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Chinese Medicine Granule Affects the Absorption and Transport of Glucose in Porcine Small Intestinal Brush Border Membrane Vesicles under Heat Stress*

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ABSTRACT : This study was conducted to investigate the effects of Chinese medicine granule (CMG, including *Cortex Phellodendron*, *Atractylodes Rhizome*, *Agastache Rugosa* and *Gypsum Fibrosum*) on absorption and transport of glucose in porcine small intestinal brush border membrane vesicles (BBMVs) under heat stress. Forty-eight 2-month-old Chinese experimental barrows were screened according to weight and litter origin, and then allotted to three groups and treated as follows: Normal temperature control group (NTCG; 23°C), high temperature control group (HTCG; 26°C for 19 h, 40°C for 5 h); Chinese medicine granule anti-stress group (CMGG; 26°C for 19 h, 40°C for 5 h) (n = 16 per group). 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 expression of SGLT1 and GLUT2 genes in the small intestine of pigs compared with the NTCG. Dietary supplementation with CMG improved the growth performance, and increased the activity of disaccharidases in duodenum and jejunum of heat stressed pigs ($p<0.05$). CMG treatment increased ($p<0.05$) the protein levels of SGLT1 and GLUT2 in the small intestine, and up-regulated ($p<0.05$) the expression of SGLT1 and GLUT2 genes in the duodenum and jejunum but without changing ($p>0.05$) them in the ileum compared with the HTCG. These results indicated that CMG treatment significantly improved porcine growth performance, and increased intestinal glucose absorption and transport by BBMVs under heat stress, in addition to up-regulating the expression of SGLT1 and GLUT2 genes in porcine duodenum and jejunum. (**Key Words :** Chinese Medicine Granule, Heat Stress, Pig, Intestinal BBMVs, Glucose Absorption)

INTRODUCTION

Heat stress causes 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 (Hyun et al., 1998; Huynh et al., 2005). The mammalian small intestine is a central organ which is very sensitive to all stressors (Nabuurs et al., 2001; Hou et al., 2006). Much research has reported that heat stress can negatively affect not only the growth performance of animals but also their nutrient utilization (Collin et al., 2001; Khajavi et al., 2003; Spencer et al., 2005), and our previous results also showed that high temperature treatment (40°C, 5 h) induced lipid peroxidation in small intestinal epithelium (Song et al., 2008) and a decrease of intestinal immunity in pigs (Hu et al., 2006). Glucose absorption in the intestine has an important role in maintaining cellular and organic functions (Jane et al., 2003), and the expressional levels of intestinal glucose transporters are crucial to the absorption and uptake of glucose in the small intestine (Rodriguez et al., 2004), but there has been little study of the effect of high temperature treatment on expression of these transporters.

Traditional Chinese medicine has been widely used to

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Table 1. Composition of experimental diet (as fed 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
DL-methionine	0.13
Vitamin-mineral mix[1]	1.0
Total	100
Chemical composition[2]	
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

[1] 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; 6,500 IU vitamin A; 750 IU vitamin D3; 20 IU vitamin E; 3.5 mg vitamin K_3; 2.8 mg vitamin B_1; 6.2 mg vitamin B_2; 33 mg niacin; 18 mg *d*-pantothenic acid; 3.5 mg vitamin B_6; 0.85 mg folic acid; 60ug biotin; 35 mg vitamin B_{12}.

[2] Calculated values.

treat a variety of diseases, and many Chinese herbal medicines have been reported to improve intestinal absorption of nutrients (Kong et al., 2007). Studies on Chinese medicine certified that the active component of *Atractylodes Rhizoma,* β-Eudesmol, has the function of preventing the evil wetness, and *Gypsum Fibrosum* is generally used to prevent and cure sthenic fever and domination disease (Chen et al., 2006). The previous study by our laboratory (Liu et al., 2002; Wang et al., 2007) demonstrated that a Chinese medicine prescription, composed of four medicines (including *Cortex Phellodendron*, *Atractylodes Rhizome*, *Agastache Rugosa* and *Gypsum Fibrosum*), improved the growth performance and immune competence of porcine small intestine in a high temperature environment (40°C). However, no information is currently available regarding the effects of the prescription on small intestinal absorption of heat stressed pigs. The current study was, therefore, designed to investigate changes in activity of functional enzymes and glucose transporter protein expression in intestinal BBMVs of a porcine heat stress model established in our laboratory, and to probe the regulative mechanism of Chinese medicine granule on growth performance and glucose absorption in porcine small intestine under heat stress.

