Bulgarian Journal of Veterinary Medicine, 2018, **21**, No 3, 292–300
ISSN 1311-1477; DOI: 10.15547/bjvm.1086

Original article

SKIN NON-SPECIFIC IMMUNE PARAMETERS IN PALOMINO RAINBOW TROUT

S. SOLTANIAN & A. GHOLAMHOSSEINI

Aquatic Animal Health & Diseases Department, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Summary

Soltanian, S. & A. Gholamhosseini, 2018. Skin non-specific immune parameters in Palomino rainbow trout. *Bulg. J. Vet. Med.,* **21**, No 3, 292–300.

Fish epidermal mucus and its components provide the first line of defense against pathogens. Little is known about mechanisms of immune defense at the mucus level in yellow phenotypes of rainbow trout (*Oncorhynchus mykiss*). The current study was undertaken to evaluate some mucosal immune parameters in the skin of a yellow phenotype, the Palomino variety, in comparison to the normally pigmented rainbow trout. Fish mucus was sampled for quantification of lysozyme, alkaline phosphatase, protease, esterase, total protein and antimicrobial activity. Both varieties were farmed together in the same ponds. Compared with wild coloured trout, Palomino individuals exhibited significantly higher total protein, lysozyme, protease, alkaline phosphatase, esterase and antimicrobial activities in mucus. The superior immune capabilities in Palomino trouts reported here may correlate positively with diseases resistance, hence; favour a chance for selective breeding programmes and genetic manipulation.

Key words: antimicrobial activity, epidermal mucus enzymes, lysozyme, *Oncorhynchus mykiss,* Palomino, skin mucosal immunity

INTRODUCTION

Colour mutations are a common phenomen in fish species. In the rainbow trout *Oncorhynchus mykiss* (Walbaum), the atypical body colour variants are classified into two main groups: the blue phenotypes, such as iridescent metallic blue and cobalt blue (Blanc *et al.*, 2006), and the yellow phenotypes, such as Palomino and albino (Dobosz *et al.*, 1999; 2000).

Yellow colour (allele a) is caused by absence of the dominant allele A controlling colour. Among the yellow fish (aa) the second gene locus allele B controls Palomino and black eye colour. Albino and red eye colour (allele b) is caused by the absence of the dominant allele B controlling colour development (Dobosz, 2007).

Recently, it was found that the genes controlling the Palomino and albino phenotype in the spring spawning rainbow trout strain have strong detrimental plei-

otropic effects on growth, vitality and innate immunity (Dobosz *et al*., 2000; 2007; Siwicki *et al*., 2003). However, there seems to be a disagreement among the scientists on the pleiotropic effect of the colour genes controlling the yellow forms phenotypes. For instance, some authors (Bridges & Limbach, 1972; Bondari, 1984) believe that albinism in commercial aquaculture species does not appear to have a significant pleiotropic effect on the mutant gene.

Fish are in constant interaction with their habitat and potential pathogens. Fish epidermis acts as the first line of defense, since mucus secreted by mucous cells plays a critical role in the animal defense being a natural, semipermeable, dynamic, physical, chemical, and biological barrier (Subramanian *et al*., 2007; Raj *et al*., 2011). Furthermore, mucus contains innate immune parameters, such as enzymes and antimicrobial proteins (Jung *et al*., 2012; Nigam *et al*., 2012). Knowledge about the mechanisms of immune defense at the skin and mucus level in fish is still scarce.

Immune molecules in fish mucus include lysozyme, immunoglobulins, complement, lectins, agglutinin, calmodulin, interferon, C-reactive protein, proteolytic enzymes, antimicrobial peptides, or vitellogenin (Jung *et al*., 2012; Nigam *et al.*, 2012). The complete repertoire of immune factors present in the skin mucus and their precise role on fish immunology and defense is poorly understood (Li *et al.*, 2013) and it is restricted to a few fish species, mainly freshwater. Little is known about the roles of enzymes in the epidermal mucus in the innate immune system of some fish species (Subramanian *et al.*, 2008). Moreover, mucus composition and immune functions varies with the fish species and with changes in the environment and its physiology (Guardiola *et al*., 2014). Taking into account these previous considerations, the aim of the present study was to investigate some non-specific immune parameters, mainly the specific activities of lysozyme, ALP, esterase and proteases in the epidermal mucus and to compare these enzymes activities between Palomino and normally pigmented rainbow trouts farmed together in the same ponds at a trout farm in Iran.

