Effects of stress simulated by dexamethasone on jejunal glucose transport in broilers


Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China, 100081

ABSTRACT Twenty-four 21-d-old Arbor Acres (AA) broilers with similar BW were randomly assigned into 4 groups to investigate the effects of stress on the absorption of glucose in the intestine of broilers and its mechanisms. In this study, dexamethasone (DEX), an analog to glucocorticoid secreted when animals suffer from stress, was chosen to simulate the effects of glucocorticoid. Broilers were injected subcutaneously with 0, 0.1, 1, or 5 mg of DEX/kg of BW into the abdomen for 7 d. To explain the nonspecific regulation of glucocorticoid on glucose transport of the jejunum in broilers, the effects of DEX on the jejunum mucosa morphology and disaccharidase activities in broilers were investigated. The results showed that DEX restrained the growth of broilers, and the extent of restraint increased with the increase of the dose of DEX (P < 0.05). However, the activities of sucrase and maltase in the jejunum of the broilers injected with DEX were not different from those of the control broilers (P > 0.05). In addition, DEX increased the crypt depth of jejunum in broilers, and decreased the villus height, absorption area, and villus height/crypt depth ratio significantly (P < 0.05), whereas DEX had no significant effect on villus width and the thickness of mucosa lamina propria (P > 0.05). To clarify the specific regulation of glucocorticoid on the glucose transport of jejunum in broilers, the absorption of glucose by jejunum brush border membrane vesicles (BBMV) was investigated, and the expression of Na+-dependent glucose transporter (SGLT1) mRNA was detected by real-time PCR. The results showed that DEX significantly inhibited the glucose absorption of jejunum BBMV (P < 0.05), and the extent of inhibition depended on the dose of DEX. In addition, DEX decreased the expression of SGLT1 mRNA significantly (P < 0.05). According to these data, we concluded that DEX restrains the glucose transport of jejunum in broilers through nonspecific and specific regulations.

Key words: dexamethasone, stress, glucose, jejunum, broiler

INTRODUCTION

Domestic animals raised intensively often suffer from several kinds of stress; for example, stress due to high or low environmental temperature, immunity, transport, oxidation, and stunning (Ali et al., 1999; Sams, 1999). Stress has many detrimental effects on animals; for example, it reduces feed intake, live weight gain, feed efficiency (Klasing et al., 1987; Donkoh, 1989; Siegel, 1995; Hicks et al., 1998), and meat quality (McKee and Sams, 1997), increases the animal’s susceptibility to disease, and impairs immune function (Kelley, 1980, 1985). Stress has caused significant economic loss to livestock husbandry because of the increased mortality of animals and decreased production (Altan et al., 2000). It has been reported that the hypothalamus-pituitary-adrenal axis is activated (Selye, 1976; Siegel, 1980) when an animal is under stress, and the adrenal gland excretes glucocorticoid, which plays a critical role in the progress of the animal’s physiological changes. Thus, the overabundance of glucocorticoid is a hallmark of stress (Munck et al., 1984; Chrousos and Gold, 1992). Glucocorticoid has been designated the stress hormone because its levels in circulation rise sharply in response to stress (Hardy et al., 2005). As one of the analogs to glucocorticoid, dexamethasone (DEX) has been used in many studies related to simulate the effects of glucocorticoid (Caldefie-Chézet et al., 2005; Eid et al., 2006; Park et al., 2005; de Vries et al., 2007). Therefore, DEX was used in this study to imitate glucocorticoid.

