



Effect of Corticosterone Administration on Small Intestinal Weight and Expression of Small Intestinal Nutrient Transporter mRNA of Broiler Chickens*

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ABSTRACT : The effects of corticosterone (CORT) administration on the weight of small intestine and the expression of nutrient transporter mRNA in the small intestine of broiler chickens (*Gallus gallus domesticus*) were investigated. One hundred and eight seven-day-old birds were randomly divided into two equal groups comprising a control group (CTRL) and an experimental group (CORT). CTRL birds were fed a basal diet and the CORT birds were fed a basal diet containing 30 mg corticosterone/kg from d 8 to 21. At 21 d of age, average daily feed intake (ADFI), serum corticosterone level, small intestinal absolute wet weight and relative weight, and relative abundance of SGLT1, CaBP-D28k, PepT1 mRNA in the duodenum and L-FABP mRNA in the jejunum were determined. The results showed that serum corticosterone level, liver weight and small intestinal relative weight (small intestinal wet weight/body weight) of CORT chickens were about 30.15%, 26.72% and 42.20% higher, respectively, than in the CTRL group ($p < 0.05$). CORT birds had relative mRNA abundance of CaBP-D28k and PepT1 in the duodenum, and L-FABP in the jejunum which was 1.77, 1.37 and 1.94 fold higher, respectively, than in the CTRL group ($p < 0.05$); the relative abundance of SGLT1 was 1.67 fold higher than in the CTRL group ($p = 0.097$). ADFI, small intestinal wet weight and length in CORT-treated broiler chickens was about 29.11%, 31.12% and 12.35% lower, respectively, than in the CTRL group ($p < 0.05$). In conclusion, corticosterone administration lowered the wet weight but increased the relative weight of the small intestine and the expression of intestinal nutrient transporter mRNA of broiler chickens. (**Key Words** : Broiler Chickens, Corticosterone, Nutrient Transporter, Small Intestine, Stress)

INTRODUCTION

The small intestine is critical for animal development and growth (see review by Ziegler et al., 2003). The intestinal epithelium of broiler chickens is responsible for the growth potential after hatching (Uni et al., 1998), and the development of intestinal morphology and function contributes to the bodyweight increase of broiler chickens (Yamauchi and Tarachai, 2000). However, the small intestine is a metabolically active organ (Spratt et al., 1990). Many factors can affect its development, especially stress (Mitchell and Carlisle, 1992; Dibner et al., 1996; Yamauchi

et al., 1996; Gal-Garber et al., 2000; Howard et al., 2004; Pinheiro et al., 2004; Thompson and Applegate, 2006).

Studies demonstrated that stress caused alterations in the intestinal architecture and weight of chickens. Mitchell and Carlisle (1992) reported that chronic heat stress decreased small intestinal villus height and wet and dried small intestinal weight of birds. Stress decreased feed intake of broiler chickens (Bottje and Harrison, 1985), and the limited nutrients decreased protein synthesis in the small intestine, which subsequently resulted in lowered small intestinal weight (Dudley et al., 1998). All kinds of stressors exist around broiler chickens and can be mimicked by glucocorticosteroid (such as corticosterone) treatment (Post et al., 2003; Pung et al., 2003; Lin et al., 2004a, b; 2006; 2007). Our previous study revealed that corticosterone administration decreased feed intake and duodenal and jejunal epithelial cell proliferation of young broilers (Hu and Guo, 2008). Decreased epithelial cell proliferation of young broilers in turn lowers duodenal and jejunal villus

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height and crypt depth (Hu and Guo, 2008). Villus height is positively related to villus surface area (Mitchell and Carlisle, 1992); reduction of villus height during villus growth would decrease the expansion of small intestinal surface area and subsequently decrease the absorptive capacity (Moran, 1985). However, Nasir and co-workers showed that CORT administration increased glucose and calcium absorption (Nasir et al., 1999), which might not be explained by the decreased small intestinal surface area (Hu and Guo, 2008) and likely to be associated with a change of nutrient transporter in the small intestinal epithelium during stress. Some studies showed that starvation stress caused the increased expression of sodium glucose co-transporter 1 (SGLT1) and peptide transporter 1 (PepT1) mRNA in the small intestine of chickens and rats (Gal-Garber et al., 2000; Naruhashi et al., 2002). Therefore, we hypothesized that corticosterone administration may induce the expression of nutrient transporter in the small intestine. Moreover CORT administration may lead to the lowered small intestinal weight of broiler chickens.

