13(3): 025-031 (2019)

Journal of FisheriesSciences.com

E-ISSN 1307-234X

Review Article

A Review on the Probiotic Effects on Haematological Parameters in Fish

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Received: 10.07.2019 / Accepted: 25.07.2019 / Published online: 31.07.2019

Abstract: Haematological indices are essential diagnostic tools used to evaluate the health status of fish. Many publications have been stated by different works that qualitative and quantitative variations in haematological parameters; for instance White Blood Cells (WBCs), Red Blood Cells (RBCs), Haematocrit (Hct), Haemoglobin (Hb) content, Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) in fish, offer an indication of the health status of the fish. The uses of probiotics as biological control agents in aquaculture have replaced the usage of chemotherapeutics, is an approach in the build-up in aquaculture environments. In the cultured fish, the use of probiotics in monospecies or multispecies forms has been reported to stimulate specific and non-specific immune parameters including lysozyme activities and phagocytic, expression of various cytokines as well as improvement of blood profiles of many fish increasing resistance diseases and to other environmental perturbations such as physiological stressors.

Interestingly, many researchers have shown that haematological indices in fish continue to offer a valuable diagnostic tool; and progress is made in establishing a reasonable range of values for blood parameters of different fish species. Also, many interventions have shown that probiotics used in aquaculture have potential in improving blood profiles of fish; although there are not many summarised information regarding the effects of probiotics on haematological parameters in fish. The purpose of this review is to synthesise the influences of probiotics on haematological parameters in fisha.

Keywords: Aquaculture; Blood cells; Haematology; Immune System; Probiotics; Physiological stressors

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Introduction

Bolliger and Everds (2010) define haematology as the study of the physiology and pathology of the cellular elements of blood. The biological and environmental factors including age, weight, sex, food, bacteria, viruses, fungi and water quality parameters can influence the importance of the haematological parameters of fish (Das and Das, 1993). Haematological indices affect physiological state of the fish and provide a significant hint on the well-being of the fish (Harikrishnan et al., 2010). Haematological indices are used as vital diagnostic tools to assess the health status of fish (Pradhan et al., 2012). Reports on haematological indices show a correlation of the physiology and evolution in organisms. However, information on fish physiology has become more essential due to diagnostic assessment and economic significance of fish (Pradhan et al., 2012). Many publications have reported on qualitative and quantitative variations in haematological/blood parameters such as White Blood Cells (WBC) count, Red Blood Cells (RBCs) count, Haematocrit (Hct) and Haemoglobin (Hb) in fish, as a sign of the wellbeing of the fish.

The status of the above haematological parameters in fish depend many factors, for instance the level of contaminants (concentration), temperature, nutrition, and fish species among others. Therefore, alteration in haematological parameters is considered as a prognostic tool as well as a primary warning signal of the disturbance in homeostatic defence abilities of fish (Sharma and Langer, 2014). Interestingly, the haematological study in fish offer valuable diagnostic tools and progress in establishing normal range values for blood parameters of diverse fish species (Pradhan et al., 2012).

In the culture of fish, the use of monospecies or multispecies have been reported to enhance fish health particularly enhancement of antibody levels, lysozymes and cytokine activities (Agrawal, 2005; Allameh et al., 2017) as well as haematological parameters in fish (Dawood et al., 2017). Extensive research and comprehensive reviews have been written on the contributions of probiotics for the enhancement of fish health regarding some of the above mentioned immune parameters. Although many interventions have been made by researchers regarding the use of probiotics in improving blood profiles of fish, (Dahiya et al., 2012; Kiron and Watanabe, 2010; da Paixão et al., 2017) there isn't any summarised information regarding the effects of probiotics on haematological parameters in fish. The purpose of the review is to reveal the influences of probiotics on haematological parameters in fish.

Haematological parameters

In the circulatory system, blood aids many transport functions. RBCs, WBCs, and plasma (extracellular space around these cells) are the three main modules with the blood cells (Farrell, 2011). The other body tissues within the blood differ in its extracellular volume, which is usually twice as large as the intracellular volume (that occupied by the cells) (Farrell, 2011). Principally, blood function as a transport medium for gases and solutes that include oxygen, carbon dioxide, nutrients, ammonia, and hormones (Farrell, 2011). Basically, the concentrations of the various components of blood are regulated while some are tightly regulated (homeostatic roles) include electrolyte (ion) concentrations, pH, arterial oxygen and carbon dioxide tensions, and osmolarity (Farrell, 2011). Relatively, arrangement of blood is represented by the relative heights of the packed layers of RBCs, WBCs, and plasma (Farrell, 2011). The term used for the percentage of the column of blood occupied by RBCs is Haematocrit (Hct), while percentage of the column of blood occupied by WBCs is leucocrit (Lct). Usually, fish blood comprises 60–80% plasma, 20–40% RBCs, and 0.5–2.0% WBCs (Farrell, 2011). Others haematological parameters including MCH, MCHC, and MCV can be calculated or derived using information from the RBCs, Haematocrit and Haemoglobin measurements (Dahiya et al., 2012).

