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Original Article

Probiotic Lactobacilli Improved Growth Performance and Attenuated Salmonella Typhimurium Infection Via Jak/Stat Signaling in Broilers

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ABSTRACT

This study aimed to investigate the effect of *Lactobacillus plantarum* DPP8 and *Lactobacillus acidophilus* C7282 in feed supplementation on growth performance, Salmonella invasion, inflammation, and mediating signaling in broilers infected with Salmonella Typhimurium (S. Typhimurium). A total of 240 broilers at day old were randomly allocated into four groups, orally infected with S. Typhimurium and supplemented with individual or combined Lactobacilli DPP8 and C7282 at doses of 0 (control), 10^{10} (individual), or 2.0×10^{10} (combination) cfu/kg of diet for 21 d. The results showed that supplementing Lactobacilli improved (p<0.05) feed intake and body weight gain and decreased (p<0.05) S. Typhimurium load in the caecum, harder gland, spleen and bursa of Fabricius. Also, the supplements decreased (p<0.05) interleukin (1β/2/4), tumor necrosis factor α and interferon γ in the serum, enhanced (p<0.05) interleukin 10, and downregulated gene expressions of inflammatory mediators including Janus kinase (Jak2/3), signal transducer and activator of transcription protein (STAT3/4/5/6) in the intestinal mucosa. In contrast, diets containing DPP8 exhibited greater effects on the inhibition of the pathogen and inflammatory response than C7282. The obtained data suggest that Lactobacilli C7282 and DPP8 can be used as feed additives to inhibit colonization and translocation of S. Typhimurium and inflammatory responses via downregulating Jak/STAT signaling in broilers.

INTRODUCTION

Salmonella infection is one of the world's most leading epidemic diseases and poses a great threat to animal production and public health (Dar *et al.*, 2017; Chen *et al.*, 2018). Poultry, a primary source for this foodborne diseases, is most susceptible to *Salmonella* strains especially *Salmonella* Typhimurium (*S.* Typhimurium) (Ding *et al.*, 2018; Liu *et al.*, 2019). With the prohibition of growth-promoting antibiotics in many countries, probiotic administration is an effective strategy to cope with pathogenic infections with few side-effects and a high safety profile (Plaza-Diaz *et al.*, 2019; Zhao *et al.*, 2020a,b; Shi *et al.*, 2020). *Lactobacilli* has long been used as a health-promoting agent in prevention or treatment of enteric infection and inflammatory diseases and its possible action mechanisms are involved in the modulation of intestinal barrier, defensin, lactic acid production and interactions with hosts and pathogens during inflammatory initiation and progress (Liu *et al.*, 2017, 2018a,c; Wang *et al.*, 2019a; Deng *et al.*, 2020a,b).

Janus kinase/signal transduction and activator of transcription (Jak/STAT) signaling pathway is implicated in the pathogenesis of inflammatory and autoimmune diseases via many cytokines to transduce intracellular signals to combat inflammatory process (Xin *et al.*, 2020). *Lactobacillus rhamnosus* GG elevated gut epithelial leptin levels which acted as a

chemokine to promote cell proliferation through the upregulation of Jak/STAT pathway in mice (Darby *et al.*, 2019), but the probiotic strain deregulated Jak/STAT, mitogen-activated protein kinase, nuclear factor-κB, and tumor necrosis factor in H_2O_2-induced Caco-2 cells (Hou *et al.*, 2019). *Lactobacillus plantarum* Lp-1s induced interferon-β, stimulated STAT1 phosphorylation and activated nuclear translocation of p-STAT1 in intestinal porcine enterocytes (Wang *et al.*, 2019b). *Lactobacillus rhamnosus* GR-1 induced IL10 production in human placental trophoblast cells and involves activation of Jak/STAT and mitogen-activated protein kinase pathways (Yeganegi *et al.*, 2010). However, information about probiotics on Jak/STAT signal in farm animals is mostly unclear.

The present study aimed to test the hypothesis that probiotic *Lactobacillus acidophilus* C7282 and *L Lactobacillus plantarum* DPP8 can attenuate *S.* Typhimurium infection by inhibiting its colonization and translocation as well as modulating inflammatory mediators via Jak/STAT signaling in broilers.

