# Effects of *Aloe vera* crude extract on growth performance and some hemato-immunological indices of *Oncorhynchus mykiss* in farm scale

## Alishahi, M.\*, Tulaby Dezfuly, Z., Mesbah, M., Mohammadian, T.

Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

### Key words:

*Aloe vera*, growth indices, hematological parameters, immune response, Rainbow trout

### Correspondence

Alishahi, M. Department of Clinical Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran Tel: +98(61) 33330072 Fax: +98(61) 33360807 Email: alishahim@scu.ac.ir

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# Introduction

With the worldwide growth of fish production and popularity of intensive cultivation systems, fish are subjected to many diseases which lead to considerable losses

### **Abstract:**

BACKGROUND: The immunostimulating effect of Aloe vera in mammals has been documented, but few works were done on effect of A. vera on fish health and immune responses. OBJECTIVES: In this study the effect of oral administration of A. vera on growth indices, hematological parameters and immune responses of rainbow trout were investigated. METHODS: One thousand five hundred rainbow trout fingerlings  $(20 \pm 2 \text{ g}, \text{Mean} \pm \text{SD})$  were divided into five groups, each in triplicate, in farm scale. Group 1 were adopted as control and fed with non-supplemented feed, groups 2 to 5 were fed with diet supplemented by 0.05%, 0.1%, 0.2% and 0.5% A. vera extract respectivly for 60 days. Growth indices (SGR, FCR, PWG, FER, PER and CF) were calculated in day 30 and 60. Blood samples were taken in day 60 and hematological parameters including: PCV, Hb, RBC, WBC, MCH, MCV, MCHC as well as immunological parameters including: Lysozyme and serum bactericidal activity, serum total protein and globulin were compared among the groups. **RESULTS:** Results showed that all calculated growth indices (except CF) and all mentioned immunological parameters were significantly increased in fish fed with 0.1% and 0.2% A. vera supplemented food (G3 and G4) compared to control group (p<0.05). Hematological parameters, HB, RBC, WBC and PCV showed a significant enhancement in G3 and G4 compared to control (p<0.05), but MCV, MCH and MCHC showed no significant changes (p>0.05). CONCLUSIONS: It can be concluded that oral administration of 0.1% and 0.2% A. vera crud extract in food (G3 and G4) can improve growth indices, stimulate non-specific immune responses and affect some hematological parameters positively in rainbow trout.

> and decrease in fish production (Phillip et al., 2006). The increasing pressure on the aquaculture to reduce or eliminate feed antibiotics as disease treatment or growth enhancers has initiated new research to find safe and efficient natural alternatives. This

new generation of feed additives includes natural sources, particularly herbs and their essential oils and extracts (Brenes and Roura, 2010).

Immunotherapy is an approach that has been actively investigated in recent years as a method for decreasing the economical loss of diseases occurrence and increasing the overall profit of aquaculture (Chi et al., 2016; Guardiola et al., 2016). Interest in the use of immunostimulants as an alternative to the drugs, chemicals and antibiotics currently being used for fish diseases is growing because immunostimulants are inexpensive, environmentally friendly, more available in different parts of the world and enhance the innate (or non-specific) immune response which has a more important role in fish immunity (Galeotti, 1998; Sakai, 1999; Guardiola et al., 2016). So the use of immunostimulants for prevention of diseases in fish is considered an alternative and promising area (Sakai, 1999). There is a growing interest in the use of medicinal herbs as immune stimulants in aquaculture (Brenes and Roura, 2010) and the immunostimulating effects of herbal medicines in various fish species has been reported (Pugh et al., 2001). Abdy et al. (2017) showed that in comparison with traditional adjuvants such as Freund's adjuvant, Aloe vera gel could be used as a natural adjuvant with similar or even greater positive effects on vaccination of common carp. Herbal additives contain substances which also increase appetite and digestion (Barreto et al., 2008). Many studies have been published that confirm that the addition of plants or their extracts in the diets has a beneficial effect to improve growth parameters and protect from diseases in aquaculture (Sasmal et al., 2005; Johnson and Banerji, 2007, Sudagar

et al., 2010; Zanuzzo et al., 2017).

*Aloe vera* inner gel consists primarily of water and polysaccharides (pectin, cellulose, hemi cellulose, glucomannan, acemannan and mannose derivatives). Acemannan is considered as the main functional component of *Aloe vera* and is composed of a long chain of acetylated mannose (Lee et al., 2001). The physiological activity of *Aloe vera*'s polysaccharides has been widely reported. (Pugh, 2001; Tan and Vanitha, 2004). The refined polysaccharide has been shown to act as an immunostimulant, displaying adjuvant activity as well as stimulate hematopoiesis (Abdy et al., 2017).

