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Study on immune-enhancing and protective effects of three Lactobacillus species on Nile tilapia (*Oreochromis niloticus*) vaccinated against *Streptococcus agalactiae*

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Abstract

Lactobacillus probiotic enriched diets can modulate host immune response. In this study, we investigated whether the three species *Lactobacillus plantarum*, *L. bulgaricus* and *L. rhamnosus* up-regulated two T-cells specific antigens and exerted the protective effect when administrated with formalin-killed *Streptococcus agalactiae* in Nile tilapia. For this purpose, a total of 180 Nile tilapia (average body weight 45.8 ± 22 g) were randomly divided into 6 experimental groups, i.e.: one control group and 5 vaccinated groups: Formalin killed cell (FKC); FKC+ Adjuvant; FKC+ *L. plantarum*; FKC+ *L. bulgaricus* and FKC+ *L. rhamnosus*. All groups were fed with normal commercial pellets and three groups were fed with pellets sprayed with three different lactobacilli. The results showed that the survival rate in the groups of Formalin killed cell (FKC) combined with oral administration of lactobacilli was from 70.0% to 75.0%. Protection in the control group amounted to 31.3% illustrating a significant difference with other experimental groups. In this research, gene expression of CD4 and CD8 which have essential functions in the immune response quantified by qRT-PCR in the head kidney, skin, and spleen was reported in the form of fold change. The analysis of fold change (Mean ± SD) related to CD4 and CD8 at 30- and 60-days post-immunization (dpi) respectively showed an increase in fold change in all probiotic groups compared to the FKC group in two of which it was significant at the level of $p<0.05$. The results revealed that vaccination with FKC administrated lactobacilli-enriched diets increased the expression of genes related to immune response which can indicate higher protection against *Streptococcus agalactiae* in the probiotic groups compared with FKC vaccination alone similar to the results observed in FKC coupled with adjuvant vaccination.

Key words: Vaccine, *Streptococcus agalactiae*, Lactobacillus, CD4, CD8

Introduction

Streptococcus agalactiae is a gram-positive bacterium that infects a variety of animals, including a wide range of fish species (Evans et al., 2002; Robinson and Meyer, 1966). This invasive pathogen causes streptococcosis in tilapia (Suanyuk

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et al., 2008; Zhang, 2021). The intensive and semi-intensive culture of tilapia makes it more vulnerable to stress and susceptible to transmissible diseases (Salama and Murray., 2011). Bacterial infections have a great impact on tilapia farming (Munang et al., 2016). Tilapia is predicted to be one of the fastest-growing farmed fish species by 2030 (World Bank, 2030). Streptococcosis affects different fish species in intensive fish farming in fresh and saltwater. The prevalence of Streptococcosis is directly related to the water temperature (Ismail et al., 2016). *Streptococcus iniae* and *Streptococcus agalactiae* are two major bacteria that affect tilapia production in the world (Evan et al., 2006; Wei et al., 2016). Clinical signs of Streptococcus spp. infection in Nile tilapia includes loss of appetite, erratic swimming, ascites, exophthalmia, external hemorrhages, pale gills, corneal opacity, and eye hemorrhage (Anshary et al., 2014; Rahman et al., 2021). Postmortem findings in tilapia characterized by splenomegaly, hemorrhagic ascites, muscle hemorrhage and meningoencephalitis (Klesius et al, 2008; Zamri-Saad et al., 2010). Vaccination is an effective way to create immunity against bacterial and viral infections. Economically sustainable aquaculture has been supported by vaccination for over 50 years (Ma et al., 2019). Different types of vaccines are approved for use in the world. Inactivated bacterial vaccines have low effective antigens, so they induce insufficient immunogenicity. Therefore, adjuvant addition to vaccine and booster doses are used to generate adequate protective immunity (Munang'andu and Evensen., 2019). Adjuvants can enhance the immune response to vaccine antigens in several ways, including enhancing the immunogenicity of weak antigens and accelerating and prolonging the specific immune response (Singh and O'Hagan et al., 1999; Petrovsky and Aguilar., 2004). Probiotics are microorganisms that provide health benefits to the host (Fouz and Amaro, 2003; Verschuere et al., 2000). Several strains of Lactobacillus have been developed as food additives for various animals as well as fish species (Zhou et al., 2012). Probiotic supplementation of *L.rhamnosus* has been shown to have protective effects against experimental *Edwardsiella tarda* infection in Nile tilapia (Pirarat et al., 2006). *Lactobacillus acidophilus* enriched diet showed a significant increase in protection against *Aeromonas hydrophila* and *Pseudomonas fluorescens* challenge in Nile tilapia (Aly et al., 2008). This research aimed to investigate the effect of the administration of three Lactobacilli probiotics when combining the vaccine on adaptive immune response and levels of protection against *Streptococcus agalactiae* by comparing them with formalin-killed cell vaccine coupled with an oil-in-water adjuvant. Here we investigated CD4 and CD8 expression after FKC vaccination combined with oral lactobacillus diet in Nile tilapia (*Oreochromis niloticus*). CD4 and CD8 are molecules expressed on T-helper cell's surfaces that interact with MHC-II and MHC-I participate in antigen recognition and coordinate the immune response (Ashfaq et al., 2019; Kato et al., 2013).