MATERIALS AND METHODS

Preparation of Chinese medicine granule

All Chinese herbal raw materials were purchased from Chinese Traditional Medicine Pharmacy Tong Ren Tang (Beijing, China). A Chinese medicine prescription was composed of four dried medicine materials, including *Cortex Phellodendron* bark (Huangbai), *Atractylodes Rhizome* (Cangzhu), the stem and leaf of *Agastache Rugosa* (Huoxiang) and *Gypsum Fibrosum* powder (Shigao), which were mixed in the dry weight ratio of 1:1:1:0.5. The mixture of material was immersed in water for 40min 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 1.21-1.27 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 dried raw medicine materials.

Animals treatment

In this trial the animals were kept at different ambient temperatures for a period 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 (NTCG) were raised under a normal environment at 23°C, and High temperature control group (HTCG) and Chinese medicine anti-stress granule group (CMGG) were raised under a high temperature environment at 40°C from 4:00 am to 9:00 am and 26°C for the remainder of the day. There were 16 pigs per treatment group. Pigs in the NTCG and HTCG were fed the basal diet (Table 1), formulated to meet the nutrient requirements of swine (NRC, 1998), and pigs in CMGG were fed the basal diet supplemented with CMG at a dose of 0.15 g/kg BW·d. All pigs were housed individually in an environmentally-controlled nursery facility, and had free access to diets and drinking water.

Experimental procedure

The experimental protocol was approved by the Committee for Experimental Animals at Nanjing Agricultural University and was conducted in accordance with the NRC Guide for the Care and Use of Laboratory animals (NRC, 1998). On days 1, 3, 6 and 10 of the trial, four barrows were selected randomly from each group, weighed and killed by exsanguinating rapidly, and then duodenum, jejunum and ileum samples were removed and immediately frozen in liquid nitrogen and stored in a freezer

Table 2. Sequences of PCR primers

Gene[1]	Accession number[2]	Primer sequence(5'→3')	Orientation	Product size (bp)
SGLT1	M34044	5'- CATCATCGTCCTGGTCGTC-3'	Forward	259
		5'- TGCCTCCTCTTCCTTGGT -3'	Reverse	
GLUT2	EF140874	5'- CAGGGGTGCTATTGGTGC-3'	Forward	275
		5'- TTCCTTGCTTTGGCTTCC-3'	Reverse	
β-actin	AY550069	5'-GCGGCATCCACGAAACTAC-3'	Forward	285
		5'- AGAAGCATTTGCGGTGGAC-3'	Reverse	

[1] SGLT1 = Na^+-dependent glucose transporter 1; GLUT2 = glucose transporter 2. [2] Genbank accession number.

at -70°C for subsequent extraction of protein and total RNA. Body weight and feed intake of individual barrows were recorded, and their average dairy growth and average dairy feed intake were analyzed.

Enzyme and protein determinations

BBMVs were prepared from frozen duodenum, jejunum and ileum segments by an ameliorated $MgCl_2$ precipitation method (Sala-Rabanal et al., 2004). The activities of lactase, maltase and sucrase in small intestinal BBMVs were measured by colorimetric enzymatic methods (Xu et al., 2002). The activity of AKP was measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The protein content of sodium-dependent glucose transporter 1 (SGLT 1) and glucose transporter 2 (GLUT 2) were determined using a commercially available porcine ELISA kit (ADI Systems, USA) according to the manufacturer's instructions. Total protein content in BBMVs suspension was examined according to the BCA method (Beyotime, China).