To test our hypothesis, the effect of corticosterone on the expression of nutrient transporter mRNA in the small intestine and on intestinal weight of broiler chickens was evaluated in this study.

MATERIALS AND METHODS

Animals, housing and experimental design

One hundred and eight 7-d old Arbor Acres (AA) male broiler chickens were distributed randomly into 18 pens (6 birds/pen) of 3-tiered, metal brooder batteries. The pens were divided into two groups (control vs. experimental group) and each group contained 9 pens. The pen served as the experimental unit. The control group (CTRL) was fed a corn-soybean basal diet containing ME 2.90 Mcal/kg; crude protein, 21.5%; calcium, 1.00%; available P, 0.45% as described previously (Hu and Guo, 2008). The experimental group (CORT) was fed the basal diet supplemented with 30 mg CORT/kg. Birds were allowed *ad libitum* access to water and diet. Twenty-four hours of artificial light was supplied. Experimental period was 2 weeks, from 8 to 21 days of age.

Sampling

At 21 days of age, blood samples ($n = 6$) were withdrawn from the wing vein, collected in 5 ml eppendorf tubes, and serum was prepared by centrifugation at 1,500 g for 10 min for the analysis of corticosterone concentration. Six birds per group were killed after being weighed, the intestine was removed, flushed with ice-cold normal saline, wiped with filter paper, then stretched naturally and the length, wet weight and relative weight were recorded. The relative weight was expressed as small intestinal wet

weight/body weight.

Another 12 birds (6 birds/group) were slaughtered, and intestinal segments (duodenum, jejunum) were removed and washed with 0.1% DEPC water, then packed with sterile and RNase-free silver paper and, after being rapidly frozen in liquid nitrogen, stored at -80°C for further analysis of SGLT1, VD-dependent calcium-binding protein 28,000 m (CaBP-D28k), PepT1 in the duodenum, and liver fatty acid-binding protein (L-FABP) in the jejunum.

Analysis

Average daily feed intake (ADFI) was calculated for the continuous 14 days.

Serum corticosterone level was determined using a RIA Kit (Diagnostic Products Corporation Los Angeles CA, USA) as described by Puvadolpirod and Thaxton (2000a).

The relative abundance of transporter mRNA was determined by quantitative real-time PCR. The frozen duodenum or jejunum was mashed in a sterile mortar, and the powder was used for total RNA extraction employing a kit (Gibco, New York, America). The integrity of the RNA was verified by optical density (OD) absorption ratio $2.0 > \text{OD}_{260} \text{ nm} / \text{OD}_{280} \text{ nm} > 1.9$ and further by electrophoresis on a 1.5% (w/v) ethidium bromide staining agarose formaldehyde gel.

A kit (Takara, DaLian, China) was employed for reverse transcription. First-strand cDNAs were synthesized from 1 μg of total RNA using oligo (dT) as primers in the presence of MML-V reverse transcriptase (RT), for 5 min at 20°C , 60 min at 42°C and 5 min at 70°C .

PCR reactions were performed in a volume of 20 μl containing 2 \times SYBR Green PCR Master Mix containing Taq DNA polymerase (Applied Biosystems, California, America) 10 μl , cDNA product 2 μl , reaction buffer, dNTP, forward primer 1 μl , reverse primer 1 μl . Primers were chosen from the conserved part of the coding regions of different nutrient transporters and the house-keeping gene β -actin (Table 1). Amplification for transporter in the duodenum occurred in a two-step procedure: denaturation at 95°C for 5 min, 40 cycles consisting of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 5 min. Amplification for transporter in the jejunum had the same steps as for the duodenum but the annealing temperature was 51°C . A standard curve was made as described by Pan et al. (2000) with slight modification: cDNA was gradient diluted 10-fold as 10^4 , 10^3 , 10^2 , 10^1 , 10^0 , all five dilutions were run as a separate PCR assay, and a standard curve was generated by plotting Ct versus log of the amount of cDNA. From the determined Ct value and the standard curve, the relative original concentration of target gene and β -actin was obtained. Target gene relative abundance was

Table 1. The primer sequence of different nutrient transporters and the house-keeping gene β -actin for quantitative RT-PCR