Materials and Methods

A total of 180 Nile tilapia with average body weight (45.8±22g) without any evidence of disease adapted to the experimental environment and were monitored in terms of their health status for 14 days. They were randomly divided into 6 experimental groups and each group was placed in polyethylene tanks with 1000 liters of water. Experimental groups were defined as 1: The control; 2: Formalin killed cell (FKC); 3: Formalin killed cell (FKC) + Adjuvant; 4: Formalin killed cell (FKC) + *L. plantarum*; 5: Formalin killed cell (FKC) + *L. bulgaricus* and 6: Formalin killed cell (FKC) + *L. rhamnosus*. All groups were fed with normal commercial pellets and three groups were fed with pellets sprayed with

three different lactobacilli. Fish were fed two commercial feed pellets containing (52% protein, and 10% fat) at first, then (48% protein, 10% fat) at the rate of 2% of the tank biomass daily. The water temperature was set at 24 ±1 °C.

The pathogenic bacterium used in our experiments, *Streptococcus agalactiae*, was isolated from naturally infected tilapia identified by 16S rDNA sequencing and stored in 2ml cryovials in the faculty of Veterinary Medicine Shahid Chamran University, Ahvaz, Iran. The bacteria were sub-cultured on tryptic soy/sheep blood agar 5% (TSA-BA) plates and incubated at 30 °C for 48 h in an aerobic environment. Several colonies grown on the TSA plate were transferred into 300 ml tryptic soy broth (TSB, Merck, Germany) and incubated at 30 °C for 48 h. *Streptococcus agalactiae* cells were centrifuged at 4000 rpm for 10 minutes, washed 3 times with phosphate-buffered saline (PBS), and adjusted to a concentration of $\sim 3\times10^9$ Cells/mL (McFarland U10). The bacterial suspension was inactivated by adding neutral buffered formalin (1%). Then, it was washed three times with PBS (pH 7.4) to obtain formalin-killed cells (Suwannasang et al., 2017). Three ml of FKC was added to 27 ml distilled water to prepare a suspension of 10^8 Cells /ml. Each fish was injected with 100 µL of bacterial suspension containing 1×10^7 cells, intraperitoneally (IP) on the first and 20 days of the experiment. Only group 3 received Montanide™ ISA 763 AVG adjuvant which was added to formalin-killed cells at a ratio of 1:1 on day 20 post-immunization (Wangkahart et al., 2023; Yao et al., 2019).

The source of the Lactobacilli seeds prepared in our research was as follows: *L. plantarum*, and *L. bulgaricus* were obtained from microbial seeds preserved in the faculty of veterinary medicine of Shahid Chamran University, Ahvaz, Iran. *L. rhamnosus* was obtained from the lyophilized microbial inventory of the Iranian Biological Resource Center (IBRC_JCM1136). All Lactobacillus species were inoculated in MRS medium and incubated at 30℃ for 48 hours, then washed with PBS 0.1 Mol, pH 7.4, adjusted turbidity equal to 0.5 McFarland standard. The solution was sprayed at a concentration of 10^8 Cells g-1 feed during the research (Ringø et al., 2018).

Three fish were randomly collected from six experimental groups in each replication. To induce euthanasia, (114mg/l) Clove Oil was used for sacrifice within 10–60 minutes (Saint-Erne, 2014). Tissue samples from the head kidney, skin, and spleen were dissected out under aseptic and cool conditions on 30- and 60-days post immunization and placed in 1.5 - ml sterile, labeled, microtubes. The samples were stored in an ultra-low freezer at -70°C. (Khaj et al., 2021).

On the 60th day after the first immunization, all fish in six experimental groups were challenged with *Streptococcus agalactiae* according to the median lethal dose (LD50). Each fish was injected with 100 µl of live bacteria suspension with 1.7×10^7 cells/mL intraperitoneally. Post-challenged fish were monitored for 14 days post- inoculation for typical signs of streptococcus infection. The skull bone was removed and the head kidney was exposed and cultured aseptically by streaking on to TSA plates. The plates were incubated at 30 ◦C for 48 hours (He et al., 2017; Bøgwald & Dalmo., 2019). The Relative Percentage Survival (RPS) was calculated according to formula: RPS = (1 - (% mortality of immunized group / % mortality of control group)) ×100 (Amend, 1981). Colonies were identified as Gram-positive cocci by Gram staining with the commercial test kit.

