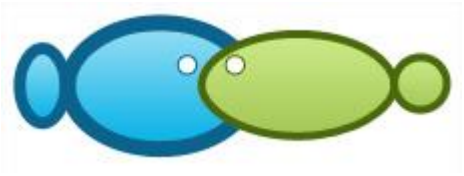

Effects of guar gum, *Lactobacillus plantarum* L-137 and phytase on the growth performance and immune responses of bighead catfish (*Clarias macrocephalus*) in Recirculating Aquaculture Systems (RAS)

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Abstract. This study was conducted to determine the effect of guar gum-GG, heat-killed *Lactobacillus plantarum* L-137 (HK L-137) and phytase on the growth, digestibility and immune response of bighead catfish (*Clarias macrocephalus*), in order to contribute to formulating effective and environmentally friendly feed for RAS systems. The experiment consisted of five diet treatments with and without additives: T1 (Control-CT), T2 (CT supplemented with GG at 5 g kg^{-1}), T3 (CT with GG at 5 g kg^{-1} and HK L-137 at 0.02 g kg^{-1}), T4 (CT with GG and 0.15 g phytase kg^{-1} of diet) and T5 (CT with GG, HK L-137 and phytase). The feeding trial was carried out for 56 days and all treatments were performed in triplicate. The results showed that the survival rate of the fish was not affected by diets supplemented with either guar gum (at 5 g kg^{-1}), phytase (0.15 g kg^{-1}) or HK L-137 (0.02 g kg^{-1}). Dietary guar gum (5 g kg^{-1}) addition did not diminish performance or feed digestibility and it did not improve the fecal characteristics or immune response of bighead catfish. On the other hand, inclusion of both phytase and HK L-137 in the diet not only increased growth performance and feed digestibility, but also the fecal characteristics and the immune response of *C. macrocephalus*. Neither phytase, nor HK L-137 had any effect on the fecal pellets size. A supplement of 5 g guar gum, 0.15 g phytase and 0.02 g HK L-137 kg^{-1} of diet appears to be suitable for raising *C. macrocephalus* in RAS systems.
Key Words: guar gum, *Lactobacillus plantarum*, phytase, RAS, bighead catfish.

Introduction. Clarias is one of the important groups of cultured fish in South-East Asia, which accounts for 70% of the total world production of Clariidae (FAO 2006-2014). The hybrid between Asian bighead catfish (*Clarias macrocephalus*) and African sharptooth catfish (*Clarias gariepinus*) has been widely cultured in Thailand and Vietnam since the 1980s (Liem 2008). In Vietnam, the culture of hybrid catfish developed rapidly from 2002 to 2010, with a 250 ha culture area that produced an average of 16,840 tons of hybrid catfish per crop in 2010 (Yen et al 2017). Currently, *C. macrocephalus* is classified as near-threatened by the Nature Conservation Union International (IUCN/FAO) (Vidthayanon & Allen 2013). In recent years, *C. macrocephalus* farming has been developing in the Mekong Delta, but production has been limited by its slow growth rate and susceptibility to disease, in culture conditions. Larvae were weaned and fed with artificial diets from day 10 after hatching and the survival rate was only 62% after 28 days of rearing, but in the growth stage, the average mortality was still high, of almost 30%, in tank conditions (Liem 2008). Low survival of *C. macrocephalus* in culture may be related to the high competition for feed, and/or fish "stress" caused by daily water exchange. In the latter case, a recirculating system reducing the water exchange could help reducing the mortality.

Recirculating Aquaculture Systems (RAS) are becoming more widely applied nowadays. The effectiveness of biofilters in RAS depends on the amount and types of fecal waste excreted by the cultured fish. One option to improve the effectiveness of biofilters in RAS is to reduce the amount of fecal waste by improving the feed quality and

the efficiency of the feed utilization. In addition, dietary binders can help to hold fecal pellets together so that they settle at the bottom of the pond or tank, rather than dissolving or remaining in suspension, thereby also reducing the pressure on the biofilter. Some studies in rainbow trout were carried out on increasing the fecal viscosity by guar gum (binder), thus enlarging the particle size of feces to settle easily (Brinker et al 2005; Brinker 2007). However, using a high level of binder, which increases the dietary viscosity, reduced the absorption of nutrients in the intestine (Meyer & Doty 1988).

