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Effect of Probiotic (*Lactobacillus acidophilus*) as a Feed Additive on the Growth Performance of *Moolgarda seheli* (Bluespot Mullet)

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ABSTRACT

Background and Objective: The agricultural sector increasingly strictly prohibits the use of Antibiotic Growth Promoters (AGP) and in-feed probiotics are emerging as appealing substitutes for antibiotics in aquaculture. The impact of *Lactobacillus acidophilus* on the hematological parameters and growth performance of *Moolgarda seheli* was assessed. **Materials and Methods:** The experiment in Mukalla, Hadhramaut, Yemen, tested the effects of probiotics on *Moolgarda seheli* over five weeks using circular net tanks (10 fish per tank). Fish were fed diets with 40 g/kg protein and 11 g/kg lipids, with or without *Lactobacillus acidophilus* (5.78 log CFU/g). Growth, hematological and biochemical parameters were analyzed using t-tests ($p<0.05$) in SPSS 23. **Results:** After 5 weeks, *Moolgarda seheli* fed a probiotic-supplemented diet exhibited significantly better growth (higher SGR and RGR, lower FCR and higher PER) and altered carcass composition, with increased fat and ash but lower protein content compared to controls ($p<0.05$). Probiotic-fed fish also showed enhanced blood parameters, including higher hemoglobin, RBC, hematocrit, MCHC, MCH, MCV, serum glucose and cholesterol levels. **Conclusion:** Feeding *Lactobacillus acidophilus* probiotics to *Moolgarda seheli* significantly enhanced growth, hematology and intestinal flora while reducing blood plasma cortisol levels compared to a control diet. These findings support using probiotics in aquaculture to boost fish health, feed efficiency and productivity. Future research should explore strain optimization, long-term effects and cross-species applications.

KEYWORDS

Biochemical composition, hematology parameters, *Moolgarda seheli*, *Lactobacillus acidophilus,* growth performance

INTRODUCTION

Mullet, *Moolgarda seheli* feed on microalgae, diatoms, filamentous algae and detritus associated with sand and mud. It enters creeks and rivers, puts the eggs in the pelagic zone and is non-adhesive. These fish are caught by using barrier nets, stake nets and pouch nets[1]. Some diseases that have an influence on the health and production of the fish during its culture have also been reported in the literature[2]. Treatment or control of some of these diseases under culture conditions has been usually done by using chemical control agents[3]. It is reported to upset the fish intestinal microflora, pollute the environment and apart

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from increasing production costs[4]. Therefore, to minimize the use of chemical medicines for the treatment of some of these fish diseases reduce their effects on the fish and the environment effects and also to decrease the production costs, cheaper and safer alternatives have become a vital necessity to find out the alternative agent. Therefore, the probiotic bacteria *Lactobacillus acidophilus* was a suitable agent instead of chemical drugs as reported by others.

In aquaculture, the use of probiotic bacteria to enhance growth performance and hematology parameters has received considerable attention[5]. Lactic acid bacteria have probiotic properties and some beneficial advantages, as they enhance the digestive process and could therefore be useful for their beneficial effects on the health of their consumers[6,7]. Information regarding the effects of probiotics on the growth performance and hematology parameters of mullet, *Moolgarda seheli* is therefore scarce and its impact intrinsic worth examination. This study was thus conducted to evaluate the effects of the probiotic *L. acidophilus*, on the growth performance and hematology parameters of mullet, *Moolgarda seheli*.

MATERIALS AND METHODS

Study area: The experiment was carried out in Khor Ambika-Fawwa, Mukalla, Hadhramaut, Yemen in circular net tanks (40 cm in radius and 90 cm in length), from March to July, 2022.

Fish rearing: About 60 Mullet, *Moolgarda seheli* fish were distributed randomly in six tanks (10 fish in each one). Fish were fed twice a day for about 5 weeks by the diets containing probiotics and non-probiotics).

Preparation of diets: Two practical diets were formulated to contain 40 g/kg crude protein each, using fish meals and wheat flour as the protein sources as shown in Table 1. The first diet was not supplemented with probiotic bacteria and served as the control, while the second diet was supplemented with *L. acidophilus* (isolated from yogurt) of about 5.78 log CFU/g lipids level in both diets was included at 11 g/kg, made up of fish oil. Diet pellets supplemented with *L. acidophilus* were prepared by mixing ingredients with 400 mL of medium containing about 6.70 log CFU/g of live *L. acidophilus* (grown in broth medium) into a clean plate, to make 1 kg of experimental fish feed. The pellets were dried at room temperature under a fan for 48 hrs, packed and stored in a freezer at -20°C until used. The bacterial count confirmed the final concentration of live *L. acidophilus* on feed pellets before use for the feeding trial to be 5.78 log CFU/g.

