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

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

Published in:
Fish and Shellfish Immunology

Link to article, DOI:
10.1016/j.fsi.2016.10.025

Publication date:
2016

Document Version
Version created as part of publication process; publisher's layout; not normally made publicly available

Citation (APA):
Hoseinifar, S. H., Zoheiri, F., & Lazado, C. C. (2016). Dietary phytoimmunostimulant Persian hogweed (Heracleum persicum) has more remarkable impacts on skin mucus than on serum in common carp (Cyprinus carpio). Fish and Shellfish Immunology, 59, 77-82. DOI: 10.1016/j.fsi.2016.10.025
Dietary phytoimmunostimulant Persian hogweed (*Heracleum persicum*) has more remarkable impacts on skin mucus than on serum in common carp (*Cyprinus carpio*).

Seyed Hossein Hoseinifar, Fazel Zoheiri, Carlo C. Lazado

PII: S1050-4648(16)30657-X
DOI: 10.1016/j.fsi.2016.10.025
Reference: YFSIM 4253

To appear in: *Fish and Shellfish Immunology*

Received Date: 20 July 2016
Revised Date: 7 October 2016
Accepted Date: 13 October 2016

Please cite this article as: Hoseinifar SH, Zoheiri F, Lazado CC, Dietary phytoimmunostimulant Persian hogweed (*Heracleum persicum*) has more remarkable impacts on skin mucus than on serum in common carp (*Cyprinus carpio*), *Fish and Shellfish Immunology* (2016), doi: 10.1016/j.fsi.2016.10.025.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Dietary phytoimmunostimulant Persian hogweed (*Heracleum persicum*) has more remarkable impacts on skin mucus than on serum in common carp (*Cyprinus carpio*).

Seyed Hossein Hoseinifar \(^a\), Fazel Zoheiri \(^a\), Carlo C. Lazado \(^b\)*

\(^a\) Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

\(^b\) Technical University of Denmark, DTU Aqua, Section for Aquaculture, The North Sea Research Centre, DK-9850 Hirtshals, Denmark

*Corresponding author: Carlo C. Lazado
Technical University of Denmark
The North Sea Research Centre
9850 Hirtshals, Denmark
Tel: + 45 35 88 32 00
Fax: + 45 35 88 32 60
E-mail: carlolazado@yahoo.com
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
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 \textit{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].

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

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>40.0</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>21.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>13.5</td>
</tr>
<tr>
<td>Gluten</td>
<td>5.5</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>6.0</td>
</tr>
<tr>
<td>Fish oil</td>
<td>6.0</td>
</tr>
<tr>
<td>Mineral premix*</td>
<td>3.0</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>2.0</td>
</tr>
<tr>
<td>Binder†</td>
<td>2.0</td>
</tr>
<tr>
<td>Anti-fungal‡</td>
<td>0.5</td>
</tr>
<tr>
<td>Antioxidant§</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Proximate composition (% dry matter basis)

<table>
<thead>
<tr>
<th>Component</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>91.12</td>
</tr>
<tr>
<td>Crude protein</td>
<td>36.81</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>11.33</td>
</tr>
<tr>
<td>Ash</td>
<td>3.50</td>
</tr>
<tr>
<td>Fiber</td>
<td>11.58</td>
</tr>
</tbody>
</table>

* Details of the premix [10].
† Amet binder ™, Mehr Taban-e- Yazd, Iran
‡ ToxiBan antifungal (Vet-A-Mix, Shenandoah, 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
were measured again. The difference in protein content was considered as the total Ig content of skin mucus.

Lysozyme activity in skin mucus was measured by a turbidimetric assay [16]. Briefly, 50 µL 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 µL 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 method described earlier [12], with minor modifications. Briefly, 100 µL of skin mucus was mixed with 100 µL 0.7 % azocasein solution (Sigma) and thereafter incubated at 30°C with constant agitation for 19 h. Then, 4.5 % trichloroacetic acid was added to stop the reaction 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 earlier seeded with 100 µL 1 N sodium hydroxide (NaOH) per well. The optical density (OD) was measured at 450 nm. Protease activity was expressed relative to a positive control (mucus replaced with trypsin solution of 5 mg mL\(^{-1}\); 100% protease activity).

