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IMPACT OF YEAST FERMENTED POULTRY BY-PRODUCT MEAL ON GROWTH, DIGESTIVE ENZYME ACTIVITIES, INTESTINAL MORPHOMETRY AND IMMUNE RESPONSE TRAITS OF COMMON CARP (*CYPRINUS CARPIO*)

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Abstract

The current study was carried out to investigate the effects of *Saccharomyces cerevisiae*-fermented poultry by-product meal (PBM) on growth performance, micromorphological, and immunological changes in common carp. Five experimental diets were prepared to include fermented PBM at 0, 5, 10, 15, and 20% level in the diet of common carp (4.91±0.01 g). The fish were reared for 90 days on these diets. The obtained results revealed that yeast fermented PBM significantly changed the final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed intake (FI), and feed conversion ratio (FCR) of the fish in a dose dependent manner (P<0.05). Fish fed 20% fermented PBM showed the highest FBW, WG, SGR, FI, and lowest FCR. However, whole body composition did not vary significantly among fish fed different diets (P>0.05). Dietary yeast fermented PBM at 10 and 20% level significantly increased the lipase, amylase, and protease activities compared to the other groups (P<0.05). The anterior, middle, and posterior intestinal villus length was significantly increased in fish fed fermented PBM at 15 and 20% level when compared to the other groups (P<0.05). The number of goblet cells was significantly increased in the middle section of intestine in fish fed yeast fermented PBM at 20% level, while in the posterior region of intestine the number of goblet cells was significantly increased in fish fed yeast fermented PBM at 15 and 20% level (P<0.05). The histomorphology of intestine showed an increased length, branching and density of intestinal villi in fish fed yeast fermented PBM diets. Most of the measured blood parameters showed insignificant (P>0.05) differences except for Hb, RBCs, WBCs, total blood protein, and globulin which were significantly affected by the inclusion of yeast fermented PBM (P<0.05). Lysozyme activity was significantly increased in fish fed yeast fermented PBM at 5, 10, and 15% level, while the phagocytic activity and phagocytic index were significantly increased at 20% level when compared to the control groups (P<0.05). It is concluded from this study that inclusion of yeast fermented PBM in the diet of common carp at 15–20% level increased digestive enzyme activities, immune function and growth of the fish.

Key words: *Saccharomyces cerevisiae*, fish, feed utilization, histomorphology, poultry by-product meal

Aquaculture offers a promising source of economic and healthy protein for humankind (Saeidi asl et al., 2017). This has raised attention to an increasing development of aquaculture industry leading to advanced production technologies and culture systems in many parts of the world (Moss et al., 2018). However, the cost of fish feeding is still the main challenge for the nutritionists, who are trying to formulate balanced diets with low cost. Fishmeal (FM) has been used as a traditional protein source in aqua feed for a long time due to the balanced nutritional value, high content of natural attractants and palatability (Dawood and Koshio, 2019). However, decrease in fisheries stocks and high price of fish meal have made it hard to meet the aquaculture consumption exigencies of this industry. In consequence, a global priority for finding alternative protein sources to replace FM in aqua feeds is essential (Dossou et al., 2018 a, b).

Because of its high nutritional value, poultry by-product meal (PBM) can be used as a protein source in the formulation of diet for fish (Gunben et al., 2014; Hekmatpour et al., 2018; Wang et al., 2015; Zhou et al., 2011). The PBM contains high amount of lipid (12–15%) and crude protein (58–65%) (Hill et al., 2019). However, PBM is lacking in some essential amino acids (mainly lysine and methionine) that can be added separately to diets. Thus, PBM seems to be a viable cost-effective alternative of FM in the formulation of diet for fish. Previous studies have demonstrated the beneficial impacts of PBM integrated diets on growth performance, immune response, oxidative status, and stress resistance in aquatic animals (Dawson et al., 2018; El-Sayed, 1998; Parés-Sierra et al., 2014). PBM can totally replace FM in practical Nile tilapia and common carp diets (El-Sayed, 1998; Gümüş and Aydin, 2013). Fermentation is based on using of microorganisms that can exhibit its beneficial role if reared under specific conditions (Dossou et al., 2019; Kaviraj et al., 2013). Poultry waste can be treated with appropriate microorganisms to preserve its nutrients and finally incorporated into the fish diet, which would reduce feed costs and environmental pollution. Fermented animal by-product meals showed better nutrient efficiency and could improve nutritional value of aqua feed (Gümüş and Aydin, 2013; Mondal, 2014; Samaddar et al., 2015). The yeast *Saccharomyces cerevisiae* is among the well-known microorganisms used in fermentation and its efficacy depends on the functional components such as "β-glucans, nucleic acids, mannan oligosaccharides and chitin" (Dawood and Koshio, 2019; Ortuño et al., 2002; Peppler, 1982). Yeast also produces different metabolites like, "enzymes, oligosaccharides, amino acids, peptides, organic acids, vitamins, and other soluble factors" (Peppler, 1982). The supplementation of *S. cerevisiae* as probiotics ameliorated the growth, immunity and disease resistance of various fish species (Dawood et al., 2019 c; El-Boshy et al., 2010). Probiotics fermentation is also beneficial for removing toxicity of dietary ingredients, which are used as alternative protein source for replacement of FM (Bairagi et al., 2002; Dawood et al., 2020 a). To the best of our knowledge, there is no document regarding inclusion of *S. cerevisiae* fermented PBM in the diet of common carp and its subsequent effects on the growth of the fish.

