Animal Nutrition 14 (2023) 334–342

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

N-acyl homoserine lactonase attenuates the virulence of *Salmonella typhimurium* and its induction of intestinal damages in broilers

Weiwei Wang [1], Yiliang Chen [1], Hui Ye, Zemin Dong, Changming Zhang, Dingyuan Feng, Qingyun Cao, Shujie Liang, Jianjun Zuo*

Guangdong Provincial Key Laboratory of Animal Nutrition Control, College of Animal Science, South China Agricultural University, Guangzhou, 510642, China

ARTICLE INFO

Article history:
Received 31 December 2022
Received in revised form
8 March 2023
Accepted 5 April 2023
Available online 12 July 2023

Keywords:
Broiler
Growth performance
Intestinal health
N-acyl homoserine lactonase
Salmonella typhimurium
Virulence

ABSTRACT

This study aimed to investigate the potential mitigating effects of N-acyl homoserine lactonase (AHLase) on the virulence of *Salmonella typhimurium* and its induction of intestinal damages in broilers. In vitro study was firstly conducted to examine if AHLase treatment could attenuate the virulence of *S. typhimurium*. Then, an in vivo experiment was performed by allocating 240 broiler chicks at 1 d old into 3 groups (8 replicates per group): negative control (NC), positive control (PC), and PC supplemented with 10,000 U/kg AHLase. All chicks except those in NC were orally challenged by *S. typhimurium* from 8 to 10 d of age. Parameters were measured on d 11 and 21. The results showed that treatment with 1 U/mL AHLase suppressed the biofilm-forming ability (including biofilm biomass, extracellular DNA secretion and biofilm formation-related gene expression), together with swarming motility and adhesive capacity of *S. typhimurium*. Supplemental 10,000 U/kg AHLase counteracted *S. typhimurium*-induced impairments ($P < 0.05$) in broiler growth performance (including final body weight, average daily gain and average daily feed intake) during either 1–11 d or 12–21 d, and increases ($P < 0.05$) in the indexes of liver, spleen and bursa of Fabricius on d 11, together with reductions ($P < 0.05$) in ileal villus height and its ratio to crypt depth on both d 11 and 21. AHLase addition also normalized the increased ($P < 0.05$) mRNA expression of ileal occludin on both d 11 and 21 in *S. typhimurium*-challenged broilers. However, neither *S. typhimurium* challenge nor AHLase addition altered ($P > 0.05$) serum diamine oxidase activity of broilers. Noticeably, *S. typhimurium* challenge caused little change in the mRNA expression of ileal inflammatory cytokines except for an increase ($P < 0.05$) in interleukin-8 expression on d 11, whereas AHLase addition normalized ($P < 0.05$) this change. In conclusion, AHLase treatment could attenuate the virulence and pathogenicity of *S. typhimurium*, thus contributing to alleviate *S. typhimurium*-induced growth retardation and intestinal damages in broilers.

1. Introduction

As one of the most common zoonotic pathogens, *Salmonella typhimurium* seriously threatens both animal health and global public health. In chicken production, *S. typhimurium* invasion causes a range of clinical symptoms and intestinal disorders, resulting in impairments of growth performance and serious economic losses worldwide (Ibrahim et al., 2021; Zhang et al., 2020). In the past few decades, antibiotics were widely employed to control or limit bacterial infection in animals. With the banning of antibiotics used in feeds due to the emergence of considerable antibiotic residues and antibiotic-resistant bacteria, non-antibiotic strategies that can obtain similar benefits to antibiotics have become crucial for

* Corresponding author.
E-mail address: zuoj@scau.edu.cn (J. Zuo).
[1] These authors contributed equally to this work.
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2023.04.010

protecting the growth and health of chickens against pathogens such as *S. typhimurium*.

Currently, using quorum sensing (QS) to regulate bacterial virulence plays a critical role in mediating the infection of host by pathogenic bacteria (Xiao et al., 2022). QS is defined as an intercellular information-exchanging mechanism among and within bacterial communities, impelling bacteria to react to the perceived changes and associate with the external environments (Whiteley et al., 2017). Through the QS system, bacteria can self-regulate their physiological activities and virulence such as growth, adhesion, biofilm formation and toxin secretion (Xiao et al., 2022). It is established that bacterial QS is mediated by some specific signal substances such as N-acyl-homoserine lactones (AHL), which are usually accumulated over bacterial proliferation and metabolism (Xiao et al., 2022). When the concentrations of these substances reach thresholds perceived by the bacteria, they can interact with their homologous receptors in bacterial cell membrane, thus activating the expression of specific genes involved in bacterial virulence and pathogenicity (Whiteley et al., 2017; Xiao et al., 2022). For *Salmonella*, it can use SdiA (a QS receptor) to recognize and bind to AHL, the primary signal substances to mediate QS of gram-negative bacteria, thereby enhancing its infectivity and aggravating host injuries (Campos-Galvao et al., 2016; Rana et al., 2021). Accordingly, AHL may be one kind of the molecular targets for restraining *S. typhimurium* infection.

Recent studies have focused on the efficacy of N-acyl-homoserine lactonase (AHLase) to degrade AHL through enzymatic hydrolysis of lactone bonds, causing a blocking of AHL-mediated QS (Sikdar et al., 2020). It has been showed that AHLase treatment could weaken QS-dependent pathogenicity (e.g. biofilm formation and toxin secretion) of gram-negative bacteria in vitro (Fan et al., 2017; Packiavathy et al., 2021). In vivo studies also confirmed that AHLase was efficient in moderating bacteria-related disorders of the host. For example, AHLase addition was reported to benefit the growth and intestinal functions in aquatic animals infected with gram-negative pathogens by inhibiting AHL-mediated bacterial QS and promoting the expression of anti-infectious and anti-inflammatory factors of host (Cao et al., 2012, 2014; Peng et al., 2021). Similar findings were obtained in laboratory animals where AHLase treatment protected mice from infection of gram-negative bacterium (*Pseudomonas aeruginosa*) and the resulting inflammatory damages (Migiyama et al., 2013; Sakr et al., 2021). However, there is still a lack of information concerning the usage of AHLase in the production of farm animals. Therefore, this study was aimed at investigating if dietary AHLase addition could be a new approach to attenuate the virulence of S. *typhimurium* and its negative effects on the growth performance and intestinal health of broilers.

2. Materials and methods

2.1. Animal ethics statement

The experimental animal protocols of the present study were approved by the Animal Care and Use Committee of the South China Agricultural University (Guangzhou, Guangdong, China).

2.2. In vitro experiments

2.2.1. Determination of biofilm biomass and the inhibition ratio of biofilm formation of S. typhimurium

The *S. typhimurium* strain ATCC14028 provided by the Food Microbial Safety Engineering Technology R&D Center of Guangdong Province (Guangzhou, China) was inoculated in *Salmonella-Shigella* agar and incubated at 37 °C for 12 h. A single colony of *S. typhimurium* was then selected and cultured in LB broth overnight, the bacterial inoculum (1.0×10^9 CFU/mL) was then added with different doses of AHLase (the final concentrations of AHLase were 0, 0.2, 0.4, 0.6, 0.8 and 1.0 U/mL, respectively). The mixed solutions were transferred to 96-well plates (6 replicates per group) and cultured at 37 °C for 24 h, followed by discard of culture medium and washing with distilled water. Thereafter, the residue in each well was incubated with 200 μL of 0.1% crystal violet for 30 min and then washed with distilled water. Finally, the residue in each well was dissolved by adding 200 μL acetic acid, followed by measurement of the absorbance at 590 nm that represents the biofilm biomass (Ning et al., 2015). The inhibition ratio (%) of biofilm formation in AHLase treatment groups was calculated by the following formula: (absorbance of blank group − absorbance of treatment group)/absorbance of blank group × 100.

