as a carminative agent. Pharmacological studies indicate that
73 *H. persicum* contains a number of bioactive compounds, many of which have been
74 previously suggested possessing antioxidant, anticonvulsant, analgesic, anti-inflammatory
75 and immunomodulatory properties [7, 8].

76 Cyprinid species are one of the major farmed fish species on a worldwide scale
77 (http://www.fao.org), and among these, the common carp (*Cyprinus carpio*) is the third
78 most important [9]. Like any other aquaculture species, carp farming is faced with
79 incessant threats, especially from infectious pathogens. Measures that can improve their
80 disease resistance are to be adopted to support its sustainable production. Extracts from
81 *H. persicum* have been earlier described possessing inhibitory activity against several fish
82 pathogens [4], making this herb a prospective dietary supplement to improve the health
83 of cultured fish. However, their immunomodulatory functions are yet to be investigated in
84 fish. Hence, this study characterized the effects of dietary *H. persicum* on immunity and
85 performance of rainbow trout. The impacts on both systemic and cutaneous mucosal
86 immunity were investigated following dietary administration of this candidate
87 phytoimmunostimulant.

88

2. Materials & methods

2.1. Preparation of plant powder and experimental diets

The whole part of Persian hogweed (*Heracleum persicum*) was collected from Qazvin (Qazvin province, Iran) during their flowering period (July–September). Botanists from the Botany Department at Golestan University (Golestan, Iran) were requested to properly identify the collected plants. The whole plant was air-dried at room temperature and powdered as previously described [4]. The plant powder was incorporated in the basal diet (Table 1) at three different inclusion levels: 2.5, 5 and 10 g kg^{-1}. The basal diet (*i.e.* 0 g kg^{-1} of *H. persicum* powder) served as the control feed. The experimental diets were prepared as detailed elsewhere [10, 11]. The dried pellets were stored in plastic bags at 4 ^{o}C until delivery.

2.2. Fish and experimental conditions

The feeding experiment was performed at the Aquaculture Laboratory of Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources (Gorgan, Iran). Common carp (*Cyprinus carpio*) were procured from a private fish farm (Mazandaran Province, Iran). The fish stock was free of disease outbreak history. Upon their arrival, they were acclimated to the laboratory conditions for 2 weeks. The following physico-chemical parameters were monitored and maintained: water temperature (26 ± 1.0 ˚C), dissolved oxygen (7.2 ± 0.01 mg L^{-1}), pH (7.6 ± 0.2) and natural photoperiod (12L:12D). Fish with an average weight of 23.5 ± 0.2 g were randomly stocked into a 150-L

tank at a density of 30 fish per tank. Twelve tanks were used in total, with three replicate tanks allocated to each of the experimental diet group.

The experimental diets were delivered manually to apparent satiation twice a day (09:00 and 15:00) over an 8-week period. All fish handling procedures employed in the study were in accordance with the internationally accepted principles on animal experimentation.

Table 1. Dietary formulation and proximate composition of the basal diet (%)

Ingredients	
Fish meal	40.0
Wheat flour	21.0
Soybean meal	13.5
Gluten	5.5
Soybean oil	6.0
Fish oil	6.0
Mineral premix [*]	3.0
Vitamin premix [*]	2.0
Binder [†]	2.0
Anti-fungal [‡]	0.5
Antioxidant [§]	0.5
Proximate composition (% dry matter basis)	
Dry matter	91.12
Crude protein	36.81
Crude lipid	11.33
Ash	3.50
Fiber	11.58

[*] Details of the premix [10].
[†] Amet binder ™, Mehr Taban-e- Yazd, Iran
[‡] ToxiBan antifungal (Vet-A-Mix, Shenan- doah, IA)
[§] Butylated hydroxytoluene (BHT) (Merck, Germany)

2.3. Immunological assays

2.3.1. Collection and preparation of skin mucus and serum

Fish were not fed 24 h prior to sample collection. Three fish (*i.e.* 9 fish per experimental feed group) were taken from each tank and were anaesthetized using clove powder (500 mg L^{-1}) dissolved in water. Thereafter, the anaesthetized fish was transferred into a polyethylene bag containing 10 mL of 50 mM NaCl (Sigma, Steinheim, Germany). Skin mucus was collected by gently rubbing the fish inside the plastic in a downward motion for 1–2 min as described earlier [12, 13]. The collected mucus sample was immediately transferred to a sterile 15 mL tubes and centrifuged at 1500 *g* at 4 ˚C for 10 min (5810R Eppendorf, Engelsdorf, Germany). The supernatant was collected and kept at -80 ˚C until analysis.