Therefore, it is necessary to add digestive enzymes or probiotics to support the digestion of fish. These additives are also necessary when using plant-based protein sources in aquafeeds, as an alternative to fishmeal, to reduce feed costs (Baruah et al 2004) by hampering feed digestibility. Plant-protein ingredients contain high levels of indigestible phosphorus (60-70%) in the form of phytate, which causes environmental pollution (Lall 1991; Ketola 1994). Phytates also reduce the digestibility of nutrients and the absorption of minerals and amino acids in fish. Thus, the adverse effects of phytates could be improved by the addition of phytase to animal feed (Truc et al 2012). A number of studies have demonstrated the benefits of dietary phytase addition for improving performance and digestibility, for example in tilapia (Schaefer & Koppe 1995), seabass *Morone saxatilis* (Papatryphon et al 1999), rainbow trout *Oncorhynchus mykiss* (Vielma et al 2002), Atlantic salmon *Salmo salar* (Sajjadi & Carter 2004) and Korean fighting fish *Sebastes schlegeli* (Yoo et al 2005) and snakehead *Channa striata* (Hien et al 2015). Furthermore, probiotics, such as bacteria, viruses, fungi and parasites, are being applied in eco-friendly aquaculture to improve fish immune responses to pathogens (Balcázar et al 2006). Both prebiotics and probiotics are now widely used in aquaculture. Probiotics are microorganisms that directly benefit the host (Salminen et al 1999), while prebiotics are non-digestible food ingredients that stimulate the growth of beneficial bacterial communities in the colon (Ringø et al 2016). Pandiyan et al (2013) suggested that the administration of prebiotics and probiotics is more environment-friendly and sustainable than using antibiotics or chemicals in aquaculture. A common commercial probiotic, *Lactobacillus plantarum* is a rod-shaped, gram-positive, catalase-negative, non-spore-forming, fermentative, facultative anaerobic lactic acid bacterium. This probiotic was reported to increase digestive enzyme activities, to improve the growth and feed utilization efficiency, to inhibit the adhesion and growth of pathogenic bacteria, as well as to improve immunity, disease resistance and survival in aquatic animals (Dash et al 2015). Heat-killed *L. plantarum* L-137 (HK L-137) has been reported to increase fish growth, to improve digestibility and to enhance immunity of shrimp and fish (Dawood et al 2019; Duc et al 2020; Nguyen et al 2019; Yang et al 2016; Hien et al 2021a,b).

Based on the foregoing, it should, in principle, be possible to formulate a diet for bighead catfish grown in RAS, that provides easy fecal waste collection, high digestibility and good fish performance. Therefore, this study aimed to evaluate the effects of dietary additives (binder, digestive enzyme and probiotics) on the performance, digestibility, fecal characteristics and immune response of *C. macrocephalus* cultured in the RAS.

Material and Method. The study was conducted with two experiments, one to evaluate fish performance and immune responses during the growth stages, and the other focusing on the feed digestibility and fecal waste characteristics of *C. macrocephalus*. The dietary additives consisted of a binder (guar gum–Treatment GG), digestive enzymes (phytase–Treatment P), and heat-killed bacteria (heat-killed *L. plantarum*–Treament HK*)*.

