

Traditional Chinese medicine decoction enhances growth performance and intestinal glucose absorption in heat stressed pigs by up-regulating the expressions of SGLT1 and GLUT2 mRNA

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ARTICLE INFO

Article history:

Received 22 April 2008

Received in revised form 4 November 2009

Accepted 4 November 2009

Keywords:

Chinese medicine decoction

High temperature stress

Pig

SGLT1

GLUT2

ABSTRACT

This study was conducted to investigate the effects of high temperature stressor on porcine growth performance, small intestinal absorption and SGLT1 and GLUT2 mRNA expressions, and probe the regulation of traditional Chinese medicine decoction (CMD) on them. Forty-eight 2-month-old Chinese experimental pigs were screened according to weight and litter origin, and then allotted to three groups and treated as follows: normal temperature Control group (Control; 23 °C), high temperature stressor group (HS; 26 °C for 19 h, 40 °C for 5 h); Chinese medicine decoction anti-stress group (CMD; 26 °C for 19 h, 40 °C for 5 h) ($n = 16$ per group). Porcine average dairy feed intake (ADFI), average dairy gain (ADG) and feed:gain ratio (F:G) were examined, and their intestinal disaccharidase activity and xylose absorption were investigated on days 1, 3, 6 and 10 of trial. A method of real-time PCR was applied to determine SGLT1 and GLUT2 mRNA expressions in the small intestine. The results showed that high temperature treatment decreased ($p < 0.05$) the growth performance and intestinal glucose absorption but there was no change ($p > 0.05$) in the expressions of SGLT1 and GLUT2 genes in the small intestine compared with the Control. Dietary supplementation with CMD improved porcine growth performance ($p < 0.05$), as well as increased total disaccharidase activity and xylose absorption in the small intestine of heat stressed pigs on days 3 and 6 ($p < 0.05$). CMD treatment up-regulated SGLT1 and GLUT2 mRNA expressions of pigs on day 6 compared to the HS and Control groups ($p < 0.05$). These results indicated that high temperature stressor decreased porcine growth performance and intestinal absorption of pigs, and CMD treatment improved the growth performance and intestinal glucose absorption of heat stressed pigs by up-regulating SGLT1 and GLUT2 mRNA expressions in the small intestine.

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1. Introduction

Environmental stressors are potential factors that contribute to the lag in BW gain that can lead to an increase in

the number of days to market (Hyun et al., 1998). Following the global warming, high temperature environment has become a major stressor that impairs the production and health of animals. Heat stress caused a series of physiological and metabolic changes in pigs such as elevated body temperature, panting and respiratory alkalosis, and changed metabolic status elicited by decreased levels of plasma triiodothyronine (Huynh et al., 2005; Patience et al., 2005). Previous studies showed that heat stress can negatively affect not only the animals' growth performance but also their immune competence and disease resistance (Morrow-

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Tesch et al., 1994; Collin et al., 2001; Khajavi et al., 2003; Spencer et al., 2005).

Small intestine in mammal is one of the central organs (Whithaker et al., 1990) that is very sensitive to all stressors (Nabuurs et al., 2001; Soderholm and Perdue, 2001). Glucose is a major source of energy for eukaryotic cells, and has significant and varied effects on cell function. Hydrolases, namely digestive enzymes of small intestinal brush-border membrane vesicles (BBMVs), perform the final digestive function on nutrition, and play a critical role on digestion and absorption (Proulx, 1991). Glucose absorption in the small intestine depends on two types of transport mechanisms. One is glucose transporter 2 (GLUT2), which serves as a facilitated diffusion system through lipid bilayers, and the other is Na⁺-dependent glucose transporter 1 (SGLT1), which mediates Na⁺/glucose co-transport function both in kidney and intestine as a secondary active transporter (Breves et al., 2007). The expression of glucose transport such as SGLT1 and GLUT2 was crucial to the absorption and transport competence of glucose in the small intestine (Rodriguez et al., 2004).

Traditional Chinese medicine has been widely used to treat a variety of disease and conditions, and many Chinese herbal prescriptions were demonstrated to be efficacious in the treatment of heat stress syndrome. Liu et al. (2002) reported that Chinese herbal prescriptions had satisfactory effect on improving the grown performance in heat stressed pigs. Chinese medicine decoction (CMD) is composed of four herbs, including *Cortex Phellodendron*, *Rhizome Atractylodes*, *Agastache rugosa* and *Gypsum Fibrosum*. According to Chinese medicine principles, this decoction is prescribed for animals with heat stress syndrome. The previous study in our laboratory (Wang et al., 2007; Song et al., 2008) demonstrated that the CMD improved porcine antioxidant status and immune competence in the small intestine under high temperature environment (40 °C). However, no investigation was done about the effects of the CMD on small intestinal absorption of heat stressed pigs. The current study will use glucose absorption as the index to investigate the effect of high temperature stressor on the growth performance and small intestinal nutritional absorption of pigs, and explore the regulative mechanism for CMD on growth performance of heat stress pigs.

