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The impact of Fermented Feed on White Leg Shrimp (*Litopenaeus vannamei)* Growth Performance, Feed Utilization, and Immune-oxidative Response in The grow out Ponds

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Abstract

THIS WORK aimed to assess the effects of fermented rice bran containing commercial probiotic bacteria in relation to culture density (150 and 200 shrimp/ m^3) and its effects on *Litopenaeus vannamei's* health, microbiota, immune response, and gene expression. The shrimp (initial weight: 0.86±0.011 g) were randomly divided into three groups, three replicate each. Group I (G-I) was fed only on formulated feed (Control group), Group II (G-II) was fed on 50 % formulated feed and 50 % fermented feed and Group III (G-III) was fed on 100 % fermented feed. Feed modification affected water dissolved oxygen, pH, total ammonia nitrogen (TAN), and nitrite but stocking density significantly affected both pH and TAN. Stocking density and feed fermentation had an impact on the phytoplankton in the culture pond. The maximum number of copepods and phytoplankton was reported in G-III. The highest growth performance was reported in G-I followed by G-II and then G-III. The application of fermented feed improved the antioxidant capacity, immune response and histomorphology of the intestine of *L. vannamei* while increasing stocking density induced a negative impact on shrimp immune-oxidative responses and intestine morphometry. In conclusion, fermented feed may serve as a sustainable substitute for commercial feed in shrimp nutrition, promoting improved growth and feed utilization while boosting stress resilience and enhancing immune-oxidative responses in shrimp.

Keywords: *Litopenaeus vannameias, feed fermentation, growth performance, immunity, micobiota*.

Introduction

According to FAO, 2020 had seen a record-breaking 122.6 million tonnes of aquaculture produced globally, valued at USD 281.5 billion [1] moreover, the worldwide production of White leg shrimp exceeded 5.4 million tons in 2019. The Pacific white shrimp (*Litopenaeus vannamei*), which makes up to more than 70% of the total output worldwide, is the most commercially farmed species of all the shrimp [2]. The relevance of this species emerged as a result of its characteristics and competency. It can deal with a wide range of environmental conditions and stocking rates of up to 400 animals per m^3 [3,4]. Shrimp aquaculture is one of the fastest growing industries of the aquaculture business. However, this progress has also been linked to a rising in health-related problems and a deterioration of the environment [5,6]. It is well recognized that feed quality and amount have a major influence on aquaculture productivity [7].

When the density of shrimp per cubic meter exceeds 100 animals, a number of production outputs, including growth and survival rates, feed conversion ratios, and growth, are compromised [2]. Appropriate management strategies need to be implemented when aquaculture production increases. The water quality and the growing species' ability to grow might both be impacted by the carbon source [8]. This is occurring due to a number of negative consequences, including waste buildup, declining water quality, and cannibalism brought on by inconsistent feed management and insufficient feed accessibility [9,10]. Together with a worldwide fish meal shortage, the cost of feed is

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thought to be the primary cause of marine-farmed species' high market prices [11]. Furthermore, according to Sarker, [7], there may be a shortfall of fishmeal of between 0.4 and 1.32 million metric tonnes by 2050, which would seriously hinder the expansion of the aquaculture sector. Alternative fish meal substitutes in fish feed are becoming more and more crucial due to the decline of fishing sources [12]. Like any other aquatic species, shrimp require a precise and balanced feed calculation to ensure they receive the right amount of protein and to diminish the release of nitrogenous compounds into the culture medium, which is greatly influenced by stocking density [13,14]. Furthermore, growth performance and water quality may be impacted by the change in the carbon supply [8]. As an illustration of how the carbon source might change, rice bran fermentation may increase digestibility while lowering the amount of anti-nutritional factors in the food of aquatic animals [15].

Additionally, including plant-based protein sources into a fish diet increases the prevalence of anti-nutritional factors, which reduces the benefits of the available proteins [16, 17]. Nutritionists are becoming more interested in the fermented diet because of its advantages. Among these benefits is the breakdown of anti-nutritional substances during fermentation, which also produces or enhances useful elements including probiotics, organic acids, short peptides, and flavonoids [18, 19]. In order to increase the nutritional content and digestibility of soybean meal, many probiotic strains have been used during the fermentation process [20]. Additionally, the fermentation process has enhanced *Litopenaeus vannamei*'s non-specific immune response while lessening the detrimental effects of soybean anti-nutritional components [21].

The protein content of rice bran ranges from 10 to 15% [22]. Prior studies have demonstrated that fermenting rice bran can increase its protein content by up to 23% [23]. Because of the fermentation's higher protein content, it may be able to partially substitute fish meal in shrimp diets. Moreover, nothing is known about how well the white leg shrimp, *L. vannamei*, perform in terms of stocking density and feeding on a diet consisting primarily of fermented agricultural waste. Therefore, the primary objective of this work was to assess the effects of fermented rice bran containing commercial probiotic bacteria in relation to the culture density, with particular attention to how these effects affected *L. vannamei*'s health, microbiota, immune system, and gene expression in grow-out ponds.

Material and Methods

Shrimp husbandry and acclimation period

From May to September 2021, the study was carried out in a private marine farm in Borg-El Arab, Alexandria, Egypt. The Ghallioun project in Egypt produced the White leg shrimp, *Litopenaeus vannamei* which had an average weight of 0.86±0.011 g. Prior to the experiment, the shrimp were acclimated to the culture environment for two weeks at 15 part per thousand (PPT) salinity and fed the control diet (40% crude protein) according to the Wabete et al. [24] protocol.

Feed fermentation

The fermented feed was prepared by fermenting the following ingredients: Twenty kilograms of rice bran, one thousand liters of water, one hundred kilograms of molasses, and one hundred grams of commercial probiotics (Sanolife PRO-W®, which includes a blend of Bacillus species, INVE Company Belgium, featuring 10^{11} CFU/gram) were mixed. Sodium bicarbonate was then used to adjust the pH to 7.9. After the mixture had been left to aerate for 48 hours, 500 liters/feddan were added daily, divided equally between feeding times, and the amount was adjusted in accordance with weekly growth monitoring.

Experimental population and treatments

The acclimatized shrimp, weighing 1.004±0.052 g at first, were split into three groups (

TABLE1), with three replicates of each group and a stocking density of 150 and 200 animals/m^3 (one feddan and water capacity 4000 m^3). Group II (G-II) was fed 50% formulated feed with CP=40 % and 50% fermented feed, Group III (G-III) was provided 100% fermented feed, and Group I (G-I) was fed just formulated feed () with CP=40 % (control group). The shrimp were given experimental diets at the following times: 8:00, 10:00, 12:00, 14:00, 16:00, 20:00, and 22:00 h, until they seemed satiated.

Physico-chemical analyses of water

Temperature and dissolved oxygen were recorded twice a day using the OxyGuard Handy Gamma (08:00 am and 3:00 pm). Using a pH meter and refractometer, the pH and salinity were monitored once a day at 8:00 am (Hach Lange HQ 40D). Every day, a portable photometer was used to measure total ammonia nitrogen (NH4+-N) using a spectrophotometric method (Martini MI 405).

Phytoplankton and zooplanktons monitoring

Using a 150-micron net to collect samples and a microscope to examine them, the experiment considered the densities of phytoplankton, copepods, and zooplankton in general. Every week at around 6 or 8 am, water samples are taken for phytoplankton examination. Four distinct locations within each pond provided composite samples, which were then placed in one-liter acid-washed polyethylene containers awaiting examination. Three distinct depths were used to measure the quantity and profile of zooplankton, and aliquots (10 L) were extracted

from the corners and middle of each pond (0.1, 0.5, and 1.0 m). After that, the zooplankton were identified and tallied in a Sedgwick-Rafter chamber using a stereoscope (Wildlife Supply Co. Buffalo, NY, USA). To detect tiny creatures (10 X and 40 X) a microscope was used.