Zanuzzo et al. (2017) found that dietary *A. vera* for 10 days prior to transport stress and infection with heat killed *Aeromonas hydrophila* either improved or prevented loss of innate immune activity in pacu (*Pi-aractus mesopotamicus*) after stressful handling and a bacterial infection. The results of research done by Mesbah & Mohammadian (2016) have demonstrated that the oral administration of *Aloe vera* (specifically 0.2%) in shirbot (*Barbus grypus*) compared with Echinacea can enhance some of the non-specific immune responses.

In another study the combination of methanolic extracts of herbal mix composed of *V. trifolia*, *S. crispus* and *A. vera* extracts in daily diet significantly improved growth of Oreochromis sp. juveniles and also reduced the mortalities post challenge with *S. agalactiae* (Manaf et al., 2016).

Although the immune modulatory potentials of *Aloe vera* in mammals, particularly in human and some other species have been well confirmed (Tan and Vanitha, 2004), few works were done on the effect of *Aloe vera* on fish (Kim et al., 1999; Alishahi et al., 2010). Iran has one of the highest rates

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of cold water fish culture in Asia and the world since 2005 and Rainbow trout is the main cultured species in Iran (FAO, 2012). So in this study the effects of Aleo vera crude extract on some growth indices, he-matological and immunological parameters of *Oncorhynchus mykiss* were investigated.

# **Materials and Methods**

**Fish:** One thousand five hundred rainbow trout fingerlings with average body weight of  $20 \pm 2$  g were obtained from a rainbow trout hatchary in Chaharmahl bakhtiyari province, Iran. The experiment was done in in Cheshmeh Sarab Rainbow trout farm in the suburb of Koohrang, Chaharmahl bakhtiyari province. In order for acclimatization of fish, they were kept in farm condition prior to the beginning of the experiment for 30 days. Water quality factors were recorded during the experiment as: temperature 11±1 °C; Dissolved oxygen 8-9.5 ppm; pH 7.9-8.5, NH3 < 0.01 mg/L, NO2 < 0.1 mg/L.

Experimental Food preparation: The commercial Rainbow trout food (Faradaneh Co, Iran) (FFT1|:40% protein, 12% lipid, 3% fiber as, 6% moisture, 7% Ash) as a basal diet and Aloe vera extract (Baridj Essence Co, Iran) were mixed. For this purpose, initially granulated food was made into paste by adding distilled water to it, then 0.05, 0.1, 0.2 and 0.5% (w/w) Aloe vera extract was added to food and homogenized with electric mixture. Finally food was pelleted by means of a special meat grinder. This method was used for Control food without supplementation with Aloe vera. Prepared experimental foods were packed in nylon bags, labeled and stored at 4 °C until use.

**Experimental design:** Fishes were randomly divided into 5 groups (each in

triplicate) and transferred into 15 pools ( $1.2 \times 10m$ ), the compositions of the feeds were as follows: Group 1: 0% *Aloe vera* as control group, Group 2: 0.05% *Aloe vera*, Group 3: 0.1% *Aloe vera*, Group 4: 0.2% *Aloe vera*, Group 5: 0.5% *Aloe vera*.

Assessment of growth performance: Percentage Weight Gain (PWG), Specific Growth Ratio (SGR), Food Conversation Ratio (FCR), Food Efficiency Rate (FER), Protein Efficiency Ratio (PER) and Condition Factor (CF) were calculated according to the following equations in day 30 and 60:

PWG (g/fish) = [Average final weight -Average initial weight] / initial weight

SGR (%/day) = [final body weight - initial body weight]  $\times$  100 / experimental period (day).

FCR = Food intake / weight gain.

FER = Body weight gain / Food intake.

PER = Body weight gain/ Total protein intake

 $CF = [Body weight / (Total length) 3] \times 100$ 

(All of the fish weights in top equations were calculated in gram unit).

**Blood and serum sampling:** At the end of experimental period, after 2 days off feeding, 20 fish from each group for biometric assay, 5 fish for hematological assay and 5 fish for immunological assay were collected from each group. Blood samples were taken from caudal vein after anesthetizing fish with MS-222 (FINQUEL, USA, Washington) by sterile syringe. Hematological parameters were measured after sampling on the same day. Remained blood samples were centrifuged (4000 rpm for 15 min), sera separated and stored at -20 °C until the desired tests were done.

