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Research Application Summary

Effect of dietary spirulina supplements on growth, haematological factors and survival of *Oreochromis shiranus* challenged with *Aeromonas hydrophila*

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Abstract

A study was conducted to determine the effect of spirulina (*Arthrospira platensis*) on growth performance and resistance of *Oreochromis shiranus* to *Aeromonas hydrophila* infection. Four diets containing 0, 3.5, 7.0 and 10.5 g spirulina/kg of the diet were fed to *O. shiranus* fingerlings twice daily for 10 weeks. After the feeding trial, fish from each treatment were divided into two groups. The first group was challenged with 0.1 ml dose of $5x10^5$cells/ml of *A. hydrophila* while the second group was given 0.1 ml of saline solution as a control and observed for 10 days. Fish fed with 7g spirulina/kg of basal diet recorded the highest growth in terms of weight gain, gain in length and specific growth rate. The highest mean total feed intake for the entire experimental period was observed in fish fed with 7g spirulina/kg. Fish in the control treatment had the highest Feed Conversion Ratio (FCR) while Treatment 3 (7g spirulina/kg) recorded the least FCR. The control treatment had the least Protein Efficiency Ratio (PER) while Treatment 3 (7g spirulina/kg) recorded the highest PER. The lowest mean survival rate was observed in the control diet. Hematological parameter of the fish increased as spirulina supplement levels increased. Cumulative mortality rate for the fish fed with the control diet was significantly higher (66.67%) than those of fish fed diets 2–4 ($P < 0.05$). The results of the present study indicate that incorporation of spirulina into the formulated diet promote immune responses in *O. shiranus* thereby increasing the resistance of the fish to *A.hydrophila* infection, hence reducing fish mortality and contributing to economic benefits.

Key words: *Aeromonas hydrophila, Arthrospira platensis, Oreochromis shiranus*

Résumé

La présente étude a été menée pour déterminer l'effet de la spiruline (*Arthrospira platensis*) sur la croissance et la résistance d'*Oreochromis shiranus* à l'infection par *Aeromonas hydrophila*. Quatre régimes contenant 0, 3, 5, 7 et 10,5g de spiruline / kg d'aliment ont été employés sur les alevins d'*O. Shiranus* deux fois par jour pendant 10 semaines. Après l'essai d'alimentation, les poissons de chaque traitement ont été divisés en deux groupes. Le premier groupe a été testé avec une dose de 0,1 ml de 5 x 105 cellules / ml d'*A. hydrophila*

tandis que le second groupe a reçu 0,1 ml de solution saline comme témoin et a été observé pendant 10 jours. Les poissons nourris avec 7 g de spiruline / kg de régime de base ont enregistré la plus forte croissance en termes de prise de poids, de gain de longueur et de taux de croissance spécifique. L'apport alimentaire total moyen le plus élevé pour toute la période expérimentale a été observé chez des poissons nourris avec 7 g de spiruline/kg d'aliment. Les poissons du traitement témoin avaient le ratio de conversion alimentaire (RCA) le plus élevé, tandis que le traitement de 7 g de spiruline par kg d'aliment avait donné le RCA le moins élevé. Le traitement témoin avait le rapport efficacité-protéine le plus faible (REP), tandis que le traitement de 7 g de spiruline par kg d'aliment avait enregistré le REP le plus élevé. Le taux moyen de survie le plus faible a été observé pour le traitement témoin. Le paramètre hématologique du poisson a augmenté au fur et à mesure que les niveaux de supplément de spiruline augmentaient. Le taux cumulé de mortalité des poissons nourris avec le régime témoin était significativement plus élevé (66,67%) que celui des poissons nourris avec des régimes 2 à 4 ($P < 0,05$). Ces résultats indiquent que l'incorporation de spiruline dans le régime de formulation renforce les réponses immunitaires chez *O. shiranus*, augmentant ainsi la résistance du poisson à l'infestation par *A.hydrophila*, réduisant donc la mortalité des poissons et apportant des avantages économiques.

Mots clés: *Aeromonas hydrophila*, *Arthrospira platensis*, *Oreochromis shiranus*

Background

Aquaculture has been reported to be one of the most rapidly growing animal food-producing sectors and has become one of the major economic activities in several nations (Samal *et al.,* 2014). It has been reported that tilapias are currently the second largest farmed finfish group in the world (FAO, 2005). The rapid development of aquaculture accompanied with intensive production systems has resulted into stress conditions which provide favorable environment for pathogenic bacterial growth (Subasinghe, 2005). This has led to economic loss due to severe mortality of tilapia fish. Although aquaculture is an industrial sector that has grown extremely fast in the last two decades, improving fish growth performance and disease resistance of cultured fish has remained a major challenge facing fish farmers (Bai *et al*., 2014). In this context, bacterial diseases are a major hindrance in the intensive production systems of cultured fish (Abdel-Tawwab *et al.,* 2008). *Aeromonas hydrophila* is a gram negative opportunist bacterium found worldwide and it causes disease in several freshwater fish species, for example, in cyprinid as explained by Bailone *et al.* (2010).