Real-time quantitative PCR analysis of gene expression

Total RNA was isolated using the TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. The RNA integrity was assessed via agarose gel electrophoresis and RNA concentration and purity were determined spectrophotometrically using A260 and A280 measurements in a photometer (Eppendorf Biophotometer). Ratios of absorption (260/280 nm) of all preparations were between 1.8 and 2.0. Reverse transcription (RT) reactions (25 μl) consisted of 2 μg total RNA, 100 U of M-MLV reverse transcriptase (Promega, Belgium), 40 U of recombinat RNAsin ribonuclease inhibitor (Promega, Belgium), 0.8 mmol/L dNTP (Promega, Belgium), and 1 μg random primers (Promega, Belgium) in RNase-free water and buffer supplied by the manufacturer. After incubation (37°C, 60 min) the mixture was heated (94°C, 5 min). Polymerase chain reaction (PCR) was performed in 20 μl containing 1 μl of the RT reaction products, 10 μl SYBR Realtime PCR Master Mix (Stratagene, USA), 0.1-0.2 mmol/L of each gene specific primer and β-actin, the internal standard (Table 2). The expression of β-actin showed no significant difference among the three groups. Thermal cycling parameters were as follows: 1 cycle 94°C for 5 min, and then 40 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 40 s on a Stratagene MX3000PTM Sequence Detection System (MXproTM QPCR software). Fluorescence data was collected in the latter stage by recording SYBR incorporation into amplified DNA. Fluorescent data were used to derive the *C (t)* for the PCR cycle at a threshold which is noted as the first significant deviation in fluorescence from a base line value. Analyses were performed in triplicate. The resultant value was expressed relative to β-actin (house keeping gene). Results (fold changes) were expressed as $2^{-\Delta\Delta C(t)}$ with $\Delta\Delta C(t)$ = ($C(t)_{ij}$-$C(t)$ β-$actin_j$)-($C(t)_{ik}$-$C(t)$ β-actink), where $C(t)_{ij}$ and $C(t)$ β-$actin_j$ are the $C(t)$ for gene i and for β-actin in a pool or a sample (named j) and where $C(t)_{ik}$ and $C(t)$ β-$actin_k$ are the *C(t)* in target gene i and in the house keeping gene in the control group (named k), respectively.

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±SE. All the

Table 3. Changes of porcine growth performance on day 6 during heat stress*

Items	NTCG	HTCG	CMGG
Initial BW (kg)	7.133±0.69[a]	7.175±0.61[a]	7.150±0.59[a]
ADFI (g)	675.0±32.3[a]	542.5±21.7[b]	630.0±12.2[a]
ADG (g)	240.5±12.5[a]	165.0±14.4[b]	230.0±20.4[a]
F:G (g/g)	2.75±0.25[a]	3.21±0.11[a]	2.76±0.28[a]

* Data are mean±SE. (n = 4). [a-b] Within a row, means without a common superscript letter differ ($p<0.05$).

[1] NTCG = Normal temperature control group, HTCG = High temperature stress group, CMGG = Chinese medicine granule anti-stress group.

[2] ADFI = Average diary feed intake, ADG = Average diary gain, F:G = Feed:gain.

