Compensation effects of coated cysteamine on meat quality, amino acid composition, fatty acid composition, mineral content in dorsal muscle and serum biochemical indices in finishing pigs offered reduced trace minerals diet

Miaomiao Bai^{1,2}, Hongnan Liu^{1,3*}, Kang Xu^{1,3}, Xiaofeng Zhang⁴, Baichuan Deng²,
Chengquan Tan², Jinping Deng^{2*}, Pingping Bing⁵ & Yulong Yin^{1,2,3*}

¹National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, Hunan Province Key Laboratory of Animal Nutritional Physiology and Metabolic Process, Hunan Provincial Engineering Research Center of Healthy Livestock, Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China;

²College of Animal Science, South China Agricultural University, Guangzhou 510642, China;

³Hunan Co-Innovation Center of Animal Production Safety, Changsha 410128, China;

⁴Hangzhou King Techina Technology Company Academician Expert Workstation, Hangzhou King Techina Technology Co., Ltd., Hangzhou 311107, China;

⁵Academics Working Station, Changsha Medical University, Changsha 410219, China

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Dear Editor,

Trace mineral elements (ME) and amino acids have been demonstrated to be essential bioelements in animal nutrition. If there is a deficiency in ME, a number of biological functions in animals may be affected, including physical growth, psychomotor development, and immunity. Supplementation of pig diets with ME is known to improve the animals' growth, reproduction, and their immunity against oxidative stress and cell damage. Pigs that are fed diets with high-dose supplemental Zn or Cu have been shown to have an increased ADG and a decreased F:G ratio (Zhu et al., 2011). However, the pigs' ability to digest and absorb trace elements is limited, which causes environmental con-

tamination and restricts the development of animal husbandry. Therefore, formulation of diets with reduced trace mineral (Cu, Fe, Mn, and Zn) supplementation may address this problem without affecting animal performance during the finishing period. However, a deficiency of Zn can lead to failure in the synthesis of RNA, DNA, and proteins. Copper is a crucial component of myoglobin, haemoglobin, and the cytochromes, and is needed in the utilization of iron for protein synthesis. In addition, manganese has been reported to elevate the expression and protein levels of manganese superoxide dismutase (Li et al., 2016). It has also been reported that deletion of minerals during the last 6 weeks before slaughter has negative effects on pork quality (Ma et al., 2016). However, the effects of deletion of ME on protein and mineral deposition have not been investigated extensively.

Cysteamine, a new type of feed additive, influences protein digestion and absorption by regulating amino acid

*Corresponding authors (Yulong Yin, email: yinyulong@isa.ac.cn; Jinping Deng, email: dengjinpj@scau.edu.cn; Hongnan Liu, email: liuhn@isa.ac.cn)

transporters. It is usually used as a feed supplement in animal production in the form of coated cysteamine (CC). Cysteamine has been shown to play two important roles, namely regulation of mTOR signalling for modifying protein metabolism and protection of cells from oxidative stress (Bai et al., 2018; Zhou et al., 2017) (Figure 1). Previous studies have shown that antioxidants can boost the utilization of iron (Lönnerdal, 2000) and demonstrated the role of cysteamine in delaying meat discoloration through enhanced anti-oxidative activity. However, the effects of CC on utilization of trace minerals in muscle have not been elucidated. The objective of the present work was to investigate the effects of reduced dietary ME on meat quality, amino acid composition, fatty acid composition, mineral content in dorsal muscle, and serum biochemical indices in finishing pigs, as well as to test the ability of supplemental CC to compensate for these effects in finishing pigs. We hypothesized that reduction of ME or addition of CC has the potential to affect mineral retention and amino acid metabolism, and that CC may have a compensatory effect in finishing pigs that have been fed a reduced trace minerals diet.

We designed the feed experiment that 360 crossbred (Duroc $\times$ Landrace $\times$ Yorkshire) finishing gilts and barrows (180 gilts and 180 barrows) with an initial body weight (BW) of 103.9 ± 0.7 kg were randomly assigned into four treatments, with six replicates/group (fifteen pigs/replicate). Each group consisted to an equal number of gilts and barrows. All diets were composed of corn, wheat and soybean throughout the experiment. And diets for pigs during the finishing phase were equivalent to nutrient requirement, and the trace mineral requirement estimate was regarded as total trace mineral elements (TME). To test the effects of reduction of ME (Cu, Zn, Fe and Mn) or supplementation with CC, the ex-

