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Effects of Dietary Supplementation with Cysteamine on Growth Hormone Receptor and Insulin-Like Growth Factor System in Finishing Pigs

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The present study was conducted to test the hypothesis that chronic cysteamine (CS) supplementation may affect serum insulin-like growth factor (IGF)-I concentrations and growth hormone (GH) receptor (GHR), IGF-I, IGF-I receptor (IGF-IR), IGF binding protein (IGFBP)-3, and insulin receptor (IR) mRNA levels in different tissues of finishing pigs. A total of 24 finishing pigs (60.05 ± 1.24 kg; 12 gilts and 12 barrows) were assigned randomly to one of the three dietary groups, with four pens/group (per pen: one gilt, one barrow). The pigs were fed a basal diet containing 0 (control), 70, or 140 mg/kg cysteamine feed additive (containing 28% cysteamine hydrochloride) for 47 days. The results indicated that CS supplementation (70 mg/kg) increased the average daily gain (ADG) and serum IGF-I level, upregulated mRNA levels of GHR and IGF-I (liver, stomach, muscle), IGF-IR (stomach, duodenum, muscle), and IGFBP-3 (liver) but downregulated IGFBP-3 (stomach, duodenum, muscle). CS supplementation (70 mg/kg) did not affect mRNA levels of GHR and IGF-I (duodenum), IGF-IR (liver), and IR (liver, stomach, duodenum, muscle). CS supplementation (140 mg/kg) downregulated GHR (duodenum), IGF-I, and IGF-IR mRNA (liver, stomach, duodenum, muscle) but upregulated IGFBP-3 and IR mRNA (liver, stomach, duodenum, muscle) and did not affect ADG and serum IGF-I concentration. Collectively, the results suggest that dietary CS supplementation modulates the growth rate, serum IGF-I concentrations, and the gene expression of GHR, IGF-I, IGF-IR, IGFBP-3, and IR in a dose-dependent manner. CS supplementation has tissue-specific regulation of GHR, IGF-I, IGF-IR, and IGFBP-3 mRNA levels. Moreover, the results also imply the possible physiologic role of the GH-IGF axis in mediating the dietary CS supplementation-supported growth of finishing pigs.

KEYWORDS: Cysteamine; finishing pigs; gene expression; growth; GH-IGF axis

INTRODUCTION

Growth in pigs is regulated in large part by the brain neuroendocrine growth hormone (GH)-insulin-like growth factors (IGFs) axis (*1*). The complex neuroendocrine control of GH release has been extensively reviewed (*2, 3*). In essence, the chief hypothalamic regulators of GH release are growth hormone releasing hormone (GHRH) and somatostatin (SS), which are subject to modulation by other hypothalamic peptides and by complex networks of neurotransmitter neurons. GHRH is able not only to stimulate pituitary GH secretion but also to promote hypothalamic SS output, thus starting an autoregulatory circuit, whereas SS inhibits both GH release from the pituitary gland and GHRH secretion from the hypothalamus.

The inhibiting action of SS may provide an alternative means of accelerating growth because the 14 or 28 amino acid residuals of SS containing an S−S bond are potent inhibitors of endogenous GH secretion (*1*). One of the more elegant techniques is represented by the active or passive immunization of animals against endogenous regulatory peptides, for instance, SS, to immunoneutralize them (*1, 4, 5*). In fact, the application of this method in endocrinology has proven to be feasible and has provided remarkable success in some tests with rats, sheep, and fish (*5–8*). However, the growth-promoting effects of anti-SS require further studies, as the results obtained to date provide reasons for optimism with regard to the application of this methodology (immunization of animals against SS) for the examination of pig growth physiology (such as body growth, muscle development, and endocrine system).