Many studies have shown that stress could affect the intestinal function of animals (Cera et al., 1988; Saunders et al., 1994; Olsen et al., 2005) and further disturb the absorption of nutrients (Thiesen et al., 2003; Shepherd et al., 2004; Garriga et al., 2006; Albin et al., 2007). The effects of stress on the absorption of
nutrients in the intestine of animals may occur in 2 ways. One is nonspecific, in which the absorption of nutrients is affected by changes in the intestinal mucosa morphology, the activities of digestive enzymes, and intestinal motility (Tsukada et al., 2002); the other is specific, in which the absorption of nutrients is affected by changing the expression of transporter that is particular to nutrients (Shepherd et al., 2004). Glucose is the main nutrient that is released through the hydrolysis of digestible enzyme to carbohydrate. Many types of carbohydrates can be utilized only when they are hydrolyzed to glucose, so glucose plays an important role in carbohydrate nutrition. The mechanism of transporting glucose into the blood from the intestine of animal has been clarified. It involves 2 transporters: one is the Na\(^+\)-dependent D-glucose transporter (SGLT1) located in the brush border membrane, the other is the glucose facilitated transporter (GLUT2) located in the basolateral membrane (Bird et al., 1996; Ferraris, 2001). Many factors affect the absorption of glucose in intestine by changing the expression levels of these 2 transporters (Cheeseman, 1997; Matekalo et al., 1998; Garriga et al., 1999, 2006; Buddington et al., 2000; Ferraris, 2001).

Therefore, the effects of the stress hormone analog DEX on the jejunal mucosa morphology, disaccharidase activities, glucose absorption by brush border membrane vesicles (BBMV), and expression levels of SGLT1 and GLUT2 mRNA were investigated in this study to determine the effects of stress on glucose absorption in the intestine of broilers and its mechanisms.

**MATERIALS AND METHODS**

**Birds**

Arbor Acres broilers were obtained from a commercial hatch farm (Beijing Huadu Broiler Inc., Beijing, China) on the day of hatch and maintained in standardized temperature and humidity conditions, with a 23L:1D cycle. The birds had free access to water and a standardized diet. At 18 d of age, 24 broilers with similar weight were chosen and assigned randomly into 4 groups. At 21 d of age, broilers of 3 groups were injected with DEX at 0.1, 1, and 5 mg/kg of BW into the abdomen subcutaneously for 7 d, respectively. The jejunum was removed and immediately flushed with ice-cold saline; it was divided into 3 segments. One segment was opened up lengthwise, and the mucosa scraped, frozen in liquid nitrogen, and stored at −80°C. Another segment, about 2 cm long, was fixed with 4% neutral-buffered formalin solution. The third segment was frozen in liquid nitrogen directly and then stored at −80°C.

**Determinations of Plasma Indexes**

At the end of trial, blood was sampled from the heart. Endogenous corticosterone concentration was determined by ELISA. The kit was obtained from RapidBio Laboratories (Calabasas City, CA). Plasma glucose concentration was measured by the glucose oxidase method (Dahlqvist, 1964).

**Determinations of Mucosa Morphology**

For each bird, 3 discontinuous paraffin-embedded sections were examined. In brief, the process was as follows: first, samples fixed in formalin solution were dehydrated, cleared, and embedded in paraffin. Then, paraffin-embedded jejunal samples were sliced to approximately 5 µm with a microtome and mounted on slides. These sections were deparaffinized in xylene, rehydrated in a graded alcohol series, and stained with hematoxylin and eosin. From these sections, 3 slides from nonconsecutive sections were examined. From each slide, 2 intact vision fields where mucosa was not destroyed were chosen for determining villus height, villus width, crypt depth, and the absorption area (2πrh, where r = villus width, h = villus height) with a microscope (Nikon E200, Hengqiao Instruments Co. Ltd., Shanghai, China) and a computer-assisted digital camera for microscope (DCM 130, Huaxin IC Technology Inc., Hangzhou, China) using MinSee 1.0 software (Huaxin IC Technology Inc.).

**Determinations of Disaccharidase Activities**

The activities of jejunal mucosa sucrase and maltase of broilers were determined by the method of Dahlqvist (1964).