2. Materials and methods

2.1. Preparation of Chinese medicine decoction (CMD)

Chinese medicine decoction was composed of *Cortex Phellodendron* (Huangbai), *Rhizome Atractylodes* (Cangzhu), *A. rugosa* (Huoxiang) and *Gypsum Fibrosum* (Shigao) in the dry weight ratio of 1:1:1:0.5. All raw materials in the decoction were bought from Chinese Traditional Medicine Pharmacy Tong Ren Tang. The mixture of material was immersed in water for 40 min and extracted in boiling water for 2 h and the aqueous extract separated by filtration (100 mesh). Then the extract was heated (50 °C) under reduced pressure to relative density 1 g/mL. The concentrated extract was dried and combined with excipient (starch) and ground into fine granules. One gram of granulated product was equivalent to 1.44 g of the raw herb.

2.2. Animals treatment

In this trial the animals were kept at different ambient temperatures for a time of 10 days. Chinese experimental minitype pigs (CEMP, Chinese Agricultural University I series) aged 2 months were bought from a commercial farm in Changping district of Beijing. Forty-eight barrows with an initial body weight of 7.15 ± 0.58 kg were selected and divided into three treatment groups according to weight and litter origin as follows: normal temperature Control group (Control) were fed under normal environment with 23 °C, and high temperature stressor group (HS) and Chinese medicine decoction anti-stress group (CMD) were fed under high temperature environment with 40 °C from 4:00 a.m. to 9:00 a.m. and 26 °C for other times within one day. Pigs in the Control and HS groups were fed the basal diet (Table 1), formulated to meet the nutrient requirements of swine and pigs in the CMD group were fed the basal diet supplementation with Chinese medicine decoction, its dose was 0.15 g kg BW⁻¹ day⁻¹. All pigs were allowed to consume both feed and water ad libitum.

2.3. Determination of growth performance

Body weights and feed intakes were measured on the beginning and end of trial. On the basis of these data, average dairy feed intake (ADFI), average dairy gain (ADG), and feed:gain (F:G) ratio were calculated during 0–1 day, 1–3 days, 3–6 days and 6–10 days.

2.4. Sampling collected and serum index assayed

The experimental protocol was approved by the Committee for Experimental Animals at Nanjing Agricultural

Table 1
Compositions of experimental diets (as feed basis).

Ingredient	(%)
Maize	51.8
Soybean meal	13.0
Fish meal	6.0
Whey	6.0
Expanded soybean	16.0
Wheat bran	3.0
Limestone	1.5
Monocalcium phosphate	1.0
Salt	0.35
Lysine-HCl	0.22
D,L-methionine	0.13
Vitamin-mineral mix ^a	1.0
Total	100
Chemical composition ^b	
Digestive energy (MJ/kg)	13.89
Crude protein (%)	20.00
Calcium (%)	0.95
Total phosphorus (%)	0.70
Available phosphorus (%)	0.49
L-Lys (%)	1.35
Met + Cys (%)	0.46

^a Vitamins and minerals were included to provide the following amounts per kilogram of diet: 180 mg Zn; 150 mg Fe; 150 mg Cu; 50 mg Mn; 0.3 mg I; 0.3 mg Se; 0.3 mg Co; 6500 IU vitamin A; 750 IU vitamin D3; 20 IU vitamin E; 3.5 mg vitamin K₃; 2.8 mg vitamin B₁; 6.2 mg vitamin B₂; 33 mg niacin; 18 mg d-pantothenic acid; 3.5 mg vitamin B₆; 0.85 mg folic acid; 60 µg biotin; 35 mg vitamin B₁₂.

^b Calculated values.

University and was conducted in accordance with the NRC Guide for the Care and Use of Laboratory animals. On days 1, 3, 6 and 10 of trial, four pigs were selected to sample from each group randomly, and poured 10 ml xylose with sucker at 8 a.m., then blood samples (15 ml per pig) were collected by venipuncture of the jugular vein at 9 a.m. Pigs were killed by anesthesia and then duodenum and jejunum of pigs were isolated and stored at liquid nitrogen within 2 h. Serum was obtained by centrifugation at 3000 rpm for 20 min with Microfuge 22® centrifuge (Beckman coulter, USA) and stored at -20°C until analyzed. The contents of glucose and xylose in serum were examined by glucose kit or xylose kit respectively (Nanjing Jiancheng, China). Xylose absorption was calculated to serum xylose content ratio 10 ml.

