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RESEARCH ARTICLE

Effects of Acute and Chronic Cold Stress on Expression of Cyclooxygenase-2 and Prostaglandin E Synthase mRNA in Quail Intestine

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ABSTRACT

The cold temperature, a common environmental stress, reduces the immunity and re-production activities of the poultry. This study aims to investigate the role of acute and chronic cold exposure in the regulation of cyclooxygenase-2 (COX-2) and prostaglandin E synthase (PTGES) expression in the duodenum, jejunum, and ileum of quail. A total of 96 quail with 15 days of age were randomly allocated into 12 groups (8 each group) for exposure to acute (up to 12 h) and chronic (up to 20 days) cold temperature (12±1°C). After that, different segments of the intestine were harvested and subjected to morphology observations under the light and electronic microscopes. qRT-PCR was performed to analyze expression of COX-2 and PTGES, and DNA sequencing was performed to analyze PCR products. The data showed that under acute cold stress, expression of COX-2 and PTGES mRNA was first decreased and then increased in the duodenum, jejunum, and ileum of quail. However, chronic cold stress induced expression of COX-2 and PTGES mRNA in the duodenum, jejunum and ileum of quail, which was then reduced after 20 days of cold exposure. Morphologically, significant changes were also observed in the duodenum, jejunum and ileum after both acute and chronic cold stresses to the animals. The data from the current study indicated that both acute and chronic cold stresses were able to induce inflammation responses in the duodenum, jejunum and ileum, which might be due to the cold-damaged intestinal morphology.

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INTRODUCTION

Various environmental factors, such as extremely hot or cold ambient temperature, can affect food intake, weight gain, and feed efficiency in the poultry (Sahin *et al.*, 2002). Cold temperature-caused stress to living animals, which is a common environmental factor in the northern region of the globe, reduces the immunity and re-production activities of animals and may cause death in newborns (Wang *et al.*, 2009). In China, quail is a key component of the Chinese poultry industry and needs high temperature (e.g., young quail with 15-30 days of age requires 28-32^{o}C for survival and growth) to maintain their activity, growth, reproduction and survival, especially in the brooding period. Furthermore, the stressful life events have long been implicated in pathogenesis of various gastrointestinal diseases, such as inflammatory bowel disease, and ulcerative colitis in humans (Collins, 2001; Muller *et al.*, 2012). The chronic cold stress induced the generation of oxidative stress in various rat tissues, particularly, in the small intestine (Kaushik and Kaur, 2003). However, there is relatively little knowledge regarding the underlying molecular mechanisms responsible for the cold-related stress in living animals, especially cold-induced inflammation in the intestine of animals, which significantly influences animal feeding and weight gain.

Cyclooxygenase (COX) is the key enzyme in the biosynthesis of prostaglandins, therefore, plays an important role in maintaining functions of the gut (Fiocchi, 1998). Specifically, COX-1 and COX-3 are expressed constitutively in broad types of tissues and cells, whereas COX-2 expression is usually induced by various cytokines, mitogens, and stresses (Yuan *et al.*, 2000). Functionally, COX-2 is pro-inflammatory (Chen *et al.*, 2012) and aberrant COX-2 expression plays a role in the pathogenesis of intestinal inflammation and different cancers (Tanabe and Tohnai, 2002). Moreover,

prostaglandin E synthase (PTGES) catalyzes the isomerization of PGH_2 to PGE_2 (Mancini *et al.*, 2001; Dakin *et al.*, 2012). In this study, we investigated the effect of cold-caused stress on the regulation of COX-2 and PTGES expression in quail, using their duodenum, jejunum and ileum as the target organs.