Blood was drawn from the caudal vein and was allowed to clot for 12 h at 4 ˚C. Thereafter, serum was collected by centrifuging the clotted blood at 5000 *g* at 4 ˚C for 5 min (International Model CL, International Equipment Co., Needham, MA, USA). The serum samples were stored at -80 ˚C until further analysis.

2.3.2. Immunological factors in skin mucus

The total Ig content in skin mucus was determined following a method described by Siwiki and Anderson [14]. Briefly, the total protein level of the samples were measured by Bradford protein assay [15]. Immunoglobulin molecules were precipitated down by a 12 % polyethylene glycol solution (Sigma) and the protein content of samples

143 were measured again. The difference in protein content was considered as the total Ig
144 content of skin mucus.

145 Lysozyme activity in skin mucus was measured by a turbidimetric assay [16].
146 Briefly, 50 µL suspension of Gram-positive bacterium *Micrococcus luteus* (Sigma) (0.3 mg
147 mL^{-1} of lyophilized cells dissolved in 40 mM sodium phosphate buffer, pH 6.5) was mixed
148 with 50 µL of the mucus sample. The reaction mixture was incubated at 30 °C and the
149 reduction in absorbance at 450 nm was measured after 0 and 15 min in a microplate
150 reader. A unit of lysozyme activity was defined as the amount of enzyme that caused a
151 decrease in absorbance of 0.001 per minute.

152 Protease activity in skin mucus was measured by the azocasein hydrolysis
153 method described earlier [12], with minor modifications. Briefly, 100 µL of skin mucus was
154 mixed with 100 µL 0.7 % azocasein solution (Sigma) and thereafter incubated at 30°C with
155 constant agitation for 19 h. Then, 4.5 % trichloroacetic acid was added to stop the reaction
156 and the supernatant was obtained by centrifuging the reaction mixture at 15 000 *g* for 5
157 min. The resulting supernatants were pipetted to a 96-well flat bottom plate that was
158 earlier seeded with 100 µL 1 N sodium hydroxide (NaOH) per well. The optical density
159 (OD) was measured at 450 nm. Protease activity was expressed relative to a positive
160 control (mucus replaced with trypsin solution of 5 mg mL^{-1}; 100% protease activity).

161

162 **2.3.3. Immunological factors in serum**

Serum total Ig and lysozyme activity were determined similarly as described above for skin mucus samples.

Serum alternative complement pathway hemolytic activity (ACH50) was determined following a method described previously using the New Zealand rabbit red blood cells (RaRBC) [17]. The volume of serum complement yielding 50 % haemolysis (ACH50) was measured and used to calculate the complement activity.

2.4. Fish performance

Fish performance was evaluated by determining weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and percentage survival (%) after the 8-week feeding period.

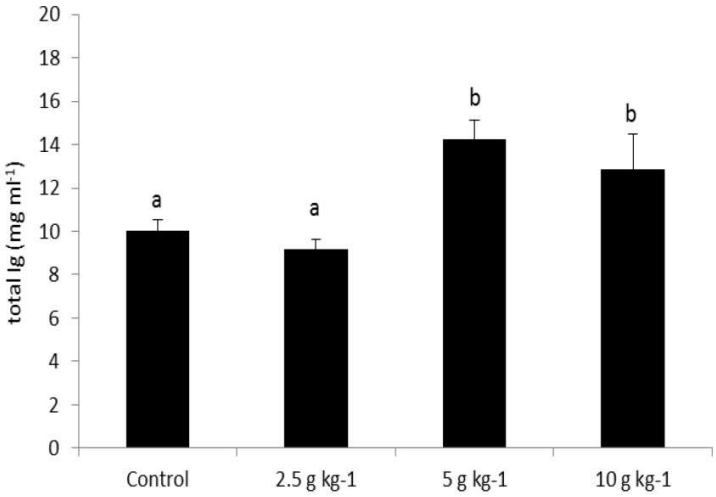
- Weight gain (g) = W_2 (g) − W_1 (g)
- Specific growth rate (SGR) = 100 (ln W_2−ln W_1)/T
- Feed conversion ratio (FCR) = feed intake (g) / weight gain (g)
- Survival rate = (N_f / N_i) × 100

Where W_1 is the initial weight, W_2 is the final weight, T is the number of days in the feeding period, N_i is initial number of fish and N_f is final number of fish.