Effect of probiotics in Haematocrit (hct)

Gallaugher (1994) defined Haematocrit (hct) as the measure of blood capacity to carry oxygen. In an organism, the higher the hct value, the higher the blood's moving ability of oxygen. Hct values in teleost have been reported to range from 0% to 70% (Fletcher and Haedrich, 1987; Graham and Fletcher, 1983; Graham and Fletcher, 1985; Wells and Baldwin, 1990; Wells and Weber, 1991). Typically, hct values have been reported to range from 17% - 44% in rainbow trout (R M G Wells and Weber, 1991), 52.5% mackerel (*Auxis rochei*), 53% tuna (*Thunnus thynnus*), 43% blue marlin (*Makaira nigricans*) 8.5% hagfish (*Eptatretus cirrhatus*) (Satchell, 1991; Fánge, 1992). Higher hct values in organism indicate healthier conditions (Gallaugher, 1994) and can also indicate a response to increase stress thus serving as an anti-stress response to compensate for increased demand for oxygen for metabolic energy (Gallaugher, 1994).

For instance supplementation with primalac probiotics (Nikandishan Farjad Commerce Corporation, Tehran, Iran) containing *Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium* and *Bifidobacterium bifidium* have been reported to increase the hct levels in Caspian roach fry subjected to salinity stress compared to those fed with the control diet (Imanpoor and Roohi, 2015). Also, infection with pathogenic bacteria *Streptococcus iniae* was shown an increase in hct and survival of Nile tilapia immersed in *Bacillus sp.*

In other research reports, the application of a mixed probiotic species of *Lactococcus rhamnosus* and *Lactococcus lactis* in red seabream (Dawood et al., 2017), *Bacillus sp.* in Nile tilapia (Feliatra et al., 2018), a combination of *B. cereus* and *B. subtilis* in Nile tilapia (Garcia-marengoni et al., 2015) and *L. rhamnosus* on rainbow trout (Kiron and Watanabe, 2010) have been reported to increase the Hct levels. These reports on fish fed probiotic-supplemented diets were indicated to have better health status compared to those fed control diets. Taken all the above into consideration, fish fed probiotic-supplemented diets might have higher hct levels and could respond better to stressors. This indicates that the anti-stress response in fish can be improved after feeding probiotic-supplemented diets as have been previously reported (Feliatra et al., 2018).

Effects of probiotics on Haemoglobin (Hb) concentration

According to Bolliger and Everds (2012), Haemoglobin (Hb) concentration is defined as the amount of total Hb per volume of whole blood and is determined by spectrophotometric method after lysis of red blood cells. Riggs (1976) reported that the purpose of Hb appears to conform to diverse metabolic needs of animals with constant environmental changes and also play a significant role in carrying oxygen from the gas-exchange organs to peripheral tissues (de Souza and Bonilla-Rodriguez, 2007). In fish, Hb form a line bet/ween the organism and the environment (Landini et al., 2002) but specifically through environmental changes and modifications in temporal and spatial variations, with oxygen availability as compared to terrestrial animals (Brix et al., 2004; de Souza and Bonilla-Rodriguez, 2007). Accounts on the utilisation of probiotic-supplemented diets hold a promise in increasing the Hb levels of fish.

For example, in contrast to the fish fed control diets, the application of a probiotic species of *Lactococcus sporogenes* in C*larius batrachus* (indian magur) (Dahiya et al., 2012) and combined dosage of these probiotics *L. sporogenes, L. acidophilus, B. subtilis, B. licheniformis, Saccharomyces cervirial* in *Cirrihinus mrigal* (Sharma et al., 2013) *Bacillus* np5 in *Oreochromis niloticus* (Tanbiyaskura et al., 2015), *Lactobacillus* and *Bifidobacterium* in *Clarias gariepinus* (catfish) (Kiron and Watanabe, 2010), *Bacillus subtilis*, *Saccharomyces cerevisiae* in mori, *L. Acidophilus* and β-glucan, in snakehead (Talpur and Ikhwanuddin, 2013), *Bacillus cereus* in juvenile nile tilapia (Garcia-marengoni et al., 2015) , *B. Pamillus* in *Labeo rohta* (Rajikkannu et al., 2015), *B. Licheniforms* and *B. Subtilis* in *Rutilis frisii* kutum (Azarin et al., 2015) have been reported to increase the Haemoglobin levels. In these reports fishes fed probiotic-supplemented diets were indicated to have better health status compared to those fed control diets.