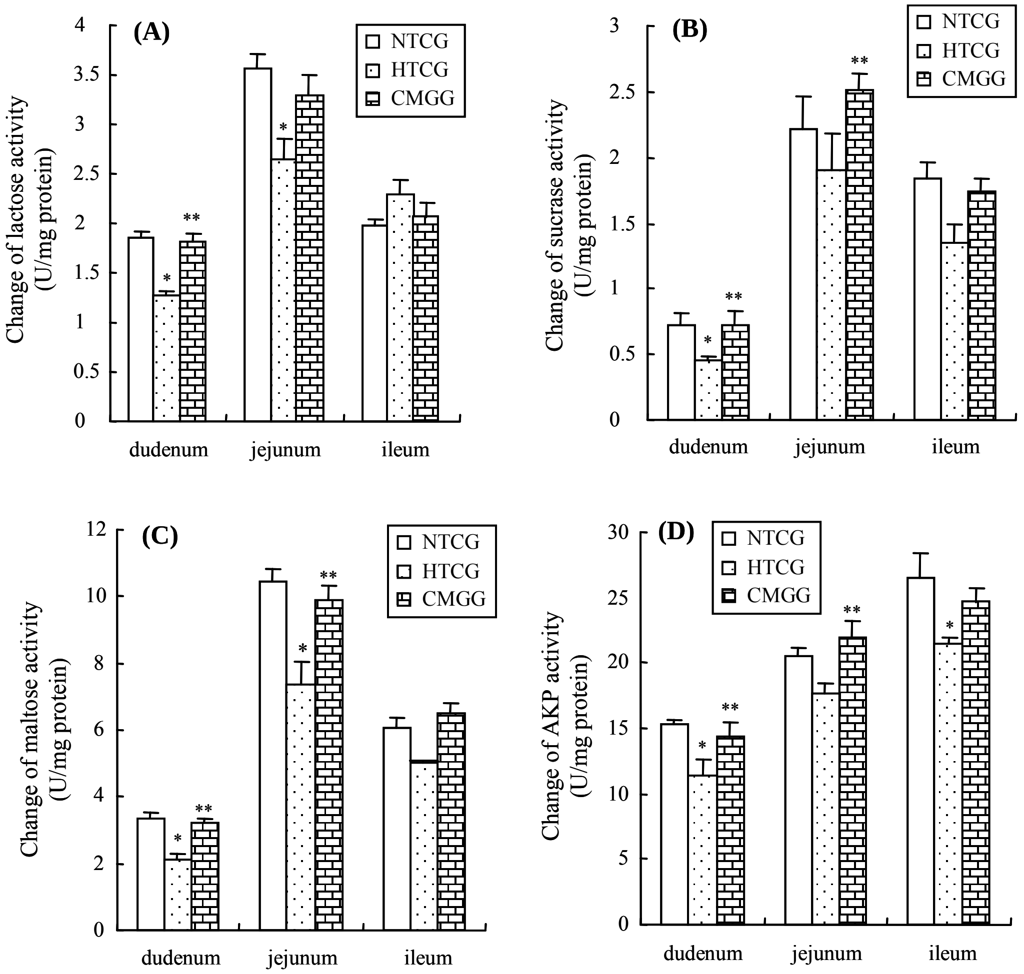

Figure 1. The activity of sucrase (A), lactose (B), maltose (C) and alkaline phosphatase (AKP, D) in porcine intestinal brush border membrane vesicles of normal temperature control group (NTCG), high temperature control group (HTCG) and Chinese medicine anti-stress granule group (CMGG) on day 6. Values are means±SE, n = 4. * $p<0.05$ (The CMGG or the HTCG vs. NTCG); ** $p<0.05$ (The CMGG vs. HTCG).

statistical analyses were performed using SPSS statistical software (Ver.11.5 for windows, SPSS).

RESULT

Changes of the growth performance in pigs during 0-6 days

Table 3 showed that the average daily gain (ADG) and average daily feed intake (ADFI) of the HTCG declined compared to the NTCG ($p<0.05$), but dietary supplementation with CMG increased ADG and ADFI of heat stressed pigs ($p<0.05$), and recovered ADG to the normal level of the NTCG ($p>0.05$). No difference was recorded in the feed:gain (F:G) ratio among three groups ($p>0.05$).

Changes of the functional enzyme activity in small intestinal BBMVs on day 6

As shown in Figure 1, the activities of sucrase, lactase and maltase of the HTCG were decreased in the duodenum and jejunum ($p<0.05$) but there was no change in the ileum, and the activity of AKP of the HTCG were decreased in the duodenum and ileum but were without change in the jejunum compared with the NTCG. However, the activities of all enzymes measured in the duodenum and jejunum of CMG-treated group were increased compared with the HTCG ($p<0.05$), and approached that of the NTCG ($p>0.05$).

Changes of SGLT1, GLUT2 expression in small intestine on day 6

Figure 2 shows that the protein levels of GLUT2 in the duodenum and ileum were decreased in the HTCG, but there was no change of the expression of SGLT1, and GLUT2 genes were increased in the duodenum, jejunum and ileum compared with the NTCG ($p<0.05$). Dietary supplementation with CMG increased the protein levels of SGLT1 and GLUT2 in the duodenum and jejunum