Name	Oligo	Primer sequence	Predicted size (bp)	Genebank accession
SGLT1	Forward primer	5'-GATGTGCGGATACCTGAAGC-3'	225	AJ236903
	Reverse primer	5'-AGGGATGCCAACATGACTGA-3'		
PepT1	Forward primer	5'-TACGCATACTGTCACCATCA-3'	205	AY029615
	Reverse primer	5'-ATGGATGGGAAGGAGCTACAA-3'		
CaBP-D28k	Forward primer	5'-ATGGATGGGAAGGAGCTACAA-3'	194	M14230
	Reverse primer	5'-TGGCACCTAAAGAACAACAGGAAAT-3'		
L-FABP	Forward primer	5'-GAAGGGTAAGGACATCAA-3'	219	NM_204192
	Reverse primer	5'-TCGGTCACGGATTTTCAGC-3'		
β -actin	Forward primer	5'-CCACCGCAAATGCTTCTAAAC-3'	175	NM_205518
	Reverse primer	5'-AAGACTGCTGCTGACACCTTC-3'		

expressed as the relative original copy of target gene \times 100/the relative original copy of β -actin. All PCR analyses were performed using 3 replicates for each sample.

Statistical analysis

Data, presented as means \pm SE, were analyzed by independent-samples T Test using the compare means procedure (SPSS13.0 software for windows, SPSS Inc., Chicago, IL, USA). Differences between means were considered to be statistically significant at $p < 0.05$.

RESULTS

Effect of CORT administration on ADFI, the weight of liver and the weight and length of small intestine of broiler chickens

ADFI, the weight of liver, the wet weight and length of small intestine are shown in Table 2. The results indicated that liver weight and small intestinal relative weight of broiler chickens in the CORT group were about 26.72% and 42.20% higher, respectively, than in the CTRL group ($p < 0.05$). ADFI, the wet weight and length of the small intestine in CORT were about 29.11%, 31.12% and 12.35% lower, respectively, than in the CTRL group ($p < 0.05$).

Effect of CORT administration on serum corticosterone level of broiler chickens

Serum corticosterone level of broiler chickens is shown in Figure 1. The data suggested that serum corticosterone concentration of broiler chickens treated with corticosterone was about 30.15% higher than in the control group ($p < 0.05$).

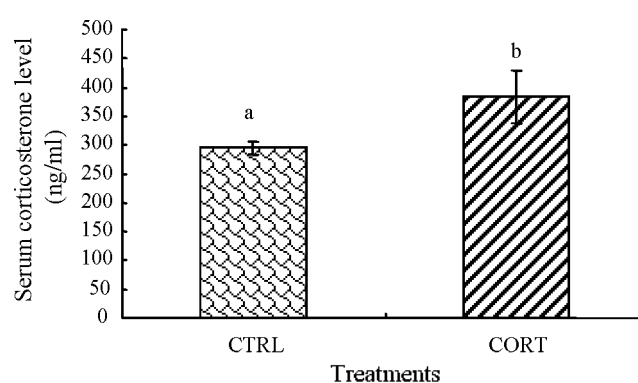


Figure 1. Serum corticosterone level of broiler chickens in different groups. Histograms with different superscripts differ significantly ($p < 0.05$).

Effect of CORT administration on nutrient transporter mRNA expression in duodenum and jejunum of broiler chickens

The relative mRNA abundance of SGLT1, CaBP-D28k, and PepT1 in duodenum and L-FABP in jejunum of broilers is shown in Figure 2. The results indicated that stress has an effect on the expression of nutrient transporter mRNA in the small intestine of birds. CORT birds had relative mRNA abundance of CaBP-D28k, PepT1 in duodenum, and L-FABP in jejunum which was 1.77, 1.37 and 1.94 fold higher, respectively, than in the CTRL group ($p < 0.05$). A statistically significant difference was not observed in the relative abundance of SGLT1 in the duodenum between the two groups, but abundance of SGLT1 in CORT-treated broiler chickens was 1.67 fold higher than in the CTRL group ($p = 0.097$).

Table 2. ADFI, liver weight, small intestinal length, small intestinal weight and relative weight of broiler chickens

Treatments	ADFI (g)	Liver weight (g)	Small intestinal wet weight (g)	Small intestine length (cm)	Small intestinal relative weight
CTRL	59.65 \pm 0.47 ^a	19.20 \pm 0.51 ^a	28.23 \pm 1.89 ^a	153.73 \pm 8.95 ^a	3.46 \pm 0.21 ^a
CORT	46.20 \pm 0.47 ^b	24.33 \pm 2.26 ^b	21.53 \pm 2.96 ^b	136.83 \pm 2.75 ^b	4.92 \pm 0.10 ^b

^{a, b} Within column, means (\pm standard error) with different superscripts differ significantly ($p < 0.05$).

