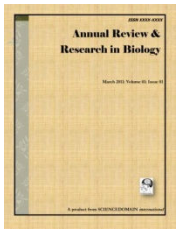

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Plasma Lipoprotein Profile in Newly Hatched Chicks Following *in Ovo* Ghrelin Administration

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Research Article

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ABSTRACT

Objective: The aim of this study was to investigation on effects of *in ovo* administrated exogenous ghrelin on plasma lipid profile in hatched broiler chicks.
Methodology: 250 eggs were obtained from commercial broiler breeder (Ross 308 strain) farm. the eggs were divided into five experimental groups; T1 or eggs without any injection (control), T2 or eggs *in ovo* injected with 50ng ghrelin at embryonic day-5, T3 or eggs *in ovo* injected with 100ng ghrelin at embryonic day-5, T4 or eggs *in ovo* injected with 50ng ghrelin at embryonic day-10, and T5 or eggs *in ovo* injected with 100ng ghrelin at embryonic day-10. Similar *in ovo* experiments were done for all of injected groups on day-5 or -10. At end of incubation, blood samples from each group were collected following chick decapitation and analyzed for determination of plasma lipoprotein concentrations.
Results: Exogenous ghrelin administration at different embryonic days couldn't have any considerable effect on low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C) or high density lipoprotein (HDL)/LDL-C. Plasma HDL-C concentration had increase follow *in ovo* injection of 100ng ghrelin at day-5 ($P<0.01$) and had slight increase at day-10 (group T3 and T5).
Conclusion: As conclusion, *in ovo* administration of 100ng ghrelin at embryonic day-5 could elevate plasma HDL-C concentrations of newly-hatched chicks without any significant effect on LDL-C, VLDL or HDL-LDL ratio.

Keywords: Ghrelin; in ovo administration; lipid; plasma lipoproteins;

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1. INTRODUCTION

Ghrelin is a multifunctional peptide produced predominantly by the X/A-like cells located in stomach that was discovered by Kojima et al., (1999). Ghrelin has been widely studied in mammalian species, and multiple physiological functions including glucose and lipid metabolism, reproduction, gastrointestinal function, cardiovascular function, cellular proliferation, immunomodulation and bone physiology in addition to GH release and food intake, have been reported (Kojima and Kangawa, 2005; Hosoda et al., 2006; Soares and Leite-Moreira, 2008). Almost all avian ghrelin peptides identified so far are 26-amino acids long, as compared to the 28-amino acid ghrelin peptides found in mammals with 54% similarity to rat ghrelin (Kaiya et al., 2007, 2009) (Figure1). Embryonic and reproductive roles of ghrelin have been cleared in mammalians (Ng et al., 2005; Imam et al, 2009). Ghrelin has been identified in albumen and yolk of fertilized chicken egg (Yoshimura et al., 2009) that this detection had create frontiers for investigation on maternal ghrelin via *in ovo* administration; related findings shows prolactin-releasing (Lotfi et al., 2011) effect of in albumen injected ghrelin.

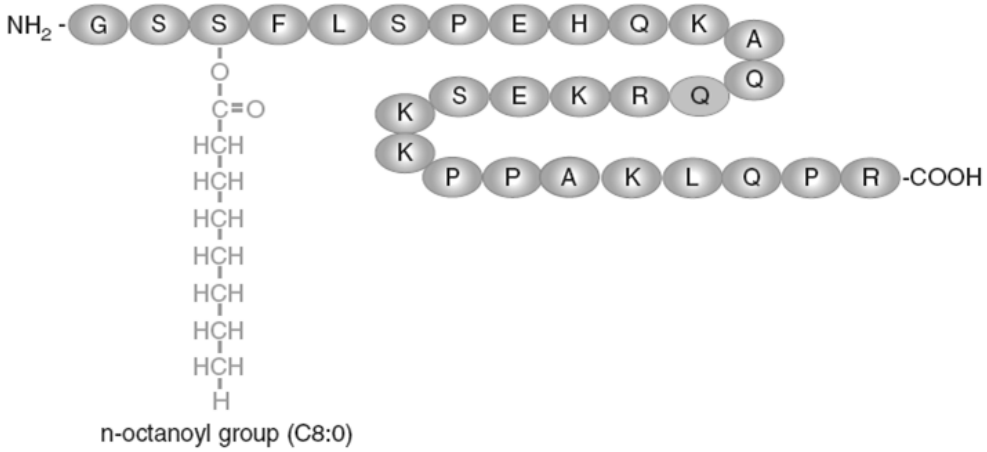

Fig. 1. Peptide structure of rat ghrelin; shows sequence of amino acids and acylation with a medium-chain fatty acid

Ghrelin in mammals is a lipogenic hormone and could cause weight gain with fat deposition without skeletal grow or muscle mass increases (Muccioli et al., 2002). Berilgen et al. (2005) had reported that patients with multiple sclerosis have higher levels of serum ghrelin, as a positive correlation between ghrelin and plasma lipid. Studies on amphibians showed that exogenous ghrelin could decrease plasma total cholesterol and low density lipoprotein (LDL-C) concentrations and increase the high density lipoprotein (HDL)-LDL-C ratio (Mustonen and Nieminen, 2006). Another evidence for ghrelin role in lipid metabolism is that ghrelin binds to plasma lipoproteins (Holmes et al., 2009). In chicken, Buyse et al. (2009) had showed chicken ghrelin had antolipogenic effect in neonatal broiler chicks that was suggested as functional difference of ghrelin in avian and mammals by Kaiya et al., (2009). Because of mentioned backgrounds, aim of this study was to investigate the effects of *in ovo* administrated exogenous ghrelin on plasma lipid profile in hatched broiler chicks.