Growth experiment

Experimental diets. The experiment lasted 60 days and was completely randomized with 5 iso-nitrogenous (45% protein) and isoenergetic (19.7 kJ g^{-1}) diet treatments: (i) treatment 1 (CT) was a formulated diet; (ii) treatment 2 (GG) was the CT diet with guar gum additive (5 g kg^{-1}); (iii) treatment 3 (GG/HK) was the CT diet with guar gum (5 g kg^{-1}) and HK L-137 (0.02 g kg^{-1}) additives; (iv) treatment 4 (GG/P) was the CT diet with guar gum (5 g kg^{-1}) and phytase (0.15 g kg^{-1}) additives; (v) treatment 5 (GG/P/HK) was the CT diet with all three additives, namely guar gum (5 g kg^{-1}), HK L-137 (0.02 g kg^{-1}) and phytase (0.15 g kg^{-1}). The Guar gum, a binder, is a product of Pakistan, and was

imported by Hoa chat Mien Nam Co. HK L-137, heat-killed *Lactobacillus*, was produced by the Japanese food company Wellness. Phytase was produced by the Roal Oy Company of Finland. Concentrations of the three additives, GG, phytase and HK L-137 in the diet were based on the research of Tu et al (2018) and Hien et al (2021a). Floating pellets of (treatments CT, GG and GG/HK) were extruded, with a size of 2.0 mm. For treatments involving phytase (GG/P and GG/P/HK), the pellets of GG and GG/HK were coated by spraying them with phytase (0.15 g kg^{-1}). All diets were stored in the freezer at -20°C during the experiment. The ingredients and chemical compositions of the diets are shown in Table 1.

Table 1

Ingredients and chemical composition of formulated diet (dry basis) for experimental *Clarias macrocephalus*

Ingredients	*%*
Fishmeal[1]	25.0
Defatted soybean[2]	35.0
Blood meal[3]	7.00
Rice bran[3]	15.0
Cassava meal[3]	15.0
Fish oil[3]	1.00
Premix mineral and vitamin[4]	1.00
Attractant[5]	1.00
Total	100
Proximate compositions	%
Crude protein	44.9
Crude lipid	6.97
Ash	11.8
Carbohydrate	36.1
Gross energy (KJ g^{-1})	19.7

[1]Ca Mau fishmeal, Vietnam; [2]Defatted soybean meal Maharashtra Solvent extraction LTD India;[3]Blood meal, fish oil, cassava, local rice bran, and premix were imported and supplied by Viet Thang feed mill, Dong Thap province, Vietnam; [4]Premix minerals and vitamins: Vitamin A. 6,000,000 UI; Vitamin D_3. 200,000 UI; Vitamin E. 50,000 mg; Vitamin K_3. 6,000 mg; Vitamin B_1. 11,000 mg; Vitamin B_2. 7,000 mg; Vitamin B_6. 8,000 mg; Vitamin B_{12}. 20 mg; Vitamin C Stay. 5,000 mg; Inositol. 5,000 mg; Folic acid. 3,000 mg; Biotin. 500 mg; Pantothenic Acid. 35,000 mg; Niacin. 60,000 mg; Iron (Fe^{2+}). 50,000 g; Copper (Cu^{2+}). 34,000 mg; Zinc (Zn^{2+}). 125,000 mg; Manganese (Mn^{2+}). 12,000 mg; Iodine (I^-). 500 mg; Cobalt (Co^{2+}). 250 mg; Se. 200 mg; [5]Attractant (shrimp soluble extract) was made by Minh Phu comp., Vietnam.

Experimental system and animal. *C. macrocephalus* fingerlings (weight of 9-11 g) were purchased from the Center for Advanced Aquaculture Research and Application (CAARA), Can Tho University. Before the experiment, the fish were acclimatized to the experimental conditions for two weeks. Healthy fish without obvious abnormalities were randomly assigned to the recirculating aquaculture system (RAS) tanks, at a density of 150 fish per tank. There were five RAS systems, each consisting of four 250 L culture tanks (200 L water), one 120 L sedimentation tank and one 350 L biofilter tank of type MBBR with a 150 L RK-Bioelement (SSA=750 m^2 m^{-3}). The biofilter was designed to remove dissolved waste produced from 1.5 kg of feed (45% protein) per day. Water flow to each culture tank was set at 2-3 L min^{-1}. The photoperiod was approximately 12 hours light and 12 hours dark. Nitrate (NO_2-N) and total ammonia nitrogen (TAN) were determined once every two weeks (APHA et al 1995) and ranged from 0.1 to 0.3 mg L^{-1}. The temperature, oxygen and pH were recorded twice a day, at 7:00 am and 3:00 pm. The mean temperature range was 27.0-27.4°C in the morning and 30.1-31.5°C in the afternoon. The pH was 7.1-7.3 in the morning and 8.0-8.3 in the afternoon.

