Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Dynamic Expression of Genes Related to Feed Intake in Broilers Regulated by Aging and Fasting Conditions

Qian Ban[1§], Dahai Liu[1§], Wenqiao Hui[2§], Fei Cheng[1] and Xiaojun Liu[3,4*]

[1]School of Life Science, Anhui University, Road Jiulong, Hefei 230601, Anhui, People's Republic of China; [2]Institute of Animal Science, Anhui Academy of Agricultural Sciences, Road Nongke, Hefei 230031, Anhui, People's Republic of China; [3]College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, 450002, Henan, People's Republic of China; [4]College of Animal Science and Technology, Shihezi University, Road Beisi, Shihezi 832003, Xinjiang, People's Republic of China
*Corresponding author: xjliu2008@hotmail.com

ARTICLE HISTORY (14-510)

Received: September 30, 2014
Revised: November 24, 2014
Accepted: January 14, 2015

Key words:
Chicken
Feed deprivation
Gene expression

ABSTRACT

This study was designed to investigate the dynamic expression of genes related to regulation of feed intake, energy homeostasis and body weight, including *AdipoR2*, *CPT-1*, *NPY*, *LEPR* and *STAT3* and the effect of fasting on their expression in growing broiler chicken with different days of age. Forty male broiler chicks were divided into two groups (the control and fasted groups). Chickens in the control group were continuously fed with nutrition regimen until the end of experiment, while their counterparts were fasted for 3 days before sampling. Chickens were humanely killed on 17, 23, 28, and 33 day-old, respectively, and the adipose and hypothalamic samples were collected. Expressions of *AdipoR2*, *CPT-1*, *LEPR* and *STAT3* in the adipose tissue, and *NPY* in the hypothalamic tissue were detected by Real-time PCR. Results showed that in the control group, expressions of *AdiopR2*, *CPT1* and *STAT3* decreased with age, but not significantly ($P>0.05$), while *NPY* and *LEPR* expression trends were not regular during the whole experiment. In addition, *AdipoR2*, *CPT1* and *NPY* expression in the fasted group was significantly higher than controls (*AdipoR2*: $P<0.05$ on day 17; *CPT1*: $P<0.01$ on day 33 and *NPY*: $P>0.05$ during the whole experiment), although their expression trend is similar in the two groups. However, *LEPR* and *STAT3* expression trends were not regular both in the feed and fasted group. It is concluded that fasting stimulated the up-regulation of genes related to fatty metabolism and feed intake, while *LEPR* and *STAT3*, two of the important components in fatty metabolism regulation pathways in mammalians, did not show regular changes in chicken. A following *in vitro* study, therefore, needs to be further carried out to study the functions of chicken *LEPR* and its downstream genes like *STAT3*.

To Cite This Article: Ban Q, D Liu, W Hui, F Cheng and X Liu, 2015. Dynamic expression of genes related to feed intake in broilers regulated by aging and fasting conditions. Pak Vet J, 35(2): 207-211.

INTRODUCTION

Living in a population, animals always experienced periodic feed deprivation during the growing phase and they have already evolved a metabolic response system allowing them to survive the difficulties. However, during feed deprivation periods, animals might be confronted with high metabolic demand, as a result, they may suffer from the cessation of growth, breakdown and utilization of stored energy reserved (Désert *et al*., 2008). It has been demonstrated that overall response to fasting operates at numerous levels, varying from macroscopic changes like body weight, to molecular mechanism, such as mRNA levels of genes related to feed intake, and transcription regulators involved in fat metabolism. In the past decades, research on molecular response to fasting have received much more attention, in a variety of model animals, ranging from mammalian animals such as mice (Bauer *et al*.,2004; Weems *et al*., 2012; Kawase *et al*., 2013), rats (Wang *et al*., 2011) and pigs (Cheon *et al*., 2005; Rakhshandehroo *et al*., 2007), to avian like chicken

§These authors contributed equally to this study.

(Higgins *et al*., 2010; Dunn *et al*., 2013; Ji *et al*., 2013) and more recently to fish such as goldfish (Tinoco *et al*., 2012) and orange-spotted grouper (Zhang *et al*., 2012).

