Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Type-dependent differential expression of neuropeptide Y in chicken hypothalamus (*Gallus domesticus*)*

CHEN Gui-qian[1,2], HU Xiu-fang[1], SUGAHARA Kunio[3], CHEN Ji-shuang[1], SONG Xue-mei[2], ZHENG Hui-chao[2], JIANG Yong-qing[2], HUANG Xin[2], JIANG Jun-fang[2], ZHOU Wei-dong[†‡1,2]

([1]*Institute of Bioengineering, Zhejiang Sci-Tech University, Hangzhou 310018, China*)

([2]*Institute of Animal Husbandry and Veterinary Science, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China*)

([3]*Laboratory of Nutritional Biochemistry, Department of Animal Science, Faculty of Agriculture, Utsunomiya University, Utsunomiya 321-8505, Japan*)

[†]E-mail: wdzhou@zjnm.cn

Received July 9, 2007; revision accepted Oct. 8, 2007

Abstract: Neuropeptide Y (NPY) is one of the most important orexigenic agents in central regulation of feeding behavior, body weight and energy homeostasis in domestic chickens. To examine differences in the hypothalamic NPY between layer-type and meat-type of chickens, which are two divergent kinds of the domestic chickens in feeding behavior and body weight, we detected mRNA levels of NPY in hypothalamic infundibular nucleus (IN), paraventricular nucleus (PVN) and lateral hypothalamic area (LHA) of these two types of chickens using one-step real time RT-PCR. The meat-type chicken had more food daily (about 1.7 folds) and greater body weights (about 1.5 folds) and brain weights than the layer-type chicken at the age of 14 d. In the meat-type of chicken, NPY mRNA levels of the IN and PVN were significantly greater than those of the LHA, and were not significantly different between the IN and PVN. However, in the layer-type of chicken, NPY mRNA levels were significantly greater in the IN than those in the LHA and PVN, and were not significantly different between the PVN and LHA. In all these hypothalamic regions, the layer-type of chicken had significantly higher NPY mRNA levels than the meat-type chicken did. These results suggest the expression of NPY in the hypothalamus has a type-dependent pattern in domestic chickens.

Key words: Neuropeptide Y (NPY), Hypothalamus, Message RNA (mRNA), Meat-type chicken, Layer-type chicken
doi:10.1631/jzus.2007.B0839 **Document code:** A **CLC number:** Q78

INTRODUCTION

The hypothalamus of the central nervous system is well documented as an important region in regulation of food intake and body weight in domestic chickens (Kuenzel *et al*., 1999; Furuse, 2002; Boswell, 2005). In the chicken hypothalamus, several nuclei including the infundibular nucleus (IN), the paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA) are involved in the regulation of food intake and body weight (Kuenzel *et al*., 1999). LHA is termed the hunger center, and contains abundant glucose-sensitive neurons (Oomura *et al*., 1967). The bilateral lesions of the LHA induce aphagia and decrease body weight (Kuenzel *et al*., 1999). The PVN acts to integrate neuropeptide signals from brain areas including the IN and brainstem. These connections between hypothalamic pathways and the caudal brainstem are essential for the long-term regulation of appetite and body weight (Kuenzel *et al*., 1999). IN is thought to play a pivotal role in integration of signals regulating appetite (Wang *et al*., 2001). Neurons in the IN are situated adjacent to the floor of the third ventricle, so they can directly respond to peripheral signals with a reduced brain-blood barrier (Kuenzel *et al*., 1999; Schwartz *et al*., 2000; Boswell, 2005), and may also have IN-PVN and IN-LHA connections

‡ Corresponding author

* Project supported by the Natural Science Foundation of Zhejiang Province (No. Y306220), and the Scientific Research Startup Fund of Zhejiang Academy of Agricultural Sciences, China

(Aste *et al*., 1991; Esposito *et al*., 2001).