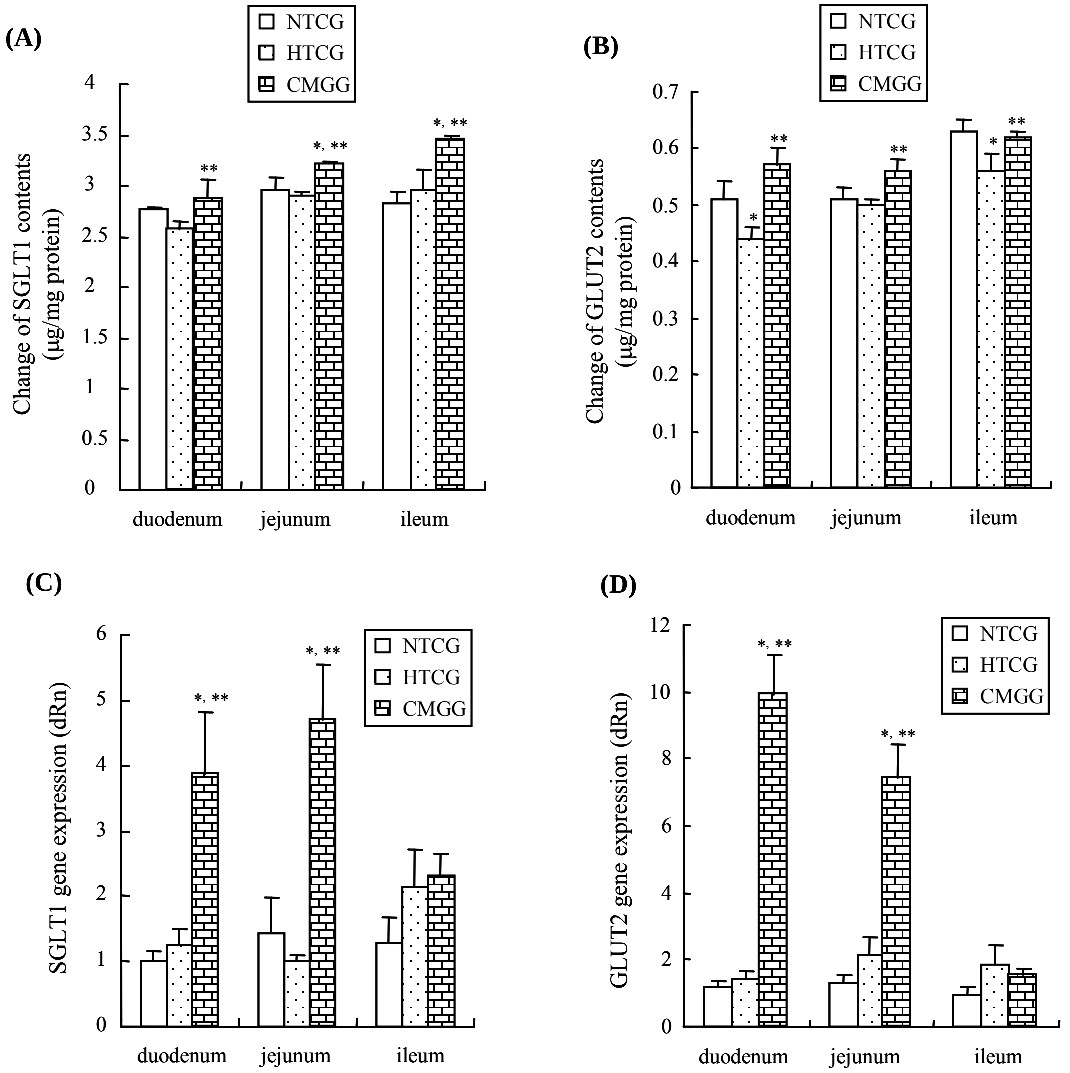

Figure 2. The protein levels of SGLT1 (A) and GLUT2 (B) in intestinal brush border membrane vesicles and the gene expression of SGLT1 (C) and GLUT2 (D) in the small intestine of normal temperature control group (NTCG), high temperature control group (HTCG) and Chinese medicine anti-stress granule group (CMGG) on day 6. SGLT1 = Na^+-dependent glucose transporter 1, GLUT2 = glucose transporter 2. Values are means±SE, n = 4. * p<0.05 (The CMGG or the HTCG vs. NTCG); ** p<0.05 (The CMGG vs. HTCG).

compared with the HTCG, and improved SGLT1 protein level in the jejunum and ileum compared with the NTCG (p<0.05). A significant elevation in the expression of SGLT1 and GLUT2 genes with the CMGG was observed in the duodenum and jejunum compared with the two control groups (p<0.05), but no difference was evident in the ileum among all treatments (p>0.05).

