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Cloning and Expression of the Duck Leptin Gene and the Effect of Leptin on Food Intake and Fatty Deposition in Mice

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ABSTRACT : Leptin is the adipocyte-specific product of the obese gene and plays a major role in food intake and energy metabolism. Leptin research was mainly focused on mammalian species, but understanding of leptin and its function in poultry is very poor. In this study, the duck leptin gene was amplified using the reverse transcription-polymerase chain reaction (RT-PCR) from duck liver RNA. The cDNA fragment was inserted into the pET-28a expression vector, and the resulting plasmid was expressed in *Escherichia coli* BL21 (DE3). Experimental mice were given an intraperitoneal injection of 10 mg/kg leptin dissolved in phosphate buffered saline (PBS), while the control mice were injected with PBS. The effect of leptin on food intake, body weight and fatty deposition in mice was detected. Sequence analysis revealed that duck leptin had a length of 438 nucleotides which encoded a peptide with 146 amino acid residues. The sequence shares highly homology to other animals. The coding sequence of duck leptin was 84 and 86% identical to human and pig leptin nucleotides sequence. Highest identity was with the rat coding sequence (95%). The identity of the amino acid sequence was 84, 82 and 96% respectively compared to that of the human, pig and rat. Results of SDS-PAGE analysis indicated that a fusion protein was specifically expressed in *E. coli* BL21 (DE3). The purified product was found to be biologically active during tests. Continuous administration of recombinant duck leptin inhibited food intake. Despite the decrease of food intake, leptin significantly induced body weight and fatty deposition. These changes were accompanied by a significant down-secretion of plasma glucose, cholesterol, triglyceride and insulin levels in mice. The observations provide evidence for an inhibitory effect of leptin in the regulation of food intake and for a potential role of duck leptin in the regulation of lipogenesis. (**Key Words** : Duck, Leptin, Expression, Food Intake, Fatty Deposition)

INTRODUCTION

Leptin, the adipocyte-specific product of the obese gene, is a recently discovered (Zhang et al., 1994) peptide hormone which regulates food intake (Halaas et al., 1995; Pelleymounter et al., 1995; Freidman et al., 1998), Reproduction (Moussavi et al., 2006) and energy balance (Mistry et al., 1997; Scarpace et al., 1997) in mammals and other vertebrates (Lin et al., 2000; Muruzábal et al., 2002). Leptin is produced and secreted by mammalian adipocytes and mediates its central effect through a specific receptor (Fei et al., 1997; Elmquist et al., 1998), thus modulating the hypothalamic neuropeptide system to suppress appetite and increase energy expenditure (Mizuno et al., 1998). Leptin is an important signaling factor that reflects body fat level. Leptin research has provided the key to understanding the molecular mechanisms underlying obesity.

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Leptin shares high conservation in vertebrates (Zhang et al., 1994). Leptin and its receptor have recently been cloned in the chicken (Taouis et al., 1998; Horev et al., 2000; Ohkubo et al., 2000). Chicken leptin is not exclusively localized in adipose tissue but is also expressed in liver (Taouis et al., 1998) and its expression is sensitive to hormonal treatment in liver but not in adipose tissue (Ashwell et al., 1999). Chicken leptin has a local potential role in the regulation of avian hepatic lipogenesis (Dridi et al., 2005). These observations are thought to be due to the role of the avian liver as the primary source of lipogenesis (Leveille et al., 1968). Reports concerning the biological role of leptin in birds are scarce (Taouis et al., 2001; McMurtry et al., 2002). The decrease in food intake observed in layer and broiler chickens injected centrally or peripherally with recombinant chicken, ovine or human leptin (Denbow et al., 2000; Dridi et al., 2000; Cassy et al., 2004), and in a wild bird species injected with chicken leptin (Lohmus et al., 2003), suggests that in birds leptin may play a similar role in regulating energy balance as it

does in mammals. Among non-mammalian species, the duck is also subjected to fattening with an increase in abdominal and subcutaneous fat deposits. Several *in vivo* and *in vitro* studies have shown a direct effect of leptin on lipolysis and lipogenesis in vertebrates (Lopez-Soriano et al., 1998; Wang et al., 1999). Such effects are still unknown in the duck. The objective of this study was to identify the duck leptin gene and evaluate the effect of intraperitoneal injections of leptin on the food intake, body weight and fatty deposition. Lipid metabolism was also assessed in the treated mice. The results will hopefully establish a foundation for study of the function and characteristics of poultry leptin.