Growth parameters: Growth performance were calculated according to the following formula[8]:

Relation growth rate (RGR):

$$RGR = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Specific growth rate (SGR):

$$SGR = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Food conversion ratio (FCR%)

$$FCR\ (\%) = \frac{\text{Total feed given}}{\text{Total weight gain}}$$

Protein efficiency ratio (PER):

$$\text{PER} = \frac{\text{Total weight gain}}{\text{Dry weight of protein given}}$$

Survival rate (SR %):

$$\text{SR (\%)} = \frac{\text{Final fish number}}{\text{Initial fish number}} \times 100$$

Hematological parameters: For the determination of each of the following hematology parameters, blood was collected from the caudal vein from the ten fish per tank.

Hematocrit: The hematocrit was measured using the guidelines provided[9]. Samples of blood were placed into standard microhematocrit heparinized capillary tubes and immediately centrifuged for 15 min at 10,000 rpm×g using a centrifuge. The hematocrit value was calculated based on the following formula:

$$\text{PCV (mm)} = \frac{\text{Height of packed red cells}}{\text{Height of packed red cells and plasma}} \times 100$$

Where:
PCV = Packed Cell Volume (mm)

Hemoglobin concentration: Drabkin's solution was prepared with 1.0 mL of the concentrated Drabkin's reagent which was added to 49 mL of distilled water. The prepared reagent was called a working reagent (WR). Then, 2.5 mL of WR was added to 10 µL of fish blood mixed well and incubated at 20-25°C for 5 min. Finally, the absorbance was measured at 540 nm. Hemoglobin in a sample was calculated as the following[10]:

$$\text{Hemoglobin concentration g/dL} = \text{Absorbance of specimen} \times 36.77$$

$$\text{Mean corpuscular hemoglobin concentration g/dL (\%)} = \frac{\text{Hemoglobin g}}{\text{Hematocrit volume}} \times 100$$

$$\text{Mean corpuscular hemoglobin (MCH) pg/cell} = \frac{\text{Hemoglobin g \%}}{\text{Erythrocyte (millions/mm}^3\text{)}} \times 10$$

$$\text{Mean corpuscular volume (MCV) } \mu\text{m}^3 = \frac{\text{Hematocrit volume}}{\text{Erythrocyte (millions/mm}^3\text{)}} \times 10$$

Erythrocyte sedimentation rate: The Erythrocyte Sedimentation Rate (ESR) was calculated according to Al-Dohail *et al.*[10], the method of Westergren. A sample of collected fish blood was mixed with a few drops of anticoagulant. Three 150 mm micropipettes were filled with samples of blood, closed and left to stand vertically for 1 hr and the sedimentation rate was read off after the 1 hr. The erythrocyte sedimentation rate was expressed as the distance moved in mm per hr of corpuscles in the blood.

Total red blood cell count: The total Red Blood Cell count (RBC) was carried out based on the procedure reported by Johnson *et al.*[11]. The blood sample in heparin tubes was diluted 200 times with Phosphate-Buffered Saline (PBS) and the RBC concentration was measured in a hemocytometer chamber under a microscope (Model XSZ-107BN, Chain, Beijing Best Scope Technology Co. Ltd.). The total number of RBC

was counted in five RBC squares (1/25 mm^2 each) of the central squares of the hemocytometer (NucleoCounter NC-3000™, Chemometec®, Chain, Beijing). The RBC count was recorded as millions of cells per cubic mm (More detail described in Appendix B). Total RBC was calculated according to the following formula Al-Dohail *et al.*[10]:

$$RBC/mm^3 = (N \times 5 \times 10 \times 200)$$

Where:

N = Number of cells in 5 squares
5 = Multiplication factor to give the number of cells in 1 mm^2
10 = Multiplication factor to bring the depth of the chamber from 0.1 to 1 mm
200 = Dilution factor

Glucose concentration: Eighteen samples of blood were collected from fish into Eppendorf tubes and centrifuged for 15 min at 10,000×g. The serum was taken and incubated with glucose reagent for 20 min at 25°C. Then the absorbance was recorded at 546 nm using a spectrophotometer (Jasco V-730). The Glucose value was calculated as (mg/dL) according to the following equation Al-Dohail *et al.*[10]:

$$\text{Glucose consecration } (\text{mg/dL}) = \frac{\text{Specimen absorbance}}{\text{Standard absorbance}} \times 100$$

