Yeast cell wall upregulated cell-mediated immune responses to Newcastle disease virus vaccine

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ABSTRACT A recent study has suggested that yeast cell wall product (**YP**) enhanced serum hemagglutination inhibition (**HI**) titers and intestinal sIgA responses in chickens immunized with Newcastle disease virus (**NDV**) vaccine. In the present study, the cell-mediated immune responses elicited by NDV and YP were investigated in commercial broilers. Broilers were fed 0 or 0.1% YP and immunized with a live NDV vaccine via an intraocular-and-intranasal route at 14 and 28 days old. After that, blood samples were collected for determination of HI titer, cytokine content, and blood analysis. Eight chickens were randomly selected from each group and sacrificed. Lymphocytes were harvested from the spleens for lymphocyte proliferation and flow cytometry analysis. Total RNA was extracted from spleen and jejunum for RT-qPCR analysis. The results showed that YP significantly increased serum concentration of IL-4, IL-6, IFN-γ, TNF-β, as well as promoted lymphocytes proliferation in broilers immunized with NDV vaccine. The enhanced cell-mediated immunity is correlated with the upregulated mRNA expression of TGF-β, IL-6, TLR5, GATA-3, and T-bet in the spleen and upregulated mRNA expression of CCR-9, J-chain, pIgR, and TLR3 in the jejunum of chickens. It is noteworthy that no significant side effect was observed after the administration of YP. Therefore, YP could be safely used as potential immunopotentiator assisting NDV vaccine for chickens.

Key words: yeast cell wall product, cytokine response, lymphocyte proliferation, mRNA expression, safety

INTRODUCTION

Newcastle disease (**ND**) is a severe disease caused by Avian avulavirus type-1 belong to the genus *Avulavirus* in the family Paramyxoviridae (Sultan et al., 2020). This disease was classified as A class infective disease by the World Organization for Animal Health (**OIE**) and its outbreak commonly results in considerable economic loss in poultry production (Megahed et al., 2018). Vaccination is an ideal approach to control contagious poultry diseases. Currently, immunization with inactivated and live Newcastle disease virus (**NDV**) vaccines is used in most chicken farms. However, the efficacy of immunization and protection for chickens remains less than satisfactory (Rauw et al., 2017; El-Dabae et al., 2018). Adjuvants including alum, cytokine, CpG DNA sequences, and plants extracts are commonly used to improve immune responses (Jiang et al., 2018; Mohan et al., 2018; Irie et al., 2020; Xiao et al., 2021). Oral administration, among various routes, is the first choice for drug delivery owing to its ease and less irritation to animals (Yan et al., 2020). Thus, increasing attention is put on immunopotentiators that are administrated orally in recent years (Van Splunter et al., 2018).

Probiotics such as *Bacillus subtilis* and *Saccharomyces* yeast have been reported effective to elicit both systemic immunity and local mucosal immunity (Li et al., 2017; Wang et al., 2019; Fazelnia et al., 2021). In addition, natural products from these probiotics showed a similar effect on immune responses in mammals and birds (Zhu et al., 2017; Abudabos, et al., 2019). In our previous investigation, a commercial yeast cell wall product (**YP**) PW220 was demonstrated effective to enhance serum hemagglutination inhibition (**HI**) titers and intestinal sIgA response in chickens immunized with NDV vaccine (Bi et al., 2020). However, the effect of YP on cell-mediated immunity and the underlying mechanism need to elucidate. The present study was designed to evaluate the effect of YP on production of Th1 and Th2 cytokines, lymphocytes proliferation, and the function of CD4 + CD8 + lymphocyte subsets. To gain a

better understanding of the mechanisms conferring enhanced immune responses after YP supplement, we examined the mRNA expression of immune related genes in spleen and jejunum.

MATERIALS AND METHODS

Birds

One-day-old Sanhuang broilers (male) were purchased from Sichuan Lihua Poultry Co., Ltd. (Zigong, China). The broilers were separated into wire cages and allowed access to feed and water ad libitum. For the first 3 d, the room temperature was kept at 35°C, then gradually lowered to 26°C. The broilers were all treated according to the guidelines set forth by the Southwest University Committee on Animal Care and Use (IAC-2021-0057).

Vaccine

Live Newcastle disease virus vaccine (Strain La Sota) was purchased from Qingdao YEBIO Bioengineering Co., Ltd. (Qingdao, China)

Reagent

YP was derived from yeast cell (*Saccharomyces cerevisiae*) walls and contains β-glucan ($\geq$30%) and mannan-oligosaccharides (**MOS**, $\geq$20%; AngelPW220, 2260-2019, Angel Yeast, Yichang, China). Chicken IL-4 (G20210824ST), IFN-γ (G20205120OS), TNF-β (G20210516RT), TGF-β (G20210422MW), and IL-6 (G20210318DR) ELISA kits were purchased from Shanghai Lengton Bioscience Co. Ltd. (Shanghai, China). The antigen and positive control sera for the detection of NDV-specific HI titers were provided by Qingdao Regen Diagnostics Development Center (Qingdao, China). Rat anti-chicken CD3-APC (C2818-T958O), CD4-FITC (D0117-WA78E), and CD8-PE (L2413-TH49T) were from Southern Biotech (Birmingham, AL).