Vibrio spp. and total bacterial count monitoring

Weekly samples were taken for total vibrio and total bacterial count enumeration. For sample examination, Villamil et al. [25] methods were utilized. The total bacterial count (TBC) was tested using nutritional agar (NA), and the total Vibrio count (TVC) was determined using trypticase soy agar (TSA) medium supplemented with 7% NaCl, and the findings were estimated as CFU/mL of water sample.

Growth performance measurements

The shrimp samples were weighted during the experiment, and the body weight gain (BGW) was measured in all groups at the end. According to Abdel-Rahim et al. [26], the values were computed as follows: BWG= W2-W1, and the specific growth rate (SGR) = 100 x [(ln W2- ln W1)/T], where W2 = final weight, W1 = initial weight, T = experiment duration (days), and ln = Natural logarithms. The shrimp body's proximate composition was determined using standard procedures (n=10) [27].

Immunological responses

Lysozyme (LSZ) activities were measured using a turbidimetric approach using *Micrococcus lysodekticus* ATCC No. 4698 (Sigma M 3770) as the substrate, as described by Leaño et al. [28]. The activity of Phenoloxidase was evaluated spectrophotometrically using L-dihydroxyphenylalanine (L-DOPA, Sigma) as substrate, as described by Hernández-López et al. [29].

Antioxidant capacity

The antioxidant capacity of the homogenized larvae was measured using spectrophotometry. Claiborne's [30] method was used to measure catalase (CAT) activity (U/mg protein). Superoxide dismutase (SOD) activity was measured using the method described by Payá et al. [31] with minor modifications made by Peixoto et al. [32]. The ferric reducing antioxidant power (FRAP) technique was used to assess the total antioxidant capacity of larval extract [33]. Glutathione (GSH) activity was measured using the technique described by Maran et al. [34]. Glutathione peroxidase (GPx) activity was determined using the Flohé & Günzler, [35] method. Finally, Lipid peroxidase (Malondialdehyde assays): Peroxidative damage of lipids was determined using of Utley et al. [36] technique.

Cytokines gene expression

Total RNA was isolated from larval homogenate using TRIzol reagents (Invitrogen, UK) according to the manufacturer's recommendations for cytokine gene expression. The reverse transcription (RT-PCR) was carried out using the SYBR green technique in an iQ5 iCycler thermal cycler (Bio-Rad).

On a 96-well plate, 1 µL of diluted (1/20) cDNA, 5 µL of 2x concentrated iQ^{TM} SYBR Green Supermix (Bio-Rad), 0.3 µM forward primer, and 0.3 µM reverse primer were mixed. Table (3) shows the sequences of specific primers used for interleukin 4 (IL-4), interleukin 12 (IL-12) and heat shock protein (HSP) respectively. The thermal profile for all reactions was 3 min at 95 °C and then 45 cycles of 20s at 95 °C, 20s at 60 °C and 20s at 72 °C. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal reference for gene expression data standardization. The $2^{-\Delta\Delta CT}$ method was used to analyze the data [37].

Antioxidant genes expression

Towards the end of experiment, the expression levels of the genes cytosolic manganese superoxide dismutase (cMnSOD), catalase (CAT), and glutathione peroxidase (GPx) were examined. Table 4 lists the primer sequences that were employed specifically for each gene in the current investigation. The housekeeping gene in this case was β-actin. The protocol for the real-time PCR was set to run at 95 °C for one minute, then 40 cycles of 95 °C for 15 seconds, 60 °C for 15 seconds, 72 °C for 45 seconds, and one step of 95 °C for 10 seconds. The double-stranded DNA was denatured by raising the temperature from 65 to 95 °C (0.5 °C/s) in order to get the melting curves. The gene expression was calculated with the comparative *C*T method ($2^{-\Delta\Delta CT}$), and the values mean n-fold difference was compared with the control [37].

Intestinal morphometric and hepatopancreas histological analysis

At the end of the trial, the intestinal tract and hepatopancreas of *L. vannamei* were removed (10 animals/treatment) from the various groups. The samples were preserved for 24 hours in 4% buffered formalin. Tissues were next passed through a series of alcohol solutions (70%, 85%, and 98%) to dehydrate them, and at last they were embedded in paraffin. Hematoxylin and eosin (H&E) were used to stain the 4-5 µm histological sections for general morphological purposes. The sections were then photographed and recorded using an Olympus BX50 microscope (Japan)[40].

Calculations and statistical analysis

Data were checked for normality and homoscedasticity. After that, data were assessed using Graph Pad Prism 7 for two-way analysis of variance (ANOVA) and Tukey's test for post hoc

comparisons (Graph Pad Prism v7.0, San Diego, CA, USA). A significant value was defined as a P-value < 0.05 for all statistical investigations. The results were shown as the replicates' mean and standard error of the mean (SEM) (n = 3).

Results and Discussion

Physico-chemical analyses of water

The effects and interactions between the stocking density and feed modification on the physicochemical characteristics of water were investigated (TABLE 5). A closer examination of the data revealed that the feed modification only exerted a considerable change to all the tested parameters except for temperature. The increased replacement of the diet resulted in a slight reduction in both DO and pH values; however, it resulted in increased concentrations of TAN and nitrite. The highest TAN was 0.485 mg/L in G-III, while nitrite was estimated to be 0.025 mg/L. Nonetheless, there was still dissolved oxygen within the required ideal range of 5.0 mg/L. The highest replacement level was where the ammonia increased. This could be caused by microbial activity of breaking down organic matters and producing byproducts. The stocking density showed a statistically significant effect on both pH value and TAN, while the change on the DO, nitrite, and temperature was not significantly affected by the stocking density. It is important to mention that the most affected parameter was the ammonia concentration, which was increased in response to both feed replacement and stocking density level increases. The change in pH might be due to the accumulation of organic acids produced by microbial activity during fermentation processes, which may be the cause of this condition. A similar pH drop was observed in the fermented soybean meal that was meant to partially replace the fish meal in goldfish [41].

Phytoplankton and zooplanktons monitoring

The main producer in fishponds where zooplankton feed is phytoplankton [42]. Table 6 displays the outcomes of the abundance of phytoplankton and zooplankton. The extent of feed replenishment significantly influenced the numbers reported per individual/L of copepod presence in the culture pond, based on the gathered data. The total was 128 individual / L copepods, with the diet replaced by 100% of the meal, at which point the number of copepods peaked. As the replacement ratio rose, the phytoplankton population grew; however, higher stocking density led to a decline. The impact of stocking density on the number of copepods was minimal. Comparable findings were noted by Silva et al. [43]. De et al. [44] mentioned that the addition of fish waste hydrolysate to *P. vannamei* resulted in enhanced growth performance and survival rates, likely due to increased abundance and diversity of phytoplankton and zooplankton.

Vibrio spp. and total bacterial count monitoring

One common pathogen found in marine water that can infect fish and shellfish is Vibrio spp. [6,45–47]. The feed replacement excreted a statistically significant effect on vibrio and total bacterial count with change happened on the opposite direction. In other words, the increase in feed replacement ratio has increased the total bacterial count while reducing the Vibrio spp. count. Table 7 clearly shows that, among the three treatments, the G-III group had the highest total bacterial count (TBC), but the total vibrio count (TVC) showed the opposite pattern, with the G-III group often having the lowest TVC. This might be because the probiotic strain in use produces an anti-Vibrio compound that inhibits Vibrio spp. or competes with vibrio for resources. Similar evidence of *Bacillus subtilis'* ability to suppress Vibrio spp. was revealed in shrimp culture [48,49].

Growth performance measurements

TABLE 8 display the shrimp's initial body weight (IBW), final body weight (FBW), weight gain (WG), daily weight gain (DWG), and specific growth rate (SGR). A closer look at the statistics in the table shows that the G-I group had the highest weight gain, followed by the G-II group, and the G-III group had the lowest weight gain. The average daily gain (ADG) and specific growth rate of G-I were higher than those of the other groups. When considering the influence of stocking density, the 150/m^3 group FBW, WG, ADG, and SGR were considerably higher than in the 200/m^3 group. The body weight changes of the treatment groups are depicted in **Fig. 1. Weekly development of the average body weight of *L. vannamei* cultivated in two different stocking densities and fed on 3 different formulated diets.** The G-I group showed responses in both densities with the greatest body weight among the three treatments. The results confirm that the basal diet is the best. Similar results were reported by Zhang et al. [50] who found that dietary supplementation of fermented diet with mixed strains improved growth performance and survival rate of shrimp.

