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Effect of high dietary copper on the expression of hypothalamic appetite regulators in weanling pigs*

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ABSTRACT

The current study evaluated appetite-related gene expression of neuropeptide Y (NPY), orexin, type 2 orexin receptor (OrexinR2), proopiomelanocortin (POMC), melanocortin-4 receptor (MC4R), AMP-activated protein kinase α2 subunit (AMPKα2) and long-form leptin receptor (LeptinRb) in hypothalamus in response to copper (Cu) supplementation. One hundred crossbred pigs were assigned to four groups of 25 pigs, each comprising five replicates of 5 animals. Groups were then randomly assigned to treatments consisted of 1. control (10 mg/kg $CuSO_4$), 2. 100 mg/kg $CuSO_4$, 3. 175 mg/kg $CuSO_4$, 4. 250 mg/kg $CuSO_4$. On d 21 of the experiment 5 pigs from each group were slaughtered and the hypothalami were collected for determination of appetite-regulating genes mRNA expression level. The results showed that average daily feed intake and average daily gain were higher ($P<0.05$) in 250 and 175, 250 mg/kg Cu supplemented groups, respectively, than in the 10 mg/kg group. Feed:gain ratio was lower in pigs fed the diets with 250 mg/kg Cu ($P<0.05$) than in the 10 mg/kg group. Furthermore, the abundances of NPY mRNA in hypothalamus were higher in 250 mg/kg Cu supplemented groups ($P<0.05$), whereas the abundances of POMC and LeptinRb mRNA were significantly reduced ($P<0.05$) in 170, 250 mg/kg and 100, 175, 250 mg/kg Cu supplemented groups, respectively. No difference was found for AMPKα2, MC4R, orexin, OrexinR2 mRNA expression ($P>0.05$). These data suggest that down-regulation of LeptinRb mRNA expression might contribute to the stimulation of feeding of high dietary Cu supplementation *via* regulation of NPY and POMC mRNA expression.

KEY WORDS: appetite, copper, hypothalamus, pigs

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INTRODUCTION

It is well recognized that high dietary copper (Cu) promotes growth performance in pigs (Cromwell et al., 1989). In addition, dietary Cu supplementation has been shown to associated with improved feed intake (Fakler, 1999). Cu could be transported through the blood-brain barrier (BBB) as a free Cu ion and participates in regulation of neuroendocrine action in the brain (Choi and Zheng, 2009). A recent study suggested that high dietary Cu appears to stimulate appetite in pigs by upregulating neuropeptide Y (NPY) mRNA expression and enhancing NPY concentration in the hypothalamus (Li et al., 2008), however, associated changes in the expression of other appetite-controlling genes such as orexin, type 2 orexin receptor (OrexinR2), proopiomelanocortin (POMC), melanocortin-4 receptor (MC4R) and long-form leptin receptor (LeptinRb) in response to high dietary Cu remain to be determined. The mechanism of appetite regulation in the hypothalamus is complex and the hypothalamic AMP-activated protein kinase (AMPK) might be involved since it has been proved to play a critical role in hormonal and nutrient-derived anorexigenic and orexigenic signals and in energy balance (Minokoshi et al., 2004).

The objective of the current study was to evaluate the effect of Cu supplementation, at a rate of 100, 175 or 250 mg/kg feed, on feed intake and expression of hypothalamic appetite-regulating genes such as orexin, OrexinR2, POMC, MC4R, LeptinRb, and AMPK α2 subunit (AMPKα2) in weanling pigs by sensitive and quantitative realtime-polymerase chain reaction (realtime-PCR) method.

MATERIAL AND METHODS

Animal care and experimental design

All experimental procedures were conducted with the approval of the Institutional Animal Care and Use Committee of Sichuan Agricultural University. One hundred crossbred (Duroc×Large White×Yorkshire) pigs, weaned at an average of 28 d of age and 7.5±0.6 kg body weight (BW), were fed a basal diet for a 2-d adjustment period. The composition of the basal diet is presented in Table 1. Following the adjustment period, animals were divided into five blocks based on BW and gender, and each block was further divided into four groups of 5 pigs per pen. Groups were then randomly assigned to treatments. Treatments consisted of: 1. control: 10 mg Cu/kg dry matter (DM) from $CuSO_4$, 2. 100 mg Cu/kg DM from $CuSO_4$, 3. 175 mg Cu/kg DM from $CuSO_4$, and 4. 250 mg Cu/kg DM from $CuSO_4$.