In chicken, molecular mechanism of response to feed deprivation mainly involved in the expression of genes related to feed intake like *NPY*, one of the most potent orexigenic factors known to date (Chee and Colmers, 2008) and genes associated with fat metabolism, such as *AdipoR*, acting as mediating the metabolism in chicken (Ramachandran *et al*., 2013), *CPT1*, a mitochondrial enzyme, which is important in many metabolic processes and weight regulation (Liu and Zhu, 2012) and the hot topic of LEPR and even its downstream pathway genes (Xu *et al*., 2013).

However, very little information is available so far about dynamic changes of genes related to feed intake, fatty metabolism and body weight, when deprived of feed at different growth period in chicken and the molecular mechanisms stimulated by fasting remain elusive. The present study, therefore, was designed to detect the dynamic expression of genes including *AdipoR2*, *CPT1*, *NPY*, *LEPR* and *STAT3* in growing broiler chicken at different growth period and also to determine the effect of fasting on expression of these genes.

MATERIALS AND METHODS

Animals and tissue collection: Forty male broiler (Aiweiyin) chicks were purchased from a local hatchery (Xinjiang Taikun Chicken Breeders Farm) at 1 day of age and reared in an environmentally controlled room. They were provided *ad libitum* access to feed and water using broiler pellet diet, prior to the experiment. The birds were randomly divided into two groups: the control and fasted groups, with 20 birds in each group. Chicken in each group were divided into 4 cages (5 individuals in each cage), numbered C1, C2, C3, C4, respectively. In the control group, the cages were named as CC1, CC2, CC3, CC4, respectively, while, in the fasted group, they were named FC1, FC2, FC3, FC4, respectively. The experiment programs are carried out as follow: i) Period 1 (days 14-17): FC1 vs. CC1; ii) Period 2 (days 20-23): FC2 vs. CC2; iii) Period 3 (days 25-28): FC3 vs. CC3; iv) Period 4(days 30-33): FC4 vs. CC4. Collectively, in each period, from the beginning days, chicken in FC group were all fasted for 3 days, while their controls CC were fed con continuously. Chicken in both the two groups were killed through cervical dislocation on 17, 23, 28, 33 day-old, respectively (n=5 for each age and group). At each sampling, the broilers were weighed. Samples of left abdominal adipose tissue and hypothalamic were removed, frozen immediately in liquid nitrogen and stored at -80^{o}C for RNA extraction.

RNA extraction and reverse transcription: RNA was extracted from up to 100 mg of above tissues, using TRIzol reagent (Invitrogen, USA) as specified by the manufacturer's protocol. Total RNA of each sample (500 ng) was reversely transcribed using the Prime Script RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China) in accordance with the manufacturer's instructions.

Real-time PCR: Real-time PCR reaction was carried out in a total volume of 20 μL, consisting of 2μL cDNA, 12.5 μL SYBR (TaKaRa), 0.5 μL each primer (Table 1), and 4.5 μL ddH_2O. The reactions were conducted on an MX3000p real-time PCR machine (Agilent Technologies, Stockport, UK) using the conditions: 94^{o}C for 5 min, followed by 35 cycles of 94^{o}C for 30 s and 72^{o}C for 30 s. All the target genes were amplified in a similar protocol with the exception of the annealing temperature illustrated in Table 1. Amplification reactions in triplicate for each sample were performed and the results were normalized to the *GAPDH* gene expression level.

Statistical analysis: The relative mRNA expression level of genes was calculated by "normalized relative quantification" method and statistical analysis was carried out using SPSS version 17.0. One-way ANOVA test and repeated measure of ANOVA were used for statistical analysis of normalized gene copy number and differences were considered significant at $P<0.05$.

