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Effect of dietary replacement of fishmeal with spent brewer's yeast on growth performance of Asian seabass (*Lates calcarifer*) in Cambodia coastal aquaculture

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Abstract

Coastal Asian seabass farming is an emerging industry in Cambodia, but most farmers depend upon trash fish to feed seabass due to the high cost of formulated diets, with inherent biosecurity and sustainability problems. Use of locally sourced, low-cost ingredients would overcome the major cost obstacle to integration of dry, formulated diets into Cambodian seabass aquaculture. We conducted two feed trials, one in tanks and one in hapas, to evaluate replacement of fishmeal in dry formulated diets with spent yeast sourced from a local brewery. Replacement of fishmeal with dry brewer's yeast at 0%, 20%, 37% and 47% did not significantly affect performance in terms of growth and feed conversion in either culture system. Brewer's yeast can thus be an affordable, locally sourced replacement for fishmeal in formulated diets for farmed seabass in Cambodia.

Keywords: *ß-glucans, fish, immune system, sustainability*

Introduction

Annual fish consumption in Cambodia is currently 63 kg per person, accounting for over 76% of total animal protein intake (compared with 20% for pork and beef and 4% for poultry) (IFReDI 2013; Baran, 2014; Nam & Leap, 2007). In comparison, the global average fish consumption in 2014 was 20 kg per person. In addition to being a protein source, fish is an important source of micronutrients and essential fatty acids (FAO, 2016). Due to overfishing, environmental destruction and utilisation of aquatic resources for other purposes, e.g. irrigation, hydropower and/or urbanisation, access to wild fish is declining. Aquaculture constitutes the most viable alternative to compensate for this reduction in fisheries (Navy, 2006; Aldin, 2008). Aquaculture production in Cambodia is increasing rapidly evidenced by the increase from 50,000 tons in 2009 to more than 110,000 tons in 2014 (Joffre et al 2016). Fish is also an important source of livelihoods and income in Cambodia, where the majority of farmers are small-scale operators (Joffre et al 2016). Consequently, the government of Cambodia has ambitious production and economic targets for aquaculture that will require investment in infrastructure and the entire value chain. Further growth will require farmers to access quality manufactured feed pellets and adopt more sustainable fish culture practices. To date, formulated feeds only represent 1% of the diet fed to farmed fish in Cambodia. This is partly explained by the high price of formulated feeds, but also by lack of access to a steady supply of formulated feeds of consistent quality. Feed supply to the aquaculture industry in coastal regions of Cambodia is currently dominated by locally obtained trash fish (Sen et al 2018), so farmers still depend on capture fisheries as the feed source for their cultured fish. This causes environmental problems both through fishing methods and when fed on-farm. It is also associated with a high risk of pathogen introduction to the farm (Bunlipatanon et al 2014) and with high nutrient input in the form of soluble and particulate organic pollutants from decayed tissue and their breakdown products (Qian et al 2001). Soybean meal and fishmeal are the main protein sources in commercial formulated aquafeed, with the fishmeal component comprising about 3.06 million tons globally (Abdur et al 2017). Besides being an excellent protein source with high digestibility, fishmeal contains natural attractants that make it highly acceptable and palatable to most fish species (Miles et al 2006).

The main aim of the present study was to promote sustainable development of aquaculture in coastal Cambodia by evaluating brewer's yeast as a locally available, hygienic and environmentally friendly source of protein to replace trash fish in the diet of farmed Asian seabass (*Lates calcarifer*).

Material and methods

Experimental conditions

Two consecutive experiments were conducted at the Marine Aquaculture Research and Development Centre (MARDeC), Preah Sihanoukville province, Cambodia. The same feed mixture was used in both experiments, but experiment 1 (in July-September 2015) was conducted using fish held in hapas (Figure 1), while experiment 2 (in January-March 2016) was conducted using fish held in tanks (Figure 2).

A randomised design was employed in both experiments. In experiment 1, 16 hapa nets of 1.5 m length x 1 m width x 1.5 m depth, equipped with individual air stones, were immersed for 60 days in two identical concrete tanks, each with volume 42 m^3 (8 hapas per tank) (Figure 1). Flow of seawater (30 psu salinity) through the tanks was maintained by pumping such that a complete water change was made every 24 h. Asian seabass fingerlings (1120, weight 40-44 g) produced at MARDeC were stocked at 70 fingerlings per hapa net and allocated to one of four treatments, with four replicates per treatment randomly distributed between the two tanks. Feed was offered by hand twice a day at 3% of fish biomass, estimated on expected growth between weighings. Amount given was recorded daily as feed intake per tank and used in calculating FCR. In this hapa experiment, uneaten feed was lost to the fish through the bottom net of the hapas.