perimental treatments was designed in a 2×2 factorial arrangement (Table S1 in Supporting Information) and was as follows: (1) a corn-soybean meal diet (control, TME); (2) control diet with 1600 mg kg^{-1} CC (TME+CC); (3) reduced trace mineral elements (RME) diet (control, RME); and (4) reduced trace mineral elements diet with 1600 mg kg^{-1} CC (RME+CC). Coated cysteamine, containing 27% cysteamine hydrochloride, was supplied by Hangzhou King Techin Technology Co., Ltd (Hangzhou, China). The pigs were weighed at the beginning of the experiment (day 0) and the day of slaughter (day 21) to compute weight gains. At the end of the feeding experiment, one pig from each pen was randomly selected and slaughtered by exsanguination after electrical stunning (250 V, 0.5 A, for 5–6 s). Serum and the longissimus dorsi muscle samples were collected for analysis.

To assess the effect of coated cysteamine on meat quality in finishing pigs fed reduced trace minerals diet, we tested drip loss, pH and meat color of longissimus dorsi (Table S2 in Supporting Information). We found that dietary RME significantly increased the pH of the longissimus dorsi at 45 min post mortem ($P < 0.05$). It indicates that the pH of the longissimus dorsi at 45 min post mortem is increased and the pH decline from 45 min to 24 h shows an increasing trend in pigs fed a diet with the deletion of ME. The increasing pH decline from 45 min to 24 h post mortem could accelerate the development of rancidity in pork (Kim et al., 2016). However, previous research found that the pH at 45 min post mortem and pH decline were not affected by the trace mineral removal. The discrepancy can be attributed both to differing amounts of trace mineral addition and to environmental stress. The pH decline from 45 min to 24 h post mortem tended to increase in pigs fed the RME diet

Figure 1 Effects of dietary cysteamine on improving antioxidant and accelerating protein deposition in the muscle of finishing pigs. (1) cysteamine could improve protein deposition by increasing the production of insulin-like growth factor (IGF-1) in the plasma and stimulating the Akt/mTOR signaling pathway; (2) cysteamine not only break the disulfide bond into cysteine for synthesizing glutathione (GSH) but also increase the activity of superoxide dismutase (SOD) for improving antioxidant status.

($P<0.10$). No significant difference related to either RME or CC was observed in other meat quality parameters. Based on the present results, the meat quality characteristics were not affected by the addition of CC or by RME diets. This was consistent with Wang et al. (2011), who indicated that cysteamine supplementation did not cause detrimental effects on meat quality. Nevertheless, Bai et al. (2018) reported that finishing pigs fed diets containing CC of 0.035 g kg^{-1} had a better meat colour at 24 h in the longissimus dorsi muscles. This difference might be related to different levels of supplementation.

We next investigated whether serum biochemical indices, amino acid composition and fatty acid composition of the longissimus dorsi muscles were affected when pigs fed supplemental coated cysteamine in reducing trace minerals diet. Amino acid content is determined to evaluate the biological value of muscle protein by following the procedure described by Lv et al. (2018). In the present study, addition of CC significantly enhanced ($P<0.05$) the concentration of TAA, Cys, Cysthi and Orn, and had a tendency to increase ($P<0.10$) the concentration of EAA and Gly (Table S3 in Supporting Information). This effect may enhance protein deposition through modifying amino acid metabolism in the pigs. As described by Zhou et al. (2015), cysteamine supplementation could promote protein deposition by stimulating the growth hormone (GH) secretion in skeletal muscle. The urea nitrogen balance indicates that adding CC to diets reduced the urea level in the longissimus dorsi muscle but increased the level in the serum. Moreover, reducing dietary TME tended to reduce ($P<0.10$) the concentration of Leu and increase the concentrations of Cit ($P<0.05$) and Tyr ($P<0.10$) in the longissimus dorsi (Table S3 in Supporting Information). The levels of ME in diets have been shown to be closely related to protein metabolism. The results indicate that deletion of TME in diet has negative effects on protein retention in muscle. Besides, reducing dietary TME decreased ($P<0.05$) the AST activity and LDH activity in the serum. And AST activity, as a biomarker for liver function and urea nitrogen and creatinine levels, is used to estimate glomerular filtration and renal functions. LDH, which catalyses the redox reaction between pyruvate and lactic acid, is an important enzyme in glycolysis and gluconeogenesis. The results indicate that the deletion of TME promotes the degradation of arginine and limits the process of amino acid synthesis, whereas supplementing an RME diet with CC enhances the concentrations of Cys and Cysthi. It seems likely that CC performs a compensatory action in finishing pigs fed an RME diet. However, further studies are required to investigate why the compensatory action of CC could be realized when reducing RME in diets.