Cysteamine (CS; mercaptoethylamine, $HS-CH_2-CH_2-NH_2$) is biologically derived from cysteine metabolism. It is an agent that works as a specific inhibitor of SS in animal production to affect the endocrine system and improve the growth rate of fish, piglets, and finishing pigs (*8–10*). Although GH and IGF-I have more direct effects in the field of animal food enhancement to improve economic returns, CS seems to be more applicable for farmed animals such as pigs. Thus, CS may become a new candidate as a growth-promoter for pigs. Previous experiments involving rats, sheep, fish, and piglets have demonstrated that

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CS increases GH secretion (*5–7, 11, 12*). The increase in GH secretion is possibly due to the decreasing levels of SS in the tissue and hypothalamus in response to the action of CS. The GH-IGF axis, which includes GH, GH receptor, IGF-I, IGF-I receptor (IGF-IR), and IGF-binding proteins that are found in animals (*13*), is believed to play a key role in the regulation of growth (*14*). The production of IGF-I depends on the actions of GH. IGF-I produced by the liver and other tissues is considered to be the prime effector of GH actions on growth and development. IGF-I produced by the liver is secreted into the circulation and has an endocrine action on target tissues (*15, 16*). In addition, IGF-I is also produced in most extrahepatic tissues and can function as an autocrine and/or paracrine growth stimulator (*15, 16*). The biological actions of IGF-I are mediated mainly through the IGF-IR and partly through the insulin receptor (IR) (*17*). Furthermore, IGF binding proteins (IGFBPs) are important modulators of the biological actions of IGF (*18*). However, there is no information about continuous long-term CS supplementation on the secretion and gene expression of the GH-IGF system in any mammalian in vivo system. As is well-known, the liver is the main source of circulating IGF and IGF-binding proteins. Moreover, the muscle growth can improve the animal body growth. The growth and maturation of the stomach and duodenum can enhance digestion and absorption of nutrients and then improve body growth. In order to explore the mechanism responsible for the growth-promoting effect of CS supplementation, we have tested the hypothesis that chronic CS supplementation may affect serum IGF-I concentrations and GHR, IGF-I, IGF-IR, IGFBP-3, and IR mRNA levels in the above-mentioned tissues of finishing pigs.

MATERIALS AND METHODS

Experimental Animals and Diets. The experimental protocols used in this study were approved by the Sichuan Agricultural University Institutional Animal Care and Use Committee. A total of 24 crossbred (*PIC* variety) finishing pigs (average initial body weight was 60.05 ± 1.24 kg; 12 gilts and 12 barrows) were used. The pigs were assigned randomly to one of the three dietary groups, with four pens/group (two pigs/pen; per pen: one gilt and one barrow). Each pen was equipped with a feeder and nipple water to allow the pigs free access to feeds and drinking water. Temperature (22−26 °C) and a cycle of 16 h light/8 h dark were maintained in the mechanically ventilated room. The pigs were fed a basal diet containing 0 (control), 70, or 140 mg/kg cysteamine feed additive (supplied by Fujian Minke Biology Co., Ltd., containing 28% cysteamine hydrochloride with starch and dextrine as carriers for stabilization. 70, the suitable quantity; 140, excess of the safety dosage; the quantity had been determined through growth performance, cysteamine dose−response and gastrointestinal tract relationship, whose data did not shown in this study). The basal diet met or exceeded nutrient requirements recommended by the National Research Council (NRC) (*19*) for pigs (**Table 1**).

Pen pig weight and feed intake were measured weekly. The experimental feeding period lasted for 47 days, during which the pigs' body weights increased from 60 kg to approximately 96 kg. Eight hours after the last feeding, blood samples were collected (0800) through the anterior vena cave puncture into 10 mL heparin-free Vacutainer tubes. Blood was centrifugated at 3500*g* for 5 min to obtain serum, which was stored at −20 °C until analysis for GH and IGF-I. One pig from each pen (per group: two gilts and two barrows) was selected randomly and killed by exsanguinations after electrical stunning. The proximate 6 cm of the duodenum beginning 2 cm distal to the pylorus, part of the fundus gland, liver, and longissimus muscle tissues were removed. The samples were quickly frozen in liquid nitrogen and were stored at −80 °C until use for RNA.

Measurement of GH and IGF-I in the Serum. The determination of serum GH was performed by using a pig GH immunoradiometric assay (RIA) kit (Shanghai Institute of Biological Products, Shanghai, China), which used a purified recombinant pig GH as the standard, a first antibody raised in guinea pigs, and a goat antiguinea pig IgG for the bound/free GH separation. A standard curve was prepared at concentrations from 0 to 50 ng/mL. All samples were analyzed in duplicate. The sensitivity of the assay was 0.24 ng/mL. The intra-assay and interassay coefficients of variation were <9.3 and <10.2%, respectively. All steps of the RIA for the serum GH were performed according to the kit protocol.