Figure 1. Experimental set up of experiment 1, with 16 hapas net divided equally between two 42 m^3 concrete tanks

In experiment 2, 12 plastic tanks (1.2 m diameter x 1 m depth) containing 1 m^3 of water were used, with three replicates per treatment (Figure 2). A total of 540 Asian seabass fingerlings at weight ranging from 22-23 g were divided between the tanks, with 45 fingerlings per tank. The fish were hand-fed twice a day at a feeding rate of 5% of fish biomass, recalculated every week based on expected growth between weighings. Also in this experiment fish fed less than the calculated feed per biomass (5% of BW) after day 45, and therefore, until day 60, feeding was commenced until apparent satiation, i.e. until fish stopped to feed actively. Then, as in the hapa experiment, amount of feed served was used to calculate FCR. The tank environment allowed the

fish to feed from the bottom. Uneaten feed was collected by syphon from each tank, per treatment and replicate, sun-dried and the weight was recorded and subtracted from feed given, to estimate the feed intake per tank.

The tank experiment was run for 60 days, with a water flow rate into the tank of 0.2 L min^{-1} using the same seawater supply as in experiment 1. The tanks were equipped with both a central and an external stand pipe to allow flushing of the tanks twice daily, at 1 h post feeding.

Figure 2. Plastic tanks with 1 m^3 water used in experiment 2. Left: external piping and stand pipe. Centre: water inlet, division of inlet water and air supply to individual air stones. Right: central stand pipe, inlet water source and air stone. Anet bag (150µm mesh size) was placed over the inlet source for water filtration and to reduce the splashing of inlet water

Experimental diets

Before formulating the diets, the ingredients were analysed for gross chemical composition and amino acids, and the content of metabolizable energy (ME) was calculated (Table 1).

Table 1. Chemical composition (% of dry matter (DM)) and metabolisable energy (ME, MJ kg^{-1}) and amino acid (% of DM) content of feed ingredients[1]. Analysis of ingredients by CelAgrid (Centre for Livestock and Agriculture Development), analysis of amino acids by National Institute of Animal Sciences, Hanoi, Vietnam

	Ingredient			
	WF	**SBM**	**FM**	**BY**
DM	88.6	91.3	91.8	93.6
Crude protein*	11.6	46.2	62.2	46.8
Crude fat	5.7	5.8	6.4	1.7
Crude fibre	0.8	3.0	4.6	2.2
Ash	0.9	6.4	2.2	5.6
ME	17.3	17.3	10.5	16.0
Essential amino acids				
Arginine	0.35	2.12	2.40	1.42
Histidine	0.12	0.51	1.07	0.43
Isoleucine	0.52	2.03	2.23	1.51
Lysine	0.34	2.94	4.32	2.74
Leucine	0.64	2.55	3.61	2.03
Methionine	0.18	0.97	1.43	0.73
Phenylalanine	0.25	1.64	1.73	0.81
Threonine	0.42	1.87	3.41	2.01
Valine	0.38	2.17	1.86	1.36
Sum	**3.20**	**16.8**	**22.1**	**13.0**
Non-essential amino acids				
Aspartic acid	0.62	2.80	3.22	2.36
Alanine	0.20	0.95	2.39	1.55
Glutamic	2.90	7.92	8.54	5.26
Glycine	0.17	0.74	1.62	0.82
Proline	0.51	2.27	2.90	1.43
Serine	0.35	1.29	1.62	1.76
Tyrosine	0.25	1.13	1.48	1.23
Sum	**5.00**	**17.1**	**21.8**	**14.4**

[1] *Wheat flour (WF), defatted soybean meal (SBM), fishmeal (FM), dry brewer's yeast (BY). *As analysed for total nitrogen and using the factor 6.25 to calculate crude protein content*

Dry brewer's yeast was obtained as beer-making by-product from the Angkor Beer company in Preah Sihanoukvill province. Four experimental diets were formulated, to comprise 45% protein and 14% lipid, with brewer's yeast (BY) replacing fish meal at a level of 0, 20, 37 and 47 %, denoted BY0 (control), BY20, BY37 and BY47, respectively (Table 2).