2.5. Determination of disaccharidase activity in intestinal BBMVs

Brush-border membrane vesicles (BBMVs) were prepared from frozen duodenum and jejunum segments by ameliorated MgCl_2 precipitation method (Sala-Rabanal et al., 2004). The activity of total disaccharidase was measured by disaccharidase kit. Protein content in BBMVs suspension was examined according to BCA method (Beyotime, China).

2.6. Determination of the expressions for SGLT1 and GLUT2 mRNA

2.6.1. RNA extraction and cDNA synthesized

Total RNA was isolated from duodenal and jejunal samples collected (on day 6 of heat stress) using the phenol and guanidine isothiocyanate based Trizol reagent (Invitrogen, USA) according to the manufacturer's instruction. The RNA purity was determined using Nanodrop ND-1000 spectrophotometer (Nano-Drop Technologies, Rockland, DE, USA) at an optical OD260 and OD260/OD280 ratio, respectively. The OD260/OD280 ratio of all samples was above 1.80. cDNA was synthesized by M-MLV reverse-transcription kit, briefly, 2.0 μg of each RNA isolated from each pig sample was added to a 25.0 μL reaction system containing 2.0 μL of Oligo-dT18 (Promega, USA), 5.0 μL of dNTPs (Sigma, Chemical), 1.0 μL of RNasin inhibitor (Promega, Madison, WI), 1.0 μL of M-MLV transcriptase (Promega, USA), 5.0 μL of M-MLV RT reaction buffer (Promega, USA) and RNase-free water. Cycle parameters for the reverse-transcription procedure were 1 cycle of 70°C , 5 min; 1 cycle of 42°C , 1 h; The RT products (cDNA) were stored -20°C for real-time PCR.

2.6.2. Real-time-polymerase chain reaction (real-time PCR)

The expressions of SGLT1 and GLUT2 mRNA were detected using relative-quantitative real-time PCR technique. Quantitative analysis of PCR was carried out in the DNA Engine Stratagene's Mx3000P® fluorescence detection system (Stratagene, USA) according to optimized PCR protocols and Brilliant SYBR Green QPCR Master Mix (Stratagene, USA), in which SYBR Green I (SGI) was a double-stranded DNA-specific fluorescent dye. β -actin as inner control was amplified in parallel with the target gene allowing gene normalization and providing quantification. The primers used for real-time PCR were designed by using the Primer Program of the Wincosin Sequence Analysis Package (Genetics Computer Group, Inc.) based on known

Table 2

Oligonucleotide primers used for a relative-quantitative real-time PCR analysis.

Primers		Sequences	Amplifiers
β -actin	Sense	5'-GCCGCATCCACGAAACTAC-3'	285 bp
	Antisense	5'-AGAAGCATTTCGGTGGAC-3'	
SGLT1	Sense	5'-CATCATCGTCTGGTCTGC-3'	259 bp
	Antisense	5'-TGCTCTCTCTCCTTGGT-3'	
GLUT2	Sense	5'-CAGGGGTGCTATTGGTGC-3'	275 bp
	Antisense	5'-TTCCTTGCTTTGGCTCC-3'	

SGLT1: Na^+ -dependent glucose transporter 1; GLUT2: glucose transporter 2.

sequences deposited in Genbank. These primers are given in Table 2. The PCR reaction system (20 μL) contained 10 μL SYBR Green qPCR mix, 0.3 μL reference dye, 1 μL primer (10 $\mu\text{mol/L}$ sense and 10 $\mu\text{mol/L}$ antisense), and 1 μL cDNA template ($<10\ \mu\text{g/L}$). For the PCR reaction, the following experimental run protocol was used: denaturation program (94°C for 5 min), amplification and quantification program repeated 40 times (94°C for 30 s, 56°C for 30 s and 72°C for 40 s), melting curve program (95°C for 1 min, $55\text{--}95^{\circ}\text{C}$ with a heating rate of 0.1°C per second and a continuous fluorescence measurement). The expressional abundance of the cDNA samples obtained from pigs of Control group (the calibrator sample) was regulated to 1, and the cDNA abundance of the other samples in the HS and CMD groups was acquired relative to this calibrator. All samples were measured in triplicate.

2.7. Statistical analysis

Data were statistically analyzed by one-way ANOVA. Duncan's multiple range test was used to compare differences among the treatment groups. A p -value of less than 0.05 was taken to indicate statistical significance ($p < 0.05$). Values were expressed as mean \pm SE. All the statistical analyses were performed using SPSS statistical software (Ver. 11.5 for windows, SPSS). The statistical analyses of real-time PCR were performed by Stratagene's Mx3000P® fluorescence detection system.

3. Results

Table 3 shows the effect of Chinese medicine decoction (CMD) on the growth performance of pigs during the trial. ADFI and ADG of pigs with HS treatment were declined ($p < 0.05$), but no difference on the feed:gain (F:G) ratio compared with the Control ($p > 0.05$). Dietary supplementation with CMD increased ADFI and ADG of pigs compared to

Table 3

Effects of heat stress and CMD treatment on porcine growth performance.

