Dietary cysteamine hydrochloride protects against oxidation, inflammation, and mucosal barrier disruption of broiler chickens challenged with *Clostridium perfringens*

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ABSTRACT: This study aimed to investigate the effect of dietary cysteamine hydrochloride (**CSH**) on the growth performance, oxidation, inflammation, and gene expression of cytoskeleton and tight junction in the intestinal mucosa of broiler chickens challenged with *Clostridium perfringens* (*C. perfringens*). A total of 360 one-day-old broiler chicks were randomly distributed into 5 groups for a negative control (**NC**, without *C. perfringens* challenge), a positive control (**PC**, with *C. perfringens* challenge), and PC plus CSH at 100, 150, or 200 mg/kg of diet. The results showed that average daily gain, gain:feed, cecal population and enterotoxin of *C. perfringens* were negatively affected ($P < 0.05$) by the *C. perfringens* challenge, but were conversely affected ($P < 0.05$) by the CSH supplementation, and G:F reached to the level of NC group. The PC group increased ($P < 0.05$) serum diamine oxidase, malondialdehyde, protein carbonyl, interleukin-6, interleukin-1β, and tumor necrosis factor-α, whereas the supplementation of CSH decreased ($P < 0.05$) these parameters. Moreover, the *C. perfringens* challenge worsened the disruption of intestinal mucosal cytoskeleton and tight junction by downregulating ($P < 0.05$) the mRNA levels of actin protein of muscle Z-line alpha, syncoilin, synemin, tubulin, claudin-1, and zona occludens protein-2, while these parameters were partially compensated ($P < 0.05$) by CSH supplementation. For the dose trends of CSH, there were linear and quadratic ($P < 0.05$) effects on gain:feed, enterotoxins, tumor necrosis factor-α, tubulin alpha 1c, syncoilin, and synemin. In conclusion, the CSH can be an alternative against *C. perfringens* infection by beneficially regulating gut pathogenic bacteria and enterotoxins, oxidation, inflammation, cytoskeleton, and tight junction in broiler chickens.

Key words: broiler chickens, *Clostridium perfringens*, cysteamine hydrochloride, inflammation, mucosal barrier

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INTRODUCTION

Enteric diseases induced by *Clostridium perfringens* (*C. perfringens*) is one of the leading causes of death and economic losses in animal production with removal of antibiotic growth promoters (Kongsted et al., 2014; Abid et al., 2016). In chickens, the occurrence of necrotic enteritis is a cascading effect of oxidative stress, inflammatory response, cytoskeletal disruption and pore formation induced by the enterotoxins of *C. perfringens* in the intestinal mucosa (Rood et al., 2016; Awad et al., 2017). The intestinal mucosal barrier is mainly composed of epithelial cells and tight junction. Disruption of intestinal epithelial cytoskeleton by enterotoxins and consequent pore formation of the junction is a primary mechanism for the invasion of pathogens (Fasano and Nataro, 2004; O'Hara and Buret, 2008).

The cytoskeleton consists of 3 major structural elements: cellular microtubules, microfilaments,

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and intermediate filaments. These elements play an important role in maintaining cellular architecture and function (Alberts et al., 2015). Cytoskeletal protein is rich in cysteine residues, and its polymerization is dependent on tubulin-thiol groups (Kuriyama and Sakai, 1974). Cellular glutathione is important for the regulation of cytoskeletal organization (Leung and Chou, 1989). Perturbing the cellular redox status by depleting intracellular glutathione provoked disruption of microfilaments in human fibroblasts (Kletsas et al., 1998). The cytoskeleton can be regulated by exogenous chemical or bioactive materials (Baumgarten et al., 2017; Imai-Sumida et al., 2017; He et al., 2018), but little is known about the effect of dietary factors or nutraceuticals on the cytoskeleton in farm animals.

