In vitro release and in vivo growth-promoting effects of coated cysteamine in broilers

Youyou Liu,[*,†] Biyue Zhao,[*] Yujing Feng,[*] Hu Zhang,[*] Qianyun Zhang,[‡] Jia Hou,[‡] Yuming Wang,[*] Renna Sa,[*] Feng Zhao,[*] and Jingjing Xie [*,1]

[*]*State Key laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China;* [†]*Animal Science Department, Hebei Normal University of Science & Technology, Qinhuangdao, China; and* [‡]*Provincial Key Agricultural Enterprise Research Institute of Encapsulated Feed Additive, King Techina Technology Co., Ltd., Hangzhou, China*

ABSTRACT The purpose of this study was to investigate the effects of coating technology on the cysteamine (**CSH**) release in the digestive tract and the growth-promoting effect of enteric-coating CSH in broilers. First, using the self-developed computer-controlled simulated digestion system to mimic the digestion process in vitro, the release of 2 coated CSH (**CSH-I** and **CSH-Ⅱ**) were studied. The results showed that less than 10% of CSH-I was released after gastric digestion and 52.35% of CSH-I was released with additional 4 h of small intestinal digestion. In contrast, 83.62% of CSH-Ⅱ was released during the gastric digestion. In order to verify the growth-promoting effects of CSH-I, a feeding trial was conducted in a completely randomized block arrangement with 3 treatments in 6 blocks, 5 chickens per replicate. Broilers were fed with corn-soybean meal diet either supplemented with 0 (**CON**), 200 mg/kg uncoated CSH (**CSH**) or 200 mg/kg CSH-I from d 7 to 42, respectively. Body weight and FI was recorded at d 21 and 42. Excreta were collected from d 39 to d 42 to determine the total tract retention (**TTR**) of dietary nutrients. In comparisons with controls, birds fed with CSH-I had greater BW, ADG, and ADFI and increased TTR of DM, gross energy (**GE**), NDF and hemicellulose ($P < 0.05$). In addition, duodenal villi height and surface area were also greater in those CSH-I-fed birds. In contrast, the growth performance of birds fed with uncoated CSH did not significantly differ from controls. Although the TTR of DM and GE was higher in birds fed with CSH than controls, no differences in small intestine morphology were noted. Thus, the type I coating (CSH-I) could be good enteric-coating technology to increase CSH release in the duodenum, improve digestion and duodenal morphology, and therefore growth performance in broilers.

Key words: coated cysteamine, in vitro digestion, release kinetics, nutrient's retention, intestinal morphology

2023 Poultry Science 102:102475
https://doi.org/10.1016/j.psj.2023.102475

INTRODUCTION

Cysteamine (H_2N-CH_2-CH_2-SH, **CSH**) contains active sulfhydryl and amino groups, which depletes somatostatin (**SS**) and indirectly promotes the secretion of growth hormones (Szabo et al., 1992). Therefore, the CSH is widely used in livestock farming as a growth-promoting feed additive. However, the depletion of SS by CSH is not tissue specific. In the hypothalamus, CSH depletes SS to promote growth, while in the stomach it acts on gastric mucosa to promote gastric acid secretion (Shi et al., 2006). High-dose CSH leads to gastric acid hypersecretion, causing gastric and duodenal ulcers (Zavy et al., 1988). In broiler chickens, the growth-promoting effect of CSH has been reported (Hu et al., 2008), but instance of ulcers caused by over-dose CSH was also noted (Drago and Montoneri, 1997). Yang et al. (2006) reported dose-dependent changes in circulating tetraiodothyronine ($\mathbf{T_4}$) and gastrin when broiler chickens were fed with diets containing 0 to 150 mg/kg CSH. However, the growth performance of those broilers was not quite consistent with T_4 and gastrin. The feed intake, average daily gain (**ADG**) and feed conversion ratio (**FCR**) of broilers decreased when dietary CSH was over 90 mg/kg (Yang et al., 2006). The reduction in the growth performance was associated with decreases in pepsin, amylase and lipase activities in the pancreas or in the digestive tract of broilers. Therefore, in order to make a full utilization of the growth-

Received September 27, 2022.
Accepted January 1, 2023.

[1]Corresponding author: xiejingjing@caas.cn

promoting effect of CSH in broilers (Yang et al., 2006), it has to avoid negative effects of high-dose CSH on excessive gastric acid secretion and nutrient digestibility in broilers.