Items	Control	HS	CMD
Initial BW (kg, $n = 4$)	7.125 \pm 0.95 ^a	7.250 \pm 0.50 ^a	7.250 \pm 0.29 ^a
ADFI (g)	710.0 \pm 54 ^a	542.5 \pm 44 ^b	705.0 \pm 42 ^a
ADG (g)	252.5 \pm 25 ^a	175.0 \pm 28.9 ^b	250.0 \pm 40.8 ^a
F:G (g/g)	2.71 \pm 0.13 ^a	3.14 \pm 0.32 ^a	2.86 \pm 0.31 ^a
Finish BW (kg)	9.75 \pm 0.87 ^a	9.00 \pm 0.71 ^b	9.75 \pm 0.50 ^a

Data are mean \pm SE. Values in a row not sharing the same superscript are different at $p < 0.05$.

Control: normal temperature control, HS: high temperature stress, CMD: anti-stress Chinese medicine decoction.

ADFI = average daily feed intake, ADG = average daily gain, and F:G = feed: gain.

Table 4

Effects of heat stress and CMD treatment on porcine intestinal xylose absorption and serum glucose levels.

Items	Control	HS	CMD
<i>Xylose absorption (m mol/L, n = 4)</i>			
Day 1	0.49 ± 0.03 ^a	0.48 ± 0.05 ^a	0.47 ± 0.04 ^a
Day 3	0.52 ± 0.02 ^a	0.45 ± 0.04 ^b	0.46 ± 0.05 ^b
Day 6	0.50 ± 0.03 ^a	0.40 ± 0.06 ^b	0.49 ± 0.04 ^a
Day 10	0.48 ± 0.03 ^{a,b}	0.42 ± 0.06 ^b	0.49 ± 0.02 ^a
<i>Serum glucose levels (m mol/L, n = 4)</i>			
Day 1	7.14 ± 0.98 ^a	5.57 ± 1.15 ^b	5.36 ± 0.60 ^b
Day 3	6.48 ± 0.72 ^a	7.36 ± 0.64 ^a	7.19 ± 0.36 ^a
Day 6	5.64 ± 0.84 ^b	7.02 ± 0.67 ^a	5.94 ± 0.45 ^b
Day 10	5.80 ± 0.74 ^a	5.48 ± 0.96 ^a	5.79 ± 0.59 ^a

Data are mean ± SE. Values in a row not sharing the same superscript are different at $p < 0.05$.

Control: normal temperature control, HS: high temperature stress, and CMD: anti-stress Chinese medicine decoction.

HS group ($p < 0.05$). No difference was noticed on ADFI and ADG between CMD treatment and Control ($p > 0.05$).

Table 4 shows the effect of CMD on serum glucose levels and small intestinal xylose absorption of heat stressed pigs at 1, 3, 6 and 10 days. Serum glucose levels with HS treatment were

increased at 6 days ($p < 0.05$), and small intestinal xylose absorption with HS treatment was decreased at 3 and 6 days ($p < 0.05$) compared to the Control. However, dietary supplementation with CMD displayed a significant reduction on serum glucose levels and xylose absorption at 6 days compared to HS group ($p < 0.05$), and no difference was noticed on them between CMD treatment and Control ($p > 0.05$).

As showed in Fig. 1, the activity of disaccharidase in the duodenum of the HS group was decreased on days 3 and 6 ($p < 0.05$), and that in jejunum was declined on days 1, 3 and 6 ($p < 0.05$). Dietary supplementation with CMD increased the activity of disaccharidase in the duodenum on days 3, 6 and 10, and improved the activity in jejunum only on day 6 compared to the HS group ($p < 0.05$).

Figs. 2 and 3 show the effect of CMD on the expression of mRNA for SGLT1 and GLUT2 in porcine duodenum (A) and jejunum (B) on day 6 of heat stressed pigs. Notably, GLUT2 mRNA expression in the duodenum and jejunum of the HS group was increased ($p < 0.05$), but no difference was noticed on SGLT1 mRNA expression compared to Control group ($p > 0.05$). A significant elevation in the expressions of SGLT1 and GLUT2 mRNA with the CMD treatment was noticed in the duodenum and jejunum compared with the HS groups ($p < 0.05$).