Effects of probiotics on RBCs

The amount of RBCs also known as Erythrocytes (ERI) in a given volume of blood is determined using an automated counter (Bolliger and Everds, 2012). In fish, RBCs are elliptical or ovoid, but species have changes in RBC dimensions and length from 10–20 mm and 6–10 mm in width (Farrell, 2011). The primary role of RBCs is the transport of oxygen by the high concentrations of the respiratory pigment Haemoglobin and pH regulation (Farrell, 2011). Fish fed with probiotic-supplemented diets can intensify the RBCs levels of fish compared to that of those fed control/unsupplemented diets (Azarin et al., 2015).

For example, the application of a mixed probiotic species of *L. sporogenes* in *C. batrachus* (Dahiya et al., 2012), a combined dosage of *L. sporogenes*, *L. acidophilus, B. subtilis, B. licheniformis* and *S. cervirial* in *C. mrigal* in (Sharma et al., 2013) and *B. licheniformis* and *B. subtilis* in *Rutilis frisii* kutum (Azarin et al., 2015), LAB in rainbow trout, *L. plantarum* in Nile tilapia (Faramaz et al., 2011) were reported to have significantly higher RBCs compared to fish feed supplemented diets. Also evidence of various scientific works depicts, the use of probiotic-supplemented improved the RBCs level in the fish species.

Effects of probiotics on WBCs count

Medicine net defines WBCs count (leukocyte count) as the number of WBCs in the blood and can be measured as part of the Complete Blood Count (CBC). Fish WBCs function importantly in cellular defense and immunity. However, the functional effects of WBCs found in tissues (thymus, spleen and kidney) outside of the blood are also production sites (Farrell, 2011). Fish regulate the number of circulating WBCs, despite their minuscule representation in the fish blood (Leucocrit (Lct) = 0.3–1.0%) but toxicants and stress can depress leucocrit (Farrell, 2011).

The practices of probiotic-supplemented diets have been testified to hold capacity in increasing the WBC levels of fish compared to that of those fed control/unsupplemented diets. In contrast to the fish fed control diets, *L. sporogenes* as probiotic in *C. batrachus* (Dahiya et al., 2012), the application of a mixed probiotic species *L. sporogenes, L. acidophilus, B subtilis, B. licheniformis* and *S. cervirial* in *C. mrigal* (Sharma et al., 2013), *B. subtilis* in rainbow trout (Kamgar and Ghane, 2014), mixed probiotic species of *B. subtilis* and *S. cerevisiae* in *C. mrigala* (Ullah et al., 2018), *L. acidophilus* and *B. subtilis* in Nile tilapia (Aly et al., 2008) have been reported to increase the WBC levels. Fish fed with probiotic supplemented diets were indicated to have better immune responses compared to those fed control diets. Also fish fed with probiotic-supplemented diets might have higher WBC levels and could respond better to stressors. Feeding fish with probiotic-supplemented diets enhanced immune defense (Munir et al., 2018).

Effects of probiotics on other blood parameters

The first blood derivatives is the Mean Corpuscular Haemoglobin Concentration (MCHC), which represents the average Haemoglobin concentration within erythrocytes and calculated by dividing the whole blood Haemoglobin value (in grams per decilitre) by the hct (as a percentage) and multiplying by 100 expressed as grams per decilitre of erythrocytes (John and Harvey, 2012). The concentration of Haemoglobin within a RBC is usually expressed as Mean Cell Haemoglobin Concentration (MCHC) and over a wide range of fish species and conditions (physiological and environmental) (Farrell, 2011). The administration of probiotics as feed in an infected *H. fossils* (Mohideen and Haniffa, 2015) elicited alteration in MCHC, a combination of *B. subtilis* and *S. cerevisiae* in a *C. mrigala* (Ullah et al., 2018), *B. subtilis* in rainbow trout (Kamgar and Ghane, 2014) and *B. pumilus* in *Labeo rohita* (Rajikkannu et al., 2015).

The second blood derivative is the Mean Corpuscular Haemoglobin (MCH). MCH is defined as the average amount of Haemoglobin found in each RBC and is determined by dividing the Haemoglobin by the RBCs (Bolliger and Everds, 2012). In general, MCH is the least useful haematology parameter, because of it's insensitive to change and provides little additional information than other RBC parameters (Bolliger and Everds, 2012).