2.2.2. Quantification of extracellular DNA (eDNA) of S. typhimurium

After the determination of biofilm biomass, the bacterioplankton in each well of control and 1.0 U/mL AHLase-treated group was discarded and the biofilm was washed, followed by supplementation with 5 μL EDTA (0.5 mol/L). After standing at 4 °C for 1 h, 700 μL Tris-EDTA-NaCl (TEN) buffer was added to each well to re-suspend the biofilm, followed by centrifugation (12,000 × *g*, 4 °C) for 5 min. A total of 100 μL of the supernatant was transferred to a tube containing 300 μL Tris-EDTA (TE) buffer, which together with an equal volume of binding buffer were then added to the absorption column (Sangon Biotech., Shanghai, China). After incubation at room temperature for 2 min, the above mixture was centrifuged (12,000 × *g*, 4 °C) for 60 s, and the absorption column was then washed and centrifuged (12,000 × *g*, 4 °C) for 30 s. Finally, sterile water was added to the absorption column, the resulting eDNA solution was quantified using an UV5Nano ultra-micro ultraviolet spectrophotometer (Mettler Toledo, Switzerland).

2.2.3. Quantification of biofilm-related gene expression of S. typhimurium

S. typhimurium inoculum (1.0×10^9 CFU/mL) was added with AHLase (the final concentrations of AHLase were 0 and 1.0 U/mL, respectively) and incubated at 37 °C for 6 h. After centrifugation, the bacteria were collected for RNA extraction using the BIOG Bacterial RNA Extraction kit (Bio-generating Biotechnology Corp., Changzhou, China). The concentration of extracted RNA was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA). After verification of RNA purity and integrity by measuring the ratio of absorbance at 260 nm to that at 280 nm and using agarose gels electrophoresis, respectively, cDNA samples were obtained by reverse transcription of RNA samples using the HiScript II qRT SuperMix (Vazyme Biotech. Co., Ltd., Nanjing, China). Real-time quantitative PCR was implemented using the ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech. Co., Ltd., Nanjing, China) in an ABI 7500 Real Time PCR Systems (Applied Biosystems, Foster City, USA). DNA gyrase subunit A (*gyrA*) served as the house-keeping gene. Primer sequences of *gyrA* and the target genes including curli subunit gene D (*CsgD*), long polar fimbriae (*Lpf*) and flagellin C (*FliC*) are listed in Table 1. The relative mRNA expression of target genes was calculated according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.2.4. Measurement of the motility of S. typhimurium

The motility of *S. typhimurium* was evaluated referring to a previous study (Cong et al., 2011). Briefly, 1 μL *S. typhimurium* inoculum (1.0×10^9 CFU/mL) was respectively instilled to the central location of swarming motility- and swimming motility-evaluating medium (semi-solid agar medium) containing 0 or 1.0 U/mL AHLase, followed by incubation at 37 °C for 24 h. Then, the

Table 1
Primer sequences for RT-PCR in vitro.

Genes	Primer sequences (5′-3′)	Product size, bp
gyrA	F: CGGGATACAGTAGAGGGATAGCGG	242
	R: CACCAACGACACGGGCAGATT	
CsgD	F: GGGCACGCCATCACTATCTT	187
	R: TCACAAACATGGGGGCATCA	
Lpf	F: TGCCTGCAGAAGAAGCCTCG	204
	R: GACGGGCTCAAAAACCTCCT	
FliC	F: CACTGCCACTCCCTGATGTT	270
	R: ACCGGTGACAGACTGGTTTC	

gyrA = DNA gyrase subunit A; *CsgD* = curli subunit gene D; *Lpf* = long polar fimbriae; *FliC* = flagellin C.

circle diameters of swarming and swimming motility were measured. The swarming motility-evaluating medium was comprised of 25 g/L LB medium, 0.5 g/L glucose and 5 g/L agar powder, while the swimming motility-evaluating medium was comprised of 10 g/L tryptone, 5 g/L NaCl, 2.5 g/L glucose and 3 g/L agar powder.

2.2.5. Assay of adhesive capacity of S. typhimurium

The intestinal epithelial cell line IPEC-J2 cells were seeded with DMEM complete medium (Gibco, USA) in 24-well plates. The *S. typhimurium* inoculum (1.0×10^9 CFU/mL) was added without or with AHLase (final concentration of 1.0 U/mL), the mixed solutions were then blended with IPEC-J2 cells based on the multiplicity of infection (MOI) of 100, followed by culture in a humidified atmosphere of 5% CO_2 at 37 °C for 1 h. Afterwards, the cells were washed 3 times and treated with 1 mL phosphate buffer solution containing 0.1% Triton-100 for 30 min. The resulting cell lysates were collected for bacterial enumeration using the spread plate method (Wu et al., 2016).

2.3. In vivo experiments

2.3.1. Animals and experimental design

A total of 240 male Lingnan yellow-feathered broiler chicks at 1 d old of age were randomly allocated into 3 treatment groups (8 replicates/group and 10 chicks/replicate). The initial body weight was similar across replicates. The treatment groups were as follows: negative control (NC, received a basal diet without challenge), positive control (PC, received a basal diet with *S. typhimurium* challenge) and AHLase group (PC chicks supplemented with 10,000 U/kg AHLase). The activity of AHLase in this supplement (Beijing Challenge Group, China) was 10,000 U/g and testified by determining the rate of substrate (N-(3-oxo-octanoyl)-L-homoserine lactone) disappearance using liquid chromatography. The additive level of AHLase in diet was selected based on our preliminary experiment. Diet was formulated based on the Chinese Feeding Standard of Yellow-feathered Chickens (NY/T 3645-2020). The composition and nutrient levels of basal diet are displayed in Table 2. According to the National Standards of China (GB/T 18246-2000), dietary lysine and threonine contents were determined using the direct hydrolysis method, dietary methionine and cystine contents were determined using the oxidation-acidolysis method, while dietary tryptophan content was measured using the alkaline hydrolysis method. The above analysis was performed on an Amino Acid Analyzer (Hitachi L-8800, Tokyo, Japan). Dietary metabolizable energy was calculated based on the nutritive value of feed ingredients listed in the Chinese Feeding Standard of Yellow-feathered Chickens (NY/T 3645-2020). All chicks were reared in wire cages and received 16 h of light per day throughout the trial period. The room temperature was kept at 34 °C during the first week and then gradually reduced to 24 °C on d 21. The feed (mash form) and fresh water were available ad libitum.

Table 2
Composition and nutrient levels of basal diet (air-dry basis).