Cysteamine or its analogues as exogenous supplements can promote the transport of L-cysteine into cells for the synthesis of glutathione. As a feed additive, cysteamine hydrochloride (**CSH**) is the simplest and the most stable among aminothiol compounds (Reid, 1958). It is well documented that thiol-containing compounds have a biological role in anti-oxidation and anti-inflammation (Morrison et al., 2005; Zhou et al., 2017; Bai et al., 2018). Recent studies have demonstrated their antimicrobial and antibiofilm activity in cystic fibrosis models (Charrier et al., 2014; Fraser-Pitt et al., 2016, 2018). Furthermore, pretreatment with *N*-acetylcystein, a glutathione precursor, significantly enhanced the intracellular glutathione level and decreased the cytotoxicity as well as cytoskeletal changes in hepatocytes induced by microcystic cyanobacteria extract (Ding et al., 2000).

Information about the effect of thiol-containing compounds on intestinal mucosal barrier in farm animals challenged with *C. perfringens* is unclear. Hypothetically, CSH can protect against the disruption of cytoskeleton and pore-forming of tight junction in the intestinal mucosa induced by gut pathogens. The aims of the present study were to investigate the effect of dietary CSH on growth performance, cecal *C. perfringens* population and enterotoxins, serum oxidative stress and inflammatory factors, and gene expression of intestinal mucosal cytoskeleton and tight junction in broiler chickens challenged with *C. perfringens*.

MATERIALS AND METHODS

Treatments, Diets, and CSH

Treatments included a negative control (**NC**) without *C. perfringens* challenge, a positive control (**PC**) with *C. perfringens* challenge, and PC plus CSH at a dose of 100, 150, or 200 mg/kg in the expense of corn in the basal diet. The nutrition levels of basal diet were recommended by China Agricultural Standard (NY/T 33-2004). All diets were isonitrogenous and isocaloric, and fed as mash on air-dried basis. No antibiotics were offered to broilers via either feed or water throughout the trial. The CSH was coated and provided by Hangzhou King Techina Technology Co., Ltd (Hangzhou, China). The compositions of ingredients and nutrients in the basal diet were listed in Table 1.

Animals, C. perfringens Challenge, and Samples

All protocols relating to the care and use of live animals were approved by the Institutional Committee for Animal Use and Ethics of Henan University of Science and Technology (Luoyang, China).

A total of 360 one-day-old male Cobb broilers were randomly distributed into 5 groups with 6 replicates (pens) of 12 chicks each. All chicks were reared on floor pens (1.2 m × 1.2 m) in an environmentally controlled facility at the Research Center of the Henan University of Science and Technology and given ad libitum access to diets and water throughout a 28-d experimental period. The room temperature was maintained at 32 °C for the first 5 d and then gradually decreased to 22 °C by 28 d old. Lighting was provided 24 h/d at first 2 d and

Table 1. Ingredients and nutrient levels of basal diet (air-dry basis)

Ingredients	Content, g/kg	Nutrients[1]	Content, g/kg
Corn	605.0	Crude protein	219.7
Soybean meal	255.0	Metabolizable energy, MJ/kg	12.53
Corn gluten meal	80.0	Ca	9.6
Soybean oil	29.0	Total P	6.9
Lysine	3.0	Nonphytate P	4.8
Methionine	1.5	Crude fat	32.1
Salt	3.0	Crude fiber	22.8
Dicalcium phosphate	21.5	Methionine	5.2
Limestone	12.0	Methionine + cysteine	8.5
Choline chloride	1.5	Lysine	12.1
Premix[2]	5.0		

[1]Calculated by Chinese Feed Database, version 21, 2010.

[2]Provided per kg diet: vitamin A (retinyl acetate), 8,000 IU; cholecalciferol, 1,000 IU; vitamin E (DL-tocopheryl acetate), 20 IU; vitamin K, 0.5 mg; thiamin, 2.0 mg; riboflavin, 8.0 mg; d-pantothenic acid, 10 mg; niacin, 35 mg; pyridoxine, 3.5 mg; biotin, 0.18 mg; folic acid, 0.55 mg; vitamin B_{12}, 0.010 mg; Mn, 120 mg; I, 0.70 mg; Fe, 100 mg; Cu, 8 mg; Zn, 100 mg; and Se, 0.30 mg.

