



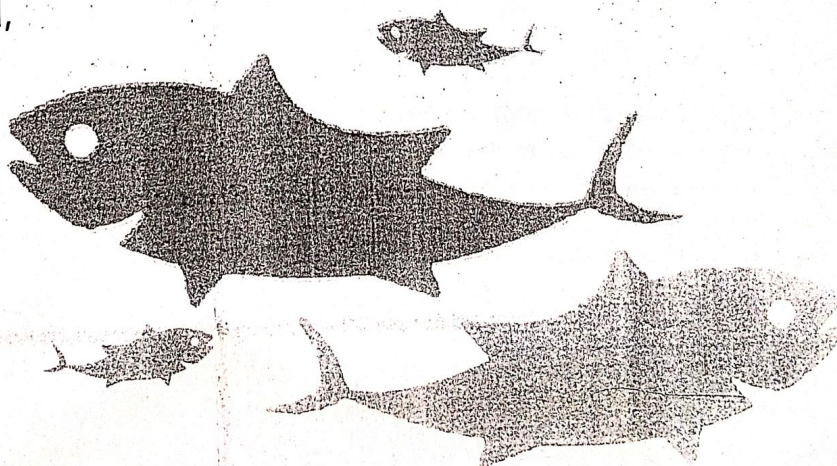
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Growth Performance and Nutrients Utilization of *Macrobrachium vollenhovenii* Fed Diets Supplemented with *Lactobacillus acidophilus*

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Abstract

Performance (growth and nutrient utilisation) of *Macrobrachium vollenhovenii* fed diets supplemented with *Lactobacillus acidophilus* were evaluated. Six diets (43% crude protein) were prepared with *L. acidophilus* which comprised 0, 10¹, 10², 10³, 10⁴, and 10⁵ CFU/mL fed to 360 *M. vollenhovenii* (10.02±0.02 g) to satiation for 84 days. Weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR) and feed intake (FI) were determined using standard methods. Data were analysed using descriptive statistics and ANOVA at p=0.05. The result showed that WG, SGR, PER, FCR and FI were significantly different among the treatments (p < 0.05). Highest growth was recorded in prawns fed 10⁴ cfu/mL *L. acidophilus* based diet. Therefore, dietary 10⁴ cfu/mL *L. acidophilus* could enhance growth and nutrients utilization of *M. vollenhovenii*.

Keywords:

Lactobacillus acidophilus;
Macrobrachium vollenhovenii; growth, nutrients; prawn; performance

Introduction

The current increase in the consumption of products from aquaculture than wild has justified the tremendous growth recorded in the sector in recent times. Thus, aquaculture has become the fastest growing sector (FAO, 2016). This achievement is unconnected with intensive farming system with improved technology currently adopted by the farmers, however, feed remains one of the major challenges of the sector. Omitoyin *et al.* (2006) reported that feed represents about 50 to 60% of total production, therefore, the need to produce feed that would enhance growth within possible time without deleterious effect on the quality and the well-being of the fish.

Macrobrachium vollenhovenii belongs to genus *Macrobrachium* in the family of Palaemonidae. It is generally recognised as an economic important prawn throughout the world. *Macrobrachium vollenhovenii* is found in freshwater body such as lakes, rivers, lagoon and ponds in Nigeria and most freshwater body in West Africa. Out of 24 identified species in the genus *Macrobrachium*, only 13 have been documented in Australia and 4 have been documented in Nigeria (New, 2000). However, *Macrobrachium vollenhovenii* is regarded as most economically important species due to its abundance and ability to attain larger size (New, 2000).

Against this background, synthetic chemicals are being used to improve growth in prawn farming. However, use of chemicals in aquaculture as growth promoters has become contentious, due to rise of drug resistant bacteria, residual effects and restraint the immune system of the fish (Ulukoy *et al.*, 2017)

leading to search for alternatives such as probiotics. Recently, there were several studies that evaluated the applicability and suitability of different materials (plants, animals and bacteria) in fish diets as total/partial replacement for conventional ingredients or as additives (Addo *et al.*, 2017; Adeshina *et al.*, 2017; Adeshina & Adewale, 2018). To this extent, evaluation of unconventional ingredients such as probiotics are been evaluated with positive results recorded so far.