Table 2. Diet formulation (% of dry matter (DM)) of Asian seabass diets replacing fishmeal with 20, 37 and 47% of brewers yeast (BY)

Ingredient	Experimental diet			
	BY0	**BY20**	**BY37**	**BY47**
Fish meal	58.2	44.4	33.4	26.5
Soybean meal (defatted)	15.0	15.0	15.0	15.0
Brewery yeast	0	20.4	36.6	46.8
Wheat flour	16.3	9.7	4.5	1.2
Oil (squid oil)	8.5	8.5	8.5	8.5
Carboxymethyl cellulose	1	1	1	1
Vitamin premix*	1	1	1	1

**Content per 500g: Vitamin A 350000 IU, vitamin D3 15000 IU, vitamin E 1350000 IU, vitamin B1 (thiamine) 500 mg, vitamin B2 (riboflavin) 500 mg, vitamin B6 (pyroxide) 500 mg, vitamin K 0.5 mg, biotin 15 mg, folic acid 150 mg, chlorine 50000 mg, d. capentothenate 1500 mg, copper 11000 mg, iron 22000 mg, zinc 11000 mg, cobalt 100 mg, calcium carbonate 150000 mg, manganese 3000 mg, nicotinamide 1000 mg, other 1000 mg*

Fishmeal and defatted soybean meal were imported from Vietnam. The experimental diets were prepared by mixing the dry ingredients together by hand, then adding squid oil and finally incorporating sufficient distilled water to form a stiff dough. The dough was then placed in a pellet press fitted with a 3.0 and 2.0 mm die for the hapa and tank experiment, respectively. All diets were dried in the shade, then stored in a freezer until use. A new batch of diet was prepared every 4 weeks, with the same ingredients as the previous batch. The complete diets were sent for analysis to the National Institute of Animal Sciences, Hanoi, Vietnam. The chemical composition of the feed used in the hapa and tank experiment is given in Table 3 and Table 4, respectively.

Table 3. Chemical composition (% of dry matter (DM)) and metabolisable energy (ME) content (MJ kg^{-1}) of the diets used in experiment 1, performed in hapas. Analyses performed by CelAgrid (Centre for Livestock and Agriculture Development)

Variable	Experimental diet with dry brewer's yeast (BY)			
	BY0	BY20	BY37	BY47
DM	85.5	85.6	84.1	86.3
Crude protein	44.5	45.9	43.8	44.4
Ash	13.6	11.2	10.2	8.92
Fibre	1.06	1.15	1.07	1.06
Crude fat	10.2	8.27	5.86	6.30
ME	14.9	15.8	15.8	16.5

Table 4. Chemical composition (% of dry matter (DM)), metabolisable energy (ME) content (MJ kg^{-1}) and amino acid content (% of DM) of the diets used in experiment 2, performed in tanks. Analyses performed by CelAgrid (Centre for Livestock and Agriculture Development) except for analysis of amino acids which was performed by the National Institute of Animal Sciences, Hanoi, Vietnam

Variable	Experimental diet with dry brewer's yeast (BY)			
	BY0	BY20	BY37	BY47
DM	85.5	86.9	88.5	88.8
Crude protein	43.9	43.7	43.3	43.7
Crude fat	12.0	11.0	10.1	10.4
Crude fibre	2.8	5.3	4.1	3.3
Ash	15.1	12.8	11.2	10.1
ME	14.5	14.7	15.4	16.3
Essential amino acids*				
Arginine	2.20	2.05	2.10	1.99
Histidine	0.75	0.79	0.70	0.65
Isoleucine	1.49	1.47	1.57	1.64
Leucine	3.00	2.98	2.91	2.95
Lysine	3.50	3.52	3.54	3.50
Methionine	1.05	0.95	0.81	0.74
Phenylalanine	1.98	1.95	1.96	1.80
Threonine	1.71	2.45	2.08	1.85
Valine	1.70	1.69	1.70	1.73
Sum	**17.4**	**17.9**	**17.4**	**16.9**
Non-essential amino acids				
Aspartic	3.69	3.58	3.72	3.78
Glutamic	6.36	6.52	6.45	6.26
Serine	1.57	1.66	1.78	1.84
Glycine	2.17	1.65	1.77	1.76
Tyrosine	1.34	1.39	1.28	1.28
Alanine	2.53	2.53	2.42	2.41
Proline	2.36	2.00	2.50	2.63
Sum	**20.0**	**19.3**	**19.9**	**20.0**

