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Hoseinifar, Seyed Hossein ; Zoheiri, Fazel; Lazado, Carlo Cabacang

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Seyed Hossein Hoseinifar, Fazel Zoheiri, Carlo C. Lazado

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1 Full length article

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3 Dietary phytoimmunostimulant Persian hogweed (Heracleum

persicum) has more remarkable impacts on skin mucus than on
serum in common carp (*Cyprinus carpio*)

- 6
- 7 Seyed Hossein Hoseinifar ^a, Fazel Zoheiri ^a, Carlo C. Lazado ^{b*}
- ⁸ ^a Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan
- 9 University of Agricultural Sciences and Natural Resources, Gorgan, Iran
- 10 ^b Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea
- 11 Research Centre, DK-9850 Hirtshals, Denmark

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- 13 *Corresponding author:
- 14 Carlo C. Lazado
- 15 Technical University of Denmark
- 16 The North Sea Research Centre
- 17 9850 Hirtshals, Denmark
- 18 Tel: + 45 35 88 32 00
- 19 Fax: + 45 35 88 32 60
- 20 E-mail: carlolazado@yahoo.com

22 Abstract

23 Immunostimulation through the use of sustainable and eco-friendly dietary additives is one of the current prophylactic strategies in fish husbandry. Plant-based 24 immunostimulants are highly considered for this intent, both for their scientific and 25 26 practical advantages. Persian hogweed (Heracleum persicum) is a flower-bearing herb that possesses interesting pharmacological importance due to its bioactive compounds. It is 27 commonly used as a spice, food additive, dietary supplement and traditional remedy. The 28 present study evaluated the potential of *H. persicum* as a dietary phytoimmunostimulant 29 30 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 31 ¹ of *H. persicum*) served as control. Experimental diets were administered to the fish for a 32 period of 8 weeks. At the termination of the feeding experiment, impacts on fish 33 34 immunity and performance were evaluated.

35 Inclusion of H. persicum in the diet significantly elevated several immunological factors such as immunoglobulins, lysozyme, protease and alternative complement 36 37 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 38 39 candidate phytoimmunostimulant. Specifically, final weight, weight gain, specific growth rate and feed conversion ratio were significantly improved in the fish that received dietary 40 H. persicum at inclusion levels 5 g Kg⁻¹ and higher. This study demonstrated the potential 41 42 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.

46

47 *Keywords*: aquaculture; fish health; immunostimulant; mucosal immunity; Persian
48 hogweed

49 **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.

56 An immunostimulant is a naturally occurring substance that has a modulatory 57 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, 58 several substances have been identified as potential immunostimulants in fish. However, 59 many candidate immunostimulants cannot be used due to high production cost and 60 61 limited effectiveness [4]. The interest has been diverted to the use of plant-based 62 products, in which many have been customarily used in traditional human medicine. Phytoimmunostimulants have little side effects, are easily degradable, and are abundantly 63 64 available, making them as cheap and sustainable alternatives to conventional microbialbased immunostimulants [5]. 65

66 *Heracleum persicum*, commonly known as Golpar or Persian hogweed, is a 67 plant belonging to the Apiaceae family and is considered native to Iran [6, 7]. This flower-68 bearing herb grows about 150 to 200 cm high with bristly haired stems of up to 50 cm in 69 thickness. They are widely distributed in Iran, but grow best in moist and nutritious areas

especially in northern mountainous regions with an altitude ranging from 1500-2500 m.
Traditionally, they are being used as a spice, food additive and supplement. They are also
widely known by the locals as a carminative agent. Pharmacological studies indicate that *H. persicum* contains a number of bioactive compounds, many of which have been
previously suggested possessing antioxidant, anticonvulsant, analgesic, anti-inflammatory
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 disease resistance are to be adopted to support its sustainable production. Extracts from 80 H. persicum have been earlier described possessing inhibitory activity against several fish 81 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 fish. Hence, this study characterized the effects of dietary H. persicum on immunity and 84 85 performance of rainbow trout. The impacts on both systemic and cutaneous mucosal immunity were investigated following dietary administration of this candidate 86 87 phytoimmunostimulant.