MATERIALS AND METHODS

RNA isolation

Total RNA was prepared from duck liver using the Trizol regent according to manufacture's recommendation (Takara, Tokyo, Japan). Pellets were suspended in DEPC-treated water. The concentration of total RNA was estimated by measuring the absorbance at 260 nm, and the purity was determined from the ratio of absorbance at 260/280 nm.

Cloning of duck leptin gene

Five micrograms of total RNA were reverse transcribed (RT) at 42°C with a Super-Script II First-Strand Synthesis System for RT-PCR (Invitrogen). A set of specific primers was prepared from human (Zhang et al., 1994), mouse (Zhang et al., 1994), chicken (Taouis et al., 1998) and other animal (Ahima et al., 2000) leptin sequence. Primers were purchased from Takara (Tokyo, Japan). DNA was amplified using PTC-200 (MJ Research USA) for 32 cycles. The conditions for PCR were denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s and a final extension at 72°C for 10 min using pairs of the sense (5'-AGGAATTCGTGCCTATCCAGGATG-3') and antisense (5'-ACAAGCTTCTCAGCATTCAGGGCT-3') primer. The PCR products were isolated by electrophoresis on 1.5% agarose gel. The amplified fragment was purified using a QIAEXIIGel Extraction Kit (QIAGEN) and cloned into pGEM-T (Promaga) vector using T_4 DNA ligase (Promaga). The sequence of gene fragments was determined and homologies of the gene fragment from other species were compared.

Phylogenetic tree

A leptin phylogenetic tree was created following alignments (Megalin Clustal W) using Phylogenetic Analysis Using Parsimony (PAUP) version 4.0 beta10 and trees were constructed using the neighbor joining method.

Expression of duck leptin gene in *Escherichia coli* BL21 (DE3)

The duck leptin gene was cloned into *EcoR* I-*Hind* III sites of the pET-28a vector to yield the pET-28a-Lep plasmid. The recombinant plasmid was confirmed by restriction analysis and transformed into *E. coli* BL21 cells. The positive clone was selected for incubation in LB culture containing 34 mg/L kanamycin. The culture was grown to OD_{600} = 0.8. Three hours after induction by 0.1 M IPTG, the culture was collected and analyzed by 12% SDS-PAGE. The fusion protein was identified with commercial goat anti-human leptin antibody (Santa Cruz Biotechnology) by western blotting.

Recombinant protein extraction and purification

After one liter bacterial culture was grown and induced as described above, the cells were collected by centrifugation, re-suspended in 100 ml pre-cooled pH 8.0 buffer A (Tris-Cl 50.0 mmol/L, EDTA 0.5 mmol/L, NaCl 50.0 mmol/L, Glycerine 5%, DTT 0.5 mmol/L) and 100 ml 1% TritonX-100. The sample was lysed by ultrasonication. The lysate was centrifuged at 12,000 rpm for 30 min and 2% sodium deoxycholate was added to the inclusion body. Inclusion body was collected by centrifugation, re-suspended in 394 ml buffer A and 6 ml 20% sodium lauryl sarcosinate. The sample was centrifuged at 12,000 rpm (4°C) for 30 min and then 20% PEG-4000, 50 mmol/L oxidized glutathione, and 100 mml/L reduced glutathione were added to the supernatant to final concentrations of 0.2%, 1 mmol/L and 2 mmol/L, respectively. The protein was then dialyzed by 10 mmol/L Tris-Cl (pH 8.0) for 3 days and concentrated by PEG-20000 (Promaga).

Effect of leptin on food intake and fatty deposition in mice

Mice were purchased from Huazhong Agricultural University Experimental Animals Center. All protocols for animal use were reviewed and approved by the Huazhong Agricultural University Committee on Laboratory Animals. Twenty Kunming mice were divided into two groups of ten. Mice were housed in individual cages in a temperature-, humidity-, and light-controlled (6-20 h) room with free access to water and a commercial diet. One week later, one group (27.96±1.7 g) was used as control and given an intraperitoneal injection with 10 mg/kg phosphate buffered saline (PBS) daily (07:00-08:30 h), the other group (27.96±1.6 g) received an equal amount (10 mg/kg) of leptin dissolved in PBS. Food intake and body weight were recorded daily. After 6 days, blood was taken (07:00-8:30 h) from the caudal vein. Blood glucose, triglycerides and cholesterol were determined by enzymatic methods (Zhongsheng Ltd, Beijing, China) using an automatic blood analyzer (Hitachi 747 auto-analyzer, Tokyo, Japan). Plasma