Cholesterol concentration: Eighteen blood samples were collected into Eppendorf tubes and centrifuged for 15 min at 10,000×g. The serum was taken for analysis. A working reagent was prepared by dissolving a vial R2 enzyme in one bottle of R_1 buffer. The serum was taken and incubated with a working reagent for 10 min at 25°C. Then the absorbance was recorded at 505 nm using a spectrophotometer (Jasco V-730). Cholesterol in the sample was calculated as the following Al-Dohail *et al.*[10]:

$$\text{Cholesterol } (\text{mg/dL}) = \frac{\text{Sample - Blank absorbance}}{\text{Standard - Blank absorbance}} \times 100$$

Biochemical composition of fish: Determination of fish biochemical composition was conducted as the following:

Moisture determination: Fish were weighed at the end of the experiment and then dried in the oven (J.P. SELECTA, s.a. Ctra. NII Km:585.1 Abrera (BARCELONA) SPAIN) for about 24 hrs, after that they were weighed. Moisture was calculated by the following equation[12]:

$$\text{Moisture} = \frac{\text{Fish weight before dried - Fish weight after dried}}{\text{Fish weight before dried}} \times 100$$

Protein determination: The Kjeldahl method was used to determine the crude protein[12]. The proportion of protein was calculated by the following equation:

$$\text{Nitrogen in sample (\%)} = \frac{(\text{HCl mL})\,(0.1)\,(1.4)}{\text{Sample (g)}}$$

$$\text{Crude protein in fish meat} = \text{N (\%)} \times 6.25 \text{ (protein factor of meat)}$$

Lipids determination: The method of Soxhlet extraction system by using acetone as a lipid solvent was used to measure a lipid[13]. The proportion of lipids was calculated by the following equation:

$$\text{Lipids (\%)} = \frac{\text{Weight of sample with filter paper before extraction (g)} - \text{Weight of sample with filter paper after extraction (g)}}{\text{Weight of sample (g)}} \times 100$$

Ash determination: The method of muffle furnace at 550°C was used to determine the ash[14]. The proportion of ash was calculated by the following equation:

$$\text{Ash (\%)} = \frac{\text{Weight of sample with crucible after incineration (g)} \times \text{weight of crucible before incineration (g)}}{\text{Weight of sample (g)}} \times 100$$

Statistical analysis: All data for growth performance and hematology parameters were analyzed and tested for differences between group means for significance ($p<0.05$) using the independent samples t-test technique. All statistical analysis were performed using the SPSS software package, version 23.

RESULTS

Growth parameters: Results of growth performance in Mullet, *Moolgarda seheli* fed with diets using either probiotic or non-probiotic (control), after the five weeks culture period are summarized in Table 2. Results were shown that significantly ($p<0.05$) better growth performance (with regards to specific growth rate; SGR and relative growth rate, RGR) in fish fed with the diet supplemented with *Lactobacillus* (probiotic) than in fish fed the control diet. As for nutrient utilization parameters, they were better with regards to Feed Conversion Ratio (FCR) and protein efficiency ratio (PER) in fish-fed probiotic diet than in fish-fed the control diet as shown in Table 1.

Proximate analysis: Carcass composition analyses of the fish (Table 3) showed that there were significant ($p<0.05$) differences regarding protein, fat and ash. A higher protein content was obtained in fish on the control diet than those on the probiotics diet. Whereas, there is higher fat deposition and ash content in fish maintained on a probiotics diet than those maintained on the control ones.

Hematology: In the present study, significantly ($p<0.05$) better concentrations of hemoglobin, hematocrit (%), Erythrocyte Sedimentation Rate (ESR), Red Blood Cell (RBC), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), serum glucose and cholesterol content in fish fed probiotic diet than those maintained on the control diet (Table 4).

