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The role of protein restriction and interaction with antibiotics in the regulation of compensatory growth in pigs: growth performance, serum hormone concentrations, and messenger RNA levels in component tissues of the endocrine growth axis

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ABSTRACT

The present study investigated the effects of protein restriction and antibiotics on the hypothalamus–pituitary–liver growth axis during the compensatory growth of growing and finishing pigs. Growth performance, serum hormones, and messenger RNA (mRNA) levels of hormones and their receptors in growth axis tissues were recorded for analyses. A total of 64 piglets (large white × Landrace × Duroc cross) with an initial weight of 10.07 ± 0.14 kg were randomly divided into 4 treatment groups of 16 piglets per group. The dietary treatments consisted of 2 protein levels (14% and 20%) and 2 antibiotic levels (no antibiotics and 20 mg/kg colistin sulfate with 50 mg/kg kitasamycin) in a 2 × 2 factorial arrangement. The study was performed over 30 d for the first stage (S1, restriction phase) and 74 d for the second stage (S2, realimentation phase). The 4 treatment diets were maintained throughout the duration in the restriction phase. The 4 groups were fed the same diet in the realimentation phase. The trial period totaled 104 d. Protein restriction decreased BW, average daily food intake, and ADG in weaning pigs ($P < 0.01$) and induced compensatory growth after feeding a normal diet during the growth of finishing pigs. Average daily gain increased during the last phase of compensatory growth ($P < 0.01$). Protein restriction increased serum GH and leptin (LEP) and the mRNA levels of liver IGF-1 receptor (IGF-1-R; $P < 0.01$) but decreased serum IGF-1 ($P < 0.01$) and the mRNA levels of liver GH receptor (GH-R; $P < 0.01$) and IGF-1 ($P < 0.05$) in weaning piglets. Serum GH was increased, but serum IGF-1 was decreased during the realimentation phase ($P < 0.05$). Antibiotics increased the mRNA levels of GHRH ($P < 0.05$) and decreased somatostatin ($P < 0.01$) in the hypothalamus of weaning pigs. Protein restriction and antibiotics had no interactions across the entire trial. In conclusion, the slowing of growth caused by early protein restriction may be compensated for in the later stages of pig raising, and the mechanism of compensation is related to the regulation of GH, IGF-1, GH-R, and IGF-1-R.

1. Introduction

Compensatory or “catch up” growth is a physiological and ecological adaptive behavior in vertebrates [1–5]. Growth is curbed when animals do not have access to enough food or are living in adverse conditions, but the

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animal grows rapidly when supplied with sufficient nutrition after restriction [6]. Bohman first called this phenomenon compensatory growth (CG) [7]. Compensatory growth increases feed conversion (the ratio of weight gain to feed) and suppresses maintenance energy. It also changes the carcass composition. Numerous studies showed that a sufficiency of dietary proteins and amino acids (AAs) after restriction is involved in the triggering of CG in growing pigs [8–11].

Antibiotics have been widely used for the treatment and prevention of bacterial infections since penicillin was introduced into medical therapy in 1942. Antibiotics transformed the modern livestock industry, which uses antibiotics for prophylaxis, meta-prophylaxis, and treatment for infection and as a growth promoter to enhance feed efficiency in healthy livestock [12]. Antibiotics are used routinely to avoid the spreading of infectious disease because of the intensity of modern animal husbandry [13]. The World Health Organization characterizes antimicrobial resistance as a global public health crisis that must be managed with the utmost urgency [14]. Stock feeding without antibiotics will become increasingly common because the European Union and Food and Drug Administration have been moving to limit the use of antibiotics in recent years.

With the rapid development of livestock husbandry, increasing numbers of organizations pay close attention to the environmental pollution created by the overuse of raw materials and feed additives, especially antibiotics and proteins, including various AAs. One important contributor to the environmental impact of pig production is the release of nitrogenous compounds [15], including protein and nonprotein nitrogen, which are undigested and excreted via feces or digested and voided in urine. Increasing concerns about the environmental impact of pig production [16], together with the limited availability and high cost of sources of dietary protein, make reductions in dietary protein content a priority objective [16,17]. Decreasing the dietary CP content while maintaining optimal standardized ileal digestibility AA concentrations successfully reduced N input per kilogram of lean meat gain [18]. Single AA deficiencies lead to an inefficient use of the other AAs, which are deaminated and excreted in urine to cause suboptimal growth. Dietary protein intake should closely match the requirements to fully exploit the genetic potential of pigs for growth without excessive intake [18].

Recent studies showed that a period of protein restriction had a positive influence on subsequent growth in pigs [19]. Amino acids play an important role in

Table 1
Composition of feed.