Histopathological analysis of fish tissues has been used as a biomarker in assessment of internal effect (nutrition) and external (aqueous environment) conditions (Barišić et al., 2018). Histological studies of the digestive system are considered to

be a good indicator of metabolic, health and nutritional status of fish (Rašković et al., 2011). The intestine absorbs proteins, lipids and carbohydrates, and is an important organ for the distribution of nutrients in the body of fish. Monitoring of the histological structure of fish intestines is already recognized as a method of choice for assessing the effects of nutrient mixtures of animal origin used as raw materials in the diet of fish (Dawood et al., 2020 d). Common carp (*Cyprinus carpio*) contributes to a large proportion of freshwater aquaculture production (Dawood et al., 2020 b; Jagruthi et al., 2014). Though the productivity of carp is rapidly increasing, profitability of the carp producer is being decreased due to the progressive increase in feed cost (Mehana et al., 2015). Therefore, the present study was conducted to evaluate the effects of the inclusion of *S. cerevisiae* fermented PBM on the growth performance, digestive enzyme activity, intestinal morphometry, and immunity of common carp.

Material and methods

Animal welfare statement

The experiment was conducted in accordance with the guideline of Kafrelsheikh University, Egypt. All animal protocols were approved by the Institutional Animal Care and Use Committee of Faculty of Agriculture, Kafrelsheikh University.

Fermentation and diet preparation

Fine powder of PBM was kindly provided by Elsodor Company (Al-Sadat City, Egypt). PBM was adjusted to a moisture content of 60–65% using de-ionized water in a 1 L of glass container with six sets of replications for each. They were autoclaved at 121°C (105 kPa) (20 min) then cooled to a room temperature. Cane molasses was collected from the local market and used as a source of fermentable carbohydrates for *S. cerevisiae* activation. To the sterilized ingredients, actively growing culture (2.27×10^{10} CFU per g) of *S. cerevisiae* (Shanghai Gosun Biotechnologies Co. Ltd, China) was inoculated and thoroughly mixed using a sterile glass rod manually. All the inoculated samples were incubated at 30±2°C for four days to carry out the fermentation process. Prior to this, the fermentation conditions were optimized for moisture (50, 55, 60, 65 and 70%), temperature (28, 30, 32, and 36°C), inoculum rate (1, 2.5, 5, 7.5 and 10%), pH (5.5, 6, 6.5, 7, and 7.5) and incubation period (1, 2, 3, 4 and 5 days). The optimized conditions of 60–65% moisture, 28–30°C temperature, 5% inoculum rate, 6.5–7 pH and four-day incubation period were applied in the present investigation. All the fermentation samples were mixed with a sterile glass rod two times every day (7.00 am and 6.30 pm) for proper homogenization. After the fourth day, all the materials were transferred to a little higher temperature of 35°C to stop the fermentation process and allowed to dry at the same temperature until the moisture content of fermented samples became <10%. The proximate and essential amino acid composition of fermented PBM is given in Table 2.

Five isonitrogenous (30.8% crude protein) and isolipidic (6.7% crude lipid) diets were formulated to include 0, 5, 10, 15, and 20% of fermented PBM (Table 1). Some amino acids are relatively low in PBM when compared to FM (Hill et al., 2019). Thus, methionine, lysine and threonine were added to obtain an equivalent indispensable amino acid profile. All ingredients were completely mixed, then added to produce a stiff dough. The dough was subsequently extruded by a pelleting machine through a die of 1 to 2 mm, then the pellets were air dried. The diets obtained were stored at –20°C until they were used. AOAC (2007) standard method was used to confirm the nutritional profile of each diet (Table 1).

Table 1. Formulation and proximate chemical composition (on dry matter basis) of experimental diets with varied levels of fermented PBM

Ingredient (%)	Test diet				
	Control	5%	10%	15%	20%
Brown fish meal (65%)[1]	10	8	6	4	2
Soybean meal (44%)[1]	36	33	28	25	21
Fermented poultry by-product meal[2]	0	5	10	15	20
Gluten[1]	5	5	5	5	5
Wheat bran[1]	12	12	12	12	12
Rice bran[1]	10	10	13	13	14
Yellow corn[1]	12	12	12	12	12
Wheat flour[1]	8.92	8.61	8.17	7.86	7.5
Fish oil[1]	3	3	2	2	2
Vitamin and mineral mix[3]	2	2	2	2	2
Dicalcium phosphate[4]	1	1	1	1	1
Vitamin C[4]	0.08	0.08	0.08	0.08	0.08
Threonine[4]	0.00	0.04	0.12	0.16	0.22
Lysine[4]	0.00	0.15	0.36	0.50	0.68
Methionine[4]	0.00	0.05	0.11	0.16	0.21
Tryptophan[4]	0.00	0.06	0.16	0.23	0.31

[1]Supplied by Feed Control Co., Ltd. (Damro, Sidi Salem, Kafrelsheikh, Egypt); fish meal, 66% crude protein, 7.9% crude lipid; soybean meal, 45.6% crude protein, 0.7% crude lipid; corn gluten, 60% crude protein, 2.1% crude lipid; wheat bran, 12.8% crude protein, 3.6% crude lipid; rice bran, 12.5% crude protein, 5.2% crude lipid; yellow corn, 7.5% crude protein, 3.5% crude lipid; fish oil (98–99% crude lipid).

[2]Poultry by-product meal was kindly provided by Elsodor company (Al-Sadat City, Egypt); fish meal, 60.2% crude protein, 7.9% crude lipid.

[3]Vitamin mixture (mg/kg premix): vitamin A (3300 IU), vitamin D_3 (410 IU), vitamin E (2660 mg), vitamin B_1 (133 mg), vitamin B_2 (580 mg), vitamin B_6 (410 mg), vitamin B_{12} (50 mg), biotin (9330 mg), choline chloride (4000 mg), vitamin C (2660 mg), inositol (330 mg), para-amino benzoic acid (9330 mg), niacin (26.60 mg), pantothenic acid (2000 mg); mineral mixture (mg/kg premix): manganese (325 mg), iron (200 mg), copper (25 mg), iodine, cobalt (5 mg).

[4]Dicalcium phosphate, vitamin C, methionine, L-lysine, threonine, tryptophan (DSM in Animal Nutrition & Health, Heerlen, the Netherlands).