Table 1. Composition of basal diets fed to weanling pigs on an as-fed basis at phase I (1-14d) and phase II (15-21d)[1]

Item	Phase I	Phase II
Ingredient,%		
maize (7.8% CP)	49.37	50.70
soyabean meal (46% CP)	9.61	14.21
extruded soyabean	8.91	14.61
whey	8.00	5.00
soya protein concentrate	5.00	5.00
glucose	5.00	3.00
homemade fish meal	4.50	-
whole egg powder	3.00	-
wheat middings	2.00	2.00
soyabean oil	2.00	2.00
dicalcium phosphate	0.75	1.46
limestone (powder)	0.66	0.73
L-lysine	0.31	0.35
salt	0.20	0.20
trace mineral mix (0.2%)[2,3]	0.20	0.20
L-threonine	0.12	0.14
choline chloride (50%)	0.10	0.10
mould inhibitor A (0.5kg/t)	0.06	0.06
antioxidant (30%)	0.06	0.06
vitamin mix[4]	0.06	0.06
aroma agent	0.05	0.06
coating VC (93%)	0.02	-
DL-methionine	0.01	0.06
L-tryptophan	-	0.04
Calculated composition		
digestible energy, MJ/kg	14.96	14.96
crude protein, %	20	20
fat, %	7.05	7.05
calcium,%	0.8	0.8
total phosphorus, %	0.62	0.62

[1] percentage as-fed basis

[2] provided per kg of diet: mg: Mn 50, I 0.3, Se 0.3, Fe 100, Zn 100

[3] trace mineral mix (0.2%) supplemented Cu in the form of $CuSO_4 \cdot 5H_2O$ at levels of 10, 100, 175, 250 mg/kg, respectively, in diets

[4] provided per kg of diet: IU: vit. A 13,500, vit. D 2,250, vit. E 22; mg: vit. K (menadione dimethylpyrimidinoe bisulphate) 3, riboflavin 6, d-pantothenic acid 15, niacin 35, d-biotin 0.l5, 1.2, folic acid 1.2; µg: vit. B_{12} 25

Feed intake and weight gain were measured on a per-pen basis. All procedures, care and handling of animals were performed according to routine management practices. The experiment lasted for 21 d. Pigs were weighed on d 14 and d 21 and feed consumption was determined daily to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed:gain ratio (F:G). On d 21, 20 pigs randomly

selected (5 pigs from each group) were slaughtered and the hypothalami was collected and quickly frozen in liquid nitrogen and stored at -70°C for determination of mRNA expression level.

Hypothalamic gene expression

Total RNA extraction. Hypothalamic gene expression was quantified using real time PCR techniques. Hypothalamus from five pigs of each group was ground in liquid nitrogen respectively, and a fraction of about 50 mg collected from each hypothalamus was used to extract total RNA with RNAiso plus kit (TaKaRa Biotechnology, Japan), according to the manufacturer's instruction. Total RNA concentration was then quantified by measuring the absorbance at 260 nm in nucleic acid/protein analyzer (Beckman Coulter DU800, USA). Ratios of absorptions (260/280 nm) were between 1.8 and 2.0 for all preparations. Aliquots of each RNA samples were subject to electrophoresis through a 1.2% agarose gel to verify their integrity.

Reverse transcription (RT). Two μg of total RNA was reverse transcribed by incubation at 37°C for 15 min in a 20 μl mixture consisting of 1×Prime Script Buffer*, 1 μl Prime Script RT Enzyme MixI*, 2.5 μmol/l random hexamer primers* and 5 μmol/l Oligo dT Primer* (* TaKaRa Biotechnology, Japan). The reaction was terminated by heating at 89°C for 15 sec. RT was performed in a Master Cycler Gradient (Eppendorf, Germany).

