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Effects of *lactobacillus* fermented brewer's yeast by-products on growth performance, innate immunity and antibacterial activity in Carp, *Cyprinus carpio*

Eun Chong Yang[*], Jae Hyeok Choi[*], Sang Mok Jung[*], Tae Won Jang[*], Jae Hoon Kim[*], Yu Jin Hwang[*], Hae In Jung[*], Chan Heun Lee[**] and Sanghoon Choi[†]

[*]*Department of Aquatic Life Medicine, College of Ocean Science and Technology, Kunsan National University, 558 Daehak-ro, Gunsan-si, Jeonbuk, Korea*
[2]*Geum Sung Sang Gong co., Ltd., 102, 2-gil Bong Hwanggongdan Gimje-si, Jeonbuk, Korea*

This study was conducted to find out the effect of yeast by-products discarded after beer production as feed additives for carp (*Cyprinus carpio*). After producing feed by adding high-temperature dried beer yeast by-products (HD), freeze-dried beer yeast by-products (FD), and freeze-dried fermented beer yeast by-products (FF) after lactobacilli fermentation, innate immunity indicators, survival rates, and challenge experiments were evaluated. Both ACH_{50} and lysozyme activity were significantly increased ($p<0.05$) in the experimental group of FF 0.2% and 0.5% compared to the control group from day 7 to day 21. In addition, phagocyte activity was significantly increased ($p<0.05$) in the group of FF 0.5% compared to the control group at all time points. Both IL-1β and TNF-α expression levels increased significantly in the FD and FF groups on day 21 compared to the control group ($p<0.05$). In addition, the FF 0.5% group showed significantly higher expression levels ($p<0.05$) at all time points. Similarly, IL-10 expression increased significantly ($p<0.05$) in FF 0.2% and 0.5% groups at all time points. SOD gene expression was significantly increased in FD 0.5% and all FF groups on day 14 and 21 ($p<0.05$). The results of a 10-day challenge experiment using *Edwardsiella piscicida (E. piscicida)* showed a higher relative survival rate than the control group at all concentrations that fed FD and FF. In summary, it is estimated that 0.5% FF can effectively improve the innate immunity, growth rate, and antibacterial properties of carp rather than using discarded beer yeast supernatant alone as a functional feed additive.

Key words: Brewer's spent yeast, Fermentation, Bactericidal activity, Immune-related gene expression, Growth performance

The aquaculture industry is rapidly becoming a part of human food production. Global fish production is estimated to be around 179 million tons in 2018, of which 156 million tons, or about 87% of them, were used for human dietary consumption, equivalent to 20.5 kg of annual supply per capita (FAO, 2020). Along with the growth of the aquaculture industry, the incidence of diseases in aquatic organisms is also increasing (Kim *et al*., 2014). Intensive feeding practices aimed at increasing fish production have led to deteriorating rearing environments, weakening the

[†]Corresponding author: Sanghoon Choi
Tel: +82-63-469-1886, Fax: +82-63-463-9493
E-mail: shchoi@kunsan.ac.kr

immune system of farmed fish and resulting in various diseases (Stentiford *et al*., 2017). To address these issues, there has been a growing effort to enhance the immune response and antimicrobial properties of cultured organisms through the use of various immune-enhancing agents, including probiotics or natural substances as feed additives (Bang *et al*., 2019; Rodrigues *et al*., 2020; Agboola *et al*., 2021).

Brewer's yeast by-products are major by-products generated in the brewing industry, and the amount of beer by-products discarded in Korea is 420,000 tons as of 2020, of which only 45% is used as feed or compost (Shin, 2022). These by-products contain various ingredients such as enzymes, nucleic acids, vitamins, minerals, amino acids, β-glucans, and mannan oligosaccharides (MOS) that beer yeast had (Ferreira *et al*., 2010 and Hibino *et al*., 2010). Brewer's yeast by-products are recognized for their potential as natural flavor ingredients extraction (Lee *et al*., 2002), skincare improvement (Lee *et al*., 2018), enhancement of alcohol-induced liver function (Shin and Rho, 2006), and pharmaceuticals and functional foods (Hibino *et al*., 2010). Moreover, they are rich in energy, protein, and fiber content, making them suitable as alternative feed and functional feed additives for animals such as poultry, livestock, pigs, and fish (Lee *et al*., 2000; Choi *et al*., 2006; Yoo *et al*., 2008; Nhi *et al*., 2018).

Probiotic strains such as *Lactobacillus (L.) plantarum* and *L. fermentum* can strengthen the intestinal barrier through the production of antimicrobial substances such as organic acids and competitive exclusion of pathogenic bacteria. Probiotic strains attached to the intestinal epithelium also interact with immune cells such as macrophages and dendritic cells, activating them and contributing to immune enhancement (Bermudez *et al*., 2012). Probiotics can ferment polysaccharides such MOS, and research has been conducted on combinations of polysaccharides and probiotics (Mohammad *et al*., 2019 and Zhang *et al*., 2022). Physiologically active substances produced through such fermentation processes, such as γ-aminobutyric acid (GABA), are known to help maintain immune-related genes and the balance of gut microbiota (Cho *et al*., 2007 and Seok *et al*., 2008).