The administration of probiotics as feed-in experimentally infected *H. fossils* elicited alteration of MCH (Mohideen and Haniffa, 2015). A combination of *B. subtilis* and *S. cerevisiae* in *C. mrigala* (Ullah et al., 2018), *B. subtilis* in rainbow trout (Kamgar and Ghane, 2014) and *B. pumilus* in *Labeo rohita* (Rajikkannu et al., 2015)

The final blood derivative is the Mean Corpuscular Volume (MCV). The average size of Red Blood Cells is known as MCV (Bolliger and Everds, 2012). When a haemocytometer determines cell counts, MCV is calculated. For instrument-generated cell counts, red blood cell volume is measured during the cell count described above, and the MCV is determined by the histogram (Bolliger and Everds, 2012). If the cells are counted using a haemocytometer, the MCV is determined by dividing the Haematocrit by the RBC (Bannerman, 1983; Bolliger and Everds, 2010; Hall, 2007). The administration of probiotics as feed-in infected *H. fossils* (Mohideen and Haniffa, 2015), *Bacillus licheniformis* and *Bacillus subtilis* fed in Kutum (*Rutilus frisii kutum*) fry (Azarin et al., 2015), *Bacillus licheniformis* and *Bacillus subtilis* in matrinxa˜ (*Brycon amazonicus*) breeders fed (Dias et al., 2012) and *B. pumilus* in *Labeo rohita* (Rajikkannu et al., 2015).

Table 1: Probiotic effects on Haematological parameters in fish.

Probiotic type		Haematological effect	Species of fish	Reference
Monospecies	Multispecies			
	Lactococcus rhamnosus and *Lactococcus lactis*	Increase hct	Red seabream	Dawood et al., (2017)
Bacillus spp		Increase hct	Nile tilapia	Feliatra et al., 2018)
	Bacillus cereus and *Bacillus subtilis*	Increase hct	Nile tilapia	Garcia-marengoni et al., (2015)
Lactococcus rhamnosus		Increase hct	Rainbow trout	Kiron and Watanabe, (2010)
Lactococcus sporogenes		Increase Hb	Indian magur (*Clarius batrachus)*	Dahiya et al., (2012)
	L. sporogenes, *L. acidophilus*, *B. subtilis*, *B. licheniformis*, *Saccharomyces cervirial*	Increase Hb	*Cirrihinus mrigal*	Sharma, Sihag and Gahlawat, (2013)
Bacillus NP5		Increase Hb	Nile tilapia	Tanbiyaskura et al., (2015)
	Lactobacillus and *Bifidobacterium*	Increase Hb	Catfish	Kiron and Watanabe, (2010)
	L. acidophilus and β-glucan	Increase Hb	Snakehead	Talpur and Ikhwanuddin, (2013)
Bacillus cereus		Increase Hb	Juvenile Nile tilapia	Garcia-marengoni et al., (2015)
Bacillus pamillus		Increase Hb	*Labeo rohta*	Rajikkannu et al., (2015)
	B. licheniforms and *B. subtilis*	Increase Hb	*Rutilis frisii kutum*	Azarin et al., (2015)
Lactococcus sporogenes		Increase RBCs	*Clarius batrachus*	Dahiya et al., (2012)
	L. sporogenes, *L. acidophilus*, *B. subtilis*, *B. licheniformis*, *Saccharomyces cervirial*	Increase RBCs	*Cirrihinus mrigal*	Sharma, Sihag, and Gahlawat, (2013)
L. plantarum		Increase RBCs	Nile tilapia	Faramaz et al., (2011)
	B. licheniforms and *B. subtilis*	Increase RBCs	*Rutilis frisii kutum*	Azarin et al., (2015)
Lactococcus sporogenes		Increase WBCs	*Clarius batrachus*	Dahiya et al., (2012)
	L. sporogenes, *L. acidophilus*, *B. subtilis*, *B. licheniformis*, *Saccharomyces cervirial*	Increase WBCs	*Cirrihinus mrigala*	Sharma, Sihag and Gahlawat, (2013)
B. subtilis		Increase WBCs	Rainbow trout	Kamgar and Ghane, (2014)
	B. subtilis and *S. cerevisiae*	Increase WBCs (Ullah et al., 2018)	*C. mrigala*	Ullah et al., (2018)
	L. acidophilus and *B. subtilis*	Increase WBCs	Nile tilapia	Aly et al., (2008)

Ultimately, the levels of MCH, MCV and MCHC in the blood serve as the determinants of Haemoglobin in the RBC.