Previous studies proved that several neuropeptides were involved in the central regulation of food intake and body weight in the domestic chicken (Furuse, 2002; 2007). Neuropeptide Y (NPY), a 36-amino acid peptide, belonging to a member of the pancreatic polypeptide family that includes peptide YY and pancreatic polypeptide (Tatemoto *et al*., 1982), is one of the most orexigenic agents in birds. Intracerebroventricular (ICV) administration of NPY in the birds has been found to elicit a marked increase in feeding (Kuenzel *et al*., 1987; Furuse *et al*., 1997) and to decrease rectal temperature (Tachibana *et al*., 2004). On the other hand, dense NPY-like immunoreactivity cells and abundant NPY mRNA expression have been identified in the hypothalamic IN of the domestic chicken (Esposito *et al*., 2001; Kameda *et al*., 2001; Wang *et al*., 2001), and dense NPY-immuno-reactivity fibers, projecting from the IN into the PVN and the LHA were observed (Aste *et al*., 1991; Boswell *et al*., 1998; Kameda *et al*., 2001). Food deprivation or restriction significantly increased feeding behavior, and also elevated messenger ribonucleic acid (mRNA) levels (Boswell *et al*., 1999), protein contents (Zhou *et al*., 2005) and neuron activity (Boswell *et al*., 2002) of the hypothalamic NPY in birds. These findings suggest that the NPY plays an important role in regulations of feeding behavior and energy homeostasis in birds.

In the modern poultry, an intensive selection produces two very divergent genotypes of domestic chickens: meat and egg production genotypes (Denbow, 1999). The meat-type chicken eats more food than the layer-type chicken does in order to support its larger daily gain in body weight (Masic *et al*., 1974; Savory, 1974). It is well proven that hyperphagia is due to the elevated hypothalamic NPY level in obese mice (Jang and Romsos, 1998) and obese Zucker rats (Hensley *et al*., 2001). However, it is not clear whether the hyperphagia of the meat-type chicken, as observed in obese mice, also is ascribed to the elevated hypothalamic NPY level, or whether there is any difference in the hypothalamic NPY between meat-type and layer-type chickens.

In the present study, we measure mRNA levels of NPY in the hypothalamic PVN, LHA and IN using one-step real time reverse transcription polymerase chain reaction (RT-PCR), and compare their differences between the meat-type and layer-type chickens at the age of 14 d.

MATERIALS AND METHODS

Animals

Two-day-old male layer-type (Hy-line) and meat-type (Ross) chickens were purchased from local farms. They were fed in an electrically heated brooder with 30 °C central temperature and 25 °C room temperature. Layer-type chickens were allowed ad libitum to access a grower diet (crude protein: 21.0%; metabolisable energy: 12.1 MJ/kg), whereas meat-type chickens were fed with broiler starter diet (crude protein, 22.0%; metabolisable energy, 12.9 MJ/kg), with water available for both throughout the experiment. At the age of 7 d, chickens were selected by body weight, and the selected chickens were moved into another room. They were individually fed in metabolism cages at the same room temperature, lighting, and feeding schedules as in the previous room. At the age of 14 d, 6 chickens were killed by decapitation after recording their body weights and daily food intakes. The brains were removed in a procedure of less than 3 min, and then frozen quickly on dry ice. The frozen brains were stored at −80 °C until used for mRNA analysis.

Isolation of hypothalamic nuclei

After weighing brain samples, the hypothalamic PVN, LHA and IN (In this study, the IN partly contained the hypothalamic inferior nucleus, median eminence and the ventromedial hypothalamus) were separated according to the method of Zhou *et al*. (2005). Briefly, the frozen brain sample was placed on a slide over dry ice, and the rostral part was cut away until the face of the anterior commissure disappeared (Kuenzel and Masson, 1988). The chiasma opticum, the third ventricle and the lateral forebrain bundle were used as landmarks for identification of the PVN (A7.8) and LHA (A7.4), and the chiasma opticum, the paraventricular organ and the median eminence were for identification of the IN (A5.6). The PVN (2 mm×2 mm×1.5 mm, length, width, depth), LHA (2 cylinders; 1 mm×1 mm×2 mm) and IN (2 mm×1 mm×1 mm) were removed quickly for subsequent homogenization and RNA isolation.

