Journal of Animal & Plant Sciences, 31(6): 2021, Page: 1542-1547
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2021.6.0357

GENETIC POLYMORPHISMS AND EXPRESSION PATTERN OF IGF-I GENE IN COMMERCIAL BROILER STRAINS

M. Fouda[1], A. Ateya[1]*, I. EL Araby[2], and A. Elzeer[1]

[1]Department of Animal Husbandry and Wealth Development, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt
[2]Department of Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt
*Corresponding author email: ahmed_ismail888@yahoo.com

ABSTRACT

Genetic polymorphisms along with expression patterns of *IGF-I* gene were studied in 300-day-old chicks of three different broiler strains (Cobb, Avian, and Ross) with 100 birds each. Blood samples were collected from chicks of each strain for DNA extraction and serum analysis of IGF-I. PCR-*Pst*I digestion of 621-bp for a fragment of 5′ untranslated region of *IGF-I* gene revealed a monomorphic pattern for genotype AA; where one undigested fragment (621-bp) was attained in all strains. Liver of randomly selected ten female chicks from each strain was collected for RNA extraction and quantitative real time PCR (RT-PCR) genetic assessment. Expression patterns of *IGF-I* gene revealed a significant up-regulation in Avian than Cobb and Ross breeds as shown by significant lower values of ΔCT. Serum analysis results were in coincidence with *IGF-I* gene expression; where Avian strain had a higher level of IGF-I than Cobb and Ross strains. It could be concluded that genetic *IGF-I-Pst*I locus polymorphism could not be used as a genetic marker (MAS) for growth traits in commercial broiler strains. However, changes in *IGF-I* expression patterns could be a proxy marker to follow up and improve growth performance of broiler chickens.

Keywords: broiler; *IGF-1* gene; PCR-RFLP; real-time PCR
Published first online March 31, 2021 Published final Nov. 20, 2021.

INTRODUCTION

Poultry industry has become of greater importance as it is a major source of animal proteins that alleviates and overcomes protein gap and malnutrition (Windhorst, 2006). In broiler strains, increasing body weight and improvement of feed utilization could be easier; this may be attributed to applications of new technologies in nutritional manipulations and genetics level. Consequently, broiler strains have higher performances than before (Tavárez and Santos, 2016).

Traditional selection methods could increase growth rate and carcass yield in poultry farming; however, this process has undesirable effects, including health issues and increased rate of sudden death (Nicholson 1998). It is suitable to use molecular markers associated with traits to improve production traits and health concurrently. Due to complex genetic control of growth, the discovery of molecular mechanisms will help in efficient selection of broiler chickens (Deeb and Lamont 2002). Molecular markers have the advantage of earlier selecting for a specific trait in animals. There is no need to wait any longer for the organism to reach a certain stage for phenotypically observing the trait, which in turn results in overwhelming a major hurdle for the animal breeder via early culling of unfavorable animals and managing to save breeding costs (El-Shafaey *et al*. 2017).

Growth is regulated by numerous endocrine pathways and sets of complex genes (Zhang *et al*. 2008). Therefore, using a candidate gene is a prevailing approach for genetic improvement. This may give rise to the best efficacy in selecting favourable traits required to ameliorate production. Insulin-like growth factor-I (*IGF-I*) gene is one of the most significant candidate genes for growth in chickens (Kaya and Yıldız 2008).

IGF-I belongs to polypeptide hormones family, prepro-insulin, which is comprised of proinsulin, IGF-I, IGF-II and C peptide with multiple metabolic and anabolic functions. It has beneficial effects during post-delivery growth and its production in the liver is under the effect of growth hormone and nutritional conditions. IGF-I can stimulate endocrine, autocrine and/or paracrine growth functions. The IGF-I biological actions are mediated by a group of cell surface receptors, IGF-I receptor, insulin receptor, and IGF-II receptor (Scanes *et al*. 1989).

Avian IGF-I was recognized as a biological candidate gene responsible for body composition, growth rate, fat deposition and metabolism (Kadlec *et al*. 2011). It regulates proliferation of satellite cell and skeletal muscle hypertrophy (Zanou and Gailly 2013). There was a positive correlation between the concentration of IGF-1 and body weight in broiler chickens as IGF-I stimulates skeletal muscle growth by increasing protein synthesis rate (Boschiero *et al*. 2013; Scanes 2009; Wen *et al*. 2014).

