Journal of Research in Ecology

An International Scientific Research Journal

ISSN No: Print: 2319 –1546; Online: 2319– 1554

Short Communication

The polymorphisms of insulin gene hormone in fragments (C1549T) and (G3971A) in hybrid chicks Ross 308 broiler

Authors:
Shayma RU and
Eman Hassan Al-Anbari

Institution:
Department of Animal Production, College of Agriculture, University of Baghdad, Iraq.

Corresponding author:
Shayma RU

ABSTRACT:

Genotypes of Insulin gene hormone was studied on 200 chicks at one day age, numbered in the leg and breeded to 35 days. The genotypes of insulin gene hormone were identified using Restriction Enzymes *Moraxella* species and PCR-RFLP technique was also implied. The results showed the presence of three genotypes of the first segment (C1549T), with the size of 529bp for insulin gene hormone, and the presence of three genotypes of the second segment (G3971A), with size of 281bp that could be used in the selection programs and to study its effect on economic traits.

Keywords:

Moraxella, Hybrid chicks, Insulin gene hormone.

Article Citation:
Shayma RU and Eman Hassan AL-Anbari
The polymorphisms of insulin gene hormone in fragments (C1549T) and (G3971A) in hybrid chicks Ross 308 broiler
Journal of Research in Ecology (2018) 6(2): 2007-2014

Dates:
Received: 25 June 2018 **Accepted:** 04 Aug 2018 **Published:** 20 Sep 2018

Web Address:
http://ecologyresearch.info/documents/EC0586.pdf

Journal of Research in Ecology

INTRODUCTION

The poultry industry has evolved rapidly in recent decades, leading to rapid genetic improvement, through the use of modern methods and technologies through which many of the genetic locations of poultry have been detected (Haley and Koning, 2006). One of the important traits in the production of broiler Chicks is the growth and carcass traits. These traits are controlled by a combination of complex genes. Therefore, It is very difficult to achieve rapid progress using the traditional methods of genetic improvement within the inbreed (Zhang *et al*., 2007). However, recent advances in molecular technology have provided new opportunities to assess genetic diversity at the level of Deoxyribo Nucleic Acid (DNA). As a result, genetic selection methods can be applied in the poultry industry.

Progress in molecular genetics of poultry has led to the recognition of genes that affect growth, carcass traits, meat quality and reproduction and this has led to an increase in the rate of growth and the quality of the carcass (Zhou *et al*., 2005; Kaya and Yıldız, 2008). The important role of candidate genes is to make them a reliable tool in chicken breeding programs because of the role that leads to genetic improvement. A number of genes have been used as candidate genes with the help of genetic markers that have been shown to have an effective role in the selection, resulting in a significant improvement in the productive performance of the birds (Li *et al*., 2010).

Among these genes is the insulin gene, which is one of the most important types of candidate genes. It has been found that the multiple forms of insulin-coding gene for insulin hormone have been broadly associated with a number of physiological and productive traits such as growth and development, body composition trait and fat deposition trait (Nie *et al*., 2005). In order to continue the productive process of domestic animals and farm animals, including poultry, it requires the modernization of methods of genetic improvement and study the genotypes of this hormone, which is pivotal in the productivity of these animals, with the remarkable development of biochemistry and molecular science, the focus has been on the genes belonging to the insulin family. It is a large family of genes under which many different types of insulin and many different genes are named (Nakashima and Ishida, 2018).

Insulin hormone is one of the various types of peptide hormones that it is secreted from the beta cells, which are located in the islets of langerhans, located in the pancreas. It consists of a group of amino acids of which 51 amino acids distributed on two chains connected by disulfide bridges (S (II)). The first series, contains 21 amino acids, while the series B, contains 30 amino acids (Fujita *et al*., 2018). The Insulin hormone works in the stimulation of liver cells, muscle and fatty tissues also by taking advantage of the available glucose to be used as an energy source. As a result, the fats stored in the body tissues are not allowed to represent and form energy by inhibiting the secretion of glucagon hormone from alpha cells within the pancreas gland in normal cases of metabolism, also contributes to the storage of glucose on the image of the glycogens in liver cells and muscles until they are returned for use when needed or when there is a lack of insulin in the blood (Simon, 1989).

