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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. acidophillus* which comprised 0, 10^1, 10^2, 10^3, 10^4, and 10^5 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 10^4 cfu/mL *L. acidophillus* based diet. Therefore, dietary 10^4 cfu/mL *L. acidophillus* could enhance growth and nutrients utilization of *M. vollenhovenii.*

Keywords:
Lactobacillus acidophilus; Macrobrachium vollenhovonii; 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 Palaeomonidae. 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, *Macrobrahium vollenhovenii* is regarded as most economically important species due to its abundance and ability to attain larger size (New, 2000).

Against this background, synthetics 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 vollenhovenii* 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 vollenhovenii.*

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, 10^1, 10^2, 10^3, 10^4 and 10^5 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 (%)	*Lactobacillus acidophilus 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 vollenhenvonii 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 x 30 x 20 cm^3) 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 o muffle furnace at 55^0 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 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:

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

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

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

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

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 ± 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 M*acrobrachium vollenhovenii* fed diets fortified with *Lactobacillus acidophilus* for 12 weeks

Lactobacillus acidophilus inclusion levels (cfu/mL)	Parameters			
	Crude protein	Ether extract (%)	Moisture (%)	Ash (%)
Control	65.47±2.35	11.63±1.19	12.69±1.40	10.21±0.35
10^1	65.92±2.19	11.60±1.04	12.14±0.78	10.34±0.21
10^2	66.86±3.71	11.42±1.27	10.94±1.16	10.78±0.98
10^3	66.95±1.11	11.41±1.33	10.87±1.09	10.77±0.15
10^4	67.02±2.16	11.38±0.95	10.95±0.95	10.65±1.26
10^5	67.24±3.20	11.38±1.32	10.86±1.13	10.52±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 vollenhovenii* fed diets fortified with *Lactobacillus acidophilus* for 12 weeks

Parameters	*Lactobacillus acidophilus 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

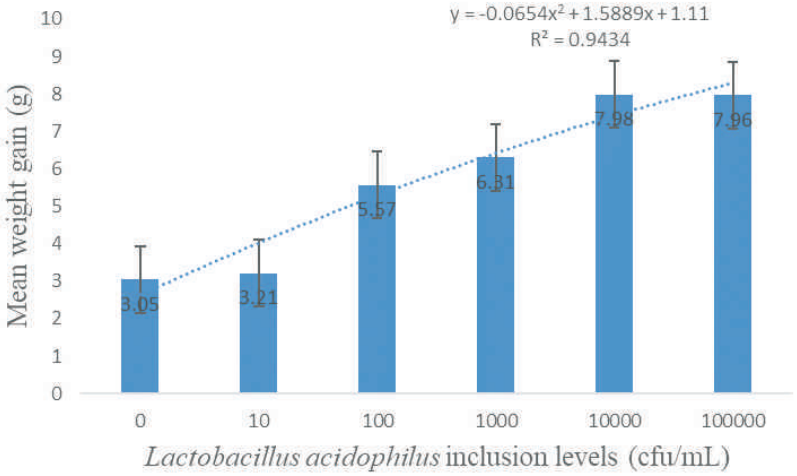

Figure 1: The mean weight gain (g) of *Macrobrachium vollenhovenii* fed increasing level of *Lactobacillus acidophilus* for 12 weeks

Discussion

The inclusion of *Lactobacillus acidophilus* in the diets of *Macrobrachium vollenhovenii* 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 vollenhovonii* 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.

References

Addo, S., Carrias, A. A., Williams, M. A., Liles, M. R., Terhune, J. S. & Davis, D.A. 2017. Effects of *Bacillus subtilis* strains on growth, immune parameters and *Streptococcus iniae* susceptibility in Nile Tilapia, *Oreochromis niloticus*. *Journal of the World Aquaculture Society* 48,:257-267.

Adeshina, I., Jenyo-Oni, A., Emikpe, B. O. & Ajani, E. K. 2018. Effect of solvents on phytoconstituents and Antimicrobial Activities of *Ocimum gratissimum* and *Eugenia caryophyllata* Extracts on Listeria monocytogenes. *Acta Veterinary Eurasia* 44, 31-38.

Adeshina, I., Adewale, Y. A. & Tiamiyu, L. O. 2017. Growth performance and innate immune response of *Clarias gariepinus* infected with *Aeromonas hydrophila* fed diets fortified with *Curcuma longa* leaf. *West African Journal of Applied Ecology* 25, 79–90.

Apun-Molina, J. P., Santamaria-Miranda, A. Luna-Gonzalez, A. Martinez-Diaz, S. F. & Rojas-Contreras, M. 2009. Effect of potential probiotic bacteria growth and survival of tilapia *Oreochromis niloticus* L., cultured in the laboratory under high density and suboptimum temperature. *Aquaculture Research* 40, 887-894.

Association of Official Analytical Chemist (AOAC) 2005. Official Methods of Analysis (18th Edition) Association of Official Analytical- Chemists International, Maryland, USA.

El-Rhman, A. M. A., Khattab, Y. A. & Shalaby, A. M. (2009): *Micrococcus luteus* and *Pseudomonas* species as probiotics for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus*. *Fish and Shellfish Immunology* 27, 175-180.

Food and Agriculture Organization of the United Nations FAO 2016. The state of world fisheries and aquaculture: contributing to food security and nutrition for all, FAO, Rome, Italy, 24.

Honsheng, M. 2010. Effects of probiotics on activities of digestive enzyme for tilapia. Journal of South *China Normal University* 2,10-15.

Lara-Flores, M., Olvera-Novoa, M.A. Guzman-Mendez, B. E. & Lopez-Madrid, W. 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 216, 193-201.

Munoz-Atienza, E., Gomez-Sala, B., Araujo, C., Campanero, C., Del Campo, R. & Hernandez, P.E. 2013. Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic

origin intended for use as probiotics in aquaculture. *BMC Microbiology* 24, 13-15.

New, M.B. 2000. Commercial freshwater prawn farming around the world. In M.B. New & W.C. Valenti, eds. *Freshwater prawn culture: the farming of Macrobrachium rosenbergii*, Blackwell Science, England, 290-325.

Omitoyin, B. O., Ajani, E. K., Adesina, B. T. & Okuagu, C. N. F. 2006. Toxicity of lindane (gamma hexachloro-cyclottexane) to *Clarias gariepinus* (Buchel, 1822). *World Journal of Zoology* 1:57-63.

Protein quality evaluation (Report of Joint FAO/WHO constitution) held in Bethside, M.D., USA, DE, 10-35.

Ringo, E. and Gatesoupe, F. J. 1998. Lactic acid bacteria in fish: a review. *Aquaculture* 160,177-203.

Ulukoy, G., Metin, S., Kubilay, A., Guney, S., Yilidrim, P., Guzel-Seydim, Z., Kok-Tas, T. & Erkan, G. 2017. The effect of kefir as a dietary supplement on nonspecific immune response and disease resistance in juvenile rainbow trout, *oncorhynchus mykiss* (Walbaum 1792). *Journal of the World Aquaculture Society* 48, 248-256.

Ehigiator, F.A.R. and Nwangwu, I. M. 2011. Comparative studies of the proximate composition of three body parts of two freshwater prawns' species from Ovia River, Edo State, Nigeria. *Australian Journal of Basic and Applied Sciences* 5, 2899-2903.

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