Experimental procedure. The initial mean weight (W_i) and final mean weight (W_f) per fish were determined before and after the experiment. During the experimental period (T), fish were fed ad libitum, twice a day, at 8:00 am and 4:00 pm. Feed consumption

was recorded daily by collecting uneaten pellets 30 minutes after feeding. At the end of the experiment, blood samples were collected from three fish in each tank for assessing immune responses by analysing red blood cells, white blood cells and lysozyme.

Digestibility experiment. At the end of the growth experiment, fish were transferred to the fecal collector system (Tu et al 2018) at a stocking density of 30 fish per tank (200 L). Dichromium trioxide (Cr_2O_3) was added as an external marker, at the rate of 1% to the same experimental diets used in the growth experiment. Each diet was ground, mixed with Cr_2O_3, and then, after adding 30% water, re-pelleted at the size of 2 mm. Pellets were dried to a moisture content of 10% and stored at -20°C. The feces samples were collected after feeding the experimental diets for two weeks. The fecal collection process was described by Hien et al (2010). Fecal samples were collected daily over a period of three weeks, and then stored in the freezer at -20ºC until analysis. At the end of the experiment, the fecal samples were collected for a further period of three consecutive days to determine the particle size of fecal waste.

Sample analysis. About 5 g of each diet were sampled for analysis at the time of pelleting, at weekly intervals throughout the experiments. The red blood cell (RBC) were counted as described by Natt & Herrick (1952), white blood cell (WBC) counts were performed according to Chinabut et al (1991) and lysozyme activity was measured according to Ellis (1990). The chemical composition of diet and fecal samples were analyzed according to standard laboratory methods (AOAC 2000). Briefly, the dry matter (DM) content was determined by drying in the oven at 105°C until constant weight and the mineral (ash) content by placing a sample of known weight in a furnace, at 560°C, for 4 hours. Crude protein was calculated from the nitrogen content (N×6.25), measured by the Kjeldahl method, and crude fat content was determined by solvent (diethyl ether) extraction. The carbohydrate (CHO) content of the sample was calculated as 100–(crude protein + crude ash + crude fat), on a dry matter basis. The gross energy (kJ g^{-1}) was calculated as [(crude protein × 23.6) + (crude fat × 39.5) + (CHO × 17.2)] / 100. The chromic oxide content was measured by a spectrophotometer at a wavelength of 350 nm after digestion of the samples by nitric acid and then oxidation with perchloric acid (Furukawa & Tsukahara 1966).

Calculations and statistical analysis. The survival rate (SR), weight gain (WG), special growth rate (SGR), feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated as follows (NRC 2011):

SR (%) = (number of fish at the end)/ (number of fish at start of experiment) × 100

WG (g $fish^{-1}$) = Wf – Wi

SGR = (% day^{-1}) = [Ln(Wf) – Ln(Wi)] x 100/T

FI (% $fish^{-1}$ day^{-1}) = 100 × consumed feed/[(Wi + Wf)/2 × T]

FCR = amount of consumed feed in dry matter/weight gain

PER = (Wf – Wi)/protein intake

Where:
T - time in days;
Wf - final mean weight;
Wi - initial mean weight.