**Preparation of BBMV**

Brush border membrane vesicles were prepared from jejunal segments by the MgCl\(_2\) precipitation method (Vazquez et al., 1997). The brief process was as follows: the intestinal segment (equivalent to 8 to 10 g) was thawed in 20 to 30 mL of buffer A containing 100 mmol/L mannitol and 2 mmol/L HEPES-Tris (pH 7.1). The segment was opened up lengthwise, and its mucosa was scraped with glass slide and placed in buffer A. The mucosal scrapings were homogenized for 3 min at high speed using a Waring blender (Waring Products Inc., Torrington City, CT); the homogenate was added to 99 mL of ice-cold distilled water, then 1 mL of 1 mol/L MgCl\(_2\) was added to reach a final concentration of 10 mmol/L. After stirring for 5 min, the suspension was left undisturbed for 15 min at 4°C and then centrifuged at 3,000 \(\times\) g for 15 min. The resulting supernatant was centrifuged at 30,000 \(\times\) g for 30 min. The resultant pellet was resuspended in buffer B containing 100 mmol/L mannitol, 2 mmol/L HEPES-Tris, and 0.1 mol/L MgCl\(_2\).
mmol/L MgSO₄ (pH 7.4). This suspension was homogenized with a glass-Teflon homogenizer (40 strokes) and centrifuged at 30,000 × g for 30 min. The final pellet containing the purified BBMV was resuspended in buffer C containing 300 mmol/L mannitol, 0.1 mmol/L MgSO₄, and 20 mmol/L HEPES-Tris (pH 7.4), and homogenized with a 1-mL syringe fitted with 27-gauge needle. The final protein concentration of BBMV was about 10 to 20 mg/mL. The suspension was frozen in liquid N₂ and stored at −80°C. The whole process was completed at −4°C.

Determinations of Enzyme and Proteins

Sucrase, the BBMV marker enzyme, was assayed by the method of Dahlqvist (1964). Ouabain-sensitive Na⁺-K⁺-ATPase, the basolateral membrane marker enzyme, was assayed by using a Na⁺-K⁺-ATPase kit (Jiancheng Bioengineering Institute, Nanjing, China). Protein was determined by a Coomassie Brilliant Blue protein assay using a bovine gamma globulin as a standard (Bradford, 1976).

Assay of D-Glucose Transport

The uptake of d-glucose was measured at 37°C by a rapid filtration technique according to the method of Vazquez et al. (1997). For time-course studies vesicles were incubated in the range from 5 s to 30 min (5 s, 10 s, 30 s, 1 min, 2 min, 30 min). For each uptake, 10 µL of BBMV suspension (equivalent to 50 to 150 µg of protein) was rapidly mixed with 100 µL of the incubation medium containing 100 mM mannitol, 0.1 mM MgSO₄, and 20 mM HEPES-Tris (pH 7.4), an aliquot of d-[³H]-glucose, and 100 mM/L NaSCN (pH 7.4). At selected time, uptake was stopped by addition of 1 mL of an ice-cold stop solution containing 300 mM/l mannitol, 0.1 mM MgSO₄, and 20 mM/L HEPES-Tris (pH 7.4). The resulting suspension was rapidly filtered under negative pressure by placing 0.9 mL of the reaction mixture on a prewetted and chilled cellulose nitrate filter (0.22-µm pore size Millipore filter, Millipore Corp., Billerica, MA), the filter was washed with 2 mL of the above ice-cold stop solution. Then the filter was dissolved in biogreen-6 cocktail (Sharlau, Barcelona, Spain), and the radioactivity was measured with a scintillation counter. Nonspecific radioactivity binding to the filters was obtained by adding the stop solution to reaction tubes immediately after addition of the vesicles. This nonspecific binding was subtracted from the total radioactivity of each sample.