Restriction Fragment Length Polymorphism (RFLP) has been a significant genetic marker used in medicine, forensic science, paternity tests and genetic studies. It was used to differentiate genetically between organisms and recognize interspecie variation by the analysis of unique patterns in DNA fragments (Cheriyedath 2018). Real time PCR (RT-PCR) is a very sensitive and flexible method used to quantify the mRNA transcriptional level (Weis *et al*. 1992). It analyses various samples attained from as little as one cell in the same experiment (Wang and Brown 1999).

Many studies explored the association between the polymorphisms in *IGF-I* gene and growth performance traits in chickens; however controversies were found (Ali *et al*. 2016; Kadlec *et al*. 2011; Promwatee *et al*. 2013; Wang *et al*. 2004). Moreover, previous studies reported *IGF-I* expression pattern in chicken tissues within the same breed (Abdalhag *et al*. 2016; Xiao *et al*. 2017); however the expression pattern comparisons among different broiler breeds/strains and the utilization of the variation in such expression as biomarker for selection of favourable growth performance breed/strain are scarcely reported (Giachetto *et al*. 2004; Pechrkong *et al*. 2017).

Therefore, this study aimed to reveal the *Pst*I polymorphism of a fragment of 5′ untranslated region of *IGF-I* gene using PCR-RFLP and to clarify the gene expression pattern as well as the serum concentration of *IGF-I* of in three broiler strains (Cobb, Avian and Ross).

MATERIALS AND METHODS

Experimental birds: A total of 300-day-old broiler chicks of both sexes from three different strains (Cobb, Avian and Ross) 100 birds each were used to conduct the study. The chicks were purchased from a commercial hatchery. The collection of samples and care of the animals used in this study followed guidelines for experimental animals established by Research Ethics Committee, Faculty of Veterinary Medicine; Mansoura University, Egypt

Experimental samples

Blood samples: Two blood samples were taken from wing vein of each bird, one sample in EDTA containing vacutainer tubes for DNA extraction and the other sample was collected without any anticoagulant into a clean tube, was left to clot at room temperature before centrifugation at 3000 rpm for 5 min for serum collection. The obtained serum was cooled and kept at -20°C until biochemically analysed.

Tissue samples: Ten female chicks from each strain were randomly selected and sacrificed for liver samples collection. Liver samples were collected under sterilization, then phosphate buffer saline (PBS) was used to wash them. Afterwards, they were snap frozen in liquid nitrogen before storing at -80°C for gene expression quantification.

PCR-RFLP for IGF-I gene: For amplification of a fragment of 5′ untranslated region of *IGF-I* gene, PCR was performed. The Amplicon size was expected to be 621-bp when the primers described by Leveque *et al*. (2003) were used.

Forward: 5′-GAC TAT ACA GA A AGA ACC CAC-3′
Reverse: 5′-TAT CAC TCA AGT GGC TCA AGT-3′

Polymerase chain reaction mixture was done in a 25 µl consisted of: 2µl DNA, 12 µl PCR master mix (Jena Bioscience, Germany), 1µl of each primer, and 9µl H_2O (distilled water). A thermal cycler was used to obtain the final reaction mixture. The temperature schedule of the PCR was programmed as described in Table 1.

Table 1. Conditions of polymerase chain reaction (PCR) for the amplified genes.

Gene	Initial denaturation	No. of Cycles	Denaturation	Annealing Temperature	Extension	Final extension
IGF-I (part of 5′ untranslated region)	94ºC /5 min	35	94ºC /45 s	57ºC /45 s	72ºC /1min	72ºC/ 10 min

*Pst*I Fast digestion enzyme (Thermo scientific) was used for digesting the amplified DNA fragments of *IGF-I* gene at a temperature of 37°C for 30 min. The volume of the reaction was about 30 µl with the following composition: 10µl PCR product, 2µl 10x fast digest green buffer, 1µl fast digest restriction enzyme, and 17µl H_2O (distilled water). Then, detection of the cleaved fragments was done using agarose gel electrophoresis for visualizing the fragment patterns under U.V using gel documentation system (Gel Doc, Alpha-Chem, Umager, USA).