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Duck                VPIQKVQDDTKTLIKTIVTRINDISHTQSVSAKQRVTGI
Sus scrofa          VPIWRVQDDTKTLIKTIVTRISDISHMQSVSSKQRVTGI
Homo sapiens        VPIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVTGI
Monkey              VPIQKVQSDTKTLIKTIVTRINDISHTQSVSSKQRVTGI
Mus musculus        VPIQKVQDDTKTLIKTIVTRINDISHTQSVSAKQRVTGI
Gallus gallus       VPCQIFQDDTKTLIKTIVTRINDISHT-SVSAKQRVTGI
Turkey              VPCQIFQDDTKTLIKTIVTRINDISHT-SVSAKQSVTGI
Rattus norvegious   VPIHKVQDDTKTLIKTIVTRINDISHTQSVSARQRVTGI
                    **   .*.**********:**.****  ***: :* *:**

Duck                YQQVLTSLPSQNVLQIADDLENLRDLLHLLAFSKSCSLH
Sus scrofa          YQQILTSLPSRNVIQISNDLENLRDLLHLLASSKSCPLH
Homo sapiens        YQQILTSMPSRNVIQISNDLENLRDLLHVLAFSKSCHLH
Monkey              YQQILINLPSRNVIQISNDLENLRDLLHLLAFSKSCHLH
Mus musculus        YQQVLTSLPSQNVLQIANDLENLRDLLHLLAFSKSCSLH
Gallus gallus       YQQVLTSLPSQNVLQIANDLENLRDLLHLLAFSKSCSLH
Turkey              YQQVLTSLPSQNVLQIANDLENLRDLLHLLAFSKSCSLH
Rattus norvegious   YQQILTSLPSQNVLQIAHDLENLRDLLHLLAFSKSCSLH
                    ***:*  . :  *:**:**:.**********:** **.* **

Duck                TEVVALSRLQGSLQDILQQLDVSPEC
Sus scrofa          TEVVALSRLQGALQDMLRQLDLSPGC
Homo sapiens        TEVVALSRLQGSLQDMLWQLDLSPGC
Monkey              TEVVALSRLQGSLQDMLWQLDLSPGC
Mus musculus        TEVVALSRLQGSLQDILQQLDVSPEC
Gallus gallus       TEVVALSRLQGSLQDILQQLDISPEC
Turkey              TEVVALSRLQGSLQDILQQLDISPEC
Rattus norvegious   TEVVALSRLQGSLQDILQQLDLSPEC
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Figure 1. Alignment of the deduced duck leptin amino acid sequence with homologues in animals. The conserved similar amino acids (*) in all animals are marked. GenBank accession numbers: *Sus scrofa*, NM_213840; *Homo sapiens*, NP-000221; Monkey, CB550068; *Mus musculus*, NM_008493;*Gallus gallus*, AF082500; Turkey, AAC32381. *Rattus norvegicus*, NM_013076.

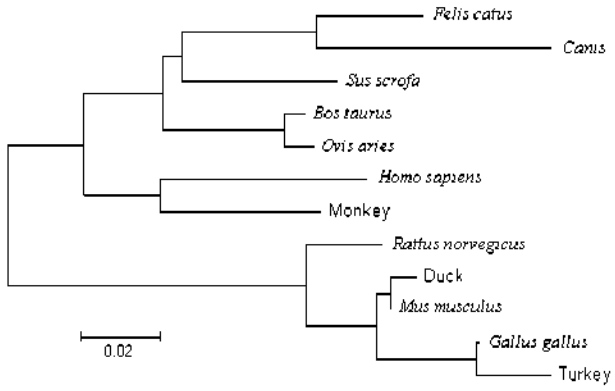

Figure 2. Phylogenetic relationships among vertebrate leptin amino acid sequence were inferred using the neighbor-joining method in Clustal W and Mega 2. GenBank accession numbers: *Felis catus*, NM_001009850; *Sus scrofa*, NM_213840; *Bos taurus*, NM_173928; *Canis*, NM_00100307; *Ovis aries*, OAU84247; *Homo sapiens*, NP-000221; Monkey, CB550068; *Rattus norvegicus*, NM_013076; *Mus musculus*, NM_008493; *Gallus gallus*, AF082500; Turkey, AAC32381.

insulin was determined by radio-immunoassay kits (Beijing Kemei Dongya biologic technique Ltd., China). The mice were killed by decapitation, and retroperitoneal fats, mesenteric fat, and other body fats were separated and weighed.

