Animal Science Papers and Reports vol. 37 (2019) no. 4, 345-353
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Expression profiling of functional candidate genes for abdominal fat weight and relative abdominal fat in liver chickens*

Rafał Parada[1], Tadeusz Malewski[2], Kazimierz Jaszczak[1], Magdalena Solka[1,]**

[1] Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Postępu 36A, 05-552 Jastrzębiec, Poland

[2] Department of Molecular and Biometric Techniques, Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 64, 00-679 Warsaw, Poland

(Accepted September 17, 2019)

This work aimed at investigating the expression of nine functional candidate genes of abdominal fat weight and percentage in chicken livers. The study was conducted on 92 medium-growing chickens from the crossing of the Cobb strain and the native Green-legged Partridge breed. Both the peroxisome proliferator-activated receptor gamma gene (*PPARG*) and the high-mobility group AT-hook 1 (*HMG1A*) in the «fatty» group were up-regulated with an average ratio of 4.26 (P≤0.01) and 3.10 (P≤0.01), respectively, relative to the «lean» group. Expression of the *HMG1A* gene was highly correlated with abdominal fat content (0.81, P≤0.01) and abdominal fat weight (0.76, P≤0.01). The correlation of the *PPARG* gene expression was significant both with the abdominal fat content (0.68, P≤0.01), and abdominal fat weight (0.61, P≤0.05). In contrast, no significant differences were detected in the expression of the *FABP1, FAPB2, FABP3, MC4R, PPARGC1A, POMC* and *PTPN1* genes. Our results show that the high mobility group AT-hook1 and *PPARG* may be candidate genes for abdominal fat deposition in the chicken and their expression may be useful markers of fat deposition in chickens.

KEYWORDS: abdominal fat / chicken / fatness traits / gene expression

*This studyies was financed by the National Science Centre (grant number 2012/07/B/NZ9/02206).
** Correspondence: m.solka@ighz.pl

One of the main purposes in poultry genetic improvement programs is to improve meat performance traits. [Kawka *et al*. 2010, 2012, Parada *et al*. 2012, Mosca *et al.* 2018]. During the last decades intensive genetic selection has resulted in increased body weight gains, growth rate and feed conversion efficiency in broiler chickens [Hocking, 2014; Wang *et al.* 2012]. However, selection for rapid growth has had unintended side effects such as excess fat deposition. The main phenotypic indicators of fat traits are abdominal fat and body fat percentage. Meat quality and carcass traits have low heritability, which hinders their genetic improvement. In turn, abdominal fat has higher heritability (h^2=0.62 for abdominal fat weight, and h^2=0.24 for abdominal fat percentage) [Chen *et al*. 2005].

Fatty acids in birds are synthesised in the liver. Then they are transported by lipoproteins or chylomicrons to fatty tissues and stored as triglycerides [Hermier, 1997]. Studies of molecular mechanisms underlying abdominal fat deposition in chicken have yielded ambiguous results. [Wu *et al*. 2016] reported that in the livers of the Northeast Agricultural University broiler lines, expression levels of the *KDR* (kinase insert domain receptor) gene, the *TUSC3* (tumor suppressor candidate 3) gene and the *PPAT* (phosphoribosyl pyrophosphate amidotransferase) gene are significantly correlated with abdominal fat weight and percentage. Completely different genes (e.g. *AATF, ABCB11, DOC2B, GRB14, LRP2, LY75, PIGW, PLA2R1, SLC25A12, TRIM37*, in total 18 genes on *GGA7* and 23 genes on *GGA19*) have been reported by [Roux *et al*. 2014] and *EGLN1, FAM120B, GGPS1, GNPAT, THBS2* genes [Moreira *et al*. 2015] as involved in the lipid metabolism. Discrepancies between the obtained results may be caused by different chicken breeds, feeding, methods of analysis and others. Our previous research performed on livers of Isa 15 broilers showed that the *HMG1A* and *PPARG* genes were up-regulated [Larkina *et al*. 2011].

The aim of the study was to analyse expression profiling of nine most probable functional candidate genes in livers of lean and fatty chickens and correlations between gene expression and chicken fatness.