**Requirement for Asian seabass (% of protein) according to Millamena (1996): Arginine 3.8%, lysine 4.5%, methionine 2.24%, and Murillo-Gurrea et al (2001): lysine and arginine 20.6 g kg^{-1} diet (4.5% of protein) and 18.2 g kg^{-1}diet (3.8% of protein), respectively*

Data collection and analysis

For both experiments, fish were acclimatised to the formulated diets for one week before initial weighing and trial commencement. The fish were fed by hand twice a day, at 08:00 h and 17:00 h. A total of 30 fish (from among 70 fingerlings) from the hapa experiment and 25 fish (from among 45 fingerlings) from the tank experiments were netted (at 15-day intervals) for measurement of body weight and total length. Daily weight gain (DWG), feed conversion ratio (FCR), condition factor (CF) and survival rate (SR) were calculated. Dissolved oxygen (DO), temperature and ammonia (NH_3) level were measured every 2 days in each tank (at 07:00-08:00 h and 13:00-14:00 h), using a DO meter (YSI 556 MSP), pH meter (ECO Test pH2) and NH_3 and NO_2 kits (Sera test kit), respectively. In experiment 1 (hapas), these water quality parameters were analysed in the large tanks containing the hapas, while in experiment 2 measurements were performed in each tank.

All growth performance data were statistically analysed by using General Linear Model (GLM) one-way (ANOVA) using SAS (Statistical analysing system, ver. 9.4). Tukey pairwise comparison was used as *post-hoc* test where the complete model was found to be significant and $p < 0.05$ was considered significant. The fixed factors were the treatments and tank. When not found significant, tank effect was omitted from the statistical model. All data were tested *a priori* for normal distribution. Data points outside the 97.5% confidential interval were omitted from the statistical analysis.

Results

Survival of fish in both experiments was high, 90-96%, and was not significantly different between diets (Table 5, 6), but a clear trend was found in that survival was nearly always higher in fish given yeast diets. In terms of growth performance, no significant ($p>0.05$) differences were found for any of the growth or feed efficiency variables between the different diets (Figures 3, Tables 5 and 6), with the exception that total feed given in the tank experiment was significantly higher in diets with higher yeast inclusion (Table 6). This increased the FCR in these treatment groups, but not significantly, over the duration of the trial (Table 6). A similar non-statistically significant difference in total feed given and FCR between low and high yeast inclusion was observed in the hapas experiment (Table 5). Furthermore, FCR was generally higher in hapas than in tanks (Tables 5 and 6). However, although care was taken not to overfeed the fish, recovery of uneaten pellets was not possible in hapas, but was undertaken in the tank experiment. Starting weight of the fish was also higher in the hapas experiment.

Table 5. Performance variables in Asian seabass reared in hapa (experiment 1), given as arithmetic mean ± standard error

Parameters	Day	Experimental diet with brewer's yeast (BY)				p-value
		BY0	BY20	BY37	BY47	
Fish number	1	70	70	70	70	–
	60	66 ± 1.34	63 ± 1.34	68 ± 1.34	68 ± 1.34	0.07
BW (g)	1	41.2 ± 3.65	41.0 ± 3.65	44.8 ± 3.65	44.6 ± 3.65	0.81
	60	114.8 ± 9.79	109.5 ± 9.79	109.7 ± 9.79	103.8 ± 9.79	0.89
DWG (g d^{-1})	1-15	1.16 ± 0.13	1.01 ± 0.13	1.04 ± 0.13	0.97 ± 0.13	0.73
	45-60	0.64 ± 0.11	0.54 ± 0.11	0.60 ± 0.11	0.63 ± 0.11	0.91
	1-60	1.23 ± 0.11	1.14 ± 0.11	1.08 ± 0.11	0.99 ± 0.11	0.50
FCR	1-60	1.5 ± 0.20	1.7 ± 0.20	1.8 ± 0.20	1.8 ± 0.20	0.54
Total feed (g)	1-60	1652 ± 107	1616 ± 107	1782 ± 107	1772 ± 107	0.61
SR (%)	1-60	94.3 ± 1.87	90.0 ± 1.87	97.3 ± 1.87	97.3 ± 1.87	0.06

***Note:** BW = Body Weight; Daily Weight Gain (DWG, g) = (W_1 - W_0/ d), where W_1 = Final weight of fish and W_0 = Initial weight of fish; Feed Conversion Ratio (FCR) = (Total feed, g / Weight gain, g); Survival Rate (SR, %) = (N_1 / N_0) * 100, where: N_o = initial fish number and N_1 = final fish number*

