

## Effects of growth hormone and pair-feeding on leptin mRNA expression in liver and adipose tissue

C.M. Ashwell<sup>a</sup>, J.P. McMurtry<sup>a,\*</sup>, X.-H. Wang<sup>b</sup>,  
Y. Zhou<sup>b</sup>, R. Vasilatos-Younken<sup>b</sup>

<sup>a</sup>*Growth Biology Laboratory, Livestock and Poultry Science Institute, USDA-ARS, Beltsville, MD 20705, USA*

<sup>b</sup>*Department of Poultry Science, College of Agricultural Sciences, The Pennsylvania State University, University Park, PA 16802, USA*

Received 4 January 1999; accepted 26 February 1999

---

### Abstract

Previous research has reported that elevations in circulating growth hormone (GH) levels in meat-type chickens depresses feed intake (FI) more than 30%. It is known that the product of the obese gene, leptin, functions to regulate FI and energy expenditure. To investigate the effect of GH on leptin gene expression, broiler chickens were infused with recombinant chicken GH. To separate any secondary effects of a GH-induced reduction in FI on leptin expression, groups of birds were pair-fed to an average level of voluntary intake similar to GH-treated birds, but received no GH treatment. GH treatment induced a dose-dependent increase in liver leptin gene expression, as measured by reverse transcriptase-polymerase chain reaction, whereas leptin expression in adipose tissue was unchanged. Conversely, in chickens pair-fed (feed-restricted) there was a decrease in leptin gene expression in both tissues. These results provide evidence of a direct effect of GH on leptin gene expression, which is independent of any effects on intake attributable to GH-treatment, and suggest differential regulation of leptin expression between adipose tissue and liver. The results of these experiments provide the first evidence of a relationship between GH and leptin in domestic birds. © 1999 Elsevier Science Inc. All rights reserved.

---

\* Corresponding author. Tel.: +301-504-8222; fax: +301-504-8623.

E-mail address: mcmurtry@lpsi.barc.usda.gov (J.P. McMurtry)

## 1. Introduction

Leptin is the polypeptide hormone product of the obese (*ob*) gene and functions to regulate energy homeostasis [1]. Leptin is produced in the periphery and acts to control food intake (FI) and energy expenditure through a specific receptor in the hypothalamus [2]. Previous studies have described decreased FI as an effect of growth hormone (GH) treatment in humans, pigs, and chickens [3–6]. Several recent studies have attempted to identify a connection between the role of leptin in FI and the role of GH in growth stimulation [7]. Treatment of young pigs with leptin administered by intracerebroventricular injection has been shown to stimulate GH production [8]. In humans, there have been several reports of decreased or unchanged circulating leptin concentrations as a result of GH treatment, likely a result of decreased total fat mass [9,10]. Even though GH has been shown to reduce FI the functional relationship between GH and FI is relatively unknown.

Our interest lies in understanding the mechanism of FI regulation in meat-type chickens. We, as well as others, have shown the presence of a leptin homolog in chickens and expression in both adipose and liver tissues [11,12]. This difference in tissue localization of leptin expression in mammals, such as rodents, may be attributable to differing avian lipid metabolism where the liver is the primary source of lipogenesis [13]. Previous studies demonstrating that increased GH decreases FI in chickens by as much as 30% [14,15] suggested a possible role for leptin in GH effects on FI. Two experiments were conducted in this study to measure leptin expression levels in both adipose and hepatic tissues of birds either treated with recombinant chicken GH (rcGH) or birds pair-fed to the average level of FI of rcGH-treated birds, but receiving no rcGH treatment.

## 2. Methods

### 2.1. Animals and GH treatment

All animal experiments were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC) under approval No. 89R1389G197. Chickens (Petersen X Arbor Acre) were hatched and reared in the Pennsylvania State University Poultry Research and Education Center. Birds were fed a commercial broiler diet and maintained under a 16-h light, 8-h dark cycle with constant temperature (23°C). Six-week-old female broiler chickens with similar body weights were prepared surgically as described previously [16] by catheterization of the right jugular vein for i.v. delivery of rcGH (Lucky Biotech Corp./LG Chemical Ltd. Science Town, Taejon, Korea). Because of practical constraints, this study was completed in two experimental replicates. The i.v. infusions were performed as described previously [5] with administration of rcGH (dissolved in 0.025 M Na<sub>2</sub>CO<sub>3</sub>, pH 9.4 with 0.1% chicken serum albumin [17]) by a microprocessor infusion pump in a pulsatile manner for 7 consecutive d. Chicken GH was administered at five dosages (0, 10, 50, 100, and 200 g/kg bwt/d) ( $n = 4-5$ ), and birds were fed ad libitum and FI measured. A significant reduction in feed intake of 7.3% and 19.2% was observed in birds treated with 100 and 200  $\mu\text{g/kg}$  bwt/d of rcGH (averaged over the last 3 d of the 7-d treatment period),