Probiotics are life microbial feed supplements that improve host's health by modifying the gastrointestinal tract of the fish. Several probiotics have been used in aquaculture but probiotics from lactic acid bacteria (LAB) and *Bacillus* species are often used (Muno-Atienza *et al.*, 2013; Ulukoy *et al.*, 2017). *Lactobacillus acidophilus* is bacteria belonging to the genus *Bacillus*. Its probiotics benefit as member of LAB group in supporting the growth of *Macrobrachium vollohovenii* has not been fully elucidated hence the need for this study. Therefore, this study investigated the effect of diets fortified with *Lactobacillus acidophilus* on growth performance and nutrients utilization of *Macrobrachium vollohovenii*.

Materials and Methods

Preparation of Experimental Diets

Feed ingredients were purchased from a reliable store in Melbourne, Australia. *Lactobacillus acidophilus* ATCC 4356 was obtained from Biotechnology Laboratory, Deakin University, Australia and prepared into 0, 10¹, 10², 10³, 10⁴ and 10⁵ cfu/mL (Table 1). Soya bean was further prepared by toasting in a hot plate for 15 minutes at 100°C. Ingredients were ground in hammer mill and mixed together to formulate 43% crude protein (Table 1) calculated according to Pearson Square Method (FAO, 1990). The mixed ingredients were pelleted through pelleting machine (Model: FOB 9001). The feeds were packed in polythene bags and stored at -4°C until use with labels on them. The feeds were reproduced fortnightly. The crude protein of the ingredients were: fish meal (72%); toasted soybean (46.2%) and white maize (9.3%) (AOAC, 2005). The feeds were reproduced after 21 days to avoid nutrients depletion.

Table 1: Ingredients and chemical composition (%; on dry matter basis) of experimental diets containing graded levels of *Lactobacillus acidophilus*

Ingredients (%)	<i>Lactobacillus acidophilus</i> inclusion levels cfu/mL					
Groundnut cake	25.00	25.00	25.00	25.00	25.00	25.00
Soya meal	24.00	24.00	24.00	24.00	24.00	24.00
Fish meal	22.15	22.15	22.15	22.15	22.15	22.15
DCP	1.00	1.00	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00	1.00	1.00
Flour	0.89	0.89	0.89	0.89	0.89	0.89
Vegetable oil	0.50	0.50	0.50	0.50	0.50	0.50
Maize	24.96	24.96	24.96	24.96	24.96	24.96
Premix	0.50	0.50	0.50	0.50	0.50	0.50
LA	0	10 ¹	10 ²	10 ³	10 ⁴	10 ⁵
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition						
Moisture	9.16	9.16	9.16	9.16	9.16	9.16
Crude protein	40.00	40.04	40.10	40.11	40.12	40.12
Ether extract	9.63	9.63	9.54	9.63	9.66	9.65
Total ash	8.27	8.27	8.27	8.27	8.27	8.27
Carbohydrates	20.17	20.13	20.16	20.06	20.02	20.03
Crude fibre	12.77	12.77	12.77	12.77	12.77	12.77

Note: DCP = Dicalcium phosphate; LA = *Lactobacillus acidophilus*

* Premixes = HI-MIX®AQUA (Fish) each one kilogram (1 kg) contains; vitamin A, 4,000,000 International Unit (IU); vitamin D3, 8,00,000 IU; vitamin E, 40, 000 IU; vitamin K3, 1,600 mg; vitamin B1, 4,000 mg; vitamin B2, 3,000 mg; vitamin B6, 3,800 mg; vitamin B12, 3 mcg; Nicotinic acid, 18000 mg; Pantothenic acid, 8000 mg; Folic acid, 800 mg; Biotin, 100 mcg; Choline chloride, 120,000 mg; Iron, 8000 mg; Copper, 800 mg; Manganese, 6000 mg; Zinc, 20,000 mg; Iodine, 400 mg; Selenium, 40 mg; Vitamin C (coated), 60,000 mg; Inositol, 10,000 mg; Colbat, 150 mg; Lysine, 10,000 mg; Methionine, 10,000 mg; Antioxidant, 25,000 mg.