Real-time RT-PCR

All real-time PCRs were carried out in triplicate on a DNA Engine thermal cycler (PTC-0200, Chromo4 Real-Time Detector, Bio-Rad, USA). Mock RT and No Template Controls (NTC) were set to monitor the possible contamination of genomic DNA both at RT and PCR. The pooled sample made by mixing equal quantity of total RT products (cDNA) from all samples was used for optimizing the PCR condition and tailoring the standard curves for each target gene, and melting curves were performed to insure a single specific PCR product for each gene. Two μl of 5-fold dilution of RT product was used for PCR in a final volume of 25 μl containning 12.5 μl SYBR Premix Ex Taq (Perfect Real Time; TaKaRa Biotechnology, Japan) and 0.2-0.8 μM of each forward and reverse primer for NPY, POMC, orexin, OrexinR2, MC4R, LepinRb and AMPKα2 (Table 2). Porcine β-actin mRNA was used as a reference gene for normalization purposes. The level of gene expression for each gene was quantified relative to the level of the housekeeping gene β-actin using the standard curve method and is presented as relative expression units. The following PCR protocols were initial denaturation (20 sec at 95°C), then a two-step amplification programme (20 sec at 95°C, 20-30 sec at 60-64°C) repeated 45 times.

Table 2. Primer sequences of target genes in porcine hypothalami

GenBank accession	Target gene	Primer sequences	PCR products (bp)
AF264083	NPY	F: 3'-ccagatactactcggcgttga-5' R: 3'-tccgtgccttctctcatcaag-5'	115
S73519	POMC	F: 3'-agtaacttgctggcgtgcat-5' R: 3'-gaagtggcccatgacgtact-5'	126
NM_001024587	LeptinRb	F: 3'-tggacagagcaagcacattc-5' R: 3'-caggaaagacccacacaactg-5'	161
AY159788	AMPKα2	F: 3'-gagttctacctcgcctctagtcc-5' R: 3'-gacatcttgctttagggctgtc-5'	133
NM_214173	MC4R	F: 3'-catcagttgtatctgggcagtc-5' R: 3'-ggacatagagagaagccatgagag-5'	134
EF434655	Orexin	F: 3'-acaccatgaatcctccttttgc-5' R: 3'-gcaggagcacgtcttttgac-5'	146
DQ321702	OrexinR2	F: 3'-agactgtgtcggtgtctgtgtct-5' R: 3'-aggagacgatccagatgatgac-5'	141
AY550069	β-Actin	F: 3'-cctcaacttccatcaaagcacc-5' R: 3'-tgtcctacgtcttcctctagtg-5'	132

NPY - neuropeptide Y, POMC - pro-opiomelanocortins, leptinRb - long-form leptin receptor; AMPKα2 - AMP-activated protein kinase α2 subunit, MC4R - melanocortin-4 receptor; OrexinR2 - type 2 orexin receptor

Statistical analysis

All statistical analyses were performed with SPSS 13.0 for Windows. One-way ANOVA was used to perform multiple comparisons. Values of mRNA abundance were expressed as the fold-change relative to that of control. All data were expressed as mean ± SEM. The level of significance was set at $P<0.05$ in all analyses.

RESULTS

Effect of dietary Cu on the growth performance of pigs

The data showed as Table 3 that, during the 0-14 d, 15-21 d and over the entire experimental period, ADFI and ADG were improved ($P<0.05$) in 250 mg/kg Cu supplemented groups than in the control group. The ADFI in 100 and 175 mg/kg Cu supplemented groups followed a similar numerical trend toward improvement but were not statistically significant. However, 100 and 175 mg/kg Cu supplementation was sufficient to increase ADG ($P<0.05$).