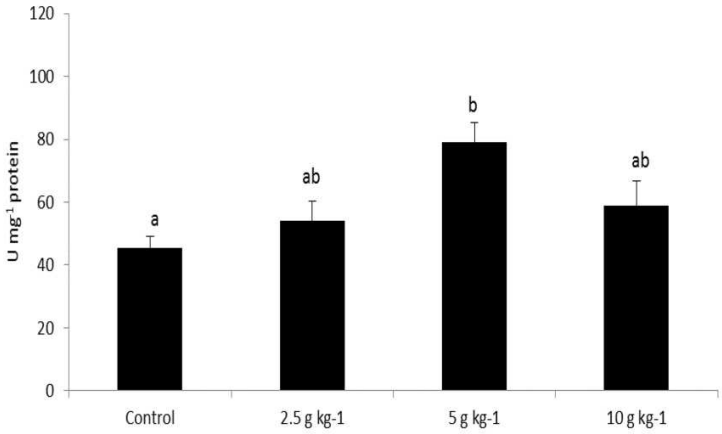
2.5. Statistical analysis

182 The homogeneity of variance and normality of the data were confirmed by
183 Leaven and Kolmogorov-Smirnov tests. Then, data were subjected to one-way analysis of
184 variance (ANOVA) followed by Duncan's multiple range tests to determine significant
185 difference at $P < 0.05$. SPSS statistical package version 16.0 (SPSS Inc., Chicago, IL, USA)
186 was used for statistical analysis.
187
188 **3. Results**
189 **3.1. Skin mucus immune responses**
190 Dietary supplementation of *H. persicum* significantly impacted the skin
191 mucosal immune responses of common carp (Figs. 1-3). Skin mucus total Ig increased
192 significantly in the fish fed with diets containing 5 and 10 g Kg^{-1} of *H. persicum*, but not at
193 inclusion level of 2.5 g Kg^{-1}. The increase in the skin mucus Ig level in the two experimental
194 groups was at least 30 % relative to the control group (Fig. 1).
195 Inclusion of the candidate phytoimmunostimulant also affected the activity of
196 skin mucus lysozyme (Fig. 2). A significant increase of at least 60 % compared with control
197 was documented in the group fed with 5 g Kg^{-1} of *H. persicum*. However, this significant
198 elevation of skin mucus lysozyme was not observed in other experimental feed groups.
199 Proteolytic activity in skin mucus was significantly influenced following feeding
200 with diets enriched with powder *H. persicum* (Fig. 3). A remarkable significant increase in
201 skin mucus protease activity was observed in all treatment groups, where the highest

202 increment was noted at inclusion level of 5 g Kg^{-1}. The percentage increase ranged from
203 75 – 200 % relative to control.



204
205 **Figure 1.** Total immunoglobulin levels in the skin mucus of common carp (*Cyprinus carpio*)
206 fed with diets supplemented with Persian hogweed (*H. persicum*) at different inclusion
207 levels for 8 weeks. Statistical differences ($P < 0.05$) between experimental groups are
208 indicated with different letter notations. The values are mean ± S.E of 9 individual fish.
209



210
211 **Figure 2.** Lysozyme activity in the skin mucus of common carp (*Cyprinus carpio*) fed with
212 diets supplemented with Persian hogweed (*H. persicum*) at different inclusion levels for 8
213 weeks. Statistical differences ($P < 0.05$) between experimental groups are indicated with
214 different letter notations. The values are mean ± S.E of 9 individual fish.
215

