◀Review▶

Ghrelin in Birds: Its Structure, Distribution and Function

Hiroyuki Kaiya, Veerle M. Darras¹ and Kenji Kangawa

Department of Biochemistry, National Cardiovascular Center Research Institute,
5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan

¹ Laboratory of Comparative Endocrinology, Zoological Institute,
K.U. Leuven, Naamsestraat 61, B-3000 Leuven, Belgium

Feeding and somatic growth are closely related to each other, and are strictly governed by several endocrine and neuroendocrine systems in animals. Endocrine control of growth is an important subject in poultry industry. Ghrelin is a recently identified, growth hormone (GH)-releasing and feeding-promoting peptide in mammals, and the major source of its release is the stomach. From the comparative endocrinological aspects, ghrelin was considered to be present in avian species. In fact, ghrelin peptide and its cDNA encoding ghrelin precursor have been identified from the chicken proventriculus in 2002, and the presence of the ghrelin molecule has by now been shown in various avian species. In this review, we summarize the recent knowledge of ghrelin structure, distribution and function in birds.

Key words : birds, function, gene expression, ghrelin, structure

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Introduction

Sufficient intake of nutrients is important for effective poultry production. Although the regulation of food intake has been extensively investigated for a long time, a number of novel neuropeptides have been identified thanks to the recent progress of molecular biology techniques and peptide chemistry, and additional networks governing feeding regulation have been clarified, especially in mammals. This review focuses exclusively on a recently in mammals identified growth hormone (GH)-releasing and feeding-promoting hormone, ghrelin, and will describe recent knowledge of its structure, distribution and function from studies of poultry and avian species with comparison to mammalian studies.

What is Ghrelin?

Ghrelin is a 28-amino acid peptide, isolated from

the rat and human stomach in 1999 (Fig. 1, Kojima *et al.*, 1999). The peptide has been identified as an endogenous ligand for an orphan G-protein coupled receptor (GPCR), namely the growth hormone secretagogue receptor (GHS-R). Growth hormone secretagogue (GHS) is the generic name for synthetic compounds that have GH-releasing activity, acting through GHS-R (Bowers, 1998). The history of GHS started earlier than that of growth hormone-releasing hormone (GHRH) isolated in 1982. Already in 1980 Bowers and co-workers reported on some opioid peptide derivatives that exhibit weak GH-releasing activity instead of opioid activity (Bowers *et al.*, 1980). This finding led to the synthesis of a number of peptidyl and non-peptidyl GHS to improve their potency to date (van der Lely *et al.*, 2004). The GH-releasing activity of GHS was known to be induced through a different pathway from that of GHRH, and the putative receptor,

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Correspondence to : Dr. H. Kaiya, Department of Biochemistry, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan

Tel : +81-6-6833-5012 x 2458 Fax : +81-6-6835-5402 E-mail : kaiya@ri.ncvc.go.jp

Fig. 1. **Structure of ghrelin and des-acyl ghrelin.** Ghrelin is a peptide consisting of 28 amino acids in mammals. This figure represents the structure of ghrelin using the amino acid sequence of chicken ghrelin consisting of 26 amino acids. The most unique feature of the ghrelin structure is the acyl-modification of a hydroxyl group of the serine residue (Ser or S) at position 3 of the N-terminal of ghrelin (Ser-3). The Ser-3 is commonly modified by *n*-octanoic acid in vertebrates, but ghrelin modified by *n*-decanoic acid is also found in chickens. The acyl-modification of des-acyl ghrelin, a form lacking acyl-modification of ghrelin, is considered to be occurred under the influence of a still unknown acyl-transferase. Therefore, ghrelin and des-acyl ghrelin are co-localized in the ghrelin-producing cells. Ghrelin released from ghrelin-producing cells in the stomach (proventriculus in chickens) circulates through the body, and exhibits multiple biological actions through GHS-R1a. However, the majority (80–90%) of circulating ghrelin is des-acyl ghrelin. A fatty acid in Ser-3 is an ester bond, an esterase, i. e., paraoxnase, in plasma may be involved in desacylation of ghrelin. At present, nothing is known about the acylation mechanism of ghrelin in plasma.

GHS-R, was indeed discovered in human and pig (Howard *et al.*, 1996). GHS increases intracellular calcium ions in cells expressing this receptor, which is distinct from GHRH that induces cAMP accumulation. Now it is known that two receptor variants, one active receptor, namely GHS-R1a, and an inactive alternative splice variant, GHS-R1b, are present (Howard *et al.*, 1996). The identified GHS-R are indeed expressed in the pituitary, hypothalamus and various peripheral tissues (Howard *et al.*, 1996 ; Guan *et al.*, 1997), and GHS can bind to them and contribute to the release of GH from the pituitary through the receptor. However, GHS are not the natural ligand but artificial compounds for the re-

ceptor. Then, Kojima and colleagues succeeded in isolating the natural endogenous ligand for the GHS-R1a, ghrelin, from stomach extracts using reverse pharmacology by the use of cells expressing rat GHS-R1a to evaluate bioactivity of the unidentified ligand during the purification process (Kojima *et al.*, 1999 ; Kojima and Kangawa, 2005). Their key of success to isolate ghrelin was to explore not only hypothalamus, where the receptor GHS-R is highly expressed, but also other peripheral tissues including the stomach to look for the natural ligand.

The name of “ghrelin” is derived from “ghre” the Proto-Indo-European root for “to grow”, and the name can also indicate the abbreviation for growth

hormone (GH), followed by “relin” a suffix meaning releasing substances. Recently, the international Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification has accepted “GHS-R1a” as the name for the ghrelin receptor and “GRLN” as the abbreviation for ghrelin (Davenport *et al.*, 2005). Concerning biological, physiological, pathophysiological, and pharmacological aspects of ghrelin in mammals, a number of excellent reviews have been published (van der Lely *et al.*, 2004 ; Anderson *et al.*, 2005 ; Broglio *et al.*, 2005 ; Kojima and Kangawa, 2005 ; Smith *et al.*, 2005 ; Tena-Sempere, 2005).

Peptide Structure

Ghrelin is a peptide containing 28 amino acids. The most unique feature of this peptide is that the third amino acid, serine (Ser³), from the N-terminus of the mature peptide is modified with a fatty acid, *n*-octanoic acid (Fig. 1). The acylation is known to be essential for ghrelin binding to the GHS-R1a and subsequent ghrelin activity (Kojima *et al.*, 1999 ; Muccioli *et al.*, 2001). This unique acylated peptide has been identified in several mammalian and non-mammalian vertebrates (Kaiya *et al.*, 2004 ; 2005 ; Kojima and Kangawa, 2005). It is interesting to note that the amino acid sequence of ghrelin is quite similar to that of a gut hormone, motilin. Based on the similarities of characteristics of both peptides and receptors, ghrelin and motilin would be categorized into the same family (Peeters *et al.*, 2005).