Body Proximate composition

Table 9 presents the proximate analysis of the shrimp body following the conclusion of the experiment. The data indicates that the diet change significantly affected the proximate composition. Following the use of fermented rice bran as a 100% replacement, the proportion of carbohydrates and total ash was increased, whereas the proportions of crude protein and fat decreased. In addition to that, the stocking density exerted the same effect on the body composition. The protein and lipid content were lower in the high stocking density, while the carbohydrate and total ash were higher in the high stocking density. Similar results were observed in the study conducted by Qiu & Davis [51].

Immunological responses

Table 10 displays a compilation of the immunological parameters that were tested. Upon closer inspection of the statistical analysis, it reveals a notable increase in THC, phagocytosis, and phagocytic index (Fig. 2) correlated with the rise in the feed replacement ratio. Along with the increase in LSZ, RB and PO activities were stimulated by the heightened diet replacement ratio. However, the measurements mentioned earlier were notably reduced due to the rise in stocking density. The LSZ's response to the analyzed parameters has remained relatively unchanged. Similar findings were recorded by Lin & Chen, [52] and Zhang et al. [50]. **Fig. 2. Phagocytic activity of hepatocytes showing different number of *candida albicans* phagocytes engulfed and digested (Giemsa stain).**This finding is coincided with that reported by Lin & Mui, [21] who found the fermentation process has enhanced *Litopenaeus vannamei*'s non-specific immune response while lessening the detrimental effects of soybean anti-nutritional components.

Antioxidant activities

The activity of antioxidant enzymes is demonstrated in Table 11. In general, there has been no noticeable change in any of the examined antioxidant parameters as the meal replacement increased, except for GPx, which was significantly decreased with total replacement. The stocking density significantly increased the production of MDA and GPx. Despite this, the production of CAT, GSR, TAC, and SOD has all diminished. The relative expression levels of several antioxidant genes, as shown in Table 12, corroborate this. With increasing stocking density, GPx expression rose, while cMn-SOD and CAT expression decreased. The increase in diet replacement displayed the contrary trend, revealing a general rise in the expression of antioxidant genes as the level of diet replacement grew. There was an unexpected outcome in G-II concerning the analyzed antioxidant, however. Increased density might raise oxidative stress because of competition for resources or the buildup of waste products. GPx is recognized as a crucial enzyme in detoxification mechanisms. This result is similar to Zhang et al. [50] who found that dietary supplementation of fermented diet with mixed strains improved antioxidant activity of shrimp.

Cytokines genes expression

TABLE 13 shows the relative gene expression of cytokines in response to an increase in stocking density as well as the feed replacement. There's a significant effect (*P*-value < 0.0001) of stocking density on the expression of all three genes. Expression was decreased with higher stocking density for all genes. The gene expression was increased in response to the increase in the ratio of diet replacement. Similar results were observed in common carp [53] and freshwater cryfish [54].

Intestine morphometric analysis and histological structure

The morphology of the intestine was examined at 90 days (Table 14). At 90 days, shrimp that consumed the G-I diet exhibited the greatest height of intestinal villi, width of villi, crypt depth, and quantity of goblet cells in the intestine (Fig. 3). Shrimp that was given the G-II and G-III diets exhibited reduced villi width and crypt depth, along with fewer goblet cells in the intestine compared to shrimp on diet G I (Fig. 4). Moreover, the shrimp that were given a low stocking density diet (Stocking density 150/1-m3) exhibited greater intestinal villi height, villi width, crypt depth, and goblet cell presence in the intestine when compared to those fed at high stocking density (200 shrimp/m^3) (Fig. 3 & 4). These results could be linked to the beneficial impacts of bacillus species and fermentation

byproducts that may improve intestinal health [55,56].

Hepatopancreas histomorphology

The hepatopancreas in shrimp plays a crucial role in the production and release of immune compounds and digestive enzymes, in addition to nutrient absorption and lipid storage. The hepatopancreas of shrimp in the G-I diet group exhibited the orderly glandular tubular structure typically found in *L. vannamei*. The tubules were tightly packed (Fig. 5). The infiltration of haemocytes was indicated by a significant quantity. The sections of hepatopancreatic tissue from *L. vannamei* that were fed the G-I diet displayed a variety of hepatopancreas tubules. Each tubule has four kinds of cells, namely B-cell (blasenzellen), F-cell (fibrillazellen), R-cell (restzellen) and E-cell (embryonalzellen) (Fig. 5).

The hepatopancreas of shrimp in the G-II diet group exhibited a reduction in the dimensions of hepatopancreas tubules, while other tissue structures appeared mostly normal. Nonetheless, shrimp that received the diet from the G-II group exhibited histological alterations such as abnormal lumens, decreased B-cells and R-cells, ruptured epithelial cells, melanization of cells, and degeneration of tubules (Fig. 6). A minor presence of haemocyte infiltration was noted.

The shrimp from the G-III diet group showed no histological alterations in the size of hepatopancreas tubules, other than a decrease in the number of R-cells (Fig. 6) when compared to those fed G-I and G-II diets. A low infiltration of haemocytes was observed. Comparable findings were observed by Novriadi et al. [57] who discovered that giving shrimp fermented feed might lead to slight alterations in the hepatopancreas, yet it does not influence growth performance.

Conclusion

The present results suggested that fermented feed could be used as an alternative for a sustainable approach to replace commercial feed in shrimp diets to have better growth and feed absorption as well as enhancing stress resistances and increasing immune-oxidative responses of shrimp through enhancing the lysozyme activities, phagocytosis assay and antioxidant enzymes in *L. vannamei*

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Data Availability Statement

All relevant data are available from the authors upon request.

Author contributions

All authors contributed equally to this work (conception, acquisition, samples analysis, statistical analysis, data interpretation, manuscript drafting, and manuscript revision).

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Conflict of interest

The authors declare that there is no conflict of interest.

TABLE1. Experimental design of the trial with different stocking densities

Group	Stocking density 150/1-m^3	Stocking density 200/1-m^3
G-I	600000 shrimp/ feddan	800000 shrimp/ feddan
G-II	600000 shrimp/ feddan	800000 shrimp/ feddan
G-III	600000 shrimp/ feddan	800000 shrimp/ feddan

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TABLE 2. Ingredients and proximate chemical composition (g/kg on dry matter basis) of the control diet

Ingredients	g/kg diet	Analysis	%
Fish meal (72% protein)	300	Dry matter	90
Soybean meal (45% protein)	300	Crude protein	40
Corn gluten (60% protein)	100	Ether extract	9
Corn grain	215	Crude fiber	3.3
Vegetable oil	40	Ash	12.7
Vitamins mixture	10	Nitrogen free extract[1]	35
Minerals mixture	10		
Di–Calcium phosphate	15	Gross energy (kcal/100g diet)[2]	458.75

Anti-aflatoxin	5
Vitamin C	5
Total	1000

[1]Nitrogen-Free Extract (calculated by difference) = 100 – (protein % + lipid% + ash% + fiber %). [2]GE (gross energy) was calculated according to NRC (1993) by factors of 5.65, 9.45 and 4.22 kcal per gram of protein, Lipid and carbohydrate, respectively.