	Restriction phase		Subsequent realimentation phase	
Stage	10–25 kg		25–60 kg	60–90 kg
Items	NP (A/NA)	RP (A/NA)	NP	NP
Ingredients composition (%)				
Corn	60.20	76.50	71.70	78.70
Extruded soybeans (46.2% CP)	19.00	11.00	25.00	18.00
Corn gluten meal	2.00	–	–	–
Puffed soybean	9.00	–	–	–
Dried whey	3.00	3.00	–	–
Fish meal (64% CP)	3.00	3.00	–	–
Soybean oil	0.50	2.00	–	–
L-Lysine (98%)	0.17	0.71	–	0.10
DL-Methionine (98%)	–	0.11	–	–
L-Threonine (98%)	–	0.28	–	–
L-Tryptophan (98%)	–	0.09	–	–
Dicalcium phosphate	0.84	0.96	1.10	1.00
Limestone	0.89	0.95	0.80	0.80
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix (1%)	1.00	1.00	1.00	1.00
Nutrient levels (%)				
Metabolizable energy (Mcal/kg)	3.14	3.14	3.08	3.09
CP	20.04	14.04	17.14	14.55
Lysine (SID)	1.23	1.23	0.91	0.80
Methionine (SID)	0.65	0.61	0.65	0.50
Tryptophan (SID)	0.23	0.23	0.21	0.16
Threonine (SID)	0.82	0.78	0.67	0.56
Leucine (SID)	1.87	1.28	1.56	1.38
Isoleucine (SID)	0.86	0.52	0.70	0.57
Valine (SID)	0.97	0.63	0.82	0.69
Calcium	0.74	0.74	0.62	0.57
Total phosphorus	0.57	0.54	0.53	0.49

Abbreviations: A, antibiotic; NA, no antibiotic; NP, normal protein; RP, protein restriction.
Composition of antibiotic: 20 mg/kg colistin sulfate with 50 mg/kg kitasamycin.
Supplied per kilogram of diet in the restriction phase: 10,000 IU vitamin A, 1,000 IU vitamin D3, 30 mg vitamin E, 5.00 mg vitamin B1, 5.00 mg vitamin B2, 5.00 mg vitamin B6, 0.02 mg vitamin B12, 24 mg pantothenic acid, 45 mg niacin, 0.05 mg biotin, 0.39 mg folic acid, 190 mg Cu, 140 mg Zn, 0.4 mg SE, 45 mg Mn, 190 mg Fe, and 0.5 mg I. Supplied per kilogram of diet in the realimentation phase: 8,000 IU vitamin A, 2,000 IU vitamin D3, 30 mg vitamin E, 1.60 mg vitamin B1, 1.60 mg vitamin B6, 12 μg vitamin B12, 20 mg pantothenic acid, 15 mg niacin, 0.05 mg biotin, 100 mg Cu, 80 mg Zn, 0.3 mg SE, 25 mg Mn, 100 mg Fe, and 0.3 mg I.

regulating the nutrition metabolism pathway [8]. Amino acids are cell signaling molecules and regulators of gene expression and the protein phosphorylation cascade. Amino Acids are also key precursors for the syntheses of hormones and low-molecular-weight nitrogenous substances, which have enormous biological importance [20]. The present study examined the effects on growth axis hormones during a protein restriction phase and subsequent realimentation phase to determine whether protein restriction could serve as a replacement for antibiotic use.

2. Materials and methods

2.1. Animals, experimental treatments, and sampling

2.1.1. Care and use of animals

Sixty-four healthy cross-bred weaning piglets (large white × Landrace × Duroc cross) weighing 10.07 ± 0.14 kg were studied. The piglets were fed separately in a test cage (1.5 m × 0.6 m × 0.8 m) via automatic troughs and drinking water nipples for free access to feed and water. The percentage composition of the basal diet is shown in Table 1. Pre-experimentation was performed before the formal test, during which the piglets were fed the basal diet. The piglets were fed the treatment diets after the start of the formal test. Feed consumption was determined daily, and pigs were weighed weekly to calculate ADG, average daily feed intake (ADFI), and feed efficiency as feed/gain (F/G). Animal care and treatment complied with the standards for the care and use of laboratory animals of Northeast Agricultural University ([2011] - 9). The animals were maintained according to the National Research Council Guide (1996) in metabolic cages and had free access to food and water. This study was performed in strict accordance with the recommendations of the National Research Council Guide (1996), and the Ethical and Animal Welfare Committee of Heilongjiang Province, China, approved all animal experimental procedures.