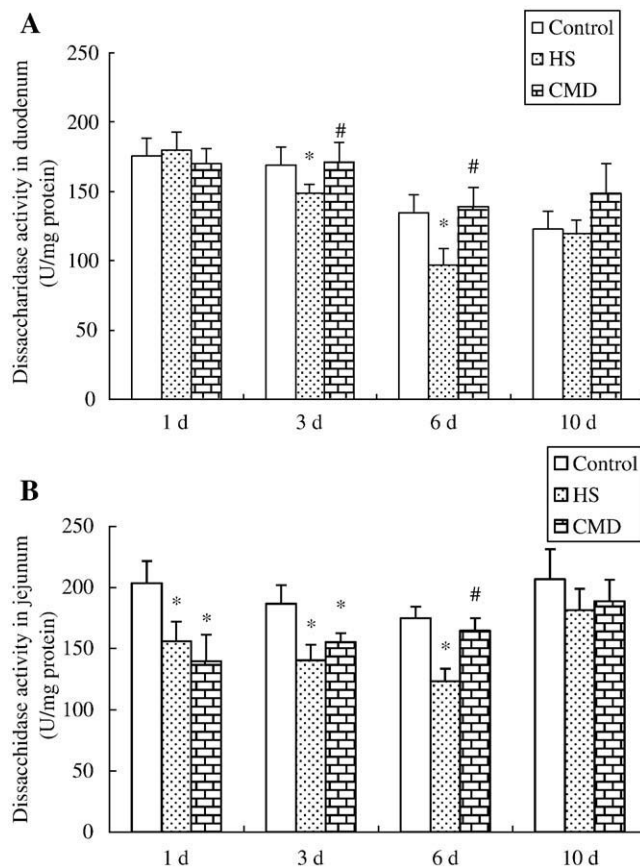


Fig. 1. Bar graphs show the effect of Chinese medicine decoction on the activity of disaccharidase in porcine duodenum (A) and jejunum (B) at 1, 3, 6, 10 days of heat stress. ($n = 4$). Control: normal temperature Control group, HS: high temperature stress group, CMD: Chinese medicine decoction anti-stress group. *: $p < 0.05$ (HS compared to the Control); #: $p < 0.05$ (CMD compared to the HS).

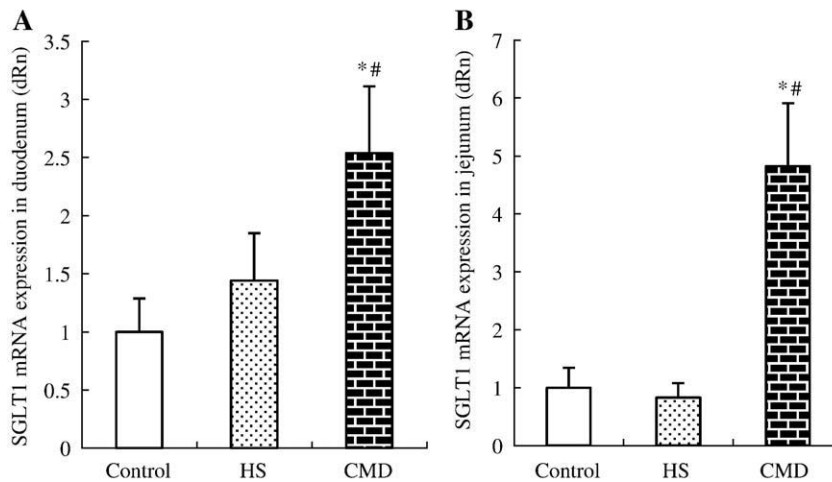


Fig. 2. Bar graphs show the relative gene expression of SGLT1 in porcine duodenum (A) and jejunum (B) in HS and CMD groups to the corresponding mRNA level of the Control group with range, after normalization with β -actin internal standard ($n=4$). SGLT1: Na^+ -dependent glucose transporter 1; Control: normal temperature Control group, HS: high temperature stress group, CMD: Chinese medicine decoction anti-stress group. *: $p<0.05$ (compared to the Control); #: $p<0.05$ (compared to the HS).

4. Discussion

4.1. Effect of high temperature stress on porcine growth performance and serum glucose level

The current result shows that high temperature stressor (40°C , 5 h/day) induced a decrease of porcine feed intake and dairy growth, but without changing feed:gain (F:G) ratio. The previous study in our laboratory reported that the same environment induced the elevation of body temperature and breath frequency of pigs, and the decrease of serum levels of cortisol, triiodothyronine, tetraiodothyronine (Li et al., 2006; Dong, 2008). These results indicated that high temperature environment (40°C , 5 h/day) induced porcine to heat stress.

Some reports on the changes of blood glucose in a hot environment were not consistent. The reduction of blood glucose level induced by heat stress was attributed to a decrease in feed intake, or to a decrease in gluconeogenesis at hot environment (Marai et al., 1995). But Dong et al. (2004) indicated that heat stress increased serum glucose levels of animals. In general, glucose is made mainly from propionic acid in liver; however, the concentration and the ratio of propionic acid in rumen fluid did not differ among the thermal treatments. In the present study, serum glucose concentration were increased on 6 days of heat stress, then recover to Control on 10 days. The result indicates that heat stress induces serum glucose level disorder, but pigs have the ability to recover from the effects of heat stress if periods of heat stress are followed by periods of thermal comfort.