**Hematological assays:** Hemoglobin (Hb) measurement was determined by the

cianometa-haemoglobin method. Packed cell volume (PCV) was determined by centrifuging micro haematocrit in 10000g for 10 min, according to the method that was used for mammals and birds (Feldman et al., 2000). Total Red Blood Cell was calculated by Neubauer haemocytometer after diluting in Natt–Herrick solution (Thrall, 2004). Mean Corpuscular Volume (MCV), Mean Corpuscular Haematocrit (MCH) and Mean Corpuscular Haematocrit Concentration (MCHC) were calculated by using the standard formulas as follow (Thrall, 2004):

MCV ( $\mu$ m<sup>3</sup> cell<sup>-1</sup>) = (Packed cell volume as percentage/RBC in millions cell mm<sup>3</sup>)× 10

MCH (pg cell<sup>-1</sup>) = (Hb in g 100 ml<sup>-1</sup>/ RBC in millions cell mm<sup>3</sup>) $\times$ 10

MCHC (g 100 ml<sup>-1</sup>Hct) = (Hb in g100 mL<sup>-1</sup>/packed cell volume as percentage)  $\times 100$ 

The blood sample was diluted with Natt– Herrick solution to determine Total White Blood Cell (TWBC) by using Neubauer haemocytometer chamber, then the Total WBC was calculated by this formula (Thrall 2004):

TWBC = (total white cell counted in 9 big square + 10%)  $\times 200$ 

For Differential count of leukocytes, the blood smear on glass microscope slides was stained with Gimsa and one hundred WBC were calculated and the percentage of different types of leucocytes was determined following the method of Schaperclaus (Schaperclaus et al., 1991).

**Immunological analysis (Serum lysozyme activity):** The lysozyme activity was measured using photoelectric colorimeter equipped with attachment for turbidity measurement. A series of dilution was prepared by diluting the standard lysozyme from hen

egg-white (Sigma) and mixed with Micrococcus lysodeikticus (Schroeter) (Sigma) suspension for establishing the calibration curve. Ten µl of standard solution or serum were added to 200 µl of micrococcus suspension (35 mg of Micrococcus dry powder/95 ml of 1/15 M phosphate buffer + 5.0 ml of 1M NaCl solution). The changes in the extinction were measured at 546 nm by measuring the extinction immediately after adding the solution which contained the lysozyme (start of the reaction) and after a 20 min incubation of the preparation under investigation at 40 °C (end of the reaction). The lysozyme content is determined on the basis of the calibration curve and the extinction measured (Thrall, 2004).

Serum bactericidal activity (SBA): Serum bactericidal activity was measured by the method described previously by Kajita et al. (1990) with slight modification. A. hydrophila AH04 (live, washed cells) was suspended in the 0.1% gelatin-veronal buffer (GVBC2) (pH 7.5, containing 0.5 mM ml-1 Mg2+ and 0.15 mM ml<sup>-1</sup> Ca2+) to make a concentration of  $1 \times 10^5$  cfu ml<sup>-1</sup>. Serum was diluted at a ratio of 3 part buffer and 1 part serum v: v, then bacterial suspension was mixed with diluted serum and incubated for 90 min at 25 °C with shaking. 5 µl of this mixture on TSA plates in triplicate was incubated at 25 °C for 24 h. The number of viable bacteria was calculated by counting the colonies and results were reported in the form of calculated bacteria colonies.

Serum total protein and globulin measurements: Total protein and albumin concentrations were determined (Zist Shimi kit, Iran) according to Nayak et al. (2008). The albumin content was estimated spectrophotometrically using a standard kit (Glaxo, India). The globulin content was estimated by

Table 1. Results of growth indices in different groups at 30 and 60 days of experiment. (Group 1: control and groups 2 to 5 were fed with diet supplemented by 0.05%, 0.1%, 0.2% and 0.5% *A. vera* extract respectively). Significant differences with control at level of 0.05 are marked by \* sign.

	Group	PWG	SGR	FCR	FER	PER	CF
Day 30	1	71.36±8.56	0.93±0.08	1.55±0.14	64.22±9.63	1.76±0.21	1.52±0.16
	2	78.52±9	1±0.08	1.47±0.1	62.74±5	1.86±0.28	1.6±0.18
	3	93.78±13.32*	1.15±0.1*	1.22±0.13*	70.86±8.5*	2.24±0.33*	$1.42 \pm 0.14$
	4	100.16±14.22*	1.2±0.12*	1.14±0.12*	75.48±11.32*	2.41±0.29*	1.43±0.12
	5	83.77±11.9	1.06±0.1	1.35±0.15	61.87±9.28	2.02±0.24	1.48±0.13
Day 60	1	162.01±19.44	$0.84{\pm}0.07$	1.77±0.16	56.44±8.46	1.61±0.19	1.77±0.2
	2	187.07±22.44*	$0.92{\pm}0.08$	1.6±0.15	62.74±9.41	1.79±0.21	1.75±0.2
	3	210±25.2*	$0.98 \pm 0.04*$	1.41±0.09*	70.86±5*	2.02±0.11*	$1.62 \pm 0.14$
	4	222±31.52*	1.01±0.1*	1.32±0.13*	75.48±12.83*	2.16±0.34*	1.55±0.19
	5	$181.28 \pm 20$	0.9±0.03	1.61±0.08	61.87±9	1.76±0.13	1.55±0.16