TABLE 3. Primers sequences for the cytokine genes expression

Genes	Primer sequence	Reference
IL-4	Forward; 5`CTATTAATGGGTCTCACCTCCCAACT`3 Reverse; 5`CATAATCGTCTTTAGCCTTTCCAAG`3	[38]
IL-12	Forward; 5`CAGCCTTGCAGAAAAGAGAGC`3 Reverse; 5`CCAGTAAGGCCAGGCAACAT`3	[38]
HSP	Forward; 5`GTGACGCGAAGATGGACAAGTC`3 Reverse; 5`CACCGTAAGCTACAGCCTCGTC`3	[38]
β-actin	Forward; 5`GCCCATCTACGAGGGATA`3 Reverse; 5`GGTGGTCGTGAAGGTGTAA`3	[39]

IL-4 =interleukin 4, IL-12 = interleukin 12, HSP` = heat shock protein, β-actin = Beta actin (a housekeeping gene)

TABLE 4. Primers sequences for the antioxidant genes expression

Gene name	Primer sequence (5'-3')	Product size (bp)	Accession no.
cMn-SOD	F: AATTGGAGTGAAAGGCTCTGGCT R: ACGGAGGTTCTTGTACTGAAGGT	153	DQ005531
CAT	(F): GCCCGTACAAGGAACTACCA (R): TGACGTTCTGCCTCATTCAG	110	AY518322
GPx	(F): AGGGACTTCCACCAGATG (R): CAACAACTCCCCTTCGGTA	117	AY973252
β-actin	(F): GCCCATCTACGAGGGATA (R): GGTGGTCGTGAAGGTGTAA	121	AF300705

cMn-SOD = Cytosolic manganese superoxide dismutase; CAT = Catalase; GPx = Glutathione peroxidase; β-actin = Beta actin (a housekeeping gene).

TABLE 5. Parameters of water quality during larval rearing (growth breeding of post larvae) feeding on fermented rice bran (90 days)

Stocking Density	Dissolved oxygen (mg/L)	pH	Temperature (°C)	TAN (mg/L)	Nitrite (mg/L)
150/1-m3	5.556	7.911[a]	29.89	0.334[a]	0.019
200/1-m3	5.300	7.744[b]	30.22	0.388[b]	0.023
Feed treatment					
G-I	5.750[a]	8.133[a]	29.67	0.200[a]	0.017[a]
G-II	5.333[ab]	7.700[b]	30.50	0.398[b]	0.022[ab]
G-III	5.200[b]	7.650[b]	30.00	0.485[c]	0.025[b]
SEM	0.154	0.062	0.782	0.012	0.002
P-value					
Stocking Density	0.065	0.007	0.611	0.0001	0.059
Feed treatment	0.009	0.0001	0.577	0.0001	0.016
Interaction	0.399	0.397	0.914	0.007	0.335

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $P < 0.05$). SEM= standard error of the mean. Group I (G-I) shrimp was fed only on formulated feed (Control group). Group II (G-II) shrimp was fed on 50 % formulated feed and 50 % fermented feed. Group III (G-III) shrimp was fed on 100 % fermented feed.

TABLE 6. Phyto and Zooplankton during larval rearing (growth breeding of post larvae) feeding on fermented rice bran (90 days)

	Copepods (individual /L)	Phytoplankton x 10^5 (cells /mL)
Stocking Density		
150/1-m3	78.22	4.278[a]
200/1-m3	66.67	3.967[b]
Feed treatment		
G-I	26.17[a]	3.600[a]
G-II	63.17[b]	4.067[b]
G-III	128.0[c]	4.700[c]
SEM	8.486	0.077
***P*-value**		
Stocking Density	0.121	0.001
Feed treatment	0.0001	0.0001
Interaction	0.723	0.135

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $P < 0.05$). SEM= standard error of the mean. Group I (G-I) shrimp was fed only on formulated feed (Control group). Group II (G-II) shrimp was fed on 50 % formulated feed and 50 % fermented feed. Group III (G-III) shrimp was fed on 100 % fermented feed.

TABLE 7.The total count of bacteria and vibrio (cfu/ml) in the rearing water during the 90-day larval rearing phase, which involves the growth and breeding of post larvae fed on fermented rice bran.

	Total Bacterial Count x 10^5 (cfu/ml)	Total Vibrio spp. x 10^2 (cfu/ml)
Stocking Density		
150/1-m^3	4.367	2.344
200/1-m^3	4.489	2.522
Feed treatment		
G-I	3.833[a]	2.783[a]
G-II	4.333[b]	2.317[b]
G-III	5.117[c]	2.200[b]
SEM	0.175	0.178
P-value		
Stocking Density	0.409	0.067
Feed treatment	0.0001	0.001
Interaction	0.959	0.816

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $P < 0.05$). SEM= standard error of the mean. Group I (G-I) shrimp was fed only on formulated feed (Control group). Group II (G-II) shrimp was fed on 50 % formulated feed and 50 % fermented feed. Group III (G-III) shrimp was fed on 100 % fermented feed.

TABLE 8.Growth performance of *L. vannamei* in the treatments during larval rearing (growth breeding of post larvae) feeding on fermented rice bran (90 days)

	IBW (g)	FBW (g)	WG (g)	ADG (g)	SGR (%/ day)
Stocking Density					
150/1-m^3	1.077	12.42[a]	11.35[a]	0.126[a]	2.72[a]
200/1-m^3	1.089	10.81[b]	9.72[b]	0.108[b]	2.55[b]
Feed treatment					
G-I	1.083	15.87[a]	14.79[a]	0.164[a]	2.98[a]
G-II	1.077	10.83[b]	9.753[b]	0.108[b]	2.56[b]
G-III	1.088	8.148[c]	7.060[c]	0.078[c]	2.24[c]
SEM	0.013	0.109	0.108	0.001	0.119
P-value					
Stocking Density	0.693	0.0001	0.0001	0.0001	0.0001
Feed treatment	0.288	0.0001	0.0001	0.0001	0.0001
Interaction	0.209	0.001	0.001	0.001	0. 001

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $P < 0.05$). SEM= standard error of the mean. Group I (G-I) shrimp was fed only on formulated feed (Control group). Group II (G-II) shrimp was fed on 50 % formulated feed and 50 % fermented feed. Group III (G-III) shrimp was fed on 100 % fermented feed.

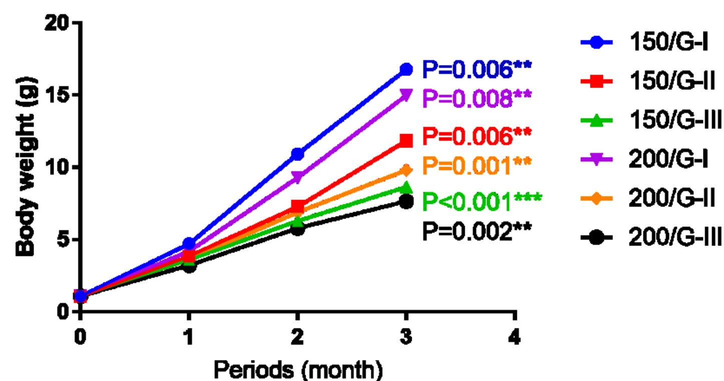

Fig. 1. Weekly development of the average body weight of *L. vannamei* cultivated in two different stocking densities and fed on 3 different formulated diets.

TABLE 9. Proximate composition (g/100 g) of L. vannamei at the end of larval rearing (growth breeding of post larvae) feeding on fermented rice bran (90 days)

	Proximate Composition				
	Crude Protein (%)	Crude lipid (%)	Moisture (%)	Carbohydrate (%)	Total Ash (%)
Stocking Density					
150/1-m^3	18.30[a]	1.506[a]	72.44[a]	5.333[a]	2.423[a]
200/1-m^3	17.33[b]	1.391[b]	72.69[b]	5.514[b]	3.079[b]
Feed treatment					
G-I	19.62[a]	1.862[a]	72.33[ab]	4.527[a]	1.668[a]
G-II	18.32[b]	1.280[b]	72.59[b]	5.733[b]	2.073[b]
G-III	15.51[c]	1.203[c]	72.76[c]	6.012[c]	4.512[c]
SEM	0.045	0.023	0.045	0.044	0.075
P-value					
Stocking Density	0.0001	0.0001	0.0001	0.002	0.0001
Feed treatment	0.0001	0.0001	0.0001	0.0001	0.0001
Interaction	0.0001	0.671	0.547	0.015	0.001

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $P < 0.05$). SEM= standard error of the mean. Group I (G-I) shrimp was fed only on formulated feed (Control group). Group II (G-II) shrimp was fed on 50 % formulated feed and 50 % fermented feed. Group III (G-III) shrimp was fed on 100 % fermented feed.