Table 1 describes the influence of monospecies and multispecies probiotics and how it affects haematological indices in some fish species.

Stress responses on probiotics in fish

Many research studies have shown that, stress is a major factor that hinders fish culture. However, the use of probiotics shows a promise in improving stress tolerance (Balcázar et al., 2006; Gatesoupe, 1999; Verschuere et al., 2003; Wang and Lin, 2008).

For instance, Taoka et al. (2006) reported commercial probiotic Alchem Poseidon (comprising a mix of *Bacillus subtilis, Lactobacillus acidophilus, Clostridium butyricum* and *Saccharomyces cerevisiae*) increased stress tolerance in *P. olivaceus*, under closed recirculating system. Hernandez et al. (2010) reported that juvenile Porthole livebearer fed with *Artemia nauplii* enriched with *L. casei* withstand stress tolerance (after 1 hour of air exposure). *D. labrax* fed with *Lactobacillus delbrueckii* decrease the cortisol levels (Carnevali et al., 2006) as well as *Sparus auratus* fed with probiotics, *Lactobacillus fructivorans* and *Lactobacillus plantarum* decrease the cortisol level under acute stress situations (Rollo et al., 2006). Pagrus major fed with probiotics, *Lactobacillus plantarum* (LP20) indicate high tolerance against low salinity stress (Dawood et al., 2017). Also Yokoyama et al. (2005) reported that the Japanese flounder fed with probiotics Lactoferrin withstand high stress tolerance when subjected to high temperature. Also, transportation of the commercial Amazonian ornamental fishes such as marbled hatchet fish *Carnegiella strigata (*Gomes et al., 2008); cardinal tetra (*Paracheirodon axelrodin*) (Gomes et al., 2009) indicate decrease in cortisol levels with the fish fed with commercial probiotic Efinol®. Fish fed with probiotic Pdp11 improve stress tolerance to HSD than control diet in *S. auratus* (Varela et al., 2010). In *S. auratus*, HSD a chronic stressor, that triggers the stress axis, creating an elevation of plasma cortisol (Mancera et al., 2008; Polakof et al., 2006; Sangiao-Alvarellos et al., 2005).

The presence of *B. subtilis* in the diet of Nile tilapia decreased the stress associated with high stock density (Telli et al., 2014). Thy et al. (2017) reported that the striped catfish (*P. hypophthalmus*) subjected to ammonia stress after *B. amyloliquefaciens* 54A, and *B. pumilus* 47B diet supplementation indicate low mortality. The mixture of *Bacillus* species (*B. subtilis, B. pumilus, B. amyloliqueficiens* and *B. licheniformis*) enhanced yellow perch's stress tolerance to hypoxia and air-exposure (Eissa et al., 2018).

Conclusion

Haematological indices including WBCs, RBCs, Hb, Hct, MCHC, MCV and MCH are used as essential diagnostic tools to assess the health status of fish. The level of these indices which depends on species, age, and environmental conditions can be used to access the physiological state of fish. Although many potential probiotic bacteria have been identified for use in aquaculture, Lactic acid and Bacillus species are common probiotics that have been reported to modulate most of these haematological indices. Besides, research data available indicate probiotic investigation on haematology is mostly based on optimal culture conditions as well as responses following pathogen infections. However many environmental perturbations such as changes in temperature, the concentration of pollutants, nutrition among others are known to cause physiological stress in fish.

Therefore, we suggest that more research will be necessary to determine the mitigation effects of probiotics on haematological indices of fish under such conditions and also how probiotics can influence the haematological parameters in the fish because haematological parameters assessed the physiological state of the fish.

Acknowledgments

The study was supported by National Natural Science Foundation of China as the funder and the award number is 31572651.

References

Agrawal, R. (2005) Probiotics: An emerging food supplement with health benefits. Food Biotechnol 19(3), 227-246.

Allameh, S.K., Noaman, V., Nahavandi, R. (2017) Effects of Probiotic Bacteria on Fish Performance. Adv Tech Clin Microbiol 1, 1-5.

Aly, S.M., Ahmed, Y.A.G., Ghareeb, A.A.A., Mohamed, M.F. (2008) Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of *Tilapia nilotica* (*Oreochromis niloticus*) to challenge infections. Fish Shellfish Immun 25(1-2), 128-136.

Azarin, H., Aramli, M.S., Imanpour, M.R., Rajabpour, M. (2015) Effect of a Probiotic Containing *Bacillus licheniformis* and *Bacillus subtilis* and Ferroin Solution on Growth Performance, Body Composition and Haematological Parameters in Kutum (*Rutilus frisii* kutum) Fry. Probiotics Antimicro 7(1), 31-37.