2.2. Experimental treatments

The 64 weaning piglets were randomly divided into 4 treatment groups. Each treatment consisted of 16 replicates (columns) with 1 piglet per replicate over a trial period of 104 d divided into a restriction phase of 30 d and a realimentation phase of 74 d. The experiment was performed using a 2 × 2 factor design (Table 1). The following treatments were administered: (1) the basal diet (20% CP without antibiotics, NANP); (2) the basal diet + antibiotics (20% CP with 20 mg/kg colistin sulfate and 50 mg/kg kitasamycin, ANP); (3) the protein restriction diet (14% CP without antibiotics, NARP); and (4) the protein restriction diet + antibiotics (14% CP with 20 mg/kg colistin sulfate and 50 mg/kg kitasamycin, ARP). During the experiment, the status and behavior of the pigs were observed and recorded. After the 30-d restriction phase, 12 weaning piglets weighing 24.10 ± 0.40 kg were slaughtered for sampling. The remaining pigs were fed a normal diet of NRC 2012 content for 74 d before slaughter.

2.3. Samples and sample collection

Blood samples were collected via an anterior vena cava puncture into 10-mL heparin-free Vacutainer tubes on Days 30 and 104. The blood was centrifuged at 3,500 × *g* for 5 min to obtain serum, which was stored at −20°C until analysis for GHRH, somatostatin (SS), GH, IGF-I, leptin (LEP), triiodothyronine (T_3), and thyroxine (T_4).

Tissues, including the hypothalamus, pituitary, liver, and skeletal muscle, were removed after slaughter, and weights were recorded. A small piece of each tissue was quickly collected, placed in liquid nitrogen, and stored in a −80°C freezer for PCR analysis.

2.4. Measurement of GHRH, SS, GH, IGF-I, LEP, T_3, and T_4 in serum

Serum hormone levels were determined according to the appropriate instructions. The determinations of serum hormone levels were performed using individual RIA kits for GH, IGF-I, GHRH, SS, LEP, T_3, and T_4 (Shanghai Jinma Institute of Biological Products, Shanghai, China).

2.5. Total RNA and quantitative real-time PCR

Total RNA from the hypothalamus, pituitary, liver, and skeletal muscle tissues was isolated using a reagent kit (EZNA Total RNA Kit; Omega Bio-Tek, Inc, Guangzhou, China) according to the manufacturer's recommended protocol. The concentration of RNA in each tissue was estimated based on its absorbance at 260 nm. Ribonucleic acid quality was determined by checking its integrity using agarose gel electrophoresis and confirming that the A260 nm/A280 nm absorbance ratio was between 1.8 and 2.0. First-strand cDNA was synthesized from 5 μg of total RNA using oligo-dT primers and superscript II reverse transcriptase according to the manufacturer's instructions (Tiangen Biotech Co, Ltd, Beijing, China).

The expression levels of genes encoding growth axis hormones and receptors in tissues, including GH and GH receptor (GH-R), IGF-1, and IGF-1 receptor (IGF-1R), GHRH and GHRH receptor (GHRH-R), SS and SS receptor 2 (SS-R_2), and β-actin were determined using quantitative real-time PCR in an ABI PRISM 7500 SDS thermal cycler apparatus (Applied Biosystems, Foster City, CA) with the following temperature program: one cycle of 95°C for 30 s; 40 cycles of 95°C for 5 s; and 61°C for 34 s. To ensure the stability of β-actin gene expression, we selected 3 reference genes using GeNorm software (Primer Design, Ltd., Southampton University, Highfield Campus, Southampton Haunts, UK). The dissociation curve for each PCR was tested using Dissociation Curve 1.0 software (Applied Biosystems) to detect and eliminate any primer-primer dimers and nonspecific amplifications. The relative expression levels of the target genes were calculated based on the efficiencies and quantification cycle (Cq) deviations of the unknown samples compared with the controls, and the results are expressed in comparison to the reference gene as described by Pfaffl [21].

Relative expression was reported as the ratio of the target gene to the control gene using the formula $2^{-(\Delta\Delta Ct)}$, where $\Delta\Delta Ct = (Ct_{Target} - Ct_{\beta\text{-actin}})_{treatment} - (Ct_{Target} - Ct_{\beta\text{-actin}})$

Table 2
The effect of protein restriction and antibiotic use in the protein restriction and realimentation phases on growth performance.