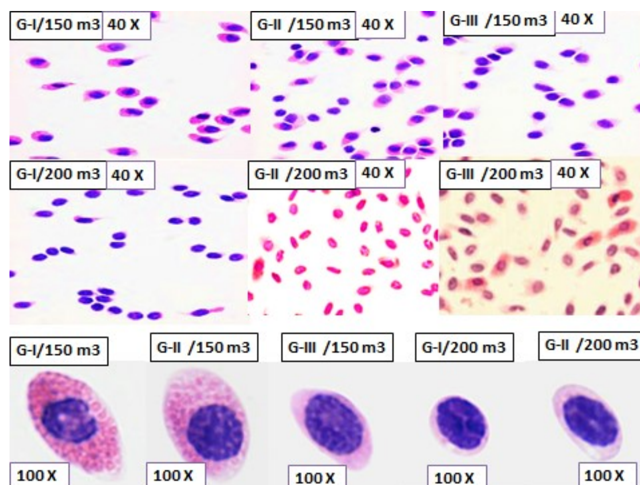

Fig. 2. Phagocytic activity of hepatocytes showing different number of *candida albicans* phagocytes engulfed and digested (Giemsa stain).

TABLE 10. Immune parameters of *L. vannamei* during larval rearing (growth breeding of post larvae) feeding on fermented rice bran (90 days)

	Parameter of immune status						
	Phagocytosis assay			Parameter in serum (hemolymph)			
	THC (X 10^6 cells µL)	Phagocytosis (%)	Phagocytic index	LSZ (UmL^{-1})	PO ($Umg^{-1}prot$)	RB	Total protein ($mg\ m\ l^{-1}$)
Stocking Density							
150/1-m^3	17.89[a]	28.22[a]	3.678[a]	2493	4.491[a]	0.357[a]	12.38[a]
200/1-m^3	14.22[b]	22.89[b]	3.089[b]	2443	3.644[b]	0.257[b]	8.400[b]
Feed treatment							
G-I	11.83[a]	18.67[a]	1.867[a]	2145	3.255[a]	0.225[a]	5.217[a]
G-II	16.67[ab]	27.83[b]	4.117[b]	2671	4.345[b]	0.332[b]	12.70[b]
G-III	19.67[b]	30.17[b]	4.167[b]	2588	4.603[b]	0.365[b]	13.25[b]
SEM	1.748	1.944	0.159	228.5	0.286	0.019	1.085
P-value							
Stocking Density	0.025	0.006	0.001	0.796	0.004	0.0001	0.001
Feed treatment	0.003	0.001	0.0001	0.085	0.001	0.0001	0.0001
Interaction	0.138	0.076	0.031	0.982	0.029	0.006	0.028

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $P<0.05$). SEM= standard error of the mean. Group I (G-I) shrimp was fed only on formulated feed (Control group). Group II (G-II) shrimp was fed on 50 % formulated feed and 50 % fermented feed. Group III (G-III) shrimp was fed on 100 % fermented feed.

TABLE 11. Antioxidation factors of *L. vannamei* ($U\ mg^{-1}$ protein) during larval rearing (growth breeding of post larvae) feeding on fermented rice bran (90days)

	SOD Umg_1 protein	CAT Umg_1 protein	MDA Umg_1 protein	GSR mmol/g wet tissue	TAC mML-1/g wet tissue	GPx, U/g tissue
Stocking Density						
150/1-m^3	32.45[a]	2.424[a]	5.048[a]	6.506[a]	2.851[a]	16.87[a]
200/1-m^3	26.65[b]	1.776[b]	5.636[b]	5.850[b]	2.309[b]	18.28[b]
Feed treatment						
G-I	26.78	1.840	5.603	5.958	2.477	18.40[a]
G-II	30.37	2.255	5.318	6.307	2.642	16.96[b]
G-III	31.49	2.205	5.103	6.268	2.622	17.37[c]
SEM	2.017	0.158	0.228	0.153	0.236	0.139
P-value						
Stocking Density	0.004	0.001	0.008	0.002	0.016	0.0001
Feed treatment	0.089	0.043	0.130	0.080	0.752	0.0001
Interaction	0.151	0.044	0.537	0.055	0.583	0.0001

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $P < 0.05$). SEM= standard error of the mean. Group I (G-I) shrimp was fed only on formulated feed (Control group). Group II (G-II) shrimp was fed on 50 % formulated feed and 50 % fermented feed. Group III (G-III) shrimp was fed on 100 % fermented feed.

TABLE 12. Relative expression of genes of antioxidants genes expression in hemolymph and hepatopancrease extract of *L. vannamei* during larval rearing (growth breeding of post larvae) feeding on fermented rice bran (90 days)

	Relative expression of genes in hemolymph extract of L. vannamei		
	cMn-SOD	***CAT***	***GPx***
Stocking Density			
150/1-m^3	1.514[a]	1.572[a]	1.516[a]
200/1-m^3	1.348[b]	1.396[b]	1.672[b]
Feed treatment			
G-I	1.350[a]	1.388[a]	1.640[a]
G-II	1.490[b]	1.570[b]	1.530[b]
G-III	1.453[b]	1.493[c]	1.612[a]
SEM	0.015	0.010	0.017
P-value			
Stocking Density	0.0001	0.0001	0.0001
Feed treatment	0.0001	0.0001	0.0001
Interaction	0.0001	0.0001	0.0001

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $P<0.05$). SEM= standard error of the mean. Group I (G-I) shrimp was fed only on formulated feed (Control group). Group II (G-II) shrimp was fed on 50 % formulated feed and 50 % fermented feed. Group III (G-III) shrimp was fed on 100 % fermented feed.

TABLE 13. Relative expression of genes of cytokine genes expression in hemolymph extract of *L. vannamei* during larval rearing (growth breeding of post larvae) feeding on fermented rice bran (90 days)

	Relative expression of genes in hemolymph extract of *L. vannamei*		
	IL-4	**IL-12**	**HSP**
Stocking Density			
150/1-m^3	1.634[a]	1.514[a]	1.703[a]
200/1-m^3	1.499[b]	1.393[b]	1.520[b]
Feed treatment			
G-I	1.493[a]	1.388[a]	1.555[a]
G-II	1.615[b]	1.502[b]	1.672[b]
G-III	1.592[b]	1.472[c]	1.608[c]
SEM	0.012	0.009	0.012
P-value			
Stocking Density	0.0001	0.0001	0.0001
Feed treatment	0.0001	0.0001	0.0001
Interaction	0.0001	0.0001	0.0001

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $P<0.05$). SEM= standard error of the mean. Group I (G-I) shrimp was fed only on formulated feed (Control group). Group II (G-II) shrimp was fed on 50 % formulated feed and 50 % fermented feed. Group III (G-III) shrimp was fed on 100 % fermented feed.

TABLE 14. Morphometric analysis of the gastrointestinal tract of *L. vannamei* during larval rearing (growth breeding of post larvae) feeding on fermented rice bran (90 days)

	Villus height, mm	*Villus width, mm*	*Crypt depth, mm*
Stocking Density			
150/1-m^3	2.267^a	0.352^a	0.218^a
200/1-m^3	2.142^b	0.290^b	0.174^b
Feed treatment			
G-I	2.177^a	0.307	0.172^a
G-II	2.208^{ab}	0.327	0.217^b
G-III	2.228^b	0.330	0.200^b
SEM	0.012	0.010	0.008
P-value			
Stocking Density	0.0001	0.0001	0.0001
Feed treatment	0.004	0.075	0.001
Interaction	0.0001	0.075	0.005

Means within a column and effect that lack common superscripts differ significantly (Tukey's multiple comparison test, $P < 0.05$). SEM= standard error of the mean. Group I (G-I) shrimp was fed only on formulated feed (Control group). Group II (G-II) shrimp was fed on 50 % formulated feed and 50 % fermented feed. Group III (G-III) shrimp was fed on 100 % fermented feed.