DISCUSSION

This trial investigated the dynamic effect of CMG on porcine growth performance and intestinal enzyme activities on day 1, 3, 6, 10 during high temperature treatment. The results indicated that high temperature treatment decreased porcine growth performance on days 6 and 10 and intestinal BBMVs enzyme activities on days 3 and 6 (data not shown). Dietary supplementation with CMG improved porcine growth performance and intestinal enzyme activities in heat stressed pigs, especially on day 6. Hence, we examined the changes in expression of porcine intestinal glucose transporters and probed the regulative mechanism of CMG on growth performance and intestinal glucose absorption under high ambient temperature treatment at 40°C for 6 days (5 h per day).

The current results show that a high ambient temperature stressor induced a decrease of porcine feed intake and daily growth, but without changing feed:gain (F:G) ratio. These results are similar to the reports of Hyun et al. (1998) and Sutherland et al. (2006), but Hyun et al. (1998) pointed out that porcine growth performance was not affected by temperature until week 3 and 4, which may be related to a lower ambient temperature stressor (38°C, 16 h per day) in their experiment than in the present study.

In recent years, small intestinal brush border membrane vesicles (BBMVs) have generally been used to study nutrient transport, assess enzyme activity, and identify and quantify various brush border proteins (Schulthess et al., 1996). The activity of disaccharidases and AKP as the functional enzymes in BBMVs affects small intestinal nutrient absorption. In the current study, the activities of disaccharidases and AKP in BBMVs were decreased by high temperature treatment, indicating that heat stress induced the reduction of intestinal digestion and nutrient absorption. The previous studies also demonstrated high temperature 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 (Patience et al., 2005; Nonaka et al., 2008). Our previous study showed that the same high temperature treatment induced decreased levels of serum thyroid hormones, either T_4 or T_3 (Dong, 2008). The stress responses in animals are excited mainly by activation of the hypothalamic-pituitary-adrenal (HPA) axis and the adrenocorticotrophic nervous system. Heat stress activates the HPA axis of animals and causes a series of physiological and metabolic changes, and changed metabolic status elicited by decreased levels of plasma thyroid hormone (Nazifi et al., 2003). Thyroid hormones, either T_4 or T_3, are known to play an important role in the animal's adaptation to environmental changes, and T_3 also plays an important role in promoting glucose absorption and utilization. The current effects of decreasing the activities of BBMVs' digestive enzymes are suggested to relate to the disturbance of hypothyroid hormone induced by heat stress.

In the present study, the CMG played an important role in improving porcine growth performance and intestinal absorption. CMG was made by a Chinese medicine prescription, which was composed of *Cortex Phellodendron*, *Rhizome Atractylodes*, *Agastache 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 *Agastache Rugosa* and *Gypsum Fibrosum* are associate medicines in this prescription. Our previous studies in chickens and pigs have demonstrated that the granule improved the growth performance and small intestinal immunity under a high temperature stressor (Liu et al., 2002; Wang et al., 2007). Other research on Chinese medicine also confirmed that a heat-stress-resistant Chinese herbals prescription composed of *Atractylodes Rhizome* and *Agastache Rugosa* improved the performance of growing-finishing pigs raised under normal temperature as well as increasing average daily gain of pigs subjected to heat stress (Jin et al., 2003). Studies have demonstrated that the extracts of *Cortex Phellodendron* and *Agastache Rugosa* show an inhibitory effect on the spasmodic contraction of the small intestine and had anti-diarrhea properties, improving overall intestinal function (Chen et al., 1998; Lu et al., 2006). 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.

From glucose uptake studies with isolated BBMVs from pig jejunum, two D-glucose transport systems were kinetically distinguished: high-affinity, low-capacity system, which is equivalent to the symporter SGLT1; and low-affinity, high-capacity system, which is a D-glucose transporter GLUT2 (Breves et al., 2007). The protein levels of SGLT1 and GLUT2 are crucial to the absorption and uptake of glucose in the small intestine, and their protein levels were up-regulated by the expression of the SGLT1 and GLUT2 genes (Rodriguez et al., 2004). In the present study, heat stress caused a reduction in the protein levels of GLUT2 in the duodenum and ileum without changing the expression of SGLT1 and GLUT2 genes in the small intestine; however, dietary supplementation with CMG increased the expression of SGLT1 and GLUT2 protein levels in the small intestine. Garriga et al. (2000) reported that D-glucose maximum transport speed in the small intestine of chickens was dependent on the protein levels of SGLT1, and the elevation of SGLT1 protein levels lead to increasing of glucose absorption. The present results indicated that CMG improved intestinal glucose absorption and transport of heat stressed pigs.