Items	Trial days	No antibiotics		Antibiotics		SEM	*P*		
		NP	RP	NP	RP		*P*	A	*P* × A
BW (kg)	Day 0	10.15	10.16	10.04	10.00	0.136	0.964	0.616	0.929
	Day 30	26.37	22.11	27.44	21.10	0.374	<0.001	0.970	0.170
	Day 66	61.55	57.01	64.79	54.60	0.909	<0.001	0.884	0.112
	Day 104	101.78	101.23	106.15	99.33	1.458	0.216	0.674	0.290
ADG (kg/d)	Days 0–30	0.58	0.43	0.62	0.40	0.011	<0.001	0.778	0.103
	Days 31–66	0.95	0.90	1.01	0.90	0.020	0.198	0.889	0.207
	Days 67–104	1.06	1.26	1.09	1.18	0.025	0.006	0.641	0.277
	Days 30–104	1.02	1.06	1.05	1.05	0.017	0.424	0.676	0.388
	Days 0–104	0.88	0.87	0.92	0.86	0.016	0.575	0.831	0.120
ADFI (kg/d)	Days 0–30	1.06	0.99	1.09	0.97	0.013	0.003	0.797	0.292
	Days 31–66	2.58	2.59	2.66	2.49	0.049	0.407	0.880	0.367
	Days 67–104	4.34	4.19	4.13	4.29	0.058	0.940	0.614	0.190
	Days 30–104	3.48	3.32	3.41	3.41	0.041	0.327	0.893	0.313
	Days 0–104	2.82	2.67	2.78	2.75	0.034	0.207	0.779	0.382
F/G	Days 0–30	1.88	2.43	1.79	2.55	0.054	<0.001	0.872	0.353
	Days 31–66	2.74	2.75	2.66	2.77	0.046	0.507	0.773	0.586
	Days 67–104	3.89	3.36	3.87	3.66	0.105	0.027	0.916	0.190
	Days 30–104	3.32	3.10	3.28	3.28	0.068	0.195	0.980	0.195
	Days 0–104	3.08	3.06	3.03	3.24	0.045	0.233	0.408	0.233

Abbreviations: A, antibiotic; ADFI, ADFI: average daily feed intake; F/G, ADFI/ADG; NA; no antibiotic; NP, normal protein; RP, protein restriction.
RP × A: the interaction of protein restriction and antibiotic use.
The *P* values represent the main effect of protein restriction, antibiotic use, and the interaction of protein restriction and antibiotics.

control. Relative expression was normalized and expressed as a ratio of the expression in the control group.

2.6. Statistical analysis

Data were analyzed using ANOVA in a 2 × 2 factorial arrangement of treatments using the general linear model procedure (SPSS 22.0; IBM-SPSS Inc, Chicago, IL) and GraphPad Prism (version 5.0, GraphPad Software Inc, San Diego, CA). The results are presented as the means and SEM for the effects of protein, antibiotics, and their interaction. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Growth performance

During the restriction phase, protein restriction significantly decreased the BW, ADG, and ADFI ($P < 0.01$; Table 2). Protein restriction significantly increased Feed/Gain ($P < 0.01$). Antibiotic use and the interaction of antibiotics and protein restriction had no effect on growth performance. At the beginning of the CG phase, the previous protein restriction limited BW. Previous protein restriction increased ADG ($P < 0.01$) but decreased F/G ($P < 0.05$) by during the late stage of the compensatory phase. During the overall trial period, protein restriction, antibiotics, and the interaction of protein restriction and antibiotics had no effect on growth performance.

3.2. Serum hormones

During the restriction phase, protein restriction increased the concentrations of serum GH and LEP but decreased the concentration of serum IGF-1 ($P < 0.01$; Table 3). During the compensatory phase, prior protein restriction increased the concentration of serum IGF-1 but decreased the concentration of serum GH ($P < 0.01$).

Table 3
The effect of protein restriction and antibiotic use in the protein restriction and realimentation phases on serum hormones of the growth axis.

Items	Trial days	No antibiotics		Antibiotics		SEM	*P*		
		NP	RP	NP	RP		*P*	A	*P* × A
GHRH (ng/mL)	Day 30	49.27	37.45	49.59	33.90	0.84	0.058	0.345	0.419
	Day 104	33.43	33.89	33.49	33.61	0.778	0.912	0.949	0.851
SS (pg/mL)	Day 30	49.11	46.78	46.02	46.61	1.449	0.770	0.589	0.629
	Day 104	65.76	66.29	65.62	65.67	2.317	0.960	0.936	0.951
GH (ng/mL)	Day 30	24.95	27.64	24.28	26.62	0.233	0.001	0.108	0.706
	Day 104	27.07	23.34	27.00[a]	25.25	0.267	0.001	0.114	0.088
IGF-1 (ng/mL)	Day 30	169.37	144.66	173.49	134.82	2.123	<0.001	0.482	0.126
	Day 104	249.65	263.04	251.60	256.21	1.009	0.001	0.249	0.051
LEP (pg/mL)	Day 30	1,785.35	1,892.58	1,776.55	1,860.92	7.601	<0.001	0.220	0.474
	Day 104	1,994.01	2,000.20	2,073.88	1,965.01	14.265	0.097	0.448	0.067
T_3 (pg/mL)	Day 30	195.77	193.99	196.51	192.83	0.596	0.051	0.861	0.448
	Day 104	959.58	964.41	961.44	960.11	0.835	0.361	0.479	0.090
T_4 (ng/mL)	Day 30	79.61	77.51	80.96	76.11	0.322	0.116	0.647	0.952
	Day 104	176.80	179.00	176.43	177.79	0.046	0.051	0.350	0.615