Concentration of IGF-I in the serum was determined by using a pig IGF-I kit (Diagnostic System Laboratories Inc., Webster, TX), which used purified recombinant pig IGF-I as the standard, a first antibody raised in guinea pigs, and a goat antiguinea pig IgG for the bound/free IGF-I separation. An acid/ethanol extraction step was added to separate IGF binding protein (*20*). A standard curve was prepared at concentrations from 0 to 1000 ng/mL. All samples were analyzed in duplicate. The sensitivity of the assay was 2.02 ng/mL. The intra-assay and interassay coefficients of variation were <6.8 and <7.1%, respectively.

Total RNA Isolation and Reverse Transcription (RT). In the experiment, the total RNA was isolated from the samples (~100 mg) using TRIzol reagent (TaKaRa, Shiga, Japan), according to the manufacturer's protocol. RNA was treated with RNase-free DNase I (TaKaRa, Shiga, Japan) to remove any contaminating DNA. The treated RNA was dissolved in RNA-free water and quantified using a Beckman DU800 spectrophotometer (Beckman Counter, Fullerton, CA) at OD260. An RNA aliquot was verified for its integrity by electrophoresis in 1% agarosis gel stained with goldview (SBS Genetech, Beijing, China). Then 2 μg of total RNA was reverse-transcribed in a 40 μL reaction mixture according to PrimerScript RT reagent kit specifications (TaKaRa, Shiga, Japan). The RT products (cDNA) were stored at −20 °C for analysis of GHR, IGF-I, IGF-IR, IGFBP-3, and IR mRNA levels by real-time PCR.

Real-Time PCR for Quantification of GHR, IGF-I, IGF-IR, IGFBP-3, and IR. Real-time quantitative PCR was performed using Bio-Rad iQ5 Optical System (Bio-Rad Laboratories, Hercules, CA) and DNAmo SYBR Green qPCR commercial kits (TaKaRa, Shiga, Japan), in which SYBR Green I was a double-stranded DNA-specific fluorescent dye. β-Actin was used as the housekeeping gene. Sequences of primers used for quantitative real-time PCR analysis are shown in **Table 2**. Gene expressions determined by real-time PCR were expressed

Table 1. Ingredient Composition of the Basal Diet (on an As-Fed Basis)

ingredient	g/kg
corn	649.7
soybean meal	191.0
rapeseed meal	80.0
whey power	45.0
rapeseed oil	11.4
calcium carbonate	8.4
dicalcium phosphate	2.2
L-lysine · HCl	3.5
sodium chloride	2.5
choline chloride	1.0
vitamin premix[a]	0.3
mineral mix[b]	5.0
cysteamine[c]	
calculated composition (g/kg)	
digestible energy (MJ/kg)	13.44
crude protein	170.0
calcium	5.0
phosphorus available	1.9
lysine	8.5
methionine	2.2
methionine + cystine	4.8

[a] Premix provided per kilogram diet: retinol acetate, 1926.4 μg; cholecalciferol, 27.5 μg; dl-α-tocopheryl acetate, 30 g; vitamin B_{12}, 30 mg; riboflavin, 15 g; niacin, 15 g; calcium pantothenate, 25 g; folic acid, 20 mg; thiamin, 12 mg; pyridoxine, 6 mg; vitamin K_3, 5 mg. [b] Mix provided per kilogram diet: Mn, 6 mg (MnO); Fe, 150 mg ($FeSO_4 \cdot H_2O$); Cu, 10.5 mg ($CuSO_4 \cdot 5H_2O$); I, 0.42 mg (ethylene diamine dihydroiodide); Se, 0.45 mg (Na_2SeO_3); Zn, 150 mg (ZnO). [c] In the cysteamine (CS)-supplemented diets, 70 or 140 mg/kg CS was added at the expense of corn.