In the study, we aimed to develop effective aquafeed additives using discarded brewer's yeast by-products. As feed additives, heat-dried brewing by-product (HD), freeze-dried brewing by-product (FD), and freeze-dried fermented brewing by-product (FF), which was obtained by fermenting brewer's yeast by-products with probiotics and subsequently freeze-drying them were added to the feed, respectively. Following 21 days of feeding, the growth rate, innate immunty and antibacterial ability in *Cyprinus carpio* were evaluated.

Materials and Methods

Experimental animals

As experimental animals, carp (*Cyprinus carpio*) with an average weight of 42.0±0.2 g were purchased from a fish farm in Wanju, Jeollabuk-do, South Korea. These fish were bathed in oxytetracycline at a concentration of 50 ppm for approximately 1 hr and then acclimated to the experimental tanks for two weeks. Square glass tanks (900 × 450 × 450 mm) were filled to a depth of 400 mm with water and the water temperature was maintained at 25±1°C. Freshwater from which chlorine was removed using sodium thiosulfatewas was daily exchanged in recirculating aquaculture system.

Preparation and feeding of brewer's yeast by-product supplemented feed

High-temperature dried brewer's yeast by-product (HD), freeze-dried brewer's yeast by-product (FD), and freeze-dried fermented brewer's yeast by-product (FF) were donated by Geumsung Sanggong Co., Ltd. (Gimje, South Korea). For manufacturing FF, each 1.0×10^7 CFU/ml of *Lactobacillus plantarum* and *L. fermentum* was added to beer yeast by-products and

incubated anaerobically at 37°C for 48 hr. HD, FD, and FF were added to the feed at concentrations of 0.1%, 0.2%, and 0.5%, respectively, based on the feed weight. To prepare the feed, 100 g of normal feed was placed in a plastic container and 15 ml of distilled water was sprayed evenly onto the feed. After adding the brewer's yeast by-product produced by each method, the feed was shaken thoroughly to ensure absorption throughout the feed. The feed was then spread out on trays and dried. A control group, where no brewer's yeast by-product was added, was prepared by spraying 15 ml of distilled water onto 100 g of normal feed. The prepared feed was stored at 4°C until use. Each experimental group received 1% of their body weight in feed twice daily (at 10:00 and 18:00) for a total of 21 days. Of the total 30 fish in each experimental group, 15 fish were used for measuring innate immune indicators and immune-related gene expression every 7 days of feeding, while the remaining 15 fish were used for measuring growth rate and antibacterial activity.

Measurement of colony-forming units (CFU) of lactic acid bacteria in FF

One gram of FF was mixed with 9 ml of PBS and then serially diluted tenfold. These dilutions were inoculated onto MRS agar and then A colony-forming unit (CFU) of lactic acid bacteria in FF was calculated after 24 hrs of incubation. The analysis revealed that the concentration of lactic acid bacteria in 1 g of FF was 5.2×10^4 CFU/g.

Pathogenic strains

The pathogenic strain used in the experiment was *Edwardsiella piscicida* (*E. piscicida*, KCTC 12267), which was obtained from the Korean Collection for Type Culture (KCTC). The strain was stored at –80°C until use. Before the experiment, the thawed strain was inoculated into brain heart infusion (BHI) broth and incubated at 25°C for 24 hrs. Subsequently, it was subcultured onto *Salmonella Shigella* (SS) agar. The strain was maintained by taking the subsequently formed black colonies and culturing them to BHI broth.

Serum collection

For serum collection, blood was collected from the caudal vein of the fish using a 26 G needle attached to a 1 ml syringe. The collected blood was kept at 4°C overnight followed by centrifugation at 1200 × g for 10 minutes to collect the serum and stored at -20°C until use.

Lysozyme activity

Serum lysozyme activity was measured using a modified turbidimetric microtiter plate technique according to Ellis (1999). Briefly, a standard *Micrococcus lysodeikticus* (Sigma) was suspended in 0.2 M citrate-phosphate buffer (pH 5.8) at a concentration of 2 mg/ml. Carp serum (10 µl) was added to 250 µl of the bacterial suspension dispensed per 96-well plate. The absorbance was measured at 0.5 and 4.5 min intervals at 450 nm in a spectro photometer (HHIMADXU UV-1600PC). One unit of lysozyme activity was defined as a reduction in absorbance of 0.001/min.

ACH_{50} Activity

The alternative pathway complement hemolysis 50 (ACH_{50}) measurement was performed using the method described by Yano (1992). The rabbit blood was harvested from the ear vein by using 500 IU of heparin treated 5 ml syringe attached with a 23 G needle. Rabbit red blood cells (RαRBCs) were separated using histopaque-1077 (Sigma, USA). The isolated Rα RBCs were washed twice with 0.01 M ethylene glycol tetraacetic acid-Mg-gelatin veronal buffer (EGTA-Mg-GVB) and diluted to a concentration of 1×10^8 cells/ml. Fish serum was diluted 12-fold with PBS and then dispensed into a 96-well plate at volumes of 200, 150, 100, and 50 µl per well. EGTA-Mg-GVB was added to each well to a final volume of 200 µl.

RαRBCs were added to each well at a volume of 100 μl and incubated at 25°C for 1 hr. The samples were then centrifuged at 220 × g for 5 min at 4°C. One hundred microlitter of the supernatant was collected and measured at 405 nm using a Sunrise microplate reader (TECAN, SWISS). The hemolysis of red blood cells (Y) was calculated using the formula Y/(1-Y), and 50% hemolysis was determined as 1/K × (reciprocal of the serum dilution) × 0.5. The results were expressed as units per ml.