Table 2. Proximate composition (%, dry matter basis) of PBM, FPBM, and the experimental diets (%) used to feed common carp with varied levels of FPBM for 90 days

Item	PBM	FPBM	Test diet					Requirements[1]
			Control	5%	10%	15%	20%	
Crude protein	60.2	65	30.06	29.90	30.00	30.08	30.10	
Ether extract	7.9	8.1	6.57	6.70	6.78	6.25	6.51	
Ash	10.6	4.6	7.26	7.49	8.27	7.46	7.30	
Fibers	27.3	14.3	4.63	5.24	5.33	4.96	5.45	
Essential amino acid								
Arginine	6.6	6.9	2.72	2.63	2.67	2.65	2.63	1.6
Histidine	1.8	2.1	1.12	0.96	0.95	0.89	0.88	0.8
Isoleucine	3.9	4.2	1.32	1.32	1.21	1.18	1.13	0.9
Leucine	7	7.3	2.04	2.02	2.14	2.31	2.43	1.3
Lysine	4.2	4.7	2.48	2.33	2.26	2.25	2.31	2.2
Methionine	1.22	1.7	1.41	1.25	1.18	1.21	1.23	0.8
Phenylalanine	3.7	4.2	1.55	1.53	1.49	1.51	1.52	1.4
Threonine	3.65	4.2	2.28	2.11	2.08	2.21	2.12	1.5
Tryptophan	0.7	1	1.01	0.95	0.93	0.94	0.92	0.3
Valine	5.22	5.7	0.96	0.92	0.89	0.82	0.85	1.4

[1]Common carp requirements based on the recommendations of NRC (2011).

Fish and experimental protocol

Specimens of common carp were obtained from a private farm located in Kafrelsheikh, Egypt. Fish were kept acclimatized for two weeks in test enclosures. During acclimatization the fish were fed the control diet (Table 1) used in this experiment. Afterwards, 375 fish with similar body weight (initial body weight, 4.91±0.01 g) were distributed into 15 Habas (enclosures) (0.5 m width × 1 m length × 0.5 m depth each) in a random manner at a rate of 25 fish per Haba so that there were three replicates for each of the diet group. The feeding rate was set at 3% of body weight per day with two feeding times of 8:00 a.m. and 3:30 p.m. for 90 days. Fish were weighed every 2 weeks and the feeding rate was readjusted accordingly. Lighting in the culture unit was set at 12:12 light: dark cycle. Parameters of water quality were regularly monitored during the experimental period. Water temperature, pH and dissolved oxygen (DO) were measured by thermometer, portable digital pH meter (Martini Instruments Model 201/digital) and waterproof Portable Dissolved Oxygen (Hanna waterproof IP67). Total ammonia-nitrogen was measured calorimetrically. The obtained water quality levels were temperature 24.2 ± 1.6°C, dissolved oxygen 6.32 ± 0.3 mg/L, pH 7.05 ± 0.6 and total ammonia 0.32 ± 0.01 mg/L. The experiment was continued for 90 days.

Sampling schedule

All fish were fasted 24 hours before the sampling. The sampled fish were immediately anesthetized by tricaine methane sulfonate (100 mg/L). Fish were measured

individually for the final body weight. Using a syringe without anticoagulant, blood samples were collected from the caudal blood vessels of 3 fish per Haba, and serum was separated by centrifuging the clotted blood at 3000 rpm/15 min at 4°C and stored at −20°C. Another group of nine sampled fish (three per Haba) for each diet group was used for histomorphological and digestive enzyme studies. Intestine from each of these fish was quickly removed and the segments from the foregut, midgut and hindgut were syringed with the ice-cold PBS at pH 7.4 and then placed in 10% formalin for intestinal morphological analysis. The intestine was then sampled from 9 fish per group for digestive enzymes analysis. The collected fish were taken and aseptically intestinal, then washed with PBS (pH 7.5; 1 g per 10 mL), homogenized and centrifuged at 8000 rpm for 5 minutes. Then the collected supernatant was reserved at 4°C.

Digestive enzymes activity

The intestinal sections were used as the substrate of the digestive enzyme activities. The total protein content was measured by following Lowry et al. (1951) method, in which BSA was used as a standard. The protease activity was evaluated using Folin phenol reagent, and amylase activity was measured using iodine solution to detect non-hydrolyzed starch according to Jiang (1982) and Worthington (1993). The activity of protease and amylase was expressed as "units per mg of protein". On the basis of the protocol described by Borlongan (1990) and Jin (1995) with olive oil as a substratum, the specific activity of lipase was assessed. The activity of lipase was expressed as intestinal content "units per gram".

Intestinal morphology

Standard paraffin embedding procedures were adopted to stain each sample (three cross-sections) using hematoxylin-eosin (Bancroft et al., 1996). Intestinal morphology including height of villus (from the tip of villus to villus crypt junction) was measured using Image J software. A total of six well and random villi and villus-associated crypts were determined for each intestinal cross-section. Goblet cells were stained with PAS and counted from same-sized villi (n=9) and the results are shown as an average (±SE).

Blood and immunological assays

Red blood cells (RBCs) and white blood cells (WBCs) were counted immediately with a hemocytometer after dilution with Natt and Herrick's solution (Houston, 1990). For differential leucocytic count, blood films were prepared and stained according to Lucky (1977), and white cells were calculated by following Jain (1986). For hemoglobin (Hb) assay Drabkin's solution was added to blood and then solution was centrifuged (3500 g for 6 min) to remove interferents, afterwards blood Hb concentration was determined with a spectrophotometer (Model RA 1000, Technicon Corporation, USA) at 540 nm using the method of Blaxhall and Daisley (1973).

Serum total proteins and albumins were determined according to Doumas et al. (1981) and Dumas and Biggs (1972). Globulins content was calculated mathematically. Activities of aspartate aminotransferase (AST) and alanine aminotransferase

(ALT) were determined calorimetrically at the wave length 540 nm (Reitman and Frankel, 1957). Serum creatinine, urea and bilirubin were calorimetrically determined according to Heinegård and Tiderström (1973) and Coulombe and Favreau (1963), respectively. Glucose (MG/100 ml) was determined using glucose enzymatic PAP kits obtained from Bio-Merieux (France) (Trinder, 1969).

