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Effect of *in ovo* ghrelin administration on serum malondialdehyde level in newly-hatched chickens

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ABSTRACT

Objective: To investigate effects of *in ovo* ghrelin administration on serum malondialdehyde (MDA) level in newly-hatched chickens. **Methods:** Fertilized eggs were divided into 7 groups: group T1 as control (without injection), group T2 (*in ovo* injected with 50 ng/egg ghrelin on day 5), group T3 (*in ovo* injected with 100 ng/egg ghrelin on day 5), group T4 (*in ovo* injected with 50 ng/egg ghrelin on day 10), group T5 (*in ovo* injected with 100 ng/egg ghrelin on day 10), group T6 (*in ovo* injected with solvent: 1% acetic acid, without ghrelin on day 5) and group T7 (*in ovo* injected with solvent without ghrelin on day 10). After hatching, serum MDA concentrations were determined. **Results:** Ghrelin administrated groups (T2, T3, T4 and T5) had lower serum MDA level in comparison with control group (T1) or solvent injected groups (T6 and T7). T2 and T3 (ghrelin injection on day 5) had significantly lower MDA concentrations (4.10 and 4.60 nmol/mL, respectively) in comparison with other groups. In T4 and T5, MDA levels were lower than T1, T6 and T7 (non-ghrelin administrated groups) (9.53 and 9.50 in comparison with 10.73, 10.03 and 10.13 nmol/mL) and were higher than T2 and T3. **Conclusions:** It can be concluded that *in ovo* administration of ghrelin can have anti-oxidative protection and reduce serum MDA level. Ghrelin administration on day 5 of incubation is more efficient.

1. Introduction

Currently, the anti-oxidant properties of some regulatory peptides are interesting research topics. For example, Zheng *et al*[1] reported that leptin has considerable role in anti-oxidant defense of body by activating super oxide dismutase. Ghrelin is another multifunctional regulatory peptide. After identification of ghrelin in mammals by Kojima *et al*[2] so many relative studies were conducted on ghrelin properties. Studies conducted during last decade suggested various specifies and functions for ghrelin, such as GH-releasing[3], osteogenesis[4,5], hematopoiesis[6,7], food intake and energy balance and regulation[8,9], endocrine/paracrine roles in pancreas[10,11], and currently anti-oxidative effects[12,13]. Peptide structure of chicken ghrelin has 26 amino acids with 54% similarity to rat ghrelin[14]. Ghrelin has been identified in albumen and yolk of fertilized chicken egg[15]. Malondialdehyde (MDA) is the organic compound with the formula $CH_2(CHO)_2$ and it is a bio-marker of oxidative stress in an organism and an increase of MDA level demonstrates oxidative stress[16]. Anti-oxidative activity of ghrelin has been reported in numerous studies[12,13,17]. Reports of Işeri *et al*[17] indicated anti-oxidative activity of ghrelin and its ameliorative ability for alendronate-induced oxidative stress, as MDA comes back to the control level after ghrelin infusion. Also, Brzozowski *et al*[18] reported its gastroprotective and hyperemic activities against ischemia-reperfusion-induced erosions and a decrease in relative MDA level. Increase in aerobic metabolism and lipid peroxidation in chick embryo makes high demands for anti-oxidative protection. Chick embryo has attempted to increase capacity of its antioxidant defenses during embryonic life[19]. Anti-oxidative effects of ghrelin have been studied on mammalian models mostly and it is unclear in birds. With attention to the presence of ghrelin in the yolk and albumen of fertilized chicken eggs[15], also because of anti-oxidative effects of mammalian ghrelin, this study was aimed to investigate the effect on

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serum MDA level in newly-hatched chicken after *in ovo* administration of ghrelin on day 5 and 10 of incubation.

2. Materials and methods

2.1. In ovo injection procedure

In the present study, 350 fertilized eggs were obtained from broiler breeder flock (Ross 308). The eggs were divided into six experimental groups: control group T1 (without injection), group T2 (*in ovo* injected with 50 ng/egg ghrelin on day 5), group T3 (*in ovo* injected with 100 ng/egg ghrelin on day 5), group T4 (*in ovo* injected with 50 ng/egg ghrelin on day 10), group T5 (*in ovo* injected with 100 ng/egg ghrelin on day 10), group T6 (*in ovo* injected with solvent, without ghrelin on day 5) and group T7 (*in ovo* injected with solvent, without ghrelin on day 10). All the eggs were incubated with normal incubation condition (37.8 ℃ and 60% relative humidity). Exogenous ghrelin was purchased from Sigma-Aldrich® (Rat Ghrelin-USA), dissolved in 1% acetic acid solvent and required concentrations of ghrelin were prepared. On day 5 of incubation, *in ovo* injection was conducted for T2, T3 and T6. Also on day 10, the same *in ovo* injection procedure was done for T4, T5 and T7. Before injection, egg shells were marked with waterproof marker for identification of air cell position and optimum in albumen injection site. The *in ovo* injection was done in hygiene room with 37 ℃ for avoiding any temperature stress for chick embryo. At this experiment, 22G needles were used for in albumen injection for all the injected eggs. After hatching, the blood samples were collected from chicks, immediately following chick decapitation. Blood samples were centrifuged and serum was obtained for MDA assay with Alcyon 300 auto analyzer (Abbott Park, IL., USA) and its commercial kits were also used for MDA assay.