Total RNA Extraction and cDNA Synthesis

The total RNA of jejunum scrapings was extracted by using an RNA extraction kit from Tiangen Biotech Co. Ltd. (Beijing, China). The optical density of RNA at 260 and 280 nm was measured by spectrophotometric analysis to evaluate the purity and concentration of RNA. The RNA samples were loaded in a formaldehyde-agarose gel (15 µg of total RNA/lane) and stained with ethidium bromide to verify RNA integrity. One to 5 µg of total RNA was reverse transcribed using super RNase H reverse transcriptase and Oligo(dT)₁₅ from Tiangen Biotech Co. Ltd.

Quantifications of SGLT1 and GLUT2 mRNA by Real-Time PCR

Real-time reverse transcription (RT)-PCR analysis was performed using the iCycler Real Time Detection System (Applied Biosystems Inc., Foster City, CA). The SYBR PCR Mastermix kit was obtained from Applied Biosystems Inc. The 25-µL PCR reaction system was as follows: cDNA, 1.5 µL; each primer (10 mmol/L), 0.3 µL; 2 × SYBR green master mix, 12.5 µL; distilled and deionized H₂O, 10.4 µL. The temperature cycles were as follows: 94°C for 5 min followed by 40 cycles at 94°C for 20 s, 58°C for 20 s, and 72°C for 30 s. The SYBR Green fluorescence was collected at the end of each cycle; that is, from 72°C, and the melting curve was recorded from 60°C. Oligonucleotide sequences of primers and the size of PCR products are shown in Table 1. The expression levels of SGLT1 and GLUT2 mRNA were presented as a ratio to 18S ribosomal RNA for correcting the differences in the amounts of cDNA.

Statistical Analysis

All results were subjected to one-way ANOVA analysis with SAS software (v8.02, SAS Inst. Inc., Cary, NC). The values were expressed as mean ± SE. Differences were considered to be significant at P < 0.05.

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Gene name¹</th>
<th>Primer sequence (5′–3′)</th>
<th>Length of product, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF173612</td>
<td>SGLT1</td>
<td>Sense: GTGAAGTGCGGATACCTGAAGC</td>
<td>229</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antisense: TAAGGGATGCGCAACATGACTGA</td>
<td>116</td>
</tr>
<tr>
<td>AJ236903</td>
<td>GLUT2</td>
<td>Sense: CCCGCGAAGGTTGATAGAAG</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antisense: GCCGTTGATCGGACAGTGAT</td>
<td></td>
</tr>
<tr>
<td>AF173612</td>
<td>18S rRNA</td>
<td>Sense: ATTCGATAACGAGACGACT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-sense: GGCATCTAAGGGCATTACA</td>
<td></td>
</tr>
</tbody>
</table>

¹SGLT1 = Na⁺-dependent glucose transporter; GLUT2 = glucose facilitated transporter; 18S rRNA = 18S ribosomal RNA.
RESULTS

Effect of DEX on BW of Broilers

According to Table 2, the initial BW of broilers between treatments and control was not significantly different (\(P > 0.05\)). At the end of the trial, however, it was found that DEX restrained the growth of broilers, and the BW of broilers in the control group was significantly greater than that of the treatments (\(P < 0.05\)). Among treatments, the BW of broilers decreased with an increase in the dose of DEX (\(P < 0.05\)).

Determination of Plasma Indexes

Table 3 shows the effects of DEX on the contents of plasma endogenous corticosterone and glucose in broilers. Compared with the broilers in the control group, DEX increased the content of plasma glucose significantly (\(P < 0.05\)). There was no significant difference between groups injected with 1 and 5 mg of DEX/kg of BW (\(P > 0.05\)). In addition, according to Table 3, DEX decreased the content of plasma endogenous corticosterone significantly (\(P < 0.05\)).

Effects of DEX on the Jejunum Mucosa Morphology and Disaccharidase Activities of Broilers

Table 4 shows the effects of DEX on the jejunum mucosa morphology of broilers. Dexamethasone decreased villus height, but increased the crypt depth significantly (\(P < 0.05\)). There was no significant difference found in villus width between control and treatments. In addition, DEX decreased the mucosa absorption area and the value of villus height/crypt depth (\(P < 0.05\)). Neither sucrase nor maltase was significantly affected by DEX (\(P > 0.05\)). Their activities were (mean ± \(SE\)) 0.097 ± 0.008 and 0.2457 ± 0.023 U, respectively, where 1 U is equivalent to millimoles of glucose discharged by per milligram of protein.