In birds, indirect evidence supported the presence of ghrelin or a ghrelin-like molecule in chicken. Indeed, GHS-R homologues had been partially cloned and appeared in database before ghrelin and its receptor have been identified. Peptidyl and non-peptidyl GHS were shown to stimulate GH secretion *in vivo* and *in vitro* (Bowers *et al.*, 1984 ; Geris *et al.*, 1998 ; 2001), suggesting the presence of putative GHS-R and endogenous ligand for the receptor in chickens. Thereafter, Ahmed and Harvey (2002) reported ghrelin-immunoreactive cells in several areas of the chicken hypothalamus, suggesting the possible presence of a ghrelin-like protein in the chicken hypothalamus. They also demonstrated that human ghrelin, not a synthetic GHS, increases plasma GH levels in young chickens (Ahmed and Harvey, 2002). This finding strongly supported the potential presence of a ghrelin-GHS-R system in chickens.

In the same year of Ahmed and Harvey's report, Kaiya *et al.* (2002) indeed demonstrated the existence of the ghrelin molecule by isolating the peptide from chicken proventriculus. Chicken ghrelin consists of 26 amino acids, which differs from mammalian ghrelin that consists of 28 amino acids (Fig. 1). The Ser³ residue in chicken ghrelin is acylated with *n*-octanoic acid. In addition, acylation with *n*-decanoic acid was also found. The mechanisms involved in the modification of ghrelin by fatty acids have not been fully elucidated. However, it has been reported that ingested and absorbed fatty acids participate in acylation of ghrelin (Nishi *et al.*, 2005 ; Yamato *et al.*, 2005). Nishi *et al.* (2005) demonstrated in mice that either medium-chain fatty acids (MCFAs) or medium-chain triacylglycerols (MCTs) increase the stomach concentration of acylated ghrelin without changing the total amount of ghrelin, being the sum of ghrelin and des-acyl ghrelin lacking acyl-modification of Ser³. The increased modification is found from 3 h after the ingestion of MCTs and the high modification level continues during feeding. Interestingly, the expression levels of stomach ghrelin mRNA do not alter by the ingestion of MCTs. These findings indicate that only modification of ghrelin is stimulated by the treatment of MCTs. In addition, *n*-heptanoyl ghrelin, which is not present as a natural form of ghrelin, could be detected in the stomach of mice after ingestion of either *n*-heptanoic acid or glycerol triheptanoate, indicating that the ingested medium-chain fatty acids are directly utilized for the acylation of ghrelin. Furthermore, ghrelin peptides modified with a *n*-butyryl or *n*-palmitoyl group could not be detected when either short- or long-chain triacylglycerides (SCTs or LCTs) were administered. This suggests that a putative enzyme specifically modifying ghrelin, i.e., ghrelin-Serine *O*-acyltransferase, may prefer MCTs that are composed of *n*-heptanoic to *n*-decanoic acids rather than SCTs and LCTs. Yamato *et al.* (2005) demonstrated similar mechanisms of ghrelin acylation in chickens. They focused on the difference in the density of ghrelin-immunopositive (ghrelin-ip) and ghrelin mRNA-expressing (ghrelin-ex) cells in chicken proventriculus during embryonic development : the density of ghrelin-ex cells was higher than that of ghrelin-ip cells in the proventriculus of embryonic stages (E18 and E20) and neonatal stages from 0-day to 3-day post-hatching (C0 and C3). They

		***** * * * *		
Chicken (Broiler)	1	MFLRVILLGILLLSILGTETALAGSSFLSPTYKNIQQQKDRKPTARLEHRRGTESFWDTD	60	
Chicken (White Leghorn)	1	-----	60	
Duck (Anas platyrhynchos)	1	---GT---F---W---G---EF-K---N-PT-T---KI---A-G---	60	
Emu (Dromaius novaehollandiae)	1	---GA---V---V-W---T---DY-K---R---P---TK---V-G-S---	60	
Goose (Anser sp.)	1	---GT---F---W---EF-K---N-PA-A---KI---G---	60	
Turkey (Meleagris gallapavo)	1	---LA---Q---A---P---	60	
Quail (Coturnix japonica)	1	---A---N---	38	
Human	1	-PSPGTVCSL---GM-WLDL-M-----EHQRV--R-ESK--P-KLQP-ALAGWLRPE	60	
		** * * * *		
Broiler	61	ETEGEDDNNVSDIKFNVPPETIGVKITEREYQBYGOALSKMLQDILAENAEETQTKS	116	
White Leghorn	61	-----R---	116	
Duck	61	KAGA--G-DGIEL-----E-----T-----KD--K--PV--	116	
Emu	61	-AWA-----IE-----EQ-----M-----G---E--TK--RM-N	116	
Goose	61	K-GA-----EL--H-----E-----T-----E--K--PV-N	116	
Turkey	61	--A-----FE--K--D	116	
Quail	39	-----	65	
Human	61	DGGQA-GAEDELEVR--A--DV-T-LSGVQ--QHS---G-F-----W-E-KEAPADK	117	

Fig. 2. **Deduced amino acid (AA) sequence of preproghrelin in avian species.** The ghrelin precursor protein is composed of a 23 AA signal peptide, the 26 AA mature ghrelin and a 67 AA C-terminal peptide. In contrast, human ghrelin is composed of a 23 AA signal peptide, the 28 AA mature ghrelin and a 66 AA C-terminal peptide. The AA sequence of the mature ghrelin, and the putative portion of obestatin are highlighted by the black and gray boxes, respectively. Asterisks indicate identical AA among all species except human. The serine residue (S) at the N-terminal position 3 of the mature ghrelin is acylated by *n*-octanoic or *n*-decanoic acid in the case of chickens. AA sequences shown in this figure were obtained from GenBank Accession Nos. AB075215 and BAC24980 (broiler), AY299454 (Leghorn), AY338466 (duck), AY338467 (emu), AY338465 (goose), AY333783 (turkey), and AB244056 (quail), AB029434 (human), respectively.

assumed that acyl modification of ghrelin in the hatching chicken may be regulated by post-transcriptional and/or post-translational mechanisms, and the amount of fatty acids available for acyl modification of ghrelin in the hatching chicken may be limited. In fact, they found that exogenous administration of *n*-octanoic acid by way of either intraperitoneal injection or oral ingestion increased the density of ghrelin-ip cells and the concentration of ghrelin having *n*-octanoic acid modification in the proventriculus. Interestingly, ghrelin mRNA levels in the proventriculus do not change after treatments with *n*-octanoic acid, indicating that only modification of ghrelin is stimulated by exogenous treatment with fatty acids in chickens as well as in mice. Next, it will be necessary to identify the enzymes involved in actual acylation to clarify the mechanisms of ghrelin acylation.