2.2. Statistical analysis

Data of 105 samples from 15 newly-hatched chickens were analyzed with SAS software (Ver. 9.1) and the differences between groups were detected via Duncan multiple test.

3. Results

Serum MDA concentration of hatched chicks following *in ovo* ghrelin administration was presented in Table 1.

In ovo administration of ghrelin in all the groups could lower serum MDA levels in newly-hatched chicks (Table 1). Group T2 and T3 had significantly lower serum MDA concentrations (4.10 and 4.60 nmol/mL, respectively) in comparison with other groups. In T4 and T5, MDA levels were lower than T1, T6 and T7 (non-ghrelin administrated groups) (9.53 and 9.50 in comparison with 10.73, 10.03 and 10.13 nmol/mL) and were higher than T2 and T3.

Table 1

Serum MDA concentrations in newly-hatched chicks after *in ovo* injection of ghrelin.

Experimental groups	Injected dosage (ng/egg)	Injection day (incubation day)	Serum MDA concentration (nmol/mL)
T1	–	–	10.73[a]
T2	50	5	4.10[c]
T3	100	5	4.60[c]
T4	50	10	9.53[b]
T5	100	10	9.50[b]
T6	Solvent (1% acetic acid)	5	10.03[a]
T7	Solvent (1% acetic acid)	10	10.13[a]

Different letters (a, b, c) shows significant difference at $P<0.05$; SEM=0.288 9; CV=5.97.

4. Discussion

Hatching time is considered to be a period of high oxidative stress due to long-chain poly unsaturated fatty acids accretion in tissues[20,21]. Due to onset of pulmonary respiration, and sudden increase in rate of oxidative metabolism[10], the hatchlings are expected to react with a compensatory induction of endogenous antioxidants. In this regard, maternal antioxidants (such as carotenoids) are obtained in the fertilized egg[9] for possible anti-oxidative protection.

In previous studies, *in ovo* administration of antioxidants including vitamin E[21], sodium selenite and vitamin C[22] had anti-oxidative effects and markedly abated oxidative damages in chick embryos.

In the present study, *in ovo* administration of ghrelin could lower serum MDA levels especially on groups injected on day 5 of incubation. Schaal[21] reported enhancement of brain tissue lipids and the antioxidant status of hatched chicks following *in ovo* administration of vitamin E. Also, Wang *et al*[22] had suggested that antioxidant defense impairment in chick embryo induced by *in ovo* exposure to *Fusarium* mycotoxin butenolide, can be eliminated by *in ovo* administration of antioxidants such as sodium selenite and vitamin C.

Results of the present study were in agreement with the findings of Işeri and Brzozowski *et al*[17,18] who reported lowering effect of ghrelin on serum MDA in mammalian

species. Also, obtained results are in accordance with *in ovo* studies of Schaal[21] and Wang *et al*[22] who have investigated other anti-oxidant compounds.

In ovo administration of ghrelin on day 5 of incubation was more efficient than day 10 administration for MDA decreasing effect (4.10 and 4.60 nmol/mL in comparison with 9.53 and 9.50 nmol/mL, respectively). It seems that *in ovo* ghrelin supplementation especially at early embryonic life (such as day 5 of incubation) could show better anti-oxidative protection during embryonic life of chicken and greater decrease in serum MDA level in newly-hatched chicks.

It is concluded that *in ovo* administration of ghrelin (as an antioxidant peptide) on day 5 and day 10 of incubation at the dosages of 50 and 100 ng/egg (T2, T3, T4 and T5 groups) can have anti-oxidative protection and decrease serum MDA level. Further studies with measuring specific enzymes (superoxide dismutase, glutathione proxidase, *etc*) activity in blood or tissues of newly-hatched chicks following *in ovo* administration of ghrelin on different incubation days are suggested for further clearness of antioxidant properties of ghrelin in chicken.

Conflict of interest statement

We declare that we have no conflict of interest.

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