Phagocytic activity

Phagocytic activity (PA) was measured following the method described by Campa (2002). Carp head kidney (HK) leucocytes were adjusted to 1×10^6 cells/200 μl/well in 5% FBS-MEM and dispensed onto a 8-well chamber slide (Thermo Scientific, USA) followed by overnight incubation at 25°C. After incubation, zymosan particles (1×10^6 cells/ml, Sigma, USA) were added at a volume of 10 μl per well and the chamber was further incubated at 25°C for 1 hr. Following incubation, the phagocytes attached at the bottom of wells were washed twice with PBS. The washed phagocytes were fixed with 70% methanol and centrifuged at 30 × g for 5 min followed by twice more washing with PBS. The chamber slide wells were thoroughly air-dried at room temperature. After drying, the cells were stained with Wright-Giemsa stain solution for 2 min. The stained cells were then thoroughly rinsed with water and dried. Subsequently, they were examined under an optical microscope. PA & PI were calculated using the following formula:

PA = (Number of phagocytic cells / Total number of macrophage cells counted) × 100

PI = Number of zymosan / Number of phagocytic cells

Quantitative PCR (qPCR) analysis of gene expression

The gene expression analysis of interleukin (IL)-1β, IL-10, tumor necrosis factor (TNF)-α, superoxide dismutase (SOD), and β-actin was conducted using the Exicycler™96 equipment from Bioneer, Korea. Total RNA was extracted from the head kidneys of the carp and then cDNA was synthesized following the protocol of the M-MLV cDNA synthesis kit (Enzynomics, Korea). For each reaction, 2 μl of cDNA, 10 μl of AccuPower® 2X GreenStar™ qPCR Master mix (Bioneer, Korea), and 1 μl each of forward and reverse primers were mixed with 6 μl of DEPC water to a total volume of 20 μl. The qPCR was carried out 40 cycles under the conditions of 95°C (10 min), 95°C (30 sec), 60°C (30 sec), and 72°C (30 sec). After PCR, a melting curve analysis was performed to confirm the generation of a single product. The expression of each target gene was normalized to β-actin gene expression and quantified using the 2-ΔΔCt method. The primers used for qPCR are presented in Table 1.

Measurement of growth rate

Initial weight of carp was measured before feeding the experimental diet. After 21 days of feeding, the final weight was measured followed by assessment of the growth performance. The feeding regime consisted of providing the experimental diet twice a day at a rate of 1% of the weight of the fish body, totaling 2% of the body weight daily. To calculate the actual feed consumption, any uneaten feed was collected and dried 30 min after providing the feed. This dried feed was then subtracted from the total feed supplied to determine the actual feed intake.

Challenge test

A challenge test was conducted using the pathogenic strain *E. piscicida*. The strain was injected intraperitoneally (i.p.) at a concentration of 1×10^7 CFU/100 μl/fish. After inoculation, each experimental diet was continuously fed and the number of surviving carp on the 10th day for each group was converted into the relative survival rate compared to the control

Table 1. Primers used for amplification of specific transcripts by quantitative PCR

Target		Sequence(5' to 3')	Product size (bp)	Accession number
IL-1β	F	AAGGAGGCCAGTGGCTCTGT	69	AB010701
	R	CTGAAGAAGAGGAGGCTGTCA		
IL-10	F	GCTGTCACGTCATGAACGAGAT	132	AB110780
	R	CCCGCTTGAGATCCTGAAATAT		
TNF-α	F	GCTGTCTGCTTCACGCTCAA	106	AJ311800
	R	CCTTGGAAGTGACATTTGCTTTT		
SOD	F	TGGCGAAGAAGGCTGTTTGT	91	JF342355
	R	TTCACTGGAGACCCGTCACT		
β-actin	F	GCTATGTGGCTCTTGACTTCGA	89	M24113
	R	CCGTCAGGCAGCTCATAGCT		

group.

Statistical analysis

Data were expressed as mean ± S.D. ANOVA analysis followed by Newman-Keuls post hoc test was used to assess the significance of differences between groups. The criterion for determining significance was determined to be significant when $p<0.05$.

Results and Discussion

For aiming to develop functional aquafeed additives using brewer's yeast by-products discarded after beer production, in the study, we compared and analyzed the differences in immune indicators, immune-related gene expression, growth rates, and antibacterial activity resulting from the feeding of carp with feed containing high-temperature dried brewer's yeast by-product (HD), freeze-dried brewer's yeast by-product (FD), and brewer's yeast by-product fermented with probiotics (FF) for three weeks. In the case of HD, the brewer's yeast by-products were quickly drum dried at a high temperature of 121°C, and FD was produced by freeze-drying brewer's yeast by-products. Therefore, it is assumed that FD has a better immune response than HD because the biologically active substances contained in brewer's yeast by-products were less destroyed than HD due to freeze-drying of FD. Furthermore, in FF, which has undergone fermentation with *L. fermentum* and *L. plantarum*, lactic acid bacteria are considered to be superior to FD in terms of innate immune response and growth rate by various metabolites produced after ingesting beer yeast by-products. Pathogen-associated molecular pattern (PAMP) components such as β-glucan, manan oligosaccharide (MOS), chitosan, peptidoglycan, and nucleic acid in beer yeast and lactobacillus are known to stimulate antigen-presenting cells to improve the host's immune system and balance immune-related genes and intestinal microbiota by various physiologically active substances (Zhang *et al*., 2020). β-glucan activates macrophages and dendritic cells expressed on the cell membrane to promote innate immunity as well as acquired immunity in which Th cells are involved (Aderem and Ulevitch, 2000; Bricknell and Dalmo, 2005). MOS is a complex carbohydrate derived from yeast cell walls and can stimulate the liver to secrete mannose binding protein that binds to the capsule of bacteria and trigger a cascade reaction of the complement, one of the innate immune responses (Abu-Elala *et al*., 2018). In addition, peptidoglycan, nucleic acid can improve the host's immune system and balance immune-related genes and intestinal microbial guns by various physiologically active substances (Dawood *et al.*, 2020).