Item	Content, %
Ingredients	
Corn	54.70
Soybean meal (43.6% CP)	38.10
Soybean oil	2.60
Limestone	1.20
Dicalcium phosphate	2.00
Sodium chloride	0.26
Lysine	0.01
DL-Methionine (98%)	0.13
Premix[1]	1.00
Total	100.00
Nutrient levels[2]	
Metabolizable energy, MJ/kg	12.55
Crude protein	21.10
Calcium	1.00
Available phosphorus	0.46
Total lysine	1.20
Total methionine	0.50
Total methionine + cystine	0.81
Total threonine	0.82
Total tryptophan	0.26

[1] Supplied per kilogram of diet: Cu, 9.5 mg; Zn, 60 mg; Fe, 70 mg; Mn, 121 mg; Se, 0.45 mg; I, 1.4 mg; vitamin A, 5000 IU; thiamin, 2.5 mg; riboflavin, 15 mg; pyridoxine, 4 mg; vitamin D, 80.75 mg; tocopherol, 31 mg; menadione, 1.6 mg; pantothenic acid, 60 mg; niacin, 15 mg; biotin, 0.5 mg; folic acid, 1.5 mg; choline, 450 mg.
[2] Values represent the analyzed contents of nutrients.

2.3.2. Oral challenge and sample collection

The *S. typhimurium* strain (ATCC14028) provided by the Food Microbial Safety Engineering Technology R&D Center of Guangdong Province (Guangzhou, China) was inoculated in LB broth and cultured in incubator shaker (37 °C, 180 r/min) overnight. The inoculum was diluted and plated on *Salmonella-Shigella* agar at 37 °C for 24 h. During three consecutive days (8, 9 and 10 d of age), each chick in PC and AHLase groups was orally gavaged with 2 mL of *S. typhimurium* culture (5×10^9 CFU/mL), while chicks in NC group was orally gavaged with the equal quantity of LB broth. At 11 and 21 d of age, one chick per replicate cage was randomly selected for blood collection from the wing vein, followed by centrifugation ($3000 \times g$, 4 °C) for 10 min to obtain serum samples. Afterwards, these chicks were sacrificed for separating thymus, liver, spleen, bursa of Fabricius and intestine. The midpoint of ileal segment of each chick was harvested and separated into 2 sections. One section was immerged in 4% paraformaldehyde solution, while the other one was put into liquid nitrogen and reserved at −80 °C. Meanwhile, ileal content of each chick was collected on d 11.

2.3.3. Measurements of growth performance, organ indexes and serum indicator

Body weight and feed consumption of each replicate cage were recorded on d 11 and 21 for calculation of the final body weight (FBW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) during 1–11 d, 12–21 d and 1–21 d. The collected thymus, liver, spleen and bursa of Fabricius were weighed for determining organ indexes, which were expressed by the ratio of organ weights (g) to body weight (kg) × 100. Diamine oxidase (DAO) activity in serum was determined by spectrophotometry using a commercial kit (Solarbio Co., Ltd., Beijing, China) with a microplate reader (Thermo Fisher Scientific, Waltham, USA).

2.3.4. Intestinal morphology examination

The ileal tissues fixed in paraformaldehyde solution were subjected to paraffin-embedding procedures and hematoxylin-eosin staining, in order to obtain 4-μm cross sections. For each section, the intact and representative villi were chosen for examining intestinal morphological structure under microscopic vision fields. The villus height (VH) was determined from the tip of villus to villus-crypt junction, while crypt depth (CD) was evaluated by measuring the emboli between adjacent villi, followed by calculation of VH to CD ratio (VCR).

2.3.5. RNA extraction and real-time quantitative PCR

Total RNA was extracted from ileal samples using the FastPure Cell/Tissue Total RNA Isolation Kit V2 (Vazyme Biotech. Co., Ltd., Nanjing, China) under the manufacturer's instructions. The confirmation of RNA concentration and quality, along with reverse transcription of RNA as well as real-time PCR were implemented according to the method described earlier in this article. Reduced glyceraldehyde-phosphate dehydrogenase (*GAPDH*) served as the house-keeping gene. Primer information of *GAPDH* and the target genes including interlukin (*IL*)-*1β*, *IL-8* and tumor necrosis factor (*TNF*)-*α*, together with tight junction (TJ) proteins claudin-1, occludin and zonula occludens 1 (*ZO-1*) are presented in Table 3. The relative mRNA expression of target genes was calculated according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.4. Statistical analysis

Data are presented as mean ± pooled standard error of the mean (SEM) and analyzed by one-way ANOVA in the SPSS 22.0. Differences among groups were analyzed by Duncan's multiple comparisons. Significance was defined as $P < 0.05$.

3. Results

3.1. In vitro experiments

3.1.1. Effect of AHLase on biofilm formation of S. typhimurium

As shown in Fig. 1A, AHLase treatment reduced ($P < 0.05$) biofilm biomass of *S. typhimurium*, with the dose of 1.0 U/mL being the most effective, which was also supported by the highest inhibition rate (86.37%) of 1.0 U/mL AHLase against biofilm formation of *S. typhimurium* (Fig. 1B). Treatment with 1.0 U/mL AHLase decreased ($P < 0.01$) the ability of *S. typhimurium* to secrete eDNA (Fig. 1C), an important component of bacterial biofilm. Besides, the expression of biofilm formation-related gene *CsgD* was down-regulated ($P < 0.05$) by the treatment with 1.0 U/mL AHLase (Fig. 1D).

Table 3
Primer sequences for RT-PCR in vivo.

Genes	Primer sequences (5′-3′)	Product size, bp
GAPDH	F: GGGCACGCCATCACTATCTT R: TCACAAACATGGGGGCATCA	187
IL-1β	F: TGCCTGCAGAAGAAGCCTCG R: GACGGGCTCAAAAACCTCCT	204
IL-8	F: TTGGAAGCCACTTCAGTCAGAC R: GGAGCAGGAGGAATTACCAGTT	120
TNF-α	F: GAGCAGGGCTGACACGGAT R: CAGGCACAAAAGAGCTGATGG	152
Claudin-1	F: CACTGCCACTCCCTGATGTT R: ACCGGTGACAGACTGGTTTC	270
Occludin	F: TTCGTCATGCTCATCGCCTC R: TCCACGGTGCAGTAGTGGTA	158
ZO-1	F: CTTCAGGTGTTTCTCTTCCTCCTC R: CTGTGGTTTCATGGCTGGATC	131

GAPDH = reduced glyceraldehyde-phosphate dehydrogenase; *IL* = interleukin; *TNF* = tumor necrosis factor; *ZO* = zonula occludens.

3.1.2. Effects of AHLase on motility and adhesion of S. typhimurium

As illustrated in Fig. 2A and B, treatment with 1.0 U/mL AHLase lowered ($P < 0.05$) the circle diameter of swarming motility rather than that of swimming motility of *S. typhimurium*. Besides, treatment with 1.0 U/mL AHLase reduced ($P < 0.01$) the adhesive capacity of *S. typhimurium* to host intestinal epithelial cells (IPEC-J2 cells) (Fig. 2C).

3.2. In vivo experiments

3.2.1. Effects of AHLase on growth performance of broilers challenged with S. typhimurium

As shown in Table 4, there were reductions ($P < 0.05$) in FBW, ADG and ADFI of broilers during 1–11 d and 12–21 d as well as the ADG and ADFI during 1–21 d in PC group versus NC group, but these parameters were better ($P < 0.05$) in AHLase group compared with PC group. In addition, broiler FCR during 1–11 d in AHLase group were lower ($P < 0.05$) than PC group and comparable to ($P > 0.05$) that in NC group.

3.2.2. Effects of AHLase on organ indexes of broilers challenged by S. typhimurium

As exhibited in Table 5, the indexes of liver, spleen and bursa of Fabricius in broilers on d 11 were all increased ($P < 0.05$) in PC group compared with NC group, however, these parameters were lower ($P < 0.05$) in AHLase group relative to PC group. Remarkably, no differences ($P > 0.05$) were detected in all organ indexes among groups on d 21.