RESULTS

Fasting retards growth: In the present study, chicken continuously fed grew significantly over the course of experiment, whereas their counterparts which relatively suffered retarded growth (Table 2). Body weight of feed deprivation chicken (FD) was significantly lower than ad lib fed broilers (AL), during different growth phase (on day 18: AL vs. FD = 610 g vs. 390 g, $P<0.05$; on day 23, AL vs. FD = 820 g vs. 670 g, $P>0.05$; on day 28, AL vs. FD = 1250 g vs. 920 g, $P<0.01$; on day 33, AL vs. FD = 1470 g vs. 1280 g $P<0.05$) (Table 2) .

Fasting altered the expression of *AdipoR2*, *CPT1*, *NPY*, *LEPR* and *STAT3*: In the present study, expressions of *AdipoR2*, *CPT-1*, *LEPR* and *STAT3* in the adipose tissue, and *NPY* in the hypothalamic tissue of growing broiler chicken both in control group and fasting group, were detected by Real-time PCR method, and expression levels were illustrated in Table 3. ***AdiopR2***: *AdiopR2* mRNA showed downward trend both in the control group and fasted group during the experiment. However, *AdiopR2* mRNA expression was higher in fasted broilers than control at each sampling days, especially on day 17 ($P<0.05$). ***CPT1***: In control broilers, a slight decrease of *CPT1* mRNA expression was noticed. However, their age-matched counterparts in the fasted group, displayed a higher *CPT1* mRNA expression, at the same age, especially at the end of the experiment (on day 33). At this time point, expression levels in the fasted group were nearly 10-fold higher as compared with control group ($P<0.01$). ***NPY*:** chicken in both fasted group and control group showed a similar expression trend during the period, although fasting birds have a relatively higher expression, however, there was no significant difference between the two groups at same growth period. ***LEPR*:** *LEPR* expression in the control group increased with age from day 17 to day 23, showing a peak expression on day 23 then, it decreased with a slight rise at the end of experiment. Reversely, *LEPR* mRNA expression in the fasted group decreased with age, although a slight increase occurred in the period from day 28 to day 33. No

clear trend was observed for *LEPR* mRNA expression in both groups. ***STAT3*:** *STAT3* mRNA expression in the control group decreased with age during the experiment, and there was no significant difference in the different growth age, although slightly higher expression abundance occurred on day 23. Most notably, levels of *STAT3* in the fasted group was lower than fed counterparts, significantly on day 17 ($P<0.05$) (Table 3).

DISCUSSION

The present study, therefore, was designed to detect the dynamic expression of genes including *AdipoR2*, *CPT1, NPY*, *LEPR* and *STAT3* in growing broiler chicken at different growth period and also to determine the effect of fasting on expression of these genes.

AdiopR2: In the present study, we found that *AdiopR2* mRNA expression was higher in fasted broilers than control at each sampling days, especially on day 17. It may be due to the fact that after 3 days' feed deprivation, chicken in fasted group suffered from a loss of weight severely, thus, resulted in an increased *AdipoR2* expression level, as *AdipoR2,* a receptor of adiponectin, which modulates a number of metabolic processes including lipogensis, glucose regulation and fatty acid oxidation in mammalian and avian, and was negatively correlated with fat synthesis (Ramachandran *et al*., 2013). The results of this study are in line with the findings of Ramachandran *et al*. (2007) who found that after 2 days feed deprivation, *AdiopR2* mRNA expression significantly increased in chicken adipose tissue. It is, therefore, partially concluded that AdiopR2 is affected by feed deprivation in broiler chicken, especially on day 17.