Abbreviations: A, antibiotic; LEP, Leptin; NA, no antibiotic; NP, normal protein; RP, protein restriction; SS, somatostatin; T_3: triiodothyronine T_4: thyroxine.
RP × A: the interaction of protein restriction and antibiotic use.
The *P* values represent the main effect of protein restriction, antibiotic use, and the interaction of protein restriction and antibiotics.

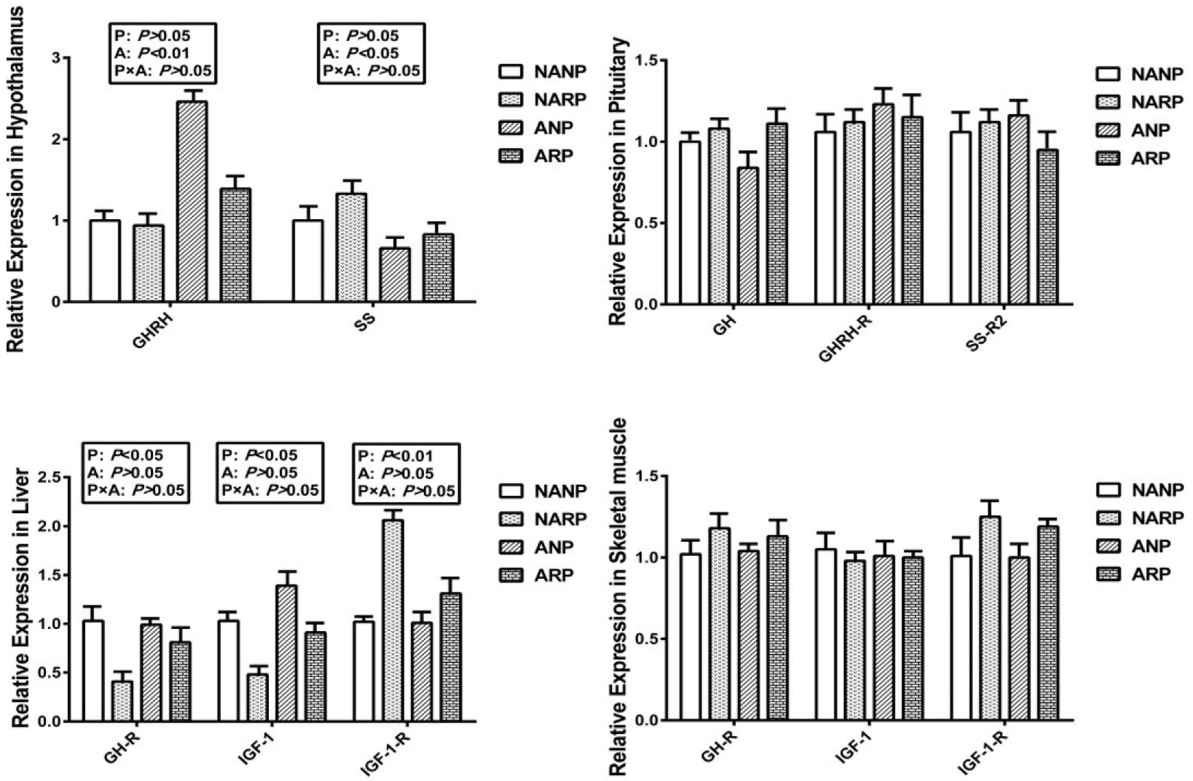

Fig. 1. The effect of protein restriction and antibiotic use on mRNA expression of the growth axis in tissues in the restriction phase. Treatment: A, antibiotic; NA, no antibiotic; NP, normal protein; RP, protein restriction; RP × A, the interaction of protein restriction and antibiotic use. NARP, no antibiotics with protein restriction; ARP: antibiotics with protein restriction; NANP: no antibiotics with normal protein; ANP, antibiotics with normal protein; SS: somatostatin; GHRH-R, growth hormone-releasing hormone receptor; SS-R_2, somatostatin receptor 2; GH-R: growth hormone receptor; IGF-1-R, insulin-like growth factor 1 receptor. The *P* values represent the main effect of protein restriction, antibiotic use, and the interaction of protein restriction and antibiotics.