Studies have shown that coating technology reduces gastrointestinal irritation of CSH (Gangoiti et al., 2010). An enteric-coating can avoid the release of CSH in the stomach when orally administrated, therefore increase the absorption of CSH into the circulation, shown as increased circulation CSH and C_{max} of circulating CSH (Gangoiti et al., 2010). Therefore, the release of coated CSH along the digestive tract is extremely important to avoid the side-effect of over-stimulation in gastric acid but increase the maximal absorption. Due to technical difficulties in measuring CSH, it is hard to study the release of CSH in the digestive tract in vivo. Iodometry and liquid chromatograph-mass spectrometry (**LC-MS**) are 2 methods often used for the CSH determination. With the practical addition levels, CSH in the chyme is far below the lower detection limit of the iodimetry method (China National Standard, 2009). Using the LC-MS, the recovery rate of CSH in the intestinal fluid was only 1.49 to 5.72% because of interferences from digesta (Liu, 2019; Liu et al., 2019a). Hence, in vitro digestion might be the only way to investigate the release kinetics of coated CSH. Our laboratory has developed a computer-controlled simulated digestion system (**CCSDS**), which can simulate the gastrointestinal digestive process of chicken in vitro. Using this system, Liu (2019) studied the release kinetics of a coated CSH for pigs in chickens. The author found that this coating technology effective in pigs hardly released CSH in the digestive tract of chickens, where only 5% of CSH was released after gastric digestion and less than 18.93% released with additional 8 h of small intestinal digestion. Accordingly, when supplemented to the diet, the growth performance of broilers was not affected by this coated CSH. Therefore, a good coating technology for CSH should resist gastric digestion but enable fast release in the small intestine. In this study, we would like to screen for an enteric-coating technology to apply CSH in broilers using the CCSDS. The effectiveness of the in vitro screening was then validated in broilers by investigating the effects of coated CSH on growth performance, nutrient's digestion and intestinal morphology in vivo.

MATERIALS AND METHODS

All experimental procedures with live animals were approved by the Animal Care and welfare committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (IAS 2022-133).

In Vitro Digestion Experiment

Two coated CSH (**CSH-Ⅰ** and **CSH-Ⅱ**) were prepared by Hangzhou King Techina Technology Co., Ltd. To form the Type I coated CSH, cysteamine was first granulated with calcium stearate, starch, β-cyclodextrin and then coated with palm oil. To form the Type II coated CSH, CSH was granulated with ZnO, NaOH, SiO2 and coated with palm oil. The CCSDS (model SDS-Ⅲ, Hunan Zhongben Intelligent Technology Development Co., Ltd., Hunan, China) was used to determine the release of these two coated CSH in the digestive tract of broilers in vitro. The CCSDS was set up according to the user's manual and the digestive enzyme kits for chicken were provided by the manufacture. Detailed parameters of the CCSDS and the in vitro digestion procedures have been described by Zhao et al. (2014). Briefly, 0.3 g of CSH-Ⅰ and CSH-Ⅱ were loaded to the CCSDS, and subjected to 3 h or 4 h of gastric digestion to investigate the gastric releases of CSH. To determine the enteric release of CSH, they were subjected to 4 h of gastric digestion and then 4 h of intestinal digestion. The digesta were filtered through a 75 μm mesh sieve. The unfiltered particles were transferred to a 250 mL conical flask, to which 25 mL of deionized water and 1 mL H_2SO_4 (6 mol/mL) were added. The solution was boiled for 2 min. After cooling, 2 mL of starch solution was added to the conical flask and shaken well. The solution was titrated using the iodine standard solution. Consumption of the iodine standard solution (V_1) was recorded accurately and the amount of coated CSH was calculated by the following Equation 1:

$$X = (V1 - V2) \times C \times 0.1136 \quad (1)$$

where X is the CSH content (g); V_1 is the volume of iodine standard solution consumed by the sample (mL); V_2 is the volume of iodine standard solution consumed in the blank (mL); C is the concentration of iodine standard titration solution (mol/L). 0.1136 is the coefficient, which indicates 1.00 mL iodine standard titration solution (C = 0.1 mol/L) equivalent to 0.1136 g of CSH (g).

Experimental Design, Experimental Diets and Birds Management

According to the in vitro determination of CSH release, only the enteric-coated CSH-Ⅰ was used in the feeding trial. The feeding trial adopted a randomized complete block design with 3 treatments in 6 blocks. Three experimental diets were formulated by supplementing with 0 (**CON**), 200 mg/kg uncoated CSH (**CSH**) or 200 mg/kg enteric-coated CSH-I (**CSH-I**), respectively (Table 1). Dietary nutrition levels were referred to Arbor Acres (**AA**) Broiler Feeding management Manual (2019). Experimental diets meet or exceed the National Research Council (1994) requirements. Uncoated CSH (75% content) was diluted with SiO_2 to 27% of CSH (Wt/Wt). 200 mg/kg of CSH were mixed directly with the premix at the expense of bentonite to prepare each of the experimental diet, respectively.

Three hundred day-old male AA broiler chicks were reared in 3-layer metabolic cages. On d 7, the chicks were divided into 6 blocks according to the body weight. A total of 15 birds from each block were randomly assigned to the 3 treatment groups. Broiler feeding and

Table 1. Ingredients and chemical composition of basal diets (air-dry basis, %).