The added feed group of FF was obtained by lyo-

philizing the fermented mixture for 48 h at 37°C by administering *L. plantarum* and *L. fermentum* to discarded beer yeast by-products at a concentration of 1.0×10^7 CFU/ml, respectively, and the concentration of 5.2×10^4 CFU/g was found when CFU were measured after drying. In additional feed papers mainly using *lactobacillus*, the concentration of 1.0×10^8 CFU/g or higher was required to be effect (Son *et al.*, 2009), but the concentration of *lactobacillus* in FF used in this experiment was thought to have been lost during the freeze-drying process, indicating that it is not enough to show effect. Nevertheless, the reason for the meaningful effect in FF can be thought of as the various metabolites produced during the by-product-based *lactobacillus* fermentation process.

Fig. 1A and B show the results of ACH_{50} and lysozyme activity measured every 7 days while feeding HD, FD, and FF additives for 21 days. ACH_{50} was observed to significantly increase ($p<0.05$) compared to controls in groups of FF 0.2% and 0.5% on day 7, HD 0.5%, FD 0.5%, and FF 0.2% and 0.5% on day 14, and FD 0.2%, 0.5% and all FF groups on day 21. Lysozyme activity significantly increased ($p<0.05$) on day 7 in the FD 0.2%, 0.5%, and all FF groups, on day 14 in all FF groups, and on day 21 in the FD 0.2%, 0.5%, and all FF groups compared

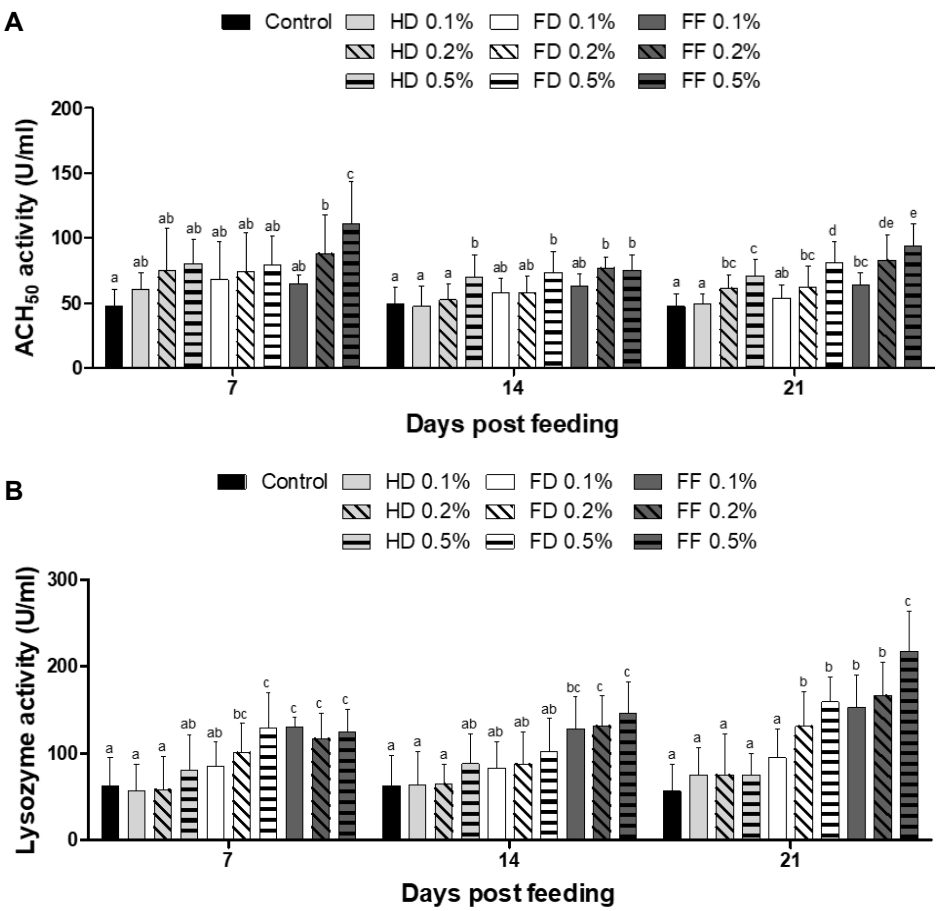

Fig. 1. Effects of dietary HD, FD and FF on ACH_{50} (A) and lysozyme activity (B) of carp. Carp were fed for 21 days with 0.1, 0.2 and 0.5%, respectively. Data represent the mean±S.D. (n=15). Different letters above the bars indicate significant differences ($p<0.05$) in different groups.