Table 2. Sequences of Primers Used for Quantitative Real-Time PCR Analysis

target gene[a]		primer sequence	product size (bp)	GeneBank accession no.
β-Actin	sense	TGCGGGACATCAAGGAGAAG	216	U_07786
	antisense	AGTTGAAGGTGGTCTCGTGG		
GHR	sense	CTCGATATTGATGACCCTGA	344	X_54429
	antisense	GATGAGTTGAGTCAGTTCCA		
IGF-I	sense	GTAACCATGAGGCTGAGAAG	254	X_64400
	antisense	AACACAGGTTCCGTCCATGA		
IGF-IR	sense	GCTGAACAGGCTCAACCC	377	NM_214172
	antisense	CGACGCCTTCATAAACCA		
IR	sense	AAACGCCAGGGACATCGTCAAGG	238	AF_102858
	antisense	CCGCAGGGAACGCAGGTAACTCT		
IGFBP-3	sense	AGCACGGACACCCAGAACTT	62	AF_085482
	antisense	CGGCAAGGCCCGTATTC		

[a] GHR, growth hormone receptor; IGF-I, insulin-like growth factor-I; IGF-IR, IGF-I receptor; IR, insulin receptor; IGFBP-3, insulin-like growth factor binding protein-3.

as ratios between the RNA for the gene of interest and that for β-Actin. Reactions were performed in a 25 μL volume with 12.5 μL of SYBR Premix Ex Taq (TaKaRa, Shiga, Japan), 2.0 μL of cDNA, 9.5 μL of double distilled H_2O, and 0.5 μL of primer pairs (10 μmol/L forward and 10 μmol/L reverse). Cycling conditions were 95 °C for 10 s, by 45 cycles (95 °C for 5 s, with different annealing temperatures for different target gene for 30 s), followed by 95 °C for 1 min and 55 °C for 1 min. The melting curve program was 55−95 °C with a heating rate of 0.5 °C/30 s and a continuous fluorescence measurement. The annealing temperatures for GHR, IGF-I, IGF-IR, IGFBP-3, IR, and β-Actin were 64, 61, 60, 63, 62, and 61 °C, respectively. To confirm specific amplifications, the PCR products from each primer pair were subjected to a melting curve. The melting curve was determined by holding the reaction at 55 °C for 30 s and then heating slowly to 95 °C with a linear rate of 0.2 °C/s while the fluorescence emitted was measured. Melting curves were generated by plotting fluorescence against temperature. The melting curve analysis demonstrated that each of the primer pairs described amplified a single product with a distinct melting temperature. The predicted length of each product was confirmed by agarose gel electrophoresis. PCR amplification products were sequenced to confirm identity. The linearity of the dissociation curve was analyzed using the iQ5 Optical system software, and the mean cycle time of the linear part of the curve was designated Ct. The β-Actin housekeeping gene was amplified for each sample to verify the presence of cDNA and as an internal control to calculate the relative levels of target gene expression for GHR, IGF-I, IGF-IR, IGFBP-3, and IR using the $2^{-\Delta\Delta Ct}$ method (*21*). In brief, each experiment was analyzed in triplicate and normalized to β-Actin using the following equation: $\Delta Ct_{GENE} = Ct_{GENE} - Ct_{\beta\text{-Actin}}$. The fold change of the target gene was presented as a value relative to the change in each respective tissue of the control pig. The fold change, relative to the control pig, was calculated using the following equation: $2^{(\Delta\Delta CtGENE)}$, where $\Delta\Delta Ct_{GENE} = \Delta Ct_{GENE}$ of each respective tissue of the control pig − ΔCt_{GENE} of the tissue of each CS-supplemented pig. Values represent the mean fold change ± SEM.

Statistical Analysis. The fold change of GHR, IGF-I, IGF-IR, IGFBP-3, or IR was presented as a value relative to the change in each respective tissue of the control pig. The data for all parameters determined were analyzed statistically by single factorial variance analysis using the general linear model procedure of SPSS 11.0 software (SPSS Inc., Chicago, IL). Data sets were further analyzed using post hoc tests (least significant difference, LSD) for multiple comparisons to determine the statistical differences between groups, which were denoted by different letter superscripts. Individual pigs were used as the experimental unit for all data except that pen was used as the unit for the average daily gain (ADG), feed intake (ADFI), and feed efficiency. Data are expressed as means ± SEM. Results were considered significant at $p < 0.05$.

RESULTS

Effects of Dietary CS Supplementation on the ADG, ADFI, Feed Efficiency, and Levels of Serum GH and IGF-I. The ADG and ADFI (70 mg/kg) were increased ($p < 0.05$) by 19.5 and 15.2%, respectively (**Figure 1A** and **B**); however, the ADG and ADFI (140 mg/kg) and feed efficiency were not affected (**Figure 1**). The serum GH and IGF-I levels (70 mg/kg) were increased ($p < 0.05$) by 43.1 and 55.2%, respectively; however, the serum GH and IGF-I levels (140 mg/kg) were not affected ($p > 0.05$) compared with the control pigs (**Figure 1D** and **E**).