The expression of CD4 and CD8 as adaptive immune-related genes were evaluated in the head kidney, skin, and spleen tissues at days 30 and 60 of the experimental period (30- and 60-days post-immunization) by qRT-PCR. Total RNA extraction kits (Parstous Biotechnology,

Iran) were used as recommended by manufacturers. To assess the purity of the extracted RNA from different organs was evaluated by measuring the ratio of absorbance at 260 and 280 nm (A260:A280) using an Eppendorf μCuvette G 1.0 microvolume measuring cell Bio photometer (Eppendorf, Germany). A ratio ~2.0 is considered acceptable quality for the purity of RNA (Imbeaud et al., 2005). Easy cDNA Synthesis Kit (Parstous Biotechnology, Iran) was used to synthesize cDNA. The primers utilized for analyzing gene expression by real-time quantitative PCR are detailed in (Table 1). Real-Time SYBR Green 2X Master mix (+ROX) (Parstous Biotechnology, Iran) was used for qRT-PCR analysis of cDNA targets. The protocol to amplify was: 94 ◦C for 5 minutes and 50 cycles of 95 ◦C for 15 seconds, 60 ◦C for 15 seconds, and 72 ◦C for 30 seconds. All samples were run in duplicate for all reactions. EF1α was used as the housekeeping gene. Two separate reactions without cDNA or with RNA were performed in parallel as controls. Validation of the assay to check whether the primers of target genes and housekeeping gene had similar amplification efficiencies. The melting curve analysis was performed to determine the specific amplification of PCR products. The results were expressed as fold change using the $2^{-\Delta\Delta Ct}$ method (Pfaffl, 2001).

Table 1: The primers utilized for analyzing genes expression

Gene Name	Forward (5′-3′)	Reverse (5′-3′)	Reference No.
EF1α	AACGGCCAGACCCGTGAG	GCAGGGTTGTAGCCGATCTT	Bayır et al, 2020
CD4	TTCAGTGGCACTTTGCTCCTAA	TGGGCGATGATTTCCAACA	Yao et al, 2019
CD8	ATGGACCAAAAATGGCTTCTG	GCTGAAAGATCCAATGAATTC	Yao et al, 2019

Statistical analysis was conducted using SPSS 26.0 for Windows. Data were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey test to determine the differences in the mean values among experimental groups. $p<0.05$ was considered significant.

Results

The expression level of CD4 and CD8 molecules in the head kidney after immunization was higher than skin and spleen. FKC immunized with lactobacillus dietary groups showed a higher expression of CD4 and CD8 fold change values compared to the group vaccinated with formalin-killed cells (Figures 1 and 2). In most vaccinated groups, CD4 and CD8 levels upregulated at 60 days compared to 30 days post immunization. Among the sampled organs, the head kidney has the highest elevation of CD4 and CD8 fold changes (Figures 1 and 2).

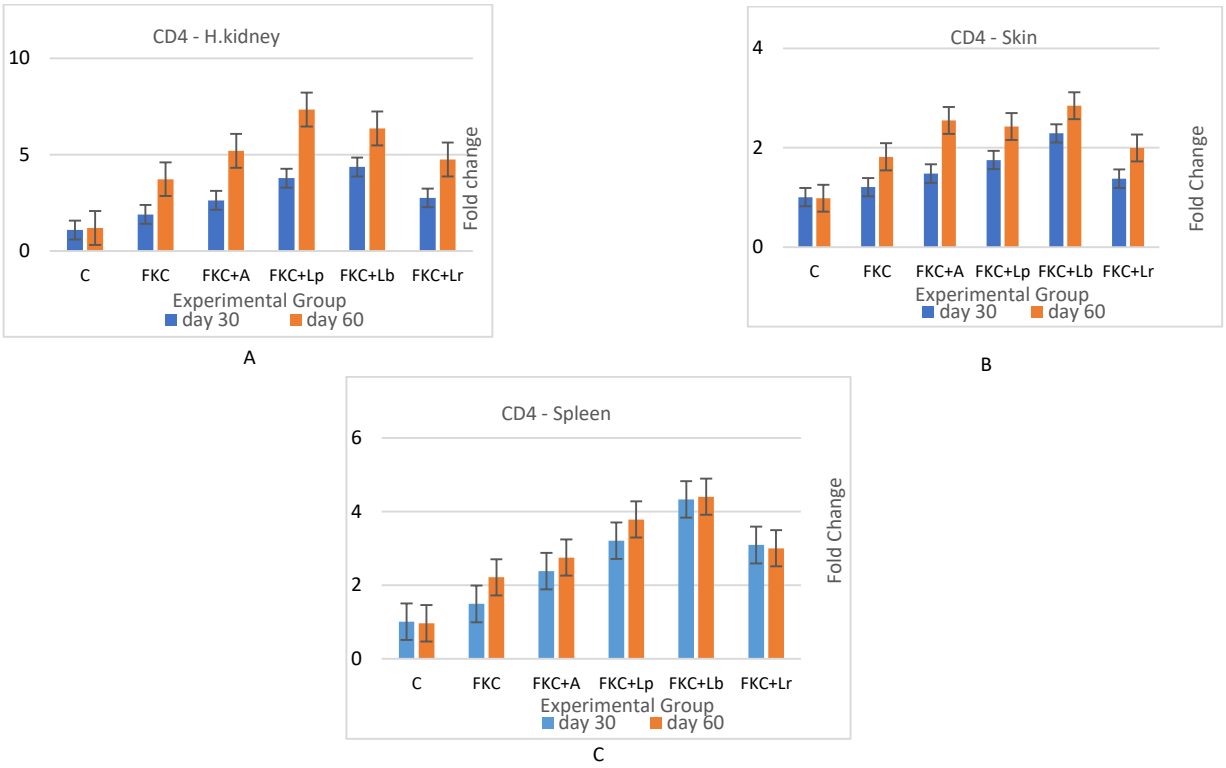


Figure 1: CD4 expression in different organs (head kidney, skin, and spleen) of Nile tilapia at 30- and 60-days post-immunization (dpi). C: control; FKC: formalin killed cell; FKC+A: formalin killed cell coupled with adjuvant; FKC+Lp: formalin killed cell and *Lactobacillus plantarum*; FKC+Lb: formalin killed cell and *Lactobacillus bulgaricus*; FKC+Lr: formalin killed cell and *Lactobacillus rhamnosus*. Data are means for three individual fish and presented as (Mean± SE).

