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, Arracacia Etilizome, 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; Huyhn et al., 2005). The mammalian small intestine is a central organ which is very sensitive to all stressors (Naburs 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 expression 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
Table 1. Composition of experimental diet (as fed basis)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>51.8</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>13.0</td>
</tr>
<tr>
<td>Fish meal</td>
<td>6.0</td>
</tr>
<tr>
<td>Whey</td>
<td>6.0</td>
</tr>
<tr>
<td>Expanded soybean</td>
<td>16.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>3.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.5</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Salts</td>
<td>0.35</td>
</tr>
<tr>
<td>Lysine-HCl</td>
<td>0.22</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.13</td>
</tr>
<tr>
<td>Vitamin-mineral mix</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

Chemical composition

- 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 g vitamin K2; 2.5 g vitamin B12; 6.2 g vitamin B6; 33 mg niacin; 18 mg d-pantothenic acid; 3.5 mg vitamin B1; 0.85 mg folic acid; 60 mg biotin; 35 mg vitamin B12.

2 Calculated values.

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 40 min and extracted in boiling water for 2 h and the aqueous extract separated by filtration (100 mesh). Then the extract was heated (55°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 minitube 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.
at -70°C for subsequent extraction of protein and total RNA. Body weight and feed intake of individual barrows were recorded, and their average daily growth and average daily feed intake were analyzed.

Enzyme and protein determinations

BBMVs were prepared from frozen duodenum, jejunum and ileum segments by an ameliorated MgCl₂ precipitation method (Sala-Rabasnal 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 (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 recombinant 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, and 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 2ΔΔCt with ΔΔCt = [C(t)β-actin]−[C(t)β-actin]−[C(t)β-actin]−[C(t)β-actin], where C(t)β-actin and C(t)β-actin are the C(t) for gene i and for β-actin in a pool or a sample (named j) and where C(t)β-actin and C(t)β-actin 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

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**Table 2. Sequences of PCR primers**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession number</th>
<th>Primer sequence (5‘→3’)</th>
<th>Orientation</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGLT1</td>
<td>M34044</td>
<td>5'-CATCATCGTCTCTGTCGTC-3'</td>
<td>Forward</td>
<td>259</td>
</tr>
<tr>
<td>GLUT2</td>
<td>EF140874</td>
<td>5'-TGCTCTGCTCTTGTG-3'</td>
<td>Reverse</td>
<td>275</td>
</tr>
<tr>
<td>β-actin</td>
<td>AY550069</td>
<td>5'-AGCCGTTCCAGAATACT-3'</td>
<td>Reverse</td>
<td>285</td>
</tr>
</tbody>
</table>

1 SGLT1 = Na⁺-dependent glucose transporter 1; GLUT2 = glucose transporter 2; *G* = Genbank accession number.

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

<table>
<thead>
<tr>
<th>Items</th>
<th>NTGC</th>
<th>HTGC</th>
<th>CMGG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW (kg)</td>
<td>7.133±0.69*</td>
<td>7.175±0.61*</td>
<td>7.150±0.59*</td>
</tr>
<tr>
<td>ADFI (g)</td>
<td>675.0±22.3*</td>
<td>542.5±12.7*</td>
<td>630.0±12.2*</td>
</tr>
<tr>
<td>ADG (g)</td>
<td>240.5±12.5*</td>
<td>165.0±14.4*</td>
<td>230.0±20.4*</td>
</tr>
<tr>
<td>F:G (g/g)</td>
<td>2.75±0.23*</td>
<td>3.21±0.11*</td>
<td>2.76±0.28*</td>
</tr>
</tbody>
</table>

* Data are mean±SE, (n = 4); * Within a row, means without a common superscript letter differ (p<0.05).
1 NTGC = Normal temperature control group, HTGC = High temperature stress group, CMGG = Chinese medicine granule anti-stress group.
2 ADFI = Average daily feed intake, ADG = Average daily gain, F:G = Feed:gain.
Figure 1. The activity of sucrase (A), lactase (B), maltase (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
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 proposed 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 (Schultheiss 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 T3 or T4 (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 T3 or T4, are known to play an important role in the animal’s adaptation to environmental changes, and T3 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 hypothryroid 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 principles of principal, associate, adjutant 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 herbal medicine prescription composed of Atractylodes Rhizome and Agastache Rugosa improved the performance of growing-fattening 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 the 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 (Rodriguez et al., 2004). 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 spatiotemporal specific manner. Dietary supplementation with CMG up-regulated disaccharidase activities and expression of SGLT1 and GLUT2 genes in the small intestine 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 BBMV's 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