Modification of the fatty acid composition of meat is strongly associated with differences in nutritional value and sensory attributes. Fatty acid composition of the longissimus

dorsi was determined by direct transesterification described by Demirel et al. (2004). We found that no significant difference in the fatty acid composition of the longissimus dorsi was observed when TME in diets was reduced ($P>0.05$). Dietary CC supplementation decreased ($P<0.05$) the amount of c20:0 and c20:1 in the longissimus dorsi (Table S4 in Supporting Information). This might be associated with a reduction in somatostatin levels and an increase in GH secretion in the serum or tissue (Ma et al., 2016). Lipid mobilization is affected by the GH and other pituitary extracts. Thus, cysteamine may regulate fatty acid composition of the longissimus dorsi muscle by stimulating GH secretion. Furthermore, there are interaction effects on the quantity of c18:1n9t and PUFA in the longissimus dorsi. A higher PUFA content was associated with an improved meat flavour and accelerated pigment and lipid oxidation in meat (Ponnampalam et al., 2012).

We next investigated the effect of minerals content of the longissimus dorsi of pigs fed with an RME diet augmented by CC. The contents of mineral substances (including K, Ca, Cu, Fe, Mn, Zn, Cd, Cr, and Pb) was determined according to Cheng et al. (2016), through a Varian ICP-OES (ICP 720 ES; Agilent, USA). The results showed that dietary RME significantly decreased ($P<0.05$) the concentration of Cd in the longissimus dorsi. The addition of CC to an RME diet had interaction effects on the concentration of Cr in the longissimus dorsi ($P<0.05$) (Table S5 in Supporting Information). Meanwhile, our findings reveal that dietary RME decreases the content of Ca in the serum and reduces the deposition of Ca in muscle (Table S6 in Supporting Information). The level of serum calcium was closely related to the functions of muscle contraction and normal nerve conduction. Moreover, an RME diet reduced the deposition of Cd, which poses a serious threat to human and animal health, in the longissimus dorsi. Therefore, the effect of RME on the deposition of beneficial trace elements in muscle was negative. Copper and Mn are involved in the synthesis of antioxidant enzymes, including cytosolic enzyme and superoxide dismutase, which cells from oxidative damage. Dietary CC significantly increased ($P<0.05$) the concentration of Cu, and had a tendency to increase ($P<0.10$) the concentration of Mn in the longissimus dorsi (Table S5 in Supporting Information). The antioxidant capacity of cysteamine may be likely attributed to increasing the deposition of Cu and Mn in the muscle. The actual mechanism need to be further proven.

Understanding the scope of compensation effects of coated cysteamine on finishing pigs offered reduced trace minerals diet is critical for basic research and applications. Here we reveal that reduction of trace minerals in pig diets increased $\text{pH}_{45\text{ min}}$ and decreased the concentration of Cd in the longissimus dorsi, and decreased serum Ca level, the activities of AST and LDH. It may cause a decrease in the deposition of minerals in the pork. But supplemented CC enhanced the

contents of TAA, Cys, Cysthi and Orn, and increased the concentrations of Cu and Mn, and reduced the levels of c20:0 and c20:1 in the longissimus dorsi. CC may have a compensatory effect on muscle nutrients deposition in finishing pigs fed a reduced trace minerals diet. We believe this contribution is theoretically and practically relevant because these findings can be applied in livestock industry.

Compliance and ethics The author(s) declare that they have no conflict of interests.

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SUPPORTING INFORMATION

Table S1 Basal diet composition for finishing pigs

Table S2 Effects of coated cysteamine on meat quality in finishing pigs fed reduced trace minerals diet

Table S3 Effects of coated cysteamine on dorsal muscles free amino acid compositions in finishing pigs fed reduced trace minerals diet

Table S4 Effects of coated cysteamine on dorsal muscles fatty acid compositions in finishing pigs fed reduced trace minerals diet

Table S5 Effects of coated cysteamine on dorsal muscles mineral content in finishing pigs fed reduced trace minerals diet

Table S6 Effects of coated cysteamine on serum biochemical parameters in finishing pigs fed reduced trace minerals diet

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