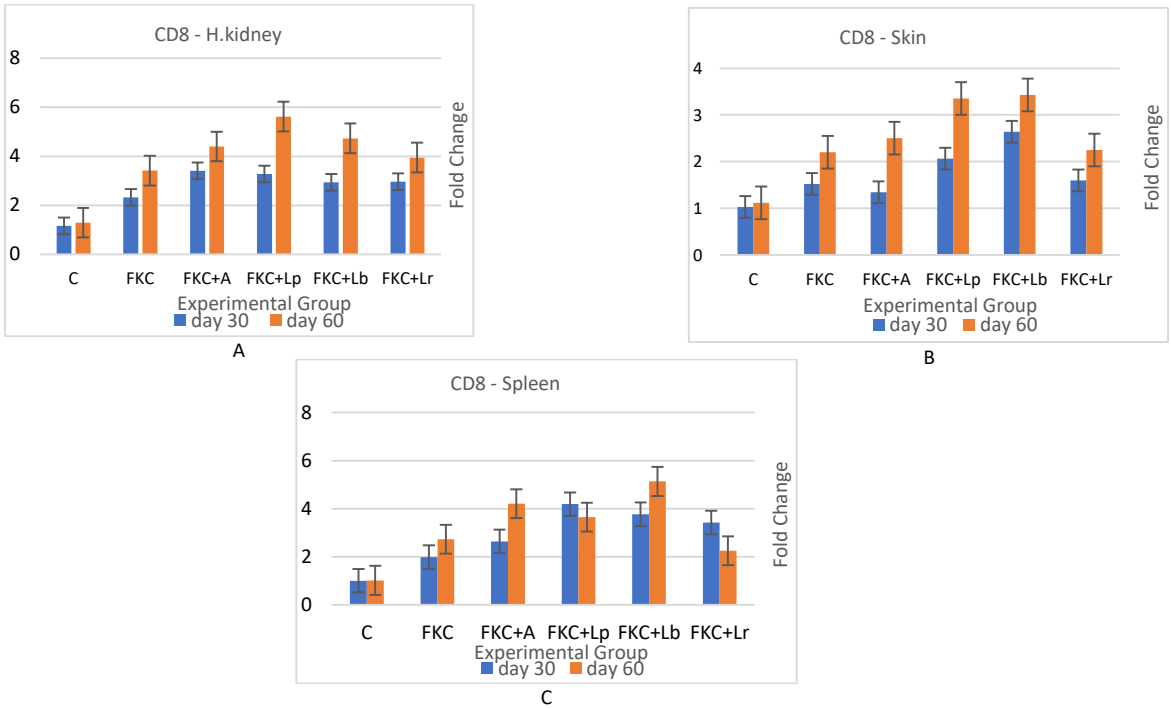
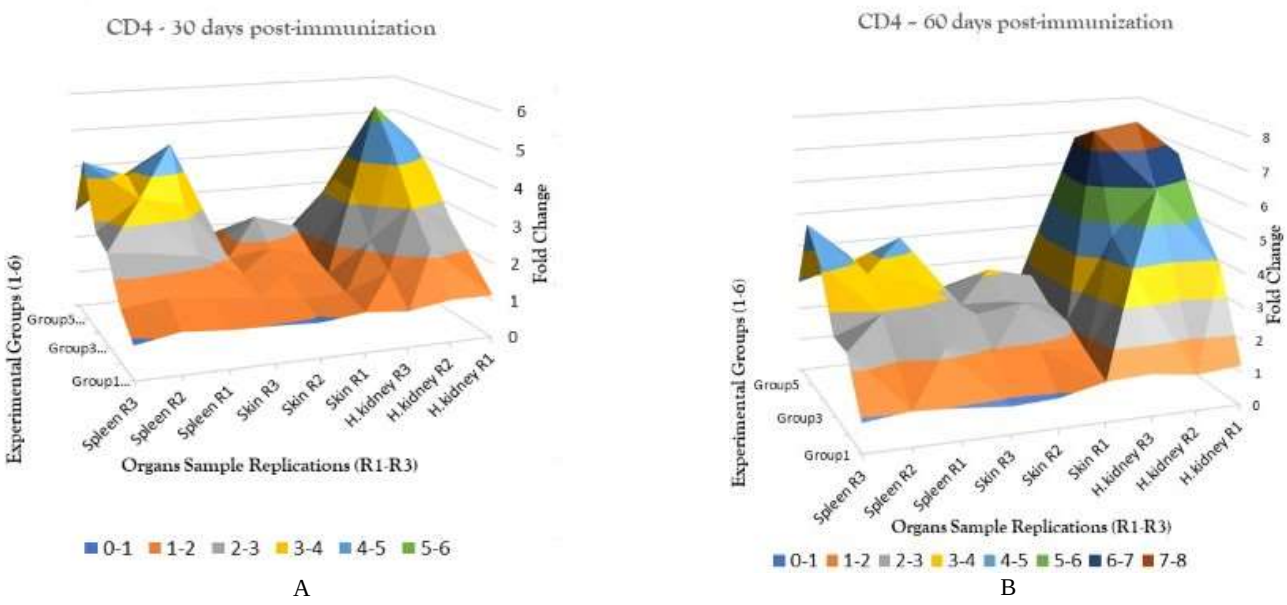
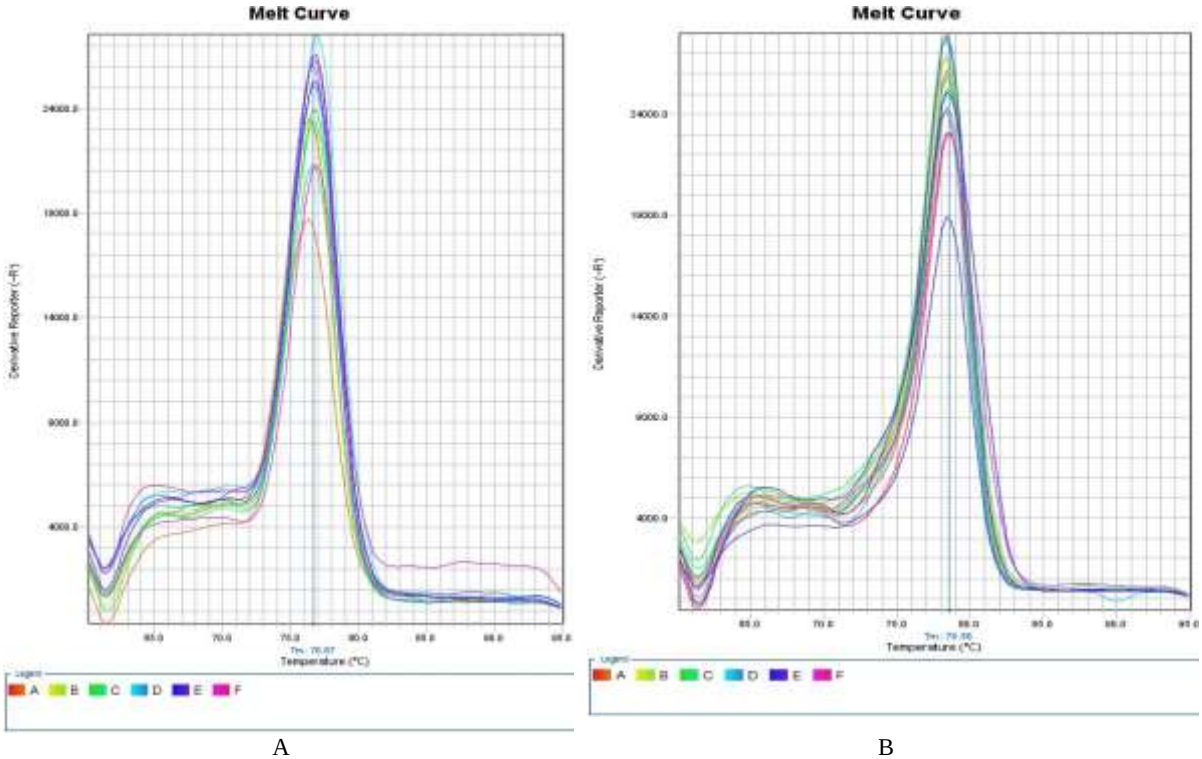


