Research Article

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Effects of dietary coated cysteamine hydrochloride on pork color in finishing pigs

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Abstract

BACKGROUND: Coated cysteamine hydrochloride (CC) was applied as a feed additive in animal production. The influence and the mechanisms of CC used as a feed additive in promoting meat quality in finishing pigs were investigated.

RESULTS: Dietary CC supplementation increased ($P < 0.05$) the a^* and H^* values and reduced ($P < 0.05$) the L^* value in the longissimus dorsi muscles at 48 h postmortem ($P < 0.05$). The deoxymyoglobin content was enhanced ($P < 0.05$) and the metmyoglobin and malondialdehyde contents were reduced ($P < 0.05$) in pigs fed the dietary CC. Pigs fed a dietary CC of 0.035 g kg^{-1} had a lower cooking loss ($P < 0.05$) and a higher a^* (24 h) value in the longissimus dorsi muscles than pigs on control treatment. The messenger RNA expression of superoxide dismutase 1 was upregulated ($P < 0.05$) in the longissimus dorsi.

CONCLUSION: Dietary supplementation with CC could improve antioxidant status and delay meat discoloration by improving glutathione levels and antioxidase activity after longer chill storage (for 48 h after slaughter). Dietary supplementation with CC at 0.035 g kg^{-1} may promote the stability of pork color by reducing oxidation.

Keywords: coated cysteamine; antioxidant status; finishing pigs; pork color

INTRODUCTION

Meat quality is an important economic trait in livestock, with meat color being the main factor that governs consumers' buying decisions.[1,2] A cherry-red color of meat is visually appealing to consumers, whereas pale or brown meat is not usually preferred by consumers and is sold at a lower price, resulting in financial losses.[3] Meat color is affected by the interaction between many factors, animals' nutritional status, postmortem environmental conditions, and factors related to meat processing, packaging, and storage conditions.[4] A pale or brown meat results from the oxidation of heme pigment and fatty acid in muscles.[5] Hence, antioxidants as functional feed additives have been applied in animal husbandry for increasing the marketable value of meat.[6]

Cysteamine, a metabolite in animals, is used as a novel feed additive in animal production in the form of coated cysteamine hydrochloride (CC) because of its growth-promoting and antioxidant activities. Cysteamine acts as a surrogate in the glutaredoxin and thioredoxin pathways in the absence of functional glutathione (GSH), which is present in muscle cell membranes and lipid depots.[7] In a previous investigation, the effect of cysteamine on GSH synthesis was confirmed (Fig. 1). Cysteamine is an excellent scavenger of the oxidants hydroxyl radical and hypochlorous acid, reacting with hydrogen peroxide (H_2O_2).[8] The central role of GSH is to catalyze the reduction of toxic H_2O_2 and hydroperoxides. Additionally, cysteamine stimulates GSH synthesis for protecting mammalian cells from oxidative stress.[9] The micro-capsule technology of the CC facilitates its release in the intestinal tract and prevents damage to the gastric mucosa.[10]

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Meanwhile, meat color is susceptible to oxidative stress and lipid peroxidation during cryopreservation and thawing.[11,12] And supplementation of cysteamine could promote the transformation of cystine into cysteine, enhancing cysteine uptake and increasing GSH synthesis.[13] In this study we examine the hypothesis that the improved meat quality of cysteamine by increasing GSH levels and antioxidase activity could protect muscles against oxidative stress and lipid peroxidation. However, only a few studies have estimated the effect of CC supplementation on pork color. Therefore, this study aimed to examine whether coated cysteamine improves growth performance and meat quality through delaying the oxidation of heme pigment and fatty acids in muscles of finishing pigs.

MATERIALS AND METHODS

Animals, experimental design, and diets

The experimental protocol was approved by the Animal Welfare Committee of the Institute of Subtropical Agriculture, the Chinese Academy of Sciences (Permit Number: 201509-10). A total of 288 crossbred finishing pigs (Duroc × landrace × Yorkshire) with an initial body weight of 88.3 ± 0.3 kg were randomly assigned into four dietary groups, with eight pens per group and nine pigs per pen. Castrated pigs had free access to feed and drinking water, and they were fed a corn–soybean meal diet containing 0 (control), 0.035, 0.070, or 0.140 g kg^{-1} of CC for 29 days. All diets met or exceeded nutrient requirements for finishing pigs recommended by the National Research Council (2012) (Table 1).[14] CC, supplied by Hangzhou King Techina Technology Co., Ltd (Hangzhou, China), contained 270 g kg^{-1} cysteamine hydrochloride. At the end of experiment, one pig was randomly selected from each pen and was slaughtered by exsanguination after electrical stunning (250 V, 0.5 A, for 5–6 s). Samples of the longissimus dorsi muscle were collected and stored at 4 °C for assessment of meat quality. After completing meat quality analysis, some samples were frozen at −20°C until further analysis for estimation of heme pigment. Moreover, some fresh samples of longissimus dorsi muscle were trimmed and snap frozen in liquid nitrogen for molecular analysis.