3.2.3. Effects of AHLase on intestinal morphology of broilers challenged by S. typhimurium

PC group showed reductions ($P < 0.05$) in ileal VH and VCR of birds on either d 11 or 21 in comparison with NC group (Table 6). However, ileal VH on d 11 along with ileal VH and VCR on d 21 were higher ($P < 0.05$) in AHLase group than PC group but similar to ($P > 0.05$) those in NC group. Besides, ileal VCR on d 11 of AHLase group were higher ($P < 0.05$) than both PC group and NC group.

3.2.4. Effects of AHLase on intestinal barrier of broilers challenged by S. typhimurium

The relative mRNA expression of ileal occludin on d 11 coupled with that of ileal occludin and *ZO-1* on d 21 were higher ($P < 0.05$) in PC group compared with NC group (Fig. 3A and B), whereas the relative mRNA expression of ileal claudin-1 and *ZO-1* on d 11 together with that of ileal claudin-1 on d 21 did not differ ($P > 0.05$) among groups. Remarkably, ileal occludin expression on d 11 in AHLase group was lower ($P < 0.05$) than that in PC group and not different ($P > 0.05$) from NC group, while ileal occludin expression on d 21 in AHLase group was similar ($P > 0.05$) to both PC and NC groups. Regarding serum DAO activity, an indicator of the degree of intestinal barrier destruction, it was not different ($P > 0.05$) among groups on either d 11 or 21 although there was a numerical increase in it in PC group versus other groups (Fig. 3C and D).

3.2.5. Effects of AHLase on intestinal inflammation of broilers challenged by S. typhimurium

As presented in Fig. 4, no differences ($P > 0.05$) were observed in the relative mRNA expression of ileal *IL-1β* and *TNF-α* on d 11 as well as the relative mRNA expression of ileal *IL-1β*, *IL-8* and *TNF-α* on d 21 among groups. In comparison, the relative mRNA

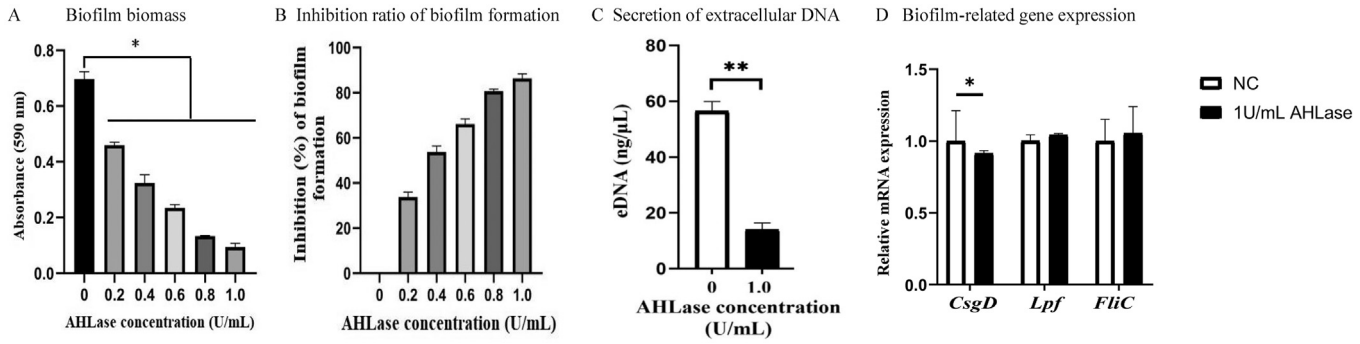

Fig. 1. Effects of N-acyl homoserine lactonase (AHLase) on (A) biofilm formation including biofilm biomass, (B) inhibition ratio of biofilm formation, (C) secretion of extracellular DNA and (D) biofilm-related gene expression of *Salmonella typhimurium*. *CsgD* = curli subunit gene D; *Lpf* = long polar fimbriae; *FliC* = flagellin C. * $0.01 \leq P < 0.05$, ** $P < 0.01$.

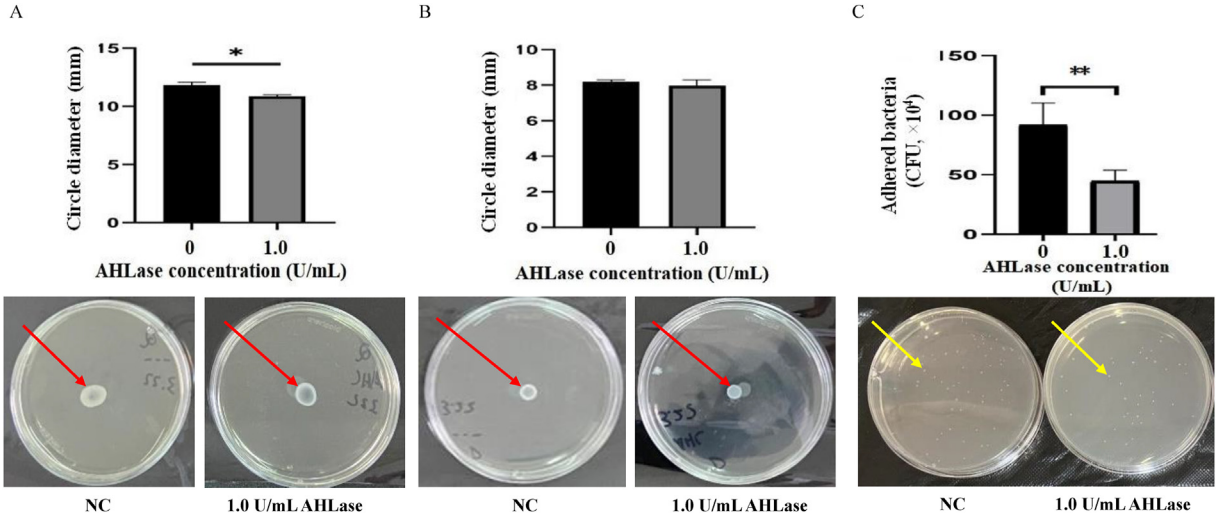

Fig. 2. Effects of N-acyl homoserine lactonase (AHLase) on motility and adherence ability of *Salmonella typhimurium*. (A) Diameter of swarming motility circle of *S. typhimurium* in agar medium (upper) and the representative graph (lower). (B) Diameter of swimming motility circle of *S. typhimurium* in agar medium (upper) and the representative graph (lower). Red arrows indicate motility circle. (C) The number of *S. typhimurium* attached to host intestinal epithelial cells (IPEC-J2 cells) (upper) and the representative graph (lower). Yellow arrows represent the bacterial colonies. * $0.01 \leq P < 0.05$, ** $P < 0.01$.

Table 4
Effects of N-acyl homoserine lactonase (AHLase) on growth performance of broilers challenged by *Salmonella typhimurium*.