CPT1: **A** mitochondrial enzyme, is important in many metabolic processes, as well as weight regulation. In this study, we detected *CPT1* mRNA expression in the adipose tissue of growing broiler chicken both in control group and fasting group. A slight decrease in *CPT1* mRNA expression was noticed, in broilers fed ad lib. This may be due to the reason that chicken experienced a significant growing time during the period of days 17-33, and the fatty acid oxidation process occurred in them was less dominant compared with the fat acid synthesis during this period. Hence, they might not need any more *CPT1*, which functions as a rate-limiting enzyme in the process of fatty acid oxidation. However, their age-matched counterparts displayed a higher *CPT1* mRNA expression, at the same age, especially at the end of the experiment (on day 33). Present study findings are similar with the previous observation reported by Song *et al*., (2012), who found that *CPT1* mRNA expression in feed deprivation chicken for 2 days was significantly higher than that of fed ad lib., suggesting that feed deprivation was associated with the up-regulation of *CPT1* mRNA. We hypothesis the reason that after feed deprivation, chicken need more fatty acid oxidation to achieve energy for survive. However, in order to survive for a long time, chicken need a negative feedback to control its oxidation, thereby resulting in the up-regulation of *CPT1* mRNA, which might play a role of rate-limiting during the process of fatty acid oxidation. However, the phenomenon in this study that fasted chicken displayed a higher *CPT1* mRNA expression than the controls, were not inconsistent with the previous study reported by Liu and Zhu (2012), who found that fasting 24h significantly decreased *CPT-1* mRNA levels in the hypothalamus tissue compared to *ad libitum* fed chickens, this difference might be derived from the tissue specific expression of *CPT-1* in chicken. In addition, in present study, we also found that feed deprivation at different age resulted in different *CPT1* mRNA expression in chicken, which might be associated with the body weight, in detail with fatty.

NPY: In mammals, *NPY* is one of the most potent orexigenic neuropeptides known to date (Schwartz, 2001; Mercer *et al*., 2011). In avian as in other vertebrates, *NPY* appears to be involved in the regulation of feeding. In this study, *NPY* mRNA expression in hypothalamic tissue of broiler chicken both in control group and fasting group were detected. *NPY* genes in control group showed a downward trend, with exception of a little rise on day 28, but not excess the peaking level on day 17. Hausman *et al*., 2012 found that in the adipose tissue of broiler chicken, *NPY* mRNA expression upregulated with age between days 9 and 33. This difference might be due to the fact that expression of *NPY*, functions in the appetite regulatory network, differed in different tissues. It might be due to the reason that chicken selected in the study, as the *NPY* expression is also influenced by chicken lines (Huang *et al*., 2010).

Likewise, fasted group broilers showed a similar *NPY* mRNA expression trend as that in controls on days 17, 23, 28 and 33, respectively. However, fasting birds have a relatively higher expression, although there was no significant difference between the two groups at same growth period. It has been demonstrated that feed deprivation is related to enhanced hypothalamic *NPY* gene expression in mammalian and avian species, including chicken (Higgins *et al*., 2010; Song *et al*., 2012) and quail (Phillips-Singh *et al*., 2003). The data represented in the current study are consistent with these findings, showing that *NPY* mRNA levels were significantly upregulated in feed deprived chickens relative to their ad libitum fed counterparts, during whole experiment, confirming that *NPY* is naturally increased in fasted chick Higgins *et al*., 2010; Song *et al*., 2012).

LEPR: *LEPR* is the receptor of leptin, which expressed in at least six isoforms in mammals, and two isoforms, in chicken (Liu *et al*., 2007). In mammals, *LEPR* plays a crucial role in *leptin* function process, which involved in a variety of regulation pathways. Currently, physiological function and utility of chicken adipose tissue *LEPR* is still unclear (Ashwell *et al*., 1999; Pitel *et al*., 2010). In the present study, *LEPR* expression increased with age from day 17 to day 23, showing a peak expression on day 23 then, it decreased with a slight rise at the end of experiment. Hausman *et al.* (2012) found that *LEPR* decreased with age during growth period of days 21-42 in broiler chicken. In the present study, *LEPR* expression decreased from day 23 onward, which was in line to the finding of Hausman *et al.* (2012). Cassy *et al.* (2004) found an increasing *LEPR* expression between days 9-33, in the brain of layer and broiler chicks. Our study was not