88

89 2. Materials & methods

90

2.1. Preparation of plant powder and experimental diets

The whole part of Persian hogweed (Heracleum persicum) was collected from 91 Qazvin (Qazvin province, Iran) during their flowering period (July-September). Botanists 92 93 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 94 95 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 96 (*i.e.* 0 g kg⁻¹ of *H. persicum* powder) served as the control feed. The experimental diets 97 were prepared as detailed elsewhere [10, 11]. The dried pellets were stored in plastic bags 98 at 4 °C until delivery. 99

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2.2. Fish and experimental conditions

102 The feeding experiment was performed at the Aquaculture Laboratory of Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources 103 (Gorgan, Iran). Common carp (Cyprinus carpio) were procured from a private fish farm 104 (Mazandaran Province, Iran). The fish stock was free of disease outbreak history. Upon 105 106 their arrival, they were acclimated to the laboratory conditions for 2 weeks. The following physico-chemical parameters were monitored and maintained: water temperature (26 ± 107 1.0 °C), dissolved oxygen (7.2 \pm 0.01 mg L⁻¹), pH (7.6 \pm 0.2) and natural photoperiod 108 109 (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 110

tanks allocated to each of the experimental diet group. 111

The experimental diets were delivered manually to apparent satiation twice a 112

day (09:00 and 15:00) over an 8-week period. All fish handling procedures employed in 113

- the study were in accordance with the internationally accepted principles on animal 114
- experimentation. 115
- 116

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 com	
(% dry matter	,
Dry matter	91.12
Crude protein	36.81
Crude lipid	11.33
Ash	3.50
Fiber	11.58
* Details of the pr	emix [10].

118 119 120

121

117

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

[†] ToxiBan antifungal (Vet-A-Mix, Shenan- doah, IA) [§] Butylated hydroxytoluene (BHT) (Merck, Germany)

122

2.3. Immunological assays

123 **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 124 experimental feed group) were taken from each tank and were anaesthetized using clove 125 powder (500 mg L⁻¹) dissolved in water. Thereafter, the anaesthetized fish was transferred 126 into a polyethylene bag containing 10 mL of 50 mM NaCl (Sigma, Steinheim, Germany). 127 128 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 129 130 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 -131 80 °C until analysis. 132

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.

- 137
- 138

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

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

Protease activity in skin mucus was measured by the azocasein hydrolysis 152 method described earlier [12], with minor modifications. Briefly, 100 µL of skin mucus was 153 mixed with 100 µL 0.7 % azocasein solution (Sigma) and thereafter incubated at 30°C with 154 155 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 min. The resulting supernatants were pipetted to a 96-well flat bottom plate that was 157 earlier seeded with 100 µL 1 N sodium hydroxide (NaOH) per well. The optical density 158 (OD) was measured at 450 nm. Protease activity was expressed relative to a positive 159 control (mucus replaced with trypsin solution of 5 mg mL⁻¹; 100% protease activity). 160

- 161
- 162

2.3.3. Immunological factors in serum

- 163 Serum total Ig and lysozyme activity were determined similarly as described 164 above for skin mucus samples.
- 165 Serum alternative complement pathway hemolytic activity (ACH50) was 166 determined following a method described previously using the New Zealand rabbit red 167 blood cells (RaRBC) [17]. The volume of serum complement yielding 50 % haemolysis 168 (ACH50) was measured and used to calculate the complement activity.
- 169
- 170

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 = $(N_f / N_i) \times 100$

178 Where W_1 is the initial weight, W_2 is the final weight, T is the number of days in the

- 179 feeding period, N_i is initial number of fish and N_f is final number of fish.
- 180
- 181 **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**

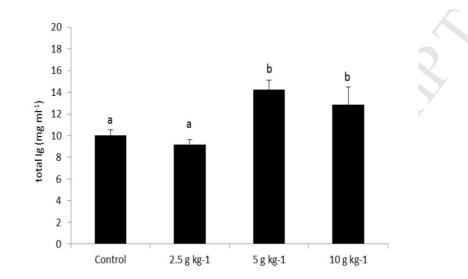
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3.1. Skin mucus immune responses

Dietary supplementation of *H. persicum* significantly impacted the skin mucosal immune responses of common carp (Figs. 1-3). Skin mucus total Ig increased significantly in the fish fed with diets containing 5 and 10 g Kg⁻¹ of *H. persicum*, but not at inclusion level of 2.5 g Kg⁻¹. The increase in the skin mucus Ig level in the two experimental groups was at least 30 % relative to the control group (Fig. 1).

Inclusion of the candidate phytoimmunostimulant also affected the activity of
skin mucus lysozyme (Fig. 2). A significant increase of at least 60 % compared with control
was documented in the group fed with 5 g Kg⁻¹ of *H. persicum*. However, this significant
elevation of skin mucus lysozyme was not observed in other experimental feed groups.