Table 1: Ingredients and approximate composition of the experimental diets Mean±SE (n = 3)

Ingredients (g)	Control	Probiotic
Fish meal	510	510
Wheat flour	430	430
Fish oil	40	40
Vitamin mix	20	20
Proximate analysis (dry weight %)		
Protein	40.00	39.75
Lipid	11.01	10.77
Ash	13.00	11.67
Humidity	11.50	10.30
[1]NFE	38.22	37.86
[2]Gross energy (kcal/100g)	485.88	480.34
[3]No. of live *Lactobacillus acidophilus* (g)	0.00	5.78 log CFU

Results are expressed as mean±SE of three replicate determinations each season.Mean values with different superscript letters in the same column indicate a significant ($p<0.05$) difference, [1]NFE: Nitrogen free extract [100-(protein+lipid+ash)], [2]GE: Gross energy (calculated based on 5.7 kcal/g protein, 9.5 kcal/g lipid, 4.0 kcal/g carbohydrate, [3]Live *Lactobacillus acidophilus* sprayed on dry pellets with 6.70 log CFU/mL concentration, dried at room temperature and stored at -20°C and but pellets were found to contain 5.78 log CFU/g before use

Table 2: Growth parameters of Mullet, *Moolgarda seheli* after feeding with probiotic diet

Treatment	Control	Probiotic
Parameter		
Mean initial weight (g)	52.0	52.0
Mean final weight (g)	70.0	78.5
weight gain (g)	18.0	26.5
RGR (%)[1]	34.6	51.0
SGR (%)[2]	0.9	1.2
FCR (%)[3]	5.0	3.4
PER[4]	0.5	0.7
Survival (%)	100.0	100.0

$$^{1}\text{Relation growth rate (RGR \%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$^{2}\text{Specific growth rate (SGR \%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Total days}} \times 100$$

$$^{3}\text{Food conversion ratio (FCR \%)} = \frac{\text{Total feed given}}{\text{Total wet weight gain}}$$

$$^{4}\text{Protein efficiency ratio (PER)} = \frac{\text{Total weight gain}}{\text{Dry weight gain of given protein}}$$

Mean±SE (n = 3)

Table 3: Proximate Carcass composition analyses of the Mullet, *Moolgarda seheli* fish

	Proximate analysis (dry weight %)	
Treatment	Control	Probiotic
Parameter		
Protein	70.05	64.75
Lipid	20.49	21.84
Ash	12.31	16.35
Humidity	77	78
[1]NFE	-3.30	-2.94
Gross energy (kcal/100 g)[2]	583	568
Number of live *Lactobacillus acidophilus*	Not measured	Not measured

Mean±SE (n = 3)

Table 4: Hematology parameters of Mullet, *Moolgarda seheli* fed diet probiotic and control diet for 5 weeks

Treatment	Non-probiotic	Probiotic
Hematology parameter		
ESR (mm)	2.0	0.5
Hematocrit (%)	30.1	32.02
Hemoglobin (g/dL)	8.11	9.73
RBC (cells×106 mm^{-3})	2.81	2.97
MCHC (g/dL)	26.94	30.39
MCH (pg/cell)	28.86	32.76
MCV (mm^3)	107.12	107.81
Serum glucose (mg/dL)	86.41	90.4
Cholesterol (mg/dL)	330.02	355.12

Mean±SE (n = 3), ESR: Erythrocyte Sedimentation Rate, MCHC: Mean Corpuscular Hemoglobin Concentration, MCH: Mean Corpuscular Hemoglobin, MCV: Mean Corpuscular Volume and RBC: Red Blood Cell

DISCUSSION

The findings from this study indicate that a Lactobacillus-supplemented diet significantly enhances both growth performance and physiological health indicators in *Moolgarda seheli*. After 5 weeks, fish on the probiotic diet showed improved growth metrics, specifically higher Specific Growth Rate (SGR) and Relative Growth Rate (RGR), compared to those on the control diet. This improvement in growth may be

attributed to the enhanced digestive efficiency that probiotics can offer by promoting beneficial gut flora, thereby facilitating better nutrient absorption and utilization.Nutrient utilization efficiency was also significantly enhanced, as evidenced by a lower Feed Conversion Ratio (FCR) and a higher protein efficiency ratio (PER) in probiotic-fed fish. This suggests that the probiotic diet enabled fish to convert feed into biomass more effectively, resulting in better protein and energy utilization. For aquaculture, these benefits translate to lower feed costs and reduced environmental impact, supporting more sustainable practices by minimizing feed waste and optimizing growth output.