Items	D7−21	D22−42		D7−21	D22−42
Corn	50.28	57.57	Nutrient content, %		
Soybean meal	38.80	31.50	ME, kcal/kg	3,039	3,183
Soybean oil	4.50	5.50	CP, %	22.5	19.51
Corn gluten meal	2.00	2.00	Lysine, %	1.44	1.15
Dicalcium phosphate	1.89	1.43	Methionine, %	0.56	0.47
Limestone	1.06	0.92	Calcium, %	0.96	0.78
Premix	0.50[1]	0.50[2]	Available phosphorus, %	0.48	0.39
Sodium chloride	0.30	0.25	Total phosphorus, %	0.73	0.62
L-lysine	0.25	0.14			
DL-methionine	0.20	0.13			
L-threonine	0.10	0.03			
L-valine	0.10	0.01			
Phytase	0.02	0.02			
Total, %	100.00	100.00			

[1]Supplied per kilogram of diet: vitamin A, 8,000 IU; vitamin D_3, 1,000 IU; vitamin E, 20.0 IU; vitamin K_3, 0.80 mg; thiamine, 3.0 mg; riboflavin, 8.0 mg; vitamin B_6, 5.0 mg; vitamin B_{12}, 20.0 μg; pantothenic acid, 10.0 mg; nicotinic acid, 40.0 mg; folic acid, 0.60 mg; biotin, 0.20 mg; Cu (as copper sulfate), 8.0 mg; Fe (as ferrous sulfate), 100 mg; Mn (as manganese sulfate), 120 mg; Zn (as zinc sulfate), 100 mg; I (as calcium iodate), 0.70 mg; Se (as sodium selenite), 0.30 mg; choline chloride 1,500 mg; bentonite, 662.4 mg.

[2]Supplied per kilogram of diet: vitamin A, 6,000 IU; vitamin D_3, 1,000 IU; vitamin E, 15.0 IU; vitamin K_3, 0.50 mg; thiamine, 2.0 mg; riboflavin, 6.0 mg; vitamin B_6, 4.0 mg; vitamin B_{12}, 10.0 μg; pantothenic acid, 10.0 mg; nicotinic acid, 35.0 mg; folic acid, 0.60 mg; biotin, 0.20 mg; Cu (as copper sulfate), 8.0 mg; Fe (as ferrous sulfate), 80 mg; Mn (as manganese sulfate), 100 mg; Zn (as zinc sulfate), 80 mg; I (as calcium iodate), 0.70 mg; Se (as sodium selenite), 0.30 mg, choline chloride 1,200 mg; bentonite, 1,383.1 mg.

management were carried out according to Manual for AA Broiler Management. Health status was monitored daily. Body weight and feed intake was taken on d 21 and d 42 and ADF, ADFI, and FCR were calculated accordingly. On d 42, two broilers were randomly selected from each replicate, sacrificed with CO_2. Tissue samples of the stomach, the small intestine and ceca were collected. The length of each intestinal segment was measured.

Total Fecal Collection

Excreta from each replicated cage were collected 3 times per day from 9:00 AM on d 39 to 9:00 AM on d 42 (Song et al., 2022). All excreta were stored in a refrigerator at −20°C, and feed intake was recorded. The collected excreta were dried in an air-force drying oven at 65°C, mixed, and grounded after placing at room temperature for 24 h. The total tract retention (**TTR**) of nutrients was calculated using the following Equation 2:

$$TTR\ of\ nutritent,\ \% = \left(1 - \frac{Excreta\ output\ (g)\ \times\ nutrient\ \%\ in\ excreta}{Feed\ intake\ (g)\ \times nutrient\ \%\ in\ feed}\right) \times\ 100\% \quad (2)$$

Chemical Analysis

Diets and excreta were grounded and passed through 0.42 mm sieve for chemical analysis. Dry matter (**DM**) was determined according to the method of 934.01 (AOAC, 1990). Gross energy (**GE**) was measured by a Parr 6400 automatic adiabatic calorimeter (Parr instrument company, Moline, IL) according to the method of ISO 9831:1998. Nitrogen content was determined according to the method of 954.01 (AOAC, 1990) by the Kjeldahl nitrogen determination apparatus (KDY-9820; Shandong Haineng Scientific Instrument Co., Ltd., Dezhou, China). Ether extract (**EE**) was measured using the method of 920.39 (AOAC, 1990). Acid detergent fiber (**ADF**) and NDF were determined using the method of 973.18 (AOAC, 1990) and the China National Standard (2006), respectively. Hemicellulose was determined by subtracting ADF from NDF.