MATERIALS AND METHODS

Experimental fish and mucus collection

The trial was conducted at a private rainbow trout farm in Shiraz, Fars province, South West of Iran. During the sampling, water temperature, pH and dissolved oxygen were monitored as 12 ºC, 7.3 and 6 ppm, respectively. The fish were fed twice daily a commercial salmon food (Table 1, Beyza Technology Co., Ltd., Iran).

Table 1. Composition of commercial diet

Nutrients	Value
Digestible energy (kcal/kg)	4400
Crude protein (%)	37–40
Crude lipid (%)	15–19
Crude fibre (%)	1.5–3
Moisture (%)	<12
Ash (%)	<10
Total volatile nitrogen (mg/100 g)	<40

After being kept 24 h without feeding, 10 healthy brooders (length 22±2.0 cm and weight 1.82±45 g) of each colour variety (wild and Palomino) were rapidly netted, carefully placed in a bathtub tank and anesthetised with clove powder (5 mg/L). The skin mucus was scraped from the dorso-lateral surface using a plastic

spatula with enough care to avoid contamination with blood and urino-genital and intestinal excretions (Palaksha *et al*., 2008).

After mucus collection, the fish were released into the brood ponds. The mucus samples were transferred to 15 mL sterile centrifuge tubes and centrifuged (1500×*g* for 10 min at 4 ^{o}C) and supernatants were stored at –80 ^{o}C for further analysis.

All the procedures involving animals were reviewed and approved by the Institutional Research Ethics Committee of the School of Veterinary Medicine of Shiraz University using non-invasive techniques and minimising stress and suffering by suitable management methods.

Skin mucus protein levels

The soluble protein concentration of mucus samples was measured by the Biuret method (Kwapinski, 1965).

Alkaline phosphatase activity

Alkaline phosphatase activity was determined through incubation of mucus supernatants with 4 mM para-nitrophenyl phosphate (Sigma) in 100 mM ammonium bicarbonate buffer containing 1 mM magnesium chloride, pH 7.8 at 30 ^{o}C (Palaksha *et al*., 2008). One unit of activity was defined as the amount of enzyme required to release 1 mmol of para-nitrophenyl product in 1 min.

Protease activity

Protease activity was determined using the azocasein hydrolysis assay according to Palaksha *et al*. (2008). Azocasein hydrolysis was assayed by incubating 50 µL of the mucus sample re-suspended in 100 mM ammonium bicarbonate, pH 7.8, with 50 µL azocasein substrate 0.25% (w/v) in the same buffer for 19 h at 30 °C. The reaction was stopped by adding 50 µL of 20% (w/v) trichloroacetic acid followed by a 5 min centrifugation at 15400×*g*. Equal volumes (100 µL) of the resultant supernatant and 0.5 M NaOH were added to a 96-well plate and the absorbance measured at 405 nm. One unit of activity was defined as the amount of enzyme that caused a change in absorbance of 0.001/min. Activity units were expressed per mg of protein (specific activity).

Esterase activity

Esterase activity was determined through incubation of mucus supernatants with 0.4 mM para-nitrophenyl myristate in 100 mM ammonium bicarbonate buffer containing 0.5% Triton X-100, pH 7.8 at 30 ^{o}C following the method given in Palaksha *et al*. (2008). The absorbance was measured continuously for 2 hours at 405 nm by ELISA reader. The activity was defined as the amount of enzyme required to release 1 mmol of para-nitrophenyl product in 1 min.