18 h/d for the remainder days. All chicks received respective experimental diets from the first day of age. Birds and feed in each pen were weighed at the beginning and end of the trial, and once mortality was occurred, average daily feed intake (ADFI), average daily gain (ADG), and gain:feed (G:F) were adjusted on a pen basis. All birds were monitored for general health twice a day.

At day 8, 9, 12, and 13 of age, each chick in PC and CSH treatments was gavaged with 1 mL of *C. perfringens* (1.0×10^8 cfu/mL). Chicks in NC group received the same volume of sterile fluid thioglycollate broth.

Six birds per pen were randomly selected at day 28, weighed, euthanized by carbon dioxide, and then dissected. Blood was immediately drawn from the heart with a syringe and aliquoted into sterile vials for the preparation for serum according to the method by Liu et al. (2008). Duodenum was dissected and flushed with phosphate buffer saline (0 to 4 °C) and mucosa was scrapped and stored at −80 °C for the analysis of gene expression. All cecal digesta was collected and pooled for gut microflora and enterotoxin analysis.

Detection of Bacteria, Spores, and Enterotoxin

Cecal digesta (1.000 g) collected above was diluted with ice-cold sterile peptone water (9 mL, 0.1%) and thoroughly mixed. The numbers of *C. perfringens* were estimated by preparing serial dilutions of the mixture, plating on sulfite polymixin sulphadiazine agar (HB0256; Qingdao Hopebio Co., Ltd, Shandong, China), and counting colonies (Liu et al., 2018). The number of colony-forming units (**cfu**) was expressed as a logarithmic ($\log_{10}$) transformation per gram of cecal content. For the enumeration of spores, 1-mL sample was heated for 20 min at 75 °C to kill vegetative cells and induce the germination of mature spores. The heat-treated culture was then serially diluted in sterile peptone, plated on sulfite polymixin sulphadiazine agar, and incubated anaerobically at 37 °C. The colonies, representing germinated spores, were counted.

The detection of enterotoxin from *C. perfringens* was carried out using an ELISA kit (TechLab, Blacksburg, VA) according to its operating manual. Briefly, fresh cecal digesta (5.0 g) was centrifuged at 12,000 × *g* for 10 min and the supernatant from each sample was diluted 1 to 5 and added to the wells. Buffered protein solution + 0.02% thimerosal was used as the negative control solution. Conjugate contains polyclonal antibody specific for enterotoxin coupled to horseradish peroxidase—in a buffered protein solution + 0.02% thimerosal. Substrate was buffered solution containing tetramethylbenzidine and peroxide. Positive control reagent was enterotoxin in a buffered protein solution containing 0.02% thimerosal. Stop solution was 0.6 mol/liter sulfuric acid. The intra-assay of the kit was CVs < 5%, and inter-assay was CVs < 9%.

Analysis of Oxidation, Inflammation, and mRNA Expression

The mucosal concentrations of oxidative products and inflammatory factors were measured using chicken speciation kits from Nanjing Jiancheng Biological Institute (Nanjing, China) for diamine oxidase (**DAO**; EC 1.4.3.6; A088), malondialdehyde (**MDA**; A003), protein carbonyl (**PCO**; A087), interleukin-6 (**IL-6**; assay range, 15.0–1000 ng/liter), interleukin-1β (**IL-1β**; assay range, 20–600 ng/liter), and tumor necrosis factor-α (**TNF-α**; assay range, 0.30–200 ng/liter). Three parallel tests with aliquots of the same sample were performed for all samples and all chemical and biochemical analyses.

