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Corticosterone Administration Alters Small Intestinal Morphology and Function of Broiler Chickens*

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ABSTRACT : Two experiments were carried out to study the effects of corticosterone (CORT) administration on intestinal morphology and function of broilers. In both experiments, birds were randomly divided into two equal groups. One group was the control group (CTRL), and the birds were fed with a basal diet. The other was the experimental group (CORT), and the birds were fed with the basal diet plus 30 mg of CORT/kg diet. At 21 days of age, performance, morphological characteristics of intestine, D-xylose level in plasma, activities of digestive enzymes in digesta, digestibility of nutrients and 5-bromo-2-deoxyuridine (BrdUrd)-labeling index of intestinal epithelial cells were determined. CORT administration decreased feed intake, daily gain and feed conversion ratio ($p<0.05$). CORT also decreased duodenal and jejunal villus height ($p<0.05$) as well as crypt depth ($p<0.05$). The D-xylose level in plasma of CORT-treated broilers was lower than that of the control ($p<0.05$). CORT treatment caused a decrease in apparent digestibility of protein ($p<0.05$), whereas fat and starch apparent digestibilities were unaffected ($p>0.05$). CORT administration increased activities of trypsin and amylase ($p<0.05$), and decreased BrdUrd-labeling index of duodenal and jejunal epithelial cells ($p<0.05$). In conclusion, CORT administration impaired the normal morphology and absorptive capacity of the small intestine of broiler chickens. (**Key Words :** Broiler Chickens, Corticosterone, Morphology, Xylose Absorption, Activities of Digestive Enzymes)

INTRODUCTION

It is well known that small bowel is not only the most important digestive organ and the main absorptive site of the gastrointestinal tract, but also immunological gut barrier to protect against invasion (translocation) of endogenous luminal microorganisms and/or their toxins (review by Ziegler et al., 2003). The gastrointestinal tract of broiler chickens is about 1.5% of BW; however, approximately 6% to 8% of the energy derived from the diets is consumed by it (Spratt et al., 1990). The small intestinal epithelium is a compound multiple cell system, which determines the growth potential of broiler after hatched (Uni et al., 1998). The development of intestinal morphology and function resulted in the development of chickens (Yamauchi and Tarachai, 2000; Yang et al., 2007).

All kinds of stressors, such as thermal conditions, crowding, immunological challenge, handling, transportation, fasting and so on, exist around broilers, which activates hypothalamic-pituitary-adrenal (HPA) axis. The activated HPA axis results in the release of adrenocorticotropic hormone (ACTH), which stimulates the adrenals to secret and release corticosterone into blood, so the increasing concentration of adrenal glucocorticosteroids (corticosterone) in circulation have long been equated with stress (Moberg and Mench, 2000). Puvadolpirod and Thaxton (2000a, b) had successfully proposed a model by continuously infusion of ACTH via osmotic pump to study stress in domestic fowl. Moreover, when chickens were treated with corticosterone (Lin et al., 2004), the plasma corticosterone level of chickens was significantly increased, so addition of the stress-related hormone to diets or injection subcutaneously may serve as a practical alternative to reproductively investigate stress in broiler chickens (Post et al., 2003; Virden et al., 2007), which would be a simple, controlled and flexible model without the need of excessive handling of the chickens.

Many documents have reported that treatment with CORT in birds shifted metabolism of protein, i.e. increased catabolism of structural proteins, and decreased the birds' BW significantly (Puvadolpirod and Thaxton, 2000a, b;

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Table 1. Ingredient and chemical composition of the basal diet for experiment 1 and 2

Ingredient (%)		Nutrients level	
Corn	55.80	ME (Mcal/kg)	2.90
Soybean meal	37.32	Crude protein (%)	21.50
Soybean oil	2.80	Calcium (%)	1.00
Dicalcium phosphate	1.94	Available P (%)	0.45
Limestone	1.17	Lysine (%)	1.08
L-lysine	0.04	Met (%)	0.50
DL-methionine	0.18		
Salt	0.35		
30% ethoxyquin	0.02		
50% choline chloride	0.16		
Vitamin premix[1]	0.02		
Mineral premix[2]	0.20		

[1] Vitamin premix provided one kilogram of diet with: vitamin A, 125,000 IU; vitamin D_3, 2,500 IU; vitamin E, 18.75 mg; vitamin K_3, 2.65 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_6, 2 mg; vitamin B_{12}, 0.025 mg; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid, 12 mg; niacin, 50 mg.