Activity of lysozyme was measured by following Parry et al. (1965). By following Kawahara et al. (1991), the phagocytic activity and phagocytic index were determined according to the following equations:

Phagocytic activity = macrophages containing yeast/total number of macrophages ×100; phagocytic index = number of cells phagocytized/number of phagocytic cells.

Growth performance calculations

All fish per tank were weighed and counted separately during the final sampling. Weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) were evaluated. The following formulas were used:

WG = (FBW– IBW) ×100/IBW; SGR (% BW/day) = 100((LnFBW -LnIBW)/T); FCR= FI/WG. Where FBW = body weight final (g), IBW = body weight initial (g), T = duration of the trial in days, WG = wet weight gain (g) and FI = estimated feed intake (g/fish/90 days).

Statistical analysis

The obtained data were subjected to one-way ANOVA to evaluate the effect of dietary fermented PBM inclusion. Differences between means were tested at 5% probability level using Duncan test as a post-hoc test. All the statistical analyses were done via SPSS version 22 (SPSS Inc., IL, USA).

Results

Growth performances and body composition

Table 3 represents the growth, feed efficiency and survival results. The survival rates ranged from 98.67% to 100% without significant alterations ($P>0.05$). It was observed that the inclusion of fermented PBM had a significant influence on fish final body weight (FBW), weight gain (WG), specific growth rate (SGR), and feed intake (FI) and feed conversion ratio (FCR) in a dose-dependent manner ($P<0.05$). Fish fed 20% fermented PBM showed the highest FBW, WG, SGR, FI, and lowest FCR ($P<0.05$).

The whole-body proximate composition of common carp fed the experimental diets for 90 days is presented in Table 4. The results showed that whole body composition of fish did not vary significantly between different dietary treatments ($P>0.05$).

Digestive enzymes activity

Lipase, amylase and protease of fish fed varying levels of fermented PBM are presented in Figure 1. Dietary yeast fermented PBM at 15 and 20% significantly increased the lipase, amylase and protease activities in relation to the control group ($P<0.05$). Protease activity showed non-significant differences in fish fed 5, 10, 15,

and 20% fermented PBM (P>0.05). The lipase activity in 15 and 20% was significantly higher than that of 10%, while no significant differences were observed in fish fed 5 or 15% (P>0.05).

Table 3. Growth performance, nutrient utilization and survival of common carp fed test diets for 90 days*

Parameter	Test diet				
	Control	5%	10%	15%	20%
Initial body weight (g)	4.91±0.01	4.92±0.01	4.90±0.01	4.91±0.02	4.91±0.01
Final body weight (g)	11.68±0.06 a	12.51±0.16 b	13.13±0.08 c	13.57±0.15 c	14.57±0.12 d
Weight gain (%)	137.64±0.91 a	154.19±2.79 b	168.04±2.08 c	176.24±2.06 c	196.44±2.49 d
Specific growth rate (% IBW/day)	1.44±0.01 a	1.55±0.02 b	1.64±0.01 c	1.69±0.01 c	1.81±0.01 d
Feed intake (g/fish/90 day)	10.95±0.15 a	12.28±0.00 ab	13.12±0.00 b	13.76±0.00 b	14.84±0.20 b
Feed conversion ratio (g FI/g WG)	1.62±0.02 b	1.62±0.03 b	1.59±0.02 b	1.59±0.03 b	1.54±0.04 a
Survival (%)	98.67±1.33	100.00±0.00	100.00±0.00	100.00±0.00	98.67±1.33

*Values expressed as means ± SE (n = 3). Different superscript letters indicate significant differences for each pairwise comparison between treatments.

Table 4. Body composition (%, as wet weight basis) in common carp fed test diets for 90 days*

Item	Test diet				
	Control	5%	10%	15%	20%
Moisture	72.89±1.04	73.67±0.75	73.25±0.66	73.94±0.48	74.81±0.33
Crude protein	17.59±0.43	17.11±0.35	17.66±0.23	16.95±0.32	16.89±0.37
Crude lipid	4.26±0.36	4.28±0.29	3.75±0.32	3.77±0.26	3.99±0.42
Ash	4.17±0.15	3.86±0.05	4.16±0.08	4.27±0.31	3.79±0.05

*Values expressed as means ± SE (n = 3). Different superscript letters indicate significant differences for each pairwise comparison between treatments.

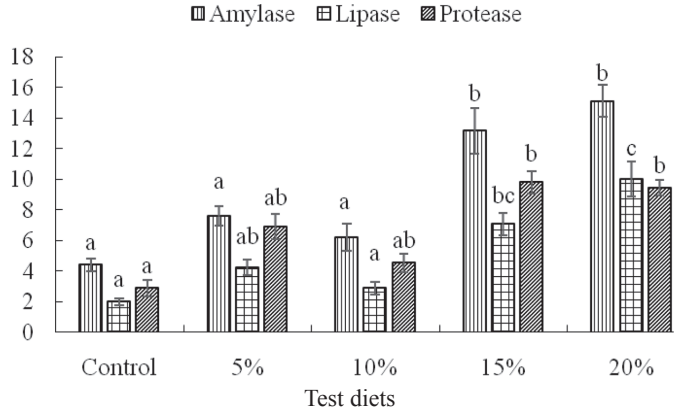

Figure 1. Intestinal digestive enzymes activities (U/mg) of common carp fed test diets for 90 days. Values are expressed as mean ± SE from triplicate groups. Bars with different superscripts are significantly different from those of control group (P<0.05)

Intestinal morphometry

The intestinal morphometry of fish fed fermented PBM was displayed in Figure 2. In the anterior segment of the intestine, villus length was significantly affected by the inclusion of yeast fermented PBM at 15 and 20% when compared to the other groups ($P<0.05$). In the middle section, only the villus length was affected significantly by the inclusion of yeast fermented PBM at 10, 15, and 20% when compared to the other groups ($P<0.05$). Posterior intestinal morphometry showed significantly increased villus length for fish fed yeast fermented PBM at 20% when compared to the control and 5% group ($P<0.05$).