Lysozyme activity

Lysozyme activity was determined using a turbidometric assay (Tukmechi *et al.,* 2011). Twenty-five mL mucus supernatants were incubated in a 96- well plate with 75 mL of lyophilised *Micrococcus lysodeikticus* cells (Sigma, 75 mg/mL) in triplicate. A unit of lysozyme activity was defined as the amount of enzyme causing a decrease in absorbance of 0.001 per min (450 nm) and expressed as U/mg of mucus sample.

Antibacterial assay

Prior to analysis, the mucus samples were thawed at room temperature. The antibacterial activity of the skin mucus samples was measured using the standard disc diffusion method (Bauer *et al*., 1966). Different bacterial species including

Aeromonas hydrophila, Yersinia ruckeri and *Lactococcus garviae* were obtained from stock cultures maintained at the Microbiology Laboratory of the Aquatic Animal Health & Diseases Department, School of Veterinary Medicine, Shiraz University. All bacterial species were grown in nutrient broth medium for 24 h at 37 ºC. Afterwards, 0.1 mL of each broth culture medium (1.5×10^8 CFU/mL) was cultured on nutrient agar. Sterile paper discs (6 mm diameter) were impregnated with 200 µL of mucus sample, placed on the medium and incubated at 37 ºC for 24 h. Thereafter, discs were checked and the diameter of the growth inhibition zone in mm (minus the diameter of paper discs) was measured with ruler. Clear zones surrounding the discs were interpreted as presence of bactericidal activity.

Statistical analysis

Mean and standard deviation (SD) for all test values were calculated. The results were subjected to independent T test to compare the statistical differences between two treatments (SPSS software v. 16) Differences in means were considered statistically significant at $P<0.05$.

RESULTS

Significant differences in measured parameters were found in the two rainbow trout varieties (wild vs. Palomino) ($P<0.05$) (Tables 2 and 3).

Total protein analysis showed significantly higher value in Palomino trout (0.66 g/L) compared with wild colored trout (0.55 g/L). A similar pattern of effects was also noted with regard to lysozyme activity (Table 2). Value for the

Table 2. Protein content and enzymes' activities (mean ±SD) in the epidermal mucus of Palomino and normally pigmented rainbow trouts (n=10)

Parameters	Normal trouts	Palomino
Protease activity (U/mg protein)	25.8±2.6	32.7±2.6*
Esterase activity (U/mg protein)	2.2±0.4	3.4±0.4*
ALP activity (U/mg protein)	10.8±1.5	12.9±1.6*
Total protein (g/L)	0.55±0.02	0.66±0.03*
Lysozyme (U/mg protein)	29.8±2.1	37.2±2.7*

* statistically significant difference at $P<0.05$.

Table 3. Bactericidal activity of epidermal mucus in Palomino and normally pigmented rainbow trout. The diameter of growth inhibition zones is expressed in mm (minus the diameter of paper discs). Values are presented as mean ±SD; n=10

Bacteria	Normal trouts	Palomino
A. hydrophila	7.2±1.9	12.3±2.0*
Y. ruckeri	6.2±2.1	10.8±2.3*
L. garviae	7.4±2.2	12.7±2.1*

* statistically significant difference at $P<0.05$.

normally pigmented rainbow trout was 29.8 U/mg whereas in Palomino trouts it was 36.9 U/mg. Values for ALP, esterase and protease activities in Palomino trouts were 12.9, 3.4, 32.7 U/mg protein respectively, significantly higher than respective values in wild coloured trouts (10.8, 2.2, 25.8 U/mg protein).

The skin mucus in Palomino trouts showed significantly higher bactericidal activity against selected pathogens compared with the normally pigmented rainbow trouts. the inhibition zones against *A. hydrophila, Y. ruckeri* and *L. garviae* in Palomino trouts were 12.3 mm, 10.8 mm and 12.7 mm respectively, significantly higher in comparison with the normally pigmented rainbow trouts (7.2 mm, 6.2 mm, 7.4 mm, respectively) (Table 3).