Balcázar, J.L., de Blas, I., Ruíz-Zarzuela, I., Cunningham, D., Vendrell, D., et al. (2006) The role of probiotics in aquaculture. Vet Microbiol 114, 173-186.

Bannerman, R. (1983) Hematology of the laboratory mouse. In: Foster HL, Small DJ, Fox JG, Editors. The Mouse Biomed Res, Amsterdam: Elsevier 3, 133-170.

Bolliger, A.P., Everds, N. (2012) Haematology of the Mouse. The Laboratory Mouse pp: 331–347.

Bolliger, A.P., Everds, N. (2010). Haematology of Laboratory rodents. Edited by Weiss D.J, Wardrop K.J. Schalms Veterinary Hematology, 6th Edition. Ames, IA: Wiley-Blackwell pp:852–862.

Brix, O., Thorkildsen, S., Colosimo, A. (2004) Temperature acclimatization modulates the oxygen binding properties of the Atlantic Cod (*Gadus morhua* L.) genotypes HbI*1/1, HbI*1/2, and HbI*2/2- by changing the concentrations of their major hemoglobin components (results from growth studies at different temperatures. Comp Biochem Physiol A Mol Integr Physiol 138(2), 241-51.

Carnevali, O., de Vivo, L., Sulpizo, R., Gioacchini, G., Olivotto, I., et al. (2006) Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax L*), with particular attention to IGF-1, myostatin and cortisol gene expression. Aquacul 258, 430–438.

Da Paixão, A., Dos Santos, J., Pinto, M., Pereira, D., De Oliveira, R., et al. (2017). Effect of commercial probiotics (*Bacillus subtilis and Saccharomyces cerevisiae*) on growth performance, body composition, hematology parameters, and disease resistance against *Streptococcus agalactiae* in tambaqui (*Colossoma macropomum*). Aquacul Int 25(6), 2035–2045.

Dahiya, T., Sihag, R.C., Gahlawat, S. (2012) Effect of probiotics on the haematological parameters of Indian magur (*Clarius batrachus L*.). J Fish Aquat Sci 7(4), 279–290.

Das, A.K., Das, R.K. (1993) A Review of the Fish Disease Epizootic Ulcerative Syndrome in India. Environment and Ecology 11(1), 134–145.

Dawood, M.A.O., Koshio, S., Ishikawa, M., Yokoyama, S., El Basuini, M.F., et al. (2017) Effects of dietary supplementation of *Lactobacillus rhamnosus* or/and *Lactococcus lactis* on the growth, gut microbiota and immune responses of red sea bream, Pagrus major. Fish Shellfish Immun 49, 275–285.

De Souza, P.C., Bonilla-Rodriguez, G.O. (2007) Fish hemoglobins. Braz J Med Biol Res 40(6), 769–778.

Dias, B.D.C., Leonardo, A.F.G., Tachibana, L., Corre, C.F. (2012) Effect of incorporating probiotics into the diet of matrinxã (*Brycon amazonicus*) breeders. J Appl Ichthyol 28(1), 40–45.

Eissa, N., Wang, H.P., Yao, H., Abou-ElGheit, E. (2018) Mixed Bacillus species enhance the innate immune response and stress tolerance in yellow perch subjected to hypoxia and air-exposure stress. Sci Rep 8.

Fánge, R. (1992) Fish blood cells. Fish Physiology.

Faramaz, I.M., Kiaalvandi, S., Lashkarbolooki, M., Iranshahi, F. (2011) The investigations of *Lactobacillus acidophilis* as Probiotics Trout, grown performance and disease resistance of rainbow trout (*Oncorhynchus mykiss*). Am Eurasian J Sci 6(1), 32-38.

Farrell, A.P. (2011) Cellular Composition of the Blood. Encyclopedia of Fish Physiology: From Genome to Environment 2, 984-991.

Feliatra, F., Elizal, L., Lukistyowati, I., Melina, D., Ramadhani, M. (2018) Effectiveness of Immersion with Probiotic in Improving the Health of Nile Tilapia (*Oreochromis niloticus*). Asian J Anim Vet Adv 13(1), 43-51.

Fletcher, G.L., Haedrich, R.T. (1987) Rheological properties of rainbow trout blood. Can J Zool 65(4), 879-883.

Gallaugher, P.E. (1994) The role of haematocrit in oxygen transport and swimming in salmonid fishes. PhD Thesis, August, Simon Fraser University, UK. pp:129.