to the control group. The alternative pathway of complement and lysozyme play a role in preventing bacterial infection by destroying the cell wall of bacteria (Punt, 2018). Previous studies have also consistent with reports that innate immune indicators such as ACH_{50} and lysozyme activity have improved when the cell wall components of beer yeast were supplied as additive feed (Welker *et al*., 2012; Abu-Elala *et al*., 2013; Torrecillas *et al*., 2014). In addition, both ACH_{50} and lysozyme activity levels increased significantly (p<0.05) in the FF 0.5% group among the all feed additive groups regardless of the duration of feeding. These results suggest that various metabolite products generated during the fermentation process of lactic acid bacteria added to brewer's yeast by-products might play a crucial role in improving both ACH_{50} and lysozyme activity, but further research is required to determine which specific ingredients are involved in promoting the innate immune activity.

Fig. 2A and B show the results of PA and PI measured every 7 days while feeding HD, FD, and FF additives for 21 days, respectively. PA significantly increased (p<0.05) only in the FF 0.5% group on day 7, but significantly increased (p<0.05) in the FD 0.2% group on day 14 and in the HD 0.5% group and all FD and FF groups on day 21. No significant differ-

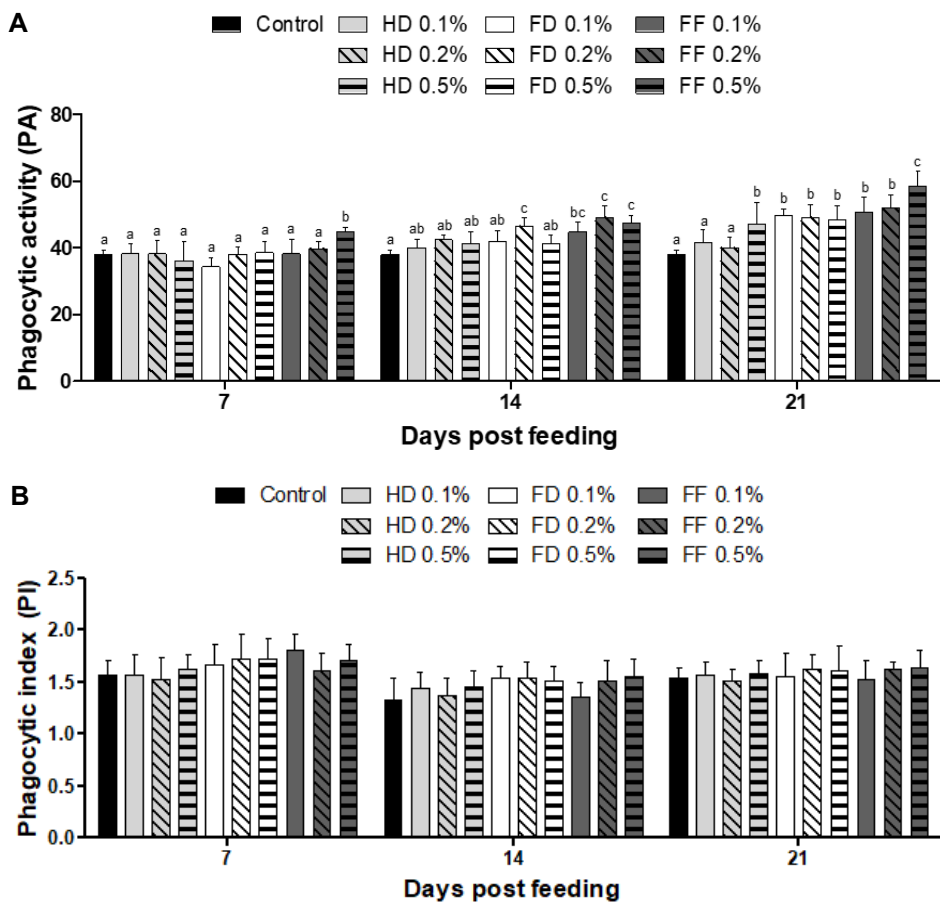

Fig. 2. Effects of dietary HD, FD and FF on phagocytic activity (A) and phagocytic index (B) of carp. Carp were fed for 21 days with 0.1, 0.2 and 0.5%, respectively. Data represent the mean±S.D. (n=15). Different letters above the bars indicate significant differences (p<0.05) in different groups.

ences were observed in PI among the feed additive groups. In the case of PI, there were fewer macrophages that consumed zymosan in the control group compared to the additional feed group, but there may be no difference between the control group and the additional feed group if a limited amount of specific macrophages in the control group ingested large quantities of zymosan (Hwang *et al*., 2019). The experiment was conducted under the assumption that PI would also rise like PA, but no significant difference was observed between the control and the experimental groups as mentioned earlier. However, we evaluated that the activity of macrophages was increased by showing a certain significant difference in PA compared to the control group. Considering the report that cellulosic polysaccharides, such as β-glucan or chitosan derived from *Saccharomyces cerevisiae*, continuously stimulate toll-like receptors (TLRs) of macrophages and other immune-related cells resulting in up-regulation of their activity (Beutler *et al*., 2004), it is assumed that the activity of phagocytes was increased by the cell wall polysaccharides in brewer's yeast by-products. Furthermore, PA consistently increased significantly ($p<0.05$) in the FF 0.5% group on day 7 and 21 post feeding compared to other experimental groups. The result is likely due to the effects of various bioactive substances produced by fermentation of brewer's yeast by-products by probiotics.