MATERIALS AND METHODS

Birds, exposure to cold stress, and tissue collections: A total of 96 French Shaweimate meat-type male quail with one day of age were purchased from Xiangyang Co. Ltd. (Harbin, China). The experiments were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University (Harbin, Heilongjiang, China). Feed and tap water were supplied freely. After they reached 15 days of age, these quail were randomly allocated into 12 groups (6 groups for the acute cold stress experiments and another 6 groups for the chronic cold stress experiments with 8 quail for each group). In the acute cold stress experiments, the birds were exposed to 12±1°C for 0.5, 1, 3, 6, and 12 h, while birds for the chronic cold stress experiments were exposed to 12±1°C for 5 d, 10 d, and 20 d. The control birds (n=32) were maintained at 28°C. The quail were killed by cervical dislocation and the duodenum, jejunum and ileum were collected immediately, tissues were fixed for electron microscope and light microscope and stored at -80°C for qRT-PCR.

Quantification of COX-2 and PTGES mRNA: Total RNA of birds tissues was isolated by using a Trizol reagent (Invitrogen, Carlsbad, CA), and the methods utilized in the present study was in line with the manufacturer. After quantification, these RNA samples were subjected to reverse transcription reaction. The expression levels of PTGES, COX-2 genes were determined by the technology of Real-time quantitative reverse transcription PCR by using SYBR Premix Ex TaqTM (Takara, China), and real time PCR system was ABI PRISM 7500 real-time PCR system (Applied Biosystems). The PCR primers were designed according to chicken PTGES (NM_001194983), COX-2 (NC_001323), and Quail's GAPDH (EU035555) from the GenBank using Oligo Primer Analysis software (version 6.0) and synthesized by Invitrogen (Shanghai, China). The primers for chicken PTGES were 5'-TTGCCATCATCACGGGACA-3' and 5'-CCACATCAGGGTCCTCACGGTA-3', for chicken COX-2 were 5'-TGTCCTTTCACTGCTTTCCAT-3' and 5'- TTCCATTGCTGTGTTTGAGGT -3', and for quail's GAPDH were 5'-TGATGCTCCCATGTTCGTGA-3' and 5'- TAAGACCCTCCACGATGCC -3'.

Statistical analysis: Statistical analyses of our experimental data were performed using SPSS for Windows system (SPSS, Chicago, IL, USA). Data are expressed as the mean±standard deviation. All data showed a normal distribution and passed equal variance testing. Differences between means were assessed using Tukey's honestly significant difference test for post hoc multiple comparisons. One-way analysis of variance was performed to generate P values for our data. A $P<0.05$ was considered statistically significant.

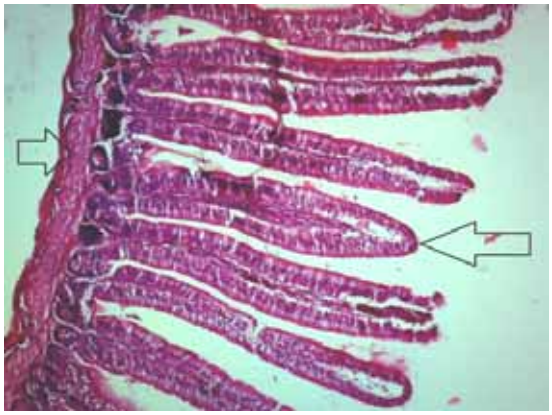
RESULTS

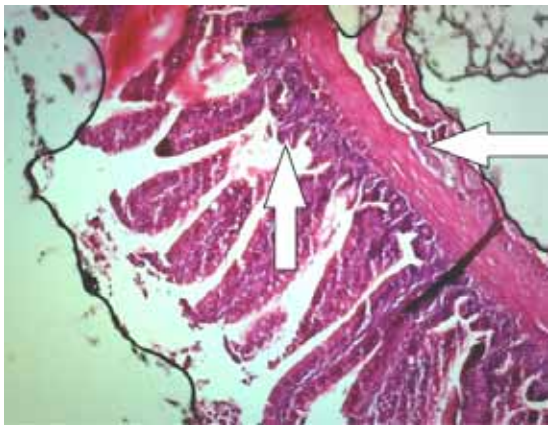
Effects of cold stress on changes in histopathology under the light microscopy: We found that the histopathological structure of the epithelium, lamina propria and muscular in mucosa layer was intact in the jejunum of the control birds and the villus appeared to have a normal arrangement, whereas the mucous layer was seriously damaged, the villus was fractured and there was disintegration in the jejunum of five day cold-stressed birds. Moreover, the villi were disordered and the mucosal epithelial cells were seriously damaged in the duodenum of five days cold-stressed birds. In addition, the muscular layer of the duodenal mucosa was hyperplasic and extended to the lamina propria. The lamina propria glands are clear and visible with inflammatory cell infiltration (Fig. 1). Similar findings were observed in ileum of these birds, such that the structure of the villi was in disorder and the mucosal epithelial cells became not clear (Fig.1).