respectively [18]. Immediately after delivery of a pulse on d 7, birds were killed and tissues (liver and abdominal fat pad) were harvested immediately, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until further analysis. Pair-feeding experiments were conducted similarly, but with no administration of rcGH, by restricting feed to FI levels of rcGH-treated birds, which exhibited significantly depressed FI relative to controls. These were 93% and 81% of controls, based on the FI of the 100 and 200  $\mu\text{g}/\text{kg}$  bwt/d rcGH treatments, respectively. On the seventh day of feed restriction, the pair-fed birds were killed and tissues were harvested as described previously.

## 2.2. RT-PCR assay for leptin expression

Total RNA was isolated from collected tissues using the TRI-Reagent procedure (Life Technologies, Rockville, MD) and quantitated spectrophotometrically at 260 nm, with acceptable 260/280 ratios of  $> 1.7$ . MMLV reverse transcriptase (RT) (Promega, Madison, WI) was used to reverse transcribe 10  $\mu\text{g}$  of total RNA isolated from both liver and adipose tissue of the rcGH-treated and pair-fed birds using an oligo dT [18] primer in a total reaction volume of 50  $\mu\text{l}$ . After the RT reaction, aliquots of 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, and 10  $\mu\text{l}$  were used as cDNA template for polymerase chain reaction (PCR) amplification, in duplicate, using an MJ Research PTC-100 programmable thermal controller (Watertown, MA) with *Taq* polymerase (Promega, Madison, WI). The thermal cycling parameters used were: 30 cycles,  $94^{\circ}\text{C}$  for 1 min,  $62^{\circ}\text{C}$  for 1 min, and  $72^{\circ}\text{C}$  for 1 min. These PCR reactions (50  $\mu\text{l}$ ) included the chicken leptin specific primer pair; sense LepUp2, 5'-CGTCGGTATCCGC CAAGCAGAGGG; antisense Lep-Dn2 5'-CCAGGACGCCATCCAGGCTCTCTGGC, that produce a 261-base pair (bp) amplification product. As an internal control for the integrity of the mRNA in each sample, additional oligonucleotide primers specific for chicken  $\beta$ -actin that produce a larger product (612 bp) were included in single (multiplexed) PCR reactions. Aliquots of 2.5 and 5.0  $\mu\text{l}$  from the RT reactions (linear range of amplification/detection) were used in the quantitative measurements for continuity of all unknown samples, which may underestimate leptin levels in birds treated with 200  $\mu\text{g}/\text{kg}$  bwt of rcGH. The reaction products were separated by agarose gel electrophoresis (2%), stained with ethidium bromide, and quantified by UV transillumination and video capture by an ALPHA INNOTECH gel documentation workstation. Samples were normalized to the quantity of  $\beta$ -actin signal produced by RT-PCR, and quantified for leptin expression by arbitrary units per  $\mu\text{g}$  of starting total RNA ( $n = 4-5$ ). Care was taken to measure DNA amplification product levels over the linear range of UV detection of ethidium bromide staining. Quantitative data obtained from this RT-PCR method has been verified previously by Northern analysis [11].

## 2.3. Statistical analyses

Statistical analyses were performed using the Student's *t* test to determine the significance of hormone-treatment differences in leptin expression levels. Nonlinear regression analysis of rcGH treatment effects on leptin expression was performed using the KaleidaGraph software package for Macintosh (version 3.0.2).