The Apparent Digestibility Coefficients (Nutrient ADC) of nutrients such as dry matter, protein, lipid and ash were estimated indirectly using the chromic oxide as inert marker (Cho & Kaushik 1985):

$$Nutrient.ADC = 1 - \left(\frac{\text{Marker}_{diet} \times \text{Nutrient}_{feces}}{\text{Marker}_{feces} \times \text{Nutrient}_{diet}}\right)$$

Where:
$Marker_{diet}$ - the concentration (g/100 g dry weight) of chromium oxide in the diet;

$Marker_{feces}$ - the concentration (g/100 g dry weight) of chromium oxide in the feces;
$Nutrient_{diet}$ - the concentration (g/100 g dry weight) of nutrient in the diet;
$Nutrient_{feces}$ - the concentration (g/100 g dry weight) of nutrient in the feces.

The percentage of feces recovery was computed using the total amount of chromium oxide in excreted feces and the total amount of chromium oxide in the consumed feed (Tu et al 2018), where the total amount of chromium oxide in excreted feces is the amount in DM (dry matter) of the collected feces multiplied by the chromium oxide concentration in the feces. The total amount of chromium oxide of consumed feed is the total amount of consumed feed in DM multiplied by the chromium oxide concentration in the feed. The particle size of fecal waste determines the potential of suspended solid formation and also the degree of nutrient leaching from the waste. Therefore, the percentage of particles larger than 2 mm was determined on the fecal waste collected over a period of 22 hours. The measured particle size distribution can be a combination of the disintegration of fecal pellets after egestion as well as the potential aggregation of fecal waste during collection. In order to prevent clogging of the mesh, sieving was done with a sieve submerged in the water. The daily collected fecal waste with water on top in the collecting containers was gently poured onto a 2 mm mesh sieve while being already submerged in water. After sieving, both fractions were dried and weighed. The percentages of the amount (in DM) of particles in the feces that were bigger or smaller than 2 mm was calculated (Tu et al 2018). The fecal waste consists of the recovered feces plus the non-recovered feces. The total amount of feces produced was computed based on the dry matter digestibility. The amount of non-recovered feces is the difference between the feces recovered from the settling tanks and the calculated amount of total feces produced.

Excel software was used to analyze the mean, standard deviation and standard error. One-way ANOVA and DUNCAN test (SPSS software) at $p<0.05$ were applied to assess the difference among sample means.

Results

Survival rate and growth of the bighead catfish. Fish survival ranged from 92 to 98% and the difference between treatments was not statistically significant ($p>0.05$) (Table 2). The results showed that the experimental treatments did not affect the survival rate of the fish. After 60 days of nursing, the highest fish growth was observed in treatment 5 (GG/P/HK, 3.26% day^{-1}) and the lowest in treatment 2 (GG, 2.97% day^{-1}), and there was a significant difference ($p<0.05$) between the GG/P/HK and other treatments (Table 2). There was no significant difference ($p>0.05$) between the control and GG treatments. The treatments with HK supplementation induced a significantly ($p<0.05$) higher growth than the treatments CT and GG.

Table 2

Initial mean weight (Wi), final mean weight (Wf), special growth rate (SGR) and survival rate (SR) of *Clarias macrocephalus*

Treatment	*Wi (g fish^{-1})*	*WG (g)*	*SGR (% day^{-1})*	*SR (%)*
CT	10.2±0.29^{a}	50.9±1.65^{c}	2.98±0.03^{c}	93.9±2.5^{a}
GG	10.1±0.07^{a}	50.2±1.61^{c}	2.97±0.03^{c}	96.8±1.4^{a}
GG/HK	10.3±0.20^{a}	55.8±1.12^{b}	3.09±0.05^{b}	95.2±5.4^{a}
GG/P	10.4±0.25^{a}	54.3±1.41^{b}	3.05±0.03^{bc}	92.4±5.8^{a}
GG/P/HK	10.3±0.01^{a}	62.5±1.48^{a}	3.26±0.03^{a}	98.5±1.4^{a}

Values were represented as mean ± standard deviation. Values with the same letters in a column were not significantly different ($p<0.05$).

