International Journal of Chemical and Biomedical Science
2015; 1(1): 6-12
Published online March 20, 2015 (http://www.aascit.org/journal/ijcbs)

AASCIT American Association for Science and Technology

Keywords
Japanese Quail,
Ghrelin,
Food Intake,
Energy Homeostasis

Received: February 3, 2015
Revised: March 3, 2015
Accepted: March 4, 2015

Effect of Peripheral Ghrelin on Energy Homeostasis in Japanese Quail

Shousha S.[1, *], Kirat D.[2], Naso T.[3]

[1]Department of Physiology, Faculty of Veterinary Medicine, Benha University, Moshtohor, Egypt
[2]Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt
[3]Department of Basic Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

Email address
Physiology2009@yahoo.com (Shousha S.)

Citation
Shousha S., Kirat D., Naso T.. Effect of Peripheral Ghrelin on Energy Homeostasis in Japanese Quail. *International Journal of Chemical and Biomedical Science.* Vol. 1, No. 1, 2015, pp. 6-12.

Abstract
Ghrelin is a peptide found in the mucosal layer of the rat stomach that exhibits growth hormone–releasing and appetite-stimulating activities. Ghrelin administration, either centrally or peripherally strongly stimulates feeding in human and rodents. Since the discovery of ghrelin in chicken in 2002, information on its structure, distribution, function, and receptors has been accumulated, mainly in poultry. In contrast to mammals and rodents, centrally injected ghrelin inhibits food intake in neonatal chickens, Japanese quail. No information is available about the mechanism and its relationship with energy homeostasis in Japanese quail. Since ghrelin is predominantly produced in the proventriculus in birds, we investigated the effect of peripherally (intraperitoneal) administered ghrelin (3 nmol/200 µl saline for each bird) on food intake and energy expenditure as measured in respiratory chambers by indirect calorimetry for 12 h in adult male Japanese quail. Plasma glucose, triglycerides, free fatty acids, total protein and T3 were measured in a separate experiment until 2 hour after peripheral administration. Food intake was measured until 12 h after ghrelin administration and showed decrease at all time points measured. The respiratory quotient in ghrelin-administered quail was reduced until 12 h after administration whereas plasma glucose and triglycerides concentrations were not changed. Free fatty acids and total protein levels also remained unchanged. Ghrelin did not influence heat production and this was supported by the absence of changes in plasma T3 levels when compared to the control values. In conclusion, peripheral ghrelin reduces food intake and therefore has a role in energy homeostasis in avian species.

1. Introduction

Ghrelin is a stomach hormone that acts as an endogenous ligand of orphan G-protein coupled receptor (GPCR). Ghrelin is a 28-amino acid peptide existing in two major forms: *n*-octanoyl-modified ghrelin, which possesses an *n*-octanoyl modification on serine-3, and des-acyl ghrelin. Fatty acid modification of ghrelin is essential for ghrelin-induced GH release from the pituitary and appetite stimulation (Kojima et al., 1999). In mammals, ghrelin stimulates the secretion of growth hormone (GH), food intake and body weight gain when administered peripherally or centrally (Arvat et al., 2000; Wren et al., 2000; Tschop et al., 2000; Wren et al., 2001). Food intake is stimulated by ghrelin through a stimulation of hypothalamic neuropeptide Y (NPY) and agouti-related peptide (AGRP) (Kamegai et al., 2000; 2001). However, this does not seem to be the case in birds.

In birds, ghrelin has been purified and identified in various tissues (such as the proventriculus, brain, intestine, lung and spleen) in six avian species (chickens, Japanese quail, duck, geese, Emu and turkey) (Kaiya et al., 2002; 2007; 2008; 2009; 2013a; 2013b; Mark et al., 2010). Ghrelin was determined to be present in various non-mammalian vertebrates, and its physiological effects were gradually revealed in chickens (Kaiya et al., 2002), Japanese quail and other avian species (Kaiya et al., 2007; 2008; 2009; 2011, 2013a; 2013b). This form is composed of 26 amino acids, has an octanoylated Ser3 and shows 54% total sequence identity and 100% N-terminal-region identity [Gly1-Pro7] with rat and human ghrelin (Kaiya et al., 2002). So chicken and Japanese quail ghrelin shows 85 % total sequence identity and 100% N-terminal-region identity [Gly1-Pro7] with rat and human ghrelin (Kaiya et al., 2002; 2008; 2011). Intracerebroventricular (icv) injection of ghrelin in neonatal chickens and Japanese quails strongly inhibits feeding under both *ad libitum* and food restriction conditions (Furuse et al., 2001; Saito et al., 2002; shousha et al., 2005). The mechanism by which this decrease in food intake is regulated is so far unknown. It also remains to be investigated whether ghrelin, which is predominantly secreted from the proventriculus, has similar effects when injected intraperitoneally. As there is also no information available on the possible effects of ghrelin on energy metabolism in birds as documented in mammalian species (Tschop et al., 2000), we investigated the effect of an intraperitoneal administration of chicken ghrelin on food intake and heat production and respiratory quotient in adult male Japanese quails. In this study, we used adult Japanese quails. This was mainly because the growth curve of young birds is steep, so food intake and consequently growth parameters vary widely from day to day, whereas adult birds have ceased growing, and therefore growth and food intake parameters are not subject to such variability. Also, we used adult male quails to avoid the effect of female reproductive hormones on food intake, energy homeostatic parameters and their interference with the injected peptides on food intake or other parameters.