Statistical analysis

All results were expressed as means±SE. The results were analyzed by one-way analysis of variance (ANOVA) with the SPSS 12.0 program. The level of significance was set at $p<0.05$.

RESULTS

Cloning and sequence analysis of duck leptin gene

RT-PCR was performed using designed primers from duck liver total RNA. The fragment comprised an open reading frame of 438 bp encoding a deduced protein of 146 amino acids (GenBank accession number AY547279). The nucleotide sequence of the duck leptin gene and the predicted amino acid sequence were aligned with those of humans, pigs, and rats (Figure 1). The sequences shared a high homology with those of other species. GenBank Blast analysis of the duck leptin gene demonstrated that this cDNA had an 84 and 86% homology to that of humans and pigs, respectively. The highest homology was with the rat coding sequence (95%). Compared to those of humans, pigs, and rats, the homology of the amino acid sequences was 84, 82, and 96%, respectively. In the phylogenetic analysis of leptin amino acid sequence, duck leptin sequence demonstrated a high conservation with the leptin amino acid sequence of other animals (Figure 2).

Expression of duck leptin gene in *Escherichia coli* BL21 (DE3)

E. coli BL21 (DE3) cells harboring pET28a-Lep were cultured. After inducing expression by 0.2 M IPTG for 3 h at 37°C, the recombinant protein was highly efficiently

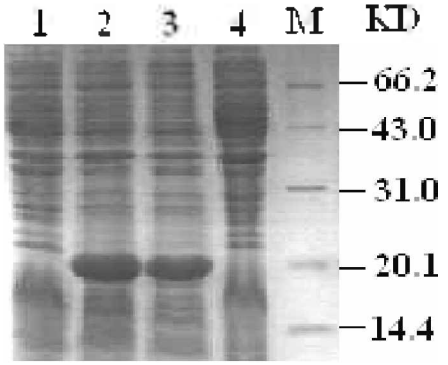

Figure 3. SDS-PAGE analysis for expression of duck leptin gene. Lane 1: pET-28a vector induced in *E. coli* BL21 (DE3); Lane 2 and 3: pET-28a-Lep induced in *E. coli* BL21 (DE3); Lane 4: pET-28a-Lep uninduced in *E. coli* BL21 (DE3); Lane M: Protein marker.

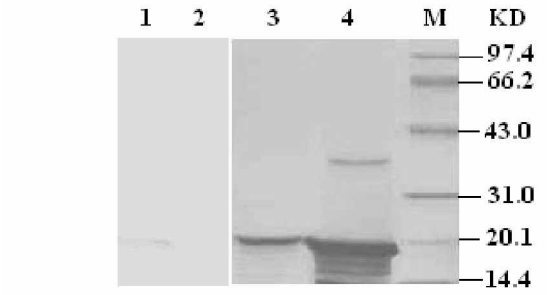

Figure 4. SDS-PAGE analysis and Western blotting for purification of recombinant protein. Lane 1: Recombinant protein by Western blotting; Lane 2: Negative control of pET-28a vector; Lane 3: Purified recombinant protein; Lane 4: Inclusion body; Lane M: Protein marker.

expressed in *E. coli* BL21 (DE3) (Figure 3). SDS–PAGE analysis of the cell lysate detected a protein band with molecular weight of about 20 kDa, which corresponded to the calculated molecular weight of recombinant leptin. The 16 kDa protein expressed by the duck leptin gene. The expressed protein was about 57% of total bacterial protein as determined by densitometric scanning (Figure 2)

Biological analysis of duck leptin

Following daily intraperitoneal injections of leptin, food intake and body weight of mice obviously decreased ($p<0.05$) (Figures 5 and 6). On the seventh day, the body fat level of the leptin-injected mice was lower than that of control mice ($p<0.05$) (Figure 7). These variations suggested that duck leptin could decrease body weight, food intake, and fatty deposition. Despite these results, the changes were accompanied by a significant down-secretion of plasma glucose, cholesterol, triglyceride and insulin levels in mice. Duck recombinant leptin protein may therefore serve an endocrine function in regulating body fat storage.