In rat des-acyl ghrelin, lacking acyl-modification of Ser³, is also present in both stomach and circulating blood (Fig. 1, Hosoda *et al.*, 2000). It is presumed that des-acyl ghrelin is also present in birds, although the actual molecule has not yet been identified to date. Des-acyl ghrelin is produced in the stomach during the process of post-translational modification and in circulating blood with an es-

terase activity (Beaumont *et al.*, 2003) ; thus, des-acyl ghrelin is considered to be either a pre-form of acylated ghrelin or the product of des-acylation from acylated ghrelin. At the beginning of ghrelin discovery, it had been recognized that des-acyl ghrelin is an inactive form of ghrelin and has no biological activity ; indeed no response was seen in GHS-R-expressing cells (Kojima *et al.*, 1999). Accumulating evidence, however, indicates that des-acyl ghrelin is also an active peptide, exerting anti-apoptotic, anti-proliferative, and adipogenic effects, and functions through an unknown receptor different from the GHS-R (Cassoni *et al.*, 2001 ; Baldanzi *et al.*, 2002 ; Cassoni *et al.*, 2004 ; Thompson *et al.*, 2004).

Amino acid sequences of preproghrelin deduced from the nucleotide sequence of cDNA or genome have been reported in chicken (Kaiya *et al.*, 2002 ; Nie *et al.*, 2004 ; Richards *et al.*, 2006), turkey (Richards *et al.*, 2006), emu, goose, duck (Zhou and Li, 2003 ; only in database) and quail with partial sequence (Yoshimura *et al.*, 2005) (Fig. 2). All preproghrelins in birds predict a protein consisting of 116 amino acids. In general, the precursor was found to consist of a 23 amino acid signal peptide, a mature ghrelin peptide of 26 amino acids, and a

C-terminal peptide of 67 amino acids. The mature peptide is processed from the precursor at the dibasic processing sequence Arg-Arg (RR) located at the C-terminal end of the mature peptide. In the turkey, however, the processing sequence is changed to Pro-Arg (PR), which is the same with mammalian ghrelin (Richards *et al.*, 2006). This may indicate a potential difference in size of the mature ghrelin in turkey with 26 and 27 amino acids. Comparison of the precursor sequence from different avian species indicates a high degree of sequence identity. Especially the N-terminal seven amino acids (GSSFLSP) of the mature ghrelin peptide including acylated Ser³ have been completely conserved. The N-terminal region of the ghrelin peptide (GSSF with acylated Ser³) is essential for receptor binding, and this portion has been named as the “active core” of ghrelin (Bednarek *et al.*, 2000 ; Matsumoto *et al.*, 2001). Nie *et al.* (2004) and Richards *et al.* (2006) found a single nucleotide polymorphism (SNP) at Gln113 Arg in the C-terminal peptide when the precursor sequences were compared between egg-laying and broiler chickens. It remains unknown whether this change influences their characteristics because it is a change not in the mature peptide but in the C-terminal peptide.

Recently, a novel anorectic peptide, namely obestatin, has been reported to be present within the C-terminal peptide of the ghrelin precursor (Zhang *et al.*, 2005). Obestatin means a contraction of obese, from the Latin “obedere”, meaning to devour, and “statin” denoting suppression. The concept is that the ghrelin precursor produces peptides having opposite effects, orexigenic (ghrelin) and anorectic (obestatin). Obestatin is a 23- or 13-amino acid amidated peptide (Fig. 2), binding to an orphan GPCR, namely GPR39, and increases intracellular cAMP as second messenger. It is interesting that obestatin is also present in the ghrelin precursor in birds, and indeed it is possible to produce a similar peptide if a processing event identical to the one for mammalian obestatin occurs in the ghrelin precursor of birds. However, several issues must be addressed before the effects can be considered further even in mammals, i.e., obestatin can not inhibit ghrelin-induced food intake ; serum levels of obestatin have no correlation with feeding.

In rats, the presence of a ghrelin-derived peptide other than 28-amino acid rat ghrelin has been

shown. Des-Gln¹⁴ rat ghrelin with 27 amino acids is derived from the single ghrelin gene by an alternative splicing of exons coding the mature ghrelin peptide (Hosoda *et al.*, 2000b). A similar variant is also found in rainbow trout ghrelin (des-V¹³R¹⁴Q¹⁵ rainbow trout ghrelin, Kaiya *et al.*, 2003). Richards *et al.* (2006) have found no evidence for potential alternative splicing events that could lead to the same types of amino acid sequence variation in the mature ghrelin peptide in the ghrelin gene of chicken and turkey. We also have no evidence of the presence of such type of ghrelin variant in our detailed sequence analyses of purified chicken ghrelin peptide, although sixteen isoforms of chicken ghrelin were identified during the course of chicken ghrelin purification (Table 1).

Ghrelin Gene

The nucleotide sequence of the ghrelin gene and intron : exon organization has been reported from chicken (Nie *et al.*, 2004 ; Richards *et al.*, 2006) and turkey (Richards *et al.*, 2006). Analysis of the draft chicken genome indicates that the ghrelin gene is located on chromosome 12 (Richards *et al.*, 2006). The ghrelin gene has five exons and four introns. Preproghrelin is encoded within exon 2 and exon 5.

Richards *et al.* (2006) have identified cDNAs from an egg-laying strain (White leghorn), a meat-type broiler chicken and the ancestral strain (red jungle fowl), and compared their nucleotide sequences. They found a particularly interesting feature : the presence of an 8-bp insertion in exon 1 of an egg-laying strain, which is a non-coding exon in the 5'-untranslated region (UTR). This insertion is also found in exon 1 of goose and emu, but is absent in meat-type broiler chicken and red jungle fowl. This unique feature has also been reported to occur at low frequency in four chicken breeds (Nie *et al.*, 2004). Given the fact that two chicken strains, egg-laying type and meat-type, markedly differ in their appetite and growth performance, there is a possibility that the insertion may reflect the difference in their characteristics. Turkeys also possess the 8-bp insertion in the same position. Furthermore, an additional 30-bp insertion is found at the junction of exons 1 and 2 of turkeys. This insertion was not found in any other avian species. Both of these sequence variations occur outside the coding region of the mRNA transcript. Nie *et al.* (2004)

Table 1. Ghrelin isoforms identified during purification process of the extract of the chicken proventriculus