2. Materials and Methods

2.1. Animals

Adult male Japanese quail (Coturnix coturnix japonica) with an average weight of 110- 120 g were used in all experiments. All birds were housed in respiratory chambers (3 birds/ respiratory chamber) with continuous lighting, and were given free access to food and water. To examine how ghrelin is anorexic in birds, chicken ghrelin (Peptide Institute, Osaka, Japan) or 0.9 % saline (vehicle control) was administered. It was administered intraperitoneally at a dose (3 nmol/200 µl saline) which was chosen to conform our former experiments (Shousha et al., 2005). Administration started at 07:00 h. Food intake, heat production and respiratory quotient determinations were performed in respiratory chambers. Six chambers were used for each experiment with three chambers for the control animals and the other three for ghrelin administered animals. Three birds were kept in each chamber (n = 9 for each group). An adaptation period of one day to the confinement in the respiratory chambers was respected before the experiment was initiated. A detailed description of the open-circuit, indirect calorimeter unit consisting of six respiratory chambers, a gas analyzing unit and data acquisition system is given elsewhere (Buyse et al., 1998). Briefly, the chambers are made of stainless steel with a floor area of 550 mm×300 mm and 500 mm height. The bottom floor consists of a wire mesh floor supported by a steel frame under which a tray can be placed for excreta collection. Each chamber is equipped with a feeder and a drinking nipple. Fresh air enters the chamber through a perforated stainless steel wall and chamber air leaves through the opposite perforated wall. Additionally, a fan improves the mixing of the air in the chamber. The carbon dioxide and oxygen concentrations in the inlet and outlet air of each respiratory chamber are measured with a double-beam infrared carbon dioxide analyzer (ADC D/8U/54/A) and a paramagnetic oxygen analyzer (ADC 02-823A), respectively. The zero and span of both analyzers are checked before starting the experiment with gas mixtures of precisely known (±0.001%) carbon dioxide and oxygen concentrations. The CO_2 production and the O_2 consumption by the quails were calculated from the differences between the gas concentrations of the outside fresh air and the chamber air. Heat production (HP) was calculated from these data according to the formula of Romijn and Lokhorst (Romijn and Lokhorst, 1961):

$$HP\ (kJ/h) = 16.18O_2\ (l/h) + 5.02CO_2\ (l/h)$$

2.2. Measurement of Food Intake

Before the feeding experiment, the birds were weighed and assigned to an experimental group (nine birds in each group) based on their body weight. The average body weight (110–120 g) in each group was kept as uniform as possible. *Ad libitum* fed birds were ip administered chicken ghrelin and then placed back into the respiratory chambers. Food intake was determined by the disappearance of food from the pre-weighted feeder. Any spillage was also collected and weighed.

2.3. Biochemical Analysis

Plasma free fatty acids, total protein, glucose and triglyceride concentrations were measured spectrophotometrically. Plasma T3 levels were analyzed in a single radioimmunoassay according to Van der Geyten et al. 2001. The intra assay coefficient of variation for the T3 assay is 2.2% and the cross reactivity with T4 is 0.1–0.5%. Birds were killed by decapitation and blood was collected from 6 birds at each time point: before (0 min) or 10, 30, 60 and 120 min after administration. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care.