Table 2. Effect of different concentration of *A. vera* on hematological parameters. (grouping is the same as Table 1). Significant differences with control at level of 0.05 are marked by \* sign.

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Group	PCV (%)	HB	WBC count	RBC count	MCV (fl)	MCH (%)	MCHC (%)
			$(\times 10^3 \text{ cell/mm3})$	$(\times 10^6 \text{ cell/mm3})$			
1	32.42±4.6	4.47±1.4	12.23±2.05	1.21±0.08	290.55±44.50	40.72±8.65	12.69±3.92
2	36.00±5.43	$4.68 \pm 0.85$	12.49±1.22	1.26±0.15	290.35±53.57	37.99±8.16	13.11±2.09
3	43.17±6.98*	5.50±1.26	13.68±1.88	1.26±0.12	322.74±46.32	43.95±9.93	12.94±2.85
4	47.33±4.48*	6.41±1.56*	15.15±1.65*	1.43±0.10*	306.18±54.48	44.75±11.13	13.48±2.77
5	39.50±7.67	5.08±1.53	13.32±2.27	1.29±0.17	293.78±67.73	37.66±12.45	12.75±2.93

subtracting the albumin content from total protein content.

**Statistical analysis:** Completely Randomized design was used in this study. For statistical analysis of data, SPSS version 16 software was used. Growth indices, haematological and immune parameters were analyzed using the one way ANOVA to determine the differences between the means and Duncan multiple range test was used to test the significance among the means, p<0.05 was accepted as significant.

# Results

**Growth indices:** Results of growth indices are shown in Table 1. Percentage Weight Gain showed a significant difference between groups (p<0.05). Group fed with 0.1 and 0.2% *A. vera* showed a significant difference with other groups in the  $30^{\text{th}}$  day and

in the end of period, Group fed with 0.05, 0.1 and 0.2% *A. vera* had significant increase (p<0.05). Other growth indices except CF were significantly improved in Groups fed with 0.1 and 0.2% *A. vera* in both phases of experiment (day 30 and 60) (p<0.05). Condition Factor did not show any significant change among different groups over the experiment period ( $p \ge 0.05$ ).

**Hematological parameters:** The results of hematological parameters are shown in Table 2. Packed cell Volume (PCV) increased significantly (p<0.05) in Group 3 and Group 4. In Hb measurement, white blood cell count and red blood cell count showed a significant difference in group fed with 0.2% *A. vera* supplemented feed. MCV, MCH and MCHC showed no significant differences in *A. vera* treated groups.

Immunological parameters (Lysozyme activity): The lysozyme activity in all

Group	Lysozyme activity (U/ml/min)	Bactericidal activity(cfu/plate)	Total protein (g/dl)	Total globulin (g/dl)
1	127.23±9.38	181.33±19.4	5.01±0.41	2.12±0.31
2	122.87±7.38	176.26±12.34	4.95±0.54	2.05±0.35
3	140.54±10.3*	171.5±14.41	5.85±0.62*	2.45±0.19
4	142.33±8.48*	156.63±15.6*	6.11±0.64*	3.13±0.37*
5	131.5±7.67	177.08±16.55	5.1±0.57	2.16±0.12

Table 3. Immunological parameters in experimental groups. (grouping is the same as Table 1). Significant differences with control at level of 0.05 are marked by \* sign.

groups fed with *Aloe vera* is shown in Table 3. Group 3 and 4 showed a significant marked increase in lysozyme activity compared with control group.

Serum bactericidal activity: The result of serum bactericidal activity is presented in Table 3. Inactivated bacterial colony percentages enhanced significantly in group 4 (p<0.05). The other group showed increase during experiment but the differences were not statically significant (p>0.05).

Total protein and Total globulin: The levels of total protein and total globulin showed significant increase in 0.1% and 0.2% *A. vera* enriched diet compared to control group. No significant differences were seen in 0.05% and 0.5% *A. vera* enriched diet and control group (Table 3). Serum albomin level was not affected by different level of *Aloe vera* (p>0.05).