Experimental Design

In experiment 1, sixty 5-day-old broilers were randomly divided into 3 groups (Table 1). Broilers were assigned to the basal diet (Table 2) or the basal diet supplemented with 0.1% YP (β-glucan, 0.3 g/kg; MOS, 0.2 g/kg) during the experiment. The broilers in group A and B were received intraocular-and-intranasal immunization with a live NDV vaccine at 14 and 28 days old. The broilers in group C were immunized with same volume of saline. Blood samples were collected before and

Table 1. Experimental design (experiment 1).

Group	N	YP	Vaccination
A	20	0.1%	+
B	20	0	+
C	20	0	

Table 2. Dietary formulation of basal diet[1].

Days	Ingredient	Percent
1−21	Crude protein, %	20.0
	Crude fiber, %	5.0
	Crude ash, %	7.0
	Ca	0.80−1.20
	Total phosphorus, %	0.45
	Digestible methionine, %	0.41
	H_2O	12.5
	Sodium chloride, %	0.20−0.80
	Phytase	500 U/kg
22−42	Crude protein, %	16.0
	Crude fiber, %	6.0
	Crude ash, %	8.0
	Ca	0.60−1.20
	Total phosphorus, %	0.40
	Digestible methionine, %	0.32
	H_2O	12.5
	Sodium chloride, %	0.20−0.80
	Phytase	500 U/kg

[1]Value were calculated from data provided by Feed Database in China (2013).

Table 3. Experimental design (experiment 2).

Group	n	YP
C	20	0
L	20	0.1%
M	20	0.3%
H	20	0.5%

1, 2, 3, 4 wk after immunization for determination of HI titer and cytokine content. At 1 and 3 wk after booster immunization, 8 chickens were randomly selected from each group and killed by cervical dislocation. Lymphocytes were harvested from the spleens for lymphocyte proliferation and flow cytometry analysis. Total RNA was extracted from spleen and jejunum for real-time quantitative PCR (**RT-qPCR**) analysis.

In experiment 2, eighty broilers were randomly divided into 4 groups (Table 3). The chickens were orally given YP by supplement at the doses of 0, 0.1 (β-glucan, 0.3 g/kg; MOS, 0.2 g/kg), 0.3 (β-glucan, 0.9 g/kg; MOS, 0.6 g/kg), and 0.5% (β-glucan, 1.5 g/kg; MOS, 1 g/kg). Weight of each chicken was recorded weekly until the end of experiment. Blood samples and serum were collected and examined for hematological and biochemical parameters.

HI Test

Serum NDV-specific HI titers were detected as previously described (Zhai et al., 2011). Briefly, a 2-fold serial dilution of serum samples (1:2 to 1:1,024) was prepared in a V-shaped bottom 96-well microtiter plate containing 25 μL saline each well, and 4 hemagglutination units (**HAU**) of NDV antigen were added to each well. Following a 40-min incubation period at 37°C, 25 μL of 1% rooster erythrocyte suspension in PBS was added and incubated for another 20 min. All of the samples were tested twice. Positive and negative serum controls were

included on each plate. The HI titer was determined as the highest dilution of serum causing complete inhibition of hemagglutination. Mean HI titers and standard error (**SE**) were calculated for each group.

Isolation of Lymphocytes

The assay was performed as previously described with minor modification (Yuan et al., 2020). Briefly, the spleens were separated into 4 mL cold PBS through a 70-μm cell strainer. Next, the cell suspension was centrifuged at 5,000 rpm for 10 min and the supernatant was removed. Equal volume of red blood cell lysis buffer was added to blow precipitate and incubated on ice for 10 min. The solution was centrifuged at 5,000 rpm for 10 min and the splenocytes were re-suspended in RPMI 1,640 complete medium (Solarbio Co., Beijing, China) containing 5% fetal bovine serum (**FBS**) (Sijiqing Co., Hangzhou, China). Finally, a chicken lymphocyte isolation kit (Tianjin Haoyang Biological Manufacture Co. Ltd., Tianjin, China) was used to isolate lymphocytes.

Lymphocyte Proliferation

A total of 5×10^5 lymphocytes were transferred to each well of 96-well microtiter plate. Inactivated NDV antigen at 4 HAU in the well was employed as a stimulator. Wells without mitogen were used as controls. Each sample was repeated 3 times. The cells and antigen were incubated for 44 h (5% CO_2, 37°C), and then 50 μL methyl thiazolyl tetrazolium (**MTT**; 2 mg/mL) was added to each well and incubated for another 4 h. The plates were centrifuged at $1,000 \times g$ for 10 min at room temperature. After that, the supernatant was removed carefully and 100 μL of DMSO was added into each well. The plates were shaken for 5 min to completely dissolve the crystals. Finally, the average optical density (**OD**) was read at 570 nm. The stimulation index (**SI**) was calculated using the formula: OD values of stimulated wells/ OD values of unstimulated wells (Cui et al., 2020).

Flow Cytometry Analysis of T Lymphocyte Subsets

The isolated lymphocyte suspension was washed twice with PBS. The cell concentration was adjusted to 10^6/mL and placed on the ice. Each sample was stained with 2 μL of rat anti-chicken CD3-APC, CD4-FITC and rat anti-chicken CD8-PE for 30 min in the condition of avoiding light, and then washed twice with PBS. The cells were determined by flow cytometry analysis on BD FACSVerse (BD Bioscience, San Jose, CA).

Cytokine Assay

The concentrations of IL-4, IFN-γ, TNF-β, TGF-β, and IL-6 were determined by specific ELISA kits from

Shanghai Lengton Bioscience Co. Ltd. (Shanghai, China) according to the manufacturer's instructions.