2.4. Statistical Analysis

All results are expressed as means ± SEM. Data were analyzed by analysis of variance and the post hoc Fisher's test. A difference with p value less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of Chicken Ghrelin on Food Intake

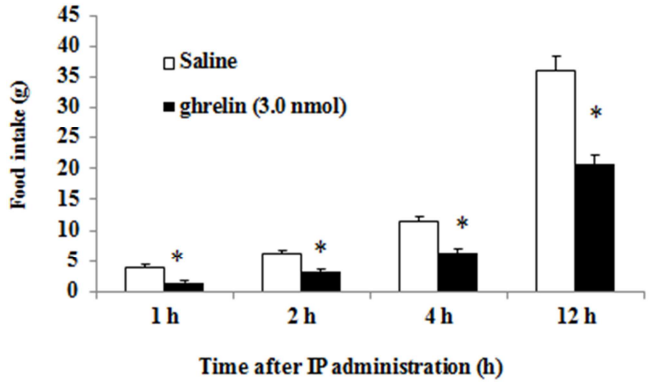


Fig. 1. *Effect of intraperitoneal (ip) administration of ghrelin on food intake in the ad libitum fed adult male Japanese quail. Saline (vehicle control) and ghrelin at a dose of (3.0 nmol/200 µl saline/bird) were injected ip at 07:00 h. Each bar and vertical line represents the mean ± SEM (n = 9). *Significantly different from the saline-treated group; P < 0.05.*

This is the first study to examine the effect of chicken ghrelin on food intake in Japanese quails. This study revealed that ip administration of chicken ghrelin to quails resulted in a significant (P<0.05) decrease in food intake in comparison with saline administered control group (Fig. 1). This reduction in food intake was apparent at 1 h (saline, 3.83 ± 0.62; ghrelin, 1.5 ± 0.18 g) after administration and continued for 12 h after ip administration of 3 nmol of chicken ghrelin (saline, 35.93±2.4; ghrelin, 20.73±1.4 g). This effect was not observed the following day (data not shown). Also, body weight gain decreased significantly (P<0.05 vs saline) after ip administration of chicken ghrelin (data not shown). The significant decrease in body weight gain was measured at 4 and 12 h after ip administration of chicken ghrelin (data not shown). Body weight gain decreased more than food intake did. By 12 h, a large decrease of body weight gain was observed in the chicken ghrelin-administered group relative to the saline-administered group (data not shown).

3.2. Effect of Ghrelin on Respiratory Quotient

Intraperitoneally administered ghrelin group showed a significant (P < 0.05) reduction in respiratory quotient in comparison with saline administered control group (Fig. 2). The values of respiratory quotient at 0 h were (saline, 1.2 ± 0.02; ghrelin, 1.14 ± 0.01). This significant reduction in respiratory quotient was consistent from the beginning of the experiment until 12 h (saline, 1.22 ± 0.02; ghrelin, 1.19 ± 0.01) after ghrelin administration. After 12 h, the respiratory quotient values were similar to control levels (data not shown).

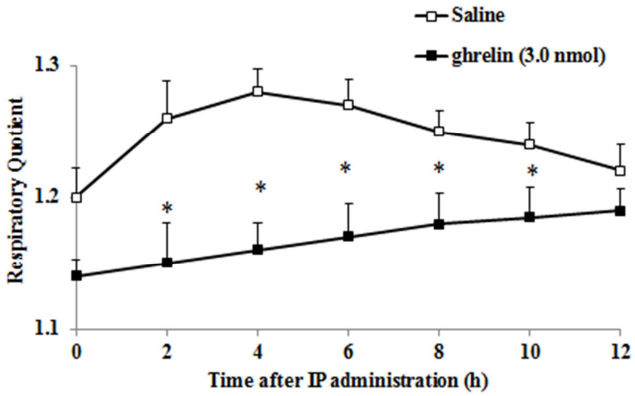
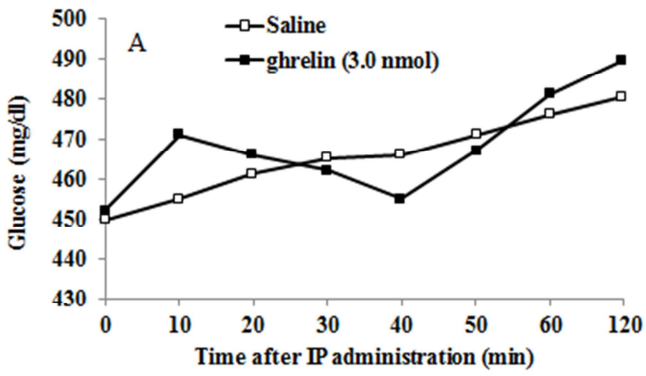
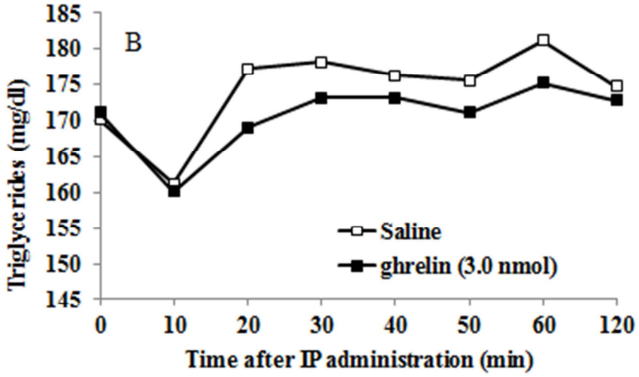