# Discussion

Since rainbow trout is the only cold water species with high economic value cultured in the Iran aquaculture industry, attempts to enhance the immune response of the fish against various diseases, especially unknown diseases is increasing. Due to various reasons, specifically the hygienic, environmental and economic disadvantages of antibiotics, lack of efficient vaccine against different pathogens and more important role of non-specific immunity than specific immunity in fish, recently a strong tendency for using the immune stimulants especially those with herbal origin has been established in the aquatic animals (Iwama, 1996; Sakai, 1999; Alishahi, 2010 and 2012).

In this study the effects of crude extract of A. vera on growth, immune and hematologic factors in Rainbow trout were investigated and the results showed that groups fed with food supplemented with 0.1 and 0.2% A. vera had positive effect on growth performance indices. The beneficial effects of A. vera extract seems to be dose dependent, as shown in our results, increasing the A. vera extract in diet up to a specific concentration (0.2%), causes the Food Conversion Ratio (FCR) to decrease, but increasing the extract in diet up to 0.5% causes declining SGR and PER and increasing FCR. Concentration of 0.5% did not induce any significant changes and it is probably because of the possible effects of A. vera on taste and appearance of diet.

No change in condition factor of fish in different groups indicates that no change in obesity has occurred. In other words, while total body weight has increased in groups 3 and 4 fishes were not obese. Effects of Immune-stimulants in the improvement of fish growth factors have been reported after administration of beta-glucan and bacterial LPS (Selvaraj et al., 2006), chitosan (Gopalakannan et al., 2006) Levamisole (Alvarez et al., 2006) and Ergosan (Gioacchini et al., 2008). Chi et al. (2014) reported the growth stimulation capacity of a medicinal plant, ryopteris crassirhizoma (a fern species in the genus Dryopteris), as a food additive in grass carp. Alishahi et al. (2012) reported the positive effect of Echinacea purpurea on the growth indices of rainbow trout. In fact, according to many reports, improvement in growth factors after oral administration of *A. vera* can be because of enhancement of immune response of fish (Chi et al., 2014).

Despite the increase in most of the blood factors in group fed on diet with 0.1% A. vera, only PCV increase was significant (p< 0.05). This result shows no effect of A. vera on the size and content of hemoglobin in red blood cells. Unlike warm-blooded animals, in cold-blooded animals, especially fish, blood factors are considerably affected by various environmental and external parameters such as stress, temperature, season, nutrition, etc. Thus there is not a completely fixed pattern for blood factors or immune status in fish (Iwama, 1996). But based on the results and by comparison of results of treatments with control group it can be claimed that A. vera extract can generally stimulate the hematopoiesis, or reduce the destruction of the blood cells by unknown mechanism. Different results about effects of immune stimulant on fish hematological parameters have been reported previously. Some researchers reported immune stimulant function on fish hematological parameters to be ineffective (Sakai, 1999); whereas conversely, the others reported changes in hematological parameters with the use of some immune stimulants such as vitamin C )Kajita, 1990; Marian, 2004). In a previous study, oral administration of A. vera gel in common carp led to increase in hematopoiesis (Alishahi and Abdy, 2013).

Increasing white blood cell counts can be caused by non-specific immune stimulation in fish. Since white blood cells, particularly Band T lymphocytes have a major role in the fish immune system, changing the number of these cells affected by immune stimulants seems reasonable. Many non-specific humoral immune components of fish are released by white blood cells. Increasing humoral factors were influenced by enhancing leukocytes. Increasing number of white blood cells in cases of vaccines administration and immunostimulants usage has been reported (Kajita et al., 1990, Marian, 2004; Sakai, 1999). Selvaraj et al. (2005) reported similar results after administration of ß-glucan in common carp. Increase in leukocyte numbers by using immunostimulants has been seen in other researches in various fishes (Khaksary Mahabady, 2006). Similar results were reported in tilapia, and many hematological indices including WBC count were increased under the effect of dietary A. vera (Gabriel et al., 2015). In contrast, although Dotta et al. (2014) reported an increase in hematocrit of Nile tilapia fed with A. vera, no significant increase was observed in WBC count.

Lysozyme is a valuable fish protein and one of the most important components of non-specific immunity. This enzyme destroys peptide glycan layer of gram positive bacteria and activates complement system and phagocytes (Sakai, 1999).