Small Intestinal Villi Morphological Analysis

Duodenum, jejunum and ileum were cut for a length of 3 to 5 cm, flushed gently with the PBS buffer to remove intestinal contents. Each intestinal segment was immersed in 4% paraformaldehyde solution (P1110, Solarbio, Beijing) for histological examination. The fixed intestinal segments were embedded in paraffin. Continuous tissue sectioning (5 μm thickness) was performed on a KEDEE paraffin slicer (model KD-3358, Zhejiang Jinhua Kedi Instrument Equipment Co., Ltd., Zhejiang, China). Sections fixed on slides were stained with hematoxylin-eosin (G1140, Solarbio, Beijing). For analysis of villi morphology, four fields of view were randomly selected for each section and photographed using an OLYMPUS microscope (model BX43, Olympus Corporation, Japan) at 40 × for duodenum and jejunum or 100 × for ileum. Intestinal villi height (**VH**), crypt depth (**CD**) and villi width (**VW**) were measured using Image J-2 (Version 1.53k, National Institutes of Health., Bethesda, America) and each image was calibrated with the microscope state micrometer calibration slide. The villi height/crypt depth ratio (**VH/CD**) and villi surface area (**VSA**) were calculated using the following Equation 3:

$$VSA = VH \times VW \times \pi \quad (3)$$

Table 2. Release rate of different coated cysteamine during gastric and intestinal digestion process in vitro.

Items	CSH-I[1]	CSH-II[1]
Gastric digestion 3 h	4.01 ± 0.75[b,y]	63.62 ± 2.93[a,z]
Gastric digestion 4 h	8.18 ± 1.88[b,xy]	83.62 ± 2.12[a,y]
Gastric digestion 4 h + Intestinal digestion 4 h	52.35 ± 2.70[b,x]	97.96 ± 0.01[a,x]
P value		
Coating technology × Digestion process	<0.001	
Coating technology	<0.001	
Digestion process	<0.001	

[a,b]Means within a row lacking a common superscript differ ($P < 0.05$).
[x,y,z]Means within a column lacking a common superscript differ ($P < 0.05$).
[1]CSH-I, type 1 coated cysteamine; CSH-II, type 2 coated cysteamine.

Statistical Analysis

All data were expressed as mean ± SEM. JMP 16.0 (SAS Inst. Inc., Cary, NC) was used for variance analysis. For in vitro analyses of CSH release kinetics, the main effects of CSH coating and digestion process and the interaction were included in the statistical model. For BW, ADFI, FCR, TTR, the main effect of dietary treatment and the random effect of block were included in the REML model. When the main effect was significant ($P < 0.05$), the Fisher's least significance method was used for multiple comparisons between treatments. The data of intestinal morphology were analyzed by JMP with animal body weight as covariance and plotted by GraphPad Prism version 9 (GraphPad Software, San Diego, CA). When $P < 0.05$, the difference between treatments was significant; when $0.05 \leq P \leq 0.1$, the trend of difference was considered.

RESULTS

Release of Coated CSH Along the Gastrointestinal Digestion In Vitro

Analysis of the release rate of CSH using CCSDS revealed a significant interaction of coating technology × digestion process ($P < 0.001$, Table 2). The release rate of CSH-I was less than 10% for a 4 h-gastric digestion. In contrast, after 3 h of gastric digestion, the release rate of CSH-II was already 63.62%, and after 4 h of gastric digestion, the release rate of this coated CSH reached 83.62%. The release rate of CSH-I was 52.35% after 4 h-gastric digestion followed by 4 h of small intestine digestion, whereas CSH-II was almost completely released. Therefore, CSH-I was selected for the feeding trial because it showed an enteric release pattern.

Growth Performance

There were no significant differences in BW, ADFI, ADG and FCR of broilers fed with 200 mg/kg CSH-I compared with either the CON group or uncoated CSH group from d 7 to 21 ($P > 0.05$, Table 3). From d 22 to 42, compared with the CON group, d 42 BW ($P = 0.035$) and ADG ($P = 0.017$) was increased in the CSH-I-fed group, but there was no significant difference in ADFI ($P = 0.183$) and FCR ($P = 0.648$). During the whole experimental period, the ADFI of d 7 to 42 ($P = 0.023$) was greater in the CSH-I-fed group than the CON group and an increased trend was also observed in ADG ($P = 0.087$). However, dietary supplementation with 200 mg/kg uncoated CSH had no effects on ADFI, ADG, and FCR of broilers compared with either the CON or those fed with the CSH-I.

Table 3. Growth performance of AA broilers fed with diets supplemented with 200 mg/kg uncoated or enteric-coated cysteamine.

Items	CON[1]	CSH[1]	CSH-I[1]	SEM	*P* value
BW, g					
D7	185.1	184.8	183.8	0.8	0.757
D21	1104.2	1090.1	1117.0	53.2	0.796
D42	2614.8[b]	2774.6[ab]	2935.1[a]	119.9	0.035
ADG, g/d					
D7−21	65.7	64.7	66.7	3.8	0.795
D22−42	74.4[b]	80.2[ab]	86.6[a]	3.8	0.017
D7−42	70.9	74.0	78.6	3.4	0.087
ADFI, g/d					
D7−21	80.0	78.7	82.3	3.8	0.448
D22−42	134.3	140.8	147.1	7.3	0.183
D7−42	112.0[b]	116.0[b]	127.6[a]	5.7	0.023
FCR					
D7−21	1.22	1.22	1.24	0.03	0.664
D22−42	1.81	1.77	1.72	0.11	0.648
D7−42	1.62	1.57	1.58	0.05	0.281

[a,b]Means within a row lacking a common superscript differ ($P < 0.05$).
[1]Abbreviation: CON, corn-soybean meal diet; CSH, uncoated cysteamine; CSH-I, type 1 coated cysteamine.