Proteolytic activity in skin mucus was significantly influenced following feeding with diets enriched with powder *H. persicum* (Fig. 3). A remarkable significant increase in skin mucus protease activity was observed in all treatment groups, where the highest

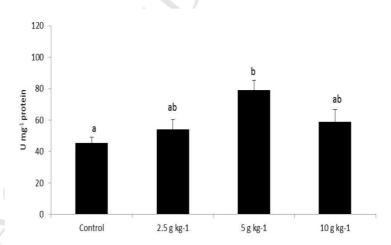


increment was noted at inclusion level of 5 g Kg⁻¹. The percentage increase ranged from

203 75 – 200 % relative to control.

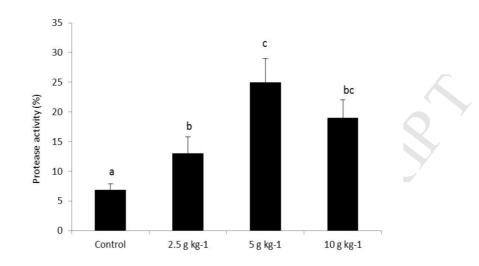
204

Figure 1. Total immunoglobulin levels 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.



210

Figure 2. Lysozyme 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.



216

Figure 3. Protease activity in the skin mucus of common carp (Cyprinus carpio) fed with 217 diets supplemented with Persian hogweed (H. persicum) at different inclusion levels for 8 218 weeks. Statistical differences (P < 0.05) between experimental groups are indicated with 219 220 different letter notations. The values are mean ± S.E of 9 individual fish. 221

- 222

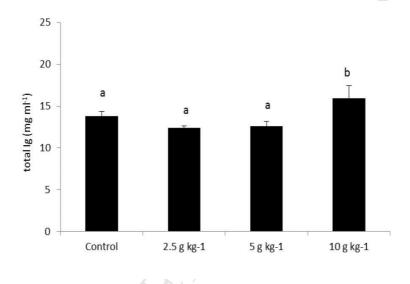
3.2. Serum humoral immune responses

223 Immunological factors in the serum were also influenced by dietary supplementation of *H. persicum*, however the changes were not as pronounced compared 224 225 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 226 increment was noted to be at least 20 % compared with control. There were no significant 227 changes in the groups fed with 2.5 and 5 g Kg⁻¹, respectively. 228

The activity level of serum lysozyme was unaltered following feeding with H. 229 persicum, regardless of the inclusion level (Fig. 5). 230

Serum alternative complement pathway hemolytic activity (ACH50) was 231 minimally influenced by the dietary manipulation (Fig. 6). A significant change was only 232

233 observed in the group that received a diet enriched with the candidate 234 phytoimmunostimulant at a level of 10 g Kg⁻¹ compared with control. ACH50 activity in 235 this group was elevated by around 8 % relative to control. In addition, the level of ACH50 236 in the three groups that received *H. persicum*-enriched diets was not significantly different 237 from one another.

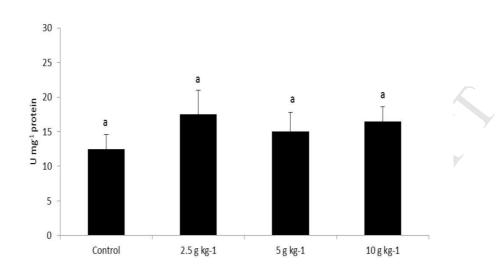


238 239

240 Figure 4. Total serum immunoglobulin levels of common carp (Cyprinus carpio) fed with

241 diets supplemented with Persian hogweed (*H. persicum*) at different inclusion levels for 8
 242 weeks. Statistical differences (P < 0.05) between experimental groups are indicated with

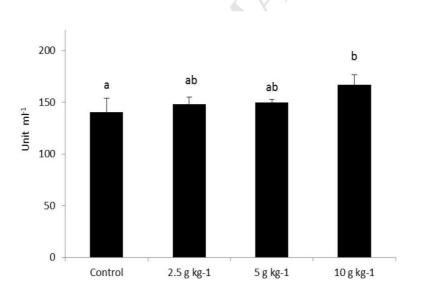
243 different letter notations. The values are mean ± S.E of 9 individual fish.



245

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.

250



251

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.