Groups	Purification processes	Yields (pmol)	Mass (M+H)	Molecular form
A	CM fr.31-32, RP fr.9-10, vy fr.48, μ Bonda fr.48-1	6.5	2747.0704	
	CM fr.31-32, RP fr.9-10, vy fr.48, μ Bonda fr.48-2	2.9	2746.9866	
B	CM fr.36-39, RP fr.4-6, vy fr.29	11.0	2877.0647	
	CM fr.36-39, RP fr.4-6, vy fr.32	18.3	2990.0876	
C	CM fr.43-46, RP fr.8-11, vy fr.37, μ Bonda fr.37-1	15.5	2902.7133	
	CM fr.43-46, RP fr.8-11, vy fr.37, μ Bonda fr.37-2	5.5	2902.5372	
	CM fr.43-46, RP fr.8-11, vy fr.40	44.6	2948.1555	
D	CM fr.49-51, RP fr.5-7, vy fr.22, μ Bonda fr.22-1	254.0	3127.1882	Chicken ghrelin (C8 : 0)
E	CM fr.52-54, RP fr.5-7, vy fr.18-19, μ Bonda fr.18/19-1	23.2	3150.4288	
	CM fr.52-54, RP fr.5-7, vy fr.18-19, μ Bonda fr.18/19-2	23.4	3151.1374	
	CM fr.52-54, RP fr.5-7, vy fr.18-19, μ Bonda fr.18/19-3	6.2	3151.2065	
	CM fr.52-54, RP fr.12-13, vy fr.13	2.0	3017.5556	
F	CM fr.57-60, RP fr.8-9, vy fr.7	13.3	3141.1996	Chicken ghrelin (C10 : 1)
	CM fr.57-60, RP fr.8-9, vy fr.8, μ Bonda fr.8-2	115.3	3153.3913	
	CM fr.57-60, RP fr.8-9, vy fr.8, μ Bonda fr.8-3	30.9	3152.8574	
G	CM fr.65-68, RP fr.11-12, vy fr.1	267.9	3157.5493	Chicken ghrelin (C10 : 0)

Abbreviation : CM, carboxymethyl ion-exchange HPLC ; fr., fraction ; RP, reverse-phase HPLC ; μ Bonda, μ Bondasphare column, Vy, vydac diphenyl column.

reported the majority of single nucleotide polymorphisms (SNP) of the chicken ghrelin gene. They reported that a SNP (C223G) in 5'-UTR, where the serum response factor binding site is present, may influence expression of the ghrelin gene. Furthermore, a number of SNPs are found in the fourth intron. However, no significant differences in these sequence variations are seen between egg-laying type and meat-type chickens. Further study is necessary to clarify the relationship between the insertion/deletion and characteristics of avian species.

Richards *et al.* (2006) have reported the 5'-flanking sequence 2000-bp upstream of the translation initiation site (ATG set) of the chicken ghrelin gene. The analysis of the putative promoter region revealed the presence of two cyclic AMP response element binding protein (CREB) sites, two AP-1 sites, one sterol regulatory binding protein-1 (SREBP-1) site, six CCAAT/enhancer binding protein (C/EBP) sites and a number of SRY and SOX-5 sites. Although nothing is known about ghrelin gene regulation in birds, the finding of CREB sites suggests a potential role in regulation of ghrelin gene expression by cAMP-producing factors such as glucagon and GHRH (Kamegai *et al.*, 2001 ; Kishimoto *et al.*, 2003). The presence of multiple SRY and SOX-5 sites could indicate their role for gene expression in testis (Tena-Sempere, 2005). The regulation of

the ghrelin gene expression certainly is a topic for future study.

Distribution of Peptide and mRNA

In mammals, ghrelin is found in various tissues (Kojima and Kangawa, 2005) ; the gastrointestinal tract is recognized as the main site for ghrelin production. The highest expression of the ghrelin gene has been found in the mucosal layer of stomach fundus. The ghrelin-producing cells have been identified as part of the X/A-like cells (Date *et al.*, 2000). Ghrelin-ip cells are also detected in the intestinal tract, but the distribution gradually declines from the duodenum to the colon (Date *et al.*, 2000). Ghrelin-ip cells are also detected in arcuate nuclei of the hypothalamus (Kojima *et al.*, 1999). Recently, a wide distribution of ghrelin-producing cells and immunopositive fibers have been observed in the brain (Cowley *et al.*, 2003), supporting the existence of interactions of ghrelin with orexigenic peptides such as neuropeptide Y (NPY), agouti-related peptide (AGRP) and orexin. Ghrelin is also produced in peripheral organs such as the pituitary, pancreas (alpha, beta and epsilon cells), kidney, placenta, and testis, suggesting autocrine/paracrine roles of ghrelin in these organs.

In the case of chicken (Fig. 3), ghrelin mRNA expression is detected in the highest level in the

Fig. 3. Tissue distribution of mRNA for ghrelin (upper panel) and GHS-R1a (lower panel) in broiler chicken. This RT-PCR was performed at the laboratory of H.K. Ghrelin is predominantly expressed in the proventriculus followed by the corpus striatum. High expression of GHS-R1a mRNA is observed in the brainstem followed by cerebellum and corpus striatum. Relatively high expression of GHS-R1a mRNA is also found in the caecum and rectum.

Fig. 4. Ghrelin-immunopositive (ghrelin-ip) and ghrelin mRNA-expressing (ghrelin-ex) cells in the chicken proventriculus. Immunohistochemistry was performed using an antiserum recognizing the N-terminal portion of rat ghrelin including acylation (Hosoda *et al.*, 2000a). This antibody can detect acylated ghrelin in chicken (Kaiya *et al.*, 2006). In situ hybridization was performed using a cRNA probe specific for chicken ghrelin (Wada *et al.*, 2003 ; Yamato *et al.*, 2005).

proventriculus, but not in the gizzard (Kaiya *et al.*, 2002). This expression level is followed by the one in brain, lung, spleen and intestine in 8-days-old layer chicken using RT-PCR analysis (Kaiya *et al.*, 2002). Richards *et al.* (2006) also found a similar expression pattern : the highest expression in the proventriculus, followed by pancreas, brain and intestine in 3-weeks-old broiler chickens. These results indicate that the major site of ghrelin synthesis is identical in broiler and layer chickens without relationship to their strains. On the other hand, in newly hatched Leghorn chicks ghrelin mRNA expression was detected only in the proventriculus, whereas it was detected in the pylorus and duode-

num as well as the proventriculus in adult Leghorn chickens (Wada *et al.*, 2003). This suggests developmental and tissue-specific changes in ghrelin mRNA expression during the hatching and growing process.

Ghrelin-ip and ghrelin-ex cells are found in the mucosal layer of the proventriculus (Wada *et al.*, 2003 ; Neglia *et al.*, 2005 ; Yamato *et al.*, 2005) and gastrointestinal tissue (Neglia *et al.*, 2005) in chickens and of the proventriculus in quails (Yoshimura *et al.*, 2005) (Fig. 4). The cells are morphologically round in shape, so called closed-type cells. It is interesting to note that mRNA and protein expression levels were similar in ghrelin-producing cells

from adult chickens, whereas mRNA expression levels was much higher in newly hatched chicks as compared to protein expression (Wada *et al.*, 2003, Yamato *et al.*, 2005). This difference in expression between mRNA and protein is related to the lack of substrate (fatty acids) for acyl modification of ghrelin (Yamato *et al.*, 2005) as explained already.