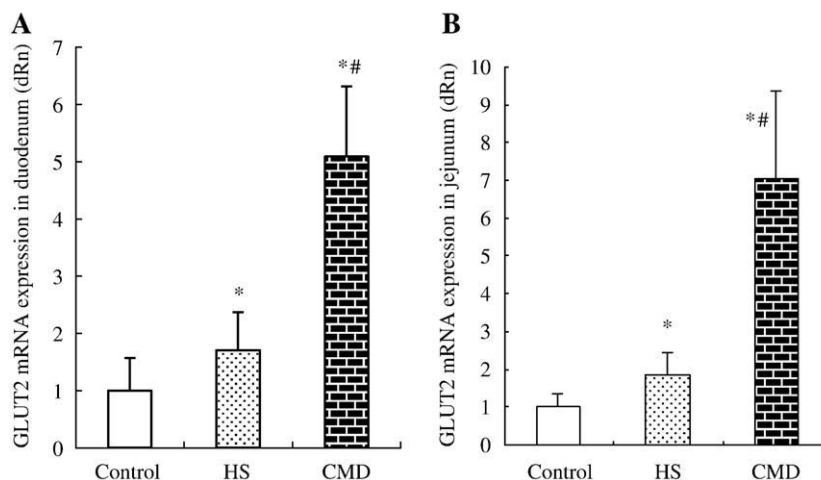


Fig. 3. Bar graphs show the relative gene expression of GLUT2 in porcine duodenum (A) and jejunum (B) in HS and CMD groups to the corresponding mRNA level of the Control group with range, after normalization with β -actin internal standard ($n=4$). GLUT2: glucose transporter 2. Control: normal temperature Control group, HS: high temperature stress group, CMD: Chinese medicine decoction anti-stress group. *: $p<0.05$ (compared to the Control); #: $p<0.05$ (compared to the HS).

4.2. Effect of high temperature stress on porcine glucose absorption and uptake in the small intestine

Disaccharidases are digestive enzymes which are distributed extensively in the mucosa and contents of the small intestine that play a key role in the utilization of carbohydrate. Xylose absorption in animals was demonstrated as an important index to evaluate the absorptive function of the small intestine (Gong et al., 2006). In the present study, high temperature stressor impaired total disaccharidase activity and xylose absorption of porcine small intestine, which may relate to the disturbance of hypothroid hormone induced by heat stress. Patience et al. (2005) and Nonaka et al. (2008) reported that heat stress altered nutrient digestibility by reducing nutrient uptake from the gut lumen or by reducing thyroid hormone levels which in turn alters gastrointestinal motility and digestive passage rates. Thyroid hormones, either T_4 or T_3 , were known to play an important role in the animal's adaptation to environmental changes, and T_3 is more concerned with thermogenesis. T_3 also plays an important role on promoting glucose absorption and utilization of animals. Patience et al. (2005) reported that plasma T_4 and aldosterone levels were not affected by temperature but T_3 levels were lower in diurnal heat stress group compared to Control pigs, and the same result was found in our previous study (Dong, 2008).

Glucose uptake is mediated by a sodium-dependent carrier (SGLT1) in the brush-border membrane (BBM), and by passive permeation through the Para cellular route. GLUT2 transports glucose and fructose out of the enterocyte across the basolateral membrane (Jane et al., 2003; Breves et al., 2007). The expressions of SGLT1 and GLUT2 were crucial to the absorption and uptake of glucose in the small intestine, the elevation of SGLT1 activity resulted in the up-regulation of glucose uptake (Rodriguez et al., 2004). Zhang et al. (2006) found that the expression of SGLT1 mRNA of piglets was significantly decreased on days 7 and 10 after weaning. Akira et al. (2002) indicated that heat shock stress increases SGLT1 activity mediated via the production of transforming growth factor- β 1, resulting in the up-regulation of glucose uptake. Noticeably, the present study indicated that high temperature stress treatment increased the expression of mRNA for GLUT2 in the duodenum and jejunum, but does not influence the expression of SGLT1 mRNA. The elevation of GLUT2 mRNA expression induced by high temperature stress may help small intestinal digestion and absorption of pigs to recover normal on day 10 of trial. However, it is unclear why high temperature stress does not affect the expression of SGLT1 mRNA in this experiment, which may relate to the extent of heat stress.