Total RNA extraction, reverse transcription and quantitative real time PCR: Total RNA was extracted from liver tissues in the one-day-old broiler chicks using Trizol reagent following the manufacturer instructions (RNeasy Mini Ki, Catalogue no.74104). The recovered RNA was reverse-transcribed into cDNA following the manufacture protocol (Thermo Fisher, Catalog number: EP0441). cDNA was amplified by real-time

PCR using SYBR Green PCR Master Mix (Quantitect SYBR green PCR kit, Catalog no, 204141). The qPCR was done with a reaction volume of 25µL consisting of 3 µl total RNA, 0.25 µl reverse transcriptase, 12.5 µl 2x Quantitect SYBR green PCR master mix, 0.5 µl of each primer, and 8.25 µl RNase free-water. A thermal cycler was used to obtain the final reaction mixture and programmed as described in Table 2. Afterwards, a melting curve analysis was done to verify the specificity of the PCR product. The β-actin gene was selected as a reference gene for expression analysis. Primer sequences are shown in Table 3. ΔCT was determined for *IGF-I* gene using threshold cycle (CT) values that was normalized to those of the *β- actin* housekeeping in each sample. Lower ΔCT indicates increased expression (Livak and Schmittgen 2001; Pfaffl 2001).

Table 2: The thermal cycler condition used during real time PCR.

Gene		*IGF1*	*ß. Actin*
Reverse transcription		50˚C 30 min.	50˚C 30 min.
Primary denaturation		94˚C 15 min.	94˚C 15 min.
Amplification (40 cycles)	**Secondary denaturation**	94˚C 15 sec.	94˚C 15 sec.
	Annealing (Optics on)	65˚C 30 sec.	51˚C 30 sec.
	Extension	72˚C 30 sec.	72˚C 30 sec.
Dissociation curve (1 cycle)	**Secondary denaturation**	94˚C 1 min.	94˚C 1 min.
	Annealing	65˚C 1 min.	51˚C 1 min.
	Final denaturation	94˚C 1 min.	94˚C 1 min.

Table 3. Primers sequence of the *IGF-I and ß. Actin* genes in real time PCR.

Gene	Primers sequence	Product length (bp)	Accession number	Reference
IGF-1	F: 5'-CAGAGCAGATAGAGCCTGCG-3' R: 5'-TCTGCAGATGGCACATTCAT-3'	655	M74176	Amills *et al*. (2003)
β-Actin	F: 5'-CCACCGCAAATGCTTCTAAAC-3' R: 5'-AAGACTGCTGCTGACACCTTC-3'	152	NM_205518.1	Yuan *et al*. (2007)

Biochemical serum analysis for determination of IGF-I: Insulin like growth factor-1 concentration was determined according to Alberti *et al*. (2011). The kit was purchased from (Siemens health diagnostics – USA). The producer depends on using IMMULITE/IMMULITE 1000 (IGF-1), which is a solid-phase, enzyme labeled chemiluminescent immunometric assay. The solid phase (bead) was coated with monoclonal murine anti-(IGF-1) antibody. The liquid phase composition was alkaline phosphatase (bovine calf intestine) in conjugation with polyclonal sheep anti-(IGF-1) antibody and alkaline phosphatase (bovine calf intestine) in conjugation with monoclonal murine anti-(IGF-1) antibody. The sample and reagent were incubated with the coated bead for 60 minutes. Meanwhile, (IGF-1) in the sample formed the antibody sandwich complex with the monoclonal murine anti-(IGF-1) antibody on the bead, enzyme conjugated polyclonal sheep anti-(IGF-1) antibody and enzyme conjugated monoclonal murine anti-(IGF-1) antibody in the reagent. After that, unbound sample and enzyme conjugate were removed by centrifugal washes. At the end, chemiluminescent substrate was added to the test unit that contained the bead and the signal was produced in proportion
n to the bound enzyme.

Statistical analysis: The standard error of the mean was used to express the results. Comparisons were made using one-way analysis of variance (ANOVA) to examine all groups' unpaired values. Differences were considered to be significant at the level of ($P \leq 0.05$). Statistical analysis was carried out using the Graph Pad Prism (SAS Institute, Inc., USA) according to the techniques described by Dawson and Trapp (2004).