3.3. The expression of mRNA levels of the growth axis

Protein restriction decreased the mRNA expression levels of GH-R and IGF-1 in liver tissue ($P < 0.05$; Fig. 1). Antibiotics increased the mRNA expression level for GHRH ($P < 0.01$) and decreased mRNA expression levels for SS ($P < 0.05$) in hypothalamus tissue. Protein restriction increased the mRNA expression levels of GH-R and IGF-1 in liver tissue ($P < 0.05$; Fig. 2).

4. Discussion

Growth is defined as an increase in weight or size (length, height, girth, or volume) of an animal over time as it develops toward maturity [22]. Animals with retarded growth due to undernutrition achieve a higher than normal growth rate for their chronological age after the removal of feed restrictions. This phenomenon was called CG by Bohman [7]. Compensatory growth is defined as a physiological process whereby an animal accelerates its growth following a period of growth restriction compared with control animals [23].

The extent and rate of CG in growing pigs varies with the type, degree, timing, and duration of nutrient intake restriction and the genotype and availability of energy and nutrients following the period of nutrient intake restriction. Animal protein requirements are based on the intake of a complete set of AAs instead of CPs. Amino acids given in excess are deaminated, and the resulting urea is excreted in the urine [24]. The diet used in the present study was protein restriction with balanced AAs, which provides a balanced nutritional intake.

One important mechanism of CG is a tendency to increase ADFI. The appetite and food intake of animals improve during the compensatory phase, which was confirmed in pigs, fish, and ruminants. Protein restriction in the restriction phase decreases ADFI and ADG but increases ADF and ADG during the subsequent realimentation phase [15,25–28]. These findings are almost consistent with our results. The differences in ADG response during the realimentation phase may be because of the lower initial BW caused by protein restriction or the duration of the protein restriction period. During the restriction period, pigs grew slowly because of the protein-insufficient diet that cannot provide balanced nutrition and led to a low ADFI and ADG compared with the control group. Branched-chain amino acids (BCAAs), including leucine, isoleucine, and valine, are essential amino acids that play an important role in the regulation of growth performance in piglets [29]. In addition to the first 4 limiting AAs, BCAA should be