DISCUSSION

Several studies have been conducted to explore the antimicrobial activity, immune components, and hydrolytic enzymes in the mucus of different fish species (Subramanian *et al*., 2007; Palaksha *et al*., 2008). However, no information is so far available on the innate immune components of mucus of Palomino rainbow trout that was examined in this study.

Lysozyme is an important defense molecule of the innate immune system, which is important in mediating protection against infectious diseases (Saurabh *et al*., 2008). Skin mucosal lysozyme was found to vary among freshwater fish species (Timalata *et al*., 2015). In the present report, higher level of lysozyme activity in the epidermal mucus of Palomino trout compared to wild coloured one was noticed. Red strain of tilapia (*Oreochromis nilloticus)* presented higher phagocytic and serum lysozyme activity than the black one (Balfry *et al*., 1999). Our findings detecting significant strain effect in immune activity supports the conclusions of previous studies (Røed *et al.*, 1989; 1993; Withler & Evelyn, 1990; Ibarra *et al.*, 1991; McGeer *et al.*, 1991; Lund *et al.*, 1995), who suggested a genetic basis for variation of lysozyme in salmonids. A higher lysozyme activity was reported in the skin mucus of rainbow trouts, administered with Ergosan and fed fermented *Saccharomyces cerevisiae* (Sheikhzadeh *et al*., 2012a,b).

In this study, Palomino trout presented higher level of epidermal mucus protease activity than wild coloured ones. In previous studies, different types of protease such as trypsin/trypsin-like protease, metallo and cysteine protease and cathepsin B and L, have been found in skin mucus of a number of fish species (Subramanian *et al*., 2007; Palaksha *et al*., 2008). Proteases could act directly on the pathogen or prevent invasion indirectly by modifying mucus consistency that results in increased sloughing of mucus and pathogen removal from body surface (Palaksha *et al*., 2008). The possible role of proteases in activating other immunological substances in the mucus, such as complement and immunoglobulin, was also demonstrated in the mammalian system (Palaksha *et al*., 2008). Furthermore, proteases are considered to be involved in the regulatory production of antimicrobial peptides (Salles *et al*., 2007). Sheikhzadeh *et al*. (2012a,b) reported a higher protease activity in the skin mucus of rainbow trout, administered with Ergosan and fed fermented *Saccharomyces cerevisiae*.

The present study revealed higher ALP activity in the skin mucus of Palomino compared to normally pigmented rainbow trouts. Alkaline phosphatase is thought to act as an antibacterial agent because of its hydrolytic activity (Iger &

Abraham, 1994). It has also a protective role in wound healing, parasitic infection and stress (Iger & Abraham, 1994; Ross *et al*., 2000). A higher ALP activity was reported in the skin mucus of rainbow trout, supplemented with Ergosan and fed fermented *Saccharomyces cerevisiae* (Sheikhzadeh *et al*., 2012a,b).

In the present study, higher esterase activity was demonstrated in epidermal mucus of Palomino trout compared to wild colored one. Higher esterase activity secreted in skin mucus was previously reported in rainbow trouts fed Ergosan and fermented *Saccharomyces cerevisiae* (Sheikhzadeh *et al*., 2012a). Even though the exact role of esterase in fish mucus is not clear it seems that this enzyme could act individually or in cooperation with other immune substances in the mucus in defending against pathogens (Sheikhzadeh *et al*., 2012b).