4.3. Effects of Chinese medicine decoction on porcine intestinal glucose absorption and uptake under heat stress

The current study demonstrated that dietary supplementation with Chinese medicine decoction improved the growth performance, increased glucose absorption and uptake in the small intestine, and regulated serum glucose levels to normal. Chinese medicine decoction was made by a Chinese medicine prescription, which was composed of *Cortex Phellodendron*, *Rhizome Atractylodes*, *A. rugosa* and *Gypsum Fibrosum* at a ratio of 1:1:1:0.5 respectively according to the principia of principal,

associate, adjuvant and messenger in Chinese medical theory. *Cortex Phellodendron* and *Rhizome Atractylodes* are principal medicine, and *A. rugosa* and *Gypsum Fibrosum* are associate medicines in this prescription. Our previous studies in chicken and pigs have demonstrated that the granule improved the growth performance and small intestinal immunity under high temperature stressor (Liu et al., 2002; Wang et al., 2007). Others on Chinese medicine (Yan et al., 2006) also certified that the active component of rhizoma *Atractylodes* as β -Eudesmol has the function of depriving the evil wetness. Berberine, the main active ingredient of phellodendron, can clear humid heat and asthenic fever, especially eliminating damp-heat in lower-JIAO (Lu et al., 2006). Studies have demonstrated that the extracts of *Cortex Phellodendron* and *A. rugosa* show an inhibitory effect on the spasmodic contraction of the small intestine and had anti-diarrheal properties, improving overall intestinal function (Chen et al., 1998). These results are consistent with our present data, indicating that the action of CMG on improving intestinal absorption may be related to the combined function of sing-medicine in the granule.

Little information is available on the effects of traditional Chinese medicine on nutrient absorption and uptake in the small intestine. The current study found that Chinese medicine decoction improved the growth performance, glucose digestion and absorption in the small intestine from the 6th day of heat stress. To probe the influencing mechanism of Chinese medicine decoction on glucose absorption of heat stressed pigs, the expressions of mRNA for SGLT1 and GLUT2 in the duodenum and jejunum on day 6 of heat stress were analyzed. As a result, Chinese medicine decoction up-regulated the expression of mRNA for SGLT1 and GLUT2, which indicated that dietary supplementation with Chinese medicine decoction improved the growth performance and intestinal glucose absorption by up-regulating of SGLT1 and GLUT2 mRNA expressions.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 30771566); Beijing Natural Science Foundation (No. 6082007) and National Eleven-five Technological Supported Plan of China (No. 2008BADB4B01, 2008BADB4B07). We appreciate all the helps from our colleagues and collaborators.

References

- Akira, I., Mika, N., Kazuya, K., Yasunobu, S., 2002. Up-regulation of sodium-dependent glucose transporter by interaction with heat shock protein 70. *Journal of Biological Chemistry* 277 (36), 33338–33343.
- Breves, G., Kock, J., Schröder, B., 2007. Transport of nutrients and electrolytes across the intestinal wall in pigs. *Livestock Science* 109, 4–13.
- Chen, X.X., He, B., Li, X.Q., Li, H.Y., Luo, J.P., 1998. Comparison of effects of three extracts of herba pogostemonis on the intestinal function. *Pharmacology and Clinics of Chinese Materia Medica* 14 (2), 31–33.
- Collin, A., Van Milgen, J., Le Dividich, J., 2001. The effect of high, constant temperature on food intake in young growing pigs. *Animal Science* 72, 519–527.
- Dong, H., 2008. Effects of traditional Chinese medicine on gut hormones in pig and rat under high temperature. Ph.D. Thesis (in Chinese, with English abstract). China Agriculture University, Beijing, China.
- Dong, S.L., Wang, Z.B., Lei, X.Q., 2004. Influence of heat stress on biochemical indexes in blood of animal. *Ecology of Domestic Animal* 25 (2), 54–56.
- Gong, J.F., Zhu, W.M., Liu, F.N., Luo, N., Tan, L., Li, N., Li, J.S., 2006. Evaluation of the absorptive area and capacity of short bowel patients using D-xylose