MATERIALS AND METHODS

Ethics statement

The trial protocol was approved by the Institutional Committee for Animal Use and Ethics of the College of Animal Science in Henan University of Science and Technology (No. 2018016).

Bacterial strains, diets and treatments

Lactobacillus acidaphilus CGMCC7282 (C7282) was isolated from the feces of swine and obtained from China General Microbiological Culture Collection Center (Beijing, China). *Lactobacillus plantarum* CCTCC M2016136 DPP8 (DPP8) from the intestine of grass carp was reserved in China Center for Type Culture Collection (Wuhan, China). Lyophilized C7282 and DPP8 were recovered and aerobically enriched in De Man, Rogosa and Sharpe (MRS) broth (HB0384-1; Qingdao Hopebio Co. Ltd., Shandong, China) at 37°C for 48 h. After bacterial enumeration, the broth loaded C7282 or DPP8 was sprayed onto corn powder (40 meshes) using a step-by-step method and was added at 0 (control), 10^{10} (C7282), 10^{10} (DPP8) and 2.0×10^{10} (combined C7282 and DPP8 at 1:1 ratio) colony forming units (cfu)/kg at the expense of corn in a basal diet (Table 1). *S.* Typhimurium (*Salmonella enterica subsp. enterica* serovar Typhimurium) SL1344 was grown overnight at 37°C in Rappaport-Vassiliadis Medium (RVM, HB4092; Qingdao Hopebio) for the establishment of animal model. Four treatments consisted of the control with *S.* Typhimurium infection, and based on the control C7282 or DPP8 was added at individual or combination as described above.

Table 1 - Ingredient and nutrition levels of basal diet (g/kg, as fed basis).

Ingredient	Content	Composition*	Content
Corn	580	Crude protein	210.5
Soybean meal	278	ME, MJ/kg	12.6
Corn gluten meal	60	Crude fiber	26.3
Soybean oil	30	Lysine	12.4
Met	2	Met	5.5
Lys	3	Met + Cys	8.7
Salt	4	Ca	10.1
Limestone	18	Non-phytate P	4.8
Dicalcium phosphate	15		
Premix**	10		

*Calculated by Chinese Feed Database, version 25, 2014.

**The premix provided the following per kg of diets: vitamin A (retinyl acetate), 9,000 IU; cholecalciferol, 4,000 IU; vitamin E (DL-tocopheryl acetate), 50 IU; vitamin K, 2 mg; thiamin, 2 mg; riboflavin, 5 mg; d-pantothenic acid, 15 mg; niacin, 40 mg; pyridoxine, 2 mg; biotin, 0.1 mg; folic acid, 0.55 mg; vitamin B12, 0.01 mg; manganese, 120 mg; iodine, 1.2 mg; iron, 40 mg; copper, 16 mg; zinc, 100 mg; and selenium, 0.3 mg.

Animal management and sample collection

A total of 240 male Arbor Acres broilers (negative for *S.* Typhimurium by rectal swab detection) at day old were randomly assigned into four groups with 6 replicates of 10 broilers each in responding to the four treatments. All broilers were raised in battery cages and had free access to the respective experimental diets and water for 21 d. Feed consumption and mortality were recorded daily. Average daily feed intake (ADFI), average daily body weight gain (ADG), and feed conversion ratio (FCR, ADFI/ADG) were calculated based on a replicate basis. On the first day of the feeding trial, each broiler was orally garaged with 1 mL of 10^3 cfu of *S.* Typhimurium to establish a clinical salmonellosis model (Liu *et al.*, 2019).

On the 21st d post administration, 6 broilers per replicate were randomly collected for blood sampling from the heart and then euthanized using CO_2 and dissected. The serum was prepared by centrifuging at 1,000 × g for 10 min (Liu *et al.*, 2018b) and stored at –20°C for the analysis of cytokines. Approximately 5 g of caecal content, caecal tonsils, liver, spleen, bursa of Fabricius, left thymus, harder gland, bone marrow from the left femurs were collected and stored at –40 °C for the enumeration of *S.* Typhimurium. Caecal mucosa was collected and stored in RNAlater for mRNA assay.