Figure 2: CD8 expression in different organs (head kidney, skin, and spleen) of Nile tilapia at 30- and 60-days post-immunization (dpi). C: control; FKC: formalin killed cell; FKC+A: formalin killed cell coupled with adjuvant; FKC+Lp: formalin killed cell and *Lactobacillus plantarum*; FKC+Lb: formalin killed cell and *Lactobacillus bulgaricus*; FKC+Lr: formalin killed cell and *Lactobacillus rhamnosus*. Data are means for three individual fish and presented as (Mean± SE).



A B

Figure 3: Graphs with 3 dimensions have illustrated the gene expression of CD4 at days 30 and 60 post-immunization in three sampled organs. Horizontal axis (Fold change values 0 to 8), Vertical axis (Organ sample replications included H. kidney 1,2 and 3; Skin 1,2 and 3; Spleen 1,2and 3), Distal axis (Experimental Groups 1,2,3,4,5 and 6).



A B

Figure 4: The qRT-PCR melt curve analysis of CD4 and CD8 genes. Derivative melting curve by use of SYBR Green to recognize the dissociation temperature of CD4 and CD8 genes amplicon and the specificity of RT-PCR reactions. A: CD4 melt curve (76.67 °C) and B: CD8 melt curve (78.56 °C)

qRT-PCR data fold change in different organs in different experimental groups showed that both the CD4 gene expression levels were significantly increased in all examined organs (H. kidney, Skin) at 60 dpi compared to 30 dpi but not in the spleen ($P<0.05$). The most level up for CD4 fold change gene expression was observed in the head kidney (Figure 3). The studied genes including CD4, CD8, and EF1α presented melting temperatures of 76.67, 78.56, and 81.81°C with a single peak indicating good specificity of studied genes amplification in the Real-time PCR test (Figure 4).