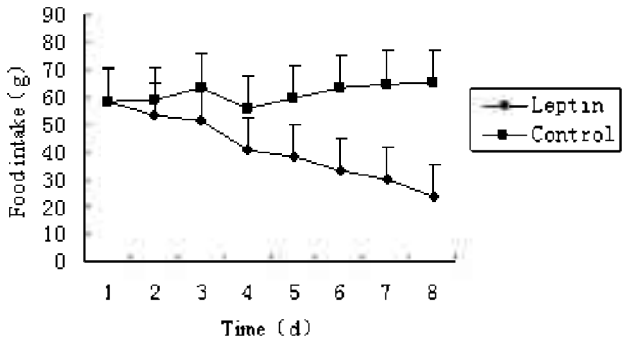

Figure 5. The effect of recombinant duck leptin on the food intake of Kunming mice. At time points marked with asterisk (*) the differences between the control and treatment were statistically significant ($p<0.05$). Each value is the mean±SE of 10 mice.

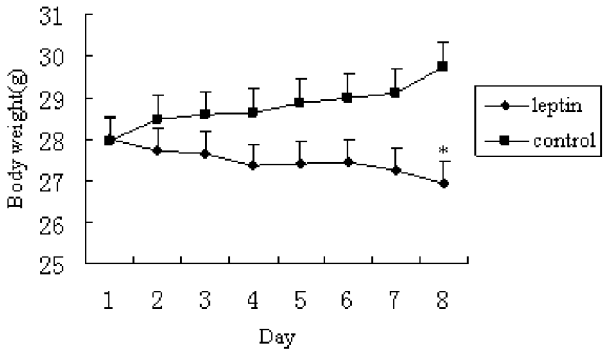

Figure 6. The effect of recombinant duck leptin on the body weight of Kunming mice. At time points marked with asterisk (*) the differences between the control and treatment were statistically significant ($p<0.05$). Each value is the mean±SE of 10 mice.

DISCUSSION

Leptin was initially discovered in mice and is highly conserved in vertebrate species. Chicken leptin and its receptor have been reported recently (Taouis et al., 1998; Horev et al., 2000; Ohkubo et al., 2000). According to the characteristics of leptin gene encoding sequences in humans, rats, pigs and other animals (Zhang et al., 1994; Taouis et al., 2000), a pair of primers was designed, and we have successfully cloned the duck leptin gene. This study demonstrates that the duck leptin gene shares a high degree of conservation with the leptin sequence of other animals. Particularly in comparison to the rat, the alignment of the nucleotide sequence and the amino acid sequence in the duck is up to 95 and 96%, respectively. From the phylogenetic relationships, the leptin gene shares a high conservation in the evolution of species.

Since its discovery in mammals (Zhang et al., 1994), leptin has been established as a regulator of multiple physiological functions including energy balance, metabolism and neuroendocrine pathways (Ahima et al.,

Table 1. Effect of recombinant duck leptin protein on plasma levels of glucose, cholesterol, triglyceride and insulin of mice

	Glucose (mmol/L)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Insulin (uIU/ml)
Control	9.89±0.54	3.72±0.10	3.5±90.14	2.39±0.25
Leptin	8.01±0.36[a]	2.86±0.15[a]	2.99±0.20[a]	1.86±0.27[a]

The data represents means±SE of the experiments. Letters with each group represent significant difference ($p<0.05$).

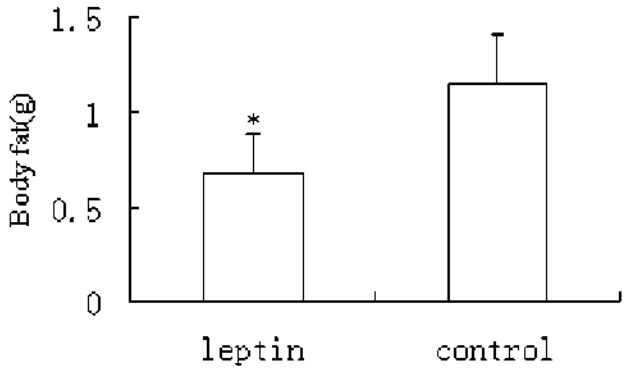

Figure 7. The effect of recombinant duck leptin on the body fat of Kunming mice. At time points marked with asterisk (*) the differences between the control and treatment were statistically significant ($p<0.05$). Each value is the mean±SE of 10 mice.