Notably, Chinese medicine granule affected the activities of functional enzymes and the expression of SGLT1 and GLUT2 genes in the small intestine in a spatio-temporal specific manner. Dietary supplementation with CMG up-regulated disaccharidase activities and expression of SGLT1 and GLUT2 genes in the duodenum and jejunum but without changing them in the ileum, which indicates that the major action target of CMG is in the duodenum and jejunum. Different parts of the small intestine show diverse abilities to digest and absorb nutrients, in which the proximal part exhibits a higher substrate digestion and uptake capacity than the distal part. Dahlqvist (1961) reported that the small intestine had powerful disaccharidases activities which showed different locations along the small intestine; the activities of sucrase and maltase were mainly localized in the distal part of the small intestine, and lactase was localized in the proximal part. However, the activity of SGLT1 in duodenum and jejunum was higher than that in the ileum (Rodriguez et al., 2004). The above findings may relate to the lower activity of SGLT1 and GLUT2 in the ileum.

In conclusion, a high temperature stressor decreased both the growth performance and the activity of BBMVs' functional enzymes, and reduced the protein levels of GLUT2 in intestinal BBMVs. Dietary supplementation with CMG improved porcine growth performance, in addition to increasing intestinal BBMVs absorption and transport of glucose in heat stressed pigs by up-regulating their expression of SGLT1 and GLUT2 genes in the duodenum and jejunum.

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REFERENCES

Breves, G., J. Kock and B. Schröder. 2007. Transport of nutrients and electrolytes across the intestinal wall in pigs. Livest. Sci. 109:4-13.

Chen, X. X., B. He, X. Q. Li, H. Y. Li and J. P. Luo. 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.

Chen, Y. M., J. Chen and G. X. Chou. 2006. Research review on chemical constituent and pharmacological action of rhizoma atractylodis. Acta Universitatis Traditionis Medicalis Sinensis Pharmacologiaeque Shanghai 20(4):95-98.

Collin, A., J. V. Milgen and J. L. Dividich. 2001. The effect of high, constant temperature on food intake in young growing pigs. J. Anim. Sci. 72:519-527.

Dahlqvist, A. 1961. The location of carbohydrases in the digestive tract of the pig. Biochem. J. 78(2):282-288.

Dong, H. 2008. Effects of traditional Chinese medicine on gut hormones in pig and rat under high temperature. Ph.D. Thesis. China agriculture university. Beijing. China.

Garriga, C., M. Moreto and J. M. Planas. 2000. Effects of resalination on intestinal glucose transport in chickens adapted to low Na^+ intakes. Experimental Physiology 85(4):371-378.

Hou, Y. Q., Y. L. Liu, J. Hu and W. H. Shen. 2006. Effects of lactitol and tributyrin on growth performance, small intestinal morphology and enzyme activity in weaned pigs. Asian-Aust. J. Anim. Sci. 19(10):1470-1477.

Hu, Y. X., R. P. She and H. Y. Zhang. 2006. Studies on the dynamic changes of the level of IL-2, IFN-γ and TNF-α in porcine serum after heat stress. Acta Veterinaria and Zootechnica Sinica 37(5):496-499.

Huynh, T. T. T., A. J. A. Aarnink and M. W. A. Verstegent. 2005. Effects of increasing temperatures on physio-logical changes in pigs at different relative humidities. J. Anim. Sci. 83(2): 1385-1396.

Hyun, Y., M. Ellis, G. Riskowski and R. W. Johnson. 1998. Growth performance of pigs subjected to multiple concurrent environmental stressors. J. Anim. Sci. 76:721-727.

Jane, D., V. Steven, P. Timothy, S. P. King and B. Shirazi. 2003. Glucose sensing in the intestinal epithelium. Eur. J. Biochem. 270:3377-3388.