Fig. 2. *Effect of intraperitoneal (ip) administration of ghrelin on the respiratory quotient in the ad libitum fed adult male Japanese quail. Saline (vehicle control) and ghrelin at a dose of (3.0 nmol/200 µl saline/bird) were injected ip at 07:00 h. Each bar and vertical line represents the mean ± SEM (n = 9). *Significantly different from the saline-treated group; P < 0.05.*

3.3. Effect of Ghrelin on Plasma Glucose, Triglycerides, Free Fatty Acids and Total Protein

Intraperitoneal administration of ghrelin showed no significant changes in plasma glucose, triglycerides, free fatty acids and total protein concentrations in comparison with saline administered control group (Fig. 3).





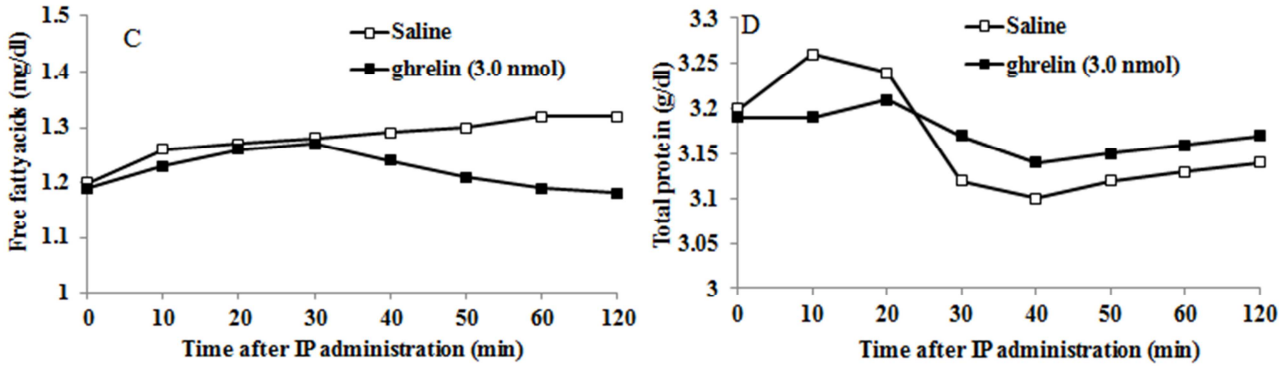


Fig. 3. Effect of intraperitoneal (ip) administration of ghrelin on the A) Plasma glucose, (B) triglyceride, (C) free fatty acids and (D) total protein in the ad libitum fed adult male Japanese quail. The dose of ghrelin was 3.0 nmol/200 µl saline/bird) or Saline (vehicle control). Each bar and vertical line represents the mean ± SEM (n = 9). No statistically significant differences were found between the two treatments.

3.4. Effect of Ghrelin on Heat Production

Heat production values at 0 h after ip administration were 880.6±24.9 and 900.3±22.5 kJ/ h for ghrelin and control group, respectively and remain non statistically significant at all time points measured (12 hours) as shown in (Fig 4).

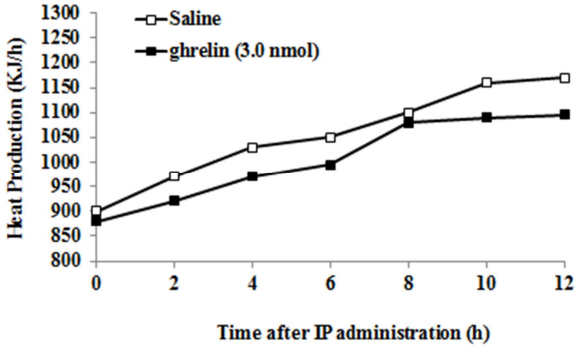


Fig. 4. Effect of intraperitoneal (ip) administration of ghrelin on the Heat production in the ad libitum fed adult male Japanese quail. Saline (vehicle control) and ghrelin at a dose of (3.0 nmol/200 µl saline/bird) were injected ip at 07:00 h. Each bar and vertical line represents the mean ± SEM (n = 9). No statistically significant differences were found between the two treatments.