[2] Mineral premix provided one kilogram of diet with: I, 0.35 mg; Se, 0.15 mg; Zn, 75 mg; Cu, 8 mg; Fe, 80 mg; Mn, 100 mg.

Post et al., 2003; Lin et al., 2004). Furthermore, the decrease of BW has shown to be independent of increased feed intake (Covasa and Forbes, 1995; Nasir et al., 1999; Puvadolpirod and Thaxton, 2000b). Virden et al. (2007) reported that CORT-induced stress had no effect on amino acid digestibility, however Puvadolpirod and Thaxton (2000b) showed that ACTH markedly decreased protein and carbohydrate digestibility of chickens, which seems to correlate with the intestinal function of broiler chickens.

Short-term fasting caused alterations in the intestinal architecture of White Leghorn hens and roosters by decreasing duodenal and jejunal villus height (Yamauchi et al., 1996; Yamauchi and Tarachai, 2000). Heat stress also significantly decreased broilers' jejunal villus height (Mitchell and Carlisle, 1992). The objective of the present study was to investigate the effect of CORT on the small intestinal morphology and function of broiler chickens.

MATERIALS AND METHODS

Animals, housing and experimental design

This study consisted of two experiments. In experiment 1, one hundred and twenty 8-day-old Arbor Acre (AA) male chickens were reared in 12 pens (10 birds/pen) of 3-tiered, metal brooder batteries. In experiment 2, one hundred and eight 8-day-old AA male chickens were reared in 18 pens (6 birds/pen) of 3-tiered, metal brooder batteries. In each experiment, the birds were randomly divided into two groups, and the pen served as the experimental unit.

One group was control group (CTRL), and fed with the basal diet based on corn-soybean meal (Table 1). The other was experimental group (CORT), fed with basal diet plus 30 mg of CORT/kg diet. Twenty-four hours artificial light was supplied. Birds were given *ad libitum* access to water and diet. Experimental period was 2 weeks, from 8 to 21 days of age.

Sampling and analysis

Feed intake (FI), daily gain (G) and feed conversion ratio (FCR) during the 14 days were calculated, respectively.

From 18 to 21 of age, excreta were collected quantitatively on plastic trays for continuous three days and stored in closed containers at -20°C. The total weight of excreta of each pen and the feed consumption in the three days were recorded. All the samples of excreta were dried to a constant weight in a forced-draft oven (65°C for two days) then kept for further analysis. Nitrogen was determined by Kjeldahl procedure, and crude protein content was calculated. Starch was determined by direct acid hydrolysis. Lipid content was determined by extraction using ethyl ether (Helrich, 1990). Nutrient digestibility (%) was calculated according to the method described by Puvadolpirod and Thaxton (2000b).

At 21 days of age, 6 birds of each group in each experiment were slaughtered. 1 cm of medial duodenum and medial jejunum were taken respectively and then kept in 4% formalin-buffered saline solution and embedded in paraffin. Histological examination was carried out according to the method described by Uni et al. (2001).

For the xylose absorption assay, after 12 h fasting, 6 birds of each group in each experiment were given a dose of 0.5 g/kg BW D-xylose solution at a concentration of 5% (w/v) via oral gavage, blood samples were withdrawn from wing vein 1 h later and collected in 5 ml heparinized eppendorf tubes, and plasma was prepared by centrifugation.The D-xylose concentration in plasma was determined using the method described by Doerfler et al. (2000), with a slight modification. To each 30 μl plasma, 3 ml of phloroglucinol (sigma) color reagent was added, then placed in boiling water for 4 min, then cooled in ice water, the absorption of each sample was read on a UV/Vis spectrometer (Perkin Elmer) set at 554 nm.

In experiment 2, in addition to those tests in experiment 1, following analysis was carried out.