NCBI-Gene Bank accession AY438372 reported with a length of 4.074 bp stated that the insulin gene in poultry, including chickens, is located in the center of the long arm of the fifth chromosome; it consists of three introns and four exons. For the purpose of covering the subject of cutting insulin gene in the broiler chicks (Ross 308) in Iraq. And for the purpose of studying the economic and productive traits within the nutritional and environmental conditions in order to benefit from the development of early selective programs and due to the importance of this gene and its relation to these traits, the present study was conducted.

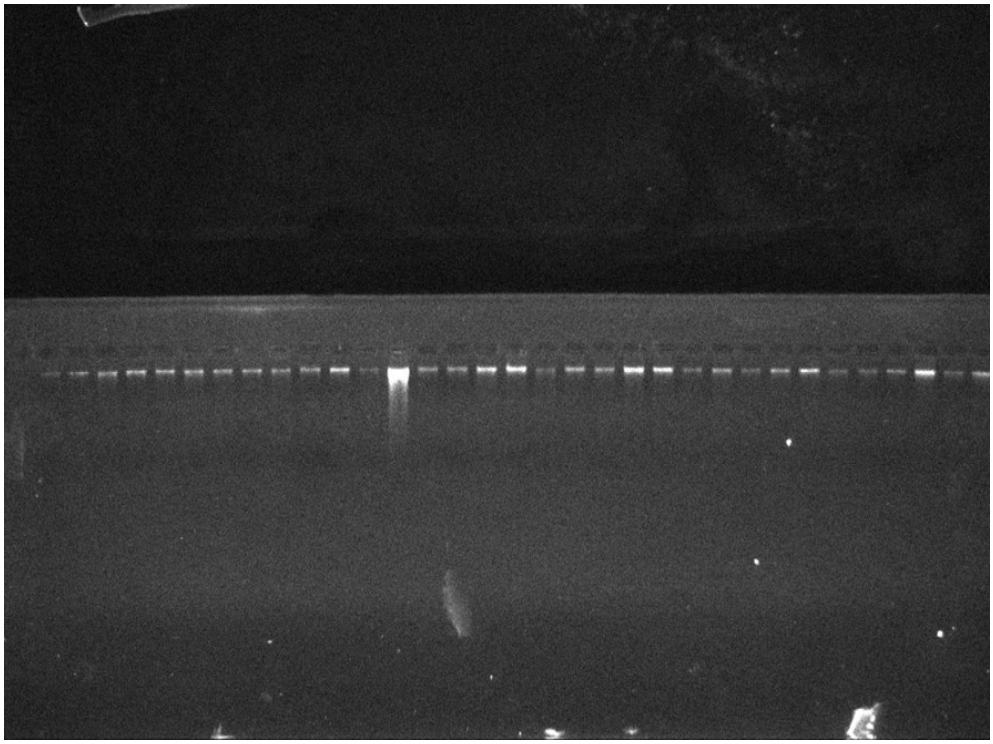