Cytokines are secretory proteins produced in immune-associated cells such as macrophages, lymphocytes, or dendritic cells and involved in the growth, differentiation, and activation of the immune response. Pro-inflammatory cytokines such as IL-1β and TNF-α induce inflammation to respond rapidly to infection of pathogens and also induce the expression of other cytokines involved in innate immunity. IL-10 is an anti-inflammatory cytokine synthesized in monocytes and serves to control the synthesis of cytokines produced by Th1 and macrophages (Reyes-Cerpa *et al*., 2012).

Fig. 3 shows the expression of the immune-related genes and SOD gene measured every 7 days while feeding HD, FD, and FF additives for 21 days, respectively. The expression of pro-inflammatory factors IL-1β and TNF-α significantly increased ($p<0.05$) on day 21 in FD 0.2%, 0.5%, and all FF groups compared to the control group. In addition, among the additive feed groups, only the FF 0.5% group showed a significant increase in expression ($p<0.05$) regardless of feeding periods. Furthermore, the expression of IL-10, an anti-inflammatory cytokine, was significantly increased ($p<0.05$) in the FD 0.5% group, FF 0.2% and 0.5% group on day 21, suggesting that the expression of anti-inflammatory cytokines was also increased as pro-inflammatory cytokines increased for sustaining the homeostasis. The results of thestudy are consistent with the findings of Meena (2013), Abu-Elala (2018), and Avramia (2021), which reported that the expression of immune-related cytokines increased in carp fed beer yeast as additive feed. It has also been reported that brewer's yeast can function as an anti-inflammatory as well as an immune-inducing effect (Stier *et al*., 2014). White (2002) reported that brewer's yeast contains immune-stimulating components such as β-glucan and MOS. Considering the results, it is evaluated that immune-stimulating substances such as β-glucan or MOS derived from brewer's yeast may have increased the expression of immune-related cytokines in carp, resulting in an increase in innate immune response.

SOD is one of the enzymes that convert reactive oxygen species into oxygen and hydrogen peroxide (Cavinato *et al*., 2020). Neutrophils and macrophages use powerful active oxygen synthesized in the cytoplasm after predating pathogenic microorganisms to cause oxidative damage to microorganisms. SOD gene expression significantly increased ($p<0.05$) in the FD 0.2% and 0.5% groups, and all FF groups on day 14 and in FD 0.2%, FF 0.2% and 0.5% group on day 21. Moreover, among the feed additive groups, on the 14th day, SOD gene expression showed the highest