There was no significant difference ($p>0.05$) in the feed intake (FI), feed efficiency (FCR) or protein efficiency (PER) between treatments (Table 3). The highest FCR (1.00), and the lowest PER (2.23) of bighead catfish were found in GG treatment, while the lowest

FCR (0.92) and the highest PER (2.60) were found in the treatment GG/P. There was almost the same FI for all treatments (2.5 to 2.6 % fish^{-1} day^{-1}) (Table 3).

Table 3

Feed intake (FI), feed conversion rate (FCR), protein efficiency rate (PER) of *Clarias macrocephalus*

Treatment	*FI (% fish^{-1} day^{-1})*	*FCR*	*PER*
CT	2.5±0.31^{a}	0.96±0.12^{a}	2.32±0.29^{a}
GG	2.6±0.26^{a}	1.00±0.09^{a}	2.23±0.20^{a}
GG/HK	2.6±0.21^{a}	0.94±0.08^{a}	2.34±0.19^{a}
GG/P	2.5±0.73^{a}	0.92±0.28^{a}	2.60±0.94^{a}
GG/P/HK	2.6±0.19^{a}	0.94±0.07^{a}	2.35±0.15^{a}

Values were represented as mean ± standard deviation. Values with the same letters in a column were not significantly different ($p<0.05$).

Feed digestibility. There was no significant difference in the digestibility of lipids or carbohydrates between diets with different additives (Table 4). However, for all other dietary components, the diet containing GG+P+HK had the highest digestibility (and usually significantly different), whereas the diet containing GG as the only additive had the lowest digestibility (Table 4). These results show that the reduction in digestibility (especially in terms of ash content), as a result of adding guar gum as a binder, can be offset by phytase or HK *Lactobacillus* and especially by a combination of both.

Table 4

The effect of dietary additives addition on the apparent digestibility coefficients (%) of *Clarias macrocephalus* fed experimental diets

Treatment	*Dry matter*	*Protein*	*Lipid*	*Ash*	*CHO*	*Gross energy*
CT	75.1±0.30^{bc}	82.8±0.32^{b}	83.3±2.03^{a}	50.1±2.24^{ab}	74.7±0.79^{a}	80.2±0.55^{b}
GG	74.5±0.78^{c}	82.3±0.85^{b}	82.0±2.13^{a}	46.3±1.91^{c}	74.6±0.92^{a}	79.9±0.59^{b}
GG/HK	75.3±0.61^{bc}	82.9±0.35^{b}	84.2±2.56^{a}	53.2±1.70^{bc}	74.6±0.43^{a}	80.2±0.68^{b}
GG/P	76.6±1.17^{b}	83.0±0.82^{b}	83.5±2.59^{a}	55.2±4.79^{b}	75.5±0.21^{a}	81.0±0.86^{b}
GG/P/HK	79.2±0.91^{a}	84.4±0.70^{a}	86.3±1.88^{a}	63.1±0.21^{a}	75.6±1.56^{a}	82.8±1.02^{a}

Values were means ± standard deviation. Values with the same letters in a column were not significantly different ($p<0.05$).

Fecal characteristics. Feces recovery was significantly higher in the two dietary treatments with HK L-137 as an additive (Table 5).

Table 5

Feces recovery (%), the amount of total feces produced (g kg^{-1} of given feed), recovered feces (g kg^{-1} of given feed), non-recovered feces (g kg^{-1} of given feed)

Treatment	*Feces recovery (%)*	*Total feces produced (g)*	*Recovered feces (g)*	*Non recovered feces (g)*
CT	4.64±0.40^{b}	249±2.98^{ab}	11.5±0.92^{b}	237±3.55^{ab}
GG	5.23±0.52^{ab}	255±7.76^{a}	13.4±1.76^{ab}	242±6.01^{a}
GG/HK	6.81±0.99^{a}	247±6.14^{ab}	16.8±2.62^{a}	230±5.58^{ab}
GG/P	5.76±1.08^{ab}	234±11.7^{b}	13.4±1.95^{ab}	221±13.6^{b}
GG/P/HK	7.15±1.72^{a}	208±9.08^{c}	14.8±3.04^{ab}	194±12.0^{c}

Values were represented as mean ± standard deviation. Values with the same letters in a column were not significantly different ($p<0.05$).