Bacterial enumeration and biochemical assay

For enumeration of C7282, DPP8, and *S.* Typhimurium, each sample was homogenized, weighed, and diluted at 1:10 (wt/vol) with phosphate buffered saline (pH 7.2) and mixed thoroughly (Liu *et al.*, 2018d). The suspension of each sample was serially diluted between 10^{-1} to 10^{-7} dilutions, and 100 µL of each diluted sample was spread in triplicate onto agars (MRS for C7282 and DPP8; RVM for *S.* Typhimurium) at 37 ºC for 24 h. The amount of bacteria was expressed as a logarithmic (log_{10}) transformation per gram of sample.

Chicken enzyme-linked immunosorbent assay kits from Cusabio Technology LLC (Distributor in Wuhan, China) were used for the detection of cytokines according to manuals, including interleukin (IL)-1β (IL1β; O73909); IL2 (O73883); IL4 (Q5W4U1); IL6 (Q90YI0); IL8 (P08317); IL10 (Q6A2H4), tumor necrosis factor-α (TNFα; CSB-E11231Ch) and interferon-γ (IFNγ; CSB-E08550Ch).

Tissue mRNA isolation and cDNA synthesis were carried out using kits from TaKaRa Co. (Dalian, China) according to manuals. Random hexamers and RNase inhibitor were utilized in the reaction. Controls without reverse transcriptase were included for the genomic DNA contamination check. The mRNA level was expressed as $2^{-\Delta\Delta Ct}$ using a housekeeping gene as a reference (Livak and Schmittgen, 2001). SYBR Green Master Mix (TaKaRa) was used for qPCR reactions by ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The qPCR reactions were set at 10 µL with 5 µL of SYBR, 1 µL of primer (Table 2), 4 µL of 10 × diluted cDNA. The conditions of the two-step qPCR were set as follows: activation for 3 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C.

Table 2 - Information of primers for quantitative real-time PCR.

		Primers (5'→3')		Length
Names	GenBank	Forward	Reverse	(bp)
Jak1	NM_204870.1	gagccagacacgagttaccc	agcttcaatgcacagctcct	269
Jak2	NM_001030538.2	gtgccgtgcatggagagg	ctgtctgcttgggtgcacta	273
Jak3	NM_204996.3	acagcttcctcacccgaaag	tagacacgtggatggcgatg	192
STAT1	NM_001012914.1	cccaagggaaacggctacatt	gctctacctttacgccgtgt	186
STAT3	NM_001030931.2	taagctctgcagctccttcc	tcgctgtaaagctggtggag	237
STAT4	NM_001267555.1	agggaccactcgtacagcat	ggatgcagcctcccaatctt	159
STAT5	AF074248.1	gaagtgctgactccaaacggg	gttgtcgaggtcgatggagt	299
STAT6	XM_025145654.1	gccgatatggtgacggagaa	cctctcgcagcgctttatct	180
GAPDH	NM_204305.1	gggcacgccatcactatctt	tcacaaacatgggggcatca	187

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Jak, Janus kinase; STAT, signal transducer and activator of transcription.

Statistical analysis

Data are represented as mean and SEM using SPSS software (IBM SPSS, version 23, Armonk, NY, USA). Statistical unit was the average mean of all broilers per replicate for growth performance and of 6 dissected broilers per replicate for tissue detection. Differences between mean values of normally distributed data were assessed by Tukey'b-test of one-way ANOVA at $p<0.05$ level of significance, and Tamhane's T2 test for parameters with heterogeneity variance.

RESULTS

Growth performance and *S.* Typhimurium load

During feeding trial, broilers with *S.* Typhimurium administration experienced mild symptoms like depression, diarrhea, dehydration and general weakness. On the 21st d post administration, the broilers in the control treatment showed worse ($p<0.05$) ADFI and ADG, whereas these parameters were recovered ($p<0.05$) by individual or combined C7282 and DPP8 addition, but there were no differences among the three treatments in growth performance (Table 3). Mortality of the control treatment was greater ($p<0.05$) than that of the other treatments.

The present study further investigated the colonization and translocation of *S.* Typhimurium (Table 4), finding that the three supplemental treatments decreased ($p<0.05$) the pathogen population in caecum, tonsils, harder gland, bursa and spleen, and the decreasing effects of diets containing DPP8 in the caecum and tonsils were more pronounced ($p<0.05$) than C7282

Table 3 - Effect of *Lactobacilli* on the growth performance of broilers from 1 to 21 days of age.