RT-qPCR Analysis

Total RNA was extracted from the spleen and jejunum using TRIzol reagent (Takara, Shiga, Japan) following the manufacturer's guidelines. PrimeScript RT Master Mix (Takara, Dalian, China) was utilized to convert RNA into cDNA on a T100 thermal cycler (Bio-Rad, Hercules, CA). The Chicken β-actin was served as the internal control gene. RT-qPCR with SYBR Premix Ex Taq II (Tli RNaseH Plus) (Takara) on selected genes was performed on a Multiple Real-Time PCR System (Applied Biosystems, Carlsbad, CA). A relative quantitative method ($2^{-\triangle\triangle CT}$) was employed to evaluate the quantitative variation (Bi et al., 2019). The primer sequences were list in Table S1.

Blood Analysis

Biochemical analysis was performed on serum samples with a Dotop 8018Vet chemistry analyzer (Shenzhen, China). Analysis items included serum concentrations of blood urea nitrogen (**BUN**, mmol/L), creatinine (**CRE**, μmol/L), serum activities (IU/L) of the enzymes alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**). Hematological analysis was determined with the BC-2600 Vet Blood Cell Analyzer (Shenzhen, China), with EDTA-K$_2$ as the anticoagulant. This analysis included the following parameters: counts of red blood cell (**RBC**, $\times 10^{12}$/L), white blood cell (**WBC**, $\times 10^9$/L), lymphocyte (**LYM**, $\times 10^9$/L), monocytes (**MON**, $\times 10^9$/L), granulocyte (**GRAN**, $\times 10^9$/L), and hemoglobin (**HGB**, $\times$ g/L; Tang et al., 2017; Manyeula et al., 2019).
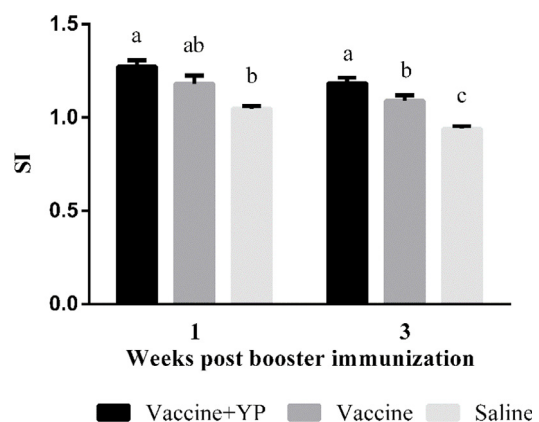
Figure 1. Lymphocyte proliferative responses. Broilers were fed with 0 or 0.1% YP and immunized with Newcastle disease virus (NDV) vaccine. Lymphocytes were harvested from the spleens at 1, 3 wk post boosting immunization for lymphocyte proliferation. The cells were incubated with inactivated NDV antigen for 48 h. MTT method was used to measure cell proliferation. The stimulation index was calculated using the formula: optical density (OD) values of stimulated wells/ OD values of unstimulated wells. The values are presented as mean ± SE. Bars with different letters are statistically different ($P < 0.05$).

A

B

C

D

E
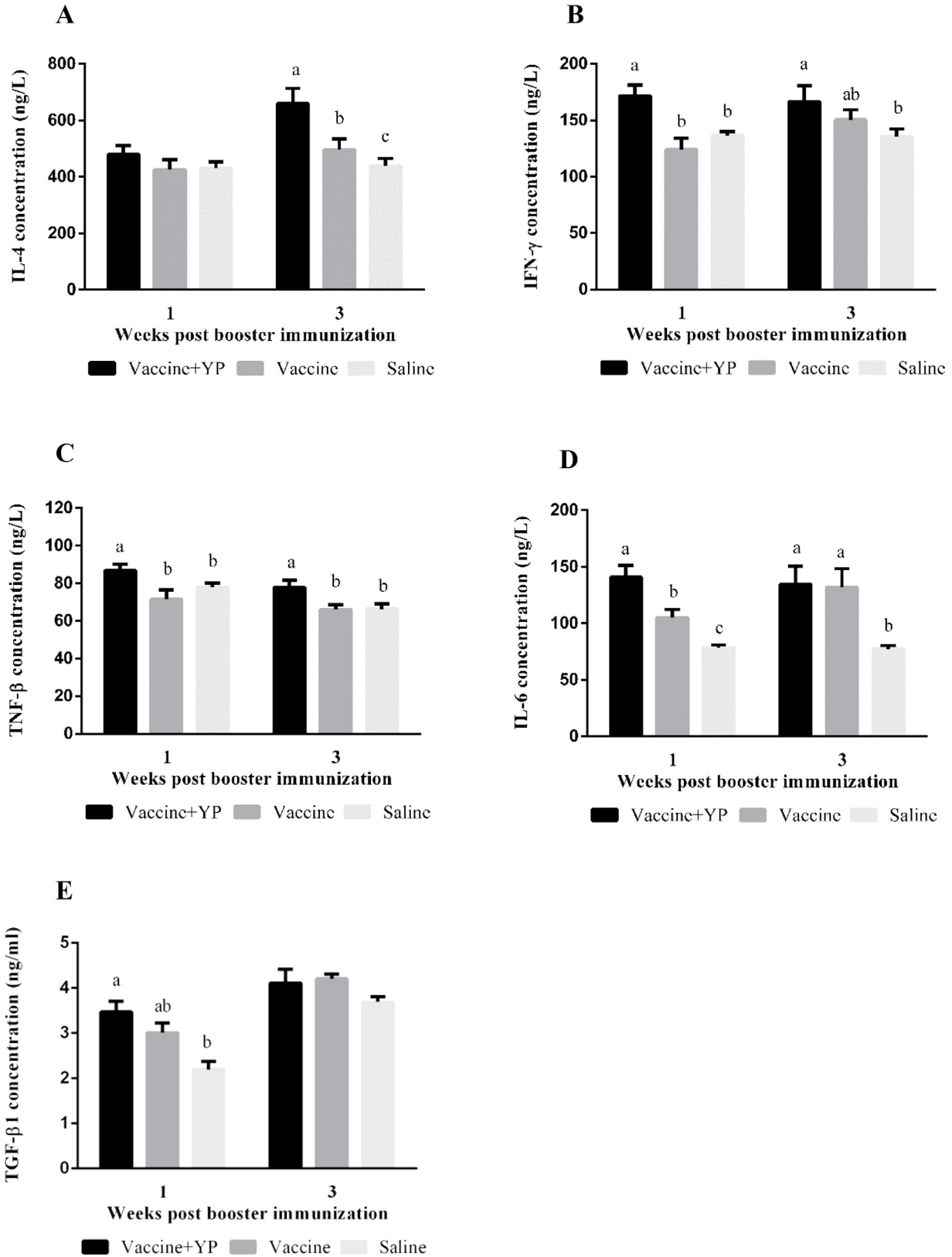
Figure 2. Cytokine production. Broilers were fed with 0 or 0.1% YP and immunized with NDV vaccine. Blood samples were collected at 1 and 3 wk post boosting immunization and the serum was used for test of IL-4 (A), IFN-γ (B), TNF-β (C), IL-6 (D), and TGF-β1 (E) contents by ELISA. The values are presented as mean ± SE. Bars with different letters are statistically different ($P < 0.05$).