Table 3. Effects of supplemental copper on growth performance in weanling pigs

Period, days	Item	Cu, mg/kg			
		10	100	175	250
0-14	ADFI, g	261.58 ± 23.06	344.50 ± 27.03	360.38 ± 42.81	404.51 ± 37.94*
	ADG, g	115.83 ± 19.83	153.37 ± 17.54	190.82 ± 20.02*	213.94 ± 18.59*
	F/G	2.36 ± 0.161	2.28 ± 0.096	1.91 ± 0.162*	1.89 ± 0.073*
15-21	ADFI, g	423.51 ± 47.67	506.09 ± 48.16	528.73 ± 63.53	627.74 ± 54.98*
	ADG, g	210.49 ± 23.58	318.64 ± 39.07*	328.74 ± 36.20*	374.70 ± 34.01*
	F/G	2.03 ± 0.147	1.66 ± 0.178	1.62 ± 0.112*	1.69 ± 0.080
0-21	ADFI, g	352.48 ± 34.94	433.76 ± 36.65	464.60 ± 49.59	544.90 ± 46.53*
	ADG, g	147.38 ± 20.41	208.46 ± 22.00	236.80 ± 23.25*	267.53 ± 23.06*
	F/G	2.44 ± 0.143	2.12 ± 0.145	1.95 ± 0.048*	2.04 ± 0.063*

* means within a row that are significantly different are denoted by an asterisk superscript ($P<0.05$)

In the overall experiment, feed gain ratio was also improved in 175 and 250 mg/kg Cu supplemented groups ($P<0.05$).

Quantification of hypothalamic mRNA expression by real-time RT-PCR

The abundances of NPY mRNA in hypothalamus was higher in 250 mg/kg Cu supplemented groups ($P<0.05$; Figure 1A), whereas the abundances of POMC and LeptinRb mRNA were significantly reduced in 175, 250 and 100, 175, 250 mg/kg Cu supplemented groups, respectively ($P<0.05$; Figures 1B and 1C). No difference was found for AMPKα2, MC4R, orexin, OrexinR2 mRNA expression ($P>0.05$; Figures 1D, 1E, 1F and 1G).

DISCUSSION

In this study, feed intake was enhanced by supplementing diets with 250 mg/kg Cu. This indicated that increased feed intake caused by high dietary Cu might underlies the observed growth stimulating effects (Cromwell et al., 1989) of dietary Cu. Moreover, it was also found that supplementing pig diets with pharmacological levels of Cu improved ADG, and F/G in weanling pigs. ADG, ADFI and feed efficiency were improved in all two phases of the nursery period when pigs were supplemented with 250 mg/kg Cu. This is consistent with earlier documented researches (Cromwell et al., 1989) that reported an increase in gain, feed intake and feed efficiency when $CuSO_4$ was added at pharmacological levels to nursery pig diets. Others reported an increase in gain and feed intake, or only gain and efficiency (Edmonds et al., 1985) when diets fed to pigs were supplemented with $CuSO_4$ at levels higher than their requirements.