2. MATERIALS AND METHODS

Present study was conducted at hatchery farm – Sufian city during spring-summer 2010. For conduction of this investigation, 250 eggs were obtained from commercial broiler breeder (Ross 308 strain) farm. the eggs were divided into five experimental groups; T1 or eggs without any injection (control), T2 or eggs *in ovo* injected with 50ng ghrelin at embryonic day-5, T3 or eggs *in ovo* injected with 100ng ghrelin at embryonic day-5, T4 or eggs in ovo injected with 50ng ghrelin at embryonic day-10, and T5 or eggs in ovo injected with 100ng ghrelin at embryonic day-10 (Table1).

2.1 *In ovo* Injection Procedure

Lyophilized exogenous Rat ghrelin (Sigma-Aldrich®, USA) were solved in to 1% acetic acid solution (according to Sigma brochure) and proposed concentrations of ghrelin were prepared, qua each in ovo injected 0.5 ml solution were include 50 ng (T2, T4) or 100ng (T3, T5) ghrelin. Next, solutions were transferred to incubation and warmed to 37℃ for avoiding to any thermal stress for embryos. For *in ovo* injection, all of eggs were candled for identification of embryo, air cell, albumen position and optimum injection point that is remarked on egg shell surface. 22G needles were used for in albumen injection. After Injection, pores were covered with special plastic agglutinate according to Iranian Razi institute© recommendations. Similar *in ovo* experiments were done for all of injected groups on day-5 or -10 (table1).

At end of incubation, blood samples from each group were collected following chick decapitation. The whole blood samples were centrifuged and obtained serum was transferred to Islamic Azad University laboratory for analysis of plasma lipoprotein concentrations. Experimental procedure was done according to recommendations of Islamic Azad Universoty-Veterinary department Animal ethics committee.

Table 1. *In ovo* injected point, dosages and volumes for experimental groups

Experimental groups	Injected dosage of ghrelin (ng)	Injected volume of solvent (1% acetic acid)	Injection day (incubation day)	Injection point Injection site
T1	0	0	-	-
T2	50	0.5 ml	5	albumen picked end of egg
T3	100	0.5 ml	10	albumen picked end of egg
T4	50	0.5 ml	5	albumen wide end of egg
T5	100	0.5 ml	10	albumen wide end of egg

2.2 Statistical Analysis

Data obtained by 15 individual samples from 15 hatched-chicks for each group were analyzed with SAS software (Ver.9.1) and the differences between groups were evaluated with Duncan multiple range test, $P<0.05$.

3. RESULTS AND DISCUSSION

According to table 2, ghrelin injection at different embryonic days couldn't have any considerable effect on LDL-C, very low density lipoprotein (VLDL-C) or HDL/LDL. Plasma HDL-C concentration had increase follow *in ovo* injection of 100ng ghrelin at day-5 ($P<0.01$) and also had slight increase at day-10 (group T3 and T5).

Table 2. Plasma lipoprotein profile in Newly-Hatched chicks after *in ovo* injection of ghrelin

Experimental groups	Injected dosage of ghrelin (ng)	HDL-C	LDL-C	VLDL-C	HDL/LDL
T1	0	199.3^{b}	500.0	24.0	0.423
T2	50	146.0^{b}	338.7	11.7	0.490
T3	100	344.7^{a}	442.7	17.6	0.818
T4	50	157.0^{b}	424.0	14.3	0.375
T5	100	245.0^{ab}	585.3	16.3	0.500
P value		0.004	0.606	0.116	0.101
SEM		29.249	109.125	2.959	0.107

**Different letters (a, b) shows significant difference*

Jeusette et al. (2005) in an investigation on dogs, observed that Correlations exist between obesity and plasma lipoproteins and ghrelin) commonly associated with obesity and in their study, obese dogs had lower peripheral ghrelin and higher HDL-C concentrations when it compared with control or non-obese dogs parameters. Katsuki et al. (2004) had shown peripheral ghrelin and HDL-C is significantly lower in obesity condition.