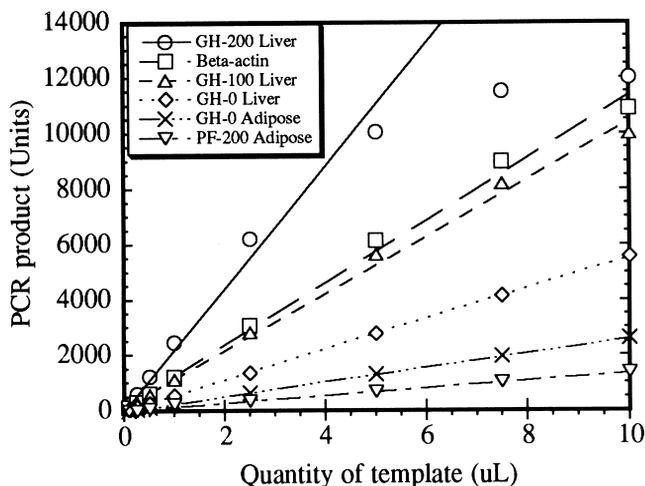


Fig. 1. RT-PCR assay for leptin expression. At 30 cycles of amplification, the linear range of leptin mRNA detection in tissues with the highest expression levels (liver from 200  $\mu\text{g}/\text{kg}/\text{d}$  rcGH-treated birds) extended from 0.5 to 5  $\mu\text{l}$  of cDNA produced by RT. Using this determination, the volumes of 2.5 and 5  $\mu\text{l}$  were used for evaluation of leptin expression because of both the need to measure the lowest leptin expression levels (PF-200) as well as mRNA levels some 20-fold higher (GH-200). Measurement of  $\beta$ -actin mRNA levels were also well within the linear range of detection from 0.5 to 5  $\mu\text{l}$  of cDNA included in the PCR reaction.

### 3. Results

Analysis of leptin expression by RT-PCR in tissues of chickens treated with rcGH indicated an increase in leptin mRNA levels. To determine the extent of the RT-PCR based assay to accurately measure leptin mRNA levels, tissues with variable leptin expression levels were examined by varying the amount of starting cDNA in the PCR amplification. The linear range of detection of leptin and  $\beta$ -actin mRNAs were from 0.5 to 5  $\mu\text{l}$  of reverse transcriptase derived cDNA for all expression levels (Fig. 1). Use of both 2.5 and 5  $\mu\text{l}$  volumes of cDNA in the standard assay PCR amplifications allowed for an average level of leptin expression to be measured for each tissue type from both high and low mRNA level tissues.

The leptin expression level in omental adipose tissue of control chickens was significantly different ( $P < 0.001$ ) and consistently about half of that found in an equivalent amount of total RNA isolated from liver as described previously [2]. Leptin expression is increased upon treatment with rcGH in the chicken liver (Fig. 2, Panel A). This increase in leptin mRNA, normalized to chicken  $\beta$ -actin expression levels, is significant ( $P < 0.05$ ) at doses of as little as 50  $\mu\text{g}/\text{kg}$  bwt/d (Table 1 and Fig. 2, Panel B). There is no change in the level of leptin expression in omental adipose tissue as a result of rcGH treatment. An apparent dose-response for rcGH treatment is observed, upon comparison of leptin mRNA levels measured in hepatic tissues (Fig. 2, Panel B).

To eliminate any secondary nutritional effects of reduced FI by rcGH treatment on leptin expression pair-feeding experiments were performed. In contrast with the results observed