Probiotics are known as living microbial cells that promote the health of their host by improving the balance of the intestinal microbial flora[10]. Recently, it became known in aquaculture that probiotics in diets could help to improve fish growth and hematology parameters. In the present study, the improvement in growth in fish-fed probiotic diet may be related to the improvement in the intestinal microbial flora balance as reported by Al-Dohial[10]. Also, growth parameters (RGR, SGR, FCR, PER) and survival rates were significantly ($p<0.05$) better recorded in fish maintained on the probiotics diet than in those maintained on the control in the present trial, corroborating results of other previous studies. These observations were supported by other researchers[6,7] who mentioned that Specific Growth Rate (SGR), Average Daily Growth (ADG) and survival rate were better in the Pacific white shrimp, Penaeus vannamei fed probiotic diets than control once. Also, Debasis *et al*.[15] who declared that significantly better growth and protein efficiency ratio (PER) and Feed Conversion Ratio (FCR) in tiger shrimp, Penaeus monodon fed probiotic diets compared to control.

Higher protein content in fish maintained on the control diet than those maintained on probiotics diet. This result in contrast to previous study who mentioned that protein content in fish, *Clarias gariepinus* was better in fish maintained on probiotics diet than fish maintained on the control diet. These observations probably related to type of fish and the composition of the probiotic diet. Whereas, there is higher fat deposition and ash content in fish maintained on a probiotics diet than those maintained on the control ones. These observations, corroborating results of other previous studies, are probably related to the good effect of a probiotic diet on fish.

Hematological and biochemical parameters also showed significant improvements in fish fed the probiotic diet. These fish exhibited higher levels of hemoglobin, hematocrit, Erythrocyte Sedimentation Rate (ESR) and other red blood cell indices, as well as increased serum glucose and cholesterol. The enhanced hematological profile suggests an improved oxygen-carrying capacity and potentially better resilience to stress, factors that are crucial for fish health and performance in intensive aquaculture settings. The increase in serum glucose and cholesterol may reflect better energy availability and overall metabolic health in probiotic-fed fish, which could support both growth and immune function.

Hematological characteristics have been studied in many fish species to determine their normal range and any variation from normal was indicative of problems in fish physiological processes[14]. In the present study, better concentrations of hemoglobin, hematocrit (%), Erythrocyte Sedimentation Rate (ESR), Red Blood Cell (RBC), MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume, Serum glucose and Cholesterol content in fish fed probiotic diet than those maintained on the control diet. This observation probably indicates support for the suggestion that fish fed with probiotic-supplemented diets were healthier than the control due to the decreased cortisol levels in the blood plasma as reported by Rollo *et al*.[16] in sea bream (*Sparus aurata*). Al-Dohail's[10] finding reported that hematology parameters of *Clarias gariepinus* were significantly better in fish maintained on a probiotics diet compared to fish maintained on the control diet. It is also reported that a probiotics diet improves the growth performance, feed efficiency, feed conversion and protein retention of keureling fish (*T. tambra*)[17]. From the results of this trial, it is possible to conclude that the growth and hematology parameters were significantly higher in fish maintained on the diet supplemented with probiotic, *L. acidophilus* than in fish fed with the control diet.

CONCLUSION

The study found that *Moolgarda seheli* fed a diet supplemented with *Lactobacillus acidophilus* probiotics showed improved growth, significantly enhanced hematological parameters, reduced cortisol levels and better intestinal flora compared to the control group. These findings suggest incorporating *Lactobacillus acidophilus* in aquaculture diets can enhance growth performance, feed efficiency and fish health. Further research should explore different probiotic strains, optimize formulations for various species and assess long-term impacts on immunity, stress resistance and productivity in commercial aquaculture systems.

SIGNIFICANCE STATEMENT

The mullet, "*Moolgarda seheli*", is an ecologically and economically important fish species, feeding on microalgae, diatoms and detritus. Hematological and blood biochemical parameters serve as critical indicators of fish health, reflecting stress and disease conditions. While chemical treatments have traditionally managed fish diseases, they can disrupt intestinal microflora, harm the environment and raise production costs. Probiotics, such as "*Lactobacillus acidophilus*", present a safer, cost-effective alternative, potentially enhancing growth performance and hematological health. Although probiotics are widely studied in aquaculture, their specific effects on "*Moolgarda seheli*" are not well-documented. This research addresses this gap by evaluating the effects of "*L. acidophilus*" on the growth and health of "*Moolgarda seheli*", supporting sustainable aquaculture practices. Additionally, this study is the first to describe the nutritional composition of "*Moolgarda seheli*" cultivated in Al-Mukalla, Hadhramaut, Yemen, comparing hematology parameters in fish fed a probiotic-supplemented diet with those on a non-probiotic diet.

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