Item	NC[1]	PC	AHLase	SEM	*P*-value
Day 1–11					
IBW, g	36.65	36.93	36.79	0.075	0.339
FBW, g	115.09^{a}	98.25^{b}	118.30^{a}	2.126	<0.001
ADG, g	7.84^{a}	6.13^{b}	8.15^{a}	0.214	<0.001
ADFI, g	11.84^{a}	10.06^{b}	11.95^{a}	0.227	<0.001
FCR	1.52^{b}	1.64^{a}	1.47^{b}	0.210	<0.001
Day 12–21					
FBW, g	318.75^{a}	283.44^{b}	315.46^{a}	4.456	<0.001
ADG, g	19.86^{a}	18.29^{b}	19.46^{a}	0.218	0.001
ADFI, g	31.14^{a}	28.93^{b}	30.96^{a}	0.342	0.007
FCR	1.57	1.61	1.58	0.021	0.708
Day 1–21					
ADG, g	13.82^{a}	12.29^{b}	13.73^{a}	0.198	<0.001
ADFI, g	21.41^{a}	19.48^{b}	21.33^{a}	0.300	0.006
FCR	1.56	1.59	1.55	0.018	0.723

SEM = standard error of the mean; IBW = initial body weight; FBW = final body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.
[a,b] Values within a row with unlike superscript letters differ significantly ($P < 0.05$).
[1] NC = negative control (broilers were free of challenge); PC = positive control (broilers were challenged with *S. typhimurium* from 8 to 10 d of age); AHLase = PC broilers supplemented with 10,000 U/kg AHLase.

Table 5
Effects of N-acyl homoserine lactonase (AHLase) on organ indexes (%) of broilers challenged by *Salmonella typhimurium*.

Item	NC[1]	PC	AHLase	SEM	*P*-value
Day 11					
Thymus	5.07	4.35	4.07	0.262	0.330
Liver	39.30^{b}	49.63^{a}	42.50^{b}	1.284	0.001
Spleen	1.12^{b}	1.68^{a}	1.19^{b}	0.815	0.004
Bursa of Fabricius	2.38^{b}	3.04^{a}	2.04^{b}	0.137	0.004
Day 21					
Thymus	4.08	4.70	4.26	0.204	0.475
Liver	30.68	30.21	30.41	0.415	0.907
Spleen	1.21	1.32	1.37	0.058	0.537
Bursa of Fabricius	3.05	2.50	2.84	0.110	0.117

SEM = standard error of the mean.
[a,b] Values within a row with unlike superscript letters differ significantly ($P < 0.05$).
[1] NC = negative control (broilers were free of challenge); PC = positive control (broilers were challenged with *S. typhimurium* from 8 to 10 d of age); AHLase = PC broilers supplemented with 10,000 U/kg AHLase.

expression of ileal *IL-8* on d 11 was higher ($P < 0.05$) in PC group than NC group but displayed no differences ($P > 0.05$) between NC group and AHLase group.

Table 6
Effects of N-acyl homoserine lactonase (AHLase) on ileal morphology of broilers challenged by *Salmonella typhimurium*.

Item	NC[1]	PC	AHLase	SEM	*P*-value
Day 11					
VH, μm	427.10[a]	305.97[b]	481.20[a]	20.231	<0.001
CD, μm	93.95	96.18	89.65	3.744	0.793
VCR	4.65[b]	3.18[c]	5.43[a]	0.224	<0.001
Day 21					
VH, μm	508.68[a]	338.24[b]	575.26[a]	29.052	<0.001
CD, μm	97.19	105.10	107.96	4.154	0.575
VCR	5.29[a]	3.25[b]	5.37[a]	0.270	<0.001

SEM = standard error of the mean; VH = villus height; CD = crypt depth; VCR = villus height to crypt depth ratio.
[a-c] Values within a row with unlike superscript letters differ significantly ($P < 0.05$).
[1] NC = negative control (broilers were free of challenge); PC = positive control (broilers were challenged with *S. typhimurium* from 8 to 10 d of age); AHLase = PC broilers supplemented with 10,000 U/kg AHLase.

4. Discussion

Bacteria are capable of secreting certain polysaccharide matrices, such as fibrin, lipoproteins and nucleic acids, which wrap themselves to form an extracellular polymer and a complex sessile community, known as biofilm, as a strategy to withstand adverse factors and survive in severe environments (Muhammad et al., 2020). Specially, biofilm greatly increases the resistance of *Salmonella* and shows a high tolerance to antibacterial agents, making biofilm formation as one of the leading causes of persistent infection and biofouling of the host (Milanov et al., 2017; Muhammad et al., 2020). In vitro studies have documented that AHLase treatment could attenuate QS-dependent biofilm formation of certain gram-negative pathogens (Fan et al., 2017; Packiavathy et al., 2021). Similarly, the current study showed that biofilm biomass of *S. typhimurium* was reduced by AHLase treatment at different dosages especially at 1 U/mL, which inhibited biofilm formation by nearly 87%. As a structural component of the extracellular matrix in biofilm, eDNA can fortify the biofilm stability and resistance of *Salmonella* by strengthening cell–cell adhesion and interacting with other matrix components in biofilm (Johnson et al., 2013; Ozdemir et al., 2018). Bacterial structures including fimbriae (e.g. the curli fimbriae and long polar fimbriae) and flagellin take important parts in the processes of *Salmonella* accessing and adhering to the surface of objects, that is, the initial phase of biofilm formation (Dong et al., 2021). In this study, AHLase treatment at 1 U/mL distinctly suppressed eDNA secretion and down-regulated the expression of curli fimbriae-encoding gene *CsgD* (a momentous determinant of biofilm formation) of *S. typhimurium* (Castelijn et al., 2012), although the expression of long polar fimbriae-encoding gene *Lpf* and flagellin-encoding gene *FliC* remained unchanged. These findings suggested that AHLase treatment compromised the biofilm-forming ability of *S. typhimurium* and consequently conduced to hinder the establishment of *S. typhimurium* infection for host (MacKenzie et al., 2017). It is known that bacteria have two forms of active movement, namely swarming and swimming motility (Swiecicki et al., 2013). Swarming motility takes place via groups of cells moving in two dimensions atop solid surface, whereas swimming motility takes place through individual cells moving in three dimensions of a liquid environment or semisolid medium (Butler et al., 2010). Motility not only helps bacteria to seek advantages and avoid disadvantages, but also benefits biofilm formation and adhesion to host cells, a prerequisite of bacterial infection of host (Harshe, 2003; Pieters, 2007). It has been proved that adherence to intestinal epithelial cells is a key step in the systemic infection and pathogenesis of *S. typhimurium* for host (Weinstein et al., 1998). In this study, the observed impairments of swarming motility of *S. typhimurium* and its adhesive capacity to host intestinal epithelial cells following AHLase treatment could be favorable for decreasing the virulence of this pathogen. Taken together, our in vitro studies verified that AHLase was efficacious in attenuating the virulence of *S. typhimurium*, probably decreasing the capability of *S. typhimurium* infecting animals. As a result, the potential protecting effects of AHLase against *S. typhimurium* challenge in broilers was then investigated.

Similar to previous studies (Ibrahim et al., 2021; Zhang et al., 2020), the present study found that *S. typhimurium* challenge resulted in impairment of broiler growth performance, as evidenced by the decrease in BW on d 11 and 21 as well as ADG and ADFI during 1–11 d, 12–21 d and 1–21 d, concurrent with an increase in FCR during 1–11 d. It was reported that aquatic animals treated with AHLase or AHL-degrading probiotics displayed improved growth performance (Ghanei-Motlagh et al., 2021; Yao et al., 2021). However, few studies are available regarding the effects of AHLase on growth performance in farm animals. In this study, AHLase addition reversed the impairments of growth performance in broilers challenge by *S. typhimurium*. This could be due to that AHLase addition suppressed AHL-mediated QS of *S. typhimurium*, which subsequently decreased its pathogenicity (as supported by the detected weakening of biofilm-forming ability, swarming motility and adhesive capacity) and alleviated infection disorders such as the observed intestinal injuries of broilers challenged by *S. typhimurium*.