Protection against pathogen

In Nile tilapia challenged with *Streptococcus agalactiae* clinical signs mostly included lethargy, darkening skin color, and unilateral or bilateral popped eye. Re-isolation of bacteria on TSA media from the brain and kidney was performed by streaking. Relative Percentage Survival for different experimental groups was 51.5(%), 55.3(%) for FKC, FKC+Adjuvant respectively and 56.3(%), 63.6(%), 63.6(%) for FKC+*L.plantarum*, FKC+*L.bulgaricus* and (FKC) + *L.rhamnosus* respectively. Immunization efficiency (survival rate) of tilapia, after challenge with lethal bacteria, *Streptococcus agalactiae*, displayed protection rates of 66.7% and 69.3% in groups of FKC and FKC coupled with adjuvant accordingly. The survival rate in the groups of FKC combined with oral administration of lactobacilli was counted from 70.0% to 75.0%. Protection in the control group amounted to 31.3% which illustrated a significant difference from other experimental groups (Figure 5).

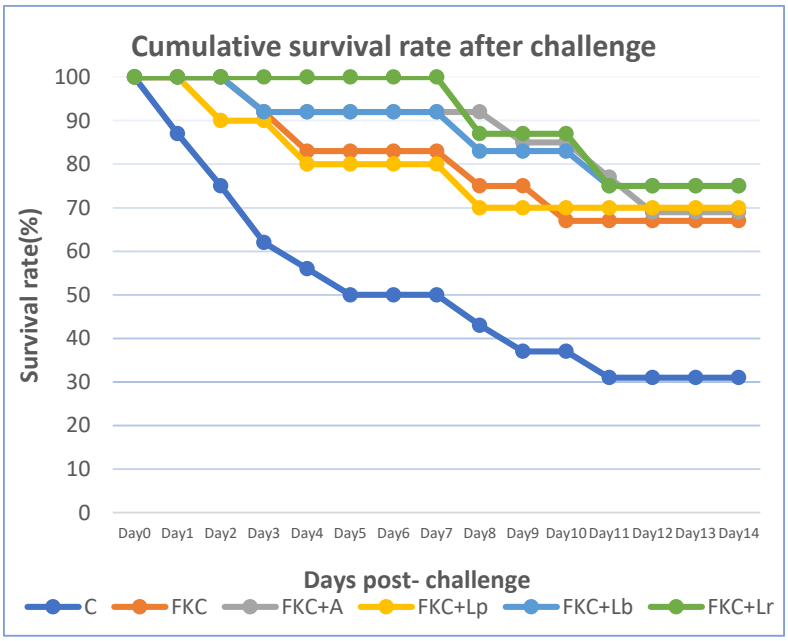


Figure 5: Cumulative survival rate of Nile tilapia after challenge through IP route with median lethal dose (LD50%=1.7× 10^6 Cells/ml/fish) against *Streptococcus agalactiae* is taken from the previous research.

Gene expression of CD4 and CD8 revealed an increase Fold changes in all immunized groups at 60 dpi compared to day 30 in all groups. In both sample points, the changes in CD4 gene expression in the adjuvant and probiotic groups increased significantly compared to the control group ($P<0.05$). No significant difference was observed between the CD4 fold change value of the FKC group and probiotic groups at day 60 whereas significant differences were observed between the FKC group and the groups FKC+Lp and FKC+Lb at 30 dpi which shows acceleration in the time to reach the higher levels in CD4 in comparison with the FKC

group as a result of increasing time (P<0.05) (table 2). The fold changes of the CD4 gene expression level in all examined organs (the head kidney, Skin, and spleen) significantly increased at 60 dpi in comparison to 30 dpi (P<0.05). The fold change of CD8 gene expression in the FKC group did not show a significant increase compared to the control group at 30 dpi whereas the FKC group showed a significant increase compared to the control group at 60 dpi (P<0.05). The information obtained in this research shows the role of selected Lactobacilli as a dietary supplement in combination with FKC in increasing the expression levels of two specific antigens, CD4 and CD8, related to T cells in H. kidney, skin and spleen.