Purity of BBMV Preparation

The purity of BBMV was determined by marker enzyme assays. In the final BBMV preparation, the activity of sucrase was highly enriched (11- to 16-fold).

The activity of Na\(^+\)-K\(^+\)-ATPase was slightly enriched (about 2-fold).

Effect of DEX on Na\(^+\)-Dependent D-Glucose Uptake by BBMV

The ratio of OD\(_{260}/OD_{280}\) of total RNA was 1.8 to 2.1, which indicated that most extraction was RNA without contamination of protein. The result of formaldehyde-agarose gel electrophoresis verified the integrity of RNA.

The expression levels of SGLT1 and GLUT2 mRNA were calculated by the 2\(^{-\Delta\Delta C_T}\) (delta delta cycle threshold) method (Livak and Schmittgen, 2001). The results are shown in Table 5. Compared with control, DEX decreased the expression of SGLT1 mRNA (\(P < 0.05\)), but there was no significant difference among treatments (\(P > 0.05\)). Treatment with DEX at 0.1 and 1 mg/kg of BW significantly increased the expression of GLUT2 mRNA (\(P < 0.05\)), but DEX at 5 mg/kg of BW had no significant effect on the expression of GLUT2 mRNA (\(P > 0.05\)).

DISCUSSION

Dexamethasone is an analog of glucocorticoid secreted when animals are under stress. It has been widely used in studies on stress by many researchers. In this study, different doses of DEX were injected subcutaneously into the abdomens of broilers to simulate the effects of glucocorticoid and investigate the effects of stress on glucose absorption in the intestine of broilers.

### Table 2. Effect of dexamethasone (DEX) on the BW (mean ± SE) of broilers

<table>
<thead>
<tr>
<th>DEX dose</th>
<th>n</th>
<th>Initial BW (kg)</th>
<th>Final BW (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (0)</td>
<td>5</td>
<td>1.187 ± 0.016</td>
<td>1.686 ± 0.023</td>
</tr>
<tr>
<td>0.1 mg/kg of BW</td>
<td>6</td>
<td>1.169 ± 0.017</td>
<td>1.587 ± 0.064</td>
</tr>
<tr>
<td>1 mg/kg of BW</td>
<td>6</td>
<td>1.162 ± 0.040</td>
<td>1.497 ± 0.042</td>
</tr>
<tr>
<td>5 mg/kg of BW</td>
<td>6</td>
<td>1.170 ± 0.026</td>
<td>1.420 ± 0.050</td>
</tr>
</tbody>
</table>

\* \*Means in columns with different superscripts differ significantly (\(P < 0.05\)).

### Table 3. Effects of dexamethasone (DEX) on the contents (mean ± SE) of plasma glucose and endogenous corticosterone

<table>
<thead>
<tr>
<th>DEX dose</th>
<th>n</th>
<th>Plasma glucose (mmol/L)</th>
<th>Plasma endogenous corticosterone (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (0)</td>
<td>5</td>
<td>16.46 ± 0.77*</td>
<td>36.22 ± 2.37*</td>
</tr>
<tr>
<td>0.1 mg/kg of BW</td>
<td>6</td>
<td>23.62 ± 1.26*</td>
<td>32.35 ± 1.56*</td>
</tr>
<tr>
<td>1 mg/kg of BW</td>
<td>6</td>
<td>27.84 ± 0.83*</td>
<td>31.61 ± 2.13*</td>
</tr>
<tr>
<td>5 mg/kg of BW</td>
<td>6</td>
<td>26.99 ± 0.39*</td>
<td>26.66 ± 1.84*</td>
</tr>
</tbody>
</table>