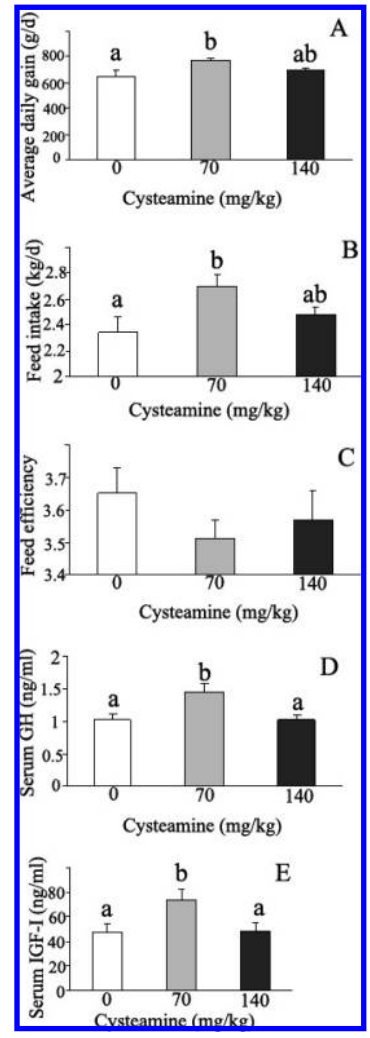

Figure 1. Effects of dietary cysteamine (CS) supplementation on average daily gain (**A**), feed intake (**B**), feed efficiency (**C**), serum growth hormone (GH) levels (**D**), and insulin-like growth factor (IGF)-I levels (**E**) in finishing pigs. Data represent the means ± SEM, $n = 4$. Different superscripts indicate significant difference ($p < 0.05$).

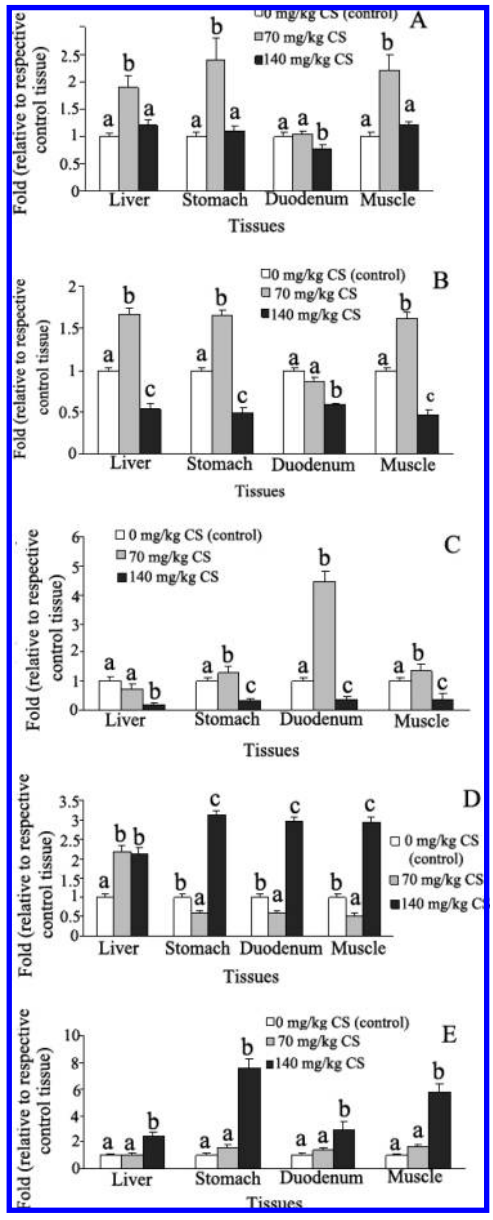

Figure 2. Effects of dietary cysteamine (CS) supplementation on growth hormone receptor (GHR) (**A**), insulin-like growth factor (IGF)-I (**B**), IGF-I receptor (IGF-IR) (**C**), IGF-binding protein (IGFBP)-3 (**D**), and insulin receptor (IR) (**E**) mRNA levels in several tissues of finishing pigs. The fold change of GHR, IGF-I, IGF-IR, IGFBP-3, or IR mRNA level was presented as a value relative to the change in each respective tissue of the control pig. Data were normalized to β-Actin. Values represent mean fold change $\pm$ SEM, $n = 4$. Different superscripts within each tissue indicate significant difference ($p < 0.05$).