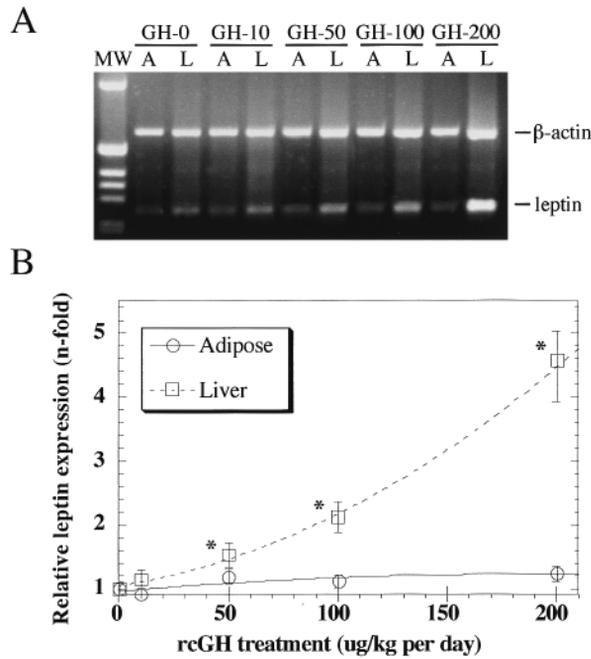


Fig. 2. Leptin expression in rcGH treated birds. (Panel A) Leptin expression was quantitated by normalization of each lane by the ethidium bromide staining of the  $\beta$ -actin RT-PCR product. The effect of rcGH on leptin expression in both liver (L) and adipose (A) tissues is indicated by the leptin band intensity for each treatment. (Panel B) The effect on leptin expression is expressed as the *n*-fold difference from that of controls for each rcGH treatment. Those rcGH treatments that caused significant differences ( $P < 0.05$ ) in leptin expression are indicated by “\*”. The dose response curve for the induction of hepatic leptin expression follows the polynomial equation  $Y = 1.055 + 0.000531x + 0.0000589x^2$ ,  $R^2 = 0.998$ .

with rcGH treatment, birds pair-fed to FI levels of those treated with 100 and 200  $\mu\text{g}/\text{kg}$  bwt/d rcGH, showed marked decreases in leptin expression in both adipose and hepatic tissues (Fig. 3, Panel A and Table 2). This decrease was approximately 50% of control leptin levels when normalized to  $\beta$ -actin expression (Fig. 3, Panel B).

Table 1  
Quantitation of leptin mRNA levels: Effects of rcGH treatment

rcGH treat. $\mu\text{g}/\text{kg}$ bwt/day ( <i>n</i> = 4)	Liver leptin (U/ $\mu\text{g}$ $\pm$ SD)	Liver Sig. Level	n-fold liver expression (relative to GH-O)	Adipose leptin (U/ $\mu\text{g}$ $\pm$ SD)	Adipose Sig. Level	n-fold adipose expression (relative to GH-O)
GH-0	2019 $\pm$ 63	–	1.0 $\pm$ 0.12	897 $\pm$ 38	–	1.0 $\pm$ 0.10
GH-10	2289 $\pm$ 178	$p < 0.23$	1.15 $\pm$ 0.14	856 $\pm$ 23	$p < 0.56$	0.91 $\pm$ 0.08
GH-50	3113 $\pm$ 220	$p < 0.04$	1.53 $\pm$ 0.19*	919 $\pm$ 44	$p < 0.11$	1.19 $\pm$ 0.12
GH-100	4761 $\pm$ 398	$p < 0.001$	2.12 $\pm$ 0.25*	941 $\pm$ 40	$p < 0.34$	1.12 $\pm$ 0.10
GH-200	9119 $\pm$ 484	$p < 0.001$	4.48 $\pm$ 0.56*	977 $\pm$ 51	$p < 0.06$	1.24 $\pm$ 0.12

\* Increasing GH treatment significantly increases leptin mRNA levels in liver, but not in adipose tissue. rcGH treatments that caused significant differences ( $P < 0.05$ ) in leptin expression are indicated by “\*”.

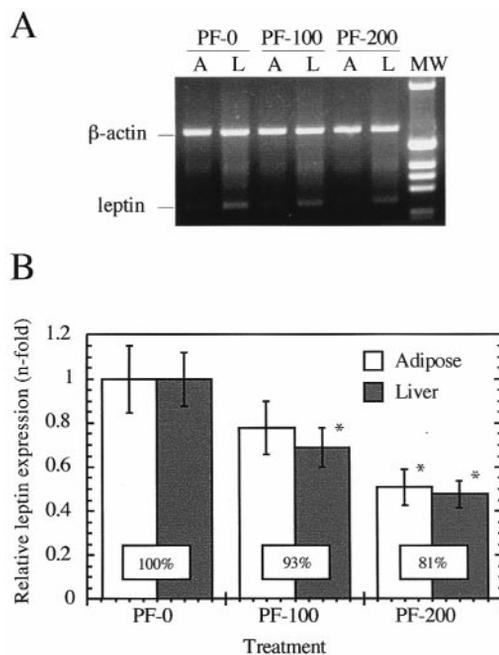


Fig. 3. Leptin expression in pair-fed birds. (Panel A) The effect of feed restriction (pair-feeding) on leptin expression in both liver (L) and adipose (A) tissues is indicated by the leptin band intensity for each treatment. (Panel B) The percent of normal feed intake is indicated for each group in boxed areas. The effect on leptin expression is expressed as the *n*-fold difference from that of controls for each rcGH treatment. Those rcGH treatments that caused significant differences ( $P < 0.05$ ) in leptin expression are indicated by “\*”.