The treatment GG/P/HK was notable for producing significantly less feces (208 g kg^{-1} of given feed) than other treatments, resulting in the lowest level of non-recoverable feces (Table 5). Surprisingly, given that GG is a fecal binder, the GG treatment had a rate of

high feces recovery but also the highest level of non-recoverable feces, despite having the highest proportion of fecal pellets larger than 2 mm (Figure 1). It means that GG improved the feces' characteristics to increase feces recovery (Table 5) and the size of fecal pellets (Figure 1). However, GG hampered digestibility (Table 4) leading to producing more feces so that releasing more non-recovered feces into the system.

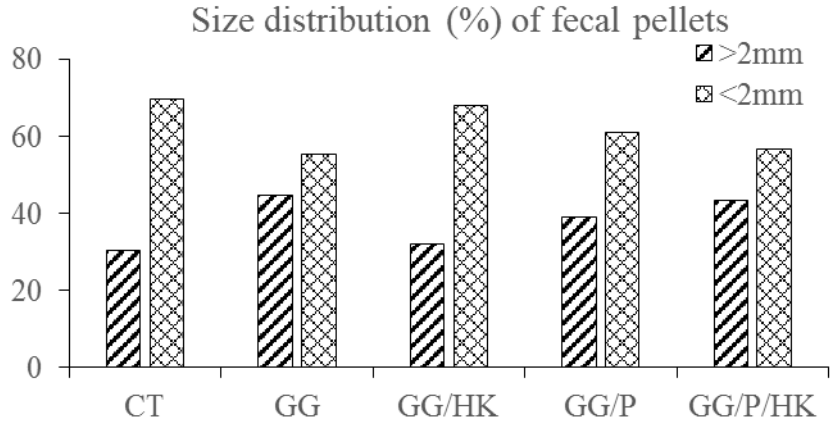

Figure 1. Size distribution of fecal pellets.

The values (n=3 per treatment) were presented as percentage of the fecal pellets with larger than and smaller than 2 mm, respectively. There was no significant difference between treatments ($p>0.05$).

Immune responses parameters. For some unknown reason, red blood cell counts in the GG/P treatment were significantly higher than in other treatments (Table 6). On the other hand, white blood cell counts and lysozyme activity were both significantly higher in the two treatments containing the probiotic HK L-137 (Treatments GG/HK and GG/P/HK) (Table 6).

Table 6

Red blood cells (RBC), white blood cells (WBC), and the immunity of Lysozyme activity of *Clarias macrocephalus*

Treatments	*RBC* (10^6 *cells* mm^{-3})	*WBC* (10^3 *cells* mm^{-3})	*Lysozyme* ($\mu g\ mL^{-1}$)
CT	2.14 ± 0.09^{bc}	96.6 ± 17.7^{b}	226 ± 8.69^{b}
GG	2.06 ± 0.06^{c}	103 ± 12.3^{b}	238 ± 11.2^{b}
GG/HK	2.24 ± 0.03^{b}	146 ± 7.89^{a}	278 ± 12.8^{a}
GG/P	2.72 ± 0.13^{a}	104 ± 24.2^{b}	232 ± 13.3^{b}
GG/P/HK	2.28 ± 0.09^{b}	145 ± 6.09^{a}	295 ± 7.55^{a}

Values were represented as mean ± standard deviation. Different letters in the same column represented a significant difference ($p<0.05$).