Statistical Analysis

One-way analysis of variance with Duncan post hoc test was used for multiple comparisons between groups using SPSS software (version 20.0, SPSS Inc., Chicago, IL). Data are presented as the mean ± SE. $P < 0.05$ or $P < 0.01$ were considered statistically significant.

A

B

C

D
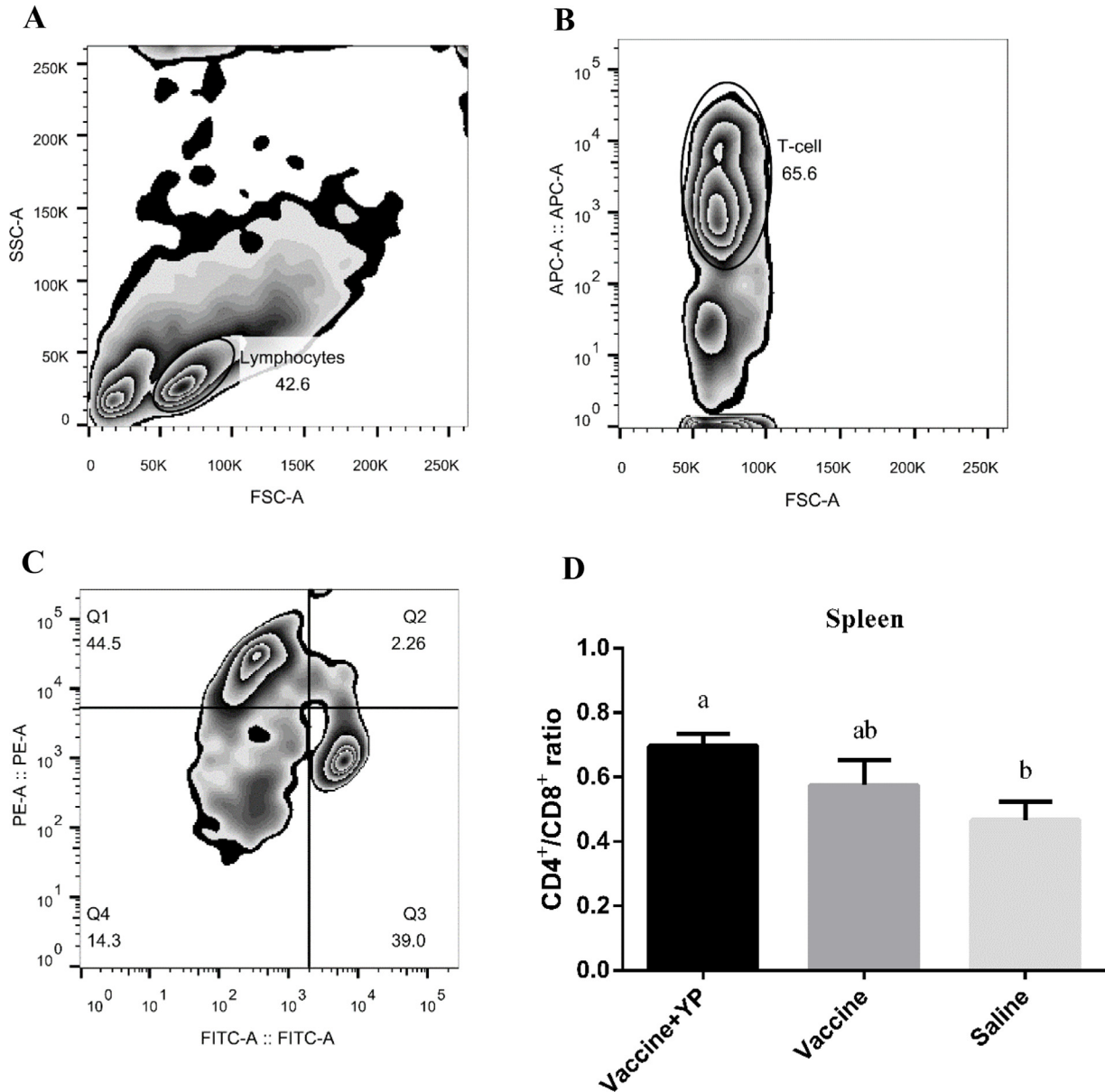
Figure 3. CD4+/CD8+ T cell ratio of the splenic lymphocytes. Broilers were fed with 0 or 0.1% YP and immunized with NDV vaccine. Lymphocytes were isolated from the spleens at 1 wk post the boosting immunization and stained with 2 μL of rat anti-chicken CD3-APC, CD4-FITC, and rat anti-chicken CD8-PE. The cells were determined by flow cytometry analysis. (A) Gate on lymphocytes; (B) gate on CD3+ T cells; (C, D) frequencies of CD3+ CD4 + CD8 + T cells. Data represent the mean ± SE. Bars with different letters are statistically different ($P < 0.05$).