Total RNA isolation

Total RNA from chicken hypothalamus was isolated using a commercial RNeasy Lipid Tissue Mini Kit (QIAGEN Sciences, Maryland, USA). Briefly, the collected tissue was quickly put into QLAzol Lysis Reagent and homogenized for about 30 s. After addition of chloroform (Zhejiang Deyer Pharmaceutical Co., Ltd., Jinhua, China) and shaking vigorously, the homogenate was separated into aqueous and organic phases by centrifuging at 12000×g for 15 min at 4 °C. Appropriate binding conditions of the aqueous phases were adjusted using 70% ethanol solution, and sample was then applied to the RNeasy Spin Column, where total RNA binds to membrane, and contaminants were efficiently washed away. Finally, the RNA was eluted using RNase-free water. Concentration of the isolated total RNA was determined by measuring the absorbance at 260 nm in a spectrophotometer, and the ratio of 260 to 280 nm was determined for RNA quality.

Real time RT-PCR

mRNA expression of the hypothalamic NPY was measured using real time RT-PCR with Taqman One-step RT-PCR Master Mix Reagent kits (Roche Molecular Systems Inc., New Jersey, USA). In the present study, ribosomal 18S RNA (18S rRNA) was used as an internal control, and results of mRNA level of NPY were presented as its ratio to 18S rRNA. Primers and Taqman probes for chicken NPY (GenBank Accession No. NM_205473) and 18S rRNA (GenBank Accession No. AF173612) were commercially synthesized (Shanghai Invitrogen Biotechnology Co., Ltd., China). NPY primers were forward 5′-CTA CTC GGC TCT GAG GCA CTA CA-3′ and reserve 5′-TCA GTG TCT CTG GGC TTG ATC TC-3′, and the probe was 5′-FAM-CAA CCT CAT CAC CAG GCA GAG ATA TGG AA-TAMRA-3′; the GAPDH primers were forward 5′-CTC CGA CTT TCG TTC TTG ATT AA TG-3′ and reserve 5′-ATT GTG CCG CTA GAG GTG AAA T-3′, and the probe was 5′-FAM-CTT TAG TTC GTC TTG CGC CGG TCC A-TAMRA-3′. In the Taqman probes, FAM (6-carboxy-fluorescein) and TAMRA (6-carboxy-tetramethyl-rhodaminie) are fluorescent dyes for reporter and quencher, respectively. The emission of the FAM at 5′-end is quenched by the TAMRA at 3′-end. During the extension of the PCR, the polymerase cleaves the Taqman probe, resulting in a release of fluorescence dye. The increasing amount of reporter dye emission was detected.

In a typical reaction, 5 μl total RNA (50 ng for NPY, and 1 ng for 18S rRNA) was mixed with 20 μl 2× Master mix w/o UNG, 10 μl 40× MultiScrible and Rnase Inhibitor (Roche Molecular Systems Inc., New Jersey, USA), 300 nmol/L from forward primer (100 μmol/L), 900 nmol/L from reverse primer (100 μmol/L), and 200 nmol/L Taqman probe (5 μmol/L) in 40 μl total volume. The reaction for one-step real time RT-PCR was performed in a 96-well Optic Reaction Plate (Applied Biosystems, California, USA) on 7500 Real Time RT-PCR System (Applied Biosystems, California, USA). The following RT-PCR protocol was used for both genes: 48 °C for 30 min for RT, one cycle of PCR initial activation at 95 °C for 10 min plus 45 cycles of PCR denaturation and combined annealing and extension, PCR denaturation stage at 95 °C for 15 s, and combined annealing and extension stage at 60 °C for 1 min.

The regression equations of NPY (y=−3.3347x+33.009, R^2=0.9996, P<0.01) and 18S rRNA (y=−3.1463x+29.125, R^2=0.9951, P<0.01) were obtained by applying the least square regression method, using logarithmic value of diluting times ($\log C_0$) as the X axis, and the threshold cycle (C_t) values as the Y axis.

Data analysis

All results are presented as means±*SEM*. Statistical differences in brain weight, food intake, body weight and mRNA level of the hypothalamic NPY between meat-type and layer-type of chickens were assessed by two-tailed Student's unpaired t-test, respectively. Data for mRNA levels within a group were analyzed by one-way ANOVA, with brain regions used as classification variables. When ANOVA showed that the brain regions had significant main effects, we performed comparisons among different brain regions using Tukey's multiple comparison tests. A confidence level of P<0.05 was considered to indicate statistical significance.