Total mRNA isolation and cDNA synthesis were carried out according to the description by Liu et al. (2008), and the transcriptional profiles of target genes were expressed as the relative expression to glyceraldehyde-3-phosphate dehydrogenase gene. The data of gene expression were expressed as $2^{-\Delta\Delta Ct}$ according to the method by Livak and Schmittgen (2001). Primer information for qPCR was listed in Table 2. Primers and qPCR reagents were provided by Dalian TaKaRa Co., Ltd (Liaoning, China). The qPCR reactions were set at 10 μL with 5 μL of SYBR Green Master Mix, 1 μL of primer, 4 μL of 10× diluted cDNA. The housekeeping gene was included when measured PCR efficiencies for all genes, and the amplification efficiencies between 90% and 100% were acceptable. All qPCRs were run in triplicates on the same thermal cycles (50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min) on the ABI Prism 7900HT Fast Real-Time PCR System. No amplification signal was detected in water or no-RT RNA samples. The PCR products for all genes were confirmed by sequencing at Dalian TaKaRa Co., Ltd.

Statistics

Data were analyzed using contrasts of 1-way ANOVA procedure (SAS Inst. Inc., Cary, NC). Linear and quadratic equation of polynomial contrasts were used for the analysis of dose trends of CSH at 100, 150, and 200 mg/kg. Pen was the

experimental unit for growth performance. The average of 6 killed birds per pen was the experimental unit for gut bacteria (log_{10} cfu) and enterotoxins, oxidative and inflammatory parameters, and gene expression. Differences of variables were separated using Tukey's studentized range test at $P < 0.05$ level of significance.

RESULTS

Growth Performance and Cecal C. perfringens Status

The *C. perfringens* challenge in the PC group depressed ($P < 0.05$) ADFI, ADG, and G:F compared to the NC group (Table 3). The supplementation of CSH at doses of 150 or 200 mg/kg increased ($P < 0.05$) ADFI. The ADG averagely increased by 13.3% ($P < 0.05$) in the 3 levels of CSH groups compared to the PC group, but did not reach the levels of NC group, whereas the G:F increased by 9.6% ($P < 0.05$) and reached the levels of NC group. There were linear ($P < 0.001$) and quadratic ($P = 0.046$) effects of CSH doses on ADG, and a linear ($P = 0.042$) effect on G:F. The mortality of broiler chickens also worsened in the PC group, and attenuated by CSH supplementation, but these influences were not significant due to the greater difference intragroup than intergroup.

The cecal *C. perfringens*, spores, and enterotoxins in the PC group were the greatest ($P < 0.05$) among treatments, and the supplementation of CSH decreased ($P < 0.05$) their levels, but did not reach the levels of NC group. Linear decreasing effects ($P < 0.001$) of CSH on the spores and enterotoxins, and a quadratic decreasing effect ($P \leq 0.012$) on enterotoxins were found. The effect of CSH at 200 mg/kg on spores and enterotoxins was more pronounced ($P < 0.05$) than that of CSH at other 2 doses.

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

		Primers (5′→3′)		
Names	GenBank	Forward	Reverse	Length (bp)
TUBA1C	NM_001305272.1	gctcgtacagggaacaatcc	attgagacacacaggagaggac	164
TUBB6	NM_001031012.2	gctactgtcttccgtggtcc	ttgccaatgaacgtggaagc	170
CAPZA1	NM_205515.1	aatggccgactttgaggacc	caccggagtgaactgatcca	196
CAPZA2	NM_001004380.1	ccgcaccaagattgactgga	gcaaaagcccatgctgactg	178
SYNM	NM_204809.3	acaagcacgagagtcgtctg	ccccactgtggtcactttca	212
SYNC	XM_015297851.1	gcacgtaaactggcgacttc	ctgggtctattgggctgcat	171
CLDN1	NM_001013611.2	acccacagcctaagtgcttc	aggtctcataaggccccact	200
ZO-2	NM_204918.1	agatgcgtttgctgcacctt	gacgtatagctgtgctccca	149
GAPDH	K01458.1	ggtggccatcaatgatccct	gcccatttgatgttgctggg	174

CAPZA, actin protein of muscle Z-line alpha; CLDN1, claudin-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SYNC, syncoilin; SYNM, synemin; TUBA1C, tubulin alpha 1c; TUBB6, tubulin beta 6; ZO-2, zona occludens protein-2.