Table 2: CD4 and CD8 gene expression of experimental groups were shown in two sampling points. The results are presented as (Mean± Standard deviation). Latin lower-case letters indicate significant differences at the (0.05) level in each column

CD8 Fold Change (Mean±SD)		CD4 Fold Change (Mean±SD)		Experimental Group	
Day 30	Day 60	Day 30	Day 60		
1.06±0.11^{a}	1.14±0.20^{A}	1.03±0.09^{a}	1.05±0.16^{A}	Control (C)	1
1.94±0.45^{ab}	2.78±0.58^{B}	1.55±0.31^{ab}	2.59±0.89^{AB}	(FKC)	2
2.46±0.99^{bc}	3.70±1.00^{BC}	2.18±0.56^{bc}	3.50±1.34^{B}	(FKC)+ Adjuvant	3
3.18±1.11^{c}	4.21±1.13^{C}	2.91±0.99^{cd}	4.51±2.22^{B}	(FKC)+ *L.plantarum*	4
3.11±0.57^{c}	4.43±0.85^{C}	3.61±1.24^{d}	4.54±1.62^{B}	(FKC)+ *L.bulgaricus*	5
2.66±0.90b^{c}	2.81±0.89^{B}	2.44±0.79^{bc}	3.25±1.25^{B}	(FKC)+*L.rhamnosus*	6

Discussion

Nile tilapia is a fast-growing fish that is farmed all over the world. The outbreak of *Streptococcus agalactiae* has been a major challenge in the tilapia fish farming industry causing great economic losses (Munang' et al., 2016). Fish vaccination has helped prevent a wide range of bacterial and viral diseases for more than 50 years (Ma et al., 2019). Autogenous vaccines are made from specific pathogen isolates from diseased fish. Relative percent survival (RPS) values differed in FKC vaccinated Japanese flounder *Paralichthys olivaceus* against *Streptococcus parauberis* with heterologous serotype isolates after challenge (Mori et al., 2012). RPS values obtained from the vaccine of formalin-killed *Streptococcus agalactiae*, did not show sufficient cross-protective efficiency against different heterologous serotypes (Suwannasang et al., 2017).

This research aim was to investigate the effect of oral supplementation of three lactobacillus bacteria on the expression of immune-related genes (CD4 and CD8) and their protective effects on Tilapia immunized with formalin-killed *streptococcus agalactiae* at two sampling points (30 and 60 dpi). This study shows that the adjuvant effects and protection rate of investigated probiotics are comparable with utilized adjuvant (Montanide™ ISA 763 AVG) which can be used through oral diet if no adjuvant is used in vaccine preparation. CD4 and CD8 molecules express on the surface of CD4+T and CD8+ T cells. Administration of a modified Lactobacillus (*Lactobacillus rhamnosus*. Gorbach Goldin) increased CD4+ and CD8+ T cells in mice (Kandasamy et al., 2011). CD4 and CD8 were used in vaccine-induced immunity studies (Ashfaq et al., 2019; Jiang et al., 2019; Kato et al., 2013; Yao et al., 2019; Vilander and Dean., 2021; Wang et al., 2022; Zeng et al., 2021).

In this study, no significant differences were observed between the FKC administrated group and the group of control at both sampling points for CD4 and the first sampling point for CD8 (Table 2) (P<0.05). Antigen vaccines such as FKC cannot provide high protection against

pathogens because they are not sufficiently immunogenic. Immunization with antigen caused level up the expression of major histocompatibility complexes (MHC-I) and (MHC-II) while Oil-adjuvanted antigen motivated a range of gene expression of MHC-I, MHC- II, CD8a, CD40, TNF-a, IL-6 and IFN-g (Jiao et al., 2010).

This research revealed that examined dietary lactobacilli generated significant increase expression of the genes encoded CD4 and CD8, compared to the control group as well as what was observed in the adjuvant group ($P<0.05$). Using autogenous vaccines in aquaculture can be a safe and effective solution for bacterial resistance to antibiotics (Barnes et al., 2021; Palić et al., 2022). Adjuvants and immunostimulants are required to enhance the vaccine efficacy which effectively potentiates the host antigen-specific immune responses (Tafalla et al., 2013; Munang'andu and Evensen., 2019). Licensed fish vaccines mostly are made from killed pathogenic microorganisms formulated with adjuvants (Ma et al., 2019). Various types of adjuvants are used to enhance the immune response (Spickler and Roth., 2003; Thim et al., 2014). There are evidences for the positive effects of probiotics with vaccine administration on the adaptive immune response (Davidson et al., 2011; Guimarães et al., 2022; Inic-Kanada et al., 2016; Kazemifard et al., 2011).

This survey showed that CD4 and CD8 gene expression levels significantly elevated in all sampled organs (head kidney, Skin, and spleen) at 60 dpi ($P<0.05$)**.** The highest change for the expression of both genes was observed in the head kidney. Teleost fish possess two CD4 genes including CD4-1 and CD4-2 containing four and two or three Ig-like domains respectively (Ashfaq et al., 2019). After inoculation Japanese flounder (*Paralichthys olivaceus*) with *Streptococcus iniae* and tuberculin, the expression level of CD4-1 mRNA lightly increased CD4-1 but CD4-2 mRNA did not increase in trunk kidney at 1-, 3-, and 6-days post-infection (Kato et al., 2013). Immune-related gene expression assayed by Real-time qPCR after oral bivalent vaccine administration in red tilapia followed by challenge against *S. iniae* and A. *hydrophila* revealed that CD4, MHC-I, and MHC-II gene expression remained significantly higher than the control groups between 24 and 72 hours post infection in organs including spleen and the head kidney ($P<0.05$) (Monir et al., 2022).