256

257

3.3. Fish performance

258	Fish that received <i>H.persicum</i> -supplemented diets at levels of 5 and 10 g kg ⁻¹ ,
259	respectively, had significantly higher final weight compared with the control group.
260	Weight gain in these groups was at least 18 % higher than the group receiving the basal
261	diet (Table 2). Performance indicators such as SGR and FCR were significantly altered as
262	well in groups receiving diets enriched with at least 5 g Kg ⁻¹ of <i>H. persicum</i> . SGR in fish fed
263	with 5 and 10 g Kg ⁻¹ of the candidate phytoimmunostimulant was at least 12.3 % higher
264	than the unsupplemented group. On the other hand, the FCR in these two groups were at
265	least 18 % better than the control. All groups registered 100 % survival rate after an 8-
266	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 (<mark>g</mark>)	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)

276 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 283 284 providing the first line of defense [18]. In particular, the skin mucus is a critical component 285 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. 286 persicum could modify some key defense molecules in the skin mucus of carp. Secretory 287 immunoglobulins play important roles in the maintenance of mucosal homeostasis [20], 288 289 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 290 291 compared with control group. Lysozyme (also known as N-acetylmuramide glucanohydrolase or muramidase) is a ubiquitous bactericidal enzyme and by far the most 292 293 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 294 tendency. Significantly elevated level of skin mucus lysozyme was only noted in the group 295 fed with 5 g Kg⁻¹ of *H. persicum;* no significant change was noted in concentration higher 296

than this. Skin mucus proteases play a protective function against pathogenic invasion 297 298 either by direct action through cleaving their proteins or indirectly by hampering their colonization and invasion mechanisms [21, 22]. Regardless of the inclusion level, dietary 299 H. persicum significantly elevated the activity of skin mucus proteases where the highest 300 increment was observed at inclusion level of 5 g Kg⁻¹. This noteworthy effect in mucosal 301 proteolytic activity following dietary H. persicum supplementation could be considered 302 303 beneficial for the protective function of the skin mucosa. Further, this is an interestingly remarkable effect because besides the protective role of proteases mentioned above, 304 305 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 peptides [23-25], hereby adding to the immunomodulatory potential of this candidate 307 308 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. Generally, significant changes were only observed at the highest inclusion level. From the 313 314 three immune parameters studied in the serum, total immunoglobulins and alternative 315 haemolytic complement activity were distinctly enhanced in the abovementioned inclusion level. While immunoglobulins identify and neutralize pathogens, the alternative 316 317 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.

325 We could implicate the bioactive compounds that have been previously 326 characterized from this plant as major contributors in the observed immunomodulatory 327 activities of dietary H. persicum. Furanocoumarins such as pimpinellin, isopimpinellin, bergapten, isobergapten and sphondin are some of the bioactive compounds being 328 attributed to the immunomodulatory activity attributed to *H. persicum* [7, 8]. In addition, 329 330 major constituents of its essential oils including hexyl butyrate, octyl acetate, hexyl 2-331 methylbutanoate and hexyl isobutyrate, have been documented with anti-inflammatory 332 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 333 endowing the immunostimulatory features observed in the current study. To explore the 334 335 definitive underlying mechanism of these beneficial actions, it is essential to extract and 336 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 337 338 elicit significant immunomodulation in carp. It is highly favourable in aquaculture that H.

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

Besides their key functions as modulators of immunity, immunostimulants are 342 343 also considered as growth promoters. In the present study, fish fed with diet supplemented with H. persicum powder demonstrated enhanced performance as 344 345 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 carp at least 5 g Kg⁻¹ or higher. The growth promoting function of plant-based 348 immunostimulant had been previously shown in a carp study, where traditional Chinese 349 medicinal plants increased body weight of fish by at least 16 % [28]. It could be possible 350 351 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].

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

360	prominent in the soluble factors in the skin mucus than in the serum. The results support
361	the exploration of traditional medicinal plants as effective, sustainable and relatively
362	cheap dietary supplements capable of enhancing the immunological robustness of farmed
363	fish.
364	
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369	
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460

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 comp (% dry matter	
Dry matter	91.12
Crude protein	36.81
Crude lipid	11.33
Ash	3.50
Fiber	11.58

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

* 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 \pm SE.

				R'
	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 (<mark>g</mark>)	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*)

Authors: Seyed Hossein Hoseinifar, Fazel Zoheiri, Carlo C. Lazado

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.