Jin, H. X. and J. S. Zhang. 2003. The effects of heat-stress-resistant Chinese herbal activity on the performance of commercial pigs. Journal of Zhengzhou College of Animal Husbandry Engineering 23(3):165-166.

Khajavi, M., S. Rahimi, Z. M. Hassan, M. A. Kamali and T. Mousavi. 2003. Effect of feed restriction early in life on humoral and cellular immunity of two commercial broiler strains under heat stress conditions. Br. Poult. Sci. 44:490-497.

Kong, X. F., G. Y. Wu, Y. P. Liao, Z. P. Hou, H. J. Liu, F. G. Yin, T. J. Li, R. L. Huang, Y. M. Zhang and D. Deng. 2007. Effects of Chinese herbal ultra-fine powder as a dietary additive on growth performance, serum metabolites and intestinal health in early-weaned piglets. Livest. Sci. 108:272-275.

Liu, F. H., Z. H. Wang and B. Li. 2002. Influence of Chinese medicine additives on performance of growing-finishing pigs under heat stress. Feed Res. 4:1-4.

Lu, Y. N., Q. Y. Qiu and Y. Wang. 2006. Effects of phellodendron and its main components on the cell membrane fluidity. Chinese J. Pathophysiol. 22(1):156-159.

Nabuurs, M. J. A., G. J. van Essen, P. Nabuurs, T. A. Niewold and M. J. vander. 2001. Thirty minutes transport causes small intestinal acidosis in pigs. Res. Vet. Sci. 70:123-127.

Nazifi, S., M. Saeb and E. Rowghani. 2003. The influences of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with triiodothyronine (T_3), thyroxine (T_4) and cortisol concentrations. Comp. Clin. Path. 12:135-139.

Nonaka, I., N. Takusari, K. Tajima, T. Suzuki, K. Higuchi and M. Kurihara. 2008. Effects of high environmental temperatures on physiological and nutritional status of prepubertal Holstein heifers. Livest. Sci. 113:14-23.

Patience, J. F., J. F. Umboh, R. K. Chaplin and C. M. Nyachoti. 2005. Nutritional and physiological responses of growing pigs exposed to a diurnal pattern of heat stress. Livest. Prod. Sci. 96:205-214.

Rodriguez, S. M., K. C. Guimaraes, J. C. Matthews, K. R. McLeod, R. L. Baldwin and D. L. Harmon. 2004. Influence of abomasal carbohydrates on small intestinal sodium-dependent glucose cotransporter activity and abundance in steers. J. Anim. Sci. 82(10):3015-3023.

Sala-Rabanal, M., M. A. Gallardo, J. Sanchez and J. M. Planas. 2004. Na-dependent D-Glucose transport by intestinal brush border membrane vesicles from gilthead sea bream (*sparus aurata*). J. Membrane Bio. l 201:85-96.

Schulthess, G., S. Compassi, D. Boffelli, M. Werder, F. E. Weber, and H. Hauser. 1996. A comparative study of sterol absorption in different small-intestinal brush border membrane models. J. Lipid Res. 37:2405-2420.

Song, X. Z., L. Lu, F. H. Liu, L. Zhang and T. Wang. 2008. Effect of high temperature stress on lipid peroxidation of small intestinal epithelium in pigs. Acta Zoonutrimenta Sinica 20 (1):75-79.

Spencer, J. D., A. M. Gained, E. P. Berg and G. L. Allee. 2005. Diet modification to improve finishing pig growth performance and pork quality attributes during periods of heat stress. J. Anim. Sci. 83:243-254.

Sutherland, M. A., S. R. Niekamp, S. L. Rodriguez-Zas and J. L. Salak-Johnson. 2006. Impacts of chronic stress and social status on various physiological and performance measures in pigs of different breeds. J. Anim. Sci. 84(3):588-596.

Wang, Z. L., T. Q. Yu, X. Y. Zhu, H. Y. Chen, F. H. Liu and J. Q. Xu. 2007. Effects of Chinese medicine granule on concentration of IL-2, IL-10 and IgA in porcine intestine after heat stress. J. Chinese Vet. Med. 9:12-15.

Xu, Z. R., W. F. Li and J. Y. Sun. 2002. Properties of gastrointestinal disaccharidase in pig. Acta Zoologica Sinia 48(2):202-207.