#### 4. Discussion

The results of this study present a novel observation that circulating GH levels affect leptin expression and may thus modulate voluntary food consumption in chickens. Correlations between leptin mRNA levels and circulating leptin have been shown in rodents [19] and require the development of a valid assay for circulating chicken leptin or a suitable antibody for Western blot analysis [20]. Previously, GH was not shown to have an effect on leptin secretion by cultured adipocytes [21]. Our observation complements previous studies

Table 2  
Quantitation of leptin expression levels: Pair-feeding

RcGH treat (% normal F1) ( <i>n</i> = 5)	Liver leptin (U/ $\mu$ g $\pm$ SD)	Liver Sig. Level	n-fold liver expression (relative to PF-0)	Adipose leptin (U/ $\mu$ g $\pm$ SD)	Adipose Sig. Level	n-fold adipose expression (relative to PF-0)
PF-0 (100%)	2019 $\pm$ 63	—	1.0 $\pm$ 0.12	842 $\pm$ 27	—	1.0 $\pm$ 0.15
PF-100 (93%)	1393 $\pm$ 58	$P < 0.003$	0.69 $\pm$ 0.09*	668 $\pm$ 51	$P < 0.06$	0.78 $\pm$ 0.12
PF-200 (81%)	963 $\pm$ 66	$P < 0.001$	0.48 $\pm$ 0.06*	427 $\pm$ 43	$P < 0.001$	0.51 $\pm$ 0.08*

\* Reduction in FI causes a significant decrease in leptin mRNA levels in both liver and adipose tissue.

that have shown a reduction in NPY as a result of rcGH treatment in chickens [18]. The regulation of NPY production in the hypothalamus by leptin has been well documented in mammals [22]. It is therefore plausible to propose that GH regulates NPY production by way of leptin induction. Studies in pigs treated with intracerebroventricular injection of leptin not only found reduced FI, but also increased GH secretion [8]. The induction of hepatic leptin expression is apparently not associated with IGF-I upregulation by GH in the chicken liver due to previous studies showing no effect of IGF-I treatment on leptin expression in chickens [11]. These findings suggest a unique inter-relationship between GH and leptin [23]. The chicken may be a suitable model in which to study this relationship, because of the robust response of leptin to GH treatment, evidence that leptin directly affects FI [24], and similarities between the feeding control mechanisms of birds and mammals [25].

Results of the pair-feeding studies reflect comparable results to many studies in rodents and humans on the leptin response to reduced FI [26]. A study in pigs has shown similar reductions in leptin expression in domestic animals as a result of food deprivation [27]. Typical decreases in leptin expression are in the range of 40–60% on deprived diets (25% of maintenance) in both pigs and rodents. The same degree of leptin mRNA depression was observed in chickens fed 80% of normal intake.

These results suggest a more precise control of FI by leptin in birds than in mammals [26]. Future studies are needed to resolve the extent of interplay between leptin and GH in regulating FI in light of their apparent costimulatory behavior.

## Acknowledgments

The authors would like to thank Donna Brocht, GBL, USDA-ARS, for technical assistance. Mention of a trade name, proprietary product, or specific equipment, does not constitute a guarantee or warranty by USDA and does not imply its approval to the exclusion of other suitable products.

## References

- [1] Gura T. Obesity sheds its secrets. *Science* 1997;275:751–3.
- [2] Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Woolf EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995;83:1263–71.
- [3] Florkowski CM, Collier GR, Zimmet PZ, Livesey JH, Espiner EA, Donald RA. Low-dose growth hormone replacement lowers plasma leptin and fat stores without affecting body mass index in adults with growth hormone deficiency. *Clin Endocrinol* 1996;45:769–73.
- [4] Nam DS, Aherne FX, He P, Weingardt R, Schaefer AL. The selection of protein intake by pigs treated with porcine somatotropin. *J Anim Sci* 1995;73:764–72.
- [5] Vasilatos-Younken R, Cravener TL, Cogburn LA, Mast MG, Wellenreiter RH. Effect of pattern of administration on the response to exogenous pituitary-derived chicken growth hormone by broiler-strain pullets. *Gen Comp Endocrinol* 1988;71:268–83.
- [6] Wray-Cahen D, Ross DA, Bauman DE, Boyd RD. Metabolic effects of porcine somatotropin: nitrogen and energy balance and characterization of the temporal pattern of blood metabolites and hormones. *J Anim Sci* 1991;69:1503–14.