At 21 day of age, 6 birds of each group were slaughtered, about 1gram digesta from upper jejunum was removed and homogenized (1:5, w/v distilled water) in 10 ml eppendorf tubes, supernatant was obtained after centrifugation at 1,500 g for 10 min at 4°C and stored at -80°C for further analysis of activities of digestive enzyme. Amylase activity was assayed using the method described by Osman (1982). Amylase activity unit was defined as the amount of amylase that hydrolyzes starch to liberate 1 μmol of maltose per min per gram of intestinal digesta at pH 7.0

Table 2. Performance of 21-day-old broilers in experiment 1 and 2

	Treatments	Food intake (g)	Daily gain (g)	FCR
Experiment 1	CTRL	66.39±1.10[a]	45.97±0.69[a]	1.44±0.01[a]
	CORT	58.69±0.74[b]	20.87±0.38[b]	2.82±0.06[b]
Experiment 2	CTRL	59.37±1.15[a]	39.42±1.60[a]	1.50±0.05[a]
	CORT	50.06±1.87[b]	17.24±0.33[b]	2.98±0.03[b]

[a, b] Within Exp. and column, means(±standard error) with unlike superscripts differ significantly ($p<0.05$).

and 37°C. Lipase activity was determined using the method of Tietz and Fiereck (1966). Lipase activity unit was equal to the volume (ml) of 0.05 M NaOH neutralized by the fatty acid liberated during a 6 h incubation with 3 ml of lipase substrate (Olive oil) at 37°C per gram of intestinal digesta. Trypsin activity was measured by the method described by Pinheiro et al. (2004) and made a little modification. Enterokinase was not used. Trypsin activity was measured from the released of P-nitroaniline from benzoyl-DL-arginine-P-nitroanilide (DL-BAPNA) at pH 7.8 and 37°C. Units are expressed as μmol of P-nitroaniline released per min per gram of intestinal digesta.

At the same day, Birds (n = 6 birds/group) were injected with 5-bromo-2-deoxyuridine (BrdUrd, sigma) intraperitoneally at a dose of 50 μg/kg BW. 1 h later, samples of medial duodenum and medial jejunum were removed and treated according to the method described by Iji et al. (2001). BrdUrd-labelled cells were identified by immunohistochemistry, in which mouse anti-BrdUrd antibody (Santa Cruz biotechnology) was used, and enterocyte proliferation labeling index also employed the method described by Iji et al. (2001).

Statistical analysis

Data, presented as means±SE, were analyzed by one-way analysis of variance (ANOVA) using the COMPARE MEANS procedure (SPSS13.0 software for windows, SPSS Inc., Chicago, IL, USA). $p<0.05$ was considered to be statistically significant.

RESULTS

Performance of broiler chickens

As shown in Table 2, in experiment 1 and 2, CORT administration, for 14 successive days, decreased feed intake, daily gain and feed conversion ratio significantly ($p<0.05$).

Morphological characteristics of small intestine of broiler chickens

The morphological characteristics of duodenum and jejunum of broilers in experiment 1 and 2 were showed in Table 3 and 4. The results indicated that CORT administration delayed the growth of the different segments of the small intestine. In experiment 1, villus heights and crypt depths of duodenum and jejunum of birds exposed to CORT were significantly lower than those of control group ($p<0.05$), as well as in experiment 2 ($p<0.05$).

Functions of small intestine of broiler chickens

The activities of digestive enzymes in small intestinal digesta were shown in Table 4. Activities of trypsin, amylase were higher in CORT treated broilers than those in control groups ($p<0.05$). Statistically significant differences were not observed in activities of lipase between CORT groups and control groups, but activities of lipase were numerically higher about 10% in CORT treated broilers than those in control groups.

In experiment 1 and 2, CORT administration significantly decreased ($p<0.05$) D-xylose level in plasma of birds, respectively (Tables 3 and 4). There was a significant effect of CORT administration on intestinal absorptive function of chickens.

Apparent digestibility of protein, fat and starch in experiment 1 and 2 were represented in Table 3 and 4. The results are expressed as percentages. Apparent digestibility of protein was lower in CORT groups than in control groups ($p<0.05$). There were no significant differences of apparent digestibility of starch between control groups and CORT groups, as well as the digestibility of fat.

BrdUrd-labeling index of epithelial cell of broilers' small intestine

Based on the results presented in Table 4, CORT administration lowered the duodenal and jejunal epithelial cell proliferation. The birds exposed to CORT had lower BrdUrd-labeling index in duodenum and jejunum than those in control groups ($p<0.05$).