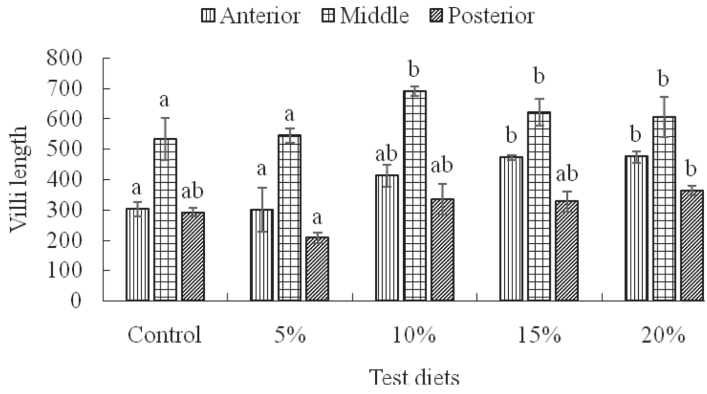

Figure 2. Intestinal villi length (μm) of common carp fed test diets for 90 days. Values are expressed as mean ± SE from triplicate groups. Bars with different superscripts are significantly different from those of control group ($P<0.05$)

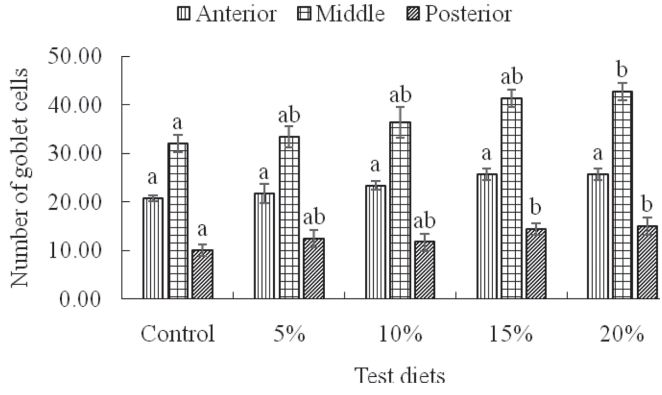

Figure 3. Number of goblet cells of common carp fed test diets for 90 days. Values are expressed as mean ± SE from triplicate groups. Bars with different superscripts are significantly different from those of control group ($P<0.05$)

The number of goblet cells was significantly increased in the middle area of intestine when fed yeast fermented PBM at 20% level, while fish fed 15 or 20% fermented PBM showed the highest number of goblet cells in the posterior region of intestine ($P<0.05$) (Figure 3).

The anterior, middle, and posterior part of fish intestine of control and yeast fermented PBM treated groups exhibited normal structure; intact tunica mucosa, submucosa, muscularis and serosa. The increase in length, branching and density of intestinal villi are evident in fish fed yeast fermented PBM diets (Figure 6).

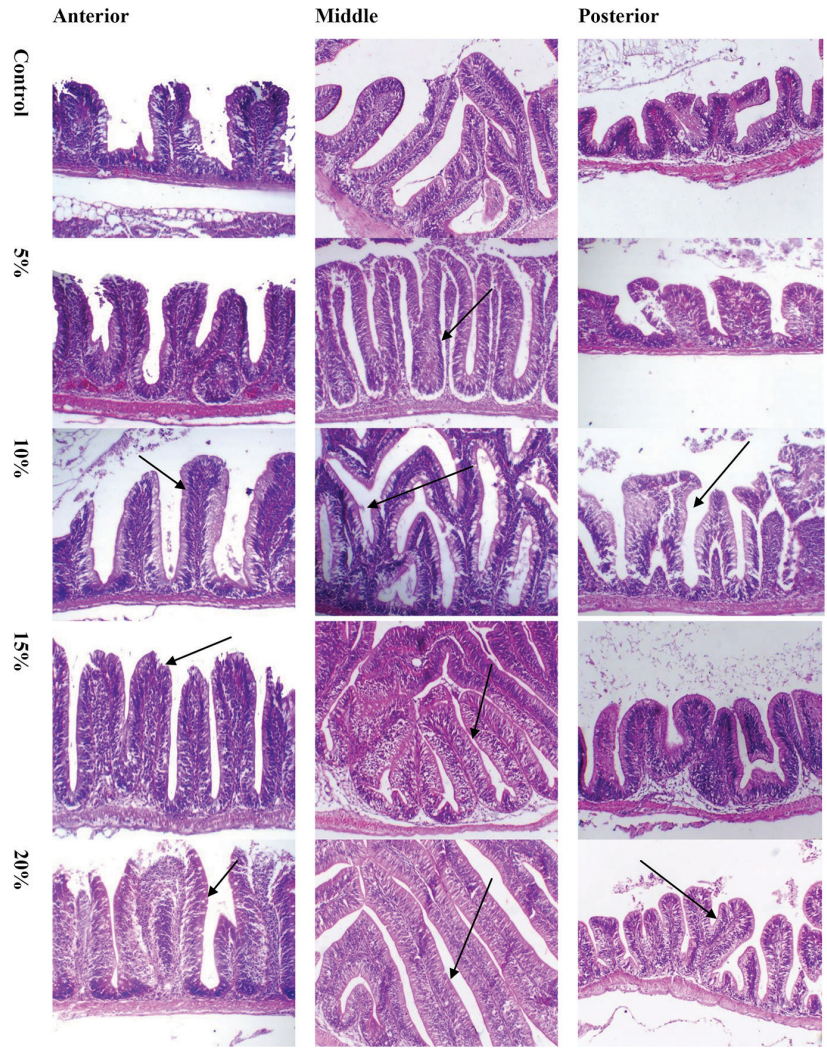

Figure 6. Intestinal morphology of fish fed the test diets (H&E, bar=100µm). The intestine of fish fed on fermented PBM showing increase the density of intestinal villi. The intestine of fish fed on fermented PBM showing increase in the length and branching intestinal villi (arrow)