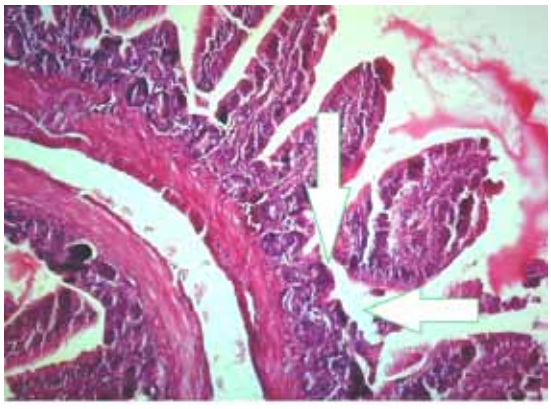
The roles of cold stress in changes in histopathology under the electron microscopy: Electron microscopically, the subcellular organelle appeared regularly and densely in the duodenum of the control birds. The nuclei of epithelial cells were clear and ovoid in shape, the mitochondrial cristae arranged regularly, and the inner and outer membranes occurred clearly. The endoplasmic reticulum and Golgi apparatus had regular morphology without any dilatations (Fig. 2A). However, in the acute cold stress groups, endoplasmic reticulum appeared abundantly and was swelled in the bird duodenum. Some of the epithelial cells were apoptotic and lysosomes were visible, while the endoplasmic reticulum parceled the damaged mitochondria (Fig. 2B). Furthermore, in chronic cold-stressed birds, the nuclei of the duodenum appeared karyorrhexis, the endoplasmic reticulum parceled damaged organelles, and formed autophagolysosome in the cytoplasm (Fig. 2C).

In the control birds, the subcellular organelle of the jejunum cells arranged regularly and densely. The nuclei of the epithelium were clear and ovoid in shape and the mitochondria were abundant. The mitochondrial cristae were arranged regularly and the inner and outer membranes were clearly visualized. The endoplasmic reticulum and Golgi apparatus had regular morphology without any dilatations (Fig. 2D). In contrast, the jejunum from the acute cold stress birds showed that the nuclei disappeared and lysosomes appeared (Fig. 2E), while the jejunum from the chronic cold stress birds showed that the mitochondria were damaged, the endoplasmic reticulum was swelled, and some nuclei became deformed (Fig. 2F).

Moreover, in the control birds, the ileum cells showed regular and dense arrangement of the subcellular organelle (Fig. 2G), whereas acute cold-exposed ileum showed that the endoplasmic reticulum appeared abundantly with slight swelling and the endoplasmic reticulum parceled the damaged mitochondria (Fig. 2H). In addition, the chronic cold stress-exposed ileum showed that the swelled endoplasmic reticulum appeared was abundant with cells, but a few cells were apoptotic (Fig. 2I).