Other than the proventriculus, ghrelin-ip cells and nerve fibers are found in the chicken hypothalamus such as the nucleus anterior medialis hypothalami, and nucleus magnocellularis preopticus pars medialis, nucleus magnocellularis preopticus pars supraopticus and in the chiasma opticus (Ahmed and Harvey, 2002). Saito *et al.* (2005) reported ghrelin mRNA levels in different regions of chicken brain. They found that the corpus striatum is the highest expression site in the brain, followed by cerebellum, optic lobes and brainstem. What types of neuron in these regions express ghrelin mRNA and what function the ghrelin has remained so far unknown. Moreover, Yoshimura *et al.* (2005) have recently reported ghrelin-ip cells in the oviduct of Japanese quail laying hens.

Regulation of Ghrelin Secretion and mRNA Expression

Generally, it has been reported in mammals that circulating ghrelin levels reflect acute feeding status. Plasma ghrelin levels are increased by 12-h fasting, in which animals are in a state of negative energy balance, and the increased plasma levels are reversed by short-term re-feeding (Toshinai *et al.*, 2001). However, the detailed mechanisms have not been fully elucidated (Sugino *et al.*, 2004 ; Mundinger *et al.*, 2006). The stomach plays the major role in the site of ghrelin release (Ariyasu *et al.*, 2001). In birds, the findings that ghrelin-ip and ghrelin-ex cells are present in the proventriculus and that high levels of ghrelin mRNA expression are detected in the proventriculus are consistent with a potential role of the proventriculus as the major source of circulating ghrelin as is the case in mammals.

Shousha *et al.* (2005) have reported that plasma ghrelin levels were approximately 10 pg/ml in free-feeding adult male Japanese quail, and were increased five-fold by 24-h fasting. The increased levels were reduced following a 3-h re-feeding period. From these findings, it is suggested that ghrelin also acts as a hunger signal in birds. However, Richards *et al.*

(2006) have reported that plasma ghrelin levels did not markedly change by 24-h fasting and following subsequent 24-h re-feeding ; an increase in plasma ghrelin level, although it is not significant, is observed after 48-h fasting in 3-week-old broiler chickens. The reason for the discrepancy may be due to different measurement systems. Indeed, Shousha *et al.* (2005) used a radioimmunoassay (RIA) for rat ghrelin using a specific antibody that recognizes the N-terminal region including the acylated Ser³ residue (N-RIA, Hosoda *et al.*, 2000a). On the other hand, Richards *et al.* (2006) used a commercially available kit for ghrelin measurement of human and rat. More recently, Kaiya *et al.* (2006) validated the N-RIA, which is the same as the system used by Shousha *et al.* (2005), for measurement of chicken ghrelin. The RIA detected several forms of acylated ghrelin but not ghrelin lacking acyl modification, des-acyl ghrelin, in extracts from plasma and stomach of chickens. Using this system, they reported that plasma ghrelin levels in 6-day-old layer chickens were increased from 20 fmol/ml to 30 fmol/ml after 12-h fasting and the increased levels returned to the control levels following 6-h of re-feeding. In addition, proventriculus ghrelin levels also increased twice from 20 fmol/mg tissue after the fasting, and the increased levels returned to control levels following re-feeding. These results suggest stimulation of biosynthesis and release of ghrelin in the proventriculus in response to feeding status. Using the same measurement system as Kaiya *et al.* (2006), Geelissen *et al.* (2006a) also reported an increase in plasma ghrelin levels following 48-h fasting in 10-day-old broiler chickens. The increased levels were declined by a short-term 4-h re-feeding. They also demonstrated the reduction of ghrelin-ip cells in the proventriculus in response to food deprivation, which is consistent with increased ghrelin levels in plasma and results in increased ghrelin release from the proventriculus. Interestingly, the reduced number of ghrelin-ip cells after re-feeding returns to the levels of time-matched free-feeding chickens, suggesting that releasing rates of ghrelin are stimulated during fasting and that ghrelin storage in the proventriculus is acutely restored after re-feeding. It is noteworthy that a different time-course of changes in plasma ghrelin levels was observed between layer and broiler chickens : an increased ghrelin level was observed 12 h after fasting in layer-type, whereas it was not 24

h but 48 h after fasting in broiler chickens. Further study needs to clarify this difference.

Ghrelin mRNA expression in the proventriculus is stimulated by 24-h of fasting in 3-week-old broiler chickens ; the increased expression does not return to pre-fasting level (Richards *et al.*, 2006). Kaiya *et al.* (2006) also observed similar changes in ghrelin mRNA expression in the proventriculus of 6-day-old layer chickens. These changes are consistent with those of plasma ghrelin, suggesting stimulation of biosynthesis in response to fasting. Geelissen *et al.* (2006a), however, observed significant reduction of ghrelin mRNA expression in the proventriculus after 24-h and 48-h fasting. They also observed a marked reduction of ghrelin-ip cells at the same time points despite increased plasma ghrelin levels. Therefore, it is assumed that the reduction of ghrelin-ip cell number may result from the reduction of ghrelin biosynthesis.

Little is known about the influence of feeding on ghrelin mRNA expression in the brain of mammals so far. In the chicken brain, no significant effects of food deprivation or re-feeding on ghrelin mRNA are seen (Richards *et al.*, 2006). It is possible that different patterns of gene expression may occur in distinct regions of the chicken brain because Saito *et al.* (2005) have reported different levels of ghrelin mRNA expression in several brain regions.

In the pancreas, a significant reduction of ghrelin mRNA level was observed 48 h, but not 24 h after fasting. Subsequent re-feeding for 24 h led to an increase in ghrelin mRNA compared to the control level (Richards *et al.*, 2006). In mammals, it is known that ghrelin regulates insulin secretion from islet tissue (Date *et al.*, 2002b ; Kageyama *et al.*, 2005). It is unclear what role ghrelin plays in pancreatic endocrine functions. However, since the changes occurred while no correlation was observed between circulating ghrelin and insulin levels, the finding of an apparent relationship between ghrelin mRNA levels in the pancreas and plasma insulin concentrations suggests possible autocrine/paracrine roles of locally produced ghrelin on insulin secretion from the pancreas (Richards *et al.*, 2006). To further clarify this fact, it is necessary to examine which type of pancreatic cells produces ghrelin.