\* \*Means in columns with different superscripts differ significantly (\(P < 0.05\)).
Table 4. Effects of dexamethasone (DEX) on the jejunal mucosa morphology (mean ± SE) of broilers

<table>
<thead>
<tr>
<th>DEX dose</th>
<th>n</th>
<th>Villus height, µm</th>
<th>Crypt depth, µm</th>
<th>Villus width, µm</th>
<th>Absorption area, 10³ µm²</th>
<th>Villus height/crypt depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (0)</td>
<td>5</td>
<td>306.20 ± 10.86a</td>
<td>27.44 ± 2.37a</td>
<td>40.56 ± 1.19</td>
<td>39.08 ± 2.04a</td>
<td>11.69 ± 1.18b</td>
</tr>
<tr>
<td>0.1 mg/kg of BW</td>
<td>6</td>
<td>243.56 ± 15.47b</td>
<td>32.97 ± 1.66ab</td>
<td>38.53 ± 2.17</td>
<td>29.18 ± 1.79b</td>
<td>7.44 ± 1.24b</td>
</tr>
<tr>
<td>1 mg/kg of BW</td>
<td>6</td>
<td>220.23 ± 23.23b</td>
<td>38.86 ± 5.94ab</td>
<td>43.16 ± 2.24</td>
<td>29.09 ± 2.58b</td>
<td>6.59 ± 1.12b</td>
</tr>
<tr>
<td>5 mg/kg of BW</td>
<td>6</td>
<td>158.31 ± 15.67c</td>
<td>45.87 ± 4.33b</td>
<td>42.29 ± 2.55</td>
<td>20.86 ± 2.49c</td>
<td>3.60 ± 0.46c</td>
</tr>
</tbody>
</table>

**Means in columns with different superscripts differ significantly (P < 0.05).**

According to Table 2, compared with the control group, DEX inhibited the growth of broilers significantly (P < 0.05). The extent of inhibition depended on the dose of DEX injected. This result is consistent with the effect of stress or other stress hormones on the growth of animals (Davison et al., 1985; Klasing et al., 1987; Siegel et al., 1989; Puvadolpirod and Thaxton, 2000a,b,c,d; Post et al., 2003). Dexamethasone increased the concentration of plasma glucose of broilers significantly (P < 0.05), but no significant difference was found in broilers injected with 1 and 5 mg of DEX/kg of BW (P > 0.05). It is well known that glucocorticoids play an important role in the process of metabolism of animals. It can enhance mobilization of carbohydrate, protein, and fat from liver and muscle tissue and increase the concentrations of metabolites. Therefore, the effects of glucocorticoids are very meaningful for protecting the functions of important tissues or organs when animals suffer from long-term or abrupt stress. This might be the reason that DEX increased the concentration of plasma glucose. The increase of plasma glucose is recognized as one signal of DEX imitating glucocorticoid successfully. The concentrations of plasma corticosterone of broilers in DEX-treated groups were observed to decrease significantly (P < 0.05). In broilers, the glucocorticoid secreted by the adrenal gland is corticosterone. Kamphuis et al. (2002) reported that DEX, as a synthetic glucocorticoid, could enhance glucocorticoid feedback inhibition of the hypothalamic-pituitary-adrenal axis response to stress and attenuate the concentrations of plasma adrenocorticotropin hormone and corticosterone in rats. The results of this study are in agreement with those in the previous study by Kamphuis et al. (2002), showing that DEX could simulate the effects of glucocorticoid very well. In addition, in this study, the phenomena of feather dishevelment and diarrhea were also observed in broilers. All of these results showed that DEX could simulate glucocorticoid-induced stress successfully.