Figure 1. Results of DNA extraction of samples

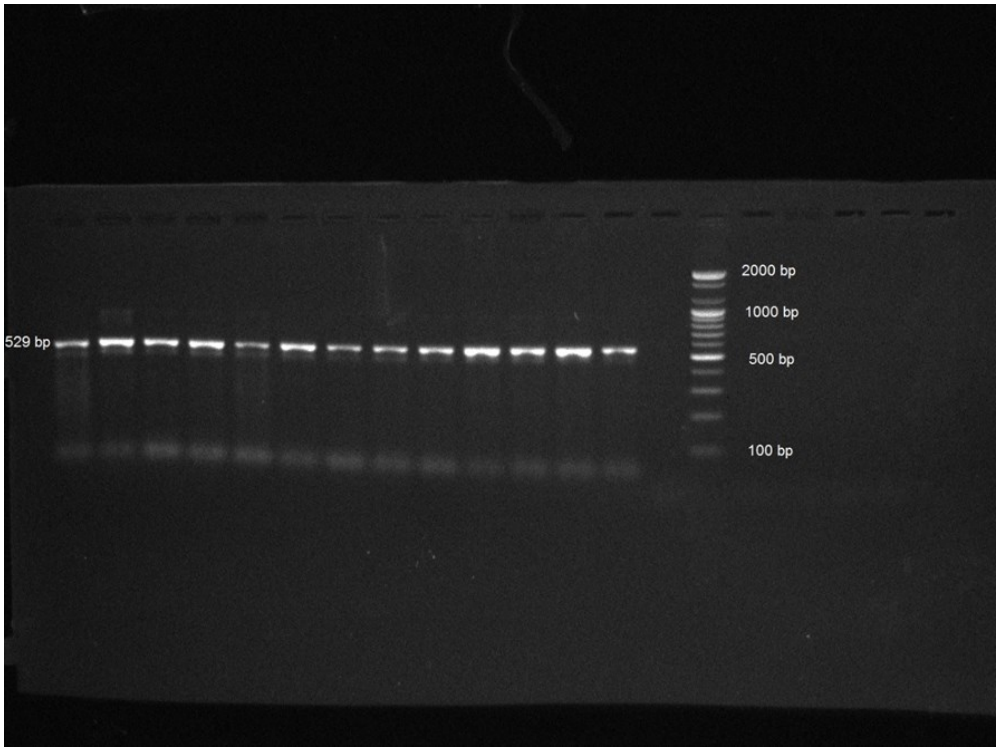

Figure 2. Results of isolation of insulin gene with size 529 bp (INS gene)

MATERIALS AND METHODS

Field experiment was conducted in the poultry field, College of Agriculture, University of Baghdad. 200 chick with one day age, numbered were breeded in a closed-ground hall to 35 days during which feed was introduced in the form of initiator, growth and final ration. They were numbered on their wings. The DNA was extracted from 20 µL blood drawn from the vena

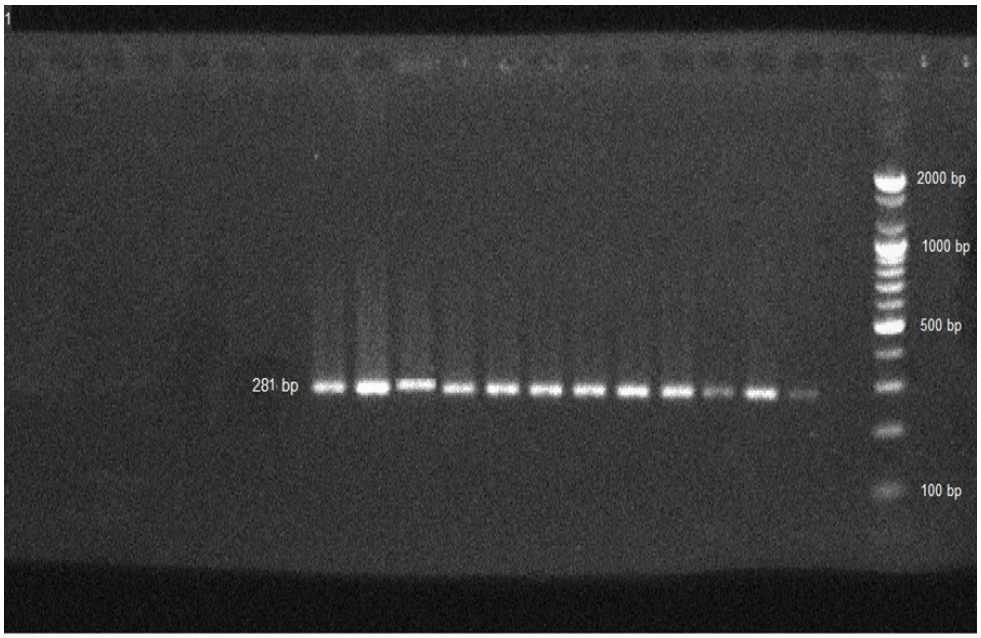

Figure 3. Results of isolation of insulin gene with size 281 bp (INS gene)