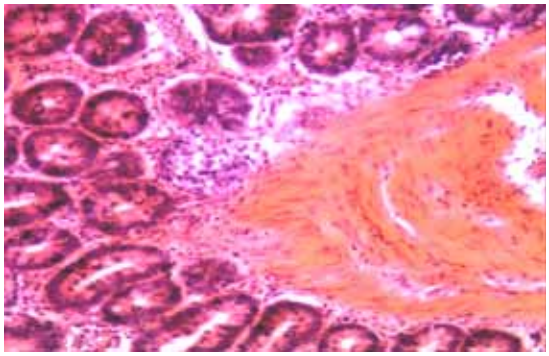


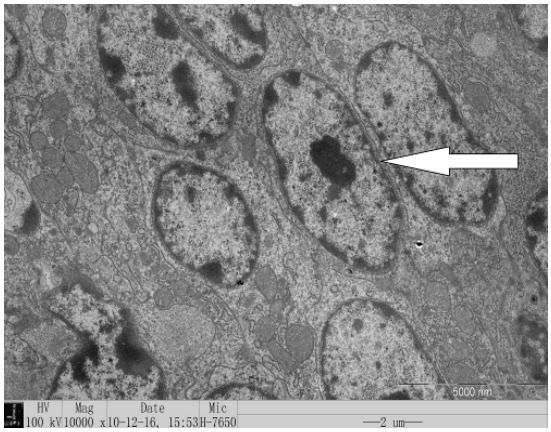
Fig. 1: Effects of cold stress on histopathologic changes of the intestine under a light microscope. A. The control jejunum. B. The duodenum from cold-exposed quail. C. The jejunum from the cold stressed quail. D. The ileum from the cold stressed quail. E, The duodenum with inflammatory cell infiltration from the cold stressed quail. The magnification of all imagines was at × 400. These were chronic exposed quail. The arrows show the changed morphology after the cold exposures.

The roles of cold stress in the alteration of COX-2 and PTGES expression in quail duodenum: Our data showed that the acute cold stress significantly decreased expression levels of COX-2 mRNA in the duodenum after 0.5-12 h of exposure to cold temperature ($P<0.05$) compared to that of the control birds (Table 1). A similar finding was observed for PTGES mRNA levels. Moreover, the chronic cold stress for 5 and 10 d significantly increased expression of COX-2 mRNA levels in the duodenum ($P<0.05$) compared to that of the corresponding control birds, while 20 d cold stress decreased the duodenum expression of COX-2 mRNA (Table 2). A similar finding was observed for PTGES mRNA levels.

Effects of cold stress on alteration of COX-2 and PTGES expression in the jejunum of quail: The roles of acute cold stress in the expression of COX-2 and PTGES mRNA levels in the jejunum are shown in Table 1. The level of COX-2 mRNA was significantly decreased in the jejunum of the 3 h acute cold exposed birds ($P<0.05$), whereas 6 h and 12 h of cold groups had a increase ($P<0.05$) in COX-2 mRNA levels compared with the control birds (Table 1). Similarly, expression of PTGES mRNA in the jejunum after 0.5-3 h exposed to cold was significantly decreased ($P<0.05$), while PTGES mRNA was increased after 12 h of cold ($P<0.05$) compared with the control group.

The roles of chronic cold stress in the expression of COX-2 mRNA levels in the jejunum are shown in Table 2. COX-2 expression in the jejunum after 5 and 10 d exposed to cold was increased ($P<0.05$) when compared with corresponding control birds, but was decreased after 20 d exposure. The levels of PTGES mRNA in the jejunum in 15, 10 and 20 d groups were all increased ($P<0.05$) compared with the corresponding controls.

Effects of cold stress on alteration of COX-2 and PTGES expression in the ileum of quail: We also found that 1, 6, and 12 h exposure to the cold increased ileum expression of COX-2 mRNA levels ($P<0.05$), with significant differences at 0.5 and 3 h ($P<0.05$) compared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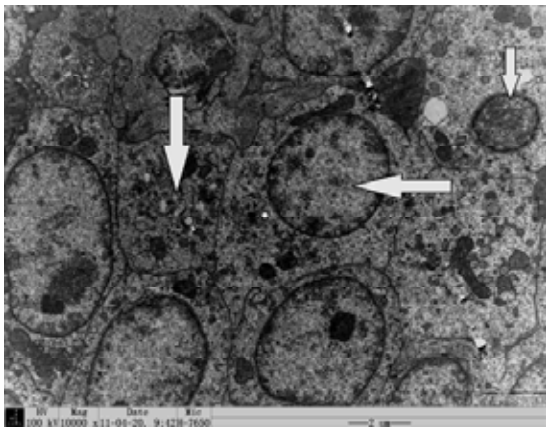