Table 1: Primer sequence of the target genes

Gene	Primer sequence 5′-3′	Product size/bp	Annealing temperature (°C)	Acc. No.
AdipoR2	F:TGGCTGAAGGACAACGATTA R:GGCAGAGGAACAACACAAAAC	145	58	NM_001007854
CPT-1	F:CTCTCGACGAGCCAAACCC R:CCGCAATGATATACGAAACGC	133	51	DQ314726
NPY	F:CGGTGCTGACTTTCGCCTT R:AATGTTTTCTGTGCTTTCCCTC	230	52	NM_205473
LEPR	F: CACACCATTACAATTCTAGCC R: ACCCACTTCATCTCCTCTTC	231	55	NM_204323
STAT3	F:CTGACCAACAACCCCAAGAAC R: CAGCCAGACCCAGAAAGAGAA	249	54	NM_001030931
GAPDH	F:GCCCAGAACATCATCCCA R:CGGCAGGTCAGGTCAACA	180	56	NM_204305

F: forward primer; R: reverse primer; Acc. No: GenBank accession number. Primers were designed according to the sequence of the target genes by their Acc. No. in GenBank.

Table 2: Effects of nutritional state on body weight in growing broiler chickens

Cage	Growing days (day)	Body weight (g)	
		Feed group	Fasted group
FC1 vs. CC1	14 (Initial)	420±10	
	17 (Feed/Fasted for 3 days)	610±21 (feed continuously)	390±15* (fasted for 3 days)
FC2 vs. CC2	20 (Initial)	700±24	
	23 (Feed/Fasted for 3 days)	820±23 (feed continuously)	670±35 (fasted for 3 days)
FC3 vs. CC3	25 (Initial)	930±40	
	28 (Feed/Fasted for 3 days)	1250±45 (feed continuously)	920±42** (fasted for 3 days)
FC4 vs. CC4	30 (Initial)	1300±74	
	33 (feed/fasted for 3 days)	1470±82 (feed continuously)	1280±70* (fasted for 3 days)

*P<0.05 (Feed Group vs. Fasted Group); **P<0.01 (Feed Group vs. Fasted Group).

Table 3: Dynamic mRNA expression of *AdipoR2, CPT1, NPY, LEPR and STAT3* in broilers regulated by aging and fasting conditions

Gene	Group	Experimental Days			
		17	23	28	33
AdipoR2	C	0.05883±0.00700	0.03249±0.00393	0.00756±0.00091	0.02236±0.00869
	F	0.21262±0.07434*	0.06210±0.03250	0.01392±0.00822	0.03844±0.0007
CPT1	C	0.01230±0.00720	0.00567±0.00270	0.00472±0.00270	0.00474±0.00170
	F	0.02388±0.00990	0.01428±0.00430	0.01439±0.00440	0.04627±0.02630**
NPY	C	0.050378±0.00411	0.024364±0.00169	0.03860±0.00498	0.02521±0.00285
	F	0.11824±0.02090	0.06319±0.00367	0.08377±0.01648	0.043617±0.00198
LEPR	C	0.00004±0.00001	0.00033±0.00010	0.00006±0.00003	0.00011±0.00003
	F	0.00018±0.00008	0.00003±0.00001	0.00007±0.00002	0.00008±0.00002
STAT3	C	0.10935±0.01307	0.03373±0.01500	0.00051±0.00003	0.00289±0.00017
	F	0.00282±0.00014*	0.02303±0.01350	0.00183±0.00007	0.00981±0.00400

C: Control group, F: Fasted group; Values (mean+SD) bearing single (P<0.05) or double asterisk (P<0.01) at a specific day between the two groups (C vs. F) differ significantly.

consistent with the results of Hausman *et al.* (2012), which may be resulted from the different tissue selected in the study.

Unlike in the control group, *LEPR* mRNA expression in the fasted group decreased with age, although a slight increase occurred in the period from day 28 to day 33. No clear trend was observed for *LEPR* mRNA expression in both groups. This phenomenon suggested that *LEPR* might have no effect on feed intake in chicken. Recently, Gertler *et al.* (2013) found that *LEPR* is not implicated in the control of appetite or adipose homeostasis in chickens. It is, therefore, necessary to carry out further study to investigate the function of *LEPR* in chicken.