RESULTS

Effect of YP on Lymphocyte Proliferation

As shown in Figure 1, increased SI in splenocytes was recorded in broilers supplemented with YP (YP + Vaccine) at 1 ($P > 0.05$) and 3 ($P < 0.05$) wk post the booster immunization, when compared with Vaccine group.

YP increased Cytokines Production in Serum

As illustrated in Figure 2A, the chickens treated with YP (YP + Vaccine) had higher IL-4 concentrations than the chickens without YP (Vaccine) at 1 ($P > 0.05$) and 3 ($P < 0.05$) wk post the booster immunization. Figure 2B showed that IFN-γ concentrations were higher in chickens treated with YP (YP + Vaccine) than the chickens with vaccine alone (Vaccine) at 1 ($P < 0.05$) and 3 ($P > 0.05$) wk post the booster immunization. As shown in Figure 2C, higher TNF-β concentrations were detected in chickens treated with YP (YP + Vaccine) than the chickens in Vaccine group at 1 ($P < 0.05$) and 3 ($P < 0.05$) wk post the booster immunization. Figure 2D showed that chickens treated with YP (YP + Vaccine) had higher IL-6 concentrations than the chickens with vaccine alone (Vaccine) at 1 ($P < 0.05$) and 3 ($P > 0.05$) wk post the booster immunization. As shown in Figure 2E, TGF-β concentrations in the chickens treated with YP (YP + Vaccine) were numerically ($P > 0.05$) higher than that in Vaccine group at 1 wk post booster immunization.
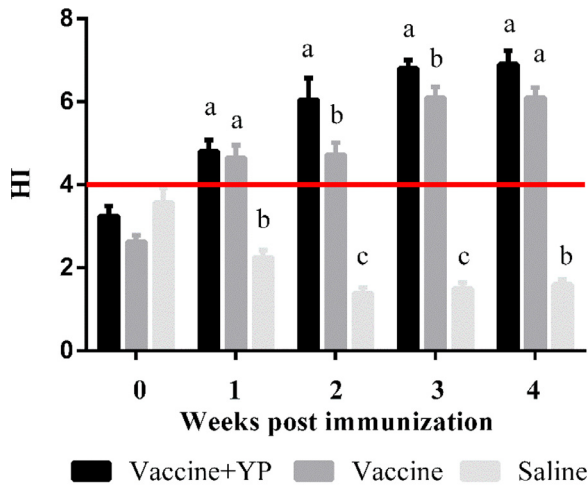
Figure 4. HI titers. Broilers were fed with 0 or 0.1% YP and immunized with NDV vaccine. Blood samples were collected before and 1, 2, 3, 4 wk post immunization for analysis of NDV-specific HI titers. The data were expressed as the $\log_2$ of the highest dilution that exhibited complete inhibition of hemagglutination. The values were represented as mean ± SE. The red line represents the minimum positive antibody titer. Bars with different letters are statistically different ($P < 0.05$).

Effect of YP on the Ratio of CD4 + /CD8 + T cell in Splenocytes

As depicted in Figure 3, there was no significant difference between the Vaccine group and the Saline group post the booster immunization ($P > 0.05$) while the ratio of CD4 + /CD8 + T cell was significantly higher in the YP group than in the Saline group ($P < 0.05$). In addition, the ratio of CD4 + /CD8 + T cell was numerically higher in YP group than in Vaccine group ($P > 0.05$).

HI Titers

The effect of YP on serum HI titers was shown in Figure 4. The results suggested that YP significantly increased HI titers at 2 ($P < 0.05$) and 3 ($P < 0.05$) wk post first immunization when compared with the chickens only immunized with vaccine.

Related Gene Expression

As illustrated in Figure 5A, increased mRNA expression of IL-6 ($P < 0.05$), IFN-γ ($P > 0.05$), and TGF-β ($P < 0.05$) was detected in spleen of broilers treated with YP (YP+Vaccine), when compared with the Vaccine group. Figure 5B showed that mRNA expression of TLR5 ($P < 0.05$) but not TLR4 and TLR3 was significantly higher in spleen of broilers supplemented with YP (YP + Vaccine) than that immunized with vaccine only. As illustrated in Figure 5C, broilers treated with YP and vaccine had higher mRNA expression of T-bet ($P < 0.05$) and GATA-3 ($P < 0.05$) in spleen than the broilers without YP. No significant difference was found in mRNA expression of NF-κB, RUN-X3, CD80 and

CD86 in spleen between YP (YP+Vaccine) and Vaccine groups ($P > 0.05$).