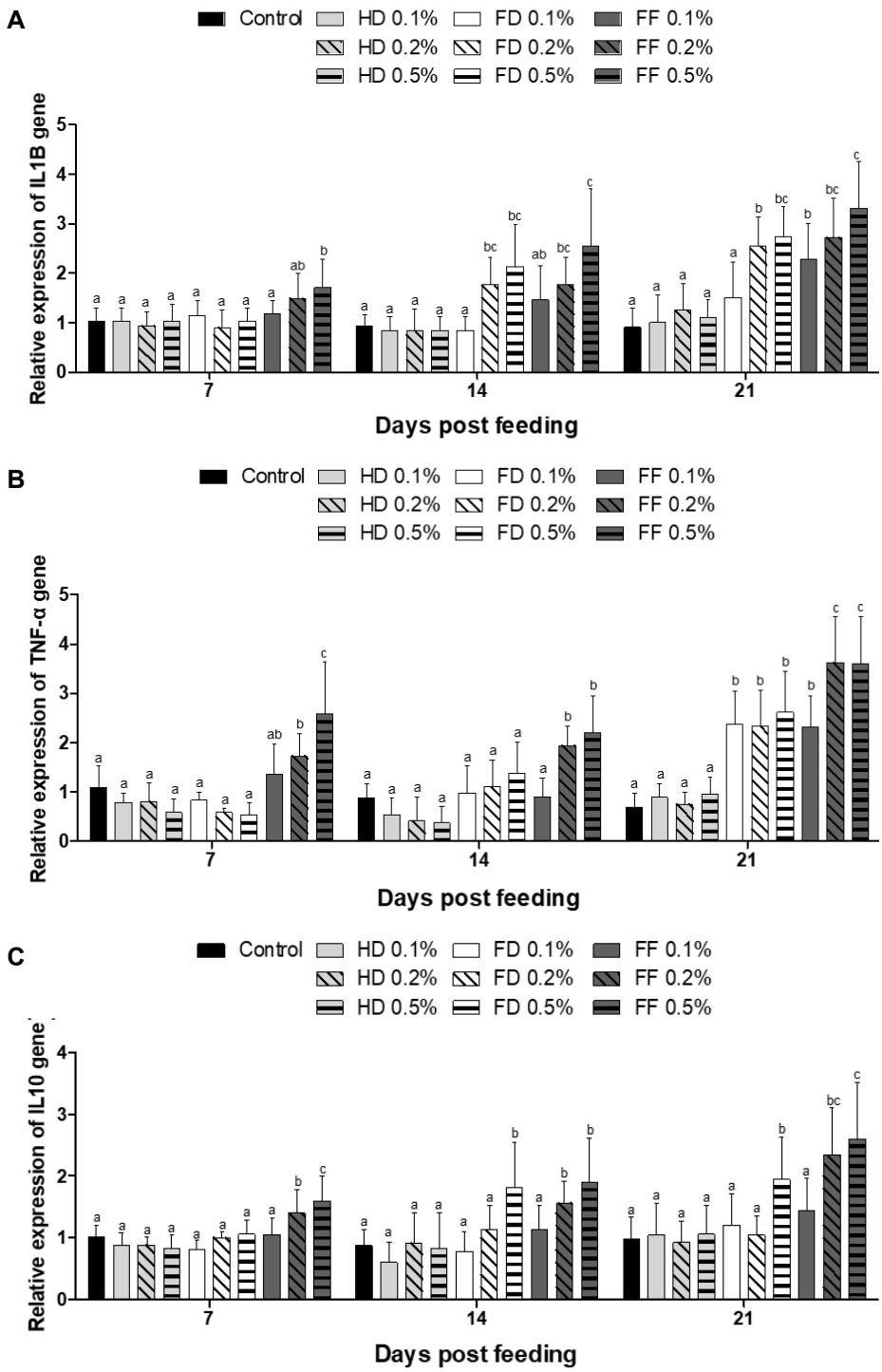

Fig. 3. Effects of HD, FD and FF on relative mRNA gene expression of TNF-α, IL-1β, IL-10 and SOD in carp. Carp were fed for 21 days with 0.1, 0.2 and 0.5%. Data represent the mean±S.D. (n=15). Different letters above the bars indicate significant differences (p<0.05) in different groups.

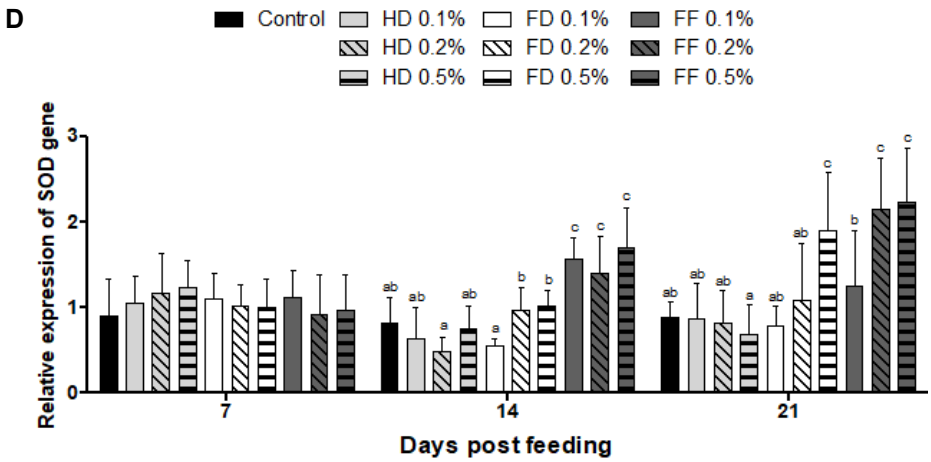

Fig. 3. Continued.

significant increase (p<0.05) in all FF groups. The result suggests that the up-regulated phagocytic activity induced the production of active oxygen species followed by the elevation of SOD synthesis.

Fig. 4 and Table 2 show the survival rate and relative survival rate of carp i.p. infected with *E. piscicida* after feeding each feed additive for 21 days. The relative survival rate was higher in all feed additive groups containing FD and FF additives than in the control group and the HD additive group. The result suggests that the β-glucan and other active ingredients in brewer's yeast by-products might enhance innate antimicrobial immune responses by binding to detectin-1 receptors present in intestinal mucosal cells

Table 2. Effect of HD, FD and FF on the relative percentage survival on 10 day of carp challenged with *E. piscicida*

Types of feed	Relative percentage survival (%)
HD 0.1%	15.9 ± 1.4
HD 0.2%	-11.1 ± 9.6
HD 0.5%	20.6 ± 6.9
FD 0.1%	36.5 ± 5.5
FD 0.2%	73.8 ± 8.6
FD 0.5%	57.9 ± 8.4
FF 0.1%	52.4 ± 4.1
FF 0.2%	78.9 ± 10.4
FF 0.5%	52.4 ± 4.1