Organ index, namely the relative weight of organs, is an important indicator to for bacterial infection and immune function status of poultry (Wu et al., 2018). As the central immune organs, thymus and bursa of Fabricius are the main sites for the development, differentiation and maturation of lymphocytes. The spleen is the primary peripheral lymphoid organ, which together with the liver, contains a large number of immune cells, thus serving as important sites for both cellular and humoral immune responses. The current study revealed increases in the indexes of liver, spleen and bursa of Fabricius in broilers owing to *S. typhimurium* challenge, which was likely ascribed to that the activation of immune system resulting from *S. typhimurium* invasion recruited considerable immune cells into these organs, thus triggering their compensatory hypertrophy (Hallstrom and McCormick, 2011). However, AHLase addition counteracted *S. typhimurium*-induced elevations of the indexes of liver, spleen and bursa of Fabricius, implying an ability of AHLase to mitigate *S. typhimurium* invasion and the resultant immune stimulation-caused enlargements of organs in broilers.

Amelioration of intestinal morphological structure such as augments in VH and VCR represents increases in villus surface area and brush border enzyme secretion as well as enhanced proliferation and maturity of intestinal epithelial cells, thus benefiting intestinal absorption, renewal and repairing (Jeurissen et al., 2002). In accordance with some previous studies (Ibrahim et al., 2021; Zhang et al., 2020), this study revealed that broilers challenged with *S. typhimurium* had damaged intestinal morphology as characterized by the reductions of ileal VH and VCR on both d 11 and 21. It was reported that AHLase treatment improved intestinal morphological structure in zebrafish by elevating intestinal VH and the density of goblet cells (Yao et al., 2021). Similarly, the present study indicated rejuvenation of intestinal morphology in challenged broilers in response to AHLase addition, which could be due to the AHLase attenuating *S. typhimurium* invasion of intestinal epithelia by decreasing the virulence especially the adherence ability.

Intraepithelial TJ are comprised of several structurally unique proteins including the transmembrane proteins (e.g. claudin family

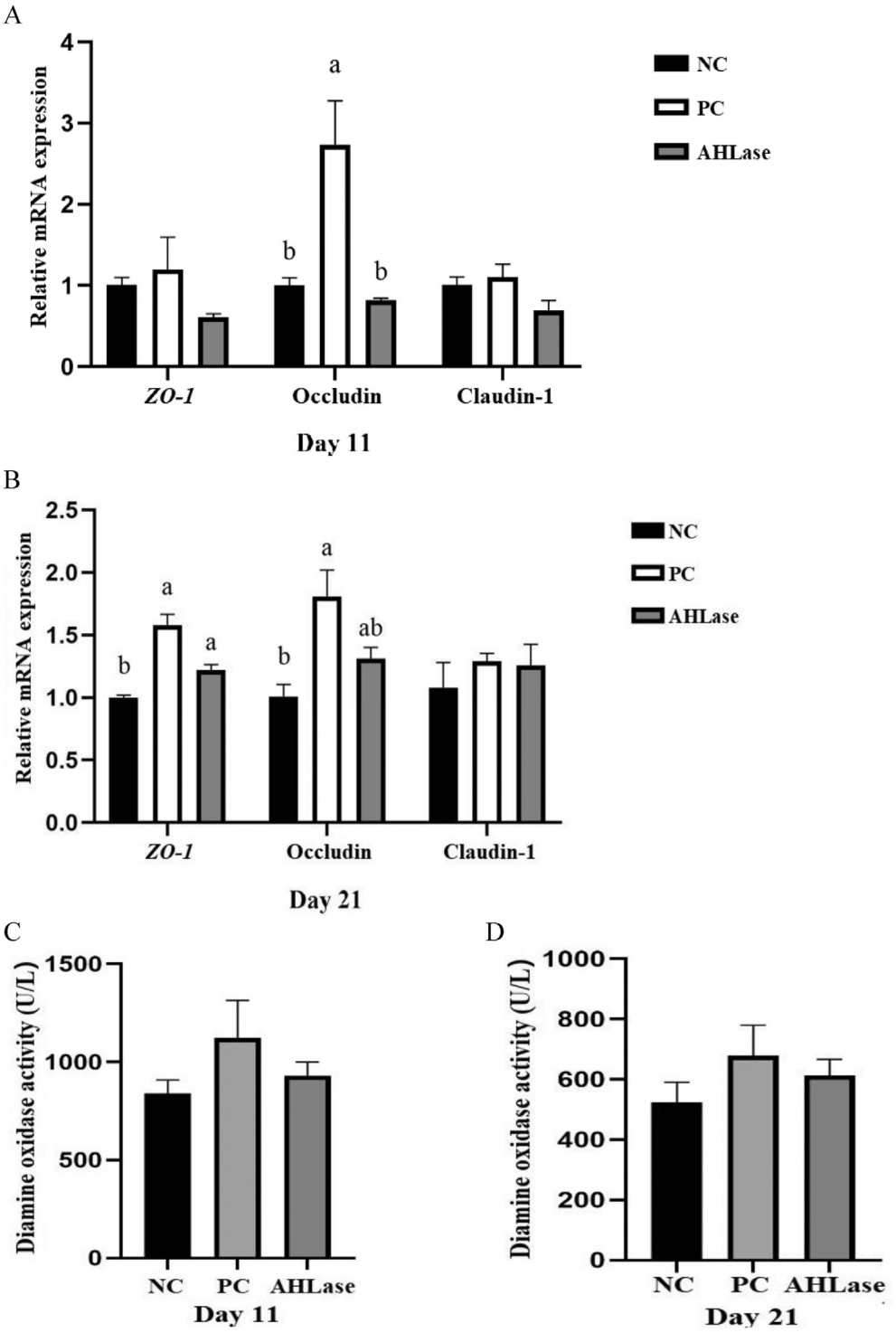

Fig. 3. Effects of N-acyl homoserine lactonase (AHLase) on intestinal barrier function including the (A, B) relative mRNA expression of ileal tight junction proteins and (C, D) serum diamine oxidase activity of broilers challenged by *Salmonella typhimurium*. NC = negative control (broilers were free of challenge); PC = positive control (broilers were challenged with *S. typhimurium* from 8 to 10 d of age); AHLase = PC broilers supplemented with 10,000 U/kg AHLase. *ZO-1* = zonula occluden 1. [a,b] Values with unlike superscript letters differ significantly ($P < 0.05$).

and occludin) and linker proteins (e.g. ZO family), which form a paracellular permeability barrier and maintain intestinal integrity, preventing translocation of macromolecules from intestinal lumen and protecting gut from enteric pathogen invasion (Buckley and Turner, 2018). DAO is a highly active intracellular enzyme in intestinal mucosal epithelia and can be released into blood once there is a disturbance of intestinal barrier (Luk et al., 1980). Thereby, serum DAO activity is viewed as a parameter reflecting the severity of intestinal injuries in chickens (Wang et al., 2016; Zhang et al., 2020). Previous studies revealed that *S. typhimurium* challenge repressed the expression of certain intestinal TJ proteins and increased serum DAO activity in broilers (Shao et al., 2013; Zhang