Receptor : Its Tissue Distribution and mRNA Expression

The receptor for ghrelin has been named growth hormone secretagogue receptor (GHS-R). In mammals, two types of GHS-R exist : GHS-R1a involved in ghrelin activity, and GHS-R1b, an alternative splice variant, of which the function is unknown. In birds, GHS-R1a and GHS-R1aV or GHS-R1c have been identified in hypothalamic cDNA of broiler chicken (Geelissen *et al.*, 2003) and in pituitary cDNA of layer-type White Leghorn chicken (Tanaka *et al.*, 2003). No difference in the amino acid sequence of the receptor is seen between broiler type and layer type. GHS-R1a has 68% identity with human GHS-R1a. Both GHS-R1aV and GHS-R1c lack the same portion of the transmembrane domain 6 by deletion of 48 bp (16 amino acids) (Geelissen *et al.*, 2003 ; Tanaka *et al.*, 2003). However, the ligand-binding ability of the receptor has not yet been examined. The cystein residues, N-glycosylation sites and potential phosphorylation sites, found in the human GHS-R1a, are conserved in the chicken isoform (Geelissen *et al.*, 2003). Tanaka *et al.* (2003) determined the organization of the chicken GHS-R gene, which is composed of two exons and one intron, and is a TATA-less gene in the 5'-UTR as human GHS-R. The position of the intron insertion is comparable to that of mammalian GHS-R genes (Petersenn *et al.*, 2002).

GHS-R1a mRNA expression is found in various tissues by RT-PCR (Fig. 3). Geelissen *et al.* (2003) have reported in 10-day-old broiler chickens that the highest levels of GHS-R1a mRNA are found in pituitary and hypothalamus and moderate levels in ovary, telencephalon, heart, adrenal gland, cerebellum and optic lobes. Low expression is found in brainstem, lung, kidney, proventriculus, duodenum and colon, and expression in skin, gizzard and muscle is negligible. Tanaka *et al.* (2003) reported GHS-R1a mRNA expression in 8-week-old Leghorn chickens : the highest expression is detected in pituitary and brain, followed by liver, spleen and intestine. Low expression levels are observed in thymus, pancreas, lung heart, kidney, proventriculus and gizzard. Saito *et al.* (2005) quantified GHS-R1a transcripts in various region of the brain of 4-day-old Leghorn chicks. The expression levels are the highest in brainstem followed by cerebellum, optic

lobes and corpus striatum. Richards *et al.* (2006) also detected expression of GHS-R1a in lung, kidney, spleen, liver, duodenum, small intestine, heart, abdominal fat pad, proventriculus and brain of 3-weeks-old broiler chickens by RT-PCR. These findings indicate that these organs are potential target sites for ghrelin in chickens.

The splice variant of GHS-R mRNA, GHS-R1aV and GHS-R1c, was detected in the same tissues where GHS-R1a mRNA was present (Geelissen *et al.*, 2003 ; Tanaka *et al.*, 2003 ; Richards *et al.*, 2006). The relative order of expression levels in tissues is almost identical to that of GHS-R1a, whereas the actual expression levels are much smaller than those of GHS-R1a. The physiological function of GHS-R1aV or GHS-R1c remains to be elucidated. It has been reported in a fish, namely in seabream, that co-expression of a seabream GHS-R variant (GHS-R1b) in HEK293 cell with seabream GHS-R1a attenuates the GHS-R1a activity (Chan and Cheng, 2004).

Geelissen *et al.* (2003) have attempted to examine acute regulation of GHS-R1a and GHS-R1c in the pituitary *in vitro*. Administration of chicken ghrelin (1 μ M) to pituitaries maintained in culture medium resulted in a down-regulation of the expression of both receptors within 15 min, and the effect disappeared by 60 min of exposure. Similar down-regulation of GHS-R expression was also observed by administration of GH and corticosterone, although the response was seen after 1 h of incubation. These results suggest a direct action of these factors on GHS-R gene expression in the chicken pituitary. It is known that ghrelin increases plasma GH and corticosterone levels following intravenous injection (Kaiya *et al.*, 2002). Therefore, GH, corticosterone as well as ghrelin itself might be involved in a feedback regulation of both GHS-Rs. However, the regulatory mechanisms underlying the feedback seem to differ among factors : ghrelin participates in the acute effect, and GH and corticosterone regulate the expression in a later phase. This may suggest a biphasic feedback regulation of GHS-R gene expression. Interestingly, TRH, known as a potent stimulator of GH release in chicken, and GHRH do not alter pituitary GHS-R mRNA expression. This may suggest that endogenous GH that was released by addition of GH secretagogues and exogenous GH regulate GHS-R expression through different mechanisms.

It is interesting to note that ghrelin down-regulates the GHS-R mRNA expression even though it is able to release GH from the pituitary *in vitro* (Ahmed and Harvey, 2002 ; Baudet and Harvey, 2003). Further study is necessary to clarify intracellular mechanisms regulating GHS-R gene expression in chickens.

Changes in GHS-R1a mRNA levels in proventriculus, pancreas and brain have been examined under different energy states created by fasting and re-feeding in 3-week-old broiler chickens (Richards *et al.*, 2006). However, no significant effects of fasting and re-feeding were seen on GHS-R mRNA expression although pancreatic GHS-R mRNA tended to be lower after 48-h fasting. It is unclear whether the receptor might play a role in regulating pancreatic functions, i.e. insulin secretion, in chickens.

Functions

Fig. 5 illustrates the physiological roles of ghrelin in birds.

GH-Releasing Effect

The primary effect of ghrelin is considered to be the stimulation of GH release based on the background of ghrelin discovery. Indeed, ghrelin acts as a potent GH-releasing peptide in mammals. In birds, an intravenous (iv) injection of ghrelin transiently increases plasma GH levels in chickens (Ahmed and Harvey, 2002 ; Kaiya *et al.*, 2002 ; Baudet and Harvey, 2003). Ahmed and Harvey (2002) showed an increase in plasma GH concentrations 10 min after iv injection of human ghrelin in 4-week-old Leghorn chickens. The effect is comparable with that of GHRH, but less potent than that of TRH. Kaiya *et al.* (2002) also showed a transient increase in plasma GH concentrations 15 min after iv injection of homologous chicken ghrelin in 8-day-old chickens. Baudet and Harvey (2003) showed dose- and time-dependent increases in plasma GH levels in 4 or 5-week-old Leghorn chickens. These findings indicate that ghrelin acts as a GH-releasing peptide in young and adult chickens. It remains unclear whether the ghrelin-induced GH release observed *in vivo* is stimulated through other hypothalamic hormones such as TRH, that is known as a potent stimulator for GH release in chickens (Harvey, 1990), and GHRH in chickens. Dispersed pituitary cells respond to ghrelin *in vitro* in a dose-dependent manner (Baudet and Harvey, 2003), in-

Fig. 5. **Physiological actions of ghrelin in birds.** Ghrelin is considered to be a brain-gut hormone. In chickens, circulating ghrelin increases plasma GH levels, although the detailed mechanisms remain unknown. A direct effect of ghrelin on GH release from the pituitary is evident. Circulating ghrelin also stimulates corticosterone release through direct or indirect pathways. Intracerebroventricular injection of ghrelin stimulates corticosterone release through the hypothalamo-pituitary-adrenal (HPA) axis. Conflicting results of ghrelin on food intake have been reported when ghrelin was injected centrally or peripherally. However, it is likely that ghrelin administered centrally inhibits food intake. It is postulated that ghrelin also participates in heat production, behavior, reproduction and energy metabolism.

dicating a direct action of ghrelin, especially on somatotrophs of the pituitary gland.