Experimental Design and Procedure

Macrobrachium vollenhovenii juveniles (mean weight = 10.02 ± 0.02 g; $n=360$; 180 prawns for each pathogen) were obtained from a reputable farm in Melbourne, Australia and acclimatized for two weeks in glass aquaria tanks before the experiment in the School of Life and Environmental Science, Faculty of Science, Engineering and Built Environment, Deakin University, Australia. Prawns were weighed and distributed into 18 glass aquaria tanks ($35 \times 30 \times 20$ cm³) in a completely randomised design with three replicates. Each tank contained twenty prawns. The tanks were constantly connected to aerator (Model: AP-60) with air blowers. The prawns were fed experimental diets for 12 weeks. The diets were fed to the *Macrobrachium vollenhovenii* to satiation throughout the experimentation. Measurements of the weight changes were measured using sensitive scale (Model: M1207).

Determination of Proximate Composition of Experimental Diets and Fish

Experimental prawn samples were subjected to proximate analysis. Moisture, ash, crude protein, crude lipid, crude fibre and nitrogen-free extracts contents were recorded using various analytical methods (AOAC, 2005). The moisture contents were determined by preweighing the samples and air-drying in a hot air-oven. The final weight was subtracted from the initial weight. Crude protein was determined using micro-Kjeldahl distillation method. The percentage protein was calculated by multiplying the nitrogen content of the sample by a factor of 6.25. Ash content was determined by burning the samples in a muffle furnace at 550°C for three hours. The samples were allowed to cool, weighed and expressed as percentage content. Ether extract was determined in a soxhlet extractor using petroleum ether ($40-60^\circ\text{C}$) for three hours. The solvents were evaporated and the ether extract was determined as the residue obtained. Crude fibre was achieved by subjecting the residual sample from the ether extraction to a successive treatment with boiling acid (0.25N sulphuric acid) and alkali of defined concentration (0.313N sodium hydroxide) under controlled condition. Also, nitrogen free extract was determined using the following expression as described by AOAC (2005).

Evaluation of Growth Performance and Nutrients Utilization of Experimental Prawn

After the feeding trial (the 12th week), prawns were collected, counted, and bulk-weighed. Growth performance was determined and feed utilization was calculated as follows:

$$\text{Weight gain (\%)} = \frac{W_2 - W_1}{W_1} \times 100; \text{ where } W_2 \text{ is final weight } W_1 \text{ is initial weight}$$

$$\text{Specific growth rate (SGR, \%g/day)} = \frac{[\ln W_2 (\text{g}) - \ln W_1 (\text{g})]}{\text{Experimental period}} \times 100; \text{ where } W_2 \text{ is final weight}$$

W_1 is initial weight, and T is the experimental period (day);

Feed intake (g) = the summation of the offered feed to fish throughout the experiment;

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake}}{\text{Weight gain}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet body weight gain (g)}}{\text{Crude protein fed}}$$

$$\text{Survival rate (SR, \%)} = \frac{\text{Initial number of prawn stocked} - \text{mortality}}{\text{Initial number of prawn stocked}} \times 100$$

Statistical Analysis

The results were presented as mean $\pm$ standard deviation of three replicates. Prior to statistical analysis, all data were tested for normality of distribution using the Kolmogorov–Smirnov test. The homogeneity of variances among different treatments was tested using Bartlett's test. Then, data were subjected to one-way ANOVA to evaluate effects of *Lactobacillus acidophilus* supplementation. Differences between means were tested at the 5% probability level using Duncan test. The optimum *Lactobacillus acidophilus* level was determined using polynomial regression analysis. All the statistical analyses were done using SPSS program version 20 (SPSS, Richmond, VA, USA).

Results

Table 2 depicts the proximate composition of prawn fed diets fortified with *Lactobacillus acidophilus* for 12 weeks. The result showed that there were no significant differences in the crude protein, ether extract, moisture and ash contents of the experimental prawn ($p > 0.05$). However, the highest crude protein was recorded in prawn fed 10^5 cfu/mL *Lactobacillus acidophilus* based diets and the least was obtained in control diet. Ether extract, moisture and ash contents were higher in fish fed control diets and least was recorded in group treated 10^5 cfu/mL.