Table 3. Effect of cysteamine hydrochloride on the growth performance and *Clostridium perfringens* in broiler chickens

			PC + CSH, g/kg of diet				Dose trends (*P*-values)	
Items	NC	PC	100	150	200	SEM	Linear	Quadratic
Growth performance at day 1 to 28								
ADFI, g per bird	47.86^{a}	45.47^{c}	46.38^{bc}	47.06^{ab}	46.97^{ab}	0.278	0.104	0.211
ADG, g per bird	35.17^{a}	30.10^{d}	33.55^{c}	34.33^{b}	34.40^{b}	0.113	<0.001	0.046
G:F	0.735^{a}	0.662^{b}	0.723^{a}	0.729^{a}	0.732^{a}	0.003	0.042	0.670
Mortality, %	2.78	8.33	4.17	4.17	2.78	1.878	0.599	0.761
Cecal status of *C. perfringens* at day 28								
Numbers, cfu/g	2.64^{c}	5.86^{a}	3.98^{b}	3.90^{b}	3.89^{b}	0.052	0.274	0.594
Spores, cfu/g	1.14^{d}	3.51^{a}	2.03^{b}	1.94^{b}	1.68^{c}	0.039	<0.001	0.114
Enterotoxin[1], U/mL	0.62^{d}	2.72^{a}	1.80^{b}	1.71^{b}	1.35^{c}	0.032	<0.001	0.012

ADG, average daily gain; ADFI, average daily feed intake; G:F, gain:feed; *C. perfringens*, *Clostridium perfringens*; CSH, cysteamine hydrochloride; NC, negative control, without *C. perfringens* challenge; PC, positive control, challenged with *C. perfringens*.

[a–d]Means within a row with no common superscripts are significantly different ($P < 0.05$).

[1]Values of optical density.

The *C. perfringens* challenge in PC group increased ($P < 0.05$) serum DAO, MDA, and PCO, while supplementation of CSH at 100, 150, or 200 mg/kg decreased ($P < 0.05$) these parameters, but did not reach the levels of NC group (Table 4). These parameters decreased linearly ($P \leq 0.006$) with the increasing doses of CSH. Additionally, the levels of DAO and PCO in the treatment of CSH at 200 mg/kg were lower ($P < 0.05$) than in the dose of 100 mg/kg.

The *C. perfringens* challenge in the PC group also caused increases ($P < 0.05$) of serum IL-1β, IL-6, and TNF-α, and the supplementation of CSH decreased ($P < 0.05$) these parameters. The doses of CSH showed a linear effect on IL-1β ($P < 0.001$), and both linear and quadratic effect on TNF-α ($P \leq 0.001$).

As shown in Table 5, the *C. perfringens* challenge in the PC group downregulated ($P < 0.05$) the mRNA expressions of tubulin alpha 1c (**TUBA1C**), tubulin beta 6 (**TUBB6**), actin protein of muscle Z-line alpha (**CAPZA1**), actin protein of muscle Z-line alpha (**CAPZA2**), syncoilin (**SYNC**), and synemin (**SYNM**) in the mucosa of intestine. The supplementation of CSH at 100, 150, or 200 mg/kg upregulated ($P < 0.05$) these gene expressions, but only the level of SYNC in the group of CSH at 200 mg/kg reached the level of NC group. There were linear effects ($P \leq 0.002$) of CSH doses on the mRNA expressions of TUBA1C, CAPZA1, CAPZA2, SYNC, and SYNM, and quadratic effects ($P \leq 0.040$) on TUBA1C, SYNC, and SYNM.