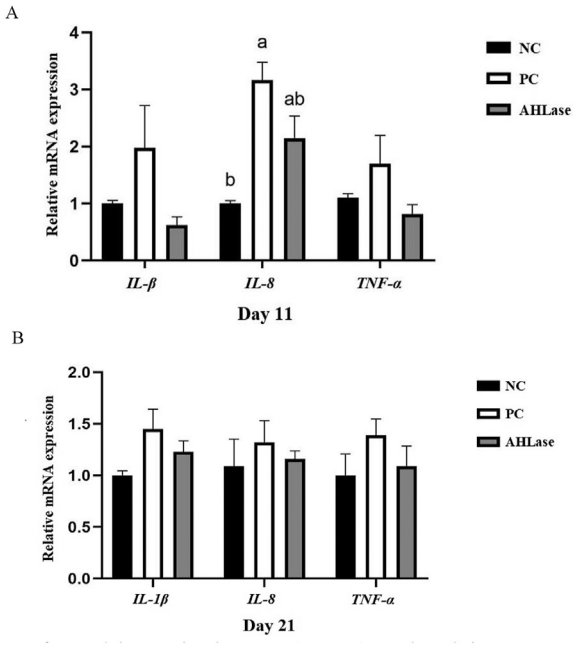

Fig. 4. Effects of N-acyl homoserine lactonase (AHLase) on the relative mRNA expression of ileal inflammatory cytokines of broilers challenged by *Salmonella typhimurium* on (A) d 11 and (B) d 21. NC = negative control (broilers were free of challenge); PC = positive control (broilers were challenged with *S. typhimurium* from 8 to 10 d of age); AHLase = PC broilers supplemented with 10,000 U/kg AHLase. *IL-1β* = interleukin 1β; *IL-8* = interleukin 8; *TNF-α* = tumor necrosis factor α. [a,b] Values with unlike superscript letters differ significantly ($P < 0.05$).

et al., 2020). However, the present study found that *S. typhimurium* challenge increased the mRNA expression of ileal occludin (a core transmembrane protein) on both d 11 and 21 as well as ileal *ZO-1* expression on d 21, but elicited little change in serum DAO activity on either d 11 or 21, implying little destruction of intestinal barrier function in challenged broilers. These might be responsible by the self-defense mechanism of the host against *Salmonella* invasion through increasing the expression of certain intestinal TJ proteins in an effort to maintain intestinal TJ structure so as to keep the *Salmonella*-induced intestinal disruption in check (Hallstrom and McCormick, 2011; Lin et al., 2016). To date, there is a scarce of information about the effect of AHLase on intestinal barrier function in animals. The present study showed that supplemental AHLase normalized *S. typhimurium*-induced increase in the mRNA expression of ileal occludin without actually modifying intestinal barrier (as supported by the unchanged DAO activity in serum) of broilers on either d 11 or 21. It was possible that the attenuated virulence and pathogenicity of *S. typhimurium* originating from AHLase reduced the invasion and burden of *S. typhimurium* to intestinal barrier, causing a feedback decrease in ileal occludin expression of broilers.

Inflammatory cytokines are well-known to be closely associated with intestinal health status of animals (Hallstrom and McCormick, 2011). On one hand, inflammatory cytokines play essential pathological roles in conducing to bacteria-related damages of intestinal TJ structure and barrier function in animals (Hallstrom and McCormick, 2011; Zhang et al., 2020). On the other hand, bacteria-induced intestinal damages render the intestine more susceptible to inflammation mediated by inflammatory cytokines (Hallstrom and McCormick, 2011). A previous study indicated a complex response of the expression profile of intestinal inflammatory cytokines in *S. typhimurium*-challenged broilers in a time-dependent manner (Fasina et al., 2008). Similarly, this study found that *S. typhimurium* challenge increased ileal *IL-8* expression of broilers on d 11 instead of d 21, but failed to modify the expression of other inflammatory cytokines, demonstrating a restriction of intestinal inflammation in challenged broilers. This could correspond to the observed restrained destruction of intestinal barrier induced by *S. typhimurium* challenge (Hallstrom and McCormick, 2011). It was reported that AHLase treatment was capable of blocking acute pneumonia by suppressing inflammatory cytokine expression in *P. aeruginosa*-challenged mice (Migiyama et al., 2013). Likewise, this study showed that supplemental AHLase normalized the *S. typhimurium*-induced increase in ileal *IL-8* expression of broilers, validating a role of AHLase in moderating ileal inflammation of *S. typhimurium*-challenged broilers probably by alleviating the virulence of *S. typhimurium* and its invasion to host intestine.

5. Conclusion

In conclusion, AHLase treatment reduced the virulence and pathogenicity of *S. typhimurium* by attenuating biofilm-forming ability, swarming motility and adhesive capacity of *S. typhimurium*, which could be responsible for the observed mitigating effects of AHLase addition on *S. typhimurium*-induced impairments in growth performance and intestinal health of broilers.

Author contributions

Weiwei Wang: writing-original draft, conceptualization. **Yiliang Chen:** investigation, methodology. **Hui Ye:** data curation. **Zemin Dong:** formal analysis, resources. **Changming Zhang:** software. **Dingyuan Feng:** validation. **Qingyun Cao:** visualization. **Shujie Liang:** project administration, writing – review & editing. **Jianjun Zuo:** funding acquisition, supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately impact our research, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (No. 32102584) and the Modern Feed Industry Innovation Team Project of Guangdong Province (No. 2021KJ115).

References

Buckley A, Turner JR. Cell biology of tight junction barrier regulation and mucosal disease. Csh Perspect Biol 2018;10:a029314.
Butler MT, Wang Q, Harshey RM. Cell density and mobility protect swarming bacteria against antibiotics. Proc Natl Acad Sci USA 2010;107:3776–81.
Campos-Galvao MEM, Ribon AOB, Araujo EF, Vanetti MCD. Changes in the *Salmonella enterica Enteritidis* phenotypes in presence of acyl homoserine lactone quorum sensing signals. J Basic Microbiol 2016;56:493–501.
Cao YA, Liu YC, Mao W, Chen RD, He SX, Gao XH, et al. Effect of dietary N-acyl homoserin lactonase on the immune response and the gut microbiota of zebrafish, Danio rerio, infected with *Aeromonas hydrophila*. J World Aquacult Soc 2014;45:149–62.
Cao YN, He SX, Zhou ZG, Zhang MC, Mao W, Zhang HT, et al. Orally administered thermostable N-acyl homoserine lactonase from Bacillus sp. strain ai96 attenuates *Aeromonas hydrophila* infection in zebrafish. Appl Environ Microbiol 2012;78:1899–908.
Castelijn GAA, van der Veen S, Zwietering MH, Moezelaar R, Abee T. Diversity in biofilm formation and production of curli fimbriae and cellulose of *Salmonella*