3.5. Effect of Ghrelin on Triiodothyronine(T3)

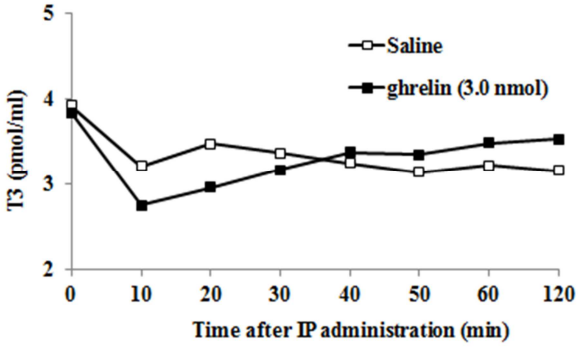


Fig. 5. Effect of intraperitoneal (ip) administration of ghrelin on the Plasma T3 concentrations in the ad libitum fed adult male Japanese quail. Saline (vehicle control) and ghrelin at a dose of (3.0 nmol/200 µl saline/bird) were injected ip. Each bar and vertical line represents the mean ± SEM (n = 9). No statistically significant differences were found between the two treatments.

Intraperitoneal administration of ghrelin did not change the values of plasma T3 concentrations in adult male Japanese quail in comparison with saline administered control group (Fig. 5).

4. Discussion

The central and peripheral regulatory mechanisms in energy homeostasis, including feed intake and energy expenditure, have been recently extensively studied in chickens in avian species (Richards 2003; Richards and McMurtry, 2010). Many of neural and endocrine mechanisms identified in mammals as involving in regulation feed intake also have this function in birds. For instance, neuropeptide Y (NPY) and agouti-related protein (AgRP) are mediators of many appetite-stimulatory peptides in central nervous system (CNS) in both groups (Zhou et al., 2005). However, there are some responses such as ghrelin that are unique to birds.

It was shown that avian ghrelin elevates GH level in dose and time dependent manner (Ahmed and Harvey, 2002; Baudet and Harvey, 2003). However, the main difference in ghrelin function between birds and mammals relates its role in the regulation of feed intake. There have been reports that centrally injected ghrelin strongly inhibit feed intake in neonatal chicks (Saito et al., 2005; Khan et al., 2006) and Japanese quails (Shousha et al., 2005). Saito et al. (2005) suggested that the inhibitory effect of central ghrelin on feeding is caused by activating the corticotropin releasing hormone (CRH) - producing neurons.

There are much interest about the role of the gastric peptide ghrelin in food intake and as a regulatory factor in energy homeostasis since its discovery by Kojima et al., 1999. Administration of this peptide has been reported to stimulate appetite, to reduce fat oxidation and to induce weight gain in mammalian models (Tschop et al., 2000; Wren et al., 2001). In mammals, ghrelin increases food intake through a stimulation of two hypothalamic hormones namely, NPY and AGRP (Kamegai et al., 2000; 2001). The effect of ghrelin on feeding is, however, clearly species-dependent as doses injected centrally inhibit food intake ranged from 0.05 to 1.5 nmol in neonatal chicks (Furuse et al., 2001; Saito et al., 2002) and from 0.5 to 1.0 nmol in Japanese quails (Shousha

et al., 2005). In avian species, ghrelin has been purified and identified in various tissues and predominantly from proventriculus in six avian species (chickens, Japanese quail, duck, geese, Emu and turkey) (Kaiya et al., 2002; 2007; 2008; 2009; 2013a; 2013b; Mark et al., 2010). Thus, the proventriculus is supposed to be the major source of circulating ghrelin levels in avian species (Wada et al., 2003). This is also true for mammals, as shown by peptide quantification in different regions in the gut and by a 70% reduction of plasma levels after gastrectomy in rodents and humans (Hosoda et al., 2003). Therefore, we studied the effect of a peripheral (intraperitoneal) ghrelin administration on food intake in Japanese quails.

From the earlier study that reported that peripheral (intravenous) ghrelin injection significantly decreased food intake until at least 1 h after administration in neonatal chickens (Geelissen et al., 2006) and up to 12 h in Japanese quail in this study after intraperitoneal administration. Therefore, our results and the earlier results (Geelissen et al., 2006; Shousha et al., 2005) support the view that ghrelin produced and secreted peripherally by the proventriculus can reach the brain and plays a direct inhibitory role in the control of food intake. The short duration of food restriction in neonatal chicks and long duration of food restriction (up to 12 h) in Japanese quails may suggest the variation in the time course of the inhibitory effect of ghrelin among bird species. The difference in the time course of the inhibitory effect of ghrelin on food intake was also reported even among different strains of the same species as seen in chicken strains (Kaiya et al., 2007; Geelissen et al., 2006; Buyse et al., 2009; Ocłon´ and Pietras, 2011; Kaiya et al., 2013a).