The mRNA expressions of intestinal mucosal claudin-1 (**CLDN1**) and zona occludens protein-2 (**ZO-2**) were also downregulated ($P < 0.05$) by *C. perfringens* challenge, while the supplementation of CSH at the 3 doses upregulated ($P < 0.05$) the mRNA levels of these genes, but they were lower ($P < 0.05$) than the NC group. Linear ($P < 0.001$) and quadratic ($P \leq 0.024$) upregulating trends of the 2 genes with the increasing doses of CSH were found. Additionally, the doses of CSH at 150 and 200 mg/kg showed a greater effect ($P < 0.05$) on the 2 genes than CSH at 100 mg/kg.

DISCUSSION

In the present study, *C. perfringens* challenge depressed the ADG and G:F of broiler chickens, which was consistent with the report by Liu et al. (2018), while these negative effects were attenuated by CSH supplementation, indicating that the CSH can partially protect broiler chickens from *C. perfringens* infection. It was reported that the CSH added at 70 mg/kg of feed improved the growth of grower or finisher pigs (Dunshea, 2007; Miller et al., 2016). In broiler chickens, cysteamine supplemented at 60 and 80 mg/kg (equal to CSH at 75 and 100 mg/kg) of feed improved feed conversion along the rearing cycle, but did not affect feed intake and weight gain (Nunes et al., 2012). However, this information is unavailable in a scenario of *C. perfringens* challenge in farm animals.

In the present study, the CSH added at 150 and 200 mg/kg improved ADFI of the broiler chickens challenged with *C. perfringens*, and supplemented at 100, 150, and 200 mg/kg improved ADG and G:F. The growth-promoting effect of CSH is attributed to its inhibitory action on somatostatin, a hormone which inhibits the GH secretion of the growth hormone (Francis et al., 1990; McLeod et al., 1995). Additionally, cysteamine occurs naturally in animal tissues, and is endogenously synthesized, metabolized, and excreted rapidly by the animal organism (Reid, 1958). Clearly, exogenous supplementation

Table 4. Effect of cysteamine hydrochloride on the serum parameters of oxidation and inflammation of broiler chickens challenged with *Clostridium perfringens*

			PC + CSH, g/kg of diet				Dose trends (*P*-values)	
Items	NC	PC	100	150	200	SEM	Linear	Quadratic
Oxidative parameters								
DAO, U/liter	0.70^{d}	2.21^{a}	1.08^{b}	0.99^{bc}	0.97^{c}	0.026	0.002	0.226
MDA, nmol/liter	2.78^{c}	5.02^{a}	4.21^{b}	4.08^{b}	3.98^{b}	0.058	0.006	0.760
PCO, nmol/g	22.91^{d}	37.57^{a}	31.33^{b}	29.43^{bc}	28.01^{c}	0.560	0.001	0.739
Inflammatory factors								
IL-1β, ng/liter	42.54^{d}	133.6^{a}	98.76^{b}	82.60^{c}	75.46^{c}	2.300	<0.001	0.077
IL-6, ng/liter	58.68^{c}	154.5^{a}	110.8^{b}	111.7^{b}	103.2^{b}	2.513	0.083	0.204
TNF-α, ng/liter	34.84^{d}	125.8^{a}	80.10^{b}	80.29^{b}	68.27^{c}	1.647	<0.001	0.001

$^{a-d}$ Means within a row with no common superscripts are significantly different ($P < 0.05$).

CSH, cysteamine hydrochloride; DAO, diamine oxidase; IL-1β, interleukin-1β; IL-6, interleukin-6; MDA, malondialdehyde; NC, negative control, without *C. perfringens* challenge; PC, positive control, challenged with *C. perfringens*; PCO, protein carbonyl; TNF-α, tumor necrosis factor-α.