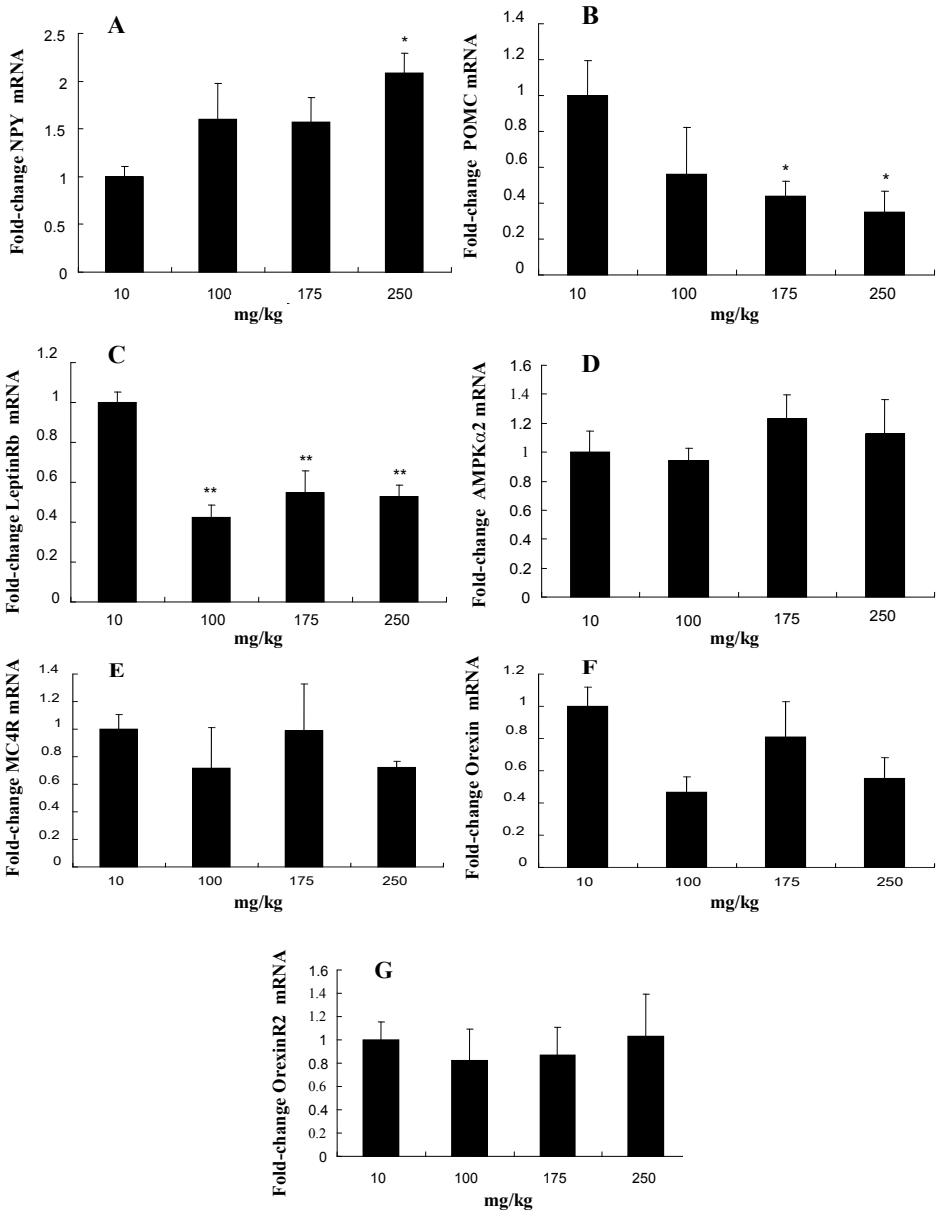

Figure 1. Expression of NPY (A), POMC (B), LRb (C), AMPKa2 (D), MC4R (E), Orexin (F), OrexinR2 (G) mRNA in the hypothalamus of pigs consuming diets supplemented with different levels of copper. β-actin was used as a reference gene. Values are the fold-change relative to control group and expressed as means ± SEM. Asterisk superscript indicates statistical difference between groups ($P<0.05$)

Of further interest in the current study was that lower dietary Cu concentrations (100 and 175 mg/kg) were as effective at stimulating gain as 250 mg/kg Cu. Up to 95% of the Cu were excreted in faeces when pigs are fed high Cu diet (Armstrong et al., 2004). Cu is stricly regulated in the body because of its essentiality to the cellular function and its cytotoxic nature in oxidative stress. Imbalance of Cu homeostasis in brain have been linked to neurologic disorders (Choi and Zheng, 2009) which suggests an important role of Cu in neurologic activity regulation in the central nervous system.

NPY, the most abundant known neuropeptides in the brain, is regarded as the most powerful feeding inducer (Gehlert, 1999). Centrally injected NPY induces feeding even in satiated animals. This function appears to be most closely associated with its action in the paraventricular nuclei of the hypothalamus (Rutkoski et al., 1999). Many factors, including Zn and Cu, have been shown to regulate the expression of NPY (Rutkoski et al., 1999; Williamson, 2002). Although some of the researches that assesses Cu's effects on energy balance have been conducted from the perspective of Cu deficiency, very little work has been done to determine the effects on appetite regulation during excessive Cu administration. Recently, it has been confirmed that porcine hypothalamic NPY mRNA was upregulated by 175 and 250 mg/kg Cu supplementation, suggesting that Cu was associated with hypothalamic NPY mRNA expression (Li et al., 2008). In the present trial, using realtime RT-PCR methods, we also found that high dietary Cu increased NPY mRNA expression levels in the pig hypothalamus. This indicated a mechanistic link between dietary Cu, NPY mRNA expression and appetite.