Item	*Salmonella* Typhimurium infection				SEM	*p*-value
	Control	C7282	DPP8	Combination		
Growth performance						
ADFI, g/bird	37.47[b]	39.48[a]	39.82[a]	40.11[a]	0.215	<0.001
ADG, g/bird	54.92[b]	56.99[a]	57.29[a]	58.28[a]	0.415	<0.001
FCR	1.466[a]	1.444[a]	1.439[a]	1.453[a]	0.012	0.441
Mortality, %	6.67[a]	1.67[b]	2.50[b]	1.67[b]	1.070	0.010

[a-b]Means with different superscripts within the same row are different (p<0.05).

ADFI, average daily feed intake; ADG, average daily body weight gain; Combination, C7282 + DPP8; C7282, Lactobacillus acidophilus CGMCC7282; DPP8, Lactobacillus plantarum DPP8; FCR, feed conversion ratio, ADFI/ADG.

alone. In thymus and bone marrow, there were no significant decreases in *S.* Typhimurium loads by the three supplements, but in the liver it was decreased (p<0.05) by DPP8. The findings indicate that individual or combined C7282 and DPP8 can attenuate the negative effect of *S.* Typhimurium infection on the growth performance by inhibiting the colonization and translocation in broilers.

Table 4 - Effect of *Lactobacilli* on the *Salmonella* Typhimurium load of broilers.

Item	*Salmonella* Typhimurium infection				SEM	*p*-value
	Control	C7282	DPP8	Combination		
Salmonella Typhimurium load, Log_{10} cfu/g						
Caecal content	4.603[a]	3.106[b]	2.647[c]	2.550[c]	0.076	<0.001
Caecal tonsil	0.420[a]	0.352[b]	0.273[c]	0.291[c]	0.010	<0.001
Harder gland	0.234[a]	0.089[b]	0.092[b]	0.080[b]	0.006	<0.001
Thymus	0.149[a]	0.134[a]	0.120[a]	0.119[a]	0.008	0.056
Liver	0.065[a]	0.053[ab]	0.041[b]	0.050[ab]	0.005	0.030
Spleen	0.404[a]	0.214[b]	0.197[b]	0.186[b]	0.007	<0.001
Bursa of Fabricius	0.180[a]	0.091[b]	0.083[b]	0.079[b]	0.007	<0.001
Bone marrow	0.032[a]	0.027[a]	0.025[a]	0.018[a]	0.007	0.494

[a-c]Means with different superscripts within the same row are different (p<0.05).

Combination, C7282 + DPP8; C7282, Lactobacillus acidophilus CGMCC7282; DPP8, Lactobacillus plantarum DPP8.

Cytokines induced by *S.* Typhimurium

Broilers in the control group had greater (p<0.05) serum levels of IL1β/2/4/6, TNFα, and IFNγ, whereas these parameters were decreased (p<0.05) by individual or combined C7282 and DPP8 (Table 5). Combined *Lactobacilli* exhibited greater decreases (p<0.05) in IL1β/8 than C7282. IL10 was increased (p<0.05) only by combination treatment compared to the control. These findings indicate that the probiotic can attenuate the inflammatory response induced by *S.* Typhimurium in broilers.

Jak/STAT signaling induced by *S.* Typhimurium

The addition of individual or combined C7282 and DPP8 downregulated (p<0.05) the mRNA levels of Jak2/3 and STAT3/4/5/6 in the intestinal mucosa compared to the control (Table 6). Diets containing DPP8 showed decreasing effects (p<0.05) on Jak1

Table 5 - Effect of Lactobacilli on serum cytokines (pg/mL) of broilers.

Item	*Salmonella* Typhimurium infection				SEM	*p*-value
	Control	C7282	DPP8	Combination		
IL-1β	202.3[a]	125.1[b]	106.8[bc]	87.71[c]	7.737	<0.001
IL-2	20.78[a]	13.90[b]	12.74[b]	11.39[b]	6.765	<0.001
IL-4	177.9[a]	125.5[b]	114.9[b]	106.5[b]	6.505	<0.001
IL-6	152.0[a]	144.5[ab]	111.4[b]	109.7[b]	9.271	0.007
IL-8	86.57[a]	75.92[ab]	66.87[bc]	57.34[c]	4.129	0.001
IL-10	39.35[b]	46.03[b]	51.49[b]	83.99[a]	3.756	<0.001
TNF-α	124.5[a]	90.19[b]	80.41[b]	77.68[b]	5.497	<0.001
INF-γ	77.75[a]	51.21[b]	43.56[b]	41.58[b]	3.776	<0.001

[a-d]Means with different superscripts within the same row are different (p<0.05).