Stress has been frequently associated with gastrointestinal manifestations; for example, changes in gastric emptying, small intestinal motility, and intestinal barrier function (Cera et al., 1988; Saunders et al., 1994; Olsen et al., 2005). The activities of digestive enzymes are such important factors affecting the absorption and utilization of nutrients in the alimentary tract that they are often detected to indicate the function of intestine to absorb nutrients. Cera et al. (1990) and Jensen et al. (1997) reported that stress of weaning decreased the activities of protease, lipase, and amylase. Disaccharidase is one of the important digestive enzymes, and is necessary in the process of carbohydrate hydrolyzed into monosaccharide. In broilers, sucrase and maltase are the main disaccharidases in the intestine. In this study, the activities of sucrase and maltase were evaluated to investigate the effects of DEX on glucose absorption. However, it was found that DEX had no effect on the activities of either sucrase or maltase. This result is unexpected and inconsistent with previous studies. Perhaps the fact that the activities of sucrase and maltase did not decrease as the previous study reported is just a compensation for the impairment of intestinal functions, which will be referred to in the subsequent discussion.

The structure of intestinal mucosa can reveal some information on the absorptive ability of intestine to nutrients and is always associated with the performance of animals. Therefore, the effects of DEX on the structure of jejunum in broilers were investigated in this study. It was found that DEX had a significant effect on jejunum mucosa morphology of broilers. It decreased villus height, absorption area, and the ratio of villus height/crypt depth, but increased the crypt depth significantly (P < 0.05). Villus height and the mucosa area are apparent indicators of the ability of intestine to absorb nutrients. Crypt depth is an indicator of the maturation of intestinal epithelium: a deeper crypt indicates a more mature intestinal epithelium. The ratio of villus height/crypt depth reflects the ability of intestine comprehensively. The results indicate that glucocorticoid may decrease nutrient absorption, including that of glucose, by regulating the nonspecific absorption of jejunum of broilers. This may be one reason that DEX decreased the BW of broilers. Also, it is possible that

Table 5. Effects of different dose of dexamethasone (DEX) on the relative expression (mean ± SE) of Na⁺-dependent glucose transporter (SGLT1) and glucose facilitated transporter (GLUT2) mRNA of jejunal mucosa

<table>
<thead>
<tr>
<th>DEX dose</th>
<th>n</th>
<th>SGLT1</th>
<th>GLUT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (0)</td>
<td>5</td>
<td>0.67 ± 0.13a</td>
<td>0.52 ± 0.24a</td>
</tr>
<tr>
<td>0.1 mg/kg of BW</td>
<td>6</td>
<td>0.31 ± 0.05b</td>
<td>8.53 ± 1.41b</td>
</tr>
<tr>
<td>1 mg/kg of BW</td>
<td>6</td>
<td>0.32 ± 0.03b</td>
<td>15.96 ± 2.00b</td>
</tr>
<tr>
<td>5 mg/kg of BW</td>
<td>6</td>
<td>0.34 ± 0.03b</td>
<td>1.79 ± 0.58b</td>
</tr>
</tbody>
</table>

**Means in columns with different superscripts differ significantly (P < 0.05).**

Table 6. Effects of dexamethasone (DEX) (5 mg/kg of BW) on the relative expression (mean ± SE) of GLUT2 mRNA of jejunal mucosa

<table>
<thead>
<tr>
<th>GLUT2 mRNA</th>
<th>n</th>
<th>Relative expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGLT1</td>
<td>5</td>
<td>0.67 ± 0.13a</td>
</tr>
<tr>
<td>GLUT2</td>
<td>6</td>
<td>0.32 ± 0.03b</td>
</tr>
</tbody>
</table>

**Means in columns with different superscripts differ significantly (P < 0.05).**
The effect of DEX on the jejunum mucosa morphology results from the inhibition of DEX on the feed intake of broilers. It deserves further investigation.