Table 5. Effect of cysteamine hydrochloride on the gene expression of cytoskeleton and tight junction in the intestinal mucosa of broiler chickens challenged with *Clostridium perfringens*

Items	NC	PC	PC + CSH, g/kg of diet			SEM	Dose trends (*P*-values)	
			100	150	200		Linear	Quadratic
Cytoskeleton (mRNA expression, $2^{-\Delta\Delta Ct}$)								
TUBA1C	4.08[a]	2.74[d]	3.36[c]	3.56[b]	3.54[bc]	0.038	<0.001	0.001
TUBB6	3.71[a]	2.47[c]	3.01[b]	2.98[b]	3.04[b]	0.073	0.699	0.492
CAPZA1	2.80[a]	1.13[c]	2.18[b]	2.20[b]	2.28[b]	0.039	0.095	0.596
CAPZA2	4.19[a]	2.90[d]	3.35[c]	3.43[bc]	3.62[b]	0.047	0.002	0.340
SYNC	1.51[a]	0.86[c]	1.33[b]	1.34[b]	1.47[a]	0.018	<0.001	0.040
SYNM	1.99[a]	0.43[d]	0.74[c]	1.20[b]	1.28[b]	0.071	<0.001	0.022
Tight junction (mRNA expression, $2^{-\Delta\Delta Ct}$)								
CLDN1	3.76[a]	0.70[d]	2.08[c]	2.52[b]	2.66[b]	0.050	<0.001	0.024
ZO-2	0.65[a]	0.42[d]	0.50[c]	0.56[b]	0.56[b]	0.008	<0.001	0.013

[a–d]Means within a row with no common superscripts are significantly different ($P < 0.05$).

CAPZA, actin protein of muscle Z-line alpha; CLDN1, claudin-1; CSH, cysteamine hydrochloride; NC, negative control, without *C. perfringens* challenge; PC, positive control, challenged with *C. perfringens*; SYNC, syncoilin; SYNM, synemin; TUBA1C, tubulin alpha 1c; TUBB6, tubulin beta 6; ZO-2, zona occludens protein-2.

may influence the physiological action of cysteamine, but more studies on this are needed.

The birds in the PC group in the present study had the greatest levels of *C. perfringens* and its spores and enterotoxin in the cecal contents, whereas CSH supplementation decreased these numbers. Generally, when *C. perfringens* is out of balance in the gut, sporulation takes place, consequently more enterotoxin is released, and disease follows. In the present study, the CSH decreased the number of spores, in most part, indicating that CSH depressed sporulation and exterotoxin secretion. Fraser-Pitt et al. (2018) reported that cysteamine sensitized *Pseudomonas aeruginosa* to killing by reactive oxygen and nitrogen species, and potentiated the antimicrobial activity of colistin and macrolide. Similarly, cysteamine showed the activity against cystic fibrosis pathogen *Burkholderia cepacia* complex (Fraser-Pitt et al., 2016).

Cysteamine is also an endogenous aminothiol produced in cells as consequence of coenzyme A metabolism. In a reducing environment in the absence of transition metals, cysteamine can act as an antioxidant, and readily form mixed disulfides with susceptible cysteine sulfhydryl groups (O'Brian and Chu, 2005), which is key for many reported biological activities. Additionally, cysteamine can be metabolized as hypotaurine and taurine via aminothiol dioxygenase in the cells of animals, but it is considered to be toxic for bacteria, fungi, and parasites because they are lack of the enzyme (Dominy et al., 2007). These reports, coupled with the present study, demonstrated the broad-spectrum antimicrobial activity of cysteamine, but the dependence on the physiological status of transition metals needs further study.

Serum DAO level is employed as a useful marker of intestinal mucosal integrity. The MDA is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Also, the higher circulatory PCO is related to the oxidative damage of proteins. In the present study, their serum levels were contrarily affected by the *C. perfringens* challenge and CSH supplementation, indicating that the lipid oxidation and protein carbonylation in the intestinal mucosa or other organs were attenuated by the supplemental CSH. The information about the effect of CSH or its analogues on the DAO and PCO is very limited. Wilmer et al. (2011) argued that cysteamine did not affect protein turnover, total glutathione levels, protein oxidation, and lipid peroxidation, but normalized glutathione redox status in cystinotic cells.