Table 2: Proximate composition of *Macrobrachium vollenhovenii* fed diets fortified with *Lactobacillus acidophilus* for 12 weeks

<i>Lactobacillus acidophilus</i> inclusion levels (cfu/mL)	Parameters			
	Crude protein	Ether extract (%)	Moisture (%)	Ash (%)
Control	65.47 $\pm$ 2.35	11.63 $\pm$ 1.19	12.69 $\pm$ 1.40	10.21 $\pm$ 0.35
10 ¹	65.92 $\pm$ 2.19	11.60 $\pm$ 1.04	12.14 $\pm$ 0.78	10.34 $\pm$ 0.21
10 ²	66.86 $\pm$ 3.71	11.42 $\pm$ 1.27	10.94 $\pm$ 1.16	10.78 $\pm$ 0.98
10 ³	66.95 $\pm$ 1.11	11.41 $\pm$ 1.33	10.87 $\pm$ 1.09	10.77 $\pm$ 0.15
10 ⁴	67.02 $\pm$ 2.16	11.38 $\pm$ 0.95	10.95 $\pm$ 0.95	10.65 $\pm$ 1.26
10 ⁵	67.24 $\pm$ 3.20	11.38 $\pm$ 1.32	10.86 $\pm$ 1.13	10.52 $\pm$ 0.82

Means with different superscripts within the same column were significantly different ($p < 0.05$), while absence of letters means not significantly different ($P > 0.05$).

Table 3 shows that final weight, weight gain, SGR, PER and FI increased significantly with increase in *Lactobacillus acidophilus* inclusion levels in the diets ($p < 0.05$). Highest final weight, weight gain, SGR, PER and FI were recorded in prawn fed 10^4 cfu/mL and the least were obtained in prawn fed control diet. Furthermore, there were significant reductions in the FCR of the prawn fed experimental diets ($P < 0.05$). The group fed 10^1 cfu/mL had highest FCR while the lowest FCR was obtained in prawn fed 10^5 cfu/mL *Lactobacillus acidophilus* supplemented diets. The relationship between prawn weight gain and dietary *Lactobacillus acidophilus* was expressed by the second-order polynomial regression ($y = -0.0654x^2 + 1.5889x + 1.11$, $R^2 = 0.9434$). From the equation, optimum inclusion level was estimated to be 10^4 cfu/mL of *Lactobacillus acidophilus* (Fig. 1). These further explained the increase in the consumption of the feed by the prawn with respect to increase in the supplement levels.

Table 3: Growth performance and nutrients utilization of *Macrobrachium vollehovenii* fed diets fortified with *Lactobacillus acidophilus* for 12 weeks

Parameters	<i>Lactobacillus acidophilus</i> inclusion levels (cfu/mL)					
	Control	10 ¹	10 ²	10 ³	10 ⁴	10 ⁵
IW (g)	10.02±0.02	10.01±0.02	10.04±0.01	10.02±0.12	10.04±0.05	10.05±0.30
FW (g)	13.07±0.01 ^b	13.22±0.14 ^b	15.61±0.20 ^{ab}	16.33±0.47 ^{ab}	18.02±0.11 ^a	18.01±0.02 ^a
WG (%)	30.4±1.8 ^c	32.1±2.1 ^c	55.5±3.4 ^b	63.0±9.0 ^b	79.5±4.6 ^a	79.2±4.3 ^a
SGR (%g/day)	0.32±0.07 ^c	0.33±0.04 ^c	0.53±0.01 ^b	0.58±0.00 ^b	0.79±0.03 ^a	0.69±0.01 ^a
FCR	2.32±0.12 ^a	2.53±0.20 ^a	2.19±0.01 ^{ab}	1.94±0.05 ^b	1.87±0.13 ^b	1.87±0.11 ^b
PER	0.07±0.00 ^b	0.07±0.01 ^b	0.13±0.01 ^a	0.15±0.02 ^a	0.19±0.01 ^a	0.19±0.01 ^a
FI (g)	7.08±0.62 ^c	8.12±0.58 ^c	12.20±0.73 ^b	12.24±0.28 ^b	14.92±0.54 ^a	14.89±0.46 ^a
SR (%)	96.9	97.8	100.0	100.0	100.0	100.0

Means with different superscripts within the same row were significantly different ($p < 0.05$), while, absence of letters means not significantly different ($P > 0.05$).