Digestive Organs Indices

There were no differences in the indices of glandular and muscular stomach weight in broilers fed with 200 mg/kg CSH both in the coated and uncoated forms (Table 4). No differences were found in the length of small intestine and cecum of broiler on d 42 or the proportion of each small intestinal segment ($P > 0.05$).

Intestinal Morphology

There weren't obvious lesions in the intestinal tissues of all treatment groups (Figure 1G). In the duodenum, the villi were higher ($P = 0.006$) but the crypts tended to be shallower ($P = 0.028$) in the CSH-I group than in the CON group. Consequently, the surface area of the duodenal villi tended to be greater in the CON group ($P = 0.078$). In the jejunum and ileum, however, no significant differences in the VH, CD, VW, VH/CD, and VSA among all treatments ($P > 0.05$).

Total Tract Retention of Nutrients

Dietary supplementation of 200 mg/kg coated or uncoated CSH increased the TTR of DM ($P = 0.014$) and GE ($P = 0.024$) in d 39-old broilers (Table 5). However, dietary supplementation with the CSH-I decreased

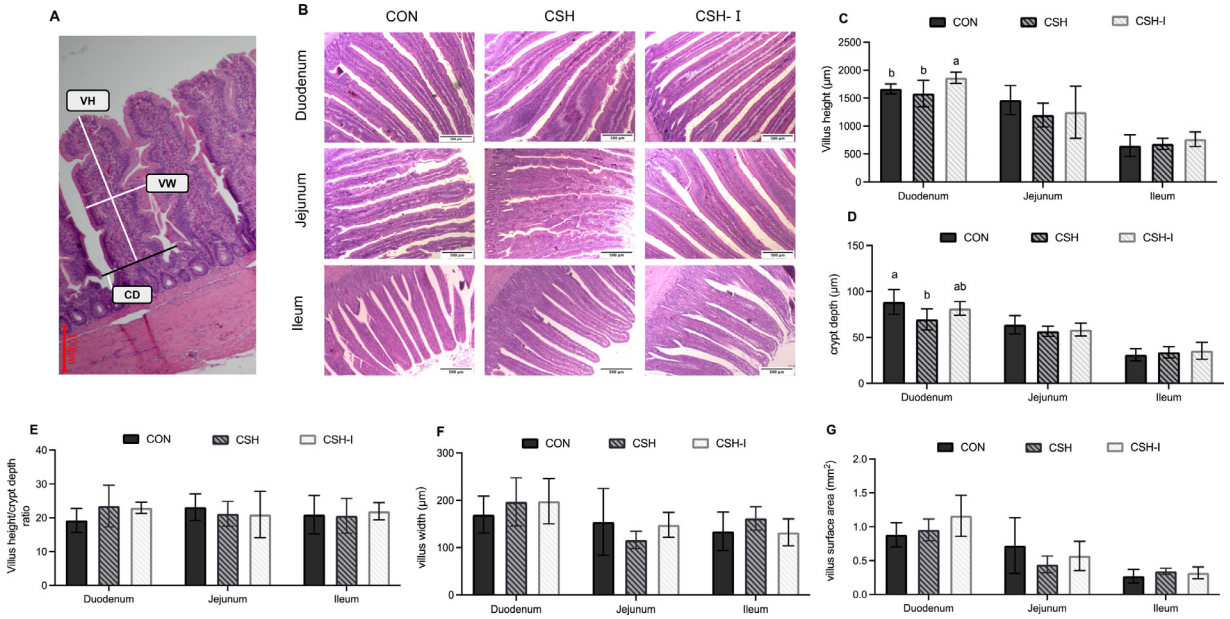

Figure 1. Morphology of the small intestine of d 42-old AA broilers fed with 200 mg/kg CSH or coated CSH-I. Sections of duodenum, jejunum, and ileum were stained with hematoxylin-eosin (H&E) and 6-10 villi and crypts per section were measured. (**A**) Illustration for villus morphology analysis (100 ×). (**B**) H&E staining sections (50 ×) for duodenum, jejunum and ileum. (**C**) Villus height (VH) for duodenum, jejunum and ileum. (**D**) Crypt depth (CD) for duodenum, jejunum and ileum. (**E**) Villus height/crypt depth (VH/CD) for duodenum, jejunum and ileum. (**F**) Villus width (VW) for duodenum, jejunum and ileum. (**G**) Villus surface area (VSA = π × VH × VW) for duodenum, jejunum and ileum. a, b means without a common letter differ ($P < 0.05$). CON, corn-soybean meal diet; CSH, uncoated cysteamine; CSH-I, type 1 coated cysteamine.