Effect of Dietary CS Supplementation on the Expression of GHR Gene. Compared with the control pigs, GHR mRNA levels (70 mg/kg) within the liver, stomach, or muscle tissue were upregulated ($p < 0.05$) by 90, 140, and 120%, respectively, but were not affected within the duodenum. GHR mRNA (140 mg/kg) was downregulated ($p < 0.05$) by 22% within the duodenum tissue. However, GHR mRNA was not affected within the liver, stomach, or muscle relative to the control pigs (**Figure 2**A).

Effect of Dietary CS Supplementation on the Expression of IGF-I Gene. Compared with the control pigs, IGF-I mRNA levels (70 mg/kg) within the liver, stomach, or muscle tissue were upregulated ($p < 0.05$) by 66, 65, and 62%, respectively, but were not affected within the duodenum. IGF-I mRNA (140 mg/kg) were downregulated ($p < 0.05$) by 46, 51, 42, and 54%, respectively, within the liver, stomach, duodenum, or muscle tissue relative to the control pigs. IGF-I (140 mg/kg) were downregulated ($p < 0.05$) by 67.5, 70.3, 33.3, and 71.6%, respectively, within respective tissues compared with 70 mg/kg CS pigs (**Figure 2B**).

Effect of Dietary CS Supplementation on the Expression of IGF-IR Gene. Compared with the control pigs, IGF-IR mRNA levels (70 mg/kg) within the stomach, duodenum, or muscle tissue were upregulated ($p < 0.05$) by 31, 346, and 38%, respectively, but were not affected within the liver tissue. IGF-IR mRNA levels (140 mg/kg) were downregulated ($p < 0.05$) by 82, 69, 63, and 64%, respectively, within the liver, stomach, duodenum, or muscle tissue relative to the control pigs. IGF-IR levels (140 mg/kg) were downregulated ($p < 0.05$) by 75, 76.3, 91.7, and 73.9%, respectively, within respective tissues compared with 70 mg/kg CS pigs (**Figure 2C**).

Effect of Dietary CS Supplementation on the Expression of IGFBP-3 Gene. Compared with the control pigs, IGFBP-3 mRNA levels (70 mg/kg) within the stomach, duodenum, or muscle tissue were downregulated ($p < 0.05$) by 41, 42, and 48%, respectively, but were upregulated ($p < 0.05$) by 119% within the liver tissue. IGFBP-3 mRNA levels (140 mg/kg) were upregulated ($p < 0.05$) by 114, 212, 196, and 194%, respectively, within the liver, stomach, duodenum, or muscle tissue relative to the control pigs. IGFBP-3 levels (140 mg/kg) within the stomach, duodenum, or muscle tissue were upregulated ($p < 0.05$) by 428.8, 410.3, and 465%, respectively, but were not affected within the liver compared with 70 mg/kg CS pigs (**Figure D**).

Effect of Dietary CS Supplementation on the Expression of IR Gene. Compared with the control pigs, IR mRNA levels (70 mg/kg) within the liver, stomach, duodenum, or muscle tissue were not affected ($p > 0.05$); however, 140 mg/kg CS pigs had upregulated ($p < 0.05$) IR mRNA levels within the liver, stomach, duodenum, or muscle tissue (**Figure 2E**).

DISCUSSION

An improvement in pig growth performance for economic purposes can be achieved by enhancing growth rate. The growth rate of animals is affected by their interactions with the environment, including food abundance and quality. The effects of these on growth rate are in turn modulated by the endocrine system. CS supplementation enhances the growth rate of pigs. However, the effects of chronic CS supplementation on the secretion and gene expression of the GH-IGF axis components have, to date, not been investigated in finishing pigs.