IW = Initial weight; FW Final weight; WG = Weight gain; SGR = Specific growth rate; FCR = Feed conversion ratio; PER = Protein efficiency ratio; FI = Feed intake

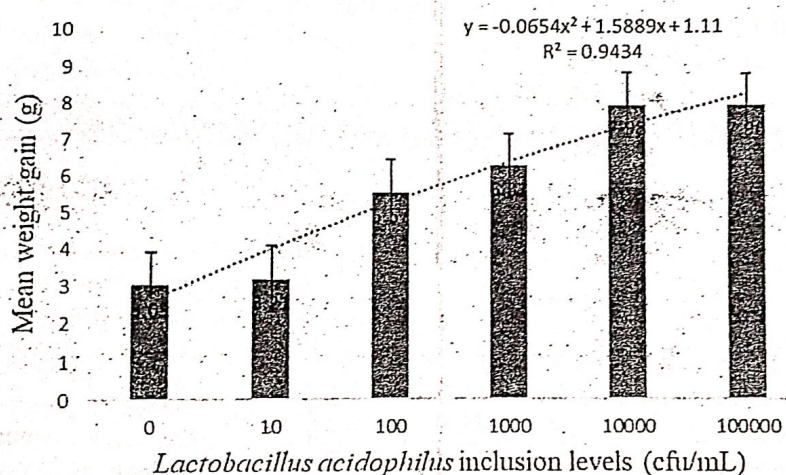


Figure 1: The mean weight gain (g) of *Macrobrachium vollehovenii* fed increasing level of *Lactobacillus acidophilus* for 12 weeks

Discussion

The inclusion of *Lactobacillus acidophilus* in the diets of *Macrobrachium vollehovenii* showed no significant difference in the proximate composition of the prawn. This result was in agreement with the findings of Ehigiator and Nwangwu (2011). Several studies have shown positive results of using probiotics to promote growth in fish. In this study, *Macrobrachium vollehovenii* fed diets supplemented with *Lactobacillus acidophilus* had improved growth. This result similar to the findings of Honsheng (2010) who documented an improved weight gain and specific growth rate in fish fed live yeast. Probiotics have ability to enhance production of digestive enzymes and modify gut ecology to promote better digestion. However, this study disagrees with the findings of El-Rhman *et al.* (2009) who reported no significant difference in the growth of fish fed with supplemented non-pathogenic *Pseudomonas* species for 21 days. The variation in the results may be attributed to cultured period. Apun-Molina *et al.* (2009) observes that significant increase in growth were observed in fish fed

probiotics supplemented diets only after 75 days which is similar to the 84-day culture periods of this study.

The nutrient utilization of the prawn fed supplemented diets were better than the control hence suggests acceptability of the feed by the prawn. The FCR and PER of the groups treated experimental diets were better than what were observed in the group fed control diet. The significantly lower FCR and higher PER observed in prawns fed fortified diets revealed that the feed were better utilized than the control diet. In addition, feed intake of the prawns fed *Lactobacillus acidophilus* based diets were significantly higher than the group fed basal diet. This further suggests the acceptability of the feed to the prawn. Also, it could be inferred that significantly higher growth recorded might not be unconnected to the feed intake. The observation of the study agrees with the findings of Ringo and Gatesoupe (1998) and Lara-flores *et al.* (2003) who reported higher FCR and PER in prawn fed probiotics. Probiotics stimulates protease level, increases food absorption and consequently, improves feed utilisation.

Conclusion

This study reveals that feed supplemented with *Lactobacillus acidophilus* had positive effect on the growth and nutrients utilization in *Macrobrachium vollenhovonii*. Also, its inclusion in diets of *Macrobrachium vollenhovonii* at 10^4 cfu/mL could be used to improve growth and nutrients utilization in prawn aquaculture.

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