Table 6. Performance variables in Asian seabass reared in tanks (experiment 2), given as arithmetic mean ± standard error

Parameters	Day	Experimental diet with brewer's yeast (BY)				p-value
		BY0	BY20	BY37	BY47	
Fish number	1	45	45	45	45	–
	60	42 ± 1.14	43 ± 1.14	43 ± 1.14	44 ± 1.14	0.67
BW (g)	1	22.9 ± 0.47	23.3 ± 0.47	22.7 ± 0.47	22.4 ± 0.47	0.56
	60	99.7 ± 2.96	97.7 ± 2.96	94.0 ± 2.96	91.2 ± 2.96	0.26
DWG (g d^{-1})	1-15	1.07 ± 0.06	0.97 ± 0.06	0.93 ± 0.06	1.00 ± 0.06	0.54
	45-60	0.77 ± 0.12	1.07 ± 0.12	0.93 ± 0.12	0.77 ± 0.12	0.30
	1-60	1.28 ± 0.10	1.23 ± 0.10	1.19 ± 0.10	1.16 ± 0.10	0.84
FCR	1-60	1.2 ± 0.08	1.3 ± 0.08	1.3 ± 0.08	1.4 ± 0.08	0.33
Total feed (g)	1-60	984 ± 81.4	1042 ± 81.4	1013 ± 81.4	1052 ± 81.4	0.93
SR (%)	1-60	93.0 ± 2.57	95.7 ± 2.57	95.7 ± 2.57	97.3 ± 2.57	0.70
CF	1	1.20 ± 0.03	1.27 ± 0.03	1.23 ± 0.03	1.23 ± 0.03	0.49
	60	1.5	1.5	1.5	1.5	–

Note: *BW = Body Weight; Daily Weight Gain (DWG, g) = (W_1 - W_0 / d); Feed Conversion Ratio (FCR) = (Total feed, g / Weight gain, g); Survival Rate (SR, %) = (N_1 / N_0) * 100, where: N_o = initial fish number; N_1 = final fish number; Condition Factor (CF) = (Body weight (g) / Body length^3 (cm)) *100*

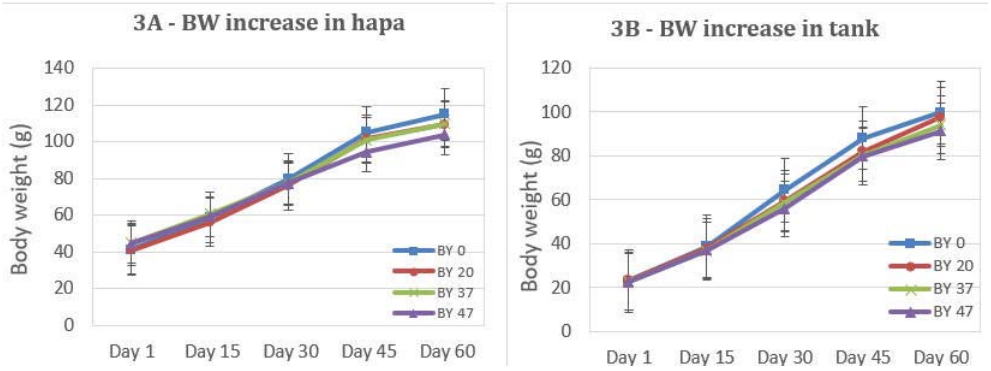

Figure 3. Body weight (BW) increase over the entire experiment, as arithmetic mean of four and three replicates in A) the hapa experiment and B) the tank experiment. Error bars indicate standard errors