Growth performance

Total body weight of each pen was recorded at the beginning (1st) and the end (30th) of the study, and the feed consumption was recorded daily throughout the experiment. Average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (FE) were calculated.

Meat quality measurements

The longissimus dorsi muscle was determined for temperature and pH value in triplicate by using a Testo 205 instrument (Testo AG, Lenzkirch, Germany) at 45 min postmortem. Drip loss was determined in duplicate according to the protocol described by Kauffman *et al.*[15] Loin samples were cut into cuboids (5 cm × 3 cm × 2 cm), weighed, and suspended on a hook from the lid of an airtight container, and then stored at 4 °C for 24 h. Subsequently, after removing surface moisture, all samples were reweighed and drip loss was calculated as

$$\text{Drip loss} = \frac{\text{Initial weight of the sample} - \text{Final weight of the sample}}{\text{Initial weight of the sample}} \times 100$$

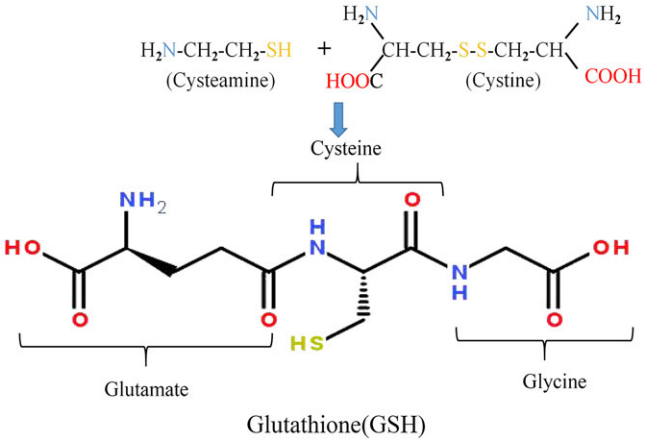

Figure 1. Mechanism of GSH synthesis by cysteamine. Cysteamine breaks the disulfide bond in cysteine. This results in formation of cysteine and improvement of GSH synthesis.

Meat color was measured with a chromameter (Konica Minota, Japan) at 1, 24, and 48 h after slaughter. Every longissimus dorsi sample was measured thrice and the values were recorded (l^*: lightness; a^*: redness; b^*: yellowness), then the measurements were averaged.[16] The hue angle H^* and chroma C^* indices were calculated as $H^* = \tan^{-1}(b^*/a^*) \times 57.29$ and $C^* = (a^2 + b^2)^{1/2}$, and their values were expressed in degrees. Cooking loss was determined by the operational method described by Josell *et al.*[17] The samples were weighed and put into cooking bags and cooked in a water bath at 80 °C until the temperature inside the samples reached 70 °C. The cooked samples were chilled to 23 ± 2 °C and reweighed. The cooking loss was calculated as

$$\text{Cooking loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Estimation of heme pigment in longissimus dorsi

The total heme pigment content comprises myoglobin (Mb), deoxymyoglobin (deoxyMb), oxymyoglobin (MbO_2), and metmyoglobin (MetMb) content.[18] The concentrations of Mb, deoxyMb, MbO_2, and MetMb in longissimus dorsi were determined by modifying the method described by Krzywicki.[19] Briefly, about 20 g of meat samples were mixed with 20 mL of 0.04 mol L^{-1} phosphate buffer (pH 6.8) using a Precellys 24 homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France). The tubes were homogenized and incubated for 1 h in ice bath. After centrifugation at 3000 × *g* for 10 min at 4 °C, the supernatants were diluted with phosphate buffer (0.04 mol $L,^{-1}$ pH 6.8) to make final volume of 25 mL. The absorbance of the extract was measured at 572, 565, 545, and 525 nm, and the relative concentrations of oxidized, oxygenated, or reduced pigment forms were calculated using the following formulas:

$$\text{Mb}\ \left[\text{mmol}/(\text{L}\ \text{L})\right] = -0.166A_{572} + 0.086A_{565} + 0.088A_{545} + 0.099A_{525}$$

$$\text{deoxyMb}\ (\%) = \left(0.369R_1 + 1.140R_2 - 0.941R_3 + 0.015\right) \times 100$$

$$MbO_2\ (\%) = \left(0.882R_1 - 1.267R_2 + 0.809R_3 - 0.361\right) \times 100$$

Table 1. Calculated composition of basal diets and nutrient content on (air-dry basis)