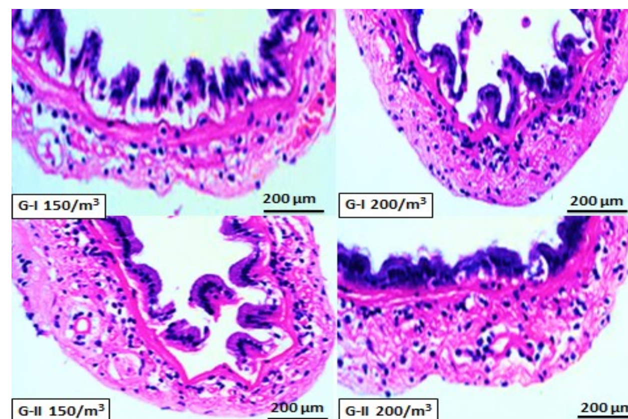

Fig. 3. Photomicrograph of gastrointestinal tract of *L. vannamei* feeding on fermented rice brain showing high intestinal villous height and increase number of goblet cell metaplasia stained with H&E

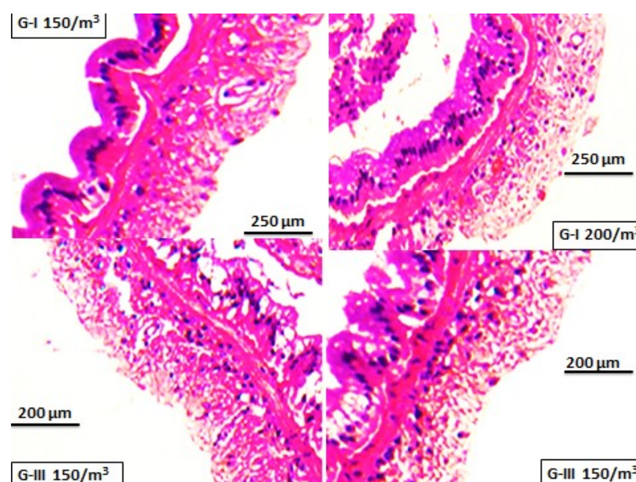

Fig. 4. Photomicrograph of gastrointestinal tract of *L. vannamei* feeding on fermented rice brain showing high intestinal villous height and increase number of goblet cell metaplasia stained with H&E

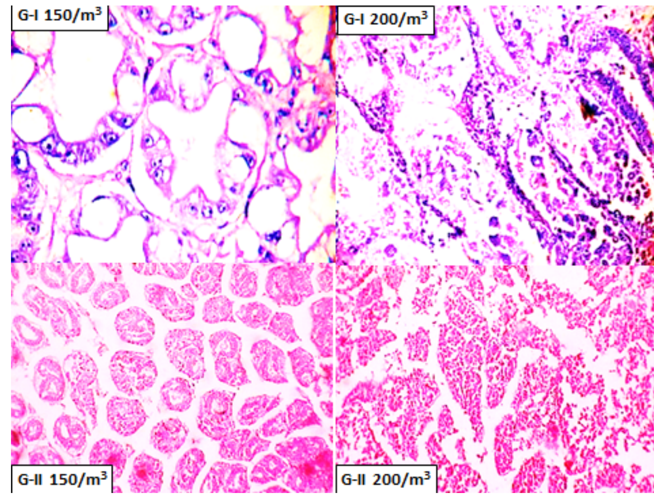

Fig. 5. Photomicrograph of hepatopancrease of *L. vannamei* feeding on fermented rice brain showing normal HP structure and tubule epithelial cells surrounded by hemolytic infiltration stained with H&E 200 X. show mild haemocyte infiltration and normal hepatopancreas lumen and tubule H&E stain magnification (×200)

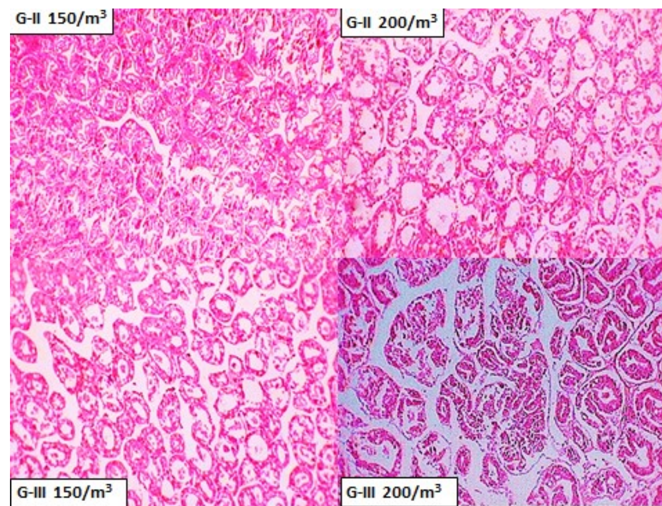

Fig. 6. Photomicrograph of hepatopancrease of *L. vannamei* feeding on fermented rice brain showing light haemocyte infiltration and normal hepatopancreas lumen and tubule H&E stain magnification (×200); show highly activation of hepatic glandular duct system H&E stain magnification (×200).

References

1. FAO. *The State of World Fisheries and Aquaculture 2022*. FAO, Rome, Italy, (2022).

2. Bardera, G., Owen, M.A.G., Façanha, F.N., Alcaraz-Calero, J.M., Alexander, M.E. and Sloman, K.A. The influence of density and dominance on Pacific white shrimp (Litopenaeus vannamei) feeding behaviour. *Aquaculture*, **531**, 735949 (2021).

3. Ray, A.J., Lewis, B.L., Browdy, C.L. and Leffler, J.W. Suspended solids removal to improve shrimp (Litopenaeus vannamei) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. *Aquaculture*, **299**, 89–98 (2010).

4. Bondad-Reantaso, M.G., Subasinghe, R.P., Josupeit, H., Cai, J. and Zhou, X. The role of crustacean fisheries and aquaculture in global food security: Past, present and future. *J. Invertebr. Pathol.*, **110**, 158–165 (2012).

5. Lauria, V., Das, I., Hazra, S., Cazcarro, I., Arto, I., Kay, S., Ofori-Danson, P., Ahmed, M., Hossain, M.A.R., Barange, M. and Fernandes, J.A. Importance of fisheries for food security across three climate change vulnerable deltas. *Sci. Total Environ.*, **640–641**, 1566–77 (2018).

6. Abdella, B., Abozahra, N.A., Shokrak, N.M., Mohamed, R.A. and El-Helow, E.R. Whole spectrum of Aeromonas hydrophila virulence determinants and the identification of novel SNPs using comparative pathogenomics. *Sci. Rep.*, **13**, 7712 (2023).

7. Sarker, P.K. Microorganisms in Fish Feeds, Technological Innovations, and Key Strategies for Sustainable Aquaculture. *Microorganisms*, **11**, 439 (2023).

8. Romano, N., Dauda, A.B., Ikhsan, N., Karim, M. and Kamarudin, M.S. Fermenting rice bran as a carbon source for biofloc technology improved the water quality, growth, feeding efficiencies, and biochemical

composition of African catfish Clarias gariepinus juveniles. *Aquac. Res.*, **49**, 3691–701 (2018).

9. Arnold, S.J., Sellars, M.J., Crocos, P.J. and Coman, G.J. Intensive production of juvenile tiger shrimp Penaeus monodon: An evaluation of stocking density and artificial substrates. *Aquaculture*, **261**, 890–896 (2006).

10. Ruiz-Velazco, J.M.J., Hernández-Llamas, A. and Gomez-Muñoz, V.M. Management of stocking density, pond size, starting time of aeration, and duration of cultivation for intensive commercial production of shrimp Litopenaeus vannamei. *Aquac. Eng.*, **43**, 114–119 (2010).

11. Chen, W. and Gao, S. Current status of industrialized aquaculture in China: a review. *Environ. Sci. Pollut. Res.*, **30**, 32278–87 (2023).

12. Kokou, F. and Fountoulaki, E. Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. *Aquaculture*, **495**, 295–310 (2018).

13. Mehrim, A.I. and Refaey, M.M. An Overview of the Implication of Climate Change on Fish Farming in Egypt. *Sustainability*, **15**, 1679 (2023).