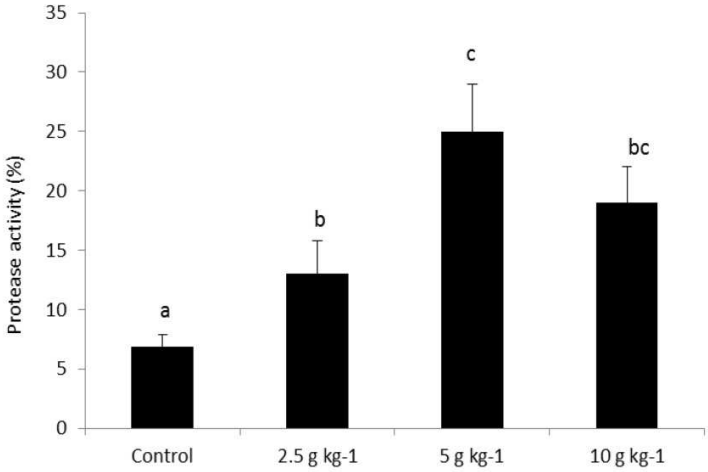


Figure 3. Protease activity in the skin mucus of common carp (*Cyprinus carpio*) fed with diets supplemented with Persian hogweed (*H. persicum*) at different inclusion levels for 8 weeks. Statistical differences ($P < 0.05$) between experimental groups are indicated with different letter notations. The values are mean ± S.E of 9 individual fish.

3.2. Serum humoral immune responses

Immunological factors in the serum were also influenced by dietary supplementation of *H. persicum*, however the changes were not as pronounced compared with the observations in skin mucus (Figs 4-6). Serum total Ig level was significantly elevated in fish fed with 10 g Kg^{-1} of the candidate phytoimmunostimulant (Fig. 4). The increment was noted to be at least 20 % compared with control. There were no significant changes in the groups fed with 2.5 and 5 g Kg^{-1}, respectively.

The activity level of serum lysozyme was unaltered following feeding with *H. persicum*, regardless of the inclusion level (Fig. 5).

Serum alternative complement pathway hemolytic activity (ACH50) was minimally influenced by the dietary manipulation (Fig. 6). A significant change was only

observed in the group that received a diet enriched with the candidate phytoimmunostimulant at a level of 10 g Kg^{-1} compared with control. ACH50 activity in this group was elevated by around 8 % relative to control. In addition, the level of ACH50 in the three groups that received *H. persicum*-enriched diets was not significantly different from one another.

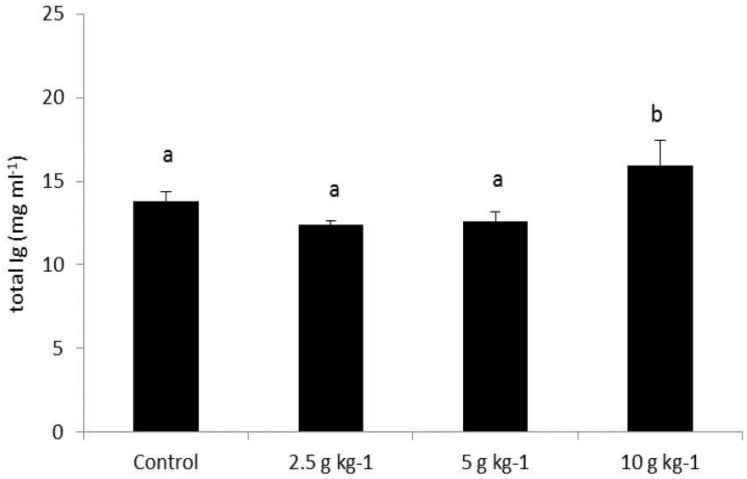


Figure 4. Total serum immunoglobulin levels of common carp (*Cyprinus carpio*) fed with diets supplemented with Persian hogweed (*H. persicum*) at different inclusion levels for 8 weeks. Statistical differences ($P < 0.05$) between experimental groups are indicated with different letter notations. The values are mean ± S.E of 9 individual fish.