Discussion. The benefits of guar gum as a dietary binder appear to depend on the type, concentration and fish species. Feed digestibility (dry matter, protein, ash and energy) was reported to be significantly reduced by the addition of 0.8% guar gum to the diet of *C. gariepinus* (Leenhouwers et al 2006). In contrast, the digestibility of dry matter, protein, ash and energy were not affected by the addition of guar gum to the diet of rainbow trout (*Oncorhynchus mykiss*) (Brinker 2007). In our study, the addition of guar gum alone to the diet had no adverse effect on digestibility of any dietary component apart from ash. Ash digestibility has been reported to be more sensitive than for other dietary components to the presence of guar gum in the diet (Morales et al 1991). Adding guar gum alone also appeared to provide no significant benefits, since it did not increase the survival or growth rates, it did not improve fecal characteristics and it did not

enhance immune responses. Perhaps it's only benefit as a binder was to increase the proportion of larger sized fecal pellets.

Phytase and/or heat-killed *Lactobacillus* were added to the diet in order to improve growth and feed digestibility, and to offset the expected (but unrealised) reduction in feed digestibility, due to guar gum. Adding the phytase digestive enzyme to the diet is reported to aid digestion by increasing the assimilation and absorption of the phosphorus in phytate or phytic acid (mainly from plant protein sources) that cannot be digested by fish (Igbasan et al 2000; Kumar et al (2012), reducing the quantity of fecal waste discharged into the environment. However, we found only a small improvement in feed digestibility by adding phytase alone to the diet (Table 4). There was no improvement in fecal characteristics (Table 5) and no significant enhancement in immune responses (Table 6).

Many recent studies have shown that phytase can improve the growth rate of many fish species, for example the American catfish *Ictalurus punctatus* (Jackson et al 1996), the African catfish, *C. gariepinus* (Van Weerd et al 1999), the common carp *Cyprinus carpio* (Nwanna et al 2005), the striped catfish *Pangasius pangasius* (Debnath et al 2005), the basa catfish *Pangasius bocourti* (Kim & Hung 2007) and *C. striata* (Hien et al 2015). However, we could not detect any improvement in the growth rate of bighead catfish from the inclusion of phytase together with guar gum in the diet.

As mentioned in the introduction, heat-killed *L. plantarum* L-137 (HK L-137) has been widely reported to increase fish growth, to improve digestibility and to enhance the immunity of both shrimp and fish (Dash et al 2015; Dawood et al 2019; Duc et al 2020; Nguyen et al 2019; Yang et al 2016; Hien et al 2021a,b). Likewise, we found that the addition of HK L-137 to the diet of the bighead catfish had a beneficial effect on the growth and feed digestibility, particularly when combined with phytase, in the diet. We also found significantly higher white blood cell counts and isozyme activity in bighead catfish fed with diets containing HK L-137, which further supports the observations previously reported in the red sea bream Pagrus major (Dawood et al 2015a,b), the Nile tilapia *O. niloticus* (Nguyen et al 2019), the white leg shrimp *Litopenaeus vannamei* (Duc et al 2017), *C. macrocephalus* (Hien et al 2021a) and *C. striata* (Hien et al 2021b). Lysozyme is an important component of the fish's innate immune system and is also an antibacterial agent attacking and destroying the outer cell wall of bacteria.

Conclusions. Collectively, the results of this study showed that the survival rate of the experimental *C. macrocephalus* was not adversely affected by any of the dietary additives (guar gum, phytase or heat-killed bacteria). While the addition of the guar gum alone (as a dietary binder) did not adversely affect performance or feed digestibility (apart from ash) compared to the control, neither did it improve the fecal characteristics or the immune response of *C. macrocephalus*. In other words, it had very little effect, negative or positive on any of the measured parameters. In contrast, a combination of guar gum, phytase and heat-killed bacteria in the diet brought significant benefits by increasing performance, improving feed digestibility and fecal characteristics, and enhancing the immune response of *C. macrocephalus*.

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