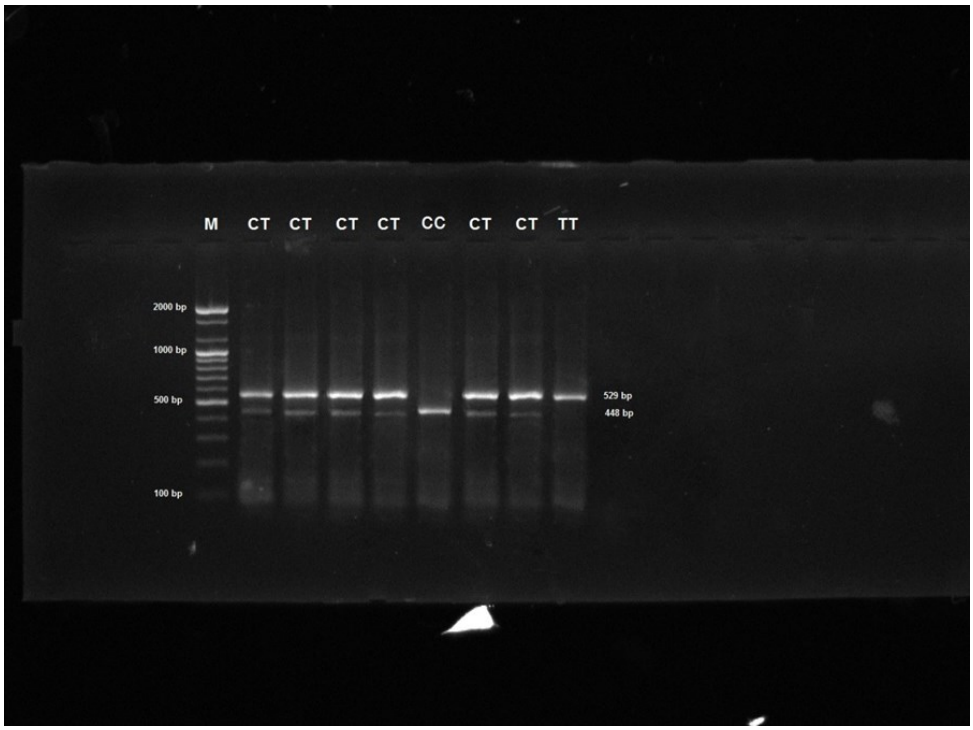

Figure 4. The C1549T segment of insulin (INS) digestion products using restriction enzymes (MSPI)

cava area of all study units using a special syringe, with a 21 day age. Then the blood was placed in tubes containing an Anticoagulant EDTA. The samples were frozen at -20°C. DNA was extracted using a special kit following the method of Khoa *et al*. (2013) after which the gene fragment under study was amplified by using primers Bioneer its sequence as follows:

First segment: No. 529bp

Primer FW: 5'.TGTTCTGCATTTGGCCCATA3'

Primer RF: 5'.CAGAATGTCAGCTTTTTGTC3'

The second segment: No. 281bp

Primer FW: 5'GGTATCTGAAAAGCGGGTCT3'

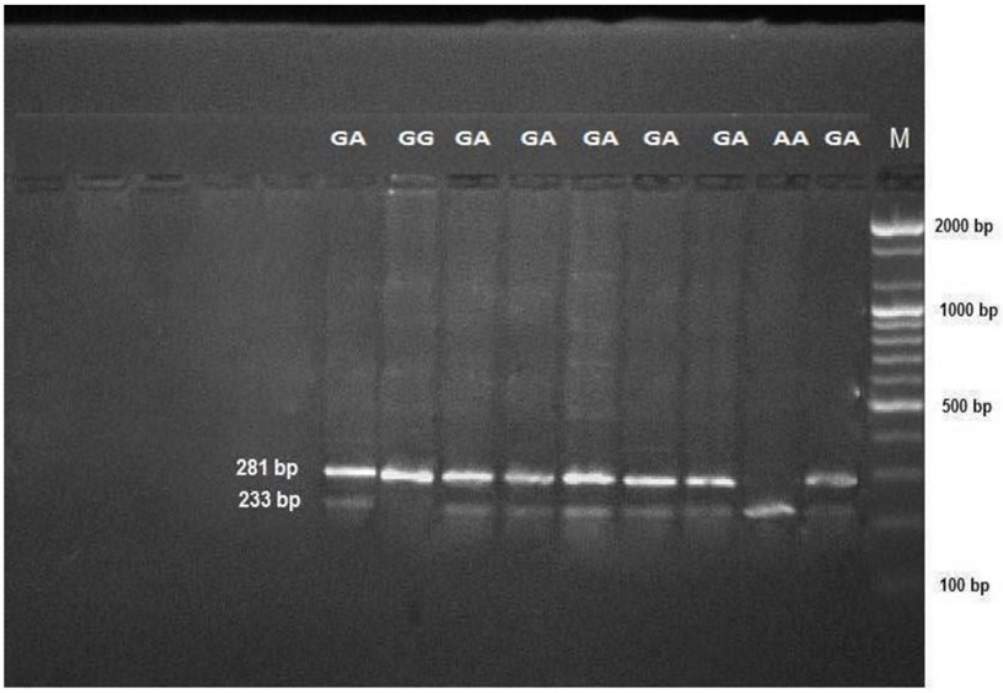

Figure 5. The G3971A segment of insulin (INS) digestion products using restriction enzyme (MSPl)