Antibiotics have frequently been utilized in aquaculture to protect the fish against *A. hydrophila* and other bacterial diseases but this practice has resulted into bacterial resistance and residual accumulation in fish tissue (Garcia *et al.,* 2007). Therefore the use of antibiotics in the control of fish diseases should be restricted due to the development of drug-resistant bacteria and concerns about environmental hazards and food safety (Kim *et al.,* 2013). Aquaculture industry is likely to benefit more if fish under culture system were conferred with enhanced growth performance, feed efficiency and disease resistance without environmental conflicts (Ragap *et al.,* 2012). The addition of probiotics such as spirulina in

the diet of cultured fish may contribute to reduction in cost and the use of chemicals and antibiotics to control diseases in aquaculture (Gudmundsdottir and Bjornsdottir, 2007).

Several studies (Abdel-Tawwab *et al*., 2008; Ragap *et al.,* 2012; Bai *et al*., 2014) on the use of spirulina as dietary supplement for different cultured fish species have shown positive effects on growth performance, feed utilization, hematological parameters and disease resistance against *A. hydrophila*. However, research on diets optimization to enhance fish health and production has not been exploited in Malawi. *Oreochromis shiranus* is one of the most important fish species for aquaculture in Malawi. Given that this species is very important for aquaculture in Malawi, a feeding trial was undertaken to examine the effects of dietary spirulina supplementation on its growth performance and disease resistance against *A. hydrophila* infection. The objective of the study was to investigate the effect of dietary spirulina supplements on growth performance and resistance of *O. shiranus to A. hydrophila* infection.

Study description

The study was conducted in a wet laboratory at Bunda Campus, Department of Aquaculture and Fisheries Science, Lilongwe University of Agriculture and Natural Resources, Lilongwe, Malawi. The fish were stocked into two 500 litre-fiber glass tanks and acclimatized for a period of one week. The fish were maintained on a basal diet containing 30.4% CP which was fed at 5% body weight during the one week period. The research set up consisted of 12 rectangular fiber glass tanks (90 x 54 cm; water depth 30 cm; water volume 146 litres), and three reservoir tanks for water aging. A total of two hundred and forty fingerlings (240), initially weighing an average of 5.86±0.06g and total length 73.07±0.3mm (Mean ± SD) were randomly distributed into 12 tanks each at a stocking rate of 20 fish per tank. Each tank was adequately aerated with compressed air via air-stones using the air pumps. Fish were cultured at room temperature under ambient photoperiod. There were four treatments that were laid out in a completely randomized design. Each treatment was replicated four times. The treatments were four different dietary levels of spirulina doses ranging from 0 (control), 3.5, 7.0 and 10.5g per kg. Each level of the diet was hand-fed to the fish in the corresponding treatment tanks twice daily (at 08.00 and 17.00hrs) for a period of 10 weeks. The feeding rate was at 5% of live body weight for the entire experimental period. Ten fish were randomly picked from each tank and each fish weighed individually to the nearest 0.1g every 2 weeks. The amount of feed given was then adjusted according to average weights of the fish after every two weeks. After the feeding trial, fish from each treatment were divided into two groups. The first group was challenged with 0.1 ml dose of $5x10^5$cells/ml of *A. hydrophila* while the second group was given 0.1 ml of saline solution as a control by intraperitoneal (IP) injection via the caudal vessels using disposable syringes and observed for 10 days.

Results and discussion

Fish fed with 7g spirulina/kg of basal diet recorded the highest growth in terms of weight gain, gain in length and specific growth rate. This was followed by fish fed with 10g spirulina/

kg of basal diet whereas the control diet produced the least growth performance ($p<0.05$). The highest mean total feed intake for the entire experimental period was observed in fish fed with 7g Spirulina/kg, followed by that of 3.5g spirulina/kg and 10g spirulina/kg of basal diet. The control treatment had the lowest mean total feed intake which was also significantly different ($p<0.05$) from the other treatments. Fish in the control treatment had the highest Feed Conversion Ratio (FCR) while Treatment 3 (7g spirulina/kg) recorded the least FCR (Table 1). The control treatment had the least Protein Efficiency Ratio (PER) while Treatment 3 (7g spirulina/kg) recorded the highest PER. The lowest mean survival rate was observed in the control diet. Hematological parameter of the fish increased as spirulina supplement levels increased. Blood glucose concentration increased with increase in spirulina levels and the highest values were obtained at 10.5g spirulina/kg diet. The highest value for red blood cells (RBC) and white blood cells (WBC) counts ware observed at 10.5g spirulina/kg of basal diet. The mean number of days to first mortality after *A. hydrophila* challenge was 1, 3, 4 and 5 for the fish fed the control diet, 3.5g, 7.0g and 10.5g spirulina/kg of basal diet, respectively. Cumulative mortality rate for the fish fed with the control diet was significantly higher (66.67%) than those of fish fed diets 2–4 ($p< 0.05$).