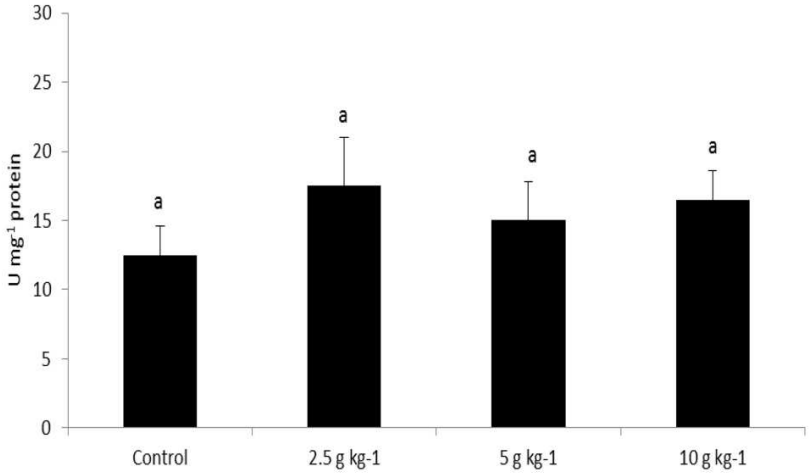


Figure 5. Serum lysozyme activity in common carp (*Cyprinus carpio*) fed with diets supplemented with Persian hogweed (*H. persicum*) at different inclusion levels for 8 weeks. Statistical differences (P < 0.05) between experimental groups are indicated with different letter notations. The values are mean ± S.E of 9 individual fish.

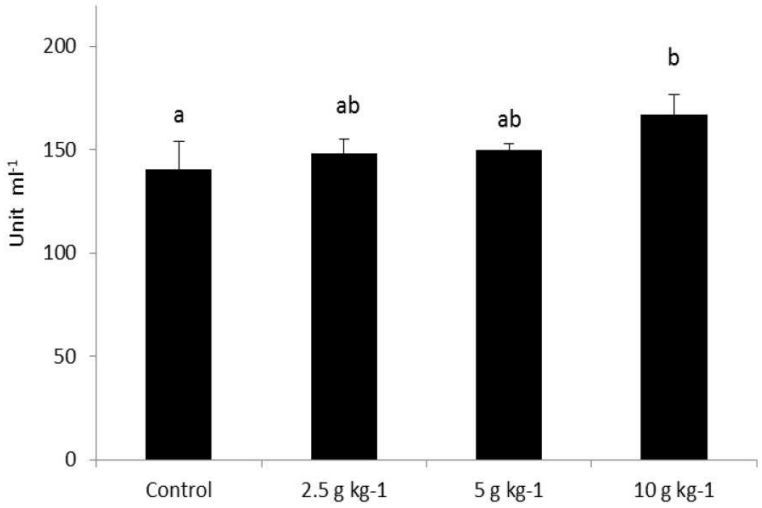


Figure 6. Serum alternative complement pathway haemolytic activity (ACH50) fed with diets supplemented with Persian hogweed (*H. persicum*) at different inclusion levels for 8 weeks. Statistical differences (P < 0.05) between experimental groups are indicated with different letter notations. The values are mean ± S.E of 9 individual fish.

3.3. Fish performance

Fish that received *H.persicum*-supplemented diets at levels of 5 and 10 g kg^{-1}, respectively, had significantly higher final weight compared with the control group. Weight gain in these groups was at least 18 % higher than the group receiving the basal diet (Table 2). Performance indicators such as SGR and FCR were significantly altered as well in groups receiving diets enriched with at least 5 g Kg^{-1} of *H. persicum*. SGR in fish fed with 5 and 10 g Kg^{-1} of the candidate phytoimmunostimulant was at least 12.3 % higher than the unsupplemented group. On the other hand, the FCR in these two groups were at least 18 % better than the control. All groups registered 100 % survival rate after an 8-week feeding period.

Table 2. Changes in the performance indicators of common carp (*Cyprinus carpio*) following feeding with diets supplemented with *H. persicum* powder at different inclusion levels. Values are presented as mean ± SE.

	Persian hogweed (g Kg^{-1})			
	0	2.5	5	10
Final weight (g)	50.24 ± 3.17 ^{a}	53.23 ± 1.02 ^{ab}	55.41 ± 1.06 ^{b}	55.93 ± 2.01 ^{b}
Weight gain (g)	26.86 ± 3.31 ^{a}	29.73 ± 1.28 ^{ab}	31.83 ± 1.07 ^{b}	32.55 ± 1.90 ^{b}
SGR	1.55 ± 0.13 ^{a}	1.66 ± 0.06 ^{ab}	1.74 ± 0.04 ^{b}	1.78 ± 0.06 ^{b}
FCR	2.37 ± 0.11 ^{a}	2.15 ± 0.10 ^{ab}	1.96 ± 0.05 ^{b}	1.90 ± 0.08 ^{b}
Survival rate (%)	100	100	100	100

Values in a row with different letter denote significant difference ($P < 0.05$)

4. Discussion

The present study demonstrated the potential of Persian hogweed (*H. persicum*) as a dietary phytoimmunostimulant in common carp (*C. carpio*). Immune responses were remarkably modulated in fish that received diets supplemented with *H. persicum* powder. In particular, both the serum humoral and skin mucosal responses were altered in the fish fed with the dietary supplement, where the latter exhibited more marked changes.