The BBMV is a good source of material for studying the specific absorption of glucose in the intestine of animals. It had been used to investigate the transport of different nutrients (Rubino et al., 1971; Vincenzini et al., 1989; Wolffram et al., 1989). The BBMV does not contain the basolateral membrane and cell organelles; therefore, glucose transported inward can neither come out nor be metabolized. In this study, we investigated the effect of DEX on the time course of d-glucose transporting into the BBMV; the results are shown in Figure 1. At the beginning of the transport, there was a typical transient increase in the intravesicular concentration of glucose in all groups. That is because there was a 100 mmol/L extravesicular-to-intravesicular Na⁺ gradient that stimulated glucose transport. As the Na⁺ gradient declined, the velocity decreased to zero. In addition, it was observed that the initial transport velocity of the control was significantly greater than that of DEX-treated groups \( (P < 0.05) \). However, there was no significant difference in the initial transport velocity among DEX-treated groups \( (P > 0.05) \), just a decreasing trend with the increasing doses of DEX \( (P < 0.05) \).

Two possible reasons are related: one is that the contents of SGLT1 per microgram of BBMV protein in the DEX-treated groups were less than that of the control; the other is that the activity of SGLT1 in BBMV of DEX-treated groups was lower than that of control. In conclusion, DEX decreased the glucose transport across membrane in the jejunum of broilers.

It has been confirmed that the changes of glucose absorption caused by many factors involve the regulation of glucose transporters SGLT1 and GLUT2 in the intestine. Thus, to investigate the specific regulation of glucocorticoid on glucose transport of jejunum in broilers, the effects of glucocorticoid on SGLT1 and GLUT2 mRNA were studied by real-time PCR. Dexamethasone decreased the expression of SGLT1 mRNA significantly \( (P < 0.05) \), but no significant difference was found among the DEX-treated groups. This result is consistent with the uptake of d-glucose by BBMV, but not consistent with the previous research (Shepherd et al., 2004). The possible reason is that the time of DEX treatment was very different and the perfusion of glucose solution was done before the treatment of DEX in the experiment of Shepherd et al. (2004). According to the current results, it can be inferred that DEX decreased the contents of SGLT1 per microgram of BBMV by decreasing the expression of SGLT1 mRNA. Unfortunately, the expression of SGLT1 protein cannot be quantified in this study, so this conclusion needs to be confirmed. In addition, DEX at 0.1 and 1 mg/kg of BW increased the expression of GLUT2 mRNA \( (P < 0.05) \). The function of GLUT2 is to transport glucose in the intestinal epithelium into blood. Therefore, the increase of GLUT2 mRNA by DEX may be a compen-

---

**Figure 1.** Effect of dexamethasone (DEX) on glucose absorption of jejunum brush border membrane vesicles (BBMV) in broilers. Control: \( y = -0.0003 + 0.2635\log_{10}(x); r = 0.6735; \) 0.1 mg of DEX/kg of BW: \( y = 0.1125 + 0.051\log_{10}(x); r = 0.6654; \) 1 mg of DEX/kg of BW: \( y = 0.0271 + 0.0414\log_{10}(x); r = 0.5606; \) and 5 mg of DEX/kg of BW: \( y = -0.0111 + 0.0289\log_{10}(x); r = 0.7623, \) where \( y \) represents the glucose in BBMV (nmol/mg of protein), and \( x \) represents incubation time (s).
sation for the decrease of uptake of glucose by brush border membrane in the intestine.

ACKNOWLEDGMENTS

This study was supported by State Key Development Program for Basic Research of China. The technical assistance of Z. X. Wang from China Agriculture University (Beijing) and X. Y. Wu from Chinese Academy of Agriculture Sciences is acknowledged. All members of the poultry laboratory of the Feed Research Institute in the Chinese Academy of Agriculture Sciences offered their help during the study.

REFERENCES


Cheeseman, C. I. 1997. Upregulation of SGLT-1 transport activity in their help during the study. in the Chinese Academy of Agriculture Sciences offered assistance of Z. X. Wang from China Agriculture University (Beijing) and X. Y. Wu from Chinese Academy