Discussion

A number of studies have examined the effects of replacing fishmeal with brewer's yeast (inactive or spent yeast), which is a natural by-product of the brewing process in commercial beer production. In previous studies, brewer's yeast has been used successfully to replace a proportion of dietary fishmeal in different fish species, including Atlantic salmon (*Salmo salar*) (Engstad et al 1992), rainbow trout (*Oncorhynchus mykiss*) (Siwicki et al 1994), and Asian catfish (*Clarias batrachus*) (Kumari and Sahoo 2006). The replacement level of up to 47% used in our experiments is broadly in agreement with findings by Oliva-Teles (2001) that brewer's yeast can replace 50% of fishmeal protein with no negative effects on growth performance of European seabass (*Dicentrarchus labrax*) juveniles. Moreover, Pongpet et al (2016) found that brewer's yeast replacement of up to 45% of fishmeal improves growth performance in Thai Panga (*Pangasianodon hypophthalmus* x *Pangasius bocourti*). However, when the inclusion rate was increased to replace 60% and 75% of fishmeal, Pongpet et al (2016) observed a significant decline in growth performance. This may reflect poor utilisation of non-starch polysaccharides by the fish (Kuz'mina 1996). The results in both experiments in the present study indicated that growth over time was not significantly different between treatments, but that fish given the highest inclusion of yeast tended to display slightly lower values for each weighing period in both experiments (Tables 5 and 6, Figure 3). This could possibly reflect slightly lower digestibility of the yeast protein compared to fishmeal protein (Sen 2019). An explanation that tallies well with the slightly numerically higher, but statistically non-significant levels of FCR and feed given with higher yeast inclusion rate. It is possible that dry brewer's yeast contains factors that incur high metabolic cost (e.g. high levels of nucleic acids), which if present in high concentrations hamper the performance of monogastric animals, including fish (Schulz et al 1976) and shrimp (Do et al 2012).

In both the hapa and tank experiments, we found no difference in weight gain of fish fed control and yeast replacement treatments. However, daily weight gain (g day^{-1}) decreased during the final period in all treatments (Tables 5 and 6). This was possibly partly caused by the defatted soybean meal content (15%) in the diets being higher than the recommended level (10%) for Asian seabass (Tantikitti et al 2005). Previous trials found that defatted soybean meal at lower than 10% supported good growth of Asian seabass, while at 20% replacement caused a significant decrease in feed intake and growth due to poor palatability and amino acid imbalance compared with fishmeal (Tantikitti et al 2005). In most cases, replacing fishmeal with plant proteins reduces feed intake, which is one of the most important factors of low growth performance of carnivorous fish species (Blaufuss and Trushenski, 2012).

We detected no significant difference in terms of fish performance or survival from inclusion of yeast in our study. However, a clear trend was discerned with higher survival in nearly all groups of fish fed yeast. Fish mortality occurred in all treatments after day 30 in both hapas and tanks, but was uneven between treatments and negatively correlated with yeast inclusion (p=0.06, in Hapa experiment Table 5). The same trend was obvious also in the tank experiment (Table 6), but due to larger tank to tank variation (Table 6) the trend was insignificant (p=0.7), in contrast to the Hapas experiment. Previously, increased survival in other fish species has been attributed to ß-glucans from yeast cell wall stimulating phagocytic function and increasing the survival after challenge with pathogenic bacteria (LaPatra et al 1998). Several studies, including those by Li and Gatlin (2003) working with striped bass (*Morone chrysops* × *M. saxatilis*) and Ortuño et al. (2002) working with gilthead seabream (*Sparus aurata*), report that dietary yeast improves the growth performance and the immune system. Those reviews concluded that feeding fish ß-glucans and other immunostimulants typically results in increased respiratory burst and macrophage, lysozyme and leucocyte activities that boost resistance to infections and stress. However, glucan structure is critical phagocyte activation and the 1-3 linkages in ß-glucans are rapidly hydrolysed under acidic conditions (Kudrenko et al 2009). With gut pH rapidly falling in sea bass post feeding (Kudrenko et al 2009), it may explain why potential effect of glucan on survival is inconsistent and was not significant in this trial. Nevertheless, brewer's yeast is rich in vitamins B, C and E, and is used as a dietary supplement for various animals, including some fish species, and may affect immune responses (Siwicki et al 1994), with potential as a health promoter for fish culture. For example, Li and Gatlin (2003) found that dietary supplementation with dry brewer's yeast at a level of 2-4% improved the immune system and resistance to bacterial infections in hybrid striped bass (*Morone chrysops* x *M. saxatilis*), with 20% higher mortality reported for fish on a control (menhaden fishmeal) diet without yeast supplementation compared with fish fed a diet containing dry brewer's yeast.

In the present trials the mortality was very low, and only occurred towards the end of the experiment. Further dietary and histopathological analysis of moribund fish are required to ascertain whether there was a nutritional or infectious disease link to this increased morality late in both trials. To conclude, our results indicate that brewer's yeast can be an affordable, locally sourced replacement for fishmeal in formulated diets for farmed seabass in Cambodia and with a possible disease resistance promotion.

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Go to top