In this study, the skin mucus in Palomino trout presented significantly higher bactericidal activity against selected pathogens compared with wild coloured one. Previously, antimicrobial activity has been shown in skin mucus of European eel (*Anguilla anguilla*), carp (*Cyprinus carpio*), tench (*Tinca tinca*) (Ebran *et al*., 1999), hag fish (*Myxine glutinosa*), haddock (*Melanogrammus aeglefinus*), koi carp (*C. carpio* subsp. Koi), Arctic char (*Salvelinus alpinus*) (Subramanian *et al*., 2008). Skin antimicrobial activity is attributed to different antimicrobial compounds present on the mucosal surface (Subramanian *et al*., 2008). Fast *et al*. (2002) showed that rainbow trouts had higher protease and lysozyme activities as well as thicker epidermis and more abundant mucous cells (mid body) than Coho salmon and Atlantic salmon (Fast *et al*., 2002). It can be assumed that the greater bactericidal activity in the skin mucus of the Palomino trouts might be partially due to an increase in the number of mucous cells and consequently accelerated release of substances with protein structure such as hydrolytic enzymes, immunoglobulins, mucins and lectins, considering the evidence provided by the present study about significantly higher total protein concentration in Palomino's skin mucus and therefore, higher bactericidal activity. In Caspian white fish (*Rutilus frisii kutum*) fry, feeding xylooligosaccharide resulted in significantly increased skin mucus antibacterial activity and protein levels (Hoseinifar *et al*., 2014).

To the best of our knowledge, this is the first attempt to investigate skin mucosal immune parameters in yellow rainbow trout phenotypes. The selection of disease resistant fish strains is one approach to improvement of the survival of cultured fish. The current study documented a significant colour variety-related difference in skin mucosal immune parameters of rainbow trout in an Iranian trout farm. In fact, very limited knowledge exists in literature regarding the impact of colour mutation on fish immunity.

Compared with normally pigmented rainbow trouts, Palomino individuals exhibited significantly higher immune potential in their epidermal mucus as seen from all the parameters evaluated in the current study. Our finding supports the Iranian trout farmers' belief that Palomino is more resistant against some infectious diseases (*e.g.* saprolegniasis) than the wild type. However, these findings did not agree with previous data which reported lower systemic innate immunity in yellow rainbow trout forms compared with the wild coloured ones. This difference could be attributed to the level of expression of colour genes controlling the Palomino phenotype having strong pleiotropic ef-

fects on innate immunity (Dobosz *et al.*, 2000; 2007). However, in fish farming, no valid comparison with other trout stocks could be done because of probable inbreeding differences. Therefore, a doubt remains about possible pleiotropic effects affecting the yellow variants harbouring different genotypes. Future research should focus on identifying the genotype of Palomino fish in different progeny families existing in various trout farms. Attempts could be made to crossbreed individuals within and between xantoric and wild colouration to obtain various hybrids or progenies with superior traits especially concerning growth rate, immune capabilities and disease resistance.

ACKNOWLEDGEMENTS

This study was supported by the Aquatic Animal Health and Diseases Department, School of Veterinary Medicine, Shiraz University, through a research grant to the first author.

REFERENCES

Balfry, S. K., M. Shariff & G. K. Iwama, 1999. Strain differences in non-specific immunity of tilapia *Oreochromis niloticus* following challenge with *Vibrio parahaemolyticus*. *Disease of Aquatic Organisms,* **30**, 77–80.

Bauer, A. W., W.M. Kirby & J. C. Sherris, 1966. Antibiotic susceptibility testing by a standard single disc method. *American Journal of Clinical Pathology,* **451**, 493–496.

Blanc, J. M., H. Poisson & E. Quillet, 2006. A blue variant in the rainbow trout, *Oncorhynchus mykiss Walbaum*. *The Journal of Heredity,* **97**, 89–93.

Bondari, K., 1984. Comparative of albino and normal pigmented channel catfish in tanks, cages and ponds. *Aquaculture,* **37**, 293–301.

Bridges, W. R. & B. Limbach, 1972. Inheritance of albinism in rainbow trout. *The Journal of Heredity,* **63**, 152–153.

Dobosz, S., K. Goryczko, K. Kohlman & M. Korwin-Kossakowska, 1999. The yellow color inheritance in rainbow trout. *The Journal of Heredity,* **90**, 312–315.

Dobosz, S., K. Kohlman, K. Goryczko & H. Kuzminski, 2000. Growth and vitality in yellow forms of rainbow trout. *Journal of Applied Ichthyology,* **16**, 117–120.