In the present study, dietary CS supplementation (70 mg/kg) caused significant increase in the growth rate and feed intake of finishing pigs. The improvement of the growth rate may result from both feed intake and CS themselves. Because Yang et al. (*10*) and Dunshea (*9*) report that CS improves the growth rate while feed intake does not affect it, the results suggest that CS itself contributes to the growth rate. Feed intake can also affect the growth rate. Here, there were no apparent pathological changes seen in the gastrointestinal tract of pigs given a diet with 70 mg/kg CS. CS supplementation (70 mg/kg) enhanced antioxidation capacity (data not shown). Thus, the results support the idea that CS is a good feeding additive in terms of food stability and long-lasting effect. Dietary CS supplementation (140 mg/kg) caused ulcers in the gastrointestinal tract of finishing pigs. This may suggest that the intake of 140 mg/kg CS exceeds the safety dosage.

It is well-known that GH secretion is pulsatile and it is not easy to detect its secretion. Thus, it is difficult to determine the relationship between GH and body growth accurately. However, IGF-I is a useful tool for estimating growth rate as IGF-I levels are positively correlated with growth rate in cattle, red deer, sheep, pigs, and guinea pigs. Thus, IGF-I is emphasized especially in this study. Dietary CS supplementation (70 mg/kg) improved serum GH level (1.46 ng/ml) and stimulated IGF-I secretion (76.5 ng/ml). The magnitude of the responses of them was considered within or slightly over the range observed previously in more pigs (GH levels, 0.98−1.26 ng/mL; IGF-I, 9.8−250 ng/mL). GH can increase muscle growth (*22*) and decrease the fat deposition of pigs, lambs, and salmonids. GH fulfills its function of growth promotion through improving the production of IGF-I. IGF-I can stimulate amino acid uptake and protein synthesis in muscles and can greatly reduce the rate of protein breakdown within muscle fibers. This is consistent with our study that CS supplementation (70 mg/kg) decreased P_2 backfat thickness and tended to improve the lean percentage of finishing pigs (data not shown). Collectively, the results also imply that CS supplementation may enhance the growth rate of finishing pigs through the GH-IGF axis.

In this study, CS supplementation (70 mg/kg) upregulated the GHR gene expression of the liver. This was consistent with the previous study that the liver GHR was found to increase with GH administration (*23*). GH may bind to the hepatic GHR to stimulate IGF-I secretion from the liver into circulation (*24*). However, no information regarding the effect of dietary CS supplementation on IGF-I gene expression has been reported in pigs. CS supplementation (70 mg/kg) upregulated IGF-I mRNA of the liver and increased serum IGF-I secretion. This was in accordance with the study that GH administration can improve the serum IGF-I concentration and liver IGF-I mRNA abundance in pigs (*25*). CS supplementation (70 mg/kg) increased the GHR and IGF-I mRNA of both stomach and muscle. This was consistent with the study that chronic CS treatment can elevate IGF-I mRNA levels (9-fold increase) in muscle of fish (*8*) and GH administration can increase GHR gene expression in muscle of pigs (*23*). GH may bind to the GHR of both stomach and muscle tissues to induce IGF-I expression, in which IGF-I works in both an endocrine and a paracrine manner to stimulate stomach development and muscle growth (*24*). However, CS supplementation (70 mg/kg) did not affect the GHR and IGF-I mRNA of the duodenum. The possible explanation was that CS supplementation (70 mg/kg) did not affect duodenum IGF-I release and then the tissue growth and development. CS supplementation (140 mg/kg) downregulated the GHR and IGF-I mRNA of the duodenum. The possible explanation was that the high dose of cysteamine (140 mg/kg) may have decreased the IGF-I release because 140 mg/kg caused ulcers in the gastrointestinal tract of finishing pigs. However, the GHR and IGF-I (140 mg/kg CS) of the liver, stomach, and muscle were not affected. Taken together, the results also suggest that dietary CS supplementation modulates the GHR and IGF-I mRNA levels in a dose-dependent manner. In addition, CS supplementation has the tissue-specific regulatory mechanisms of GHR and IGF-I.