The results of this study have shown that dietary spirulina supplements have a positive impact on the growth performance in terms of final body weight, weight gain, specific growth rate and increase in length. The higher growth rate is likely to be due to the improved feed intake as the level of dietary spirulina increases. Furthermore, spirulina improves nutrient digestibility and it contains several nutrients such as protein, lipids, vitamins and minerals that promote growth (Krishnaveni *et al*., 2013). It is, however, interesting to note that fish fed on 7g spirulina/kg of basal diet had higher growth rate than fish fed on the highest level of spirulina (10.5g/kg basal diet) in the present study. The negative effects of higher levels of spirulina dietary inclusion on fish growth can be attributed to insufficient phosphorous and decreased feed palatability (Kim *et al.,* 2013). The dietary administration of spirulina in the present study significantly increased the survival rate compared to fish fed the control diet. These results suggest that spirulina may have enhanced the nonspecific immune system of *O. shiranus* making the fish to generally become more resistant to disease infection. This could also be due to the stimulation of the host immunological responses and the expression of some immune-related genes (Hamdan *et al.,* 2016).

Haematological analysis of blood often provides important information that can be used to assess the health of cultured fish (Ayoade *et al*., 2014). The analysis also provides useful indices of dietary sufficiency, pathological status and physiological response to environmental stress (Vazquez and Guerrero, 2007). The present study focused on the effects of spirulina supplemented diet on the hematological parameters of the fish before being infected by *A. hydrophila.* The present study results show that fish fed on diets containing spirulina exhibited higher blood glucose concentration and higher RBCs and WBCs counts when compared with fish fed on the control diet. It has been reported that blood glucose levels decrease when the fish are unhealthy (Harikrishnan *et al*., 2003). Therefore, the increase in blood glucose with the level of dietary spirulina is an indication of better health in fish as influenced by spirulina supplemented diet in the present study. According to Bailone *et al. (*2010), a decrease in the RBC number and hemoglobin concentration may be as a result of a bacterial

Table 1. Growth performances of *O. shiranus* (Mean ± SE) fed experimental diets containing different levels of spirulina for 10 weeks

Parameters	Levels of spirulina (g/kg of basal diet)				Contrast		
	T 1 Control (0.0)	T 2 (3.5 g/kg)	T 3 (7 g/kg)	T 4 (10.5 g/kg)	Linear	Quadratic	cubic
Initial weight (g)	5.98±0.08[a]	6.03±0.10[a]	5.72±0.09[a]	5.73±0.16[a]	ns	ns	ns
Final weight (g)	11.23±0.27[a]	12.97±0.17[b]	14.68±0.05[c]	13.65±0.67[b]	*	*	ns
Weight gain (g)	5.25±0.27[a]	6.94±0.11[b]	8.96±0.04[c]	7.93±0.54[b]	*	*	*
Initial total length (mm)	73.53±0.19[a]	73.90±0.44[a]	72.53±0.53[a]	72.32±0.80[a]	ns	ns	ns
Final total length (mm)	89.40±1.31[a]	94.97±0.33[b]	98.97±0.28[b]	95.93±1.97[b]	*	*	ns
Gain in length (mm)	15.87±1.29[a]	21.07±0.32[b]	26.43±0.49[c]	23.62±1.43[bc]	*	*	ns
Specific growth rate (SGR) (%/day)	0.35±0.01[a]	0.43±0.01[b]	0.52±0.01[d]	0.48±0.02[c]	*	*	*

infection. Likewise, a decrease in the number of RBC and hematocrit percentage suggests that RBC were destroyed by leukocytic activity. Since higher RBCs and WBCs counts is an indication of good health (Abdel-Tawwab *et al*., 2008), it can be suggested that spirulina supplemented diet had a positive effects on the health of fish in the present study.

The present study show an increase in hematocrit value with increase in the levels of spirulina diets, which is an indication of improvement in the fish health. Low hematocrit values are said to cause reduction in fish activity and are possibly the consequence of anemia and haemodilution caused by massive erythrocytosis (Ayoade *et al*., 2014). Therefore higher hematocrit values suggest better health in fish. The results of the challenge test indicate that spirulina inclusion in the formulated diet provide a significant level of protection of *O. shiranus* against *A. hydrophila.* The results indicated a decrease in fish mortality with increase in the level of spirulina doses. It has been reported that spirulina has several bioactive pigments such as phycocyanin, phycobilins, xanthophylls and allophycocyanin which are antioxidants (Abdel-tawwab *et al*., 2008; Bai *et al*., 2014). The results of the present study indicate that incorporation of spirulina into the formulated diet promote immune responses in *O. shiranus* thereby increasing the resistance of the fish to *A.hydrophila* infection, hence reducing fish mortality and contributing to economic benefits.

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