Fish have interestingly complex defense system, with the integument providing the first line of defense [18]. In particular, the skin mucus is a critical component of the integumentary system and is characterized by a collection of potent defense molecules [13, 19]. The present study demonstrated that the dietary administration of *H. persicum* could modify some key defense molecules in the skin mucus of carp. Secretory immunoglobulins play important roles in the maintenance of mucosal homeostasis [20], and the candidate dietary phytoimmunostimulant could potentiate their level in the skin mucus of carp by at least 30 % especially at inclusion levels 5 g kg^{-1} and higher when compared with control group. Lysozyme (also known as N-acetylmuramide glucanohydrolase or muramidase) is a ubiquitous bactericidal enzyme and by far the most studied defense molecule in fish skin mucus [20]. It was observed that the effect of dietary *H. persicum* in the skin mucus lysozyme activity demonstrated a ceiling effect-like tendency. Significantly elevated level of skin mucus lysozyme was only noted in the group fed with 5 g Kg^{-1} of *H. persicum;* no significant change was noted in concentration higher

297 than this. Skin mucus proteases play a protective function against pathogenic invasion
298 either by direct action through cleaving their proteins or indirectly by hampering their
299 colonization and invasion mechanisms [21, 22]. Regardless of the inclusion level, dietary
300 *H. persicum* significantly elevated the activity of skin mucus proteases where the highest
301 increment was observed at inclusion level of 5 g Kg^{-1}. This noteworthy effect in mucosal
302 proteolytic activity following dietary *H. persicum* supplementation could be considered
303 beneficial for the protective function of the skin mucosa. Further, this is an interestingly
304 remarkable effect because besides the protective role of proteases mentioned above,
305 they have been shown as well influencing the production of other innate immune
306 components present in fish mucus such as complement, immunoglobulins or antibacterial
307 peptides [23-25], hereby adding to the immunomodulatory potential of this candidate
308 phytoimmunostimulant.

309 Aside from the immune molecules in the skin mucus that provide the first line
310 of defense, soluble mediators of immunity are constantly present as well in fish circulatory
311 system as serum factors. It is interesting to highlight that the effect of dietary *H. persicum*
312 in serum was not as striking compared with the changes observed in the skin mucus.
313 Generally, significant changes were only observed at the highest inclusion level. From the
314 three immune parameters studied in the serum, total immunoglobulins and alternative
315 haemolytic complement activity were distinctly enhanced in the abovementioned
316 inclusion level. While immunoglobulins identify and neutralize pathogens, the alternative
317 complement system is composed of soluble plasma proteins, which is antibody

independent, that play key roles in the opsonization and killing of pathogens [26]. It could be speculated that the potentiation of the recognition and presentation capability for foreign antigenic factors was the main immunomodulatory impact of dietary *H. persicum* in the serum. This deduction could be fully substantiated with more mechanistic approaches in future studies. Further, the minimal changes in serum immune parameters posit a possibility that the immunostimulatory functions of dietary *H. persicum* were higher towards the mucosal rather than the systemic arms of the host immunity.

We could implicate the bioactive compounds that have been previously characterized from this plant as major contributors in the observed immunomodulatory activities of dietary *H. persicum*. Furanocoumarins such as pimpinellin, isopimpinellin, bergapten, isobergapten and sphondin are some of the bioactive compounds being attributed to the immunomodulatory activity attributed to *H. persicum* [7, 8]. In addition, major constituents of its essential oils including hexyl butyrate, octyl acetate, hexyl 2-methylbutanoate and hexyl isobutyrate, have been documented with anti-inflammatory and analgesic properties [27]. It is likely that these compounds that have been previously characterized and tested in mammalian models may also be the same compounds endowing the immunostimulatory features observed in the current study. To explore the definitive underlying mechanism of these beneficial actions, it is essential to extract and characterize the bioactive components of *H. persicum* in future studies. On the other hand, it is a practical advantage that even the crude powder extract of the plant could elicit significant immunomodulation in carp. It is highly favourable in aquaculture that *H.*

339 *persicum* could be used as a phytoimmunostimulant without undergoing tedious and
340 expensive extraction and purification procedures which eventually present significant
341 production cost making its use impractical.