Ingredients (g kg^{-1})		Nutrient component[a] (g kg^{-1})	
Corn (4.52% crude protein)	580.0	Digestible energy (MJ kg^{-1})	3.12
Soybean meal (8.84%)	200.0	Crude protein	164.0
Wheat bran	80.0	Calcium	8.0
Rice bran	100.0	Total P	6.0
Vitamin premix[b]	20.0	Available P	4.0
Mineral premix[b]	20.0	Lysine	17.7
		Methionine	3.2
Total	1000	Methionine + cysteine	6.0

[a] Based on the composition of ingredients provided by the National Research Council.[14]
[b] Provided per kilogram of diet: vitamin A, 80 000 IU; vitamin D_3, 20 000 IU; vitamin E, 300 mg; vitamin K, 30 mg; vitamin B_1, 30 mg; vitamin B_2, 60 mg; vitamin B_6, 30 mg; biotin, 0.2 mg; folic acid, 10 mg; niacin, 300 mg; pantothenic acid, 300 mg; Cu ($CuSO_4 \cdot 5H_2O$), 12 mg; Fe ($FeSO_4 \cdot 7H_2O$), 150 mg; Mn ($MnSO_4 \cdot H_2O$), 5 mg; Se ($NaSeO_3$), 0.45 mg; Zn (ZnO), 150 mg.

Table 2. Primers used for quantitative real-time polymerization (qPCR)

Gene	Accession no.	Primer 5′–3′	Size (bp)
β-Actin	DQ452569	F: GGACCTGACCGACTACCTCAT R: GGGCAGCTCGTAGCTCTTCT	181
SOD1	NM_001190422	F: ACCTGGGCAATGTGACTG R: TCCAGCATTTCCCGTCT	176
SOD2	NM_214127	F: GGACAAATCTGAGCCCTAACG R: CCTTGTTGAAACCGAGCC	159
GSH-Px	NM_001115136	F: CAAGTCCTTCTACGACCTCA R: GAAGCCAAGAACCACCAG	184

GSH-Px: glutathione peroxidase; *SOD1*: superoxide dismutase 1; *SOD2*: superoxide dismutase 2; F: forward; R: reverse.

Table 3. Effects of dietary CC (g kg^{-1}) on growth performance in finishing pigs

	Dietary level of CC					
Item	0	0.035	0.070	0.140	SEM[a]	*P*-value
Initial body weight (kg)	88.06	88.07	88.62	88.3	1.56	0.999
Final body weight (kg)	107.65	109.38	111.36	110.33	1.76	0.906
ADG (kg day^{-1})	0.69	0.75	0.80	0.77	0.02	0.284
ADFI (kg day^{-1})	2.62	2.61	2.74	2.68	0.04	0.757
FE[b]	0.26	0.29	0.29	0.28	0.06	0.268

[a] SEM: standard error of the mean; $n = 8$.
[b] FE = ADG/ADFI.

$$\text{MetMb}\ (\%) = \left(-2.541R_1 + 0.777R_2 + 0.800R_3 + 1.098\right) \times 100$$

where $R_1 = A_{572}/A_{525}$, $R_2 = A_{565}/A_{525}$, and $R_3 = A_{545}/A_{525}$.

Lipid oxidation analysis (thiobarbituric acid reactive substances)

The values for thiobarbituric acid reactive substances (TBARSs) were expressed as milligrams of malondialdehyde (MDA) per kilogram of muscle. The concentration of MDA in longissimus dorsi was determined using commercial reagents (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The level of TBARS was calculated as nanomoles of MDA formed per milligram of protein. The concentration of protein was also determined using commercial reagents (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) for the calculation of MDA.

Determination of glutathione levels

The GSH content in longissimus dorsi at 48 h was also measured using the aforementioned commercial reagents according to the manufacturer's instructions. About 0.1 g frozen muscle sample was weighed and homogenized on ice in 900 μL of 9 g mL^{-1} sodium saline solution and then centrifuged at 3800 × *g* for 10 min at 4 °C. The supernatant was prepared to determine concentration of protein for calculating the content of GSH.

RNA extraction, complementary DNA synthesis, and quantitative real-time polymerase chain reaction

Total RNA was isolated from longissimus dorsi samples using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The extracted RNA was dissolved in diethylpyrocarbonate (DEPC)-treated water, and its concentration was assessed using an Eppendorf Biophotometer (Eppendorf AG, Hamburg, Germany) and its integrity verified by electrophoresis on a 1% agarose gel. After DNase I treatment (Takara, Otsu, Japan), 1 μg of total RNA was used as a template for complementary DNA (cDNA) synthesis using an Oligo(dT) primer (Takara, Japan). The resultant cDNA was diluted and used for evaluating gene expression.