Garcia-marengoni, N., Moura, M.C., Tavares, N., de Oliveira, E. (2015) Short Communication Use of probiotics *Bacillus cereus* var. toyoi and *Bacillus subtilis* C-3102 in the diet of juvenile Nile tilapia cultured in cages. Lat Am J Aquat Res 43(3), 601-606.

Gatesoupe, F.J. (1999) The use of probiotics in aquaculture. J of Appl Microbiol 119(4), 917-935.

Gomes, L.C., Brinn, R.P., Marcon, J.L., Dantas, L.A., Brandao, F.R., et al. (2009) The benefits of using Efinol® during transportation of cardinal tetra, Paracheirodon axelrodi (Schultz) in the Amazon. Aquacul Res 40, 157-165.

Graham, M.S., Fletcher, G.L. (1985) On the low viscosity blood of two cold water, marine sculpins - A comparison with the winter flounder. J of Comparative Physiol B 155(4), 455-459.

Graham, M.S., Fletcher, G.L. (1983) Blood and plasma viscosity of winter flounder: influence of temperature, red cell concentration, and shear rate. Can J Zool 61, 2344-2350.

Hall, R.L. (2007) Clinical pathology of laboratory animals. Animal Models in Toxicology, 2nd Edition, New York: CRC Press. pp: 789–829.

Harikrishnan, R., Balasundaram, C., Heo, M.S. (2010) *Lactobacillus sakei* BK19 enriched diet enhances the immunity status and disease resistance to streptococcosis infection in kelp grouper, *Epinephelus bruneus*. Fish Shellfish Immun 29(6), 1037-1043.

Hernandez, L.H.H., Barrera, T.C., Mejia, J.C., Mejia, G.C., Del Carmen, M., et al. (2010) Effects of the commercial probiotic *Lactobacillus casei* on the growth, protein content of skin mucus and stress resistance of juveniles of the Porthole livebearer *Poecilopsis gracilis* (Poecilidae). Aquacul Nutrition 16(4), 407-411.

Imanpoor, M.R., Roohi, Z. (2015) Influence of Primalac Probiotic on Growth Performance , Blood Biochemical Parameters , Survival and Stress Resistance in the Caspian Roach (*Rutilus rutilus*) Fry. Turk J Fish Aquat Sc 15, 917-922.

John, W., Harvey, D.V.M. (2012) Concomitant Interpretation of Hematocrit (Hct) and Total Plasma Protein (TPP) Concentration. Veterinary Hematology Chapter 4 pp:49-121.

Kamgar, M., Ghane, M. (2014) Studies on *Bacillus subtilis*, as Potential Probiotics, on the Hematological and Biochemical Parameters of Rainbow trout, *Oncorhynchus mykiss* (Walbaum). J of Appl Env Microbiol 2(5), 203-207

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Kiron, A.P.V., Watanabe, S.S.T. (2010) Probiotic bacteria *Lactobacillus rhamnosus* influences the blood profile in rainbow trout *Oncorhynchus mykiss* (Walbaum). Fish Physiol Biochem 36(4), 969-977.

Landini, G.F., Schwantes, A.R., Schwantes, M.L. (2002) *Astyanax scabripinnis* (Pisces: Characidae) haemoglobins: structure and function. Braz J Biol 62, 595-599.

Mancera, J.M., Vargas-Chacoff, L., García-López, A., Kleszczyńska, A., Kalamarz, H., et al. (2008) High density and food deprivation affect arginine vasotocin, isotocin and melatonin in gilthead sea bream (*Sparus auratus*). Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology 149(1), 92-97.

Mohideen, M., Haniffa, M. (2015) Effect of Probiotic on Microbiological and Haematological Responsiveness of Cat fish (*Heteropnuestes fossilis*) Challenged with Bacteria *Aeromonas hydrophila* and Fungi *Aphanomyces invadans*. J Aquac Res Development 6(12).

Munir, M.B., Hashim, R., Nor, S.A.M., Marsh, T.L. (2018) Effect of dietary prebiotics and probiotics on snakehead (*Channa striata*) health: Haematology and disease resistance parameters against Aeromonas hydrophila. Fish Shellfish Immun 75, 99-108.

Polakof, S., Arjona, F.J., Sangiao-Alvarellos, S., Martín Del Río, M.P., Mancera, J.M., et al. (2006) Food deprivation alters osmoregulatory and metabolic responses to salinity acclimation in gilthead sea bream Sparus auratus. J Comp Physiol B 176(5), 441-452.

Pradhan, S.C., Patra, A.K., Sarkar, B., Pal, A. (2012) Seasonal changes in hematological parameters of *Catla catla*. Comp Clin Path 21(6), 1473-1481.