342 Besides their key functions as modulators of immunity, immunostimulants are
343 also considered as growth promoters. In the present study, fish fed with diet
344 supplemented with *H. persicum* powder demonstrated enhanced performance as
345 indicated by higher final weight and weight gain, as well as improved SGR and FCR values.
346 Observed changes indicate that in order to elicit significant improvement in growth
347 performance, the candidate phytoimmunostimulant must be included in the basal diet of
348 carp at least 5 g Kg^{-1} or higher. The growth promoting function of plant-based
349 immunostimulant had been previously shown in a carp study, where traditional Chinese
350 medicinal plants increased body weight of fish by at least 16 % [28]. It could be possible
351 that the growth-promoting property of the phytoimmunostimulant may be through
352 stimulation of appetite, modulation of the digestive enzymatic physiology, supply of
353 beneficial vitamins and minerals or by providing substrate for microbial activity in the gut.
354 These mechanisms have been proposed in several earlier studies in fish [5, 29, 30].

355 In conclusion, the present study revealed the immuno-nutritional benefits
356 from dietary Persian hogweed (*H. persicum*), hence, designating the herb as an interesting
357 candidate phytoimmunostimulant to improve the health status of carp. Remarkably, the
358 inclusion of the crude powder form of the plant in the diet stimulated both the skin
359 mucosal and serum humoral defense molecules. The extent of stimulation was more

prominent in the soluble factors in the skin mucus than in the serum. The results support the exploration of traditional medicinal plants as effective, sustainable and relatively cheap dietary supplements capable of enhancing the immunological robustness of farmed fish.

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Table 1. Dietary formulation and proximate composition of the basal diet (%)

Ingredients	
Fish meal	40.0
Wheat flour	21.0
Soybean meal	13.5
Gluten	5.5
Soybean oil	6.0
Fish oil	6.0
Mineral premix *	3.0
Vitamin premix *	2.0
Binder †	2.0
Anti fungi ‡	0.5
Antioxidant §	0.5
Proximate composition (% dry matter basis)	
Dry matter	91.12
Crude protein	36.81
Crude lipid	11.33
Ash	3.50
Fiber	11.58

* Details of the premix [10].

† Amet binder ™, Mehr Taban-e- Yazd, Iran

‡ ToxiBan antifungal (Vet-A-Mix, Shenan- doah, IA)

§ Butylated hydroxytoluene (BHT) (Merck, Germany)

Table 2. Changes in the performance indicators of common carp (*Cyprinus carpio*) following feeding with diets supplemented with *H. persicum* powder at different inclusion levels. Values are presented as the mean ± SE.

	Persian hogweed (g Kg^{-1})			
	0	2.5	5	10
Final weight (g)	50.24 ± 3.17 [a]	53.23 ± 1.02 [ab]	55.41 ± 1.06 [b]	55.93 ± 2.01 [b]
Weight gain (g)	26.86 ± 3.31 [a]	29.73 ± 1.28 [ab]	31.83 ± 1.07 [b]	32.55 ± 1.90 [b]
SGR	1.55 ± 0.13 [a]	1.66 ± 0.06 [ab]	1.74 ± 0.04 [b]	1.78 ± 0.06 [b]
FCR	2.37 ± 0.11 [a]	2.15 ± 0.10 [ab]	1.96 ± 0.05 [b]	1.90 ± 0.08 [b]
Survival rate (%)	100	100	100	100

Values in a row with different letter denote significant difference ($P < 0.05$)

Title: Dietary phytoimmunostimulant Persian hogweed (*Heracleum persicum*) has more remarkable impacts on skin mucus than on serum in common carp (*Cyprinus carpio*)

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HIGHLIGHTS

- Persian hogweed is a flower-bearing herb rich in bioactive compounds.
- Carp diet was supplemented with the powder form of the herb at 3 inclusion levels.
- Immunological defences were altered following an 8-week feeding trial.
- Changes in immune factors were more pronounced in skin mucus than in serum.
- *H. persicum* is a potential phytoimmunostimulant in carp aquaculture.