Mustonen and Nieminen (2006) had shown ghrelin efficiency in decreasing LDL-C and increases HDL/LDL ratio. Present observation for increasing effect of *in ovo* injected ghrelin (100ng/egg at day-5 or -10) and non-significant increasing in HDL-LDL ratio is in agreement with Mustonen and Nieminen, 2006) findings in amphibian and is apposite to findings on mammalian models (Jeusette et al., 2005). Present findings with *in ovo* ghrelin administration supports Kaiya et al.'s (2009) suggestions about possible differences for ghrelin role in mammalian and birds. Also this is completive and supportive finding for Buyse et al., (2009) that reported anti-lipogenic (lower plasma triglyceride concentration) effects of injected ghrelin in neonatal broiler chicks, because in present study slightly lower VLDL-C concentrations was observed for all of *in ovo* ghrelin administrated groups (T2, T3, T4 or T5) when they are compared with control or non-injected group (T1). Possible reason of mentioned increase in HDL-C may be because of Perez-Tilve et al. (2010) findings that

stated, reducing HDL-C uptake by the liver independent of food intake or by ghrelin effect is an endocrine mechanism for increased circulating HDL-C.

4. CONCLUSION

As conclusion, *in ovo* administration of 100ng ghrelin at embryonic day-5 (and somewhat at day-10) could elevate plasma HDL-C concentrations of newly-hatched chicks, without any significant effect on LDL-C, VLDL or HDL-LDL ratio. It is suggested that ghrelin can affect avian lipid metabolism via increase HDL-cholesterol.

REFERENCES

Berilgen, M.S., Bulut, S., Ustundag, B., Tekatas, A., Ayar, A. (2005). Patients with multiple sclerosis have higher levels of serum ghrelin. Neuro Endocrinol. Lett., 26, 819-822.

Buyse, J., Janssen, S., Geelissen, S., Swennen, Q., Kaiya, H., Darras V.M., Dridi, S. (2009). Ghrelin modulates fatty acid synthase and related transcription factor mRNA levels in a tissue-specific manner in neonatal broiler chicks. Peptides, 30, 1342-1347.

Holmes, E., Davies, I., Lowe, G., Ranganath, L.R. (2009). Circulating ghrelin exists in both lipoprotein bound and free forms. Ann. Clin. Biochem., 46, 514-516.

Hosoda, H., Kojima, M., Kangawa, K. (2006). Biological, physiological and pharmacological aspects of ghrelin. J. Pharmacol. Sci., 100, 398–410.

Imam, S.S., Kandil. M.E., Shoman, M., Baker, S.I., Bahier R. (2009). Umbilical cord ghrelin in term and preterm newborns and its relation to metabolic hormones and anthropometric measurements. Pak. J. Biol. Sci., 12, 1548-1555.

Jeusette, I.C., Lhoest, E.T., Istasse, L.P., Diez, M.O. (2005). Influence of obesity on plasma lipid and lipoprotein concentrations in dogs. Am. J. Vet. Res., 66, 81-86.

Kaiya, H., Darras, V.M., Kangawa, K.J. (2007). Ghrelin in birds: Its structure, distribution and function. J. Poult. Sci., 44, 1-18.

Kaiya, H., Furuse, M., Miyazato, M., Kangawa, K. (2009). Current knowledge of the roles of ghrelin in regulating food intake and energy balance in birds. Gen. Comp. Endocrinol., 163, 33-38.

Katsuki, A., Urakawa, H., Gabazza, E.C., Murashima, S., Nakatani, K. (2004). Circulating levels of active ghrelin is associated with abdominal adiposity, hyperinsulinemia and insulin resistance in patients with type 2 diabetes mellitus. Eur. J. Endocrinol., 151, 573-577.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., Kangawa K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature, 402, 656–660.

Kojima, M., Kangawa, K. (2005). Ghrelin: structure and function. Physiol. Rev. 85: 495–522.

Lotfi, A., Shahryar, H.A., Ghaleh-Kandi, J.G., Kaiya, H., Ahmadzadeh, A. (2011). *In ovo* administration of ghrelin and subsequent prolactin level in newly hatched chicks. J. Poult. Sci., 48, 130-132.

Muccioli, G., Tschop, M., Papotti, M., Deghenhi, R., Heinman, M., Ghigo, E. (2002). Neuroendocrine and peripheral activities of ghrelin: Implications in metabolism and obesity. Eur. J. Pharmacol., 440, 235–254.

Mustonen, A.M., Nieminen, P. (2006). Human ghrelin modifies lipid metabolism of the common frog Rana temporaria. Folia Zool., 55, 145 –149.

Ng, P.C., Lee, C.H., Lam, C.W., Chan, I.H.S., Wong, E., Fok, T.F. (2005). Ghrelin in preterm and term newborns: Relation to anthropometry, leptin and insulin. Clin. Endocrinol., 63, 217-222.

Perez-Tilve, D., Hofmann, S.M., Basford, J., Nogueiras, R., Pfluger, P.T., Patterson, J.T., et al. (2010). Melanocortin signaling in the CNS directly regulates circulating cholesterol. Nat. Neurosci., 13, 877-882.

Soares, J.B., Leite-Moreira, A.F. (2008). Ghrelin, des-acyl ghrelin and obestatin: Three pieces of the same puzzle. Peptides, 29, 1255–1270.

Yoshimura, Y., Tsuyuki, C., Subedi, K., Kaiya, H., Sugino, T., Isobe N. (2009). Identification of ghrelin in fertilized eggs of chicken. J. Poult. Sci., 46, 257-259.