Table 4. Gastrointestinal indices of AA broiler fed with diets supplemented with 200 mg/kg uncoated or enteric-coated cysteamine.

Items	CON[3]	CSH[3]	CSH-I[3]	SEM	*P* value
Proventriculus, g[1]	3.13	2.43	2.86	0.23	0.111
Gizzard, g[1]	6.53	5.88	6.52	0.03	0.127
Small intestine, cm[1]	59.57	64.40	63.58	3.75	0.634
Cecum, cm[1]	5.33	5.00	5.40	0.37	0.731
Duodenum, %[2]	14.85	14.20	14.55	0.65	0.783
Jejunum, %[2]	45.00	44.79	45.44	0.42	0.561
Ileum, %[2]	40.15	41.02	40.01	0.51	0.357

[1]Relative to 1000 g liver weight.
[2]Relative to the full length of small intestine.
[3]Abbreviation: CON, corn-soybean meal diet; CSH, uncoated cysteamine; CSH-I, type 1 coated cysteamine.

Table 5. Total tract retention of nutrients in AA broiler fed with diets supplemented with 200 mg/kg uncoated or enteric-coated cysteamine (dry matter basis, %).

Items	CON[2]	CSH[2]	CSH-I[2]	SEM	*P* value
DM, %	69.74[b]	71.74[a]	71.27[a]	0.41	0.014
AME/GE[2], %	73.95[b]	75.59[a]	75.86[a]	0.80	0.024
N, %	44.23	50.40	48.76	3.84	0.131
EE[2], %	79.37	81.20	84.13	3.54	0.172
ADF[2], %	16.10	15.89	20.45	3.29	0.117
NDF[2], %	36.38[a]	38.34[a]	32.26[b]	2.17	0.013
Hemicellulose, %	56.68[a]	60.64[a]	45.03[b]	3.50	<0.001

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).
[2]Abbreviation: CON, corn-soybean meal diet; CSH, uncoated cysteamine; CSH-I, type 1 coated cysteamine; N, nitrogen; EE, ether extract.

the TTR of NDF ($P = 0.013$) and hemicellulose ($P < 0.001$) compared with the other two groups. There were no differences in TTR of N, EE, and ADF among the three treatment groups.

DISCUSSION

The coating process can effectively cover the pungent odor of CSH and enhance the palatability of this additive (Atallah et al., 2020a). Coated CSH can reduce the over-stimulation on gastric acid release and improve the utilization rate of CSH (Liu et al., 2019b). At the same time, the oxidation of active sulfhydryl group in the CSH and the complexation with metal ions in the feed is largely avoided by the coating process and therefore the stability of CSH is improved (Atallah et al., 2020a,b). Therefore, coating is a common process for feed additive CSH. It was noted that bio-efficacies of the same coated CSH can greatly differed in pigs and poultry (Liu, 2019). As known, the environment greatly differs in the intestine of pigs and poultry. First of all, the jejunal pH of the pig is 6.42, while the pH of the chicken is 8.12 (Hu et al., 2010; Ren et al., 2012). Secondly, activities of digestive enzymes, including pepsin (pig: 890 U/ml vs. chicken: 1500 U/ml), amylase (pig: 195.64 U/ml vs. chicken: 430.5 U/ml), trypsin (pig: 62.93 U/ml vs. chicken: 50.2 U/ml) and chymotrypsin (pig: 7.52 U/ml vs. chicken: 13.7 U/ml), were greater in the chicken than the pig (Ren et al., 2012; Wang et al., 2015). Lastly, the total passage time through the digestive tract was about 36.5 to 44.8 h in the pig (Gao et al., 2018) but 7.59 to 10.16 h in the chicken (Yu et al., 2021a). All these differences could contribute to different release patterns of the same coated CSH along the digestive tract of pig and

poultry as mentioned above. Therefore, it is important to design or evaluate those coated CSH according to the physiological conditions in the digestive tract of target animals.

In current study, the release of coated CSH in the digestive tract of broilers was investigated in the CCSDS, which simulates the gastroenteric digestion of chickens in vitro. The CCSDS was designed based upon parameters of digestion fluid, body temperature and movement of digestive tract in chickens, mimicing the enzymatic digestion process in vivo (Zhao et al., 2014). Using this CCSDS, in vitro digestible energy (IVDE) of feed materials were not only close to the measured ME in chickens but also highly correlated with the in vivo values (R^2 = 0.9998) (Zhao et al., 2014; Yu et al., 2021b). These results indicated the in vitro digestion of CCSDS well mimics the in vivo process in chickens. Using this in vitro digestion system, we found that type I coating could better resist the gastric digestion, where less than 10% CSH-I was released in the stomach; in contrast, over 80% CSH-Ⅱ was released. At half of the total passage time (4 h) in the small intestine, about 50% of CSH-I was released. Thus, the type 1 coating was considered as an enteric-coating technology for chicken and its growth-promoting effect was further verified.