The biological action of IGF-I by CS supplementation is not only dependent on interaction with IGF-IR and IR but is also influenced by the IGFBP-3 in the local cellular environment, which can potentially inhibit or enhance IGF-I actions. In other words, CS supplementation-related changes in IGF-IR, IR, and IGFBP-3 may be secondary to changes in tissue IGF-I expression, and they occur to either increase or decrease IGF-I activity in the tissues. Thus, it is worth examining how dietary CS supplementation affects the expression of IGF-IR, IR, and IGFBP-3 mRNAs in several tissues. IGF-IR mRNA levels (70 mg/kg CS) were upregulated in the stomach, duodenum, or muscle. However, IGF-IR was not affected in the liver compared with each respective tissue of the control pigs. The IGF-IR mRNA of both stomach and muscle (70 mg/kg CS) had a positive response to IGF-I. It is well-established from in vitro systems that the IGF system acting through the IGF-IR has acute anabolic effects on metabolism and has longer-term effects on cell replication and differentiation (*26*). The data imply that the IGF system by CS supplementation may play an important role in the development of both stomach and muscle of finishing pigs. CS supplementation (70 mg/kg) caused an increase in the IGF-I mRNA level with no change in the IGF-IR level in the liver and an increase in the IGF-IR mRNA level with no change in the IGF-I mRNA level in the duodenum. The results demonstrate that the effect of CS supplementation on IGF-I and IGF-IR is quantitatively different among tissues and suggest that the regulation of IGF-I and IGF-IR mRNA by CS supplementation is discoordinate. CS supplementation (140 mg/kg) downregulated IGF-IR mRNA levels in the liver, stomach, duodenum, or muscle. The drop in IGF-IR expression may be linked to a decrease or no change in the IGF-I mRNA levels in order to regulate the IGF-I activity in the tissues. Taken together, the results also suggest that dietary CS supplementation modulates IGF-IR mRNA levels in a dose-dependent manner. CS supplementation has tissue-specific regulatory mechanisms of IGF-IR. Compared with IGF-I gene expression, the expression of IR gene (70 mg/kg CS) was less sensitive to changes for CS supplementation. CS supplementation (70 mg/kg) did not affect the expression of IR gene in the liver, stomach, duodenum, or muscle tissue. However, IR mRNA levels (140 mg/kg CS) were upregulated in the liver, stomach, duodenum, or muscle. The results suggest that dietary CS supplementation regulates IR mRNA levels in a dose-dependent manner. The interaction of IGF-I with the IGF-IR may be modulated positively or negatively by IGFBP-3. CS supplementation (70 mg/kg) upregulated the IGFBP-3 mRNA level in the liver but downregulated IGFBP-3 levels in the stomach, duodenum, or muscle. In the liver subjected to CS supplementation (70 mg/kg), the change in IGFBP-3 mRNA level was consistent with that in IGF-I mRNA; the possible explanation was that IGFBP-3 can dramatically increase the IGF-I release in the liver cell. The changes in IGFBP-3 mRNA levels (stomach and muscle) were contrary to those of IGF-I.and IGF-IR mRNA; the possible explanation was that the interaction of IGF-I with the IGF-IR may be regulated positively by IGFBP-3. CS supplementation (140 mg/kg) upregulated IGFBP-3 mRNA in the liver, stomach, duodenum, or muscle tissue relative to each respective tissue of the control pigs. The possible explanation was that the upregulation of IGFBP-3 mRNA levels may preserve IGF-I activity in above-mentioned tissues. Collectively, the results also suggest that dietary CS supplementation modulates IGFBP-3 mRNA levels in a dose-dependent manner. CS supplementation has tissue-specific regulatory mechanisms of IGFBP-3.

In conclusion, the results presented here clearly suggest that dietary CS supplementation modulates the growth rate, serum IGF-I concentration, and the gene expression of GHR, IGF-I, IGF-IR, IGFBP-3, and IR in several tissues of finishing pigs. CS supplementation has tissue-specific regulation of GHR, IGF-I, IGF-IR, and IGFBP-3 mRNA levels. The elevation in the levels of GHR, IGF-I, IGF-IR mRNA, and the resulting increase in serum IGF-I concentration may well imply the growth

acceleration in CS-supplemented animals. To our knowledge, this is the first in vivo report on the effects of CS supplementation on GH-IGF axis components. Therefore, further analysis is warranted to elucidate the mechanisms by which CS supplementation influences the expression of these genes. Here, GH-IGF axis components may be affected by both feed intake and CS. However, to completely separate the effects of feed intake from the effects of CS on somatotrophic axis, pair-feeding should be considered in future studies.

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