All primers were developed previously for amplification of mRNA sequences of pig (*Sus scrofa*) (Table 2). The qPCR for three target genes (*SOD1*, *SOD2*, and *GSH-Px*) and the housekeeping gene (*β*-actin) were performed in a 10 μL reaction volume including 1 μmol L^1 of each forward and reverse primer, 2 μL of cDNA, 2 μL of DEPC-treated water, and 5 μL of SYBR Premix Ex Taq (Takara Bio Inc., Japan). The qPCR was carried out (Lightcycler-480I I, Roche Diagnostics GmbH, Mannheim, Germany) with the following conditions: 95 °C for 30 s, 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and elongation at 72 °C for 30 s, followed by a melting curve analysis. The relative expression of target genes was expressed as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct_{Target} - Ct_{\beta\text{-actin}})_{treatment} - (Ct_{Target} - Ct_{\beta\text{-actin}})_{control}$.

Table 4. Effects of dietary CC (g kg^{-1}) on meat quality in finishing pigs

Item[1]	Dietary level of CC				SEM[2]	*P*-value
	0	0.035	0.070	0.140		
pH (45 min)	5.89	5.88	5.76	5.85	0.052	0.812
Drip loss (%)	2	2.73	1.91	3.18	0.32	0.463
Cooking loss (%)	33.20^{a}	30.60^{b}	32.40^{ab}	31.94^{ab}	0.41	0.156
$l^*_{1\,h}$	49.55	47.20	48.44	49.70	0.615	0.464
$a^*_{1\,h}$	13.20	13.78	13.98	13.60	0.156	0.342
$b^*_{1\,h}$	4.56	3.99	4.53	4.61	0.156	0.472
$H_{1\,h}$	162.27	199.32	180.57	164.33	6.363	0.136
$C_{1\,h}$	13.97	14.36	14.73	14.37	0.176	0.531
$l^*_{24\,h}$	56.52	53.98	54.45	54.87	0.440	0.193
$a^*_{24\,h}$	15.00^{b}	16.42^{a}	15.54^{ab}	15.73^{ab}	0.221	0.151
$b^*_{24\,h}$	8.84	8.79	8.66	8.70	0.214	0.992
$H^*_{24\,h}$	86.43	99.52	93.97	93.46	2.862	0.472
$C^*_{24\,h}$	17.42	18.65	17.82	17.99	0.260	0.421
$l^*_{48\,h}$	56.48^{a}	53.05^{b}	54.18^{b}	54.81^{ab}	0.395	0.012
$a^*_{48\,h}$	14.81^{b}	16.04^{a}	16.00^{a}	14.77^{b}	0.219	0.039
$b^*_{48\,h}$	9.40	8.76	9.84	8.88	0.214	0.257
$H^*_{48\,h}$	77.87^{b}	96.78^{a}	81.67^{b}	84.42^{b}	2.325	0.017
$C^*_{48\,h}$	17.54	18.30	18.79	17.24	0.269	0.162

Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).
[1] l^*, lightness; a^*, redness index; b^*, yellowness index; H^*, hue angle; C^*, chroma.
[2] SEM: standard error of the mean; $n = 8$.

Statistical analysis

The data were expressed as means, whereas the meat color values, the pigments content, TBARS, and GSH were analyzed using the mixed procedure for repeated measures based on adjusted degrees of freedom solution. All data were analyzed statistically by one-way analysis of variance using SPSS 20 (SPSS Inc., Chicago, IL, USA). Growth performance was analyzed with pen as the experimental unit ($n = 8$). Meat traits and messenger RNA (mRNA) abundance were analyzed with pig as the experimental unit ($n = 8$). Duncan's multiple-range test was performed for indicating differences between significant mean values. The differences were declared significant at $P < 0.05$ and a trend at $0.05 < P < 0.10$ in all analyses.

RESULTS

Growth performance

The effect of dietary CC on growth performance of finishing pigs is shown in Table 3. Dietary CC levels did not affect final body weight, ADG, ADFI, or FE.

Meat quality traits

No significant differences in meat pH, drip loss, loss, b^*, and C^* values among the treatments were observed (Table 4 and Fig. 2). Compared with control treatment, dietary 0.035 g kg^{-1} CC had lower ($P < 0.05$) cooking loss in the longissimus dorsi muscles. However, pig fed the dietary 0.035 g kg^{-1} CC had the highest a^* value at 24 h ($P < 0.05$), while those fed the dietary CC levels of 0, 0.070 and 0.140 g kg^{-1} exhibited low ($P < 0.05$) a^* values. The l^* value at 48 h was decreased ($P < 0.05$) in the longissimus dorsi muscles of pig fed the dietary CC level of 0.035 and 0.070 g kg.$^{-1}$ In addition, dietary treatments supplemented with 0.035 and 0.070 g kg^{-1} of CC increased significantly ($P < 0.05$) the a^* value in the longissimus dorsi muscles. The highest ($P < 0.01$) H^* value was observed in pigs fed the dietary CC level of 0.035 g kg^{-1} at 48 h postmortem.