A decreased mRNA abundances of anorexigenic POMC in hypothalamus was also observed to company with the elevated mRNA expression of NPY in high dietary Cu supplemented groups. POMC is the precursor molecule of the anorexigenic neuropeptides α-melanocyte stimulating hormone (α-MSH) which acts *via* MC4R, a key molecule underlying appetite control and energy homeostasis, to inhibit feeding and reduce body weight. In the arcuate nucleus (ARC), POMC expression is decreased by NPY (Garcia de Yebenes et al., 1995). Because POMC and NPY have opposing effects on feed intake regulation, this result seems predictable. Moreover, it has been proved that when the ARC registers an energy deficit, the neuropeptide balance is shifted to high NPY expression and low POMC expression to promote appetite (Pinto et al., 2004). The observation that elevated mRNA expression of NPY was accompanied by reduced mRNA expression of POMC suggested that decreased POMC mRNA expression might also contribute to the appetite stimulating effect of high dietary Cu. Numerous observations support this model of how hypothalamic energy balance is regulated by leptin. For example, absence of leptin in obese (Lep ob/Lep ob) mice reduces POMC expression in the ARC (Thornton et al., 1997) while increases levels of

NPY (Schwartz et al., 2000), mimicking the hypothalamic response to starvation. Similarly, absence of a-MSH in POMC knockout mice causes hyperphagia and obesity (Yaswen et al., 1999), and blockade of neuronal melanocortin signaling diminishes the response to central leptin administration (Seeley et al., 1997). The LeptinRb is a class 1 cytokine receptor that regulates gene transcription *via* activation of the Janus kinase–signal transducer and activator of transcription (JAK–STAT) pathway. In hypothalamic neurons, leptin also activates the insulin receptor substrate-phosphatidylinositol 3 kinase (IRS–PI3K) pathway (Niswender et al., 2001). STAT3 activation *via* LeptinRb in response to leptin is proposed to stimulate gene transcription of POMC while inhibit gene transcription of orexigenic neuropeptide. On the other hand, PI(3)K activation inhibits FOXO1-mediated gene transcription and also result in increased POMC gene transcription and reduced orexigenic neuropeptide gene transcription (Morton et al., 2006). In addition, it was found that leptin increases PI(3)K activity and firing rate in POMC neurons while inhibiting both parameters in NPY neurons (Morton et al., 2006). LeptinRb mRNA was significantly reduced in 100, 175 and 250 mg/kg Cu supplemented groups, which fited well with its expected role to regulate NPY and POMC mRNA expression as mentioned above. Thus, we hypothesize that down-regulation of LeptinRb mRNA expression might play a pivotal role in mediating the well-known feeding stimulating effect of high dietary Cu *via* alleviating the appetite-suppressing regulation of NPY and POMC mRNA expression, given the observation that mRNA expression of NPY was elevated while POMC mRNA expression was reduced.

However, we did not see significant changes in AMPKα2 subunit, melanocortin-4 receptor (MC4R), orexin, type 2 orexin receptor mRNA expression. It was possible that nucleic specific changes of these mRNA expressions were masked because we used complete hypothalamic samples. There might exist another possibility that changes in these appetite regulator gene expression might not be detectable when evaluated at different time point if their gene expressed in a circadian manner (Adrian et al., 2007). Furthermore, in a ARC NPY and POMC neurons lesion rat model, hyperphagia occurs without increase in expression of the genes for the lateral hypothalamic orexigenic peptides, MCH and orexin (Ai-Jun et al., 2008). Thus, the source of the stimulatory drive for feeding in weanling pigs consuming high Cu supplemented diet needs further investigation.

CONCLUSIONS

Supplementing diets with 250 mg/kg Cu stimulates feed intake and results in an enhancement in weanling pigs' growth. Furthermore, the shifted hypothalamic

appetite-regulating genes expression profile suggests that down-regulation of LeptinRb mRNA expression might contribute to the stimulation of feeding of high dietary Cu supplementation *via* regulation of neuropeptide Y and propiomelanocortin mRNA expression. However, refined nucleic specific studies is needed to investgate the further mechanism of the feed stimulating effects of high dietary Cu.