2000). The effects of leptin on food intake have given rise to the hypothesis that leptin has a role in the feedback regulation of adipose mass on food intake. Daily intraperitoneal injection of mice with recombinant leptin lowered their body weight, body fat level, and food intake (Pelleymounter et al., 1995; Houseknecht et al., 1998). With the aim of studying duck leptin function, we constructed the expression vector of the duck leptin gene, and it was highly efficiently expressed in *E. coli* BL21. The amino acid residues of about 4 kDa of the pET-28a vector and the target protein formed the fusion protein. The molecular mass of the fusion protein was about 20 kDa. A clear effect of injected leptin on the food intake, body weight, and fatty deposition was demonstrated. The administered recombinant duck leptin depressed body weight and fatty deposition and inhibited food intake, thereby confirming its biological activity. Surprisingly, despite the decrease in food intake, body weight and fatty deposition, leptin depressed the levels of glucose, cholesterol, triglyceride and insulin in plasma indicating a potential role of leptin in the regulation of duck lipid metabolism. Experimental evidence has shown that leptin could inhibit lipogenesis in adipose and hepatic tissues (Bryson et al., 1999). In addition, leptin specifically inhibits fatty acid synthase (FAS) gene expression by converging on the insulin/glucocorticoid response element (Fukuda et al., 1999). Muller et al. (1997) demonstrated leptin inhibition of the metabolic actions of insulin including insulin stimulation of lipogenesis. Chicken leptin can inhibit food intake and significantly induce the expression of FAS in chicken liver. The results suggest a local potential role of leptin in the regulation of avian hepatic lipogenesis (Dridi et al., 2005).

In conclusion, the present study reports the duck leptin gene and shows the leptin sequence has high identity with the leptin gene of other animals. Daily intraperitoneal injections of duck leptin protein reduced the body weight of mice. The leptin protein reduced food intake and increased energy consumption in mice, suggesting that the leptin gene protein serves an endocrine function in regulating body fat storage (Lin et al., 2000). The results demonstrate that the duck leptin may share the same function with that in other animals. The cloning and expression of the duck leptin gene has established the foundation for further studies on the function and application of duck leptin.

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REFERENCES

Ahima, R. S and J. S. Flier. 2000. Leptin. Annu. Rev. Physiol. 62:413-437.

Ashwell, C. M., S. M. Czerwinski, D. M. Brocht and J. P. McMurtry. 1999. Hormonal regulation of leptin expression in broiler chickens. Am. J. Physiol. 276:R226-232.

Bryson, J. M., J. L. Phuyal, V. Swan and I. D. Caterson. 1999. Leptin has acute effects on glucose and lipid metabolism in both lean and gold thioglucose-obese mice. Am. J. Physiol. 270:E417-422.

Cassy, S., M. Picard, S. Crochet, M, Derouet, D. H. Keisler and M. Taouis. 2004. Peripheral leptin effect on food intake in young chickens is influenced by age and strain. Domest. Anim. Endocrinol. 27:51-61.

Denbow, D. M., S. Meade, A. Robertson, J. P. McMurtry, M. Richards and C. M. Ashwell. 2000. Leptin-induced decrease in food intake in chickens. Physiol. Behav. 69:359-362.

Dridi, S., J. Buyse, E. Decuypere and M. Taouis. 2005. Potential role of leptin in increase of fatty acid synthase gene expression in chicken liver. Domest. Anim. Endocrinol. 29:646-660.

Dridi, S., N. Raver, E. E. Gussakovsky, M. Derouet, M. Picard, A. Gertler and A. Taaouis. 2000. Biological activities of recombinant chicken leptin C4S analog compared with unmodified leptons. Am. J. Physiol. Endocrinol. Metab. 279:E116-123.

Elmquist, J. K., R. S. Ahima, C. F. Elias, J. S. Flier and C. B. Saper. 1998. Distributions of leptin receptor mRNA isoforms in the

rat brain. J. Comp. Neurol. 395:535-547.

Fei, H., H. J. Okano, C. Li, G. H. Lee, C. Zhao, R. Darnell and J. M. Friedman. 1997. Anatomic localization of alternatively splicedleptin receptors (ob-R) in mouse brain and other tissues. Proc. Natl. Acad. Sci. USA. 94:7001-7005.

Freidman, J. M. and J. L. Halaas. 1998. Leptin and the regulation of body weight in mammals. Nature. 395:763-770.