As shown in Figure 6A, significantly elevated mRNA expression of CCR9 ($P < 0.05$), J-chain ($P < 0.01$), and pIgR ($P < 0.05$) but not CCR-7 in jejunum was detected in the broilers supplemented of YP, when compared with the broilers immunized with vaccine only. Significantly higher mRNA expression of TLR3 ($P < 0.05$) but not TLR4 and TLR5 was found in jejunum of broilers treated with YP than that treated without YP (Figure 6B). Figures 6C and 6D showed that there is no significant difference in mRNA expression of NF-κB, Myd88, CD40, CD80 and CD86 in jejunum between YP (YP+Vaccine) and Vaccine groups ($P > 0.05$).

The Safety of YP

As depicted in Table S2, supplement of YP-H ($P < 0.01$) or YP-M ($P < 0.01$) or YP-L ($P < 0.01$) significantly decreased body weight of broilers at 4 wk. However, there is no apparent difference between YP-H, M, L groups and the Control birds in body weight at 1, 2, 3 wk and the end of experiment. As shown in Table S3, no significant toxic effect was observed between YP-H, M, L groups and the Control birds in hematological parameters. As shown in Table S4, serum biochemical analysis of broilers showed that significantly decreased serum ALT was detected in YP-H ($P < 0.05$) and YP-M ($P < 0.05$) groups, significantly decreased serum AST was found in YP-H ($P < 0.05$), as well as significantly decreased serum CR was detected in YP-M ($P < 0.05$), when compared with the Control. However, the alteration of these biochemical indexes does not suggest pathological change in clinical diagnosis.

DISCUSSION

The previous study demonstrated that supplement of YP could increase humeral and intestinal mucosal immunity in chickens immunized with NDV vaccine (Bi et al., 2020). Nevertheless, cell-mediated immunity also plays a vital role in protection against virus infection (Yuan et al., 2020). In this study, supplement of YP enhanced lymphocyte proliferation response to ND virus, and promoted the production of IL-6, IL-4, IFN-γ, and TNF-β in broilers immunized with NDV vaccine. Quantitative Real-time PCR analysis revealed that YP stimulated cell-mediated immunity by upregulating mRNA expression of TGF-β, IL-6, TLR5, GATA-3, T-bet in spleen and CCR-9, J-chain, pIgR, and TLR3 in jejunum.

Lymphocytes will expand or produce antibody when in response to mitogen. Stimulation index of lymphocyte is often used to evaluate lymphocytes in humoral and cellular immune responses (Ma et al., 2019). In this study, supplemented with YP increased proliferative responses to NDV of lymphocytes, suggesting
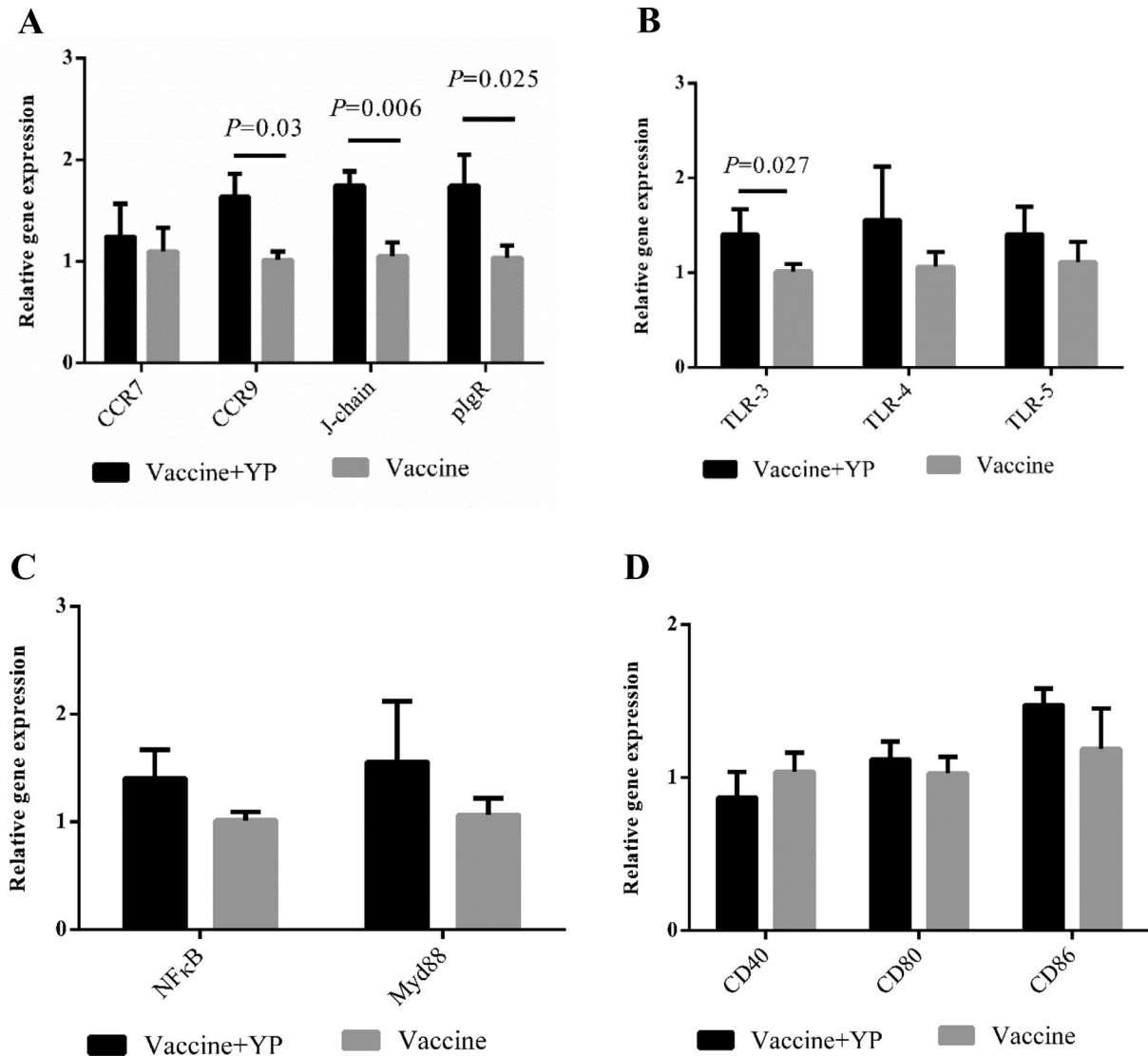
A