RESULTS

Fig.1 shows brain weight, daily food intake and body weight of the meat-type and layer-type chickens

at the age of 14 d. The meat-type chickens ate more daily food (about 1.7 folds), resulting in a greater body weight (about 1.5 folds) than the layer-type chickens. Brain weight in the meat-type chickens was also significantly greater than that in the layer-type chickens.

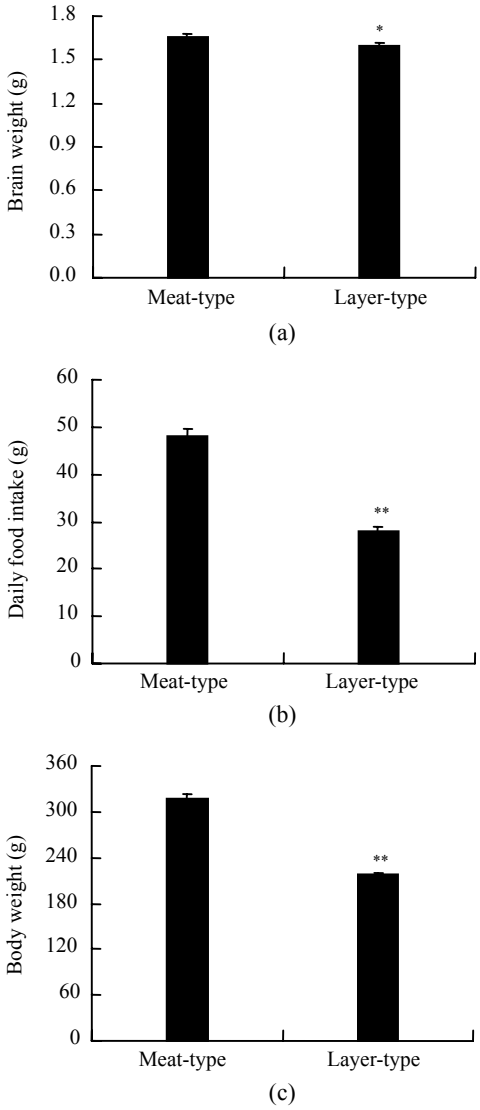

Fig.1 Brain weight (a), daily food intake (b) and body weight (c) of the meat-type and layer-type chickens at the age of 14 d

Values are shown as means±*SEM* (n=5~6). $^{*}P<0.05$, $^{**}P<0.01$ between two types of chickens

mRNA levels of the hypothalamic NPY and 18S rRNA (as an internal control) in the two types of chickens were determined using one-step real time RT-PCR. Fig.2 shows mRNA levels in the hypothalamic PVN, IN and LHA of the meat-type and layer-type chickens at the age of 14 d. In the meat-type of chicken, NPY mRNA levels were significantly greater in the IN and PVN than those in the LHA, and were not significantly different between IN and PVN. However, in the layer-type of chicken, NPY mRNA levels were significantly greater in the IN than those in the LHA and PVN, and were not significantly different between PVN and LHA. In all these brain regions, NPY mRNA levels were significantly greater in the layer-type chicken than those in the meat-type chicken.

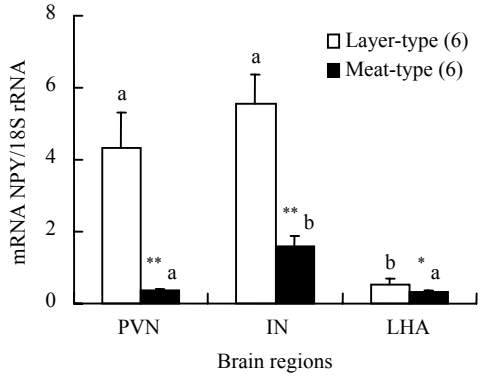

Fig.2 The hypothalamic mRNA levels in the meat-type and layer-type chickens at the age of 14 d