Richards *et al.* (2006) reported that no correlations were observed between endogenous plasma ghrelin and GH concentrations in response to changes in feeding status of 3-week-old chickens. However, since a close functional entanglement exists between the somatotrophic, thyrotrophic, and corticotrophic axis which influence the regulation of GH release in chickens (Kühn *et al.*, 2005), it is not easy to rule out a relationship between changes in endogenous ghrelin and GH. Growth hormone released by ghrelin could participate in a negative feedback on the ghrelin-induced GH response.

Appetite-Regulatory Effect

It is known that ghrelin acts as an orexigenic peptide in mammals when injected centrally and

peripherally (Nakazato *et al.*, 2001 ; Date *et al.*, 2002a). However, in neonatal chicks, intracerebroventricular (icv) administration of ghrelin or GHS potently inhibits food intake in a dose-dependent manner (Furuse *et al.*, 2001 ; Saito *et al.*, 2002a ; Saito *et al.*, 2005 ; Khan *et al.*, 2006). This effect is opposite to that of mammals. Interestingly, NPY-induced food intake is attenuated by co-administration of ghrelin (Saito *et al.*, 2005). In the case of mammals, orexigenic activity of ghrelin is mediated by activation of other orexigenic peptides such as NPY, AGRP and orexin (Nakazato *et al.*, 2001, Toshinai *et al.*, 2003). However, it is likely that icv ghrelin does not link to orexigenic NPY in chicks (Saito *et al.*, 2005). In contrast, corticotropin-releasing factor (CRF) mediates the anorectic effects of ghrelin (Saito *et al.*, 2005) (Fig.

Fig. 6. A potential network of ghrelin for anorexia and hyperactivity after intracerebroventricular (icv) injection of ghrelin. Icv injection of ghrelin induces hyperactivity and inhibits food intake in neonatal chicks. It is known that ghrelin stimulates neuropeptide Y (NPY) neurons in the arcuate nuclei of the hypothalamus in rodents. However, in chicks, ghrelin stimulates the release of corticotropin-releasing factor (CRF)-containing neurons instead of activating NPY neurons. The released CRF mediates hyperactivity as well as corticosterone (CORT) release through the hypothalamo-pituitary-adrenal (HPA) axis. The effects of ghrelin on food intake and CORT release are blocked by a CRF receptor antagonist, astressin, but other endogenous CRF-like systems including utocortin and urotensin may mediate these effects. It is not known whether ghrelin that injected peripherally activates this network in the central nervous system.

6). Consistent with the effect of ghrelin on food intake, GHS does not promote motility of the digestive tract from the crop to the gizzard (Khan *et al.*, 2006), which is also opposite to the effect in mammals (Masuda *et al.*, 2000). Recently, conflicting effects of ghrelin on food intake were reported in adult Japanese quail (Shousha *et al.*, 2005). Icv injection of ghrelin inhibited food intake with both high and low doses (0.04, 0.4 and 0.8 nmol/100 g BW), while ip injection of a high dose (2.4 nmol/100 g BW) of ghrelin inhibited, low doses (0.4 and 0.8 nmol/100 g BW) of ghrelin stimulated food intake irrespective of light and dark conditions. More recently, similar conflicting effects of peripheral ghrelin on food intake have been reported in chickens. Geelissen *et al.* (2006b) reported that an iv injection of chicken ghrelin (1 nmol/100 g BW) inhibited food intake during the first 1 h after injection in 7-day-old broiler chickens. On the other

hand, Kaiya *et al.* (2006) found no effect on food intake in 8-day-old Leghorn chickens during a 2 h experimental period when chicken ghrelin (0.6 nmol/100 g BW) was injected intraperitoneally (ip). The reason of the conflicting effects of peripheral ghrelin may reflect the differences of species/strain, developmental stage and dose of injection. Shousha *et al.* (2005) have discussed that the inhibitory effect of a high dose of peripheral ghrelin may be caused centrally by ghrelin that passed through the blood-brain barrier (Banks *et al.*, 2002). It is known in rodents that peripheral ghrelin signals concerning stimulation of GH release and appetite conveys to the central nervous system through the nucleus of the solitary tract from the vagal afferent nerves (Date *et al.*, 2002a). It is assumed that birds also have a similar signal transduction system in their evolutionary process. Taking this into consideration, however, it is evident that the central effect of

ghrelin on food intake differs between mammals and birds : central ghrelin in birds acts as an inhibitor for food intake. Further study needs to elucidate the physiological relevance of the inhibitory effect of central ghrelin in birds.

In mammals, researchers recently started to recognize that next to ghrelin, des-acyl ghrelin is also a biologically active peptide (Cassoni *et al.*, 2001 ; Baldanzi *et al.*, 2002 ; Cassoni *et al.*, 2004 ; Thompson *et al.*, 2004). Concerning food intake, conflicting findings have been reported. Asakawa *et al.* (2005) reported an inhibitory effect of des-acyl ghrelin in mice, and this effect is mediated through urocortin and cocaine and amphetamine regulated transcript (CART) (Chen *et al.*, 2005). In contrast, Toshinai *et al.* (2006) recently reported a stimulatory effect of des-acyl ghrelin on food intake in rodents. In birds, des-acyl ghrelin injected icv did not change food intake in neonatal chicks (Saito *et al.*, 2002b). Further study needs to elucidate the effects of des-acyl ghrelin on food intake as well as on other physiological actions such as the regulation of GH release.

Corticosterone Release

In mammals, ghrelin injection results in an increased release of steroid hormones from the adrenal gland, but the effect is not so potent (Nagaya *et al.*, 2001). However, in 8-day-old chickens, a marked increase in plasma corticosterone (CORT) levels is observed by 30 min after iv injections of chicken ghrelin and the effect is clearly dose-dependent (Kaiya *et al.*, 2002). Saito *et al.* (2005) found time- and dose-dependent increases in plasma CORT levels after icv injection of chicken ghrelin. These findings suggest involvement of ghrelin in the hypothalamo-pituitary-adrenal (HPA) axis. GHS-R mRNA has been detected not only in the hypothalamus and pituitary, but also in the adrenal gland (Geelissen *et al.*, 2003). This opens the possibility that CORT release could be stimulated indirectly via CRF, arginin vasotocin and ACTH release from hypothalamus/pituitary but also directly at the level of the adrenal gland. The fact that the effect of icv injected ghrelin on CORT release is completely blocked by co-administration of the CRF antagonist, astressin, indicates that at least CRF is involved in the stimulation of CORT release via the HPA axis (Saito *et al.*, 2005).