Blood biochemical and hematological parameters

The blood hematological and biochemical parameters of fish fed yeast fermented PBM were displayed in Tables 5 and 6. Most of the measured parameters showed insignificant (P>0.05) differences except for Hb, RBCs, WBCs, total blood protein, and globulin which were significantly affected by the inclusion of yeast fermented PBM (P<0.05). Significantly the highest Hb and RBCs levels were observed in fish fed yeast fermented PBM at 10 and 15% level, while WBCs and globulin levels were significantly increased in fish fed yeast fermented PBM at 20% level when compared to the other groups (P<0.05). Furthermore, blood total protein was significantly increased in fish fed yeast fermented PBM at all levels when compared to the control groups (P<0.05) (Table 6).

Table 5. Hematological parameters of common carp fed test diets for 90 days*

Item	Test diet				
	Control	5%	10%	15%	20%
Hb (g/100ml)	12.20±0.07 a	12.89±0.48 ab	13.73±0.10 b	15.29±0.10 c	12.37±0.03 a
RBCs (10/mm^3)	4.13±0.07 a	4.25±0.08 ab	4.45±0.03 b	4.90±0.01 c	4.10±0.05 a
WBCs (10/mm^6)	34.77±0.69 a	32.35±1.37 a	35.74±0.26 a	40.50±0.81 b	34.62±1.23 a
Heterophils (%)	5.00±0.58	11.00±2.31	4.50±0.29	6.50±0.29	5.50±0.87
Lymphocyte (%)	87.50±0.29	78.50±4.33	86.00±0.00	87.00±0.58	86.00±1.15
Eosinophils (%)	1.00±0.00	4.50±2.02	1.50±0.29	0.00±0.00	2.00±0.58
Basophils (%)	1.00±0.00	0.50±0.29	0.50±0.29	0.50±0.29	1.50±0.29
PCV (%)	39.50±1.44	41.00±0.58	44.00±0.58	47.50±0.29	39.00±0.58
MCV (mm^3)	95.55±1.89	96.49±0.35	98.87±0.53	97.04±0.30	95.33±2.55
MCH (Pg)	29.54±0.33	30.29±0.59	30.85±0.03	31.23±0.11	30.21±0.44
MCHC (%)	30.98±0.96	31.40±0.72	31.20±0.19	32.18±0.02	31.72±0.39

*Values expressed as means ± SE (n = 3). Different superscript letters indicate significant differences for each pairwise comparison between treatments.

Table 6. Blood biochemical parameters of common carp fed test diets for 90 days*

Item	Test diet				
	Control	5%	10%	15%	20%
ALT (U/L)	4.25±0.02	4.44±0.07	4.10±0.01	4.27±0.05	4.31±0.09
AST (U/L)	77.75±0.87	104.06±14.40	75.19±0.00	78.38±0.28	76.23±2.05
Total protein (mg/dl)	5.00±0.02 a	5.76±0.58 b	5.89±0.03 b	5.83±0.04 b	5.90±0.10 b
Albumin (g/dl)	3.70±0.00	2.72±0.69	3.56±0.01	3.30±0.11	3.86±0.02
Globulin (g/dl)	2.07±0.03 a	2.28±0.11 ab	2.33±0.03 ab	2.53±0.07 b	2.04±0.12 a
Urea (mg/dl)	5.61±0.21	5.79±0.39	6.01±0.00	5.73±0.14	5.50±0.18
Creatinine (mg/dl)	0.27±0.01	0.31±0.00	0.26±0.02	0.26±0.01	0.31±0.00
Uric acid (g/dl)	2.04±0.03	2.01±0.07	2.12±0.01	2.02±0.07	2.04±0.02
Glucose (mg/dl)	15.17±0.04	15.10±0.14	14.59±0.12	14.95±0.03	23.80±8.69
Cholesterol (mg/dl)	72.62±1.52	77.60±0.78	72.25±2.75	73.75±2.46	80.64±11.51
Triglyceride (mg/dl)	133.93±0.79	133.25±2.31	131.94±2.34	132.01±2.73	108.68±22.83

*Values expressed as means ± SE (n = 3). Different superscript letters indicate significant differences for each pairwise comparison between treatments.

Immune responses

Blood immune responses of fish fed varying levels of yeast fermented PBM are presented in Figures 4 and 5. Dietary yeast fermented PBM significantly altered the lysozyme activity, phagocytic activity, and phagocytic index in a dose dependent manner (P<0.05). Lysozyme activity was significantly increased in fish fed yeast fermented PBM at 5, 10, and 15% level, while the phagocytic activity and phagocytic index were significantly increased by feeding fermented PBM up to 20% level when compared to the control group (P<0.05).

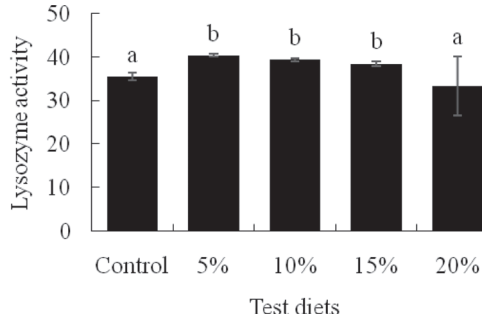

Figure 4. Lysozyme activity in common carp fed test diets for 90 days. Values are expressed as mean ± SE from triplicate groups. Bars with different superscripts are significantly different from those of control group (P<0.05)