14. Abdella, B., Shokrak, N.M., Abozahra, N.A., Elshamy, Y.M., Kadira, H.I. and Mohamed, R.A. Aquaculture and Aeromonas hydrophila: a complex interplay of environmental factors and virulence. *Aquac. Int.*, (2024).

15. Liñan-Vidriales, M.A., Peña-Rodríguez, A., Tovar-Ramírez, D., Elizondo-González, R., Barajas-Sandoval, D.R., Ponce-Gracía, E.I., Rodríguez-Jaramillo, C., Balcázar, J.L. and Quiroz-Guzmán, E. Effect of rice bran fermented with Bacillus and Lysinibacillus species on dynamic microbial activity of Pacific white shrimp (Penaeus vannamei). *Aquaculture*, **531**, 735958 (2021).

16. Francis, G., Makkar, H.P.. and Becker, K. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, **199**, 197–227 (2001).

17. Magbanua, T.O. and Ragaza, J.A. Selected dietary plant-based proteins for growth and health response of Nile tilapia Oreochromis niloticus. *Aquac. Fish.*, **9**(1), 3-19(2022).

18. Yang, H., Bian, Y., Huang, L., Lan, Q., Ma, L., Li X. and Leng, X. Effects of replacing fish meal with fermented soybean meal on the growth performance, intestinal microbiota, morphology and disease resistance of largemouth bass (Micropterus salmoides). *Aquac. Reports*, **22**, 100954 (2022).

19. Mukherjee, R., Chakraborty, R. and Dutta, A. Role of Fermentation in Improving Nutritional Quality of Soybean Meal — A Review. *Asian-Australasian J. Anim. Sci.*, **29**, 1523–9 (2015).

20. Huang, J., Dai, Y., Huang, T., Du, T. and Xiong, T. Comparison of nutritional value, bioactivity, and volatile compounds of soybean meal–corn bran mixed substrates fermented by different microorganisms. *Lett. Appl. Microbiol.*, **76**(2), ovad012 (2023).

21. Lin, Y.-H. and Mui, J.-J. Comparison of dietary inclusion of commercial and fermented soybean meal on oxidative status and non-specific immune responses in white shrimp, Litopenaeus vannamei. *Fish Shellfish Immunol.*, **63**, 208–212 (2017).

22. Fabian, C. and Ju, Y.-H. A Review on Rice Bran Protein: Its Properties and Extraction Methods. *Crit. Rev. Food Sci. Nutr.*, **51**, 816–827 (2011).

23. Chinma, C.E., Ilowefah, M. and Muhammad, K. Optimization of Rice Bran Fermentation Conditions Enhanced by Baker's Yeast for Extraction of Protein Concentrate. *Niger. Food J.*, **32**, 126–32 (2014).

24. Wabete, N., Chim, L., Pham, D., Lemaire, P. and Massabuau, J.-C. A soft technology to improve survival and reproductive performance of Litopenaeus stylirostris by counterbalancing physiological disturbances associated with handling stress. *Aquaculture*, **260**, 181–193 (2006).

25. Villamil, L., Tafalla, C., Figueras, A. and Novoa, B. Evaluation of Immunomodulatory Effects of Lactic Acid Bacteria in Turbot (Scophthalmus maximus). *Clin. Vaccine Immunol.*, **9**, 1318–1323 (2002).

26. Abdel-Rahim, M., Bahattab, O., Nossir, F., Al-Awthan, Y., Khalil, R.H. and Mohamed, R. Dietary Supplementation of Brown Seaweed and/or Nucleotides Improved Shrimp Performance, Health Status and Cold-Tolerant Gene Expression of Juvenile Whiteleg Shrimp during the Winter Season. *Mar. Drugs*, **19**, 175 (2021).

27. AOAC. *Association of Official Analytical Chemists.* sixteenth ed., Association of Official Analytical Chemists, Virginia, USA, (1995).

28. Leaño, E.M., Lavilla-Pitogo, C.R. and Paner, M.G. Bacterial flora in the hepatopancreas of pond-reared Penaeus monodon juveniles with luminous vibriosis. *Aquaculture*, **164**, 367–374 (1998).

29. Hernández-López, J., Gollas-Galván, T. and Vargas-Albores, F. Activation of the prophenoloxidase system of the brown shrimp Penaeus californiensis Holmes). *Comp. Biochem. Physiol. Part C Pharmacol. Toxicol. Endocrinol.*, **113**, 61–66 (1996).

30. Claiborne, A. Catalase activity. *Handbook of Methods for Oxygen Radical Research*. (Greenwald RA ed.), CRC Press, Boca Raton, Florida, USA, pp.283–4 (1985).

31. Payá, M., Halliwell, B. and Hoult, J.R.S. Interactions of a series of coumarins with reactive oxygen species. *Biochem. Pharmacol.*, **44**, 205–214 (1992).

32. Peixoto, F., Alves-Fernandes, D., Santos, D. and Fontaínhas-Fernandes, A. Toxicological effects of oxyfluorfen on oxidative stress enzymes in tilapia Oreochromis niloticus. *Pestic. Biochem. Physiol.*, **85**, 91–96 (2006).

33. Benzie I.F.F., Strain J.J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal. Biochem.*, **239**, 70–76 (1996).

34. Maran, E., Fernández, M., Barbieri, P., Font, G. and Ruiz, M.J. Effects of four carbamate compounds on

antioxidant parameters. *Ecotoxicol. Environ. Saf.*, **72**, 922–930 (2009).

35. Flohé, L. and Günzler, W.A. Assays of glutathione peroxidase. *Methods in enzymology*. Vol. 105, pp.114–120 (1984).

36. Utley, H.G., Bernheim, F. and Hochstein, P. Effect of sulfhydryl reagents on peroxidation in microsomes. *Arch. Biochem. Biophys.*, **118**, 29–32 (1967).

37. Livak, K.J. and Schmittgen, T.D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods*, **25**, 402–408 (2001).

38. Moustafa, E.M., Saad, T.T., Khalil, R.H., Dawood, M.A.O. and Lolo, E.E. The Ameliorative Role of Synbiotic Culture Techniques Application in White Shrimp (Litopenaeus vannamei) During Nursery Stage. *Adv. Anim. Vet. Sci.*, **8**(3), 277 (2020).

39. Huang, W., Ren, C., Li, H., Huo, D., Wang, Y., Jiang, X., Tian, Y., Luo, P., Chen, T. and Hu, C. Transcriptomic analyses on muscle tissues of Litopenaeus vannamei provide the first profile insight into the response to low temperature stress. *PLoS One*, **12**, e0178604 (2017).

40. Eldessouki E.A.A., Diab A.M., Selema T.A.M.A., Sabry N.M., Abotaleb M.M., Khalil R.H., El-Sabbagh N., Younis N.A. and Abdel-Tawwab M. Dietary astaxanthin modulated the performance, gastrointestinal histology, and antioxidant and immune responses and enhanced the resistance of Litopenaeus vannamei against Vibrio harveyi infection. *Aquac. Int.*, **30**, 1869–1887 (2022).

41. da Cunha, L., Besen, K.P., de Oliveira, N.S., Delziovo, F.R., Gomes, R., da Cruz, J.M., Picoli, F., Gisbert, E., Skoronski, E., El Hadi Perez Fabregat, T. Fermented soybean meal can partially replace fishmeal and improve the intestinal condition of goldfish juveniles reared in a biofloc system. *Aquac. Res.*, **53**, 6803–6815 (2022).

42. Sadique, K.J., Pandey, A., Khairnar, S.O. and Naveen Kumar B.T. Effect of Fermented Water Hyacinth Leaf Meal on Plankton Productivity and Gut Content Analysis of Common Carp (Cyprinus carpio). *Int. J. Curr. Microbiol. Appl. Sci.*, **7**, 1947–1959 (2018).

43. Silva, D.A., Lima, P.C.M., Silva, A.E.M., Oliveira, Filho, P.R.C., Silva, S.M.B.C., Olivera Gálvez, A. and Brito, L.O. Effects of adding rotifers on the water quality, plankton composition and growth of Pacific white shrimp, Litopenaeus vannamei juvenile, when cultured with biofloc technology. *Aquac. Res.*, **52**, 4380–4393 (2021).