Typhimurium strains of different origin in high and low nutrient medium. Biofouling 2012;28:51–63.
Cong Y, Wang J, Chen Z, Xiong K, Xu Q, Hu F. Characterization of swarming motility in *Citrobacter freundii*. FEMS Microbiol Lett 2011;317:160–71.
Dong T, Wang WW, Xia MH, Liang SJ, Hu GZ, Ye H, et al. Involvement of the heat shock protein HtpG of *Salmonella Typhimurium* in infection and proliferation in hosts. Front Cell Infect Microbiol 2021;11:758898.
Fan XJ, Liang MJ, Wang L, Chen R, Li H, Liu XL, et al. Aii810, a novel cold-adapted n-acylhomoserine lactonase discovered in a metagenome, can strongly attenuate *Pseudomonas aeruginosa* virulence factors and biofilm formation. Front Microbiol 2017;8:1950.
Fasina YO, Holt PS, Moran ET, Moore RW, Conner DE, McKee SR. Intestinal cytokine response of commercial source broiler chicks to *Salmonella Typhimurium* infection. Poultry Sci 2008;87:1335–46.
Ghanei-Motlagh R, Mohammadian T, Gharibi D, Khosravi M, Mahmoudi E, Zarea M, et al. Quorum quenching probiotics modulated digestive enzymes activity, growth performance, gut microflora, haemato-biochemical parameters and resistance against *Vibrio harveyi* in Asian seabass (*Lates calcarifer*). Aquaculture 2021;531:735874.
Hallstrom K, McCormick BA. *Salmonella* interaction with and passage through the intestinal mucosa: through the lens of the organism. Front Microbiol 2011;2:88.
Harshey RM. Bacterial motility on a surface: many ways to a common goal. Annu Rev Microbiol 2003;57:249–73.
Ibrahim D, Abdelfattah-Hassan A, Badawi M, Ismail TA, Bendary MM, Abdelaziz AM, et al. Thymol nanoemulsion promoted broiler chicken's growth, gastrointestinal barrier and bacterial community and conferred protection against *Salmonella* Typhimurium. Sci Rep 2021;11:7742.
Jeurissen SHM, Lewis F, van der Klis JD, Mroz Z, Rebel JMJ, ter Huurne AAHM. Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity, and functionality. Curr Issues Intest Microbiol 2002;3:1–14.
Johnson L, Horsman SR, Charron-Mazenod L, Turnbull AL, Mulcahy H, Surette MG, et al. Extracellular DNA-induced antimicrobial peptide resistance in *Salmonella enterica serovar Typhimurium*. BMC Microbiol 2013;13:115.
Lin ZJ, Zhang YG, Xia YL, Xu XL, Jiao XN, Sun XJ. *Salmonella enteritidis* effector AvrA stabilizes intestinal tight junctions via the JNK pathway. J Biol Chem 2016;291:26837–49.
Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. Methods 2001;25:402–8.
Luk GD, Bayless TM, Baylin SB. Diamine oxidase (histaminase)-a circulating marker for rat intestinal mucosal maturation and integrity. J Clin Invest 1980;66:66–70.
MacKenzie KD, Palmer MB, Köster WL, White AP. Examining the link between biofilm formation and the ability of pathogenic *Salmonella* strains to colonize multiple host species. Front Vet Sci 2017;4:138.
Migiyama Y, Kaneko Y, Yanagihara K, Morohoshi T, Morinaga Y, Nakamura S, et al. Efficacy of AiiM, an N-acylhomoserine lactonase, against *Pseudomonas aeruginosa* in a mouse model of acute pneumonia. Antimicrob Agents Chemother 2013;57:3653–8.
Milanov D, Ljubojević D, Čabarkapa I, Karabasil N, Velhner M. Biofilm as risk factor for *Salmonella* contamination in various stages of poultry production. Eur Poult Sci 2017;81.
Muhammad MH, Idris AL, Fan X, Guo Y, Yu Y, Jin X, et al. Beyond risk: bacterial biofilms and their regulating approaches. Front Microbiol 2020;11:928.
Ning Y, Cheng LJ, Ling M, Feng XR, Chen LL, Wu MX, et al. Efficient suppression of biofilm formation by a nucleic acid aptamer. Pathog Dis 2015;73:ftv034.
Ozdemir C, Akcelik N, Akcelik M. The role of extracellular DNA in *Salmonella* biofilms. Mol Genet Microbiol 2018;33:60–71.
Packiavathy IASV, Kannappan A, Thiyagarajan S, Srinivasan R, Jeyapragash D, Paul JBJ, et al. AHL-lactonase producing *Psychrobacter* sp. from palk bay sediment mitigates quorum sensing-mediated virulence production in gram negative bacterial pathogens. Front Microbiol 2021;12:634593.
Peng MF, Tong WT, Zhao Z, Xiao L, Wang ZY, Liu XM, et al. Attenuation of *Aeromonas hydrophila* infection in carassius auratus by YtnP, a N-acyl homoserine lactonase from *Bacillus licheniformis* T-1. Antibiotics-basel 2021;10:631.
Pieters RJ. Intervention with bacterial adhesion by multivalent carbohydrates. Med Res Rev 2007;27:796–816.
Rana K, Nayak SR, Bihary A, Sahoo AK, Mohanty KC, Palo SK, et al. Association of quorum sensing and biofilm formation with *Salmonella* virulence: story beyond gathering and cross-talk. Arch Microbiol 2021;203:5887–97.
Sakr MM, Elkhatib WF, Aboshanab KM, Mantawy EM, Yassien MA, Hassouna NA. In vivo evaluation of a recombinant N-acylhomoserine lactonase formulated in a hydrogel using a murine model infected with MDR *Pseudomonas aeruginosa* clinical isolate, CCASUP2. Amb Express 2021;11:109.
Shao YJ, Guo YM, Wang Z. beta-1,3/1,6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with *Salmonella enterica serovar Typhimurium*. Poultry Sci 2013;92:1764–73.
Sikdar R, Elias M. Quorum quenching enzymes and their effects on virulence, biofilm, and microbiomes: a review of recent advances. Expert Rev Anti-Infect Ther 2020;18:1221–33.
Swiecicki JM, Sliusarenko O, Weibel DB. From swimming to swarming: *Escherichia coli* cell motility in two-dimensions. Integr Biol 2013;5:1490–4.
Wang WW, Li Z, Han QQ, Guo YM, Zhang B, D'inca R. Dietary live yeast and mannan-oligosaccharide supplementation attenuate intestinal inflammation and barrier dysfunction induced by *Escherichia coli* in broilers. Br J Nutr 2016;116:1878–88.
Weinstein DL, O'Neill BL, Hone DM, Metcalf ES. Differential early interactions between *Salmonella enterica serovar Typhi* and two other pathogenic *Salmonella serovars* with intestinal epithelial cells. Infect Immun 1998;66:2310–8.
Whiteley M, Diggle SP, Greenberg EP. Progress in and promise of bacterial quorum sensing research. Nature 2017;551:313–20.
Wu QJ, Zheng XC, Wang T, Zhang TY. Effects of dietary supplementation with oridonin on the growth performance, relative organ weight, lymphocyte proliferation, and cytokine concentration in broiler chickens. BMC Vet Res 2018;14:34.
Wu SC, Fu BD, Chu XL, Su JQ, Fu YX, Cui ZQ, et al. Subinhibitory concentrations of phloretin repress the virulence of *Salmonella typhimurium* and protect against *Salmonella typhimurium* infection. Antonie Leeuwenhoek 2016;109:1503–12.
Xiao YP, Zou HC, Li JJ, Song TX, Lv WT, Wang W, et al. Impact of quorum sensing signaling molecules in gram-negative bacteria on host cells: current understanding and future perspectives. Gut Microb 2022;14:2039048.
Yao YY, Xia R, Yang YL, Gao CC, Zhang FL, Hao Q, et al. Dietary quenching enzyme AiiO-AIO6 promotes fish growth through intestinal microbiota: zebrafish as a model. Aquacult Rep 2021;22:100947.
Zhang BB, Li G, Shahid MS, Gan LP, Fan H, Lv ZP, et al. Dietary L-arginine supplementation ameliorates inflammatory response and alters gut microbiota composition in broiler chickens infected with *Salmonella enterica serovar Typhimurium*. Poultry Sci 2020;99:1862–74.