Values are shown as means±*SEM* and the number of chickens is shown in parentheses. $^{*}P<0.05$, $^{**}P<0.01$ between two types of chickens; Means with a, b are significantly different among brain regions in the same type of chicken ($P<0.01$)

DISCUSSION

In the modern poultry, the meat-type chicken has been intensively selected for rapid body weight gain and high meat yield, whereas layer-type chicken has been selected for high egg production. The difference in growth rate could be due partly to the difference in food intake between two types of chickens (Savory, 1974; Denbow, 1999). As a result, in the present study, body weight and daily food intake of the meat-type chicken were 1.5 and 1.7 folds greater than those of the layer-type chicken at the age of 14 d, respectively. Significant difference in brain weight was observed between the layer-type and meat-type of chickens. This result is in accordance with our previous findings in the embryonic stage of chicken (Zhou *et al*., 2006).

In the present study, the highest mRNA level of

NPY was observed in the hypothalamic IN of two types of chickens. This result agrees with previous studies, in which the denser NPY-immunoreactive cell bodies and higher NPY mRNA expression (Esposito *et al*., 2001; Kameda *et al*., 2001; Wang *et al*., 2001) were located, and the higher NPY content was determined in the hypothalamic IN of chickens (Zhou *et al*., 2005). However, in the meat-type chicken, NPY mRNA level in the PVN was significantly greater than that in the LHA, and was not significantly different between the IN and PVN. In the layer-type chicken, NPY mRNA level in the PVN was similar to that in the LHA. These results are slightly different from our previous data, where the order of NPY protein content amount is: IN> PVN>LHA in the meat-type chicken at the age of 14 d (Zhou *et al*., 2005). These findings suggest that patterns of NPY expression in the different regions vary with chicken types, and NPY plays different roles in the hypothalamic regions.

Significantly higher mRNA levels of NPY in the PVN, IN and LHA in the layer-type chicken than those in the meat-type chicken at the age of 14 d were observed. These results agree with our previous data in the embryonic stage, in which the hypothalamic NPY peptide content was significantly greater in the layer-type chicken than that in the meat-type chicken (Zhou *et al*., 2006). It could not directly explain that the different food intake and body weight between two types of chickens are associated with different mRNA levels of NPY in the hypothalamic PVN, IN and LHA. Cassy *et al*.(2004) suggested that the hyperphagic feeding of the meat-type chicken might be less sensitive to respond to leptin (an anorectic factor), but not to higher NPY level (an orexigenic factor). On the other hand, NPY exerts its actions via multiple receptor subtypes, and receptors Y1 and Y5 may mediate the orexigenic action in chickens (Kuenzel and Fraley, 1995). Thus, it could be considered that the expressions of hypothalamic Y1 and Y5 receptors differ between two types of chickens. However, knowledge about a possible different expression in NPY receptors between meat-type and layer-type chickens is limited.

Compared with the layer-type chicken, the behavior of the meat-type chicken was less active, and had less capability to adapt to a novel environment or was much less sensitive to changes in environment (Saito *et al*., 2005; Furuse, 2007). NPY administrated into PVN significantly increased searching behaviors for food in rats (Stanley and Leibowitz, 1984). These findings, together with our results, suggest that the higher mRNA level of hypothalamic NPY in the layer-type chicken is due to increased behaviors, including feeding behaviors. On the other hand, growth hormone inhibits the hypothalamic NPY in chickens (Wang *et al*., 2000), and the peak of growth hormone secretion appears at the age of 14~28 d (Burnside and Cogburn, 1992). Body weight of the meat-type chicken was significantly greater than that of the layer-type chicken at the same age, suggesting a rapid growing rate in the meat-type chicken. Therefore, the lower mRNA level of NPY in the PVN, IN and LHA may be due partly to a rapid increase of growth hormone in the meat-type chicken.

In conclusion, the meat-type chicken ate more food daily and had a greater body weight and brain weight than the layer-type chicken did at the age of 14 d. The orders of NPY mRNA levels were: IN>PVN=LHA in the meat-type of chicken, and IN=PVN>LHA in the layer-type of chicken. NPY mRNA levels in the IN, PVN and LHA were significantly greater in the layer-type chicken than those in the meat-type chicken. These results suggest the expression of NPY in the hypothalamus has a type-dependent pattern in the domestic chicken.