Rajikkannu, M., Natarajan, N., Santhanam, P., Deivasigamani, B., Janani, S. (2015) Effect of probiotics on the haematological parameters of Indian major carp (*Labeo rohita*). Int J Fish Aquat Stud 2(5), 105-109.

Riggs, A. (1976) Factors in the evolution of haemoglobin function. Fed Proc 35, 2115-2118.

Rollo, A., Sulpizio, R., Nardi, M., Silvi, S., Orpianesi, C., et al. (2006) Live microbial feed supplement in aquaculture for improvement of stress tolerance. Fish Physiol and Biochem 32(2), 167-177.

Alvarellos, S., Guzmán, J.M., Laiz-Carrión, R., Miguez, J.M., Martín del Río, M.P., et al. (2005) Interactive effects of high stocking density and food deprivation on carbohydrate metabolism in several tissues of gilthead sea bream *Sparus auratus*. J Exp Zool A 303, 761-775.

Satchell, G.H. (1991) In Physiology and Form of Fish Circulation. Cambridge University Press, Cambridge, England. pp:233.

Sharma, J., Langer, S. (2014) Effect of Manganese on haematological parameters of fish, *Garra gotyla gotyla*. J Entomol Zool Stud 2(3), 77-81.

Sharma, P., Sihag, R.C Gahlawat, S.K. (2013) Effect of probiotic on haematogical paramaters of diseased fish (*Cirrihinus mrigal*). J Fish Sci 7(4), 323-328.

Talpur, A.D., Ikhwanuddin, M. (2013) *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, Lates calcarifer challenged with Vibrio harveyi. Fish Shellfish Immun 34, 254-264.

Taoka, Y., Maeda, H., JO, J., Kim, S., Park, S., et al. (2006) Use of live and dead probiotic cells in tilapia *Oreochromis niloticus*. Fish Sci 72, 755-766.

Telli, G.S., Ranzani-Paiva, M.J.T., Dias, D.D.C., Sussel, F.R., Ishikawa, C.M., et al. (2014) Dietary administration of *Bacillus subtilis* on hematology and nonspecific immunity of Nile tilapia *Oreochromis niloticus* raised at different stocking densities. Fish Shellfish Immunol 39, 305-311.

Thy, H.T.T., Tri, N.N., Quy, O.M., Fotedar, R., Kannika, K., et al. (2017) Effects of the dietary supplementation of mixed probiotic spores of *Bacillus amyloliquefaciens* 54A, and *Bacillus pumilus* 47B on growth, innate immunity and stress responses of striped catfish (*Pangasianodon hypophthalmus*). Fish Shellfish Immun 60(1), 391-399.

Ullah, A., Zuberi, A., Ahmad, M., Bashir, A., Younus, N., et al. (2018) Dietary administration of the commercially available probiotics enhanced the survival, growth, and innate immune responses in Mori (*Cirrhinus mrigala*) in a natural earthen polyculture system. Fish Shellfish Immun 72, 266-272.

Varela, J.L., Ruiz-Jarabo, I., Vargas-Chacoff, L., Arijo, S., León-Rubio, J.M., et al. (2010) Dietary administration of probiotic Pdp11 promotes growth and improves stress tolerance to high stocking density in gilthead seabream *Sparus auratus*. Aquaculture 309(1-4), 265-271.

Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete, W. (2000) Probiotic Bacteria as Biological Control Agents in Aquaculture. Microbiol Mol Biol Rev 64(4), 655-671.

Wang, Y.B., Li, J.R., Lin, J. (2008) Probiotics in aquaculture: challenges and outlook. Aquaculture 281, 1-4.

Wells, R.M.G., Baldwin, J. (1990) Oxygen transport potential in tropical reef fish with special reference to blood viscosity and haematocrit. J Exp Mar Biol Ecol 141(2-3), 131-143.

Wells, R.M.G., Weber, R. (1991) Is there an optimal haematocrit for rainbow trout, *Oncorhynchus mykiss* (Walbaum)? An interpretation of recent data based on blood viscosity measurements. J Fish Biol 38(1), 53-65.

Yokoyama, S., Koshio, S., Takakura, N., Oshida, K., Ishikawa, M., et al. (2005) Dietary bovine lactoferrin enhances tolerance to high temperature stress in Japanese flounder, *Paralichthys olivaceus*. Aquaculture 249, 367-373.

Nguyen, A.T., Hien, L.T. (2015) Study of motion sickness incidence in ship motion. Sci Tech Dev J 18(4), 102-109.