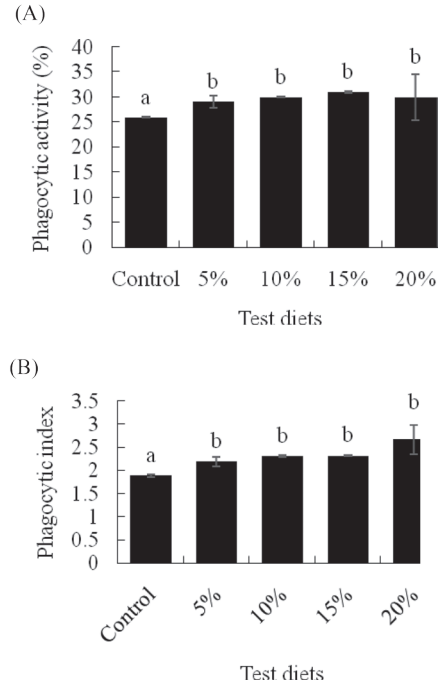

Figure 5. Phagocytic activity (A) and phagocytic index (B) of common carp fed test diets for 90 days. Values are expressed as mean ± SE from triplicate groups. Bars with different superscripts are significantly different from those of control group (P<0.05)

Discussion

Data of PBM, FPBM, and formulated diets analysis (Table 2) revealed that crude protein and ether extract contents are higher in FPBM (65 and 8.1%) compared to non-fermented PBM (60.2 and 7.9%). Though experimental diets were formulated to be balanced in protein (29.9–30.1%) and lipid (6.51–6.78%), the analyzed essential amino acids showed relatively higher values in FPBM compared to non-fermented PBM. The dietary change had a slight deviation in essential amino acids quantity, the nutritional requirement of common carp in essential amino acids was fulfilled even at higher substitution of FM in our study.

Several trials were applied to investigate the possibility of using PBM to replace FM in fish diets, including common carp (*C. carpio*) (Gümüş and Aydin, 2013), Nile tilapia (*Oreochromis niloticus*) (Dawood et al., 2020 c; El-Sayed, 1998; Hernández et al., 2010), gibel carp (*Carassius auratus gibelio*) (Yang et al., 2006), African catfish (*Clarias gariepinus*) (Abdel-Warith et al., 2001), chinook salmon (Fowler, 1991), red sea bream (*Pagrus major*) (Takagi et al., 2000), red drum (Kureshy et al., 2000), sunshine bass (Webster et al., 1999), gilthead seabream (*Sparus aurata*) (Karapanagiotidis et al., 2019), and rainbow trout (*Oncorhynchus mykiss*) (Parés-Sierra et al., 2014). In all the above-mentioned studies, FM had been replaced partially or totally with PBM in carnivore fish species, while omnivore and herbivore fish species required low levels of FM.

In this study, the use of FM as an animal protein source did not give superior growth performance compared to fermented PBM diets. However, no growth depression was observed by the inclusion of fermented PBM up to 20% replacement level. Similarly, the utilization of PBM as protein source has been reported in several fish species, including Nile tilapia (El-Sayed, 1998), gibel carp (Yang et al., 2006), African catfish (Abdel-Warith et al., 2001), chinook salmon (Fowler, 1991), gilthead sea bream (Nengas et al., 1999), rainbow trout (Steffens, 1994). However, improved growth performance of common carp fed high levels of fermented PBM in this study can be attributed to the increased digestive enzyme activity. Furthermore, the lowest FCR was observed for fish fed the diet with fermented PBM where 20% was included in the current study, suggesting beneficial role of using *S. cerevisiae* to ferment PBM. Fermentation process can increase the apparent digestibility of nutrients in the gastrointestinal tract of fish (Dawood and Koshio, 2019; Dawood et al., 2020 c; Upadhaya and Kim, 2015). The improved feed efficiency in fermented PBM groups could be related to the increased levels of peptides produced by enzymatic degradation during the fermentation process. Protein fractions are broken down into small-size peptides which are known to have independent absorption mechanisms and can therefore be more efficiently absorbed by intestinal cells (Hong et al., 2004).

In this study, the survival rate of fish fed diets with fermented PBM remained high during the trial. This agrees with numerous studies that tested fermented animal protein source in the diets of common carp (Gümüş and Aydin, 2013), *Labeo rohita* (Samaddar et al., 2015), and *Catla catla* (Mondal, 2014).

The high activity of digestive enzymes increases the feed utilization in fish intestine (Dawood et al., 2014). High levels of inclusion may weaken the digestion

and use of feed by affecting digestive enzyme activity. In the current study, fish fed diets with 20% fermented PBM showed higher feed efficiency ratio compared to that of fish given the FM-based diet (0% fermented PBM), suggesting beneficial role of using *S. cerevisiae* to ferment PBM. Thus, the growth performance can be linked to improved digestibility of nutrients and feed efficiency. It is believed that the production of metabolites like enzymes, antimicrobial substances is not only higher in quantity during fermentation and would also be more beneficial in enhancing the nutritive values of ingredients (Dawood and Koshio, 2019; Ortuño et al., 2002; Peppler, 1982). The effect of experimental diets, in relation to fermented PBM inclusion, on the activity of digestive enzymes is scarce. Probiotics could participate in digestive processes by producing extracellular enzymes (amylases, proteases, and lipases) (Dawood and Koshio, 2016; Jannathulla et al., 2019; Plaipetch and Yakupitiyage, 2014).

Morphometric measurement of heights and width of intestinal villi, as well as the number of goblet cells can help in predicting the absorption and digestion mechanism in the fish gut (Rašković et al., 2011). The measured villous length of the three intestinal sections was enlarged in fish fed yeast fermented PBM in the present study. The villi surface area for nutrient absorption in animals can be enhanced if the size of tunnels, the number of villi and the height of villus of intestine are increased (Caspary, 1992). The enhanced feed utilization of fish fed yeast fermented PBM can be elucidated to the content of intestinal cells from protein that improved the metabolism and absorption of nutrients (Mello et al., 2013). The beneficial effects of yeast on intestinal morphology in the present study might be due to the presence of mannooligosaccharides (MOS) found in the yeast cell wall, which showed an improvement in the morphology of villi and microvilli in various fish species, indicating that MOS may prevent harmful bacteria from becoming attached to mannose residues on intestinal epithelial cells, thereby alleviating atrophy of villi and microvilli due to inflammation caused by pathogens and associated toxins (Peppler, 1982; Ran et al., 2015). Taking together, fermented PBM positively increased the ability of fish to digest diets more efficiently which in turn increased the growth performance.