Fukuda, H., N. Iritani, T. Sugimoto and H. Ikeda. 1999. Transcriptional regulation of fatty acid synthase gene by insulin/glucose, polyunsaturated fatty acid and leptin in hepatocytes and adipocytes in normal and genetically obese rats. Eur. J. Biochem. 260:505-511.

Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley and J. M. Friedman. 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Sci. 269:543-546.

Horev, G., P. Einat, T. Aharoni, Y. Eshdat and M. Friedman-Einat. 2000. Molecular cloning and properties of the chicken leptin-receptor gene. Mol. Cell. Endocrinol. 162:95-106.

Leveille, G. A., E. K. O'hea and K. Chakrabarty. 1968. *In vivo* lipogenesis in the domestic chicken. Proc. Soc. Exp. Biol. Med. 128:398-401.

Lin, X., V. H. Volko., Y. Narnaware, N. J. Bernier, P. Peyon and R. E. Peter. 2000. Review: Brain regulation of feeding behavior and food intake in fish. Comp. Biochem. Physiol. A. 126:415-434.

Lohmus, M., L. F. Sundstrom, M. El Halawani and B. Silverin. 2003. Leptin depresses food intake in great tits (Parus major). Gen. Comp. Endocrinol. 13:57-61.

Lopez-Soriano, J., N. Carbo, F. J. Lopez-Soriano and J. M. Argil´es. 1998. Short-term effects of leptin on lipid metabolismin the rat. FEBS Lett. 431:371-374.

McMurtry, J. P., C. Ashwell, M. Richards and R. Vasilatos-Younken. 2002. Leptin: molecular biology and physiology (Ed. A. Dawson, C. M. Chaturvedi). Avian endocrinology. New Dehli, India: Narosa Publishing House.pp. 317-328.

Mistry, A. M., A. G. Swick and D. R. Romsos. 1997. Leptin rapidly lowers food intake and elevates metabolic rates in lean and ob/ob mice. J. Nutr. 127:2065-2072.

Mizuno, T. M., S. P. Kleopoulos, H. T. Bergen, J. L. Roberts, C. A. Priest and C. V. Mobbs. 1998. Hypothalamic proopiomelanocortinm RNA is reduced by fasting in ob/ob and db/db mice, but is stimulated by leptin. Diabetes. 47:294-297.

Moussavi, A. H., M. Ahouei, M. R. Nassiry and A. Javadmanesh. 2006. Association of Leptin Polymorphism with Production, Reproduction and Plasma Glucose Level in Iranian Holste in cows. Asian-Aust. J. Anim. Sci. 19(5):627-631.

Muller, G., J. Ertl, M. Gerl and G. Preibish. 1997. Leptin impairs metabolic actions of insulin in isolated rat adipocytes. J. Biol. Chem. 272:10585-10593.

Muruzábal, F. J., G. Fruhbeck, J. Gomez-Ambrosi, M. Archanco and M. A. Burrel. 2002. Immunocytoc-hemical detection of leptin in non-mammalian vertebrate stomach. Gen. Comp. Endocrinol. 128:149-152.

Ohkubo, T., M. Tanaka and K. Nakashima. 2000. Structure and tissue distribution of chicken leptin receptor (cOb-R) mRNA. Biochim. Biophys. Acta. 1491:303-308.

Pelleymounter, M. A., M. J. Cullen, M. B. Baker, R. Hecht, D. Winters, T. Boone and F. Collins. 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. Sci. 269:540-543.

Houseknecht, K. L., C. A. Baile, R. L. Matteri and M. E. Spurlock. 1998. The biology of leptin: a review. J. Anim. Sci. 76:1405-1420.

Scarpace, P. J., M. Matheny, B. H. Pollock and N. Tumer. 1997. Leptin increases uncoupling protein expression and energy expenditure. Am. J. Physiol. 273:E226-230.

Taouis, M., J. W. Chen, C. Daviaud, J. Dupont, M. Derouet and J. Simon. 1998. Cloning the chicken leptin gene. Gene. 208:239-242.

Taouis, M., S. Dridi, S. Cassy, Y. Benomar, N. Raver, N. Rideau, M. Picard, J. Williams and A. Gertler. 2001. Chicken leptin: properties and actions. Domest. Anim. Endocrinol. 21:319-327.

Wang, M. Y., Y. Lee and R. H. Unger. 1999. Novel form of lipolysis induced by leptin. J. Biol. Chem. 274:17541-17544.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425-432.