In this study, serum lysozyme activity levels in fish fed on concentrations of 0.1 and 0.2% *A. vera* showed a significant increase compared to control group. It seems that increasing concentration of lysozyme in blood serum in fish is related to white cell stimulation because the origin of lysozyme is leukocytes (Alvarez, 2006). Increasing lysozyme activity after administration of immune stimulants, vaccines and some probiotics in fish has been reported ) Swain et al., 2006; Yuan et al., 2007). The lysozyme activity levels in Carassius auratus (Chen et al., 2003), yellow croaker (Jian and Wu, 2003) and common carp (Jian and Wu, 2004) have been enhanced after administration of herbal stimulant. Alishahi et al. (2010) reported that oral administration of *A. vera* extract in the level of 0.5% significantly increases serum Lysozyme activity in common carp.

Lower number of counted live bacteria in groups fed with 0.2% A. vera is than the control group means less survival of the bacteria in vitro and shows higher serum bactericidal activity. There are some similar studies that indicate the increasing serum bactericidal activity after administration of immune stimulant that matches the results of present study. In common carp enhanced serum bactericidal activity after oral administration of A. vera extract was reported in a study conducted by Alishahi et al. (2010), in addition Divyagnaneswari et al. (2007) in tilapia, Misra et al. (2006) in Indian major carp and Katija et al. (1990) in rainbow trout reported increase of serum bactericidal activity after administration of biological immunostimulants.

Serum total protein and globulin are a good indicator for determining the activation of immune system (Siwicki et al., 1994). The levels of total protein and Ig increased in 0.1% and 0.2% *A. vera* enriched diet compared to control group. Some herbal immunostimulants were reported to increase total protein as well as total globulin in fish (Sukumaran et al., 2016), in contrast, there are some reports which indicate lack of any influence of immunostimulant on serum proteins (Ispir and Mustafa 2005; Misra et al., 2006). The increase in serum pro-

tein content might be related to an increase of WBC and proteins like serum lysozyme, complement factors and bactericidal peptides (Misra et al., 2006).

As a general conclusion, based on these results it can be argued that the oral administration of 0.1- 0.2% concentration of the crude extract of *A. vera* improved investigated growth factors, stimulated non-specific immune and had a good effect on hematological factors.

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### References

- Abdy, E., Alishahi, M., Tollabi, M., Ghorbanpour, M., Mohammadian, T. (2017) Comparative effects of *Aloe vera* gel and Freund's adjuvant in vaccination of common carp (*Cyprinus carpio* L.) against *Aeromonas hydrophila*. Aquacult Int. 25: 727–742.
- Alishahi, M., Abdy, E. (2013) Effects of different levels of *Aloe vera* L. extract on growth performance, hemato-immunological indices of *Cyprinus carpio* L. Iran J Vet Sci Technol 5: 33–44.
- Alishahi, M., Ghorbanpour, M., Peyghan, R. (2012) Effects of Viscum album Linnaeus and Nigella sativa Linnaeus extracts on Some immune responses of Common carp Cyprinus carpio Linnaeus. Asian Fish Sci. 25: 15-28
- Alishahi, M., Ranjbar, M.M., Ghorbanpour, M., Peyghan, R., Mesbah, M., Razijalali, M. (2010) Effects of dietary *Aloe vera* on specific and nonspecific immunity of Common carp (*Cyprinus carpio*). J Vet Res. 4: 85-91.
- Alvarez-pellitero, P., Stija Bobadilla, A., Bermuolez, R., Quiroga, M.I. (2006) Levamisole

activates several innate immune factors in *Scophthalmus moximus* (Teleostei). Int J Immunopathol Pharmacol. 19: 727-738.

- Barreto, M.S.R., Menten, J.F.M., Racanicci, A.M.C., Pereira, P.W.Z., Rizzo, P.V. (2008)
  Plant extracts used as growth promoters in broilers. Braz J Poultry Sci. 10: 109-115.
- Brenes, A., Roura, E. (2010) Essential oils in poultry nutrition: Main effects and modes of action. Anim Feed Sci Tech. 158: 1-14.
- Chen, X., Z. Wu, Z., Yin. J., Li, L. (2003) Effects of four species of herbs on immune function of *Carassius auratus gibelio*. J Fish Sci China. 10: 36–40.
- Chi, C., Giri, S.S., Jun, J.W., Kim, H.J., Yun, S., Kim, S.G., Kim, G., Chang, S.P. (2016) Immunomodulatory effects of a bioactive compound isolated from *Dryopteris crassirhizoma* on the Grass Carp *Ctenopharyngodon idella*. J Immunol Res. 1: 1–10.
- Divyagnaneswari, M.D., Christybapita, A., Dinakaran, R. (2007) Enhancement of nonspecific immunity and disease resistance in *Oreochromis mossambicus* by *Solanu mtrilobatum* leaf fractions. Fish Shellfish Immunol. 23: 249-259.
- Dotta, G., de Andrade, J.I., Tavares Gonc, alves,
  E.L., Brum, A., Mattos, J.J., Maraschin, M.,
  Martins, M.L. (2014) Leukocyte phagocytosis and lysozyme activity in Nile tilapia fed
  supplemented diet with natural extracts of
  propolis and Aloe barbadensis. Fish Shellfish
  Immunol. 39: 280–284.
- Feldman, B.F., Zinkl, J.G., Jain, N.C. (2000)
   Schalm's Veterinary Hematology. (5<sup>th</sup> ed.)
   Lippincott Williams & Wilkins. London, UK.
- Food and Agriculture Organization, FAO. (2012) FAO Statitical Yearbooks. FAO Pub.
- Gabriel, N.N., Qiang, J., He, J., Ma, X.Y., Kpundeh, M.D., Xu, P. (2015) Dietary *Aloe vera* supplementation on growth performance, some haemato-biochemical parameters and