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
The homogeneity of variance and normality of the data were confirmed by Levene and Kolmogorov-Smirnov tests. Then, data were subjected to one-way analysis of variance (ANOVA) followed by Duncan’s multiple range tests to determine significant difference at $P < 0.05$. SPSS statistical package version 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

3. Results

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 75 – 200% relative to control.

**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.

**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.
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](image)

**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.

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

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-acetylmuramidase 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
than this. Skin mucus proteases play a protective function against pathogenic invasion 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 \textit{H. persicum} significantly elevated the activity of skin mucus proteases where the highest increment was observed at inclusion level of 5 g Kg$^{-1}$. This noteworthy effect in mucosal proteolytic activity following dietary \textit{H. persicum} supplementation could be considered 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, they have been shown as well influencing the production of other innate immune components present in fish mucus such as complement, immunoglobulins or antibacterial peptides [23-25], hereby adding to the immunomodulatory potential of this candidate phytoimmunostimulant.

Aside from the immune molecules in the skin mucus that provide the first line of defense, soluble mediators of immunity are constantly present as well in fish circulatory system as serum factors. It is interesting to highlight that the effect of dietary \textit{H. persicum} 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 three immune parameters studied in the serum, total immunoglobulins and alternative haemolytic complement activity were distinctly enhanced in the abovementioned inclusion level. While immunoglobulins identify and neutralize pathogens, the alternative 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.*
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 also considered as growth promoters. In the present study, fish fed with diet supplemented with *H. persicum* powder demonstrated enhanced performance as indicated by higher final weight and weight gain, as well as improved SGR and FCR values. Observed changes indicate that in order to elicit significant improvement in growth 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 immunostimulant had been previously shown in a carp study, where traditional Chinese medicinal plants increased body weight of fish by at least 16 % [28]. It could be possible that the growth-promoting property of the phytoimmunostimulant may be through stimulation of appetite, modulation of the digestive enzymatic physiology, supply of beneficial vitamins and minerals or by providing substrate for microbial activity in the gut. 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
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.

Acknowledgements

The author would like to thank the research affairs of Gorgan University of Agricultural Sciences and Natural Resources (GUASNR) for financing this research. The technical assistance of the staff at the Aquaculture Laboratory of GUASNR is also appreciated.

References


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

<table>
<thead>
<tr>
<th>Ingredients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>40.0</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>21.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>13.5</td>
</tr>
<tr>
<td>Gluten</td>
<td>5.5</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>6.0</td>
</tr>
<tr>
<td>Fish oil</td>
<td>6.0</td>
</tr>
<tr>
<td>Mineral premix*</td>
<td>3.0</td>
</tr>
<tr>
<td>Vitamin premix*</td>
<td>2.0</td>
</tr>
<tr>
<td>Binder †</td>
<td>2.0</td>
</tr>
<tr>
<td>Anti fungi‡</td>
<td>0.5</td>
</tr>
<tr>
<td>Antioxidant§</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proximate composition (% dry matter basis)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>91.12</td>
</tr>
<tr>
<td>Crude protein</td>
<td>36.81</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>11.33</td>
</tr>
<tr>
<td>Ash</td>
<td>3.50</td>
</tr>
<tr>
<td>Fiber</td>
<td>11.58</td>
</tr>
</tbody>
</table>

* 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.

<table>
<thead>
<tr>
<th>Persian hogweed (g Kg⁻¹)</th>
<th>0</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final weight (g)</td>
<td>50.24 ± 3.17a</td>
<td>53.23 ± 1.02ab</td>
<td>55.41 ± 1.06b</td>
<td>55.93 ± 2.01b</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>26.86 ± 3.31a</td>
<td>29.73 ± 1.28ab</td>
<td>31.83 ± 1.07b</td>
<td>32.55 ± 1.90b</td>
</tr>
<tr>
<td>SGR</td>
<td>1.55 ± 0.13a</td>
<td>1.66 ± 0.06ab</td>
<td>1.74 ± 0.04b</td>
<td>1.78 ± 0.06b</td>
</tr>
<tr>
<td>FCR</td>
<td>2.37 ± 0.11a</td>
<td>2.15 ± 0.10ab</td>
<td>1.96 ± 0.05b</td>
<td>1.90 ± 0.08b</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

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.