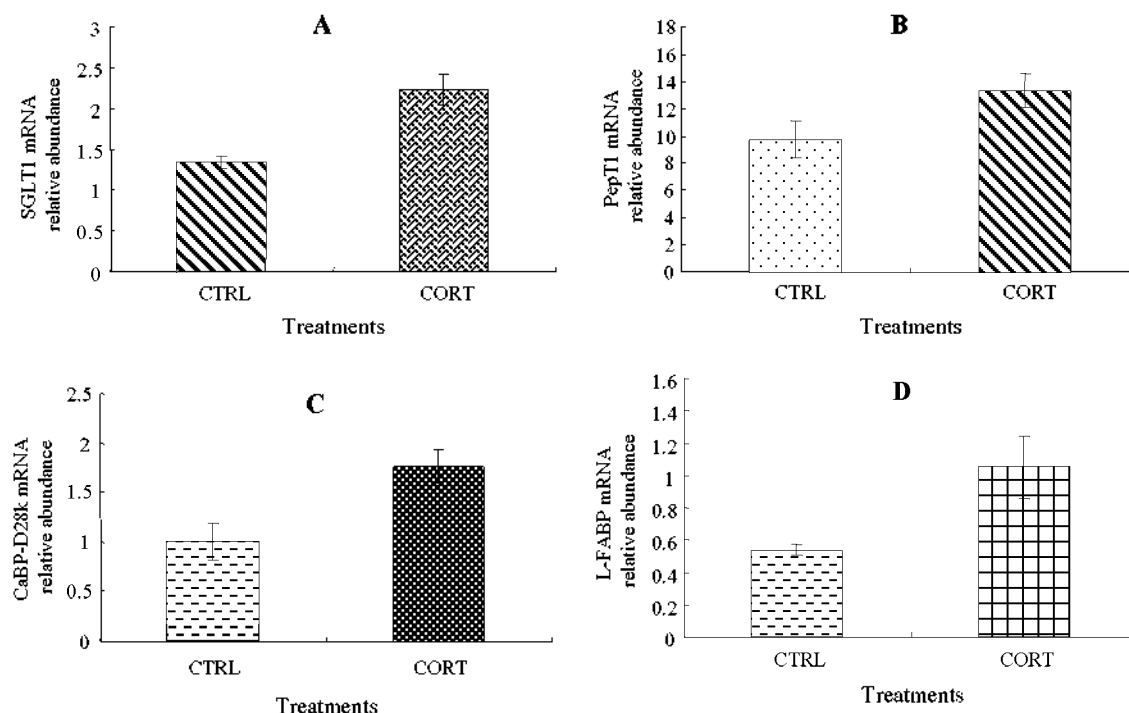


Figure 2. Relative mRNA abundance of nutrient transporters in small intestine of broiler chickens in different groups. A. SGLT1 mRNA abundance in duodenum. B. PepT1 mRNA abundance in duodenum. C. CaBP-D28k mRNA abundance in duodenum. D. L-FABP mRNA abundance in jejunum. Histograms with different superscripts differ significantly ($p < 0.05$).

DISCUSSION

In the present experiment, CORT administration significantly increased the liver weight of broiler chickens. This result agrees with previous studies (Covasa and Forbes, 1995; Puvadolpirod and Thaxton, 2000b; Malheiros et al., 2003). Moreover, increased liver weight is always regarded as one index of stress conditions (Puvadolpirod and Thaxton, 2000a, b, c). CORT administration increased CORT level in the blood of broiler chickens. This effect accords with results reported by others (Morrow et al., 1993; Mickey et al., 1996; Puvadolpirod and Thaxton, 2000a, b, c; Lin et al., 2004a; Davis et al., 2005; Olanrewaju et al., 2006). Increased serum corticosterone level is another index of stress (Moberg and Mench, 2000). The two indices indicated that CORT administration induced physiological stress successfully in this study.

CORT administration lowered small intestinal weight and shortened small intestinal length in the present experiment. This result is, partly, in agreement with previous research (Mitchell and Carlisle, 1992), in which heat stress lowered the wet and dry weight of small intestine. In the present study, CORT administration decreased feed intake, which is in accordance with previous reports (Malheiros et al., 2003; Lin et al., 2004a; Hu and Guo, 2008). The decreased feed intake leads to limited nutrients in the small intestine, which selectively decreases

protein synthesis in the small intestine and delays the full growth of small intestine, and subsequently results in lowered small intestinal weight (Dudley et al., 1998). Starvation or total parenteral nutrition (TPN) rats also had a declined weight of small intestine (Chance et al., 1997; Ihara et al., 2000; Howard et al., 2004). Corticosterone administration increased the relative weight of the small intestine, which may indicate that the small intestine utilized the limited nutrients for its growth with higher priority over body weight increase during stress.