Heme pigment estimations, lipid oxidation, and glutathione levels

There was no significant difference in Mb content in the longissimus dorsi at 48 h postmortem compared with the control (Table 5). However, dietary CC increased the deoxyMb content ($P < 0.05$) and decreased the level of MetMb ($P < 0.05$). Furthermore, a significant difference in the MDA content ($P < 0.01$) in the longissimus dorsi muscle was measured among the treatments supplemented with different levels of dietary CC. The MDA content was higher ($P < 0.05$) in pigs fed the dietary CC of 0.140 g kg^{-1} than in those fed the other three treatment diets. The GSH content was highest ($P < 0.05$) in the group fed with the dietary CC of 0.140 g kg.$^{-1}$

Expression of antioxidant-related genes

The mRNA levels of antioxidant-related genes were determined in longissimus dorsi, as detailed in Fig. 3. The expression of *SOD1* in longissimus dorsi was significantly increased in pigs fed diets supplemented with 0.035 g kg^{-1} CC ($P < 0.01$).

DISCUSSION

In the study, dietary CC did not affect growth performance of finishing pigs. Among finishing pigs fed diets supplemented with different levels of CC, the numerically highest ADFI was observed in dietary treatments with 0.070 g kg^{-1} CC. Studies reported that dietary cysteamine supplementation increases the ADFI, with optimal responses occurring at 0.070 g kg^{-1} cysteamine.[20] A low dose of cysteamine increases the ADFI, while the higher doses