REFERENCES

Adrian M.S., Staszkiewicz J., Ptitsyn A., Argyropoulos G., 2007. Circadian expression of genes regulating food intake. Obesity 15, 607-615

Ai-Jun L., Thu T.D., Sue R., 2008. Hyperphagia and obesity produced by arcuate injection of NPY-saporin do not require upregulation of lateral hypothalamic orexigenic peptide genes. Peptides 29, 1732-1739

Armstrong T.A., Cook D.R., Ward M.M., Williams C.M., Spears J.W., 2004. Effect of dietary copper source (cupric citrate and cupric sulfate) and concentration on growth performance and fecal copper excretion in weanling pigs. J. Anim. Sci. 82, 1234-1240

Choi B.S., Zheng W., 2009. Copper transport to the brain by the blblood-brain barrier and blood-CSF barrier. Brain Res. 1248, 14-21

Cromwell G.L., Stahly T.S., Monegue H.J., 1989. Effects of source and level of copper on performance and liver copper stores in weanling pigs. J. Anim. Sci, 67, 2996-3002

Edmonds M.S., Izquierdo O.A., Baker D.H., 1985. Feed additive studies with newly weaned pigs. efficacy of supplemental copper, antibiotics, and organic acids. J. Anim. Sci. 60, 462-469

Fakler T.M., 1999. Copper complexes improve nursery pig performance. Trace Miner Focus 5, 1-2

Garcia de Yebenes E., Li S., Fournier A., St-Pierre S., Pelletier G., 1995. Regulation of proopiomelanocortin gene expression by neuropeptide Y in the rat arcuate nucleus. Brain Res. 674, 112-116

Gehlert D.R., 1999. Role of hypothalamic neuropeptide Y in feeding and obesity. Neuropeptides 33, 329-338

Li J.,Yan L., Zheng X., Liu G., Zhang N.S., Wang Z., 2008. Effect of high dietary copper on weight gain and neuropeptide Y level in the hypothalamus of pigs. J.Trace Elem. Med. Biol. 22, 33-38

Minokoshi Y., Alquier T., Furukawa N., Kim Y.B., Lee A., Xue B., Mu J., Foufelle F., Ferré P., Birnbaum M.J., Stuck B.J., Kahn B.B., 2004. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature 428, 569-574

Morton G.J., Cummings D.E., Baskin D.G., Barsh G.S., Schwartz M.W., 2006. Central nervous system control of food intake and body weight. Nature 443, 289-295

Niswender K.D., Morton G.J., Stearns W.H., Rhodes C.J., Myers M.G., Schwartz J.W., 2001. Intracellular signalling. Key enzyme in leptin-induced anorexia. Nature 413, 794-795

Pinto S., Roseberry A.G., Liu H., Diano S., Shanabrough M., Cai X., Friedman J.M., Horvath T.L., 2004. Rapid rewiring of arcuate nucleus feeding circuits by leptin. Science 304, 110-115

Rutkoski N.J., Fitch C.A., Yeiser E.C., Dodge J., Trombley P.Q., Levenson C.W., 1999. Regulation of neuropeptideY mRNA and peptide concentrations by copper in rat olfactory bulb. Brain Res. Mol. Brain Res. 65, 80-86

Schwartz M.W., Woods S.C., Porte Jr. D., Seeley R.J., Baskin D.G., 2000. Central nervous system control of food intake. Nature 404, 661-671

Seeley R.J., Yagaloff K.A., Fisher S.L., Burn P., Thiele T.E., van Dijk G., Baskin D.G., Schwartz M. W., 1997. Melanocortin receptors in leptin effects. Nature 390, 349

Thornton J.E., Cheung C.C., Clifton D.K., Steiner R.A., 1997. Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. Endocrinology 138, 5063-5066

Williamson P.S., Browning J.D., Sullivan M.J., 2002. Neuropeptide Y fails to normalize food intake in zinc-deficient rats. Nutr. Neurosci. 5, 19-25

Yaswen L., Diehl N., Brennan M.B., Hochgeschwender U., 1999. Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. Nature Med. 5, 1066-1070