STAT3: *STAT3* is the downstream component of leptin/LEPR pathway. In the present study, it was notable that *STAT3* mRNA expression in the fed group decreased with age, during the experiment, and there was no significant difference in the different growth age, although slightly higher expression abundance occurred on day 23. Most notably, levels of *STAT3* in the fasted group were lower than fed counterparts, significantly on day 17. Bergan *et al.* (2012) found that fasting resulted in the deactivation of *STAT5* gene in adipose tissue of rainbow trout, which is consistent with our results, although the species are different.

Conclusion: In the present study, *CPT1*, *AdipoR2* and *NPY* expression in the fasted group was higher than control group, while *LEPR* and *STAT3* expression trends were not regular in growing broiler chicken both groups. It is, therefore, concluded that fasting stimulated the up-regulation of genes related to fatty metabolism and feed intake, in chicken, while *LEPR* and *STAT3*, two of the important components in fatty metabolism regulation pathways in mammalians, did not show regular changes in chicken. A following *in vitro* study, therefore, need to be further carried out to study the chicken *LEPR* function and its downstream genes, like *STAT3*.

Acknowledgement: This work was supported by Research Fund for the Oasis scholars Program of Shihezi University; and Research Fund for the Doctoral Program of Anhui University (J10113190031).

REFERENCES

Ashwell CM, SM Czerwinski, DM Brocht and JP McMurtry, 1999. Hormonal regulation of leptin expression in broiler chickens. Am J Physiol-Reg I, 276: R226-R232.

Bauer M, AC Hamm, M Bonaus, A Jacob, J Jaekel, H Schorle, MJ Pankratz, and JD Katzenberger, 2004. Starvation response in mouse liver shows strong correlation with life-span-prolonging processes. Physiol Genomics, 17: 230-244.

Bergan HE, JD Kittilson, and MA Sheridan, 2012. Nutrition-regulated lipolysis in rainbow trout (Oncorhynchus mykiss) is associated with alterations in the ERK, PI3K-Akt, JAK-STAT, and PKC signaling pathways. Gen Comp Endocrinol, 176: 367-376.

Cassy S, M Picard, S Crochet, M Derouet, DH 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.

Chee MJS, and WF Colmers, 2008. Y eat? Nutrition, 24: 869-877.

Cheon Y, TY Nara, MR Band, JE Beever, MA Wallig, and MT Nakamura, 2005. Induction of overlapping genes by fasting and a peroxisome proliferator in pigs: evidence of functional PPAR alpha in non-proliferating species. Am J Physiol Regul Integr Comp Physiol, 288: R1525-1535.

Désert C, MJ Duclos, P Blavy, F Lecerf, F Moreews, C Klopp and S Lagarrigue, 2008. Transcriptome profiling of the feeding-to-fasting transition in chicken liver. BMC Genomics, 9: 611.

Dunn IC, PW Wilson, TV Smulders, V Sandilands, RB D'Eath and T Boswell, 2013. Hypothalamic agouti-related protein expression is affected by both acute and chronic experience of food restriction and re-feeding in chickens. J Neuroendocrinol, 25: 920-928.

Gertler A, D Shinder, S Yosefi, M Shpilman, CI Rosenblum, M Ruzal, and M Friedman-Einat, 2014. Pegylated leptin antagonist with strong orexigenic activity in mice is not effective in chickens. J Exp Biol, 217: 180-184.

Hausman GJ, CR Barb, BD Fairchild, J Gamble and L Lee-Rutherford, 2012. Expression of genes for interleukins, neuropeptides, growth hormone receptor, and leptin receptor in adipose tissue from growing broiler chickens. Domest Anim Endocrinol, 43: 260-263.

Higgins SE, LE Ellestad, N Trakooljul, F McCarthy, J Saliba, LA Cogburn, and TE Porter, 2010. Transcriptional and pathway analysis in the hypothalamus of newly hatched chicks during fasting and delayed feeding. BMC Genomics, 11: 162.

Huang JX, L Lu, L Xi, XG Luo and B Liu, 2010. Effects of age and strain on the expression of leptin receptor, neuropeptide Y and pro-opiomelanocortin in the hypothalamus of young chickens. Brit Poult Sci, 51: 696-702.