Nutrient transporters play a critical role in the absorption of luminal substrate. In the small intestine of animals, PepT1 is responsible for the transportation of di- and tri-peptides arising from digestion of dietary protein (reviewed by Daniel, 2004). SGLT1 is the key factor that affects glucose absorption by the small intestine (Garriga et al., 2000; Kellett, 2001; Wood and Frayhum, 2003), and is expressed mainly in the duodenum (Garriga et al., 2002). Two types of fatty acid binding protein (FABP) are found in intestinal epithelium, intestinal FABP (I-FABP) and liver FABP (L-FABP) (Banaszak et al., 1994). The functions of L-FABP and I-FABP are different; the former participates in the uptake of long-chain fatty acids from digesta in the small intestine into intestinal epithelial cells, and the latter transports fatty acids from the cells to the organism (Prows et al., 1995). Calcium is absorbed in the duodenum and jejunum by calcium-binding protein (CaBP), which is

manipulated by 1, 25(OH)-D3 and has a molecular weight of 28,000 daltons, so the transporter is named CaBP-D28k (Craviso et al., 1987).

Stress affects the expression of nutrient transporters in the small intestinal epithelium. Starvation or TPN positively regulates the expression of PepT1 mRNA (Ogihara et al., 1999; Ihara et al., 2000; Naruhashi et al., 2002; Howard et al., 2004). In the present study, CORT administration increased the expression of PepT1, L-FABP, CaBP-D28k mRNA significantly ($p < 0.05$) and of SGLT1 mRNA ($p = 0.097$).

CORT treatment increases the small intestinal absorptive ability for glucose of broiler chickens (Nasir et al., 1999). Physiological stress induces a higher glucose level in blood (Puvadolpirod and Thaxton, 2000a, b, c; Odihambo et al., 2006; Olanrewaju et al., 2006; Lin et al., 2007). These results are likely to correlate with increased SGLT1 expression in the intestine of chickens (Garriga et al., 2000). Previous study showed that starvation stress increased the expression of SGLT1 mRNA in the small intestine of chickens (Gal-Garber et al., 2000).

Stress enhances the absorption of calcium by the small intestine (Nasir et al., 1999); this might be due to the upregulation of CaBP-D28k transport activity in chick duodenum induced by stress. However, heat stress decreases the absorption of calcium by the intestine of hens (Hansen et al., 2004). These different results may arise from the strain and age of chicken, or the type and extent of stress. Olanrewaju and co-worker showed that 35 d Ross×Cobb male broiler chickens, infused with ACTH 8 IU/kg BW/d continuously for 7 d, had the same plasma Ca^{2+} level as the controls (Olanrewaju et al., 2006). Moreover, there is more than one pathway that is responsible for the absorption of calcium (Bronner, 1987; Wasserman and Fullmer, 1995).

Stress increases the plasma high-density lipoprotein (HDL) and triglyceride (TRI) level of broiler chickens (Mickey et al., 1996; Puvadolpirod and Thaxton, 2000a, b; Odihambo et al., 2006; Olanrewaju et al., 2006). Non-esterified fatty acid is a component of HDL and TRI (Richards et al., 2003). In the present study, L-FABP mRNA expression was increased by CORT-induced stress, which may enhance the absorption of non-esterified fatty acid by intestinal epithelium from digesta.

CORT administration causes decreased small intestinal villus height (Hu and Guo, 2008), which leads to a decline of absorptive area (Yamauchi and Tarachai, 2000), and the shortened small intestine length induced by CORT administration further lowered total absorption area of the small intestine, and subsequently decreased the small intestinal absorptive capacity. The increased expression of nutrient transporter mRNA during stress is likely to be a compensation for the loss of absorptive area of the small

intestine.

It was concluded that stress induced by CORT administration lowered the small intestinal absolute weight. The small intestine utilized the limited nutrients for its growth with priority over body weight increase during stress. CORT treatment caused an increased expression of SGLT1, CaBP-D28k, PepT1 mRNA in the duodenum and L-FABP mRNA in the jejunum as a compensation for the loss of absorptive area of the small intestine.

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