Zhou et al. (2017) reported that cysteamine increased glutathione and its peroxidase, and decreased MDA in pigs. Also, the CSH improved antioxidant status and delayed pig meat discoloration by improving glutathione levels and anti-oxidase activity (Bai et al., 2018). Thus, in the present study, besides the inhibition of *C. perfringens* by CSH, the enhanced glutathione activity in the CSH treatments also contributed to the lower oxidative stress, because the CSH or derivatives act through sulfhydryl-disulfide exchange reactions in glutathione redox cycle, and the providing of sulfhydryl group can theoretically facilitate the synthesis and turnover of glutathione (Courtney-Martin and Pencharz, 2016).

Thiol-containing compounds also influence inflammation and immunity by intracellular

glutathione and cysteine levels. Cysteamine is a U.S. FDA-approved drug with anti-inflammatory and mucolytic properties (Kopp et al., 2017). Indeed, in the present study, the serum TNF-α, IL-1β, and IL-6 were increased by *C. perfringens* challenge, and were decreased by CSH. In farm animals, cysteamine supplementation increased the concentrations of secretory IgA, IgM, and IgG in the jejunal mucosa of pigs (Zhou et al., 2017), and induced proliferation and differentiation of IgA-positive cells and intraepithelial lymphocytes in the intestinal mucosa of chickens by reducing the number of somatostatin-positive cells (Yang et al., 2007).

In the present study, the supplementation of CSH protected the architecture and function of intestinal mucosal cells by upregulating the mRNA profiles of cytoskeletal protein. The findings indicate that exogenous providing of CSH facilitates the synthesis of cellular glutathione and consequently protects cytoskeleton that is suffering attack from the toxic protein of *C. perfringens*, as shown in studies that cellular glutathione was important to counteract the reorganization of cytoskeleton (Leung and Chou, 1989; Kletsas et al., 1998).

Moreover, the thiol group contributes not only to the cellular glutathione level but also to the component of microtubules and tubulin polymerization (Kuriyama and Sakai, 1974), and intracellular and extracellular thiol redox status modulates the proliferative potential of epithelial cells (Perez et al., 2017). The information about the effect of CSH on cytoskeleton is unavailable, but the tunability of cytoskeleton by *N*-acetylcystein or other bioactive materials (Ding et al., 2000; Baumgarten et al., 2017; Imai-Sumida et al., 2017; He et al., 2018) indirectly supported the present study, and its mechanistic signaling pathway will be an interesting topic for the future study.

The disruption of cytoskeleton is usually companied by the dysfunction of tight junction with the invasion of pathogens. In the present study, *C. perfringens* challenge downregulated CLDN1 and ZO-2, indicating the disruption of tight junction barrier, and this was attenuated by the supplementation of CSH. Similar effects were found in studies that cysteamine supplementation increased the mRNA abundance of tight junction, including occludin, claudin-1, and zonula occludens protein-1, in the jejunum mucosa of finishing pigs (Zhou et al., 2017) and *N*-acetyl-L-cysteine protected occludin, zona occludens-1, claudin-3, E-cadherin, and beta-catenin, as well as the actin cytoskeleton in mouse colon (Shukla et al., 2016).

CONCLUSIONS

The *C. perfringens* challenge negatively affected ADG, G:F, enterotoxin, lipid peroxidation, protein carbonylation, and inflammatory factors, but these properties were attenuated by the supplementation of CSH, and G:F reached the level of NC group. Moreover, the *C. perfringens* challenge disrupted intestinal mucosal barrier by downregulating the mRNA levels of cytoskeleton and tight junction, whereas CSH upregulated these parameters. By contrasts, linear and quadratic dose trends of CSH on G:F, enterotoxins, TNF-α, tubulin alpha 1c, syncoilin, and synemin were found. The results suggest that the CSH can protect against *C. perfringens* infection by reducing enterotoxins, oxidation, inflammation, and disruption of cytoskeleton and tight junction in broiler chickens.

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