Materials and methods

Animals

Research was conducted on medium-growing crossbreed Cobb (C) x native Green-legged Partridge (GP) chickens, that came from the BIOFOOD project (innovative, functional products of animal origin) agreement of the Ethical Committee no. 27/2009. All birds were kept under the same controlled environmental conditions and received the same diets. Chickens received a starter diet of 3080 kcal ME/kg and 21.3% crude protein until 2 weeks of age. Between 3 and 4 weeks of age they received a grower 1 feed of 3160 kcal ME/kg and 20.8% CP and next (in 4 and 5 weeks of age) a grower 2 diet of 3180 kcal ME/kg and 19.9% CP. From 6 weeks of age birds were fed a finisher diet of 3200 kcal ME/kg and 19% CP. Feed and drinking water were offered *ad libitum*. The two broiler lines with a total of 425 birds (48 in the «fatty» group and

44 in the «lean» group) were randomly selected in accordance with a high (4-6.6%) and low (0.88-2.54%) percentage contents of abdominal fat. At the 63rd day chickens from each group were taken according to mean body weight for the group. After fasting for 12 h they were sacrificed by electrical stunning in a water bath (120 mA, 50 Hz) for 2 s, and slaughtered by cutting the cervical blood vessels and bled out for ca. 3-4 min.

Samples were collected from each bird during slaughter and next frozen and stored at -80°C. The characteristics of the two groups of birds used for expression quantification are given in Table 1.

RNA isolation

Total RNA was extracted with a TRI Reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's recommendations [Farrell 1998]. About 100 mg of liver tissue were homogenised in 1 ml of TRI Reagent and then incubated for 5 minutes at room temperature. Next, 0.2 ml of chloroform was added, the sample was vortexed and again incubated at room temperature for 15 minutes. The obtained mixture was centrifuged at 12.000 g for 15 minutes at 4 ˚C. The upper phase was transferred to a fresh tube and RNA was precipitated with isopropanol. Obtained RNA was stored at -80°C. The concentration and purity of the extracted RNA was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). RNA integrity was checked electrophoretically in a 1.5% agarose gel [Malewski *et al.* 2015]. Only samples with high integrity were used in further experiments.

An enhanced Avian HS RT-PCR Kit (Sigma-Aldrich, St. Louis, MO, USA) was used for reverse transcription, following the manufacturer's instructions. The total RNA was treated with RNase free DNase I (Sigma-Aldrich, St. Louis, MO, USA). 1 µg of RNA was treated with 1 U of DNase I for 15 min at room temperature. The reaction was stopped by adding a stop solution and DNase I was inactivated at 70°C for 10 min. After incubation for 10 min at 70°C all the remaining components were added and the reaction was run at 45°C for 50 min. The obtained cDNA was used immediately in the PCR or stored at -20°C.

qPCR

Expression profiling of nine functional candidate genes: fatty acid binding proteins (*FABP1, FABP2, FABP3*), the high mobility group AT-hook 1 (*HMG1A*), melanocortin 4 receptor (*MC4R*), pro-opiomelanocotin (*POMC*), peroxisome proliferator-activated receptor gamma (*PPARG*), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PPARGC1A*) and tyrosine phosphatase, non-receptor type 1 (*PTPN1*), was performed by qPCR. Primers were the same as in a study of Larkina *et al*. [2011]. *GAPDH* was used as a reference gene, analogously as in our previous experiments. The RotorGene 6000 system and the LuminoCt SYBR Green qPCR Master Mix (Sigma-Aldrich, Milwaukee, WI, USA) were used for real-time PCRs. Cycle threshold (Ct) estimates were obtained using the relative quantification module in the software package. The final PCR product was obtained

from 20 µl reactions using 1 µl of cDNA sample, 2.0 µl of the primer mix (5 µM of each primer), 10 µl of the 2 x LuminoCt SYBR Green qPCR Master Mix, and 7 µl of H_2O. All PCR reactions were performed as follows: the initial denaturation step at 95°C for 3 min, followed by 40 cycles at 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec. After amplification the melting-curve analysis was performed as follows: initial denaturation at 95°C for 60 sec, cooling to 72°C followed by gradual raising of temperature to 95°C with increments of 0.5°C in each step. Fluorescence data were analysed using the Tm calling module in the RotorGene 6000 software.

The qRT-PCR analysis was performed in triplicate. The $2^{\Delta\Delta Ct}$ method [Schmittgen and Livak 2008] was used to calculate the relative ratio, with correction for amplification efficiency. To compute the final efficiency value of the PCR reaction, nonlinear regression and weighted average analysis were used (Real-time PCR miner; www.miner.ewindup.info) [Zhao and Fernald 2005]. Differences between groups in target gene expression were estimated byStudent's t-test. The Relationships between gene expression and abdominal fat content were estimated with the use of the Pearson correlation.. Results with $P<0.05$ were statistically significant, while those with $P<0.01$ were highly significant.