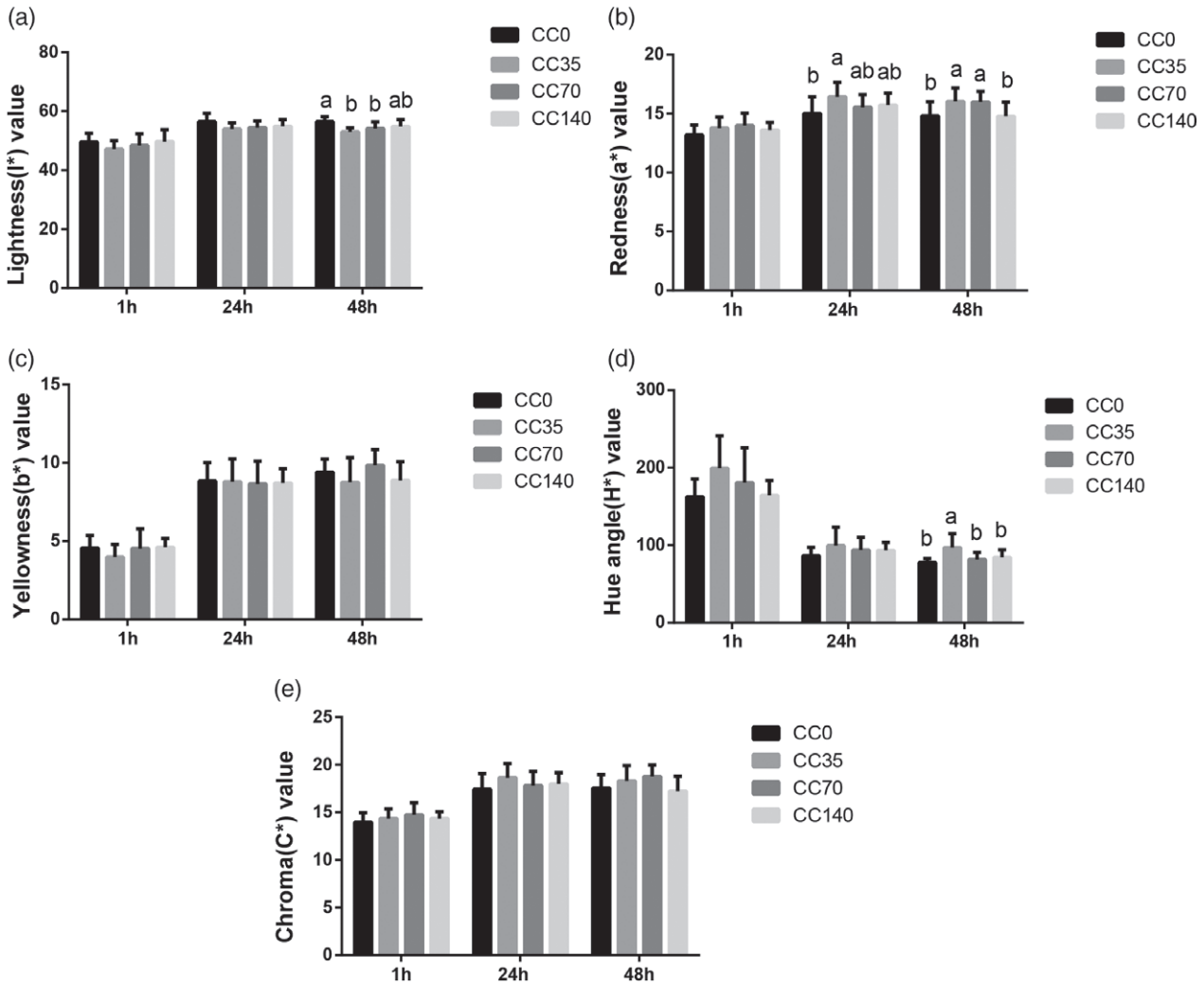

Figure 2. Evolution of instrumental color of the longissimus dorsi muscle: (a) lightness, (b) redness index, (c) yellowness index, (d) hue angle, and (e) chroma. Letters a, b above the columns indicate significant differences ($P < 0.05$) among treatments ($n = 8$). The error bars represent standard error. CC0, CC35, CC70, CC140: corn–soybean diet supplemented with 0 (control), 0.035 g kg,$^{-1}$ 0.070 g kg,$^{-1}$ and 0.140 g kg^{-1} of cysteamine respectively.

Table 5. Effects of dietary CC (g kg^{-1}) supplementation on longissimus dorsi heme pigments and lipid oxidation in finishing pigs

	Dietary level of CC					
Item	0	0.035	0.070	0.140	SEM[1]	*P*-value
Mb (mmol L^{-1})	0.03	0.03	0.04	0.05	0.002	0.466
DeoxyMb (%)	50.98^{b}	53.04^{a}	53.27^{a}	52.67^{a}	0.29	0.013
MbO_2 (%)	2.56^{b}	5.03^{a}	5.37^{a}	4.06^{ab}	0.42	0.067
MetMb (%)	21.5^{a}	14.19^{b}	13.37^{b}	17.98^{ab}	1.14	0.036
MDA (nmol/mg protein)	0.25^{b}	0.19^{b}	0.38^{b}	0.74^{a}	0.06	0.002
GSH (mg/g protein)	4.61^{b}	5.77^{ab}	6.05^{ab}	7.80^{a}	0.49	0.136

Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).
[1] SEM: standard error of the mean; $n = 8$.

of cysteamine have no effect on growth performance, or they may even have a negative impact.[21] In many studies, the positive correlation between cysteamine and growth performance and FE has been found in fishes, rats, pigs, and broilers.[22–24] In our study, no significant difference was found in feed conversion ratio, thus corroborating the study by Liu *et al.*,[25] who also found no significant difference in FE between treatments with 0.070 g kg^{-1} cysteamine and control in finishing pigs. These results indicate that increasing the feed conversion ratio by at least 8% through dietary CC might improve economic returns in swine industry.

Meat quality traits, such as pH (at 45 min postmortem), drip loss (at 24 h postmortem), and cooking loss and meat color values (at 1 and 24 h postmortem) are mainly unaffected by dietary CC in finishing pigs. Several workers have reported the rate and