disease resistance against *Streptococcus iniae* in tilapia (GIFT). Fish Shellfish Immunol. 44: 504–514.

- Galeotti, M. (1998) Some aspects of the application of immunostimulants and a critical review of methods for their evaluation. J Appl Ichthyol. 14: 189–199.
- Gioacchini, G., Smith, P., Carnevali, O. (2008) Effects of Ergosan on the expression of cytokine genes in the liver of juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to enteric red mouth vaccine. Vet Immunol Immunopathol. 123: 215–222
- Gopalakannan, A., Arul, V. (2006) Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. Aquaculture. 255: 179–187.
- Guardiola, F.A., Porcino, C., Cerezuela, R., Cuesta, A., Faggio, C., Esteban, M.A. (2016)
  Impact of date palm fruits extracts and probiotic enriched diet on antioxidant status, innate immune response and immune-related gene expression of European seabass (*Dicentrarchus labra*), Fish Shellfish Immunol. 52: 298–308.
- Ispir, U., Mustafa, D.M. (2005) A Study on the Effects of Levamisole on the Immune System of rainbow trout (*Oncorhynchus mykiss*, Walbaum). Turk J Vet Anim Sci. 29: 1169-1176.
- Iwama, G., Nakanishi, T. (1996) The Fish Immune System. Chapter 3: Innate Immunity in Fish. Academic Press, London, UK.
- Johnson, C., Banerji, A. (2007) Influence of extract isolated from the plant *Sesuviumportulacastrumon* Growth and Metabolism in Freshwater Teleost, *Labeo rohita* (Rohu). Fishery Tech. 44: 229-234.
- Kajita, Y., Sakai, M., Atsuta, S., Kobayash, M.(1990) The immunonodulatory effects of levamisole on rainbow trout, *Oncorhynchus*

mykiss. Fish Pathol. 25: 93-98.

- Khaksary Mahabady, M., Ranjbar, R., Arzi, A., Papahn, A.A., Najafzadeh, H. (2006) A comparison study of effects of Echinacea extract and levamisole on phenytoin-induced cleft palate in mice. Regul Toxicol Pharmacol. 46: 163-166.
- Kim, K.H., Hwang, Y.J., C. Bai, S. (1999) Resistance to Vibrio alginolyticus in juvenile rockfish (Sebastes schlegeli) fed diets containing different doses of aloe. Aquaculture. 180: 13–21.
- Lee, J.K., Lee, M.K., Yun, Y.P., Kim, Y., Kim, J.S., Kim, Y.S., Kim, K., Han, S.S., Lee, C.K. (2001) Acemannan purified from *Aloe vera* induces phenotypic and functional maturation of immature dendritic cells. Int Immunopharmacol. 1: 1275–1284.
- Manaf, S.R., Daud, H.M., Alimon, A.R., Mustapha, N.M., Hamdan, R.H. (2016) The Effects of *Vitex trifolia*, *Strobilanthes crispus* and *Aloe vera* herbal-mixed dietary supplementation on growth performance and disease resistance in red hybrid Tilapia (*Oreochromis* sp.), J Aquac Res Dev. 7(425), 2.
- Marian, M.P. (2004) Growth and immune response of juvenile greasy groupers (Epinephelus tauvina) fed with herbal antibacterial active principle supplemented diets against Vibrio harveyi infections. Aquaculture. 237: 9-20.
- Mesbah, M., Mohammadian, T. (2016) Effects of dietary *Aloe vera* and Echinacea on some nonspecific immunity in shirbot (*Barbus grypus*), Iran J Aquat Anim Health. 2: 24-36.
- Misra, C.K., Das, B.K., Mukherjee, S.C., Meher, P.K. (2006) The immunomodulatory effects of tuftsin on the non-specific immune system of Indian Major carp, Labeo rohita. Fish Shellfish Immunol. 20: 728-738.
- Nayak, S.K., Swain, P., Nanda, P.K., Dash, S., Shukla, S., Meher, P.K., Maiti, N.K. (2008)

Effect of endotoxin on the immunity of Indian major carp, *Labeo rohita*. Fish Shellfish Immunol. 24: 394–399.