Dobosz, S., 2007. Xantoric variety of rainbow trout: Studies of inheritance and breeding value. *Archives of Polish Fisheries,* **15**, 5–69.

Ebran, N., S. Julien, N. Orange P. Saglio C. Lemaître & G. Molle, 1999. Pore-forming properties and antibacterial activity of proteins extracted from epidermal mucus of fish. *Comparative Biochemistry and Physiology,* **122**, 181–189.

Fast, M. D., N. W. Ross, A. Mustafa, D. E. Sims, S. C. Johnson, G. A. Conboy, D. J. Speare, G. Johnson & J. F. Burka, 2002. Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtherirus salmonis*. *Diseases of Aquatic Organisms,* **52**, 57–68.

Guardiola, F. A., A. Cuesta, M. Arizcun, J. Meseguer & M. A. Esteban, 2014. Comparative skin mucus and serum humoral defense mechanisms in the teleost gilthead seabream (*Sparus aurata*). *Fish and Shellfish Immunology,* **36**, 545-551.

Hoseinifar, S. H., M. Sharifian, M. Khalili, M. J. Vesaghi, & M. Á. Esteban, 2014. The effects of dietary xylooligosaccharide on mucosal parameters, intestinal microbiota and morphology and growth performance of Caspian white fish (*Rutilus frisii kutum*) fry. *Fish and Shellfish Immunology,* **39**, 231–236.

Ibarra, A. M., G. A. E. Galland & R. P. Hednck, 1991. Susceptibility of two strains of rainbow trout *Oncorhynchus mykiss* to experimentally induced infections with the

myxosporean *Ceratomyxa shasta*. *Diseases of Aquatic Organisms,* **10**, 191–194.

Iger, Y. & M. Abraham, 1994. The process of skin healing in experimentally wounded carp. *Journal of Fish Biology,* **36**, 421–437.

Jung, T. S., C. S. Del Castillo, P. K. Javaregowda, R. S. Dalvi, S. W. Nho, S. B. Park, I. S. Cha, H. W. Sung, J. Hikima & T. Aoki, 2012. Seasonal variation and comparative analysis of non-specific humoral immune substances in the skin mucus of olive flounder (*Paralichthys olivaceus*). *Developmental and Comparative Immunology,* **38**, 295–301.

Kwapinski, J. B., 1965. Methods of Serological Research. John Wiley and Sons, New York. pp. 5–26.

Li, C., R. Wang, B. Su, Y. Luo, J. Terhune, B. Beck & E. Peatman, 2013. Evasion of mucosal defenses during *Aeromonas hydrophila* infection of channel catfish (*Ictalurus punctatus*) skin. *Developmental and Comparative Immunology,* **39**, 447–455.

Lund, T., T. Gjedrem, H. B. Bentsen, D. M. Eide, H. J. S. Larsen & K. H. Raed, 1995. Genetic variation in immune parameters and associations to survival in Atlantic salmon. *Journal of Fish Biology,* **46**, 748–758.

McGeer, J. C., L. Baranyi & G. K. Iwama, 1991. Physiological responses to challenge tests in six stocks of coho salmon (*Oncorhynchus kisutch*).*Canadian Journal of Fisheries and Aquatic Sciences,* **498**, 1761–1771.

Nigam, A. K., U. Kumari, S. Mittal & A. K. Mittal, 2012. Comparative analysis of innate immune parameters of the skin mucous secretions from certain freshwater teleosts, inhabiting different ecological niches. *Fish Physiology and Biochemistry,* **38**, 1245–1256.

Palaksha, K. J, G. W. Shin, Y. R. Kim & T. S. Jung, 2008. Evaluation of non-specific immune components from the skin mucus of olive flounder (*Paralichthys olivaceus)*. *Fish and Shellfish Immunology,* **24**, 479–488.

Raj, V. S., G. Fournier, K. Rakus, M. Ronsmans, P. Ouyang, B. Michel, C. Delforges, B. Costes, F. Farnir, B. Leroy, R. Wattiez, C. Melard, J. Mast, F. Lieffrig & A. Vanderplasschen, 2011. Skin mucus of *Cyprinus carpio* inhibits cyprinid herpesvirus 3 binding to epidermal cells. *Veterinary Research,* **42**, 92.