To clear the role of ghrelin in energy homeostasis, we investigated the effect of ghrelin on energy expenditure. The respiratory quotient was always higher than unity, which implicates the presence of de novo lipogenic activity (Ferrannini 1988). Although still exceeding unity, a single intraperitoneal administration of chicken ghrelin significantly decreased the respiratory quotient values for up to 12 h as shown in figure 2. The reduction in food intake was of the same duration (12 h) as the change in respiratory quotient (12 h). It is therefore suggested that ghrelin has also peripheral actions on nutrient metabolism of Japanese quails, the same as reported previously in chickens (Geelissen et al., 2006). Although yet speculative, it might be that ghrelin is also involved in the preference of use of substrates for oxidation and storage in the Japanese quail, as was clearly reported for rodents and chickens. Indeed, in rodent models (Tschop et al., 2000; 2002), the respiratory quotient was increased after subcutaneous ghrelin or GHS injection, which is in good agreement with the opposite effect of ghrelin on food intake in mammals compared to birds. Also, our study reported that IP administration of ghrelin showed no significant changes in plasma glucose, triglycerides, free fatty acids and total protein concentrations in Japanese quails. Geelissen et al., (2006) reported that IV administration of ghrelin decreases the respiratory quotient in broiler chicks. In addition to the results of Geelissen et al., (2006) in broiler chicks, our results revealed that IP administration of ghrelin decreased the respiratory quotient in Japanese quails, suggesting that birds use lipids or proteins rather than glucide as an energy source after ghrelin administration. This anti-lipogenic effect is opposite to the effect of ghrelin in mammals such as rats, which promotes the accumulation of fat, suggesting that the actions of peripheral ghrelin in birds are fundamentally different from those in mammals. This difference is natural, given that these animals have different life cycles and that birds have unique behaviors such as flying, migration, and brooding that are likely supported by an avian-specific physiology and metabolism.

Ghrelin induced a positive energy balance in rodents by decreasing fat utilization without changing heat production (Tschop et al., 2000). This lack of change in heat production after ghrelin treatment was also observed in Japanese quails in the present experiments. In addition, the circulating T3 levels were not altered. T3 has been reported to have a role in thermoregulatory mechanisms in birds by stimulating heat production (Decuypere et al., 1981; Silva 1995). In an earlier study (Geelissen et al., 2006), iv administration of chicken ghrelin resulted in unchange in heat production and T3 in neonatal chicks, that completely agreed with our study in Japanese quails. The enhancement of the avian uncoupling protein mRNA (Collin et al., 2003), together with the increases in T3 β-receptor mRNA expression, β -oxidation and cytochrome oxidase activities, mitochondrial respiration and ATP synthesis observed by Mouillet (Mouillet 2000) in muscle of ducklings treated with thyroid hormones, could contribute to the thermogenic action of thyroid hormones in birds. The unchanged heat production in chicken corresponds with the unchanged T3 values in plasma. In an earlier study, it is reported that peripherally injected ghrelin inhibited food intake and decreased mRNA expression of fatty acid synthase, PPAR-γ, and SEREBP-1 in the liver (Buyse et al. (2009) suggesting with our results that ghrelin has an anti-lipogenic action.

In conclusion, opposite to the situation in mammals, peripheral (as well as central) administration of ghrelin decreases food intake in Japanese quail. Intraperitoneal administration of ghrelin reduced respiratory quotient without affecting plasma glucose and triglyceride levels. Moreover, ghrelin has no effect on heat production in Japanese quails, the same as was observed already in mammals**.**

References

[1] Ahmed, S.; Harvey, S., 2002: Ghrelin: a hypothalamic GH – releasing factor in domestic fowl (Gallus domesticus). *Journal of Endocrinology* 172, 117–125.

[2] Arvat, E.; Di Vito, L.; Broglio, F.; Papotti, M.; Muccioli, G.; Dieguez, C.; Casanueva, F. F.; Deghenghi, R.; Camanni, F.; Ghigo, E., 2000: Preliminary evidence that ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. *Journal of Endocrinological Investigation* 23, 493–495.

[3] Baudet, M. L.; Harvey, S., 2003: Ghrelin – induced GH secretion in domestic fowl in vivo and n vitro. *Journal of Endocrinology* 179, 97–105.