Results and discussion

In mammals lipogenesis takes place in the adipose tissue and the liver, whereas in birds the main lipogenic site is the liver. Expression patterns of adipogenic transcription factors are different *in vitro* and *in vivo* between mammals and avian species [Matsubara *et al*. 2005]. The four proteins are part of the HMGA family: HMGA1a, HMGA1b, HMGA1c and HMGA2. The first three are products of the *HMGA1* gene [Fedele *et al*. 2001]. The increase of *HMGA1* protein levels is associated with adipocyte differentiation in the 3T3 L1 cell line, while the block of *HMGA1* synthesis suppresses adipocyte differentiation [Pierantoni *et al*. 2003]. High-mobility group A1 protein can interact with peroxisome proliferator-activated receptor gamma in vascular cells [Bloch *et al*. 2012]. The *HMGA1* gene was up-regulated in livers of the "fatty" group from the Isa 15 breed about threefold and its expression correlated with relative abdominal fat content and abdominal fat weight [Larkina *et al*. 2011], which is consistent with our results. Obtained data suggest that up-regulation of *HMGA1* in the liver is associated with metabolism rather than chicken breed.

Characteristics of the fatty and lean groups of chickens are presented in Table 1. Deposition of abdominal fat was about two-fold higher in the fatty group, while the percentage of abdominal fat over 2.5-fold higher comparing with the lean group of chickens.

Expression of nine functional candidate genes was investigated by qPCR in livers of fatty and lean chicken groups. Results obtained in our study showed that all the investigated genes were expressed in the liver (Fig. 1). The *HMGA1* and *PPARG* genes were differentially expressed between the lean and fatty groups, with the expression

Table 1. Characteristics of the two groups of chicken used in the experiment (means and their standard deviations)

Trait	Fatty group		Lean group	
	mean	SD	mean	SD
Live body weight (g)	2840	215.40	2516	241.55
Carcass weight (g)	2102	211.55	1862	254.65
Abdominal fat (g)	89.0	20.32	41.0	8.98
Abdominal fat of carcass (%)	4.2	0.77	2.2	0.49

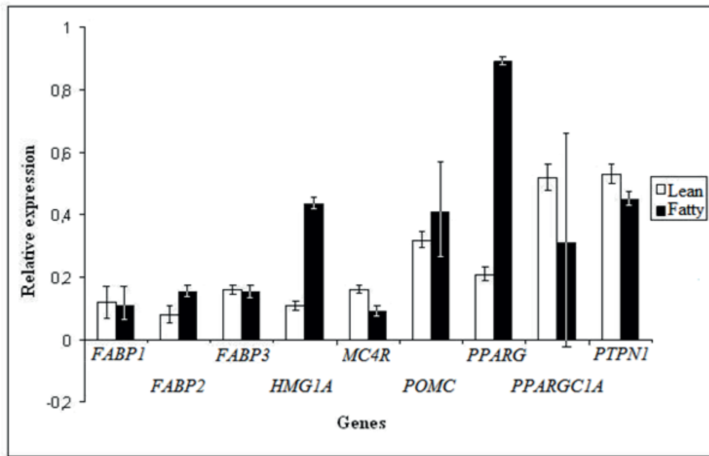

Fig. 1. Comparison of expression profiling in liver tissue between fatty and lean chickens.

levels higher in fat chickens. Only in the case of the *PPARGC1A* gene, the expression level in fatty birds was significantly lower than that of lean chickens. For the other studied genes: *FABP1, FAPB2*, *FABP3, MC4R, POMC* and *PTPN1*, no significant differences in the mRNA level of these genes were observed in the liver tissue between the fatty and lean groups. The HMGA proteins are involved among others in the regulation of chromatin structure [Cleynen and Van de Ven 2008]. They code for a small, nonhistone, chromatin-associated protein that can modulate transcription by altering the chromatin architecture. The *HMGA1* gene encodes a non-histone chromatin protein involved in many cellular processes, among others regulation of inducible gene transcription, DNA replication and heterochromatin organization. In our study the *HMGA1* gene in chicken livers was up-regulated with an average ratio of 3.10 ($P \leq 0.01$) in the fatty group relative to the lean group. As shown in Table 1, in the fatty chickens abdominal fat content was 4.2±0.77 %, while abdominal fat weight 89.0±20.32 g, whereas in the lean group of birds the abdominal fat content was 2.2±0.49% and abdominal fat weight 41.0±8.98 g. Similarly, the *PPARG* gene expression was up-regulated in the liver with an average ratio of 4.26 ($P \leq 0.01$) in the fatty group relative to the lean group.