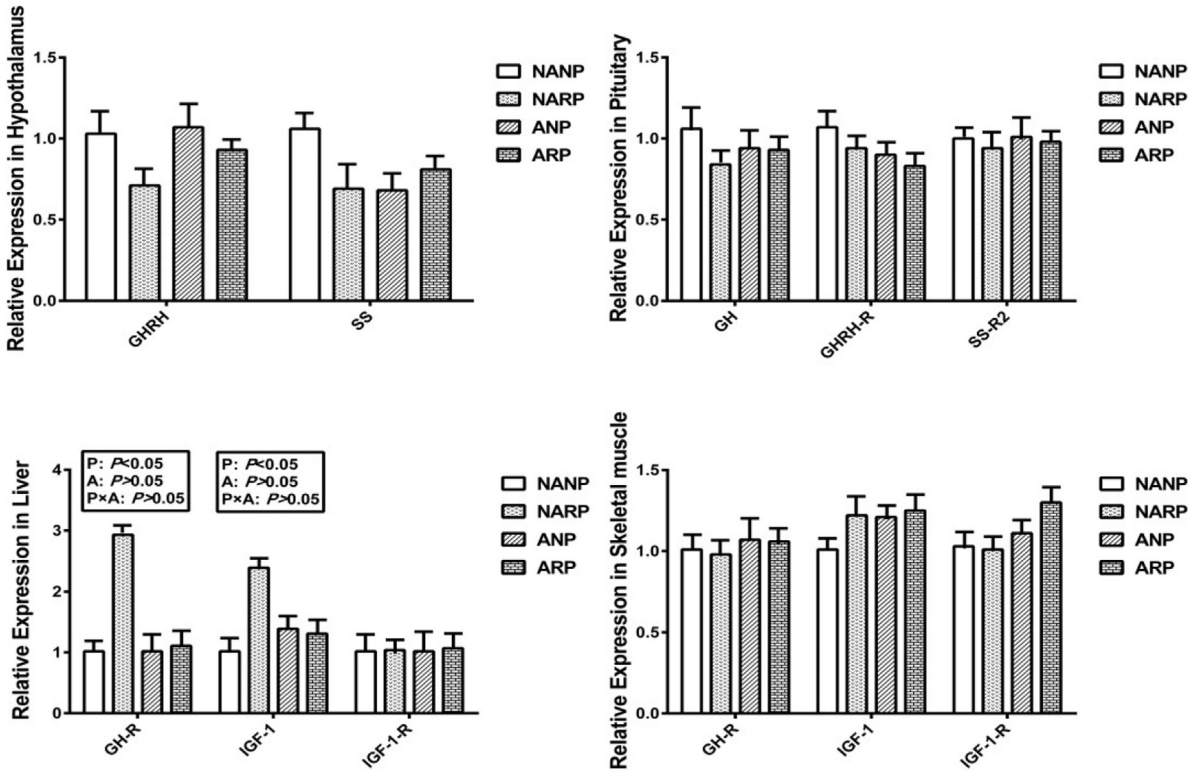

Fig. 2. The effect of protein restriction and antibiotic use on mRNA expression of the growth axis in tissues in the realimentation phase. Treatment: A, antibiotic; NA, no antibiotic; RP, protein restriction; NP, normal protein. RP×A: the interaction of protein restriction and antibiotic use; NARP, no antibiotics with protein restriction; ARP, antibiotics with protein restriction; NANP, no antibiotics with normal protein; ANP, antibiotics with normal protein; SS, somatostatin; GHRH-R, growth hormone-releasing hormone receptor; SS-R_2, somatostatin receptor 2; GH-R, growth hormone receptor; IGF-1-R, insulin-like growth factor 1 receptor. The *P* values represent the main effect of protein restriction, antibiotic use, and the interaction of protein restriction and antibiotics.

supplemented when the CP reduction is below 6% to ameliorate the poor growth performance of piglets [30]. Current research demonstrated that the supplementation of BCAAs to low-protein diets increased feed intake and skeletal muscle growth in piglets and restored growth performance to normal levels [31–33]. However, the isoleucine and valine contents did not meet the requirements of NRC (2012) in protein restriction diets in this study, which may further lead to the discrepancies of ADG and ADFI of pigs. Many studies showed that antibiotics improved growth performance in pigs. During the present study, antibiotics had no effect on BW, ADFI, or ADG. Our results showed that protein restriction induced CG in pigs, which grew faster after returning to a normal diet.

Serum hormone levels reflect the growth of animals. Previous research showed that changes in IGF-1 were connected with the changes in growth [28,34]. IGF-1 mediates the growth-promoting effects of GH. Recent studies suggest that protein restriction increases serum GH but depresses serum IGF-1 during the restriction phase, but it has no effect on return to a normal diet compared with the control group [15,26]. Growth hormone-releasing hormone is secreted by the hypothalamic arcuate and ventromedial nuclei, and it binds to GHRH-R in the cell membrane to promote the secretion of GH. Somatostatin is secreted by the periventricular nucleus, hypothalamic arcuate nucleus, and other tissues, and it binds to SS-R in the cell membrane to inhibit the secretion of GH. High levels of serum GH and IGF-1 accelerate the release of SS. Growth hormone promotes the target cell secretion of somatomedin, which is also called IGF. The present study found that protein restriction significantly increased GH and LEP in serum and decreased IGF-1. The level of GH was decreased, and IGF-1 increased significantly during the realimentation phase. We speculate that GH and IGF-1 play important roles during the process that stimulates CG. Growth hormone has a negative feedback effect on hypothalamic GHRH and pituitary GH. Insulin-like growth factor −1 increases SS and the release or depression of GH, which creates negative feedback loops for hypothalamic and pituitary function. However, we did not find significant changes in the level of GHRH or SS in serum in either phase. Triiodothyronine (T_3) and thyroxine (T_4) promote animal growth together with GH in body, but our results were inconsistent. Shi et al found that serum hormone levels were not significantly affected when finishing pigs were fed a low-protein diet [35]. Further experiments are needed to research the effects of protein restriction on serum hormones. Notably, our results showed that antibiotics had no