- [7] Considine RV. Weight regulation, leptin and growth hormone. *Horm Res* 1997;48:116–21.
- [8] Barb CR, Yan X, Azain MJ, Kraeling RR, Rampacek GB, Ramsay TG. Recombinant porcine leptin reduces feed intake and stimulates growth hormone secretion in swine. *Domest Anim Endocrinol* 1998;15:77–86.
- [9] Janssen YJH, Frolich M, Deurenberg P, Roelfsema F. Serum leptin levels during recombinant human GH therapy in adults with GH deficiency. *Eur J Endocrinol* 1998;137:650–4.
- [10] Wolthers T, Grofte T, Norrelund H, Poulsen PL, Andreasen F, Christiansen JS, Jorgensen JOL. Differential effects of growth hormone and prednisolone on energy metabolism and leptin levels in humans. *Metabolism* 1998;47:83–8.
- [11] Ashwell CM, Czerwinski SM, Brocht DM, McMurtry JP. Hormonal regulation of leptin expression in broiler chickens. *Am J Physiol* 1999;45:226–32.
- [12] Taouis M, Chen JW, Davidaud C, Dupont J, Derouet M, Simon J. Cloning the chicken leptin gene. *Gene* 1998;208:239–42.
- [13] O’Hea EK, Leurille CA. Lipogenesis in isolated adipose tissue of the domestic chick. *Comp Biochem Physiol* 1968;26:111–20.
- [14] Rosebrough RW, McMurtry JP, Vasilatos-Younken R. Effect of pulsatile or continuous administration of pituitary-derived chicken growth hormone (p-cGH) on lipid metabolism in broiler pullets. *Comp Biochem Physiol* 1991;99:207–14.
- [15] Vasilatos-Younken R, Tsao PH, Foster DN, Smiley DL, Bryant H, Heiman ML. Restoration of juvenile baseline growth hormone secretion with preservation of the ultradian growth hormone rhythm by continuous delivery of growth-hormone releasing factor. *J Endocrinol* 1992;135:371–82.
- [16] Cravener TL, Vasilatos-Younken R. A method for catheterization, harnessing, and chronic infusion of undisturbed chickens. *Lab Anim* 1989;23:270–4.
- [17] Peel CJ, Bauman DE, Gorewit RC, Sniffen CJ. Effect of exogenous growth hormone on lactational performance in high yielding dairy cows. *J Nutr* 1981;111:1662–71.
- [18] Wang XH, Day JR, Beard JL, Zhou Y, Vasilatos-Younken R. Evidence for mediation of the depressive effect of growth hormone on voluntary feed intake by neuropeptide Y and monoamines. *Poult Sci* 1998;77(suppl):1:33.
- [19] Patel BK, Koenig JI, Kaplan LM, Hooi SC. Increase in plasma leptin and Lep mRNA concentrations by food intake is dependent on insulin. *Metabolism* 1998;47:603–7.
- [20] Richards MP, Caperna T, McMurtry JP. Development of a polyclonal peptide antiserum for the universal detection of leptin. *FASEB J* 1998;12:A835.
- [21] Hardie LJ, Guilhot N, Trayhurn P. Regulation of leptin production in cultured mature white adipocytes. *Horm Metab Res* 1996;28:685–9.
- [22] Houseknecht KL, Baile CA, Matteri RL, Spurlock ME. The biology of leptin: a review. *J Anim Sci* 1998;76:1405–20.
- [23] Heiman ML, Chen Y, Caro JF. Leptin participates in the regulation of glucocorticoid and growth hormone axes. *J Nutr Biochem* 1998;9:553–9.
- [24] Raver N, Taouis M, Simon J, Robinzon B, Arnon E, Djiane J, Gertle A. Recombinant chicken obese protein (leptin) and its C4S analogue. Proceedings of the Endocrinology Society Meeting, P3–224. Bethesda, MD: Endocrine Society, 1998. p. 433.
- [25] Denbow DM. Peripheral regulation of food intake in poultry. *J Nutr* 1994;124:1349S–54S.
- [26] Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS. Role of leptin in the neuroendocrine response to fasting. *Nature* 1996;382:250–2.
- [27] Spurlock ME, Frank GR, Cornelius SG, Ji S, Willis GM, Bidwell CA. Obese gene expression in porcine adipose tissue is reduced by food deprivation but not by maintenance or submaintenance intake. *J Nutr* 1998;128:677–82.