PPARs (peroxisome proliferator-activated receptors) are transcription factors activated by a ligand. They belong to the nuclear hormone receptor family, comprising three isoforms (α, β or δ, γ) [Michalik *et al*. 2006]. *PPARG* is involved in adipose development and function [Gray *et al*. 2005, Lefterova *et al*. 2008], among others insulin sensitivity, lipid storage and energy dissipation [Koutnikova *et al*. 2003, Rangwala and Lazar 2004]. The *PPARG* gene encodes PPAR gamma participating in adipocyte differentiation. Several studies have showed that SNPs in the *PPARG* gene are linked with obesity in the Han Chinese [Chen *et al*. 2009]. *PPARG* mediates in the expression of fat-specific genes and activates the adipocyte differentiation program [Hindle *et al*. 2009]. *PPARG1* expression was also detected at lower levels in the liver, spleen and the heart. Vidal-Puig *et al*. [1996] showed a marked effect of fasting to reduce PPAR gamma protein levels in adipose tissue. Wang *et al.* [2008] showed that transfection of *in vitro* synthesised small-interference *PPARG* RNA (*siPPARG*) in cultivated preadipocytes of 12 d chicken significantly inhibited differentiation and stimulated proliferation of preadipocytes. Sato *et al*. [2004] also reported s connection of *PPARG* expression with fat deposition in broilers. This gene was also found in adipose tissue in different broiler lines [Wang *et al*. 2009]. It was shown that *PPARG* affects chicken fat metabolism and could be used in marker assisted selection (MAS) [Meng *et al*. 2005]. Up-regulated expression of *PPARG* was also found in livers of the Isa 15 breed [Larkina *et al*. 2011].

Correlation coefficients between nine gene expression levels and abdominal fat weight and percentage in livers of chickens are given in Table 2. Expression levels of four genes: *FABP2, HMGA1,* and *PPARG*, in liver tissue of studied chickens were positively correlated both with abdominal fat weight and abdominal fat percentage. Up-regulated expression of *HMGA1* and *PPARG* was significantly positively correlated with abdominal fat content (0.81, P≤0.01 and 0.68, P≤0.01, respectively) and with abdominal fat weight (0.76, P≤0.01 and 0.61, P≤0.05, respectively) (Tab. 2). In contrast, *FABP1, FABP3, MC4R* and *PTPN1* expression levels in liver tissues were

Table 2. Correlation of gene expression with abdominal fat weight and abdominal fat percentage in liver of chickens

Gene	Pearson correlation coefficient	
	With abdominal fat weight	With abdominal fat percentage
FABP1	-0.04	-0.01
FABP2	0.38	0.51
FABP3	-0.12	-0.42
HMGA1	0.81**	0.76**
MC4R	-0.52	-0.43
POMC	0.12	-0.08
PPARG	0.68**	0.61*
PPARGC1A	-0.12	0.02
PTPN1	-0.43	-0.26

*P≤0.05, **P≤0.01.

negatively correlated with abdominal fat weight and abdominal fat percentage. In turn, the expression level of the *POMC* gene was positively correlated with abdominal fat weight, but negatively with abdominal fat percentage. The opposite was true for the *PPARGC1A* gene, which was negatively correlated with abdominal fat weight and positively correlated with abdominal fat percentage.

Our study provides insight into gene expression levels in liver tissue in chickens coming from two different breeds. We know that the adipose tissue of humans and chickens have certain similar physiological characteristics and gene homology. Results obtained in our study regarding chicken liver tissue may be potentially useful for research on obesity in humans.

Our results show that the high mobility group AT-hook 1 and peroxisome proliferator-activated receptor gamma may be candidate genes for abdominal fat deposition in chickens. Expression of the *HMG1A* and *PPARG* genes may serve as useful markers of chicken fat deposition. Both these candidate genes are responsible for adipocyte proliferation.