Besides its role in the digestion process, the intestinal goblet cells can excrete mucous and antibacterial substances that protect the mucosal layer from dehydration, damage, and pathogens (Noga, 1996; Pirarat et al., 2015). These antibacterial substances contain glycoproteins and low molecular weight fatty acids that affect the lysozyme activity (Junqueira and Carneiro, 2005). The number of goblet cells in fish intestines is affected by the richness of diets and its nutritional value as well as the environmental stressors (e.g. pathogens and rearing conditions) (Schwarz et al., 2010). Inclusion of yeast fermented PBM in this study resulted in an increased number of goblet cells which may improve the immunity and resistance against infectious diseases.

Hematological, biochemical and physiological blood markers could be utilized to recognize probable nutritional effects on the health of aquatic animals (Dawood et al., 2016 b; Doan et al., 2019; Saeidi asl et al., 2017). The improved hematological (Hb, RBCs and WBCs) values indicate that fish are free of anemia due to fermented PBM feeding. Inclusion of yeast fermented PBM improved hematological param-

eters in comparison with the control in this study. It has been assumed that dietary yeast fermented PBM can positively ameliorate most of the blood indices in fish as a result of improved immunity. The presence of fermented PBM helped to improve the immune function in fish blood by activating the immunity of intestinal barriers. Blood total protein and its derivatives including albumin and globulin are well-thought-out as an effective indicator of humoral immunity and wellbeing of fish as the most significant composites of blood serum (Abdel-Daim et al., 2019; Alexander et al., 2011). In the current study, blood total protein and globulin exhibited high values in fish fed yeast fermented PBM which might be due to improved health condition resulted from using *S. cerevisiae* in fermentation. Similarly, increased blood total protein in Nile tilapia fed probiotics, suggested immunomodulatory effects of *S. cerevisiae* on Nile tilapia (Dawood et al., 2019 a, b). It has been assumed that dietary probiotics can positively alter most of the blood indices in fish (Dawood et al., 2016 a; Yu et al., 2017). The presence of digested components produced by probiotics helped to improve the immune function in fish blood (Panigrahi et al., 2007; Yu et al., 2017). The observed changes in hematological parameters might be because of relatively long-term influence of the probiotic supplementation. Similar results were observed in Oscar (*Astronotus ocellatus*) fingerlings fed diets supplemented with probiotics (Firouzbakhsh et al., 2011). The increased total number of RBCs in the fingerlings fed *S. cerevisiae* compared to the control was apparently caused by a high, probiotic-driven metabolism with consequent increase in oxygen requirements (Firouzbakhsh et al., 2011). The increased RBCs, therefore, intensifies the concentration of Hb and eventually leads to a high oxygen-carrying capacity in fish. Such fishes, hence, may be more capable of supplying oxygen to tissues in situations where oxygen is highly required. Irianto and Austin (2002) presented evidence that use of probiotic could result in raised RBCs and WBCs levels in rainbow trout. Similarly, the present study revealed significant rise of average total number of WBCs. Elevated number of WBCs is a reinforcement of non-specific immune system resulting from *S. cerevisiae* consumption (Nayak, 2010). As lymphocytes were the most numerous in WBCs, and because interactions of lymphocytes B and T as well as macrophages are necessary for an immune response to occur (Borges et al., 2004), it can, accordingly, be concluded that fish immune system can be substantially stimulated by elevation of lymphocytes following an increase in WBCs. In other words, an appropriate dose of probiotic additives entering the digestive tract of fish is considered as aliens, provoking the defense system and multiplying WBCs and other immunizing compounds. The increased levels of globulin in the current study is in line with the increased total protein, suggesting immunomodulatory effects of yeast fermented PBM on common carp.

In the present study, the serum lysozyme activity significantly increased in fermented PBM fed groups compared to the control. Saurabh and Sahoo (2008), linked the increase in the lysozyme activity in fish to the abundance of cells related to the immune system, such as monocytes, macrophages, and polymorphonucleated granulocytes, which are the main source for such proteolytic enzyme. Also, the increase in lysozyme in the blood of stimulated fish is associated either with the proliferating phagocytes or the increased amounts of lysozymes produced from the lysosomes.

The nearly similar results were recorded in fish fed fermented plant protein sources (Dossou et al., 2018 b; Kim et al., 2009). A similar improvement in blood phagocytosis was observed in the current study by fermented PBM feeding. Phagocytosis is recognized as an immune defensive component that helps fish to avoid the risk of infectious diseases (Harikrishnan et al., 2011). The obtained results confirmed the vital role *S. cerevisiae* in activating the immune responses (lysozyme activity and phagocytosis) that may improve the resistance against pathogens. Through the increase of phagocytosis, our study has revealed that *S. cerevisiae* promotes heightened immune responses and provides greater tolerance against infectious diseases. Likewise, Tellez-Bañuelos et al. (2009) and El-Boshy et al. (2010) reported enhanced phagocytosis index values following probiotic. Engstad and Robertsen (1993) discovered that Atlantic salmon has specific-BG-receptor on phagocytes membrane which might be influenced by using of *S. cerevisiae* in fermentation.

Conclusion

It is obvious that yeast fermented PBM has beneficial effects on the digestive enzymes, intestinal morphometry, immune functions and growth performance of common carp. Considering the low price of PBM and the beneficial effects of *S. cerevisiae*, the results obtained showed the importance of fermented PBM as dietary supplement in the formulation of feed for common carp.

Conflict of interest declaration

The authors declare no conflict of interest.

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