Combination, C7282 + DPP8; C7282, Lactobacillus acidophilus CGMCC7282; DPP8, Lactobacillus plantarum DPP8.

whereas diets containing C7282 reduced (p<0.05) STAT1 compared to the control. For Jak2, treatments DPP8 and combination showed a more pronounced decrease (p<0.05) than C7282, but there was no difference between treatments DPP8 and combination.

Table 6 - Effect of *Lactobacilli* on mRNA expression ($2^{-\Delta\Delta Ct}$) of Jak/STAT signaling in broilers.

Item	*Salmonella* Typhimurium infection				SEM	*p*-value
	Control	C7282	DPP8	Combination		
Jak1	0.702[a]	0.610[ab]	0.593[b]	0.557[b]	0.025	0.009
Jak2	0.607[a]	0.349[b]	0.293[c]	0.255[c]	0.015	<0.001
Jak3	0.664[a]	0.513[b]	0.472[b]	0.441[b]	0.019	<0.001
STAT1	0.476[a]	0.384[bc]	0.429[ab]	0.344[c]	0.017	0.002
STAT3	0.710[a]	0.587[b]	0.568[b]	0.547[b]	0.017	0.001
STAT4	0.721[a]	0.603[b]	0.595[b]	0.594[b]	0.025	0.013
STAT5	0.628[a]	0.486[b]	0.503[b]	0.457[b]	0.013	0.001
STAT6	0.641[a]	0.430[b]	0.457[b]	0.415[b]	0.024	<0.001

[a-c]Means with different superscripts within the same row are different (p<0.05).

Combination, C7282 + DPP8; C7282, Lactobacillus acidophilus CGMCC7282; DPP8, Lactobacillus plantarum DPP8; Jak, Janus kinase; STAT, signal transducer and activator of transcription.

DISCUSSION

Poultry is the primary source of typical or nontypical *Salmonella* outbreaks due to rapidly growing from genetic and feeding practices. In the present study, broilers in the control treatment with *Salmonella* infection showed worse growth performance and the greatest load of *S.* Typhimurium, but these were significantly reversed by supplemental *Lactobacilli*. In laying hens, Upadhaya *et al.* (2016) reported that probiotics decreased intestinal and fecal *Salmonella* counts, whereas Adhikari *et al.* (2019) found that *Salmonella* was the lowest in the ovary and the highest in the spleen, however, probiotics did not cause statistical differences in the internal organ loads among the treatments. Additionally, the reduction of *S.* Typhimurium in the intestinal colonization by probiotics was related to competition for iron in mice (Deriu *et al.*, 2013). Whether *Lactobacilli* strains used in the present study inhibit *S.* Typhimurium through some nutrients deserves further study. Also of note was that the lack of a negative group in the present study did not invalidate the obtained results, but make it not so strong.

In the present study, the effect of probiotics on attenuating inflammatory response is a consequence of inhibiting S. Typhimurium colonization and translocation in the digestive tract and other visceral organs, namely the less pathogen count, the less oxidative stress and inflammatory response. For relationship between probiotics and expression of cytokines in Salmonella-induced chicken models, Hu *et al.* (2015) reported that Lactobacillus zeae LB1, Lactobacillus plantarum S8 and Lactobacillus reuteri S64 attenuated lipopolysaccharide-induced TNF factor, IL6, IL8, IL12, IFNγ and IL4; Haghighi *et al.* (2008) argued that only IL12 and IFNγ were repressed by Lactobacillus acidophilus, Bifidobacterium bifidum and Streptococcus faecali in chicken intestinal colonization, but not for IL6; Wang *et al.* (2018) found that Lactobacillus plantarum LTC-113 prevented the increase of inflammatory meditators myeloperoxidase, IL1β, IL6 and inflammation scores; and also Lactobacillus based probiotics reduced IL1β and lipopolysaccharide-induced TNF factor (Penha Filho *et al.*, 2015).