In this present study, the active CSH compound is 54 mg/kg. Neither the CSH-I nor the uncoated CSH has negative effect on the feed intake and intestinal morphology of broilers. With this level of CSH inclusion, Yang et al. (2006) found that CSH could increase the activities of amylase, protease and lipase in the small intestine, suggesting CSH can improve nutrients' digestion. Here in the present study, although the TTR of N and crude fat did not significantly differ between the CSH-adding groups and the control group, the TTR of dietary DM and GE were greater in the two groups of broilers fed with CSH. It was thought that nutrients' digestion would be improved by CSH supplementation. Further analysis in digestive enzyme activity on amylase should be conducted. Interestingly, the BW, ADG and ADFI of broilers on d 42 was only increased in the CSH-I group ($P < 0.05$), rather than the uncoated CSH group. As shown by the release kinetics determined in the CCSDS, this enteric-coated CSH-I may release 80% of the CSH in the small intestine, where it can target at the small intestine to eliminate SS expression but induce expression of neuropeptides, such as Ghrelin, to promote feed intake in broilers (Seoane et al., 2000).

As an important organ of digestion and absorption, intact epithelial structure and larger surface area of small intestine are closely related to the growth performance of animals. Previous studies have shown that adding appropriate amounts of CSH to broiler diets can reduce intestinal pathogens and enterotoxins, alleviate intestinal inflammation, enhance intestinal integrity, and improve feed intake of broilers (Liu et al., 2018a,b). Although no effects of dietary CSH were noted on the organ indices for the stomach and small intestine, addition of the CSH-I indeed increased villus height, decreased crypt depth in the duodenum and tended to increase the VSA of duodenal villi. It is consistent with the findings that CSH increased duodenal V/C and muscularis thickness in goats (He et al., 2007). Hence, CSH-I technology would improve the absorption in the duodenum as well. It explained a better growth performance in broilers fed with CSH-I. It is important to note that this coating technology only affect the duodenum but not other small intestinal segments. This evidence supported the idea that the coated CSH-I was mainly released in the duodenum.

CONCLUSIONS

The current study demonstrated that the release kinetics of coated CSH in the digestive tract can be comprehensively investigated using the self-developed in vitro digestion system CCSDS to simulate the digestive tract environment and digestive process of broilers. An enteric coating technology for CSH (coated CSH-I in the present study) can assure the release of CSH mainly occurs in the small intestine, resulting in improving the morphology of the small intestine, nutrients' digestibility, and feed intake in broilers. Therefore, the release kinetics of coated CSH must be evaluated to ensure the targeted release of CSH in the small intestine to avoid over-stimulation of gastric acid secretion and achieve a greater growth-promoting effect.

ACKNOWLEDGMENTS

This work was supported by Poultry Research Team of Beijing Innovation Consortium of Agriculture Research System (BAIC06-2022) and Cooperative Project between IASCAAS-King Techina (2021-YF-15).

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Association of Official Analytical Chemists (AOAC). 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Washington, DC.

Atallah, C., C. Charcosset, and H. Greige-Gerges. 2020a. Challenges for cysteamine stabilization, quantification, and biological effects improvement. J. Pharm. Anal. 10:499–516.

Atallah, C., H. Greige-Gerges, and C. Charcosset. 2020b. Development of cysteamine loaded liposomes in liquid and dried forms for improvement of cysteamine stability. Int. J. Pharm. 589:119721.

China National Standard. 2006. Method for Determination of Neutral Detergent Fiber in Feedstuffs (GB/T 20806-2006). General Administration of Quality Supervision., Inspection and Quarantine, Beijing, P. R. China.

China National Standard. 2009. Method for Determination of Feed Additives- Cysteamine Hydrochloride Beta-Cyclodextrin Beadlets (GB/T 24832-2009). General Administration of Quality Supervision., Inspection and Quarantine, Beijing, P. R. China.

Drago, F., and C. Montoneri. 1997. Influence of growth hormone on cysteamine-induced gastro-duodenal lesions in rats: the involvement of somatostatin. Life Sci 61:21–28.

Gangoiti, J. A., M. Fidler, B. L. Cabrera, J. A. Schneider, B. A. Barshop, and R. Dohil. 2010. Pharmacokinetics of enteric-coated cysteamine bitartrate in healthy adults: a pilot study. Br. J. Clin. Pharmacol. 70:376–382.

Gao, Q. T., F. Zhao, H. Zhang, Y. Wang, and B. L. Liu. 2018. Effects of dietary crude fiber level and feeding time on passage rate of digesta in Intestinal tract of growing pigs. Chin. J. Anim. Nutr. 30:5230–5237.

He, J., Z. M. Shen, and Q. Yang. 2007. Effects of cysteamine on growth and antioxidation of small intestine in weaned goat lambs. Chin. J. Vet. Sci. 27:887–892.

Hu, Y., Y. Ni, L. Ren, J. Dai, and R. Zhao. 2008. Leptin is involved in the effects of cysteamine on egg laying of hens, characteristics of eggs, and posthatch growth of broiler offspring. Poult. Sci. 87:1810–1817.