Primer RF: 5'AATGCTTTGAAGGTGCGATA3'

With the addition of the Taq polymerase enzyme, under the conditions. Thermal cycler PCR product rotation was conducted and the results were predicted using electrophoreses with 1% agarose gel at 70 volts for 30 min after the packages were observed. Under the device photocopy of UV rays, the size of the segment is determined at 529 bp and the size of the second segment is 281bp. The product was then digested at 37°C with 10 µL of Restriction Enzymes MSPI for 3 h. The samples were then migrated on the gel and tested at 3% agarose in, 100 V for an hour and a half with the volume index, examined under UV photodetector.

RESULTS AND DISCUSSION

Figure 1 shows the results of the extraction and migration of DNA samples of the broiler chicks (Ross 308). Figure 2 shows the amplification and migration of the first segment (C1549T) using PCR from the second intron at 529 bp volume with the volumetric index. Figure 3 amplification and migration of the third segment (G3971A) PCR of the second intron at 281 bp, representing CC wild, CT heterozygous, and TT mutant with volumetric index. Figure 4 shows insulin gene digestion

Figure 6. Insulin gene segment for G3971A segment

Table 1. Number and percentages of genotypes and first insulin gene frequencies - first segment C1549T

Genotypes	Number	Percentage (%)
CC	1	0.50
CT	36	18.00
TT	163	81.50
sum	200	100 %
χ^2 value	----	346.34**
index	Frequencies	
C	0.09	
T	0.91	
(P<0.01)**		

products of the C1549T using the MSPI enzyme in RFLP technology. The RDLP products showed two genotypes of the Insulin gene in the broiler chicks (Ross 308). The CT genotypes showed three introns that had 81 bp, 448 bp and 529 bp size, which resulted from the C cutting location in one of the alleles and not in the other, the second genotype TT was at the 529 bp size, which did not have a cutting location by the MSPI enzyme. These results were consistent with (Kadlec *et al*., 2011; Khoa *et al*., 2012). Figure 5 shows the INS insulin gene digestion products of G3971A with the restriction enzyme MSPl. There are two genotypes of the Insulin gene in the second intron of the broiler Chicks (Ross 308), where the GA appears in three introns 48 bp, 233 bp and 281 bp, which resulted from the location of G in one of the alleles and not in the other. The second genotypes AA represented the size of the original segment 281 bp, representing GG Wild, GA Heterozygous and AA Mutant. In which, the location was not cut by the MSPI enzyme and these results were compatible with (Kadlec *et al*., 2011; Khoa *et al*., 2012). Figure 6 shows the insulin gene for G3971A segment, with a size of 281 bp which were studied in the study units. The region represented by the red color is exons. The sequence of the enzyme cutting location is CC GG and GG Wild, GA Heterozygous and AA are Mutant.

Table 2. Number and percentages of genotypes and first insulin gene frequencies - second segment G3971A

Genotypes	Number	Percentage (%)
CC	3	1.50
CT	96	48.00
TT	101	50.50
sum	200	100%
χ^2 value	----	96.520**
index	Frequencies	
C	0.25	
T	0.75	
(P<0.01)**		

Table 1 shows that the percentages of genotypes in broiler chicks (Ross 308) showed significant differences (P<0.01) for various genotypes which reached 0.50, 18.00, 81.50% for CC, CT, TT, respectively, for the first C1549T segment. This means that there is a clear dominance of pure individuals carrying the genotypes followed by those carrying the hybrid genotype CT. This result is in agreement with Khoa *et al*. (2012) while studying the genotypes of the multiple manifestations of insulin gene in local Vietnamese chicken inbreeds and cobb commercial hybrids. The proportion of pure genotype TT were high (36-51%) and genotype CT reached (50-40%), which also showed an increase compared with the genotype CC reaching (0.16-0.08%). Which showed a decline from its predecessors. The alleles were calculated based on the presence or absence of cutting locations in the different alleles. The allele which has one cutting location at 529 bp location is called the allele T while in the case of two cutting locations at 81 bp location and 448 bp location, which is called the C allele. As a follow-up to the results shown in the table above, we find that the allelic frequencies were 0.09, 0.91 for both allele C and T, respectively. The results of the current study were consistent with Khoa *et al*. (2012). This convergence can be attributed to the fact that the studied species may have a similar genetic base considering as commercial inbreeds with a single genetic base resulting from the continuation of the selection.