REFERENCES

1. BLOCH M., PROCK A., PAONESSA F., BENZ V., BÄHR IN., HERBST L., WITT H., KAPPERT K., SPRANGER J., STAWOWY P., UNGER T., FUSCO A., SEDDING D., BRUNETTI A., FORYST-LUDWIG A., KINTSCHER U., 2012 – High-mobility group A1 protein: a new coregulator of peroxisome proliferator-activated receptor-γ-mediated transrepression in the vasculature. ***Circulation Research*** 110, 394-405.
2. CHEN H.H., LEE W.J., FANN CS., BOUCHARD C., PAN W.H., 2009 – Severe obesity is associated with novel single nucleotide polymorphisms of the ESR1 and PPARgamma locus in Han Chinese. ***The American Journal of Clinical Nutrition*** 90, 255-62.
3. CHEN J.L., WEN J., ZHAO G.P., ZHENG M.Q., YANG N., 2005 – Genetic parameter estimation for inosine-5-monophosphate and intramuscular fat contents and other meat quality traits in chicken muscle. ***Yi Chuan*** 27, 898-902.
4. CLEYNEN I., VAN DE VEN W.J., 2008 – The HMGA proteins: a myriad of functions. ***International Journal of Oncology*** 32, 289-305.
5. GRAY S. L., DALLA N. E., VIDAL-PUIG A. J., 2005 – Mouse models of PPARγ deficiency: dissecting PPARγ's role in metabolic homoeostasis. ***Biochemical Society Transactions*** 33, 1053-1058.
6. FARRELL R.E., Jr., RNA Methodologies. 2nd ed. New York: Academy Press; 1998.
7. FEDELE M., BATTISTA S., MANFIOLETTI G., CROCE C.M., GIANCOTTI V., FUSCO A., 2001 – Role of the high mobility group A proteins in human lipomas. ***Carcinogenesis*** 22 1583-1591.
8. HERMIER D., 1997 – Lipoprotein metabolism and fattening in poultry. ***Journal of Nutrition*** 127, 805-808.
9. HINDLE A. K., KOURY J., MCCAFFREY T., FU S.W., BRODY F., 2009 – Dysregulation of gene expression within the peroxisome proliferator activated receptor pathway in morbidly obese patients. ***Surgical Endoscopy*** 23, 1292-7.
10. HOCKING P.M., 2014 – Unexpected consequences of genetic selection in broilers and turkeys: problems and solutions. ***British Poultry Science*** 55, 1, 1-12.
11. KAWKA M., SACHARCZUK, M., COOPER R.G., 2010 – Identification of genetic markers associated with laying production in ostriches (*Struthio camelus*) – a preliminary study. ***Animal Science Papers and Reports*** 28, 95-100.