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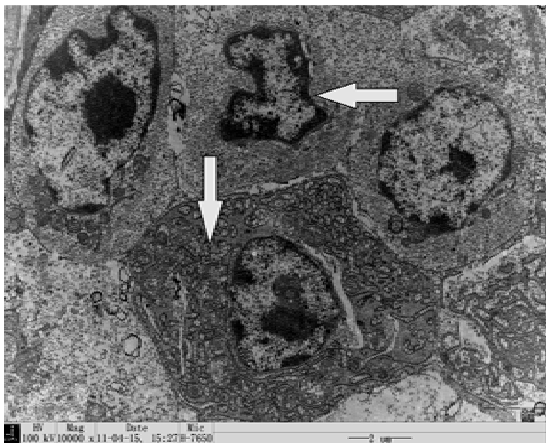
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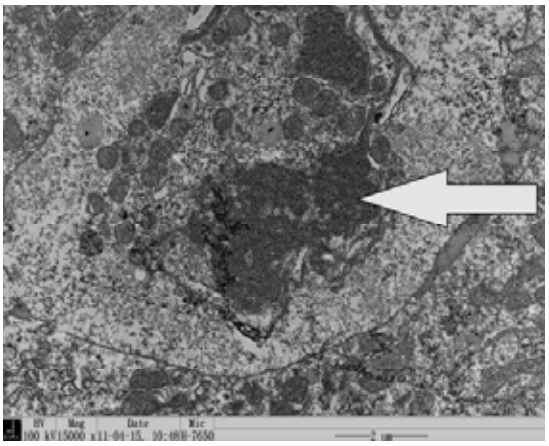
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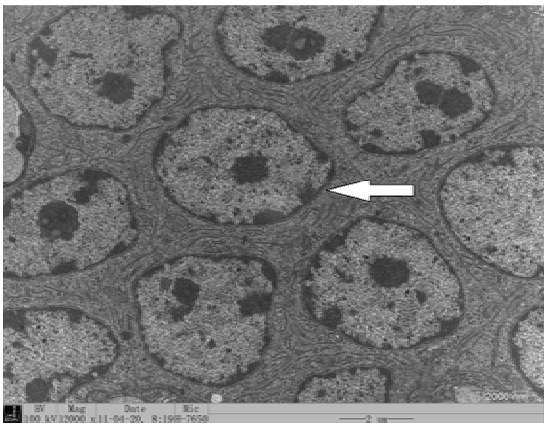
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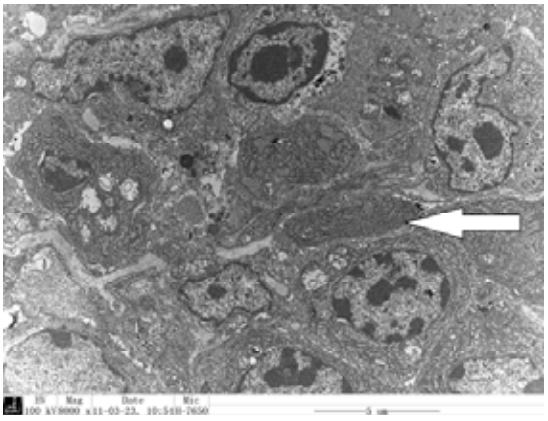
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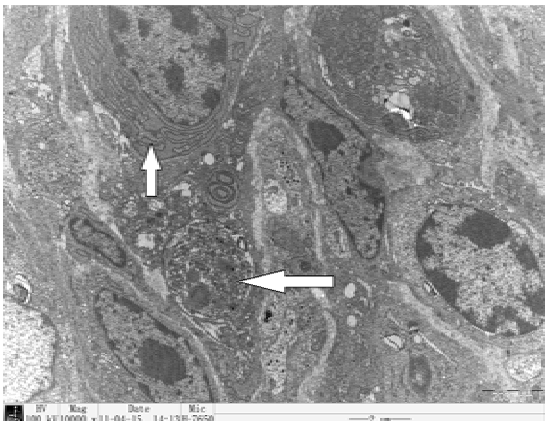