Table 2 shows that the genotypes percentage of the second segment G3971A in broiler chicks (Ross 308) showed significant differences (P<0.01) for the different genotypes which reached 1.50, 48.00, 50.50% for genotypes GG, GA, AA respectively. The results agree with Khoa *et al*. (2012) who studied the genotypes of the multiple manifestations of the insulin gene in the local vietnamese chicken inbreeds and cobb commercial hybrids. The percentage of pure genotypes AA was found (25 to 22%) and GA genotypes (64-53%), showed an increase in the comparison to the genotypes GG (20-18%), which showed a decline from its predecessors. The presence or absence of cutting locations in different alleles where the allele which has one cutting location at 281 bp location called allele A, while in the case of two cutting locations at locations of 48 bp and 233 bp. In addition, following the results shown in the above table, the allele frequencies were 0.25 and 0.75 for G and A allele, respectively. Figure 6 shows the location of the third introns of the segment that did not show clearly that the results of the present study were consistent with Khoa *et al*. (2012), that this convergence and compatibility may be attributed to the fact that the studied species may have a genetic base similar to the commercial inbreeds.

CONCLUSION

Selective programs based on the studies depending on the traits of these inbreeds, such as chest width, body length, Length of the leg bone, primary and final live body weight and genetic gain. The molecular analysis for many genes can be related with this traits in many ways. The purpose of which is to adopt the selection at an early stage, depending on the genetic structure which is characterized by good traits and required.

REFERENCES

Fujita S, Yamaguchi M, Hiramoto D, Saneyasu T, Honda K and Kamisoyama H. 2018. Effects of Fasting and refeeding on the mRNA levels of insulin-like growth factor-binding proteins in chick liver and brain. *The Journal of Poultry Science*, 55(4): 269-273.

Haley C and Koning DJ. 2006. Genetical genomics in livestock potentials and pitfalls. *Animal Genetic*, 37 (Supplement 1): 10 -12.

Kadlec J, Hosnedlová B, Rehout V, Čítek J, Večerek L and Hanusová L. 2011. Insulin-like growth factor-I gene polymorphism and its association with growth and slaughter characteristics in broiler chickens. *Journal of Agrobiology*, 28(2): 157-163.

Khoa DVA, Khang NTK, Ngu NT, Matey J, Loan HTP and Thuy NTD. 2012. Single nucleotide polymorphisms in Gh, Ghr, Ghsr and insulin candidate genes in chicken breeds of Vietnam. *Green Journal of Agricultural Science,* 3(10): 716-724.

Kaya M and Yıldız MA. 2008. Genetic diversity among Turkish native chickens, Denizli and Gerze, estimated by microsatellite markers. *Biochemical Genetics*, 46(7-8): 480-491.

Li H, Zhu W, Chen K, Song W, Shu J and Han W. 2010. Effects of the polymorphisms of GHR gene and IGF-1 gene on egg quality in wenchang chicken. *Research Journal of Poultry Sciences*, 3(2): 19-22.

Nakashima K and Ishida A. 2018. Regulation of autophagy in chick myotubes: effects of insulin, insulin-like growth factor-I, and amino acids. *The Journal of Poultry Science*, 55(4): 257-262.

Nie Q, Sun B, Zhang D, Luo C, Ishag NA, Lei M, Yang G and Zhang M. 2005. High diversity of the chicken growth hormone gene and effects on growth and carcass traits. *Journal of Heredity*, 96(6): 698-703.

Simon J. 1989. Chicken as a useful species for the comprehension of insulin action. *Critical Reviews in Poultry Biology*, 2(2): 121–148.

Zhang XL, Jiang X, Liu YP, Du HR and Zhu Q. 2007. Identification of AvaI polymorphisms in the third intron of GH gene and their associations with abdominal fat in chickens. *Journal of Poultry Science*, 86(6): 1079-1083.

Zhou H, Mitchell AD, McMurtry JP, Ashwell CM and Lamont SJ. 2005. Insulin-like growth factor-1 gene polymorphism associations with chicken body composition, skeleton integrity and metabolic traits in chickens. *Poultry Science,* 84(2): 212-219.