DISCUSSION

CORT administration decreased daily gain and feed conversion ratio of the broiler chickens in the present study, and this indicated that CORT administration induced physiological stress. This result was in agreement with those of other studies (Eid et al., 2003; Malheiros et al., 2003; Lin et al., 2004; Virden et al., 2007). CORT administration lowered broilers' feed intake significantly, this effect was in accordance with the results reported by

Lin et al. (2004) and Malheiros et al. (2003), but was contrast to the results reported by Covasa and Forbes (1995) and Nasir et al. (1999) and Virden et al. (2007). The different results of feed intake in different experiment were likely due to the difference of strain, age of birds, dose of corticosterone and the time exposed to corticosterone. Covasa and Forbes (1995) used 2 wk and 5 wk old broilers and the experiment lasted only 5-d period, Nasir et al. (1999) tested 10 wk old White Leghorn for 5 days, and Virden et al. (2007) used 21-day-old broilers of Ross 708 and Ross 308. Lin et al. (2004) reported that, in 2-wk-period experiment, the absolute feed intake of birds was not significantly affected during the first week, but markedly decreased in the second week after CORT administration. Malheiros et al. (2003) also reported that 45 mg of CORT/kg diet decreased feed intake of broiler chickens, however 30 mg of CORT/kg diet increased feed intake of birds (in this experiment, the period was from 21 to 35 day of age of male broilers), in the present study, the birds were only 1wk old, and the experimental period was 2 weeks. Corticosterone administration slowed food passage (Nasir et al., 1999), which would decrease feed intake of broilers exposed to CORT.

When feed intake of chickens declines or diet is deprived, villus heights of small intestinal epithelium decrease subsequently (Mitchell and Carlisle, 1992; Yamauchi et al., 1996; Yamauchi and Tarachai, 2000). So the decreased duodenal and jejunal villus heights in the present study would be expected.

D-xylose, a poorly metabolized pentose, is absorbed from the upper small intestine primarily by passive diffusion and, to a lesser extent, by the same active transport system responsible for absorbing glucose and galactose. Xylose absorption test has been proven to be a reliable indicator of intestinal absorptive function (Doerfler et al., 2000). Histologically, the malabsorptive condition was reflected as villus atrophy such as decreased villus surface and, subsequently, a loss of absorptive surface area.

Table 3. The small intestinal morphological characteristics and function of 21-day-old broilers in experiment 1

	CTRL	CORT
morphological characteristics		
Duodenum villus height (μm)	716.00±54.02[a]	572.26±37.68[b]
Duodenum crypt depth (μm)	97.18±3.89[a]	80.92±1.26[b]
Jejunum villus height (μm)	686.34±29.21[a]	498.25±24.60[b]
Jejunum crypt depth (μm)	106.89±3.79[a]	83.92±2.63[b]
Nutrients apparent digestibility		
Protein (%)	60.31±2.29[a]	32.04±0.06[b]
Fat (%)	67.33±0.87	68.50±0.91
Starch (%)	85.03±0.38	84.63±0.41
D-xylose level in plasma (mmol/L)	3.17±0.28[a]	2.48±0.18[b]

[a, b] Within line, means (±standard error) with unlike superscripts differ significantly ($p<0.05$).

Table 4. The small intestinal morphological characteristics, function and cell proliferation characteristics of 21-day-old broilers in experiment 2

	CTRL	CORT
Morphological characteristics		
Duodenal villus height (μm)	756.96±52.74[a]	563.89±37.19[b]
Duodenal crypt depth (μm)	84.98±4.29[a]	70.28±1.24[b]
Jejunal villus height (μm)	647.07±7.94[a]	456.25±12.90[b]
Jejunal crypt depth (μm)	87.66±11.50[a]	69.92±7.50[b]
Nutrients apparent digestibility		
Protein (%)	62.32±1.19[a]	34.58±1.78[b]
Fat (%)	73.01±2.87	74.85±0.73
Starch (%)	84.34±0.11	84.95±0.28
D-xylose level in plasma (mmol/L)	1.32±0.12[a]	1.00±0.06[b]
Digestive enzyme activities		
Trypsin (U/g)	0.68±0.16[a]	1.45±0.29[b]
Lipase (U/g)	228.76±17.31	251.84±11.37
Amylase (U/g)	29.29±1.43[a]	33.23±0.71[b]
BrdUrd-labeling index		
Duodenum	20.67±0.67[a]	17.25±0.75[b]
Jejunum	20.25±1.89[a]	15.50±1.94[b]

[a, b] Within line, means (±standard error) with unlike superscripts differ significantly ($p<0.05$).