[4] 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.

[5] Buyse, J.; Michels, H.; Vloeberghs, J.; Saevels, P.; Aerts, J. M.; Ducro, B.; Berckmans, D.; Decuypere, E., 1998: Energy and protein metabolism between 3 and 6 weeks of age of male broiler chickens selected for growth rate or improved food efficiency. *British Poultry Sciences* 39, 264–272.

[6] Collin, A.; Taouis, M.; Buyse, J.; Ifuta, N. B.; Darras, V. M.; Van As, P.; Malheiros, R. D.; Moraes, V. M.; Decuypere, E., 2003: Thyroid status, but not insulin status, affects expression of avian uncoupling protein mRNA in chicken. *American Journal of Physiology, Endocrinology and Metabolism* 284, E771–777.

[7] Decuypere, E.; Hermans, S. C.; Michels, H.; K¨uhn, E. R.; Verheyen, J., 1981: Thermoregulatory response and thyroid hormone concentrations after cold exposure in young chicks treated with iopanoic acid and saline. *Advances in Physiological Sciences* 33, 291–298.

[8] Ferrannini, E., 1988: The theoretical bases of indirect calorimetry: a review. *Metabolism* 37, 287–301.

[9] Furuse, M.; Tachibana, T.; Ohgushi, A.; Ando, R.; Yoshimatsu, T.; Denbow, D. M., 2001: Intracerebroventricular injection of ghrelin and growth hormone releasing factor inhibits food intake in neonatal chicks. *Neuroscience Letters* 301,123–126.

[10] Geelissen, S. M.; Swennen, Q.; Geyten, S. V.; Kühn, E. R.; Kaiya, H.; Kangawa, K.; Decuypere, E.; Buyse, J.; Darras, V. M., 2006: Peripheral ghrelin reduces food intake and respiratory quotient in chicken. *Domestic Animal Endocrinology* 30, 108-116.

[11] Hosoda, H.; Kojima, M.; Mizushima, T.; Shimizu, S.; Kangawa, K., 2003: Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-transcriptional processing. *Journal of Biological Chemistry* 278, 64–70.

[12] Kaiya, H.; Darras, V. M.; Kangawa, K., 2007: Ghrelin in birds: its structure, distribution and function. *Journal of Poultry Sciences* 44, 1–18.

[13] 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. *General and Comparative Endocrinology* 163, 33–38.

[14] Kaiya, H.; Kangawa, K.; Miyazato, M., 2013a: Update on ghrelin biology in birds. *General and Comparative Endocrinology* 190, 170–175.

[15] Kaiya, H.; Kangawa, K.; Miyazato, M., 2013b: Ghrelin receptors in non-mammalian vertebrates. *Frontiers in Endocrinology* 4, 1–16.

[16] Kaiya, H.; Miyazato, M.; Kangawa, K., 2011: Recent advances in the phylogenetic study of ghrelin. *Peptides* 32, 2155–2174.

[17] Kaiya, H.; Miyazato, M.; Kangawa, K.; Peter, R. E.; Unniappan, S., 2008: Ghrelin: a multifunctional hormone in non-mammalian vertebrates. *Comparative Biochemistry and Physiology, Part A*149, 109–128.

[18] Kaiya, H.; Van Der Geyten, S.; Kojima, M.; Hosoda, H.; Kitajima, Y.; Matsumoto, M.; Geelissen, S.; Darras, V. M.; Kangawa, K., 2002: Chicken ghrelin: purification, cDNA cloning, and biological activity. *Endocrinology* 143, 3454–3463.

[19] Kamegai, J.; Tamura, H.; Shimizu, T.; Ishii, S.; Sugihara, H.; Wakabayashi, I., 2001: Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and agouti-related protein mRNA levels and body weight in rats. *Diabetes* 50, 2438–2443.

[20] Kamegai, J.; Tamura, H.; Shimizu, T.; Ishii, S.; Sugihara, H.; Wakabayashi, I., 2000: Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic gene expression. *Endocrinology* 141, 4797–4800.

[21] Khan, M. S. I.; Dodo, K.; Yahata, K.; Nishimoto, S.; Ueda, H.; Taneike, T.; Kitazawa, T.; Hosaka, Y.; Bungo, T., 2006: Intracerebroventricular administration of growth hormone releasing peptide – 6 (GHRP - 6) inhibits food intake, but not food retention of crop and stomach in neonatal chicks. *Journal of Poultry Sciences* 43, 35–40.

[22] 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.