B

C

D
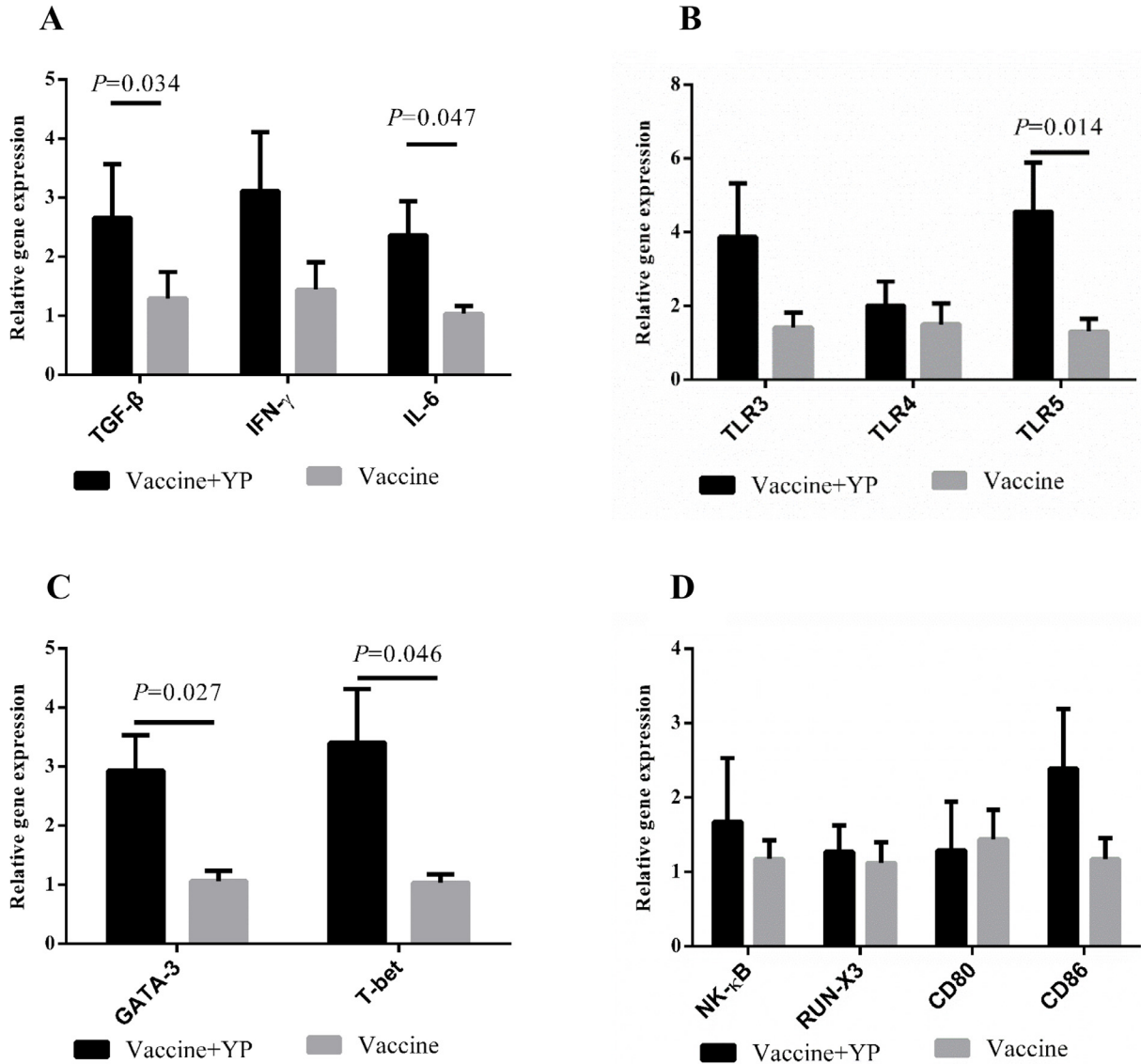
Figure 5. Relative mRNA expression in the spleen. Broilers were fed with 0 or 0.1% YP and immunized with NDV vaccine. Total RNA was extracted from spleen at 1 wk post boosting immunization. Expression of (A) TGF-β, IFN-γ, IL-6; (B) TLR3, TLR4, TRL5; (C) gata-3, T-bet and (D) NF-κB, RUN-X3, CD80, CD86 mRNA were assessed by real-time quantitative PCR. A relative quantitative method ($2^{-\triangle\triangle C_T}$) was employed to evaluate the quantitative variation. Data are represented as mean $\pm$ SE.