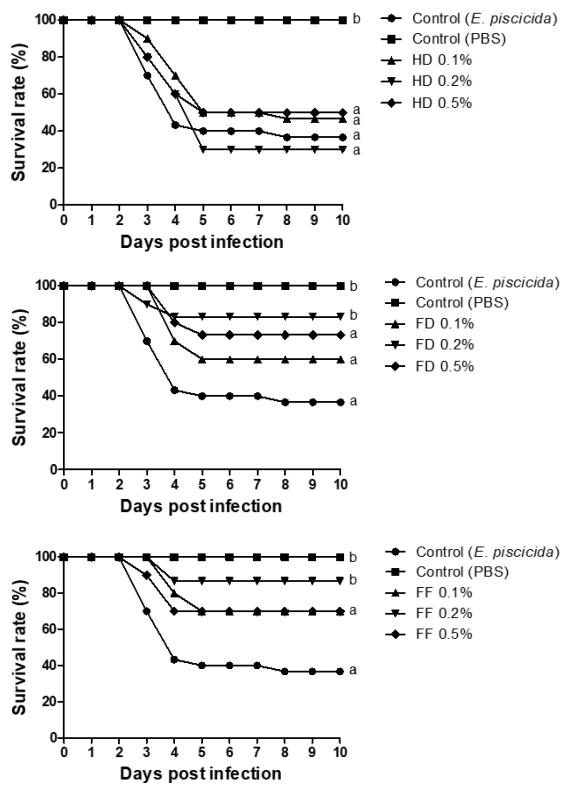

Fig. 4. Survival rates of carp for 10 days following *E. piscicida* (1×10^8 CFU/ml) challenge. Carp were fed for 21 days with 0.1, 0.2 and 0.5% of fermented HD, FD and FF, respectively, followed by i. p. injection with *E. piscicida.* Normal diet was fed as a control. Different letters above the bars indicate significant differences (p<0.05) in different groups.

Table 3. Growth performances and feed utilization of carp fed different levels of dietary HD, FD and FF

Parameters	Types of feed									
	Control	HD 0.1%	HD 0.2%	HD 0.5%	FD 0.1%	FD 0.2%	FD 0.5%	FF 0.1%	FF 0.2%	FF 0.5%
Initial body weight (g)	42.0±0.2	41.9±0.0	42.0±0.0	41.5±0.2	41.9±0.1	41.9±0.1	41.9±0.0	41.6±0.0	42.0±0.2	41.9±0.1
Final body weight (g)	50.2±1.3^{a}	50.6±2.4^{a}	50.0±2.6^{a}	51.2±0.3^{a}	51.2±0.2^{a}	52.3±0.9^{ab}	49.8±1.4^{a}	50.5±1.1^{a}	52.0±1.9^{ab}	53.1±1.1^{b}
Weight gain (g)	8.2±1.1^{a}	8.7±2.4^{ab}	8.3±2.5^{ab}	9.2±0.3^{ab}	9.7±0.0^{ab}	10.4±0.8^{ab}	7.9±1.4^{a}	8.9±1.1^{ab}	10.0±1.8^{ab}	11.2±1.1^{b}
Weight gain (%)	19.5±2.6^{a}	20.8±5.7^{ab}	19.9±5.8^{a}	22.0±0.8^{ab}	23.3±0.1^{ab}	24.9±1.9^{ab}	18.9±3.4^{a}	21.4±2.6^{ab}	23.8±4.1^{a}	26.7±2.7^{b}
Specific growth Rate (%)	0.8±0.1^{a}	0.9±0.2^{a}	0.9±0.2^{a}	0.9±0.0^{ab}	1.0±0.0^{ab}	1.1±0.1^{ab}	0.8±0.1^{a}	0.9±0.1^{ab}	1.0±0.2^{ab}	1.1±0.1^{b}
Feed conversion rate	1.9±0.0^{a}	1.9±0.3^{a}	2.0±0.3^{a}	1.7±0.3^{ab}	1.6±0.2^{ab}	1.5±0.1^{ab}	2.0±0.1^{a}	1.7±0.0^{ab}	1.6±0.1^{ab}	1.4±0.1^{b}

The numerical numbers in initial and final body weight (g) represent an average weight of 10 fish per each group from three repeated experiments.
Weight gain (g) = Final weight (g) - Initial weight (g)
Percent weight gain (PWG) = [100×(Final weight-Initial weight) / Initial weight]
Specific growth rate (SGR) = [{LNFinal weight (g)-LNInitial weight (g)} / DAY] × 100
Feed conversion ratio (FCR) = Total feed taken (g) / Weight gain (g)
Different superscript letters indicate significant differences (p<0.05) in different groups.

(Stier *et al*., 2014). In the case of HD, it is assumed that the antibacterial effect has been reduced by drying at a high temperature of 130℃ or higher and destroying the active ingredients affecting the innate immune responses. Moreover, the relative survival rate of the FF 0.1% group is about 16% higher than that of the FD 0.1% group. The result indicates that the metabolites such as lactic acid, bacteriocins, hydrogen peroxide, or polysaccharides produced by lactic acid bacteria in brewer's yeast by-products can enhance antibacterial activity (Bermudez *et al*., 2012, Kechagia *et al*., 2013, Lee *et al*., 2014).

Table 3 presents the growth rate and feed conversion ratio of carp after supplying feed additives for 21 days. Interestingly, all evaluated indicators significantly increased (p<0.05) only in the FF 0.5% group compared to the control group and other feed additive groups. *Lactobacillus sp.* ingests polysaccharides and proteins and can produce various digestive enzymes such as lactase, proteases, peptides, fructanases, and amylases (Maske *et al*., 2021), consistent with reports that these factors have improved the digestion ability of fish that have ingested lactic acid bacteria, thereby increasing the growth rate (Wu *et al*., 2012; Giri *et al*., 2013; Dilip *et al*., 2015). Therefore, it is assumed that the growth rate was improved in the FF 0.5% group, which contains the various digestive enzymes produced by lactic acid bacteria during anaerobic fermentation of beer yeast by-products.

Considering the results obtained from the study, in conclusion, it is assumed that when freeze-dried brewer's yeast by-products fermented with probiotics are supplemented at a concentration of 0.2-0.5% to standard feed, the growth rate of carp as well as innate immune functions including antibacterial activity might be effectively enhanced.

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