References

Aste, N., Viglietti-Panzica, C., Fasolo, A., Andreone, C., Vaudry, H., Pelletier, G., Panzica, G.C., 1991. Localization of neuropeptide Y-immunoreactive cells and fibers in the brain of the Japanese quail. *Cell and Tissue Research*, **265**(2):219-230. [doi:10.1007/BF00398070]

Boswell, T., 2005. Regulation of energy balance in birds by the neuroendocrine hypothalamus. *The Journal of Poultry Science*, **42**(3):161-181. [doi:10.2141/jpsa.42.161]

Boswell, T., Millam, J.R., Li, Q., Dun, I., 1998. Cellular localization of neuropeptide Y mRNA and peptide in the brain of the Japanese quail and domestic chicken. *Cell and Tissue Research*, **293**(1):31-38. [doi:10.1007/s004410051095]

Boswell, T., Dunn, I.C., Corr, S.A., 1999. Hypothalamic neuropeptide Y mRNA is increased after food restriction in growing broilers. *Poultry Science*, **78**(8):1203-1207.

Boswell, T., Li, Q., Takeuchi, S., 2002. Neurons expressing neuropeptide Y mRNA in the infundibular hypothalamus of Japanese quail are activated by fasting and co-express agouti-related protein mRNA. *Molecular Brain Research*, **100**(1-2):31-42. [doi:10.1016/S0169-328X(02)00145-6]

Burnside, J., Cogburn, L.A., 1992. Developmental expression

of hepatic growth hormone receptor and insulin-like growth factor-1 mRNA in the chicken. *Molecular and Cellular Endocrinology*, **89**(1-2):91-96. [doi:10.1016/0303-7207(92)90214-Q]

Cassy, S., Picard, M., Crochet, S., Derouet, M., Keisler, D.H., Taouis, M., 2004. Peripheral leptin effect on food intake in young chickens is influenced by age and strain. *Domestic Animal Endocrinology*, **27**(1):51-61. [doi:10.1016/j.domaniend.2004.01.004]

Denbow, D.M., 1999. Food intake regulation in birds. *Journal of Experimental Zoology*, **283**(4-5):333-338. [doi:10.1002/(SICI)1097-010X(19990301/01)283:4/5<333::AID-JEZ3>3.0.CO;2-R]

Esposito, V., Pelagalli, G.V., DeGirolamo, P., Gargiulo, G., 2001. Anatomical distribution of NPY-like immunoreactivity in the domestic chick brain (*Gallus domesticus*). *Anatomical Record*, **263**(2):186-201. [doi:10.1002/ar.1089]

Furuse, M., 2002. Central regulation of food intake in the neonatal chick. *Animal Science Journal*, **73**(2):83-94. [doi:10.1046/j.1344-3941.2002.00014.x]

Furuse, M., 2007. Behavioral regulators in the brain of neonatal chicks. *Animal Science Journal*, **78**(3):218-232. [doi:10.1111/j.1740-0929.2007.00429.x]

Furuse, M., Matsumoto, M., Mori, R., Sugahara, K., Kano, K., Hasegawa, S., 1997. Influence of fasting and neuropeptide Y on the suppressive food intake induced by intracerebroventricular injection of glucagon-like peptide-1 in the neonatal chick. *Brain Research*, **764**(1-2):289-292. [doi:10.1016/S0006-8993(97)00623-9]

Hensley, I.E., Lawler, J.E., Alemzadeh, R., Holshouser, S.J., 2001. Diazoxide effects on hypothalamic and extra-hypothalamic NPY content in Zucker rats. *Peptides*, **22**(6):899-908. [doi:10.1016/S0196-9781(01)00415-6]

Jang, M., Romsos, D.R., 1998. Neuropeptide Y and corticotropin-releasing hormone concentrations within specific hypothalamic regions of lean but not ob/ob mice respond to food-deprivation and refeeding. *Journal of Nutrition*, **128**(12):2520-2525.