12. KAWKA M., HORBANCZUK J.O., JASZCZAK K., PIERZCHALA M., COOPER R.G., 2012 – A search for genetic markers associated with egg production in the ostrich (*Struthio camelus*). ***Molecular Biology Reports*** 39, 7881-7885.
13. KOUTNIKOVA H., COCK T.A., WATANABE M., HOUTEN S.M., CHAMPY M.F., DIERICH A., AUWERX J., 2003 – Compensation by the muscle limits the metabolic consequences of lipodystrophy in PPAR gamma hypomorphic mice. In Proc. Nat. Acad. Sci., United States of America 100. Pages 14457-14462.
14. LARKINA T. A., SAZANOVA A.L., FOMICHEV K.A., BARKOVA O.Y., MALEWSKI T., JASZCZAK K., SAZANOV A.A., 2011 – HMG1A and PPARG are differently expressed in the liver of fat and lean broilers. ***Journal of Applied Genetics*** 52, 225-228.
15. LEFTEROVA M. I., ZHANG Y., STEGER J.D., 2008 – PPARγ3 and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. ***Genes & Development*** 22, 2941-2952.
16. MALEWSKI T., BOGDANOWICZ W., DURSKA E., ŁOŚ M., KAMIŃSKI M., KOWALEWSKA K., 2015 – Expression profiling of heat shock genes in a scuttle fly Megaseliascalaris (*Diptera, Phoridae*). ***Journal of Experimental Zoology*** Part A 323A, 704-713.
17. MATSUBARA Y., SATO K., ISHII H., AKIBA Y., 2005 – Changes in mRNA expression of regulatory factors involved in adipocyte differentiation during fatty acid induced adipogenesis in chicken. ***Comparative Biochemistry & Physiology*** Part A 141, 108-115.
18. MENG H., ZHAO J.G., LI H., LI Z., 2005 – Single nucleotide polymorphism on peroxisome proliferators-activated receptor genes associated with fatness traits in chicken. ***Asian Australasian Journal of Animal Sciences*** 18, 1221-5.
19. MICHALIK L., AUWERX J., BERGER J. P., CHATTERJEE V. K., GLASS C. K., GONZALEZ F. J., GRIMALDI P. A., KADOWAKI T., LAZAR M. A., O'RAHILLY S., PALMER C. N. A., PLUTZKY J., REDDY J. K., SPIEGELMAN B. M., STAELS B., WAHLI W., 2006 – International union of pharmacology. LXI. peroxisome proliferator-activated receptors. ***Pharmacological Reviews*** 58, 726-741.
20. MOREIRA G. C., GODOY T. F., BOSCHIERO C., GHEYAS A., GASPARIN G., ANDRADE S. C., PADUAN M., MONTENEGRO H., BURT D. W., LEDUR M. C., COUTINHO L. L., 2015 – Variant discovery in a QTL region on chromosome 3 associated with fatness in chickens. ***Animal Genetics*** 46, 141-7.
21. MOSCA F., ZANIBONI L., STELLA S., KUSTER C.A., IAFFALDANO N., CEROLINI S., 2018 – Slaughter performance and meat quality of Milanino chickens reared according to a specific free-range program. ***Poultry Science*** 97, 4, 1148-1154.
22. PARADA R., KSIAZKIEWICZ J., KAWKA M., JASZCZAK K., 2012 – Studies on resources of genetic diversity in conservative flocks of geese using microsatellite DNA polymorphic markers. ***Molecular Biology Reports*** 39, 5291-5297.
23. PIERANTONI G. M., BATTISTA S., PENTIMALLI F., FEDELE M., VISONE R., FEDERICO A., SANTORO M., VIGLIETTO G., FUSCO A., 2003 – A truncated HMGA1 gene induces proliferation of the 3T3-L1 pre-adipocytic cells: a model of human lipomas. ***Carcinogenesis*** 24, 1861-9.
24. RANGWALA S. M., LAZAR M. A., 2004 – Peroxisome proliferator-activated receptor γ in diabetes and metabolism. ***Trends in Pharmacological Sciences*** 25, 331-336.
25. ROUX P.F., BOUTIN M., DESERT C., DJARI A., ESQUERRE´ D., KLOPP CH., LAGARRIGUE S., DEMEURE O., 2014 – Re-Sequencing Data for Refining Candidate Genes and Polymorphisms in QTL Regions Affecting Adiposity in Chicken. PLoS ONE 9(10), e111299. doi:10.1371/journal.pone.0111299.
26. SATO K., FUKAO K., SEKI Y., AKIBA Y., 2004 – Expression of the chicken peroxisome proliferator-activated receptor-gamma gene is influenced by aging, nutrition, and agonist administration. ***Poultry Science*** 83, 1342-7.

27. SCHMITTGEN T. D., LIVAK K. J., 2008 – Analyzing real-time PCR data by the comparative C-T method. ***Nature Protocols*** 3, 1101-1108.
28. VIDAL-PUIG A., JIMENEZ-LIÑAN M., LOWELL B. B., HAMANN A., HU E., SPIEGELMAN B., FLIER J. S., MOLLER D. E., 1996 – Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. ***Journal of Clinical Investigation*** 97, 2553-2561.
29. WANG D., WANG N., LI N., LI H., 2009 – Identification of differentially expressed proteins in adipose tissue of divergently selected broilers. ***Poultry Science*** 88, 2285-92.
30. WANG S. Z., HU X. X., WANG Z. P., LI X. C., WANG Q. G., WANG Y. X, TANG Z. Q., LI. H., 2012 – Quantitative trait loci associated with body weight and abdominal fat traits on chicken chromosomes 3, 5 and 7. ***Genetic and Molecular Research*** 11, 956-965.
31. WANG Y., MU Y., LI H., DING N., WANG Q., WANG Y., WANG S., WANG N., 2008 – Peroxisome proliferator-activated receptor-gamma gene: a key regulator of adipocyte differentiation in chickens. ***Poultry Science*** 87, 226-32.
32. WU X., ZHANG Q., XU S., JIN P., LUAN P., LI Y., CAO Z., LENG L., WANG Y., WANG S., 2016 – Differential expression of six chicken genes associated with fatness traits in a divergently selected broiler population. ***Molecular and Cellular Probes*** 30, 1-5.
33. ZHAO S., FERNALD S. D., 2005 - Comprehensive algorithm for quantitative real-time polymerase chain reaction. ***Journal of Computational Biology*** 12, 1045-1062.