

Superoxide dismutase and glutathione peroxidase activities in chicken model following *in ovo* ghrelin administration

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Abstract

Ghrelin is a regulatory peptide with specific anti-oxidative effects. The aim of this study was to investigate the activity of two antioxidant enzymes includes superoxide dismutase (SOD) and glutathione peroxidase (GPx) in erythrocyte of newly-hatched chicken following *in ovo* administration of ghrelin. The fertilized eggs were divided into four experimental groups; control G1 (without injection), group G2 (*in ovo* injected with 50 ng/egg ghrelin), group G3 (*in ovo* injected with 100 ng/egg ghrelin), group G4 (*in ovo* injected with 150 ng/egg ghrelin). At d 10 of incubation, *in ovo* injections of ghrelin were conducted. SOD and GPx activity in erythrocytes were determined by laboratory analysis of whole blood taken from newly-hatched chicks. There were no significant changes in SOD activity following *in ovo* administration of different concentrations of exogenous ghrelin. But, in groups G3 and G4 (100 ng and 150 ng ghrelin/ egg), significant elevation in activity of GPx has been observed ($P < 0.05$). It was concluded that *in ovo* administrated ghrelin can increase antioxidant defense by increase in GPx activity in newly-hatched chickens.

Key words: ghrelin, antioxidant enzymes, oxidative damage, *in ovo* injection.

1. Introduction

Ghrelin is a multifunctional regulatory peptide with anti-oxidative effect [1-4]. Following identification of ghrelin in mammals by Kojima et al. [5], so many relative studies were published on ghrelin properties and its physiological importance. Studies conducted during last decade suggests various specifies and functions for ghrelin, such as GH-releasing [6], hematopoiesis [7, 8], food intake and energy balance and regulation [9], endocrine/paracrine roles in pancreas [10-12], and the anti-oxidative effects [1-4]. The chicken ghrelin has 26 amino acids with 54% homology to rat ghrelin [13]. The maternal ghrelin has been identified in albumen and

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yolk of fertilized chicken egg [14]. So, chicken ghrelin may has numerous functional effects in embryo development, similar with mammalian ghrelin. In this regard, our *in ovo* studies show considerable regulatory effects of ghrelin on some of endocrine hormones (includes prolactin and insulin) [10, 15], hematopoiesis [8], and intestinal development [16] in chicken.

The chicken embryo has challenges with oxidative stress, and during the final 2 weeks of embryonic development, notable changes in expression of superoxide dismutase (SOD) and glutathione peroxidase (GP_x) were occurred [17]. Investigations of Kheradmand et al. [18, 19] had mentioned specific anti-oxidant effect for rat ghrelin. But this effect was not studied in chicken or for chicken ghrelin.

So, the aim of this study was to investigation on activity of two antioxidant enzymes (SOD and GP_x) in erythrocyte of newly-hatched chicken follownig *in ovo* administration of ghrelin.

2. Materials and Methods

In present study, 200 fertilized eggs were obtained from broiler breeder flock (Ross 308). The eggs were divided into four experimental groups; control G1 (without injection), group G2 (*in ovo* injected with 50 ng/egg ghrelin), group G3 (*in ovo* injected with 100 ng/egg ghrelin), group G4 (*in ovo* injected with 150 ng/egg ghrelin). All of eggs were incubated with normal incubation condition (37.8 °C and 60%: RH). Exogenous ghrelin (Rat Ghrelin – Sigma-Aldrich, USA), dissolved in 1% acetic acid solvent (according to company brochure) and required concentrations of ghrelin were prepared. At d 10 of incubation, *in ovo* injection was conducted for G2, G3 and G4. Before injection, egg shells were marked with waterproof marker for identification of air cell position and optimum site for *in albumen* injection with 22G needles. The *in ovo* injection was done in hygiene room with 37 °C for avoiding any thermal stress for embryo. After hatching, the blood of newly-hatched chickens was collected in EDTA coated

tubes. The activities of SOD (EC: 1.15.1.1) and GP_x (EC: 1.11.1.9) were measured by Alyson auto-analyzer and commercial kits. Enzymatic activities were expressed as U/l. Obtained data were analyzed with SAS software (Ver. 9.1) and the differences between means were detected via Duncan multiple range test ($P < 0.05$).

3. Results and Discussion

According to table1, there were no any significant changes for SOD activity following *in ovo* administration of different concentrations of exogenous ghrelin. But there was drastic elevation in GP_x activity following ghrelin administration, so in groups G3 and G4 (100 ng and 150 ng ghrelin/ egg), significant elevation in activity of GP_x has been occurred ($P < 0.05$), whereas difference between G2 (50 ng ghrelin/ egg) and G1 (control or intact group) for GP_x was not significant.

Table. 1. Activity of superoxide dismutase (SOD) and glutathione peroxidase (GP_x) in erythrocyte of newly-hatched chicken following *in ovo* ghrelin administration

Experimental groups	Injected dosage (ng/egg)	SOD (U/L)	GP _x (U/L)
G1 (intact)	-	0.93	169.03 ^b
G2	50	1.03	175.70 ^{ab}
G3	100	0.80	181.07 ^a
G4	150	0.73	177.33 ^a
P value	-	0.4471	0.0399
SEM ¹	-	0.3203	2.3756

- Different letters (a or b) shows significant difference.

¹ SEM, based on pooled estimate of variance.

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]. The pulmonary respiration and subsequent increase in rate of oxidative metabolism and the hatchlings process are expected to react with a compensatory induction of endogenous antioxidants [22]. For the purpose of elimination of oxidative damages for embryo and at hatching process, *in ovo* administration of antioxidant compounds such as sodium selenite and vitamin C [23] had markedly abated oxidative damages in chick embryo or newly-hatched chicks.

It was suggested that ghrelin has free radical scavenging capacity [4]. In a previous study [18], it was reported that GPx activity in rat testis was increased and SOD activity was unchanged by administration of 1 nmol kg⁻¹ ghrelin for 10 d. They had suggested that greater concentrations of ghrelin may be able to activate SOD enzyme. In their other experiment [19] conducted on rat ovary, it reported that SOD is major antioxidant enzyme affected by administrated ghrelin, and GPx is not affected significantly. Cetin et al. [4] had stated that ghrelin pretreatment enhances antioxidant defense via activation of antioxidant enzymes, against oxidative injury. In present study (table1), according to Kheradmand et al. [18] findings, *in ovo* administration of ghrelin caused increased rate of GPx in comparison with control (G1). Also present finding is in agreement with Barazzoni et al. [24] that reported on elevation in GPx activity of rat muscle due to injection of Acyl-ghrelin. In present study *in ovo* administration of ghrelin (100 ng or 150 ng/egg) cause increased GPx activity, but there was not significant change for SOD (table 1). It seems that ghrelin has antioxidant effect due to activation of both or one of major antioxidant enzymes (SOD and GPx). In chicken, *in ovo* or maternal ghrelin may has antioxidant role, and make antioxidant defense in post-hatch and neonatal life.

4. Conclusion

It was concluded that *in ovo* administrated ghrelin (100 ng or 150 ng/ egg) can increase antioxidant defense by increase in glutathione peroxidase activity in newly-hatched chickens, although the superoxide dismutase is not increased.

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