Ji B, B Ernest, J Gooding, S Das, A Saxton, J Simon and B Voy, 2012. Transcriptomic and metabolomic profiling of chicken adipose tissue in response to insulin neutralization and fasting. BMC genomics, 13: 441.

Kawase A, T Ohgami, I Yoshida, Y Tsunokuni and M Iwaki, 2013. Diurnal variation of nuclear receptors in mice with or without fasting. Pharmacol Pharm, 4: 240-243.

Liu X, IC Dunn, PJ Sharp and T Boswell, 2007. Molecular cloning and tissue distribution of a short form chicken leptin receptor mRNA. Domest Anim Endocrinol, 32: 155-166.

Liu L and L Zhu, 2012. Effect of 24^{h} fasting on gene expression of AMPK, appetite regulation peptides and lipometabolism related factors in the hypothalamus of broiler chicks. Asian-Aust J Anim Sci, 25: 1300-1308.

Mercer RE, MJS Chee and WF Colmers, 2011. The role of NPY in hypothalamic mediated food intake. Front Neuroendocrinol, 32: 398-415.

Phillips-Singh D, Q Li, S Takeuchi, T Ohkubo, PJ Sharp, and T Boswell, 2003. Fasting differentially regulates expression of agouti-related peptide, proopiomelanocortin, prepro-orexin, and vasoactive intestinal polypeptide mRNAs in the hypothalamus of Japanese quail. Cell Tissue Res, 313: 217-225.

Pitel F, T Faraut, G Bruneau and P Monget, 2010. Is there a leptin gene in the chicken genome? Lessons from phylogenetics, bioinformatics and genomics. Gen Comp Endocrinol, 167: 1-5.

Rakhshandehroo M, LM Sanderson, M Matilainen, R Stienstra, C Carlberg, PJ de Groot, M Muller and S Kersten, 2007. Comprehensive analysis of PPAR alpha-dependent regulation of hepatic lipid metabolism by expression profiling. PPAR Res, 2007: 26839.

Ramachandran R, OM Ocón-Grove and SL Metzger, 2007. Molecular cloning and tissue expression of chicken AdipoR1 and AdipoR2 complementary deoxyribonucleic acids. Domest Anim Endocrinol, 33: 19-31.

Ramachandran R, S Maddineni, O Ocón-Grove, G Hendricks, R Vasilatos-Younken and JA Hadley, 2013. Expression of adiponectin and its receptors in avian species. Gen Comp Endocrinol, 190: 88-95.

Schwartz MW, 2001. Brain pathways controlling food intake and body weight. Exp Biol Med, 226: 978-981.

Song Z, L Liu, Y Yue, H Jiao, N Everaer, E Decuypere, and J Buyse, 2012. Fasting alters protein expression of AMP-activated protein kinase in the hypothalamus of broiler chicks (*Gallus gallus domesticus*). Gen Comp Endocrinol, 13: 546-555.

Tinoco AB, LG Nisembaum, E Isorna and M Delgado, 2012. Leptins and leptin receptor expression in the goldfish (*Carassius auratus*) regulation by food intake and fasting/overfeeding conditions. Peptides, 34: 329-335.

Wang P, FJ Yang, H Du, YF Guan, DF Su, and CY Miao, 2011. Involvement of leptin receptor (LepRb)-STAT3 signaling pathway in brain FTO down regulation during energy restriction. Mol Med, 17: 523-532.

Weems JC, BA Griesel and AL Olson, 2012. Class II histone deacetylases downregulate GLUT4 transcription in response to increased cAMP signaling in cultured adipocytes and fasting mice. Diabetes, 61: 1404-1414.

Xu Z, Q Nie and X Zhang, 2013. Overview of genomic insights into chicken growth traits based on genome-wide association study and microRNA regulation. Curr Genomics, 14: 137-146.

Zhang H, H Chen, Y Zhang, S Li, D Lu, H Zhang and H Lin, 2013. Molecular cloning, characterization and expression profiles of multiple leptin genes and a leptin receptor gene in orange-spotted grouper (*Epinephelus coioides*). Gen Comp Endocrinol, 181: 295-305.