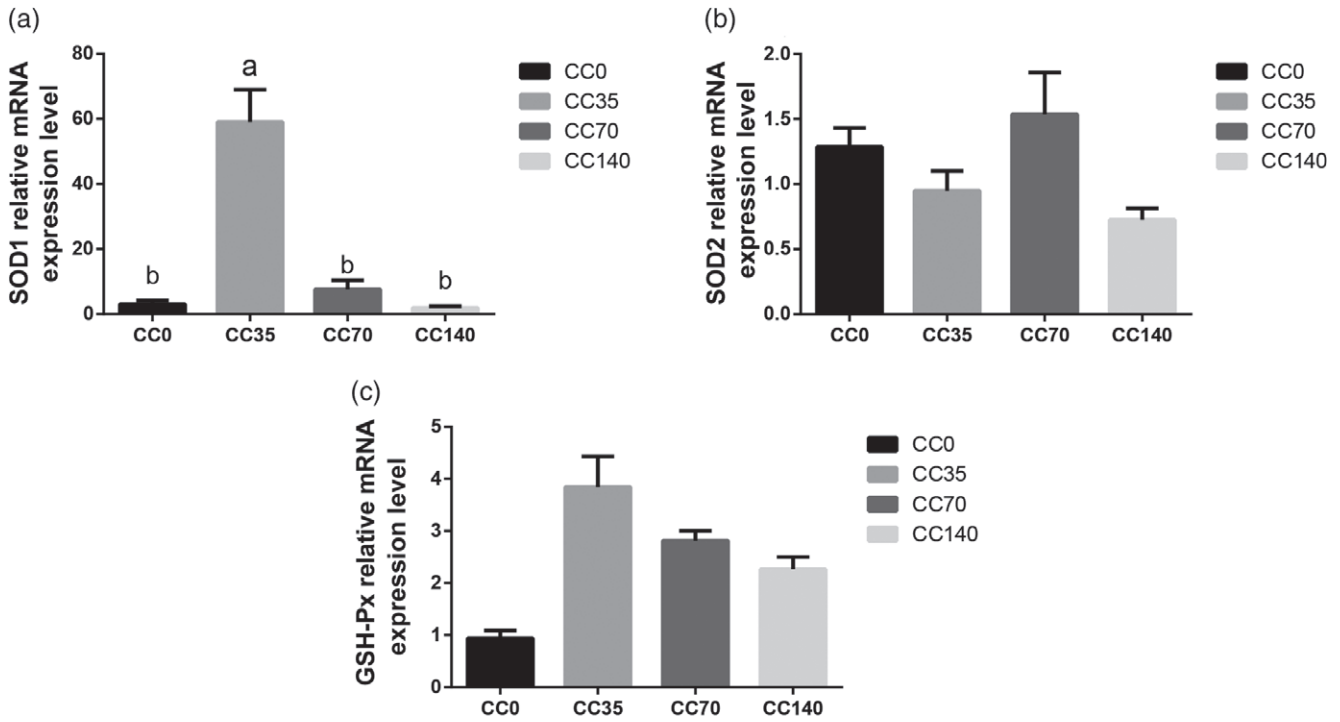

Figure 3. Effect of dietary CC on expression of antioxidant-related genes in longissimus dorsi in finishing pigs: (a) *SOD1*, (b) *SOD2*, and (c) *GSH-Px*. The relative expression was calculated as the ratio of target gene to internal reference gene. Letters a, b above the columns indicate significant differences ($P < 0.05$) among treatments ($n = 8$). The error bars represent standard error. CC0, CC35, CC70, CC140: corn–soybean diet supplemented with 0 (control), 0.035 g kg,$^{-1}$ 0.070 g kg,$^{-1}$ and 0.140 g kg^{-1} of cysteamine respectively.

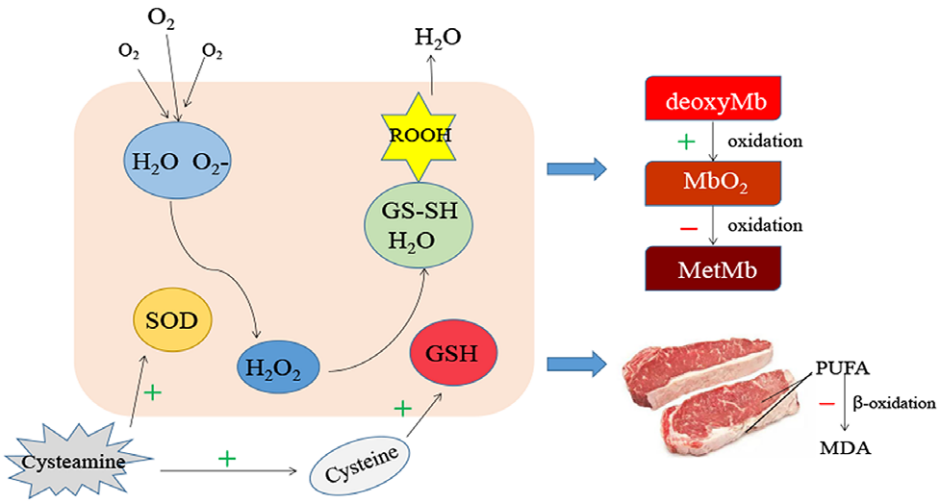

Figure 4. Proposed mechanism for preserving the pork color after long chill storage among antioxidant capacity, heme pigments, and lipid oxidation in the dietary supplementation of cysteamine.

extent of changes in meat quality due to the decline in muscle pH postmortem.[26,27] However, little information is available about the effect of CC supplement on meat quality.