44. De, D., Sandeep, K.P., Kumar, S., Raja, R.A., Mahalakshmi, P., Sivaramakrishnan, T., Ambasankar, K. and Vijayan, K.K. Effect of fish waste hydrolysate on growth, survival, health of Penaeus vannamei and plankton diversity in culture systems. *Aquaculture*, **524**, 735240 (2020).

45. Abdella, B., El-Wazzan, E., El-Sersy, N.A., Sabry, S.A. and El-Helow, E.R. Pathogenicity and antibiotic susceptibility of two bacterial pathogens associated with the clam Tapes decussatus in some Egyptian fisheries. *Ege J. Fish. Aquat. Sci.*, **34**, 383–389 (2017).

46. El-Wazzan, E., Ghareeb, D.A. and Abdella, B. Pre-induction of Hsp70 expression to protect the grooved carpet shell clam, Ruditapes decussatus, against Micrococcus luteus: A trained immunity strategy. *Egypt. J. Aquat. Res.*, **46**, 79–84 (2020).

47. Mahmoud, N.H., Elsherbiny, B.A., Moffit, S.M., Mohamed, J.H., Abouelkheir, S.S. and Abdella, B. Cell Biology and Microbial Interactions in Algal Cells. *Handbook of Research on Algae as a Sustainable Solution for Food, Energy, and the Environment*. (El-Sheekh Mostafa M , Abdullah Norhayati , Ahmad Imran ed.), IGI, pp.84–108 (2022).

48. Vaseeharan, B. and Ramasamy, P. Control of pathogenic Vibrio spp. by Bacillus subtilis BT23, a possible probiotic treatment for black tiger shrimp Penaeus monodon. *Lett. Appl. Microbiol.*, **36**, 83–87 (2003).

49. Wang, D., Li, J., Zhu, G., Zhao, K., Jiang, W., Li, H., Wang, W., Kumar, V., Dong, S., Zhu, W. and Tian, X. Mechanism of the Potential Therapeutic Candidate Bacillus subtilis BSXE-1601 Against Shrimp Pathogenic Vibrios and Multifunctional Metabolites Biosynthetic Capability of the Strain as Predicted by Genome Analysis. *Front. Microbiol.*, **11**, 581802(2020).

50. Zhang, M., Pan, L., Fan, D., He, J., Su, C., Gao, S. and Zhang, M. Study of fermented feed by mixed strains and their effects on the survival, growth, digestive enzyme activity and intestinal flora of Penaeus vannamei. *Aquaculture*, **530**, 735703 (2021).

51. Qiu, X. and Davis, D.A. Evaluation of dried fermented biomass as a feed ingredient in plant-based practical diets for juvenile Pacific white shrimp Litopenaeus vannamei. *Aquac. Nutr.*, **24**, 383–391 (2018).

52. Lin, Y.-H. and Chen, Y.-T. Lactobacillus spp. fermented soybean meal partially substitution to fish meal enhances innate immune responses and nutrient digestibility of white shrimp (Litopenaeus vannamei) fed diet with low fish meal. *Aquaculture*, **548**, 737634 (2022).

53. Wang, R.-F., Wang, Y., Zhang, J., Weng, M.-Q., Liu, Y.-H., Cheng, Q.-Y., Song, M., Yang, Y.-P., An, X.-P. and Qi, J.-W. The effects of dietary fermented wheat bran polysaccharides on mucosal and serum immune parameters, hepatopancreas antioxidant indicators, and immune-related gene expression of common carp (Cyprinus carpio) juveniles. *Aquac. Int.*, **30**, 1835–1853 (2022).

54. Siddik, M.A.B., Fotedar, R., Chaklader, M.R., Foysal, M.J., Nahar, A. and Howieson, J. Fermented Animal Source Protein as Substitution of Fishmeal on Intestinal Microbiota, Immune-Related Cytokines and Resistance to Vibrio mimicus in Freshwater Crayfish (Cherax cainii). *Front. Physiol.*, **10**, 01635 (2020).

55. Wang, L., Zhou, H., He, R., Xu, W., Mai, K. and He, G. Effects of soybean meal fermentation by Lactobacillus plantarum P8 on growth, immune

responses, and intestinal morphology in juvenile turbot (Scophthalmus maximus L.). *Aquaculture*, **464**, 87–94 (2016).

56. Liu, W.-C., Zhou, S.-H., Balasubramanian, B., Zeng, F.-Y., Sun, C.-B. and Pang, H.-Y. Dietary seaweed (Enteromorpha) polysaccharides improves growth performance involved in regulation of immune responses, intestinal morphology and microbial community in banana shrimp Fenneropenaeus merguiensis. *Fish Shellfish Immunol.*, **104**, 202–212 (2020).

57. Novriadi, R., Herawati, V.E., Prayitno, S.B., Windarto, S., Mertz, K. and Nguyen Duy, H. Effect of fermented corn protein concentrate on growth performance, haemocyte counts, histological structure of hepatopancreas and intestinal condition of pacific white shrimp Litopenaeus vannamei. *Aquac. Fish Fish.*, **2**, 82–93 (2022).

تأثير الأعلاف المخمرة على أداء نمو الجمبري ذو الساق البيضاء (*Litopenaeus vannamei*) والاستفادة من الأعلاف والاستجابة المناعية وحالة الاكسدة في أحواض التربية

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الملخص

كان الهدف من هذه الدراسة هو تقييم تأثيرات نخالة الأرز المخمرة المحتوية على بكتيريا بروبيوتيك تجارية فيما يتعلق بكثافة الاسكان وتأثيراتها على صحة الجمبري ذو الساق البيضاء (*Litopenaeus vannamei*) وميكروبيوتا الجسم والاستجابة المناعية والتعبير الجيني. تم الحصول على الجمبري ذو الساق البيضاء الوزن الأولي: 0.86 ± 0.011 جم (من مفرخ غليون وتم تربيته بكثافة اسكان 150 و 200 حيوان / م 3. تم تقسيم الجمبري إلى ثلاث مجموعات: ثلاث مكررات لكل مجموعة. تم تغذية المجموعة الأولى (G-I) فقط على الأعلاف المصنعة (المجموعة الضابطة)، وتم تغذية المجموعة الثانية (G-II) على 50٪ من الأعلاف المصنعة و 50٪ من الأعلاف المخمرة وتم تغذية المجموعة الثالثة (G-III) على 100٪ من الأعلاف المخمرة. أثر تعديل العلف على الأكسجين المذاب في الماء، ودرجة الحموضة، والامونيا الكلية(TAN) ، والنتريت، لكن كثافة الاسكان أثرت بشكل كبير على كل من درجة الحموضة و .TANكان لكثافة التسكين وتخمير العلف تأثير على الفرد/لتر من وجود العوالق النباتية في حوض الاستزراع. مع استبدال الوجبة بنسبة 100٪، لوحظ الحد الأقصى لعدد القشريات والعوالق النباتية. تم الحصول على أعلى أداء للنمو في G-I تليها G-II ثم .G-III أدى تطبيق الأعلاف المخمرة إلى تحسين قدرة مضادات الأكسدة والاستجابة المناعية وشكل الأمعاء لدى *L. vannamei* بينما أدى زيادة كثافة التسكين إلى إحداث تأثير سلبي على الاستجابات المناعية للأكسدة للجمبري ومورفومترية الأمعاء. في الختام، يمكن استخدام الأعلاف المخمرة كبديل لنهج مستدام لاستبدال الأعلاف التجارية في أنظمة الجمبري الغذائية للحصول على نمو أفضل وامتصاص أفضل للأعلاف بالإضافة إلى تعزيز مقاومة الإجهاد وزيادة الاستجابات المناعية للأكسدة في الجمبرى.

الكلمات المفتاحية: *Litopenaeus vannameias*، تخمير الأعلاف، أداء النمو، المناعة، الكائنات الحية الدقيقة.