Kameda, Y., Miura, M., Nishimaki, T., 2001. Localization of neuropeptide Y mRNA and peptide in the chicken hypothalamus and their alterations after food deprivation, dehydration and castration. *Journal of Comparative Neurology*, **436**(3):376-388. [doi:10.1002/cne.1074.abs]

Kuenzel, W.J., Masson, M., 1988. A Stereotaxic Atlas of the Brain of the Chick (*Gallus domesticus*). The Johns Hopkins University Press, Baltimore, p.1-10.

Kuenzel, W.J., Fraley, G.S., 1995. Neuropeptide Y: its role in the neural regulation of reproductive function and food intake in avian and mammalian species. *Avian and Poultry Biology Reviews*, **6**(3):185-209.

Kuenzel, W.J., Douglass, L.W., Davison, B.A., 1987. Robust feeding following central administration of neuropeptide Y or peptide YY in chicks, *Gallus domesticus*. *Peptides*, **8**(5):823-828. [doi:10.1016/0196-9781(87)90066-0]

Kuenzel, W.J., Mary, M.B., Teruyama, R., 1999. Neural sites and pathways regulating food intake in birds: a comparative analysis to mammalian systems. *Journal of Experimental Zoology*, **283**(4-5):348-364. [doi:10.1002/(SICI)1097-010X(19990301/01)283:4/5<348::AID-JEZ5>3.0.CO;2-5]

Masic, B., Wood-Gush, D.G.M., Duncan, I.J.H., McCorquodale, C., Savory, C.J., 1974. A comparison of feeding behavior of young broiler and layer males. *British Poultry Science*, **15**:281-286.

Oomura, Y., Ooyama, H., Yamamoto, T., Naka, F., 1967. Reciprocal relationship of the lateral and ventromedial hypothalamus in the regulation of food intake. *Physiology and Behavior*, **2**(2):97-115. [doi:10.1016/0031-9384(67)90020-0]

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**(1-3):201-208. [doi:10.1016/j.regpep.2004.09.003]

Savory, C.J., 1974. A comparison of the feeding behaviour of young broiler and layer males. *British Poultry Science*, **15**(15):499-505.

Schwartz, M.W., Woods, S.C., Porte, D.Jr, Seeley, R.J., Baskin, D.G., 2000. Central nervous system control of food intake. *Nature*, **404**(6778):661-671.

Stanley, B.G., Leibowitz, S.F., 1984. Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. *Life Science*, **35**(26):2635-2642. [doi:10.1016/0024-3205(84)90032-8]

Tachibana, T., Saito, S., Tomonaga, S., Takagi, T., Saito, E.S., Nakanishi, T., Koutoku, T., Tsukada, A., Ohkubo, T., Boswell, T., Furuse, M., 2004. Effect of central administration of prolactin-releasing peptide on feeding in chicks. *Physiology and Behavior*, **80**(5):713-719. [doi:10.1016/j.physbeh.2003.12.005]

Tatemoto, K., Carlquist, M., Mutt, V., 1982. Neuropeptide Y—a novel brain peptide with structural similarities to peptide YY and pancreatic. *Nature*, **296**(5858):659-660. [doi:10.1038/296659a0]

Wang, X., Day, J.R., Zhou, Y., Beard, J.L., Vasilatos-Younken, R., 2000. Evidence of a role for neuropeptide Y and monoamines in mediating the appetite-suppressive effect of GH. *Journal of Endocrinology*, **166**(3):621-630. [doi:10.1677/joe.0.1660621]

Wang, X., Day, J.R., Vasilatos-Younken, R., 2001. The distribution of neuropeptide Y gene expression in the chicken Brain. *Molecular and Cellular Endocrinology*, **174**(1-2):129-136. [doi:10.1016/S0303-7207(00)00436-6]

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 & Integrative Physiology*, **141**(2):146-152. [doi:10.1016/j.cbpb.2005.04.015]

Zhou, W., Aoyama, M., Yoshizawa, F., Sugahara, K., 2006. Developmental increases in hypothalamic neuropeptide Y content with the embryonic age of meat- and layer-type chicks. *Brain Research*, **1072**(1):26-29. [doi:10.1016/j.brainres.2005.11.029]