An interesting development has been observed in this study: supplementation with CC affected the stability of pork color during chill storage. The values of *l**, *a**, and *H** of longissimus dorsi at 48 h postmortem were significantly affected by CC supplement as observed through different levels of color change. The lightness increased with time, although the changes in *l** during storage indicate that chill storage plays a minimal role in meat color stability.[28,29] The *a** values are frequently associated with the concentration of heme pigments and Mb oxidation in muscles,[30] whereas the *H** values are a comprehensive indicator of discoloration in meat.[31,32] Similar properties of *a** and *H** values were also found in longissimus dorsi heme pigment estimations. The results indicate that CC acted as a meat color retarder, thus further improving meat quality. The results of meat parameters indicated that the highest meat quality was achieved by adding 0.035 g of CC per kilogram feed in finishing pigs.

Meat color as a consumer's first impression of meat product has a major impact on purchase decision.[33,34] Color intensity is affected by many factors, with the majority being related to the

amount of Mb.[35] To further explain the improvement of meat color in finishing pigs after dietary supplementation with CC, heme pigment contents and lipid oxidation status played substantial roles. More specifically, different chemical forms (deoxyMb, MbO_2, and MetMb) of Mb oxygenation determine the final meat color.[36] Oxygenation of deoxyMb results in bright-red meat due to the formation of MbO_2, while oxidation of deoxyMb to MetMb causes brown discoloration.[37] The reduction of MetMb influences meat color stability and increases the shelf life of fresh meat color.[38] In this study, dietary CC increased the content of deoxyMb and decreased the content of MetMb, suggesting that the inhibition on MetMb generation by appropriate CC supplement could delay meat discoloration, although excess of CC may accelerate oxidation. Similarly, lipid oxidation was highly correlated with the levels of CC supplement. Huff-Lonergan *et al.*[39] reported that meat oxidation increased water loss from meat, resulting in increased lightness. Lipid oxidation was associated with increasing cell membrane permeability and juice loss.[40] An important role of cysteine was observed to retain the fresh meat color by slowing down the rate of conversion of MetMb into MbO_2.[41,42]

Keeping in view the oxidative damage to meat quality, especially meat color, that mainly result in the impairment of biological systems which control reactive oxygen species levels, including enzymatic (SOD, GSH-Px and catalase) and nonenzymatic antioxidant agents (GSH, α-tocopherol, and others). Alterations in GSH concentration and SOD activity have been associated with oxidative stress.[43] Thus, as the main nonenzymatic defense system in myocytes, GSH promotes both the detoxification of lipid peroxides and the removal of H_2O_2. Similarly, Rocha-Frigoni *et al.*[44] also found that cysteamine could increase intracellular GSH levels. Cysteamine, as a low molecular weight compound, enhanced cysteine-mediated GSH synthesis.[45] Furthermore, cysteamine supplementation in finishing pigs upregulated GSH synthesis and improved production of reactive oxygen species under high oxygen tension.

As is known to all, SOD is one of the most important antioxidative defense enzymes. In this study, qPCR for gene expression was conducted for examining whether dietary CC supplementation is related to the activity of antioxidases. The results indicated that SOD1 and SOD2 mRNA levels in longissimus dorsi increased significantly with the addition of dietary CC supplement. Higher levels of SOD1 mRNA expression imply improved antioxidative capacity in muscles. The antioxidative defense enzymes could scavenge intracellular and extracellular superoxide radical and protect plasma membrane against lipid peroxidative damage.[46] Meanwhile, the *SOD1* gene expression is negatively correlated with MDA values. Deleuze and Goudet[47] have reported that MDA, as a biomarker of lipid oxidation, is important for assessing oxidative stress. In addition, dietary CC supplementation prevents Mb oxygenation since CC supplementation increases SOD mRNA expression, subsequently improving antioxidative ability and ameliorating meat color. This is in agreement with Adeyemi *et al.*,[48] who reported a positive correlation between the antioxidant enzyme, SOD expression, and meat color. Collectively, coat cysteamine, as an antioxidant, improved meat color via retarding lipid oxidation and improving antioxidative ability of the muscles. Based on the results of antioxidant-related genes expression level, the best enhancement of antioxidative ability in muscles is achieved by adding 0.035 g of CC per kilogram feed in finishing pigs. A similar trend was observed for maintaining meat color.

CONCLUSIONS

Dietary supplementation with CC had a significant effect on meat color that was observed 48 h after slaughter. Optimal dosage of CC improves meat quality, especially by preserving the meat color after long chill storage, through improving antioxidative ability and slowing down lipid oxidation (Fig. 4). On the basis of the present observation, the appropriate level of dietary CC, as a meat color promoter, is 0.035 g kg^{-1} of basal diet for finishing pigs. Such dietary supplementation will be useful in the livestock industry to improve meat quality and reduce financial losses results from peroxidation in pork under 48 h storage.

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