In this study, Relative percentage survival (RPS) values for administered lactobacillus species varied from 55.3 to 63.6 %, whereas RPS for FKC coupled with adjuvant, was 51.5% which indicates that probiotics as dietary supplements effectively protected fish against *streptococcus agalactiae*. The RPS value in rainbow trout- fed diet enriched with *Lactobacillus sakei* 2-3 for 21 days after the challenge against *Lactococcus garvieae* was 80% (Didinen et al., 2017). Sixteen days after starting *Lactobacillus rhamnosus* feeding diet, rainbow trout were challenged *with Aeromonas salmonicida* subsp. *salmonicida,* which causes furunculosis, mortality was reduced significantly compared to the control group (Nikoskelainen et al., 2001).

This research revealed that dietary Lactobacilli administered with inactivated antigen enhanced the efficiency and immunogenicity of parenteral vaccination against *streptococcus agalactiae* and accelerated immune response.

The results indicated that the gene expression of immune-related antigen-specific T cells (CD4 and CD8) was significantly increased in the immunized groups in which FKC was administered in combination with probiotics compared to the control group. The challenge with *Streptococcus agalactiae* resulted a protection rate of 69.3% in the FKC group coupled with adjuvant, 70.0% to 75.0% in the FKC groups with oral administration of lactobacilli, which was drastically different

from the control group with survival rate of 31.3%. Considering the immunomodulation and protective effects of the three Lactobacillus species used as probiotics in this study, it is recommended that they be used in combination with *Streptococcus agalactiae* killed-vaccines where they are without adjuvant. Lactobacilli studied in this research improved the efficiency of vaccination in Nile tilapia and shortened the time to reach the higher levels of CD4 and CD8 in comparison with the FKC group.

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Conflict of Interest

The authors of this article declare no conflict of interest.

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بررسی اثرات تقویت کننده ایمنی و حفاظتی سه گونه لاکتوباسیلوس بر روی ماهی تیلاپیا نیل (*Oreochromis niloticus*) واکسینه شده علیه *استرپتوکوکوس آگالاکتیه*

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چکیده

رژیم غذایی غنی شده از پروبیوتیک لاکتوباسیلوس می‌توانند پاسخ ایمنی میزبان را تعدیل کنند. در این مطالعه، بررسی نمودیم که آیا سه گونه *Lactobacillus plantarum*، *L. bulgaricus* و *L. rhamnosus* دو آنتی‌ژن اختصاصی سلول T را تنظیم کرده و اثر محافظتی را هنگام تجویز با استرپتوکوکوس آگالاکتیه کشته شده با فرمالین در تیلاپیای نیل اعمال می‌کنند. برای این منظور، در مجموع ۱۸۰ ماهی تیلاپیای نیل (میانگین وزن بدن ۲۲ ± ۴۵/۸ گرم) به طور تصادفی به ۶ گروه آزمایشی تقسیم شدند، یعنی: یک گروه کنترل (گروه درمان نشده)، و پنج گروه واکسینه شامل: واکسینه شده با سلول‌های کشته شده با فرمالین(FKC) ، FKC + ادجوانت، *L. plantarum* +FKC ، *L. bulgaricus* +FKC و *L. rhamnosus*+FKC همه گروه‌ها با غذای پلت معمولی تجاری و سه گروه با غذای پلت اسپری شده با سه لاکتوباسیل فوق تغذیه شدند. نتایج نشان داد که میزان بقا در گروه‌های واکسینه شده با سلول کشته شده با فرمالین (FKC) همراه با تجویز خوراکی لاکتوباسیل‌ها از ۷۰ درصد تا ۷۵ درصد بود. میزان حفاظت در گروه کنترل ۳۱/۳ درصد بود که نشان دهنده تفاوت معنی‌داری با سایر گروه‌های آزمایشی بود. در این تحقیق بیان ژن CD4 و CD8 که دارای عملکردهای اساسی در پاسخ ایمنی هستند با استفاده از qRT-PCR در کلیه قدامی، پوست و طحال اندازه‌گیری و به صورت fold change گزارش شد. تحلیل نتایج fold change مطالعه (میانگین ± انحراف معیار) CD4 و CD8 به ترتیب در روزهای ۳۰ و ۶۰ پس از ایمن‌سازی (dpi) در همه گروه‌های پروبیوتیکی نسبت به گروه FKC افزایش نشان داده است که در دو گروه از سه گروه پروبیوتیکی افزایش‌ها در سطح $P<0.05$ معنی‌داری بوده‌اند. این امر نشان‌دهنده افزایش بیان ژن‌های وابسته به پاسخ ایمنی در گروه‌های پروبیوتیکی نسبت به گروه FKC است. نتایج این مطالعه نشان داد که Fold change در واکسیناسیون با FKC همراه با رژیم‌های غذایی غنی شده با لاکتوباسیلوس‌ها، بیان CD4 و CD8 را افزایش داد که می‌تواند نشان دهنده محافظت بالاتر در برابر استرپتوکوکوس آگالاکتیه در مقایسه با واکسیناسیون FKC به تنهایی باشد، مشابه نتایجی که در گروه FKC همراه با ادجوانت مشاهده شد.

کلمات کلیدی: واکسن، *استرپتوکوکوس آگالاکتیه*، لاکتوباسیلوس، CD4، CD8

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