Fig. 2: Electron microscopic detection after the cold stress. A. The duodenum in the control birds (10000 ×). B. The duodenum after the birds were exposed to the acute cold (15000 ×). C. The duodenum after the birds were exposed to the chronic cold temperature (15000 ×). D. The jejunum in the control birds (10000 ×). E. The jejunum after the birds were exposed to the acute cold (10000 ×). F. The jejunum after the birds were exposed to the chronic cold temperature (10000 ×). G. The ileum in the control birds (10000 ×). H. The ileum after the birds were exposed to the acute cold (10000 ×). I. The ileum after the birds were exposed to the chronic cold temperature (10000 ×). The arrows show the changed morphology after the cold exposures.

to that of the control birds (Table 1). Similarly, ileum expression of PTGES mRNA levels significantly increased after 1, 6, and 12 h exposure to the cold ($P<0.05$) compared to that of the control group (Table 1).

After 5 and 10 exposure to the cold temperature, ileum expression of COX-2 mRNA levels was significantly increased ($P<0.05$), but 20 d exposure significantly decreased COX-2 expression in the ileum ($P<0.05$) compared to that of the corresponding controls (Table 2). A similar finding was observed for PTGES mRNA levels (Table 2).

DISCUSSION

In this study, we investigated the effects of the acute and chronic cold exposure on the regulation of COX-2 and PTGES expression in various intestinal tissues from quail. We found that acute cold stress first decreased and then increased expression of COX-2 and PTGES mRNA in the duodenum, jejunum and ileum, whereas chronic cold stress induced expression of COX-2 and PTGES mRNA in these tissues, but they were reduced after 20 d of cold exposure. Morphologically, we also demonstrated significant changes in the cold-exposed duodenum, jejunum, and ileum. Our current study suggests that cold stress could induce an inflammatory response in the intestine, leading to a change in food intake by the quail.

Physiological and biochemical knowledge regarding the growth and development of organism is fundamental to understanding the behavior of the species in response to different environmental conditions (Valente *et al.*, 2012). Previous studies have demonstrated that environmental stress induces pathological alterations and symptoms in the intestine of poultry, changing the course of chronic intestinal diseases (Hart and Kamm, 2002). Cold-induced stress resulted in alteration of epithelial cell proliferation in rat small intestinal mucosa, which supported the hypothesis that chronic stress can be an initiating factor in inducing inflammation in the small intestine (Kaushik and Kaur, 2003). Indeed, in our current study, we found that acute and chronic cold exposure induced morphological alterations in these different segments of the intestine under both light and electronic microscopes. These stress-induced damages could further trigger host defense, such as inflammation reactions.

Inflammation is induced by various genes. Previous studies showed that COX-2 and its products, prostanoids, were important factors in gut homeostasis and inflammation (Vichai *et al.*, 2005). Inflammatory cytokines (e.g., IL-1β, IL-10 and IL-13) can promote COX-2 expression, resulting in high levels of prostaglandins at the site of inflammation. COX-2 inhibitors reduce mucosal damages, which further indicate the role of prostaglandins in tissue damage and inflammation reactions (Zheng *et al.*, 2012). Our current study linked altered COX-2 expression to cold exposure-induced stress in the intestine. We found that acute cold exposure first decreased and then induced COX-2 expression in quail' intestine, where the chronic cold stress promoted COX-2 expression for up to 15 d. After 20 d of cold exposure, COX-2 expression was reduced

Table 1: Effects of acute cold stress on regulation of COX-2 and PTGES mRNA levels in the duodenum, Jejunum and ileum.