- Phillip, H.K., Evans, J. J., Shoemaker, A.C., Pasnik, D.J. (2006) A vaccination and challenge model using calcein marked fish. Fish Shellfish Immunol. 20: 20-28.
- Pugh, N., Ross, S.A., ElSohly, M.A., Pasco, D.S. (2001) Characterization of aloeride, a new highmolecular-weight polysaccharide from with potent immunostimulatory activity. J. Agric Food Chem. 49: 1030-1034.
- Sakai, M. (1999) Current research status of fish immunostimulants. Aquaculture. 172: 63-92.
- Sasmal, D., Babu, C.S., Abraham, T.J. (2005) Effect of garlic (*Allium sativum*) extract on the growth and disease resistance of Carassius auratus. Indian J Fish. 52: 207-214.
- Schaperclaus, W., Kulow, H., Schreckenbach, K. (1991) Hematological and serological technique. In: Fish Disease, Oxonian Press. Kothekar, V.S. (ed.). New Delhi, India. p. 71-108.
- Selvaraj, V., Sampath, K., Sekar, V. (2005) Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*. Fish Shellfish Immunol. 19: 293-306.
- Selvaraj, V., Sampath, K., Sekar, V. (2006) Adjuvant and immunostimulatory effects of  $\beta$ -glucan administration in combination with lipopolysaccharide enhances survival and some immune parameters in carp challenged with *Aeromonas hydrophila*. Vet Immunol Immunopathol. 114: 15–24
- Sudagar, M., Hosseinpoor, Z., Hosseini, A. (2010) The use of citric acid as attractant in diet of grand sturgeon (*Huso huso*) fry and its effects on growing factors and survival rate. AACL Bioflux. 3: 311-316.
- Sukumaran, V., Park, S.C., Giri, S.S. (2016) Role

of dietary ginger Zingiber officinale in improving growth performances and immune functions of *Labeo rohita* fingerlings, Fish Shellfish Immunol. 57: 362–370.

- Swain, P., Dash, S., Sahoo, P.K., Routray, P., Sahoo, S.K., Gupta, S.D., Meher, P.K., Sarangi, N. (2006) Non-specific immune parameters of brood Indian major carp *Labeo rohita* and their seasonal variations. Fish Shellfish Immunol. 22: 38-43.
- Tan, B.K., Vanitha, J. (2004) Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: a review. Curr Med Chem. 11: 1423–1430.
- Thrall, M.A. (2004) Veterinary Hematology and Clinical Chemistry. Lippincott Williams & Wilkins, USA. p. 241, 277-288, 402.
- Yuan, C., Li, D., Chen, W., Sun, F., Wu, G., Gon, Y., Tang, J., Shen, M., Han, X. (2007) Administration of a herbal immunoregulation mixture enhances some immune parameters in carp (*Cyprinus carpio*). Physiol Biochem. 33: 93- 101.
- Zanuzzo, F.S., Sabioni, R.E., Montoya, L.N.F., Favero, G., Urbinati, E.C. (2017) Aloe vera enhances the innate immune response of pacu (*Piaractus mesopotamicus*) after transport stress and combined heat killed Aeromonas hydrophila infection, Fish Shellfish Immunol. 65: 198-205.

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اثر عصاره خام الوئه ورا بر شاخصهای رشد و برخی شاخصهای خونی ایمنی قزلآلای رنگین کمان (Oncorhynchus mykiss) در مقیاس مزرعه

مجتبی علیشاهی<sup>•</sup> زهرا طولابی دزفولی مهرزاد مصباح تکاور محمدیان گروه علوم درمانگاهی، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران (دریافت مقاله: ۲۳ خرداد ماه ۱۳۹۶، پذیرش نهایی: ۱۳ شهریور ماه ۱۳۹۶)

*چکید*ہ

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\*) نویسنده مسؤول: تلفن: ۲۳۳۳۰۰۷۲ (۹۱) ۳۳۳۶۰۸۰۷ نمابر: ۳۳۳۶۰۸۰۷ (۹۱) ۳۳۳۶۰۸۰۷ Email: alishahim@scu.ac.ir