Villus height was positively related to villus surface area (Mitchell and Carlisle, 1992), the expansion of surface area that occurs with villus growth has been used to explain the increased absorptive capacity, whereas the decreased villus height lowered the absorptive capability of small intestine (Yamauchi et al., 1996). CORT-induced stress decreased villus height of intestine of broiler chickens in the present study, so the absorptive capacity of small intestine declined subsequently, and then the lowered D-xylose level in plasma of broilers in CORT groups could be resulted from.

The activities of digestive enzyme reflect the capacity of small intestine to digest the dietary nutrient (Yuan et al., 2008). In the present study, trypsin and amylase activities were significantly increased by CORT, and lipase activity was also numerically increased by CORT. Pinheiro et al. (2004) reported that feed restriction increased the activities of pancreatic and intestinal enzymes in broilers, which resulted in the elevated activities of digestive enzymes in digesta. The elevated activities of digestive enzymes would increase digestive function of small intestine and provide more available nutrients for birds to utilize under stressed condition.

CORT decreased the digestibility of protein and had no effect on the digestibility of fat in the present study, which were in agreement with Puvadolpirod and Thaxton (2000b). The digestibility of starch was unaffected by CORT, however Puvadolpirod and Thaxton (2000b) showed that ACTH treatment decreased the digestibility of carbohydrate, in their experiment the objectives were 5-wk-old Peterson×Arbor Acre chicks, and ACTH treated for 7 days.

The digestibility of protein could have been compounded by nonprotein nitrogen levels, corrections for uric acid content in fecal matter were not attempted in this study. Previous studies found that CORT administration increases creatine kinase activity and thereafter increases skeletal muscle proteolysis, and then increased plasma uric acid (Lin et al., 2004). Virden et al. (2007) reported that CORT-induced stress had no effect on apparent ileal amino acid digestibility. So the decrease of the digestibility of protein may be related to muscle oxidation induced by CORT. Positive regulation of peptide transporter 1 (PepT1) mRNA has been observed during animal starvation or total parenteral nutrition (Ogihara et al., 1999; Howard et al., 2004). The expression of intestinal specific amino acid transporter mRNA also increased by total parenteral nutrition (Howard et al., 2004). Gal-Garber et al. (2000) showed that starvation increased the expression of sodium glucose cotransporter 1 (SGLT1) mRNA in small intestine of chickens. We also found that CORT administration increased the mRNA expression of PepT1, SGLT1 and fatty acid binding protein (FABP) in small bowel (data unpublished). PepT1 plays an important role in transporting small peptides arising from digestion of dietary protein in the small intestine (reviewed by Daniel, 2004). Amino acids enter the intestinal epithelial cell also need amino acid transporters. SGLT1 is the key factor that affects the glucose absorption by intestine (reviewed by Hediger and Rhoads, 1994). Intestinal FABP (I-FABP) are believed to participate in the uptake of long-chain fatty acids (LCFAs) (reviewed by Weisiger, 1996). The increased expression of intestinal nutrient transporters mRNA and the elevated activities of digestive enzymes in digesta caused by CORT are likely to compensating for the loss of absorptive surface area caused by CORT, which may result in the fact that CORT administration has no effect on fat and starch digestibility in the present study.

BrdUrd is a uridine analogue, which can be incorporated into cells at the S phase, and BrdUrd incorporation into proliferating cells can be used to study the cell kinetics (Thoolen, 1990). The increase of intestinal epithelial cell BrdUrd-labeling index may reflect an increase rate of cell proliferation to support both crypt and villus growth (Iji et al., 2001). In the present study, CORT administration significantly decreased the BrdUrd-labeling index of duodenum and jejunum of broilers, so villus height and crypt depth of the two sections significantly decreased subsequently.

In conclusion, CORT administration delays proliferation of intestinal epithelial cell, which in turn lowers intestinal villus height and crypt depth, and then subsequently impairs absorptive (Xylose absorption) capacity of small intestine of broilers.

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