lymphocytes comprise memory T cells might proliferate faster in YP group than control broilers upon stimulation with recall antigens. We further investigated T cells and cell subsets by staining lymphocytes with CD3-APC, CD4-FITC, and CD8-PE antibodies. The results showed that increased proportion of CD4+/CD8+ T cells was detected in YP group, suggesting that more CD4+ T cells were activated by YP. Similarly, significantly higher proportion of peripheral CD4+ T cell was found in mammals that supplement of yeast cell wall than the control (Aung et al., 2020). However, the subset of virus-specific T cells is still unknown. In the future, we will use more chickens to detect the frequency of CD4 + CD8 + double positive T cells after stimulation by ND virus using cytometric analysis. Interestingly, the increased cell proliferative response to NDV stimulation paralleled the enhanced production of IL-4, IL-6, IFN-γ,

and TNF-β. Cytokines are critical to developing a cellular immune response against NDV. Generally, IL-12 and IFN-γ belong to Th1 cytokines while IL-4 and IL-10 belong to Th2 cytokines (Gagliani and Huber, 2017). In an in vitro experiment, yeast cell wall polysaccharides upregulated expression of cytokines IL-4, IL-10, IFN-γ, and IL-12 profile in chicken B lymphocytes exposed to LPS (Echeverry et al., 2021). In an in vivo experiment, supplement of glucan from yeast increased serum IL-6 and IFN-γ as well as decreased TGF-β in chickens with immunosuppression (Wang et al., 2019). In the present study, the chickens administrated with YP had significantly higher IL-4, IFN-γ, IL-6, and TNF-β content in serum than that immunized with vaccine only, suggesting that YP triggers both Th1 and Th2 immune responses.

Cytokines such as IL-4, IFN-γ, IL-12, and TGF-β will promote CD4+ T cells to differentiate into Th1 or Th2

Figure 6. Relative mRNA expression in the jejunum. Broilers were fed with 0 or 0.1% YP and immunized with NDV vaccine. Total RNA was extracted from jejunum at 1 wk after boosting immunization. Expression of (A) CCR-7, CCR-9, J-chain, pIgR; (B) TLR3, TLR4, TLR5; (C) NF-κB, Myd88 and (D) CD40, CD80, CD86 mRNA were assessed by real-time quantitative PCR. A relative quantitative method ($2^{-\triangle\triangle CT}$) was employed to evaluate the quantitative variation. Data are represented as mean ± SE.

cells, which are responsible for host defense against intracellular pathogens and adaptive immune responses (Kaiko et al., 2008). Furthermore, production of these cytokines was mainly regulated by the master regulator genes of Th1 (T-bet) and Th2 (GATA-3) (Lazarevic et al., 2013; Zhu, 2018). In this study, both T-bet and GATA-3 in spleen were upregulated in broilers treated with YP and vaccine, indicating that a balanced Th1/Th2 response was induced by YP. Consistently, a previous study reported that supplemented with yeast product increased the expression of T-bet/GATA-3 in fish with crowding stress (Reyes-Cerpa et al., 2018). As Jak-STAT signaling pathway is primarily involved in the production of numerous cytokines and transcription of T-bet/GATA-3, we speculate that YP might stimulate Th1/Th2 immune responses through JAK-STAT pathways (Kaiko et al., 2008; Villarino et al., 2017).

CCR9 was a tissue-homing marker expressed in Peyer patch and appeared critical for gut mucosal lymphocytes

homing (Wang et al., 2018). Upregulation of CCR-9 was generally associated with increased T cells and IgA+ plasma cells in mucosa (Huang et al., 2019). Effective secreting IgA is a chimeric molecule that needs both IgA produced by IgA+ plasma cells and pIgR produced by epithelial cells (Mallikarjunappa et al., 2019; Pabst et al., 2020). In this study, higher mRNA expression of CCR-9, J-chain and pIgR in jejunum was detected in the broilers after YP treatment. The findings may explain the enhanced intestinal sIgA responses and IgA+ cells in our previous study (Bi et al., 2020).

Toll-like receptors (**TLRs**) are membrane-bound receptors that play an important role in innate immunity by detecting pathogen-associated molecular patterns, activating nuclear transcription factor and producing cytokines through downstream signaling pathways (Monlish et al., 2016). Previous research reported that diets containing yeast cell wall upregulated the relative expression of TLR21 but not affected TLR2 and TLR4 in broilers, which could be considered

as one of active sites for yeast cell wall products (Alizadeh et al., 2016). In the present study, increased mRNA expression of TLR5 in spleen and TLR3 in jejunum was observed after supplement of YP. Activating of TLRs and downstream signaling pathways induce transcription of cytokines (Jang et al., 2020; Sanchez-Tapia et al., 2020). Our results showed that YP treatment enhanced the mRNA expression of genes IL-6, IFN-γ, and TGF-β in spleen, confirming that YP increased cell-mediated immunity by upregulating TLRs and inducing cytokines production.

In conclusion, enhanced cell-mediated immune response to NDV by YP was demonstrated. Considering its excellent activity on mucosal and systemic immunity and its safety for commercial broilers, YP deserves further study as a candidate immunopotentiator used in poultry industry.

ACKNOWLEDGMENTS

DISCLOSURES

The authors declare no competing financial interests.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2022.101712.

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