Rombout, J. H., N. Taverne, M. Van De Kamp & A. J. Taverne-Thiele, 1993. Differences in mucus and serum immunoglobulin of carp (*Cyprinus carpio* L.). *Developmental and Comparative Immunology,* **17**, 309–317.

Røed, K. H., K. Fjalestad & A. Stromshein, 1993. Genetic variation in lysozyme activity and spontaneous haemolytic activity in Atlantic salmon (*Salmo salar* L.). *Aquaculture,* **114**, 19–31.

Ross, N. W., K. J. Firth, A. Wang, J. F. Burka & S. C. Johnson, 2000. Changes in hydrolytic enzyme activities of naïve Atlantic salmon *Salmo salar* skin mucus due to infection with the salmon louse *Lepeophtheirus salmonis* and cortisol implantation. *Diseases of Aquatic Organisms Journal,* **41**, 43–51.

Salles, C. M. C., P. Gagliano, S. A. T. Leitão, J. B. Salles, H. L. M. Guedes, V. P. F. Cassano & S. Giovanni De-Simone, 2007. Identification and characterization of proteases from skin mucus of tambacu, a Neotropical hybrid fish. *Fish Physiology and Biochemistry,* **33**, 173–179.

Saurabh, S. & P. K. Sahoo, 2008. Lysozyme: An important defense molecule of fish innate immune system. *Aquaculture Research,* **39**, 223–239.

Sheikhzadeh, N., A. Karimi Pashaki, K. Nofouzi, M. Heidarieh & H. Tayefi Nasrabadi, 2012a. Effects of dietary Ergosan on cutaneous mucosal immune response in rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology,* **32**, 407–410.

Sheikhzadeh, N., M. Heidarieh, A. Karimi Pashaki, K. Nofouzi, M. Ahrab Farshbafi & M. Akbari, 2012b. Hilyses® fermented

Saccharomyces cerevisiae, enhances the growth performance and skin non-specific immune parameters in rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology,* **32**, 1083–1087.

Subramanian, S., S. MacKinnon & N. A. Ross, 2007. Comparative study on innate immune parameters in the epidermal mucus of various fish species. *Comparative Biochemistry and Physiology – Part B: Biochemistry and Molecular Biology,* **148**, 256–263.

Subramanian, S., N. W. Ross & S. L. MacKinnon, 2008. Comparison of the biochemical composition of normal epidermis mucus and extruded slime of hagfish (*Myxine glutinosa* L.). *Fish and Shellfish Immunology,* **25**, 625–632.

Siwicki, A., S. Dobosz, K. Goryczko, H. Kuźmiński, K. Kohlmann, S. Trapkowska & B. Kazun, 2003. Cell mediated immunity in yellow forms of rainbow trout. *Polish Journal of Veterinary Science*, **6**, 49–50.

Timalata, K., K. Marimuthu, R. Vengkades, R. Xavier, M.A. Rahman, S. Sreeramanan, M. V. Arasu, N. A. Al-Dhabi & J. Arockiaraj, 2015. Elucidation of innate immune components in the epidermal mucus of different freshwater fish species. *Acta Ichthyologica et Piscatoria,* **3**, 221–230.

Withler, R. E. & T. P. T. Evelyn, 1990. Genetic variation in resistance of bacterial kidney disease within and between two strains of coho salmon from British Columbia. *Transactions of the American Fisheries Society*, **119**, 1003–1009.

Paper received 22.11.2016; accepted for publication 06.02.2017

Correspondence:

Siyavash Soltanian DVM, PhD
Aquatic Animal Health & Diseases Department, School of Veterinary Medicine, Shiraz University, 71441-69155 Shiraz, Iran
Phone: 0098 713 6138906
Fax: 0098 713 32286940
e-mail: siyavashsoltanian@yahoo.com