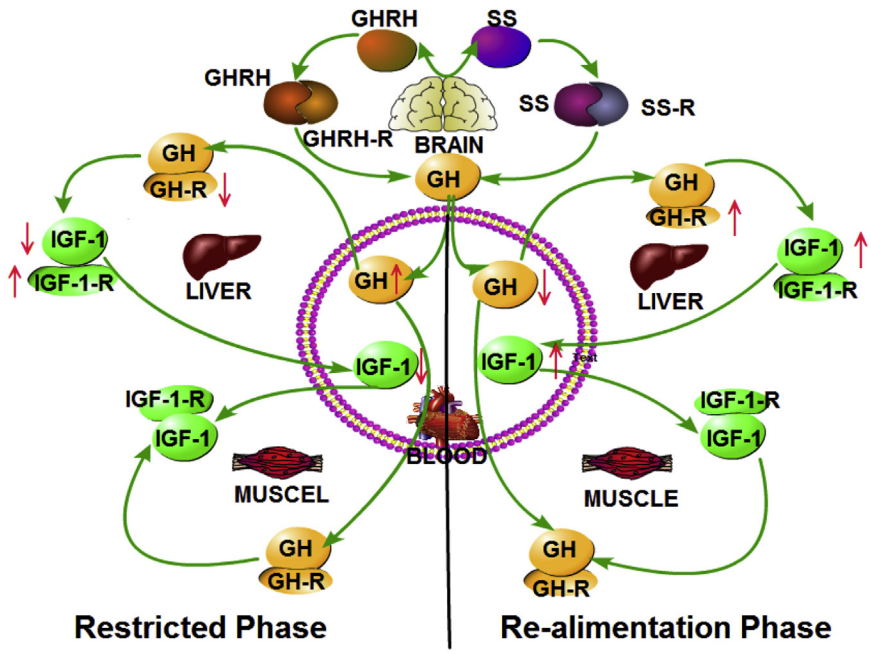

Fig. 3. The mechanism of the endocrine growth axis. GHRH-R, growth hormone-releasing hormone receptor; SS, somatostatin; SS-R, somatostatin receptor; GH-R, growth hormone receptor' GHBP, growth hormone binding protein; IGF-1-R, insulin-like growth factor-1 receptor.

effect on serum hormones during the restricted phase or the realimentation phase, and the interaction of protein restriction and antibiotics was also not significant.

Protein restriction increased the mRNA concentration of IGF-1-R in liver tissue during the restriction phase in the present study. This result is consistent with many other studies [36–38]. GH accumulation negatively correlated with a reduction in liver GH-R. The reduction of GH-R inhibits the synthesis of liver IGF-1, which decreases the synthesis of protein in other peripheral tissues, such as skeletal muscle. The GH-R in peripheral tissue was increased, and the sensitivity of peripheral tissue IGF-1-R to IGF-1 was increased, which made full use of IGF-1 and the promotion of protein synthesis. Protein restriction increased IGF-1 mRNA expression in the liver in the realimentation phase. The concentration of GH-R in the liver tissue surged when pigs were returned to a normal diet. Many GH molecules bind to GH-R to promote the synthesis of IGF-1 in liver tissue [39]. This interaction provides an explanation of why serum GH was depressed, but IGF-1 was increased after the return to a normal diet. A large number of IGF-1-R bind with IGF-1 to improve protein synthesis, which promotes growth. We also found that antibiotics increased GHRH mRNA but depressed SS mRNA abundance in the hypothalamus. However, the interaction of protein restriction and antibiotics was not significant for tissue mRNA in this research.

The endocrine growth axis includes the hypothalamus, pituitary, target organs, hormones, and their receptors, which promote animal growth. The main target organs are the liver and skeletal muscles. Relevant molecular factors are listed as follows: GHRH and SS, which are secreted by the hypothalamus; GH, which is released by the pituitary; and IGF-1, which is produced by the liver and other target tissues and are the receptors for these hormones [40,41]. Figure 3 shows the mechanism of the neuroendocrine growth axis, which includes GHRH-R that binds to GHRH in the pituitary to promote the release of GH. Our results showed that protein restriction significantly increased the level of GH serum and decreased the abundance mRNA of GH-R and IGF-1 in the liver, which further decreased the release of IGF-1. During the realimentation phase, the level of GH was decreased significantly in serum, and the abundance of GH-R and IGF-1 mRNA increased in the liver, which further increased the release of IGF-1. Somatostatin receptor binds to SS to restrain the pituitary release of GH. Growth hormone receptor combined with GH in liver tissue promotes liver secretion of IGF-1. Nevertheless, we did not find changes in the abundance of SS-R and GH-R mRNA in pituitary in either phase. All these hormones are transported by the circulatory system to combine with their respective receptors and regulate body growth.

5. Conclusions

Protein restriction induced CG via decreasing BW, ADF and ADG in weaning pigs, and restricted pigs grew faster during CG. Protein restriction may regulate the CG of pigs by changing serum hormone concentrations and the gene expression of components of the growth axis. Antibiotics had no effect on growth performance. The interaction between protein restriction and antibiotics had no effect on

the growth of pigs. In conclusion, the slowing of growth caused by early protein restriction may be compensated for in the CG stage, and the mechanism of compensation is related to the regulation of GH, IGF-1, GH-R, and IGF-1-R.

CRediT authorship contribution statement

D. Ju: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **T. Teng:** Validation, Formal analysis, Investigation, Resources, Data curation. **G. Bai:** Validation, Formal analysis, Investigation, Resources, Data curation. **H. Fu:** Investigation, Resources, Data curation, Writing - original draft. **S. Qiu:** Investigation, Resources, Data curation, Writing - original draft. **X. Zhao:** Conceptualization, Methodology, Software, Project administration. **Y. Sun:** Conceptualization, Methodology, Software, Project administration. **B. Shi:** Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Funding acquisition.

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