- absorption test. *Parenteral & Enteral Nutrition* 13 (2), 88–91 (in Chinese, with English abstract).
- Huynh, T.T.T., Aarnink, A.J.A., Verstegen, M.W.A., 2005. Effects of increasing temperatures on physio-logical changes in pigs at different relative humidities. *Journal of Animal Science* 83 (2), 1385–1396.
- Hyun, Y., Ellis, M., Riskowski, G., Johnson, R.W., 1998. Growth performance of pigs subjected to multiple concurrent environmental stressors. *Journal of Animal Science* 76, 721–727.
- Jane, D., Steven, V., Timothy, P., King, S.P., Shirazi, B., 2003. Glucose sensing in the intestinal epithelium. *European Journal of Biochemistry* 270, 3377–3388.
- Khajavi, M., Rahimi, S., Hassan, Z.M., Kamali, M.A., Mousavi, T., 2003. Effect of feed restriction early in life on humoral and cellular immunity of two commercial broiler strains under heat stress conditions. *British Poultry Science* 44, 490–497.
- Li, X.G., Zhu, X.Y., Liu, F.H., 2006. Effect of Qingliang electuary on the amounts of CD⁴⁺ and CD⁸⁺ T-cells under high temperature in chickens. *Journal of Chinese Veterinary Medicine* 6, 5–8 (in Chinese, with English abstract).
- Liu, F.H., Wang, Z.H., Li, B., 2002. Influence of Chinese herbs additives on performance of growing-finishing pigs under heat stress. *Feed Research* 4, 1–4 (in Chinese, with English abstract).
- Lu, Y.N., Qiu, Q.Y., Wang, Y., 2006. Effects of phellodendron and its main components on the cell membrane fluidity. *Chinese Journal of Pathophysiology* 22 (1), 156–159 (in Chinese, with English abstract).
- Marai, I.F.M., Habeeb, A.A., Daader, A.H., Yousef, H.M., 1995. Effects of Egyptian subtropical summer conditions and the heat stress alleviation technique of water spray and a diaphoretic on the growth and physiological functions of Friesian calves. *Journal of Arid Environments* 30, 219–225.
- Morrow-Tesch, J.L., Mcglone, J.J., Salak-Johnson, J.L., 1994. Heat and stress effects on pig immune measures. *Journal of Animal Science* 72, 2599–2609.
- Nabuurs, M.J.A., van Essen, G.J., Nabuurs, P., Niewold, T.A., Vander, M.J., 2001. Thirty minutes transport causes small intestinal acidosis in pigs. *Research in Veterinary Science* 70, 123–127.
- Nonaka, I., Takusari, N., Tajima, K., Suzuki, T., Higuchi, K., Kurihara, M., 2008. Effects of high environmental temperatures on physiological and nutritional status of prepubertal Holstein heifers. *Livest. Sci.* 113, 14–23.
- Patience, J.F., Umboh, J.F., Chaplin, R.K., Nyachoti, C.M., 2005. Nutritional and physiological responses of growing pigs exposed to a diurnal pattern of heat stress. *Livestock Production Science* 96, 205–214.
- Proulx, P., 1991. Structure function relationships in intestinal brush border membranes. *Biochimica et Biophysica Acta* 1071, 255–271.
- Rodriguez, S.M., Guimaraes, K.C., Matthews, J.C., McLeod, K.R., Baldwin, R.L., Harmon, D.L., 2004. Influence of abomasal carbohydrates on small intestinal sodium-dependent glucose cotransporter activity and abundance in steers. *Journal of Animal Science* 82 (10), 3015–3023.
- Sala-Rabanal, M., Gallardo, M.A., Sanchez, J., Planas, J.M., 2004. Na-dependent D-Glucose transport by intestinal brush border membrane vesicles from gilthead sea bream (*sparus aurata*). *J. Membrane Bio.* 120, 85–96.
- Soderholm, J.D., Perdue, M.H., 2001. Stress and the gastrointestinal tract II. Stress and intestinal barrier function. *American Journal of Physiology* 280 (10), C7–C13.
- Song, X.Z., Wang, Z.H., Mao, S., Liu, F.H., Wang, T., 2008. Effect of Chinese medicine additives on porcine serum antioxidant status under high temperature stress. *Animal Husbandry & Veterinary Medicine* (5), 5–8.
- Spencer, J.D., Gained, A.M., Berg, E.P., Allee, G.L., 2005. Diet modification to improve finishing pig growth performance and pork quality attributes during periods of heat stress. *Journal of Animal Science* 83, 243–254.
- Wang, Z.L., Yu, T.Q., Zhu, X.Y., Chen, H.Y., Liu, F.H., Xu, J.Q., 2007. Effects of Chinese medicine decoction on concentration of IL-2, IL-10 and IgA in porcine intestine after heat stress. *Journal of Chinese Veterinary Medicine* 9, 12–15 (in Chinese, with English abstract).
- Whithaker, J.S., Ryan, C.F., Buckley, P.A., 1990. The effects of refeeding on peripheral and respiratory muscle function in malnourished chronic obstructive pulmonary disease patients. *American Review of Respiratory Disease* 142, 283–288.
- Yan, M.C., Chen, J., Chou, G.X., 2006. Research review on chemical constituent and pharmacological action of rhizoma atracylodes. *Acta Universitatis Traditionis Medicis Sinensis Pharmacologiaeque Shanghai* 20 (4), 95–98 (in Chinese, with English abstract).
- Zhang, L., Wang, L.N., Shi, Z.M., Wei, X.H., Chen, J., Zhao, R.Q., 2006. Expression of SGLT1 mRNA in duodenum, jejunum and ileum of weaning pigs and the effect of cysteamine on it. *Journal of Agricultural Biotechnology* 14 (6), 850–854 (in Chinese, with English abstract).