Hu, G. Y., F. Zhao, H. F. Zhang, Y. X. Zhong, and Z. K. Liu. 2010. Effects of the source and level of dietary protein on the composition of jejunal fluid in growing pigs. Chin. J. Anim. Nutr. 22:1220–1225.

Liu, B. L. 2019. The study of encapsulated cysteamine release evaluation with gastric-small intestine simulated digestion. Master Thesis. Hebei University of Engineering. China.

Liu, H. N., M. M. Bai, B. Tan, K. Xu, R. Yu, R. L. Huang, and Y. L. Yin. 2019b. Influence of supplemented coated-cysteamine on morphology, apoptosis and oxidative stress status of gastrointestinal tract. BMC Vet. Res. 15:328.

Liu, N., L. Lin, J. Wang, F. Zhang, and J. P. Wang. 2018a. Dietary cysteamine hydrochloride protects against oxidation, inflammation, and mucosal barrier disruption of broiler chickens challenged with Clostridium perfringens. J. Anim. Sci. 96:4339–4347.

Liu, N., J. Q. Wang, Z. Y. Liu, Y. K. Chen, and J. P. Wang. 2018b. Effect of cysteamine hydrochloride supplementation on the growth performance, enterotoxic status, and glutathione turnover of broilers fed aflatoxin B_1 contaminated diets. Poult. Sci. 97:3594–3600.

Liu, B. L., F. Zhao, Z. F. Li, K. X. Chen, H. Zhang, W. Zhao, L. Yang, and H. F. Zhang. 2019a. Study on improved method of iodimetry for determination of cysteamine hydrochloride in diets,intestinal digesta and excreta. China Anim. Husb. Vet. Med. 46:395–403.

National Research Council. 1994. Nutrient Requirements for Poultry. 9th rev. ed Natl. Acad. Press, Washington, DC.

Ren, L. Q., F. Zhao, H. Z. Tan, J. T. Zhao, J. Z. Zhang, and H. F. Zhang. 2012. Effects of dietary protein source on the digestive enzyme activities and electrolyte composition in the small intestinal fluid of chickens. Poult. Sci. 91:1641–1646.

Seoane, L. M., S. Tovar, R. Baldelli, E. Arvat, E. Ghigo, F. F. Casanueva, and C. Dieguez. 2000. Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats. Eur. J. Endocrinol. 143:7–9.

Shi, Z. M., G. M. Du, J. Chen, and R. Q. Zhao. 2006. Effects of cysteamine hydrochloride on H^+-K^+-ATPase activity and somatostatin mRNA expression in gastric mucosal cells of piglets *in vitro*. Chin. J. Vet. Sci. 26:411–414.

Song, M. Q, F. Zhao, X. M. Ye, J. J. Xie, R. N. Sa, G. M. Zhang, and Y. M. Wang. 2022. A comparative evaluation on the energetic values and digestibility of fatty acids in rice bran oil and palm oil for broilers. Poult. Sci. 101:101954.

Szabo, S., S. Reichlin, J. A. Bollinger-Gruber, and A. Brown. 1992. Somatostatin depleting potency of cysteamine-related thiols and amines in the rat: structure-activity relation. J. Pharmacol. Exp. Ther. 263:752–756.

Wang, Y. M., F. Zhao, R. Liao, L. Zhang, H. Zhang, and H. F. Zhang. 2015. Correlation study between digestive enzyme activity of jejunal fluid and nutrient digestibility of diets in growing pigs. Chin. J. Anim. Nutr. 27:3033–3040.

Yang, C. M., A. G. Chen, Q. H. Hong, J. X. Liu, and J. S. Liu. 2006. Effects of cysteamine on growth performance, digestive enzyme activities, and metabolic hormones in broilers. Poult. Sci. 85:1912–1916.

Yu, Y., F. Zhao, J. Chen, Y. Zou, Y. Wang, S. Liu, and H. Tan. 2021a. Research note: effect of dietary cottonseed meal and soybean oil concentration on digesta passage time and amino acids digestibility in roosters. Poult. Sci. 100:101446.

Yu, Y., F. Zhao, J. Chen, Y. Zou, S. L. Zeng, S. B. Liu, and H. Z. Tan. 2021b. Sensitivity of in vitro digestible energy determined with computer-controlled simulated digestion system and its accuracy to predict dietary metabolizable energy for roosters. Poult. Sci. 100:206–214.

Zavy, M. T., T. O. Lindsey, and R. D. Hedde. 1988. Gizzard ulceration in chicks fed cysteamine alone or in combination with a histamine H_2-receptor antagonist. J. Vet. Pharmacol. Ther. 11:362–373.

Zhao, F., L. Zhang, B. M. Mi, H. F. Zhang, S. S. Hou, and Z. Y. Zhang. 2014. Using a computer-controlled simulated digestion system to predict the energetic value of corn for ducks. Poult. Sci. 93:1410–1420.