Organ	COX-2/ PTGES expression	Exposure Time (hours)					
		0	0.5	1	3	6	12
duodenum	1	1.000±0.000^{a}	0.416±0.012^{b}	0.048±0.007^{c}	0.093±0.011^{d}	0.102±0.008^{d}	0.107±0.009^{d}
	2	1.000±0.000^{a}	0.566±0.043^{b}	0.349±0.015^{cd}	0.336±0.009^{c}	0.411±0.032^{d}	0.827±0.024^{e}
Jejunum	1	1.000±0.000^{a}	0.763±0.041^{a}	0.721±0.043^{a}	0.051±0.004^{b}	2.944±0.248^{c}	1.461±0.122^{d}
	2	1.000±0.000^{a}	0.352±0.011^{b}	0.494±0.043^{c}	0.157±0.002^{d}	1.135±0.091^{a}	1.291±0.066^{e}
Ileum	1	1.000±0.000^{a}	0.518±0.032^{b}	3.296±0.294^{c}	0.406±0.0618^{b}	5.5±0.241^{e}	6.679±0.105^{f}
	2	1.000±0.000^{ab}	1.293±0.12^{bd}	1.802±0.267^{d}	0.588±0.053^{ac}	4.773±0.478^{e}	1.905±0.061^{df}

Values (mean±SD) bearing different letters in a row differ significantly ($P<0.05$). COX-2=1; PTGES expression=2

Table 2: Effects of chronic cold stress on regulation of COX-2 and PTGES mRNA levels in the duodenum, Jejunum and ileum.

Organ	COX-2/ PTGES expression	Exposure Time (days)					
		5		10		20	
		Control	Stress	Control	Stress	Control	Stress
duodenum	1	1.000±0.000	1.247±0.007*	1.000±0.000	1.36±0.031*	1.000±0.000	0.727±0.153
	2	1.000±0.000	1.163±0.002*	1.000±0.000	1.163±0.081	1.000±0.000	0.067±0.001*
Jejunum	1	1.000±0.000	1.197±0.035*	1.000±0.000	4.757±0.324*	1.000±0.000	0.206±0.035*
	2	1.000±0.000	1.686±0.061*	1.000±0.000	7.235±0.222*	1.000±0.000	6.052±0.2482*
Ileum	1	1.000±0.000	1.553±0.279	1.000±0.000	3.779±0.278*	1.000±0.000	0.17±0.007*
	2	1.000±0.000	1.497±0.236	1.000±0.000	5.515±0.46*	1.000±0.000	0.386±0.01*

Values (mean±SD) bearing different letters in a row at an exposure time differ significantly ($P<0.05$). COX-2=1; PTGES expression=2

again in the intestine, for a reason that remains to be determined. However, a previous study showed that the deleterious effects of COX-2 inhibitors on the intestinal epithelium suggest a protective role of COX-2 in intestinal homeostasis (Bento *et al.*, 2012). Thus, these paradoxical effects of cold stress on the regulation of COX-2 expression cause the role of COX-2 in cold stress reaction to be more complicated.

COX-2 is the key enzyme in production of different prostaglandins; however, various types of these prostaglandins have different functions in the tissues and cells (Grundemann *et al.*, 2012). PTGES catalyzes the isomerization of PGH2 to PGE2 and the latter plays an important role in inflammation (Liu *et al.*, 2012), cell growth, and transformation (Sasaki *et al.*, 2012). Thus, in the current study, we also detected PTGES expression after the quail were exposed to acute and chronic cold stresses. We found that the altered PTGES expression was similar to that of COX-2 mRNA after cold exposure-induced stress, suggesting that PGE_2 may not participate in acute responses of the cold stress, but may play a role in chronic cold stress to induce inflammation reaction or cell growth. However, the mechanism of cold stress is very complicated and the data from different studies are not consistent (Wang and Xu, 2008). These could be attributed to the duration and temperature of cold exposure, the genetic background of experimental animals, or others (Wang and Xu, 2008). Thus, the mechanisms responsible for cold stress-induced intestinal damages require further study and future investigation will focus on how COX-2 and PTGES regulate stress response and cell growth and differentiation in the intestine of quail.

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