[23] Mark, P.R.; McMurtry, J. P., 2010: Review Article: The Avian Proghrelin System. *International Journal of Peptides* Volume 2010, Article ID 749401, 14 pages. doi:10.1155/2010/749401.

[24] Mouillet, L., 2000: Iodothyronine setm´etabolisme ´energ´etique chez le caneton en croissance au froid. PhD thesis. University Claude Bernard-Lyon I, France.

[25] Ocłon´, E.; Pietras, M., 2011: Peripheral ghrelin inhibits feed intake through hypothalamo-pituitary-adrenal axis-dependent mechanism in chicken. *Journal of Animal Feed Sciences* 20, 118–130.

[26] Richards, M. P., 2003: Genetic regulation of feed intake and energy balance in poultry. *Poultry Sciences* 82, 907–916.

[27] Richards, M. P.; McMurtry, J. P., 2010: The avian proghrelin system. *International Journal of Peptides* 10, 401–414.

[28] Romijn, C.; Lokhortst, W., 1961: Some aspects of energy metabolism in birds. In: Proceedings of the Second Symposium on Energy Metabolism. European Association for Animal Production. Lunteren 49–59.

[29] Saito, E. S.; Kaiya, H.; Tachibana, T.; Tomonaga, S.; Denbow, D. M.; Kangawa, K.; Furuse, M., 2005: Inhibitory effect of ghrelin on food intake is mediated by the corticotropin - releasing factor system in neonatal chicks. *Regulatory Peptides* 125, 201–208.

[30] Saito, E.; Kaiya, H.; Takagi, T.; Yamasaki, I.; Denbow, D. M.; Kangawa, K.; Furuse, M., 2002: Chicken ghrelin and growth hormone-releasing peptide-2 inhibit food intake of neonatal chicks. *European Journal of Pharmacology* 453, 75–79.

[31] Shousha, S.; Nakahara, K.; Kojima, M.; Miyazato, M.; Hosoda, H.; Kangawa, K.; Murakami, N., 2005: Different effects of peripheral and central ghrelin on regulation of food intake in the Japanese quail. *General and Comparative Endocrinology* 141, 178–183.

[32] Silva, J. E., 1995: Thyroid hormone control of thermogenesis and energy balance. *Thyroid* 15, 481–492.

[33] Tschop, M.; Smiley, D. L., 2000: Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 407, 908–913.

[34] Tschop, M.; Statnick, M. A.; Suter, T. M.; Heiman, M. L., 2002: GH-releasing peptide-2 increases fat mass in mice lacking NPY: indication for a crucial mediating role of hypothalamic agouti-related protein. *Endocrinology* 143, 558–568.

[35] Van der Geyten, S.; Segers, I.; Gereben, B.; Bartha, T.; Rudas, P.; Larsen, P. R.; Kühn, E. R.;Darras, V. M., 2001: Transcriptional regulation of iodothyronine deiodinases during embryonic development. *Molecular and Cellular Endocrinology* 183, 1–9.

[36] Wada, R.; Sakata, I.; Kaiya, H.; Nakamura, K.; Hayashi, Y.; Kangawa, K.; Sakai, T., 2003: Existence of ghrelin-immunopositive and -expressing cells in the proventriculus of the hatching and adult chicken. *Regulatory Peptides* 28, 123–128.

[37] Wren, A. M.; Seal, L. J.; Cohen, M. A.; Brynes, A. E.; Frost, G. S.; Murphy, K. G.; Dhillo, W. S.; Ghatei, M. A.; Bloom, S. R., 2001: Ghrelin enhances appetite and increases food intake in humans. *Journal of Clinical Endocrinology and Metabolism* 86, 5992.

[38] Wren, A. M.; Small, C. J.; Abbott, C. R.; Dhillo, W. S.; Seal, L. J.; Cohen, M. A.; Batterham, R. L.; Taheri, S.; Stanley, S. A.; Ghatei, M. A.; Bloom, S. R., 2001: Ghrelin causes hyperphagia and obesity in rats. *Diabetes* 50, 2540–2547.

[39] Wren, A. M.; Small, C. J.; Ward, H. L.; Murphy, K. G.; Dakin, C. L.; Taheri, S.; Kennedy, A. R.; Roberts, G. H.; Morgan, D. G.; Ghatei, M. A.; Bloom, S. R., 2000: The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 141, 4325–4328.

[40] Zhou, W.; Murakami, M.; Hasegawa, S.; Yoshizawa, F.; Sugahara, K., 2005: Neuropeptide Y content in the hypothalamic paraventricular nucleus responds to fasting and refeeding in broiler chickens. *Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology* 141, 146–152.