Heat Production

Either peripheral or central injection of ghrelin causes a dose-dependent increase in body temperature in adult Japanese male quails (Shousha *et al.*, 2005). The time-course of changes is somewhat different depending on the route of administration : the increase was observed 5–20 min after ip injection, whereas it appeared 10–60 min after icv injection. This suggests that ghrelin may affect energy expenditure in birds although the mechanism underlying these thermal changes is unclear. In contrast, no effect was seen on thermogenesis during 24 h after iv injection of ghrelin in 1-week-old male broiler chickens (Geelissen *et al.*, 2006b). The difference of these observations may be due to the difference of methodology used. Shousha *et al.* (2005) measured the temperature with the sensor tip inserted into the cloaca, whereas Geelissen *et al.* (2006b) measured heat production using respiratory cells.

Reproduction

In mammals, it is known that ghrelin is produced in reproductive organs, although the function remains unknown (Tena-Sempere, 2005). In birds, Yoshimura *et al.* (2005) detected ghrelin-ip cells in the mucosal folds in the infundibulum and magnum of 80-day-old Japanese quail at the pre-ovulation state. The intensity of ghrelin-ip cells was reduced at 5-h post-ovulation compared with the pre-ovulation stage. In contrast, no immunoreactive ghrelin was observed in these tissues in immature 20-day-old quails. These results indicate that ghrelin synthesis is initiated in association with development and differentiation of the oviduct, and suggest that ghrelin may be secreted during the passage of eggs through the oviduct. Ghrelin transferred into albumen of fertile eggs may participate in the regulation of embryonic development through GH since postnatal development of chicks is improved by injection of GH into fertile eggs (Decuypere and Buyse, 2005). Further study is needed to clarify the function of ghrelin in reproduction and embryonic development.

Regulation of Behavior

Ghrelin is known to induce both sleep-like behavior and hyperactivity in neonatal chicks after icv injection under *ad libitum* and food-deprived condition (Tachibana *et al.*, 2001 ; Saito *et al.*, 2002b). These behaviors are induced with a different time-course ; hyperactivity is seen during the first 30 min, and motionless and narcoleptic behaviors are ob-

served during the next 30 min. This result suggests that the decreased food intake by ghrelin partially results in this ghrelin-induced sleep-like behavior. CRF also induces hyperactivity and inhibits food intake when injected icv (Ohgushi *et al.*, 2001). Saito *et al.* (2005) demonstrated that the inhibitory effect of ghrelin on food intake is mediated by the CRF system. Thus, it is suggested that hyperactivity and inhibition of feeding are mediated by ghrelin-induced CRF. Iv injection of chicken ghrelin did not induce compensatory hyperphagia in 7-day-old broiler chickens (Geelissen *et al.*, 2006b). It has not been examined whether compensatory hyperphasia is caused after icv injection of ghrelin in chickens.

Energy Metabolism

Geelissen *et al.* (2006b) have reported the effects of ghrelin on energy metabolism in chickens. Iv injections of chicken ghrelin decreased the respiratory quotient (RQ) until 16 h post-injection in 7-day-old chickens, suggesting a reduced de novo lipogenic activity. An opposite effect has been reported in rodents where ghrelin increases RQ after subcutaneous injection (Tschöp *et al.*, 2000). This difference may be due to the opposite effect of ghrelin on food intake between rodents and chickens. Geelissen *et al.* (2006b) also observed no effects of ghrelin on glucose, triglyceride, fatty acids, protein and triiodothyronine (T3) levels in plasma as well as on thermogenesis.

Others

Accumulating evidence indicates that ghrelin is a multifunctional peptide in mammals and non-mammalian species (van der Lely *et al.*, 2004 ; Anderson *et al.*, 2005 ; Broglio *et al.*, 2005 ; Kojima and Kangawa, 2005 ; Smith *et al.*, 2005). Unfortunately, there are no reports exploring possible effects of ghrelin on cardiovascular system, immune function, and regulation of hypophyseal hormones other than GH in birds.

Perspective

Based on the current knowledge of ghrelin in mammals, physiological actions of ghrelin appear to be in accordance with the induction of a positive energy balance : ghrelin increases food intake, decreases fat deposition, and suppresses heat production of the body. The actions may have been developed by evolutionary selection processes for survival in times of reduced caloric supply (van der Lely *et*

al., 2004). From this point of view, the current status of poultry that was artificially generated seems to fail in establishing a natural response for the regulation of energy balance. This may be one of the reasons for the different response of peripheral ghrelin on food intake between domestic chickens and quails (Shuosha *et al.*, 2005 ; Geelissen *et al.*, 2006b ; Kaiya *et al.*, 2006). However, an increase in plasma ghrelin levels is found in response to fasting in the chicken and quail (Shuosha *et al.*, 2005 ; Geelissen *et al.*, 2006b ; Kaiya *et al.*, 2006). This suggests that the release of ghrelin in chickens is also regulated by similar mechanisms as in mammals although it has not been fully elucidated (Sugino *et al.*, 2004 ; Mundinger *et al.*, 2006). Additionally it is assumed that ghrelin is capable of stimulating food intake in chickens under certain conditions, i.e. under scheduled feeding, food-deprived or food-restricted condition. Further study needs to elucidate the role of peripheral ghrelin on food intake and GH release, and interaction with other neuropeptides in domestic chickens (Boswell, 2005 ; Decuyper and Buyse, 2005). In sheep, ghrelin secretion is regulated by cephalic mechanisms, and is modified by scheduled feeding regimens (Sugino *et al.*, 2004). Interestingly, the pre-prandial ghrelin surge is reduced by repeated feeding, and plasma ghrelin levels in sheep fed *ad libitum* do not show pre-prandial and post-prandial changes but maintain a constant level (Sugino *et al.*, 2004). Recently, similar results have also been reported in rats and humans (Arosio *et al.*, 2004 ; Drazen *et al.*, 2006). These findings suggest that ghrelin is not released when the nutrient condition is sufficient. This may apply in daily care of domestic chickens because they keep feeding under continuous light condition for all day. Therefore, scheduled meal and lighting programs would produce a natural rhythm of ghrelin release and the released ghrelin may play an effective role in absorbing ingested nutrients to the body and optimize the level of function of several hormones regulating body homeostasis. Therefore, ghrelin may have a potential to improve the production of poultry.

The exact role played by the ghrelin system in birds still remains largely unknown. Further study is necessary to understand ghrelin physiology in avian species, i.e., difference in the effect of ghrelin on food intake in neonatal and adult chickens,

effects of ghrelin on cardiovascular system, immune function, and regulation of hypophyseal hormones other than GH.

Some avian species do not feed at certain times such as during migration or brooding. Ghrelin may be involved in suppression of food intake during these fasting conditions. Increased ghrelin may suppress appetite, stimulate GH release and enhance metabolism during migration and brooding. The relationship among ghrelin levels and avian-specific behaviors such as feeding, flying or nesting should be studied further.

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