The Jak family members are intracellular, non-receptor tyrosine kinases that transduce cytokine -mediated signals via the Jak/STAT pathway. Jak inhibition is a therapeutic strategy for immune and inflammatory diseases (Schwartz *et al.*, 2017). In the present study, the addition of C7282, DPP8 or their combination downregulated the transcriptional levels of Jak family, which further implicates the anti-inflammatory effect of the two probiotics. Similar findings were reported in literature that dietary intake of probiotic kimchi ameliorated IL6 secretion and Jak2 activation in mice (An *et al.*, 2019). *Bifidobacterium* spp reduced the levels of lipopolysaccharide-induced IL1 and TNFα in macrophage cells (Yeşilova *et al.*, 2012). Probiotic mixture restored phosphorylated Jak2 protein levels to the control condition compared to paclitaxel-induced increase in hybridoma cells (Castelli *et al.*, 2018). However, another probiotic mixture increased Jak2 phosphorylation in the hypothalamus of diet-induced-obese mice (Bagarolli *et al.*, 2017). Therefore, further studies are needed for the effect of Jak2 signal on inflammatory responses induced by pathogens in farm animals.

Members of STAT protein family are intracellular transcription factors that mediate many aspects of cellular immunity, proliferation, apoptosis and differentiation. STAT1 gene provides instructions for making a protein involved in the generation of Th17 cells and cytokines such as interferon and IL12. STAT3 also mediates cellular responses to a variety of cytokines including IL10/6. Similar with Jak family, STAT1 and STAT3 were deregulated by probiotics in inflammatory models (Bagarolli *et al.*, 2017; Castelli *et al.*, 2018). The literature was consistent with findings in the present study that mRNA levels of STAT3/4/5/6 were also downregulated by individual or combined C7282 and DPP8, and this is also in accordance with the decreases of IL1β/2/4, TNFα and IFNγ in probiotic treatments.

Interestingly, IL10 was elevated by individual or combined probiotics in the present study, indicating that IL10 acts as an anti-inflammatory cytokine in the *S.* Typhimurium-induced model. However, Haghighi *et al.* (2008) and Hu *et al.* (2008) argued that probiotics reduced or did not affect IL10 expression in *Salmonella*-induced chickens. Hutchins *et al.* (2013) found that IL10/STAT3-mediated anti-inflammatory response represented an essential homeostatic mechanism that controlled the degree and duration of inflammation. Furthermore, Kang (2019) reported that STAT3's DNA binding activity was not required for IL10 to inhibit pro-inflammatory gene expression, however, IL10 required STAT3-mediated transcription to enhance anti-inflammatory M2 macrophage polarization. Additionally, $CD4^+$ T cells used STAT1 to drive intestinal inflammation and STAT1 played a critical role in shielding T cells from natural-killer-cell-mediated cytotoxicity (Kang, 2019). As such, how probiotics influence the T cell and its subsets via STAT signaling in a *S.* Typhimurium-induced scenario deserves further study.

As a chain of interactions between proteins in a cell, the Jak/STAT pathway plays a major role in many fundamental processes, including apoptosis and inflammation. Information about the effect of probiotics on Jak/STAT pathway is mainly from *in vitro* or rodent models, however, literature from farm animals is unavailable. Truong *et al.* (2017a,b) found that necrotic enteritis induced differential expression of Jak/STAT in the spleen and intestinal mucosa of chickens. Similarly, in the present study, *S.* Typhimurium induced the expression of Jak/STAT, and importantly, this was reversed by probiotic addition. Paradoxically, IL2 signaling and Jak/STAT signaling were downregulated in response to *Salmonella enteritidis* infection by the investigation into chicken hepatic global gene expression (Coble *et al.*, 2013). Therefore, the relationship between pathogen-induced Jak/STAT pathway and probiotic protection in farm animals needs further exploration.

It was concluded that supplementing individual or combined C7282 and DPP8 restored growth performance of broilers infected with *S.* Typhimurium, decreased the pathogenic colonization and translocation in tissues, and attenuated inflammation by influencing cytokines and inflammatory mediators. The effects of diets containing DPP8 were more pronounced than C7282 on elimination of *S.* Typhimurium. The results suggest that C7282 and DPP8 can be used as feed additives to prevent *S.* Typhimurium infection and attenuate inflammatory responses via Jak/STAT signaling blockage in broilers.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

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