RESULTS

Effect of IGF-I gene polymorphism: Genomic DNA of chicks from each breed was used for amplifying specific DNA fragments 621-bp of 5′ untranslated region of *IGF-I* gene. Restriction analysis of 621-bp PCR products digested with *Pst*I gave a monorphic undigested fragment (621-bp) for one genotype AA in all studied strains (Figure 1).

Expression pattern of IGF-I gene: Gene expression level of liver *IGF-I* in the Avian was significantly higher than Cobb and Ross strains. This is shown by the significantly lower value of ΔCT indicating increased expression (Figure 2).

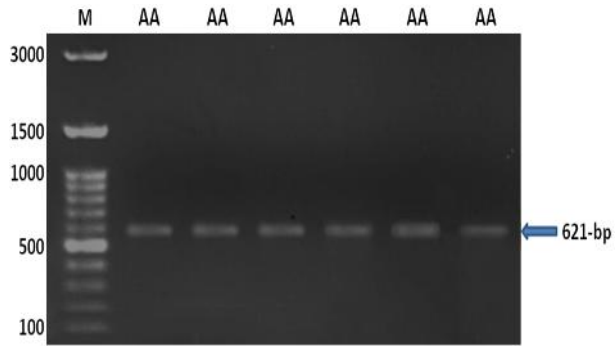

Figure 1: Representative samples of RFLP banding pattern of *IGF-I* gene (621 bp) from three strains (Cobb 500, Ross 308 and Avian 48) after digestion with *Pst I* enzyme, M: 100 bp ladder.

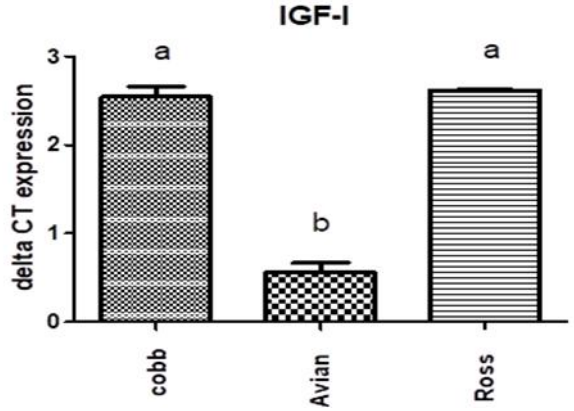

Figure 2: Relative expression of *IGF-I* gene in liver of chicken strains. The β-actin gene was used as a reference gene to normalize the data and shown as ΔCT ± SE. *Lower ΔCT values indicate increased expressions*.

Biochemical serum analysis of IGF-I: Data shown in figure 3 illustrated serum level of *IGF-I* in three different broiler strains. The data revealed that, there is a significant difference; where Avian was higher in serum level of *IGF-I* than Cobb and Ross strains.

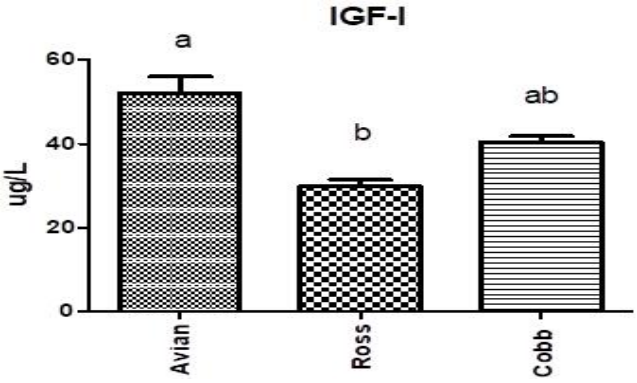

Figure 3: Serum level of *IGF-I* in the Cobb, Ross and Avian strains shown as means ±SE.

DISCUSSION

In this study, *IGF-I* gene polymorphism in the studied chicken breeds using PCR-RFLP-*Pst*I revealed a monomorphic undigested fragment (621-bp) representing one genotype AA. This is contrary to the findings of Ali *et al*. (2016) who investigated the polymorphism in *IGF-I* gene in Pakistani native Desi chicken using PCR-RFLP-*Pst*I and found three genotypes (AA, AB, and BB) with frequencies 0.12, 0.64, and 0.24, respectively. AA individuals had the highest body weight followed by AB and BB genotypes, respectively. The *IGF-I-Pst*I genetic polymorphism could be attributed to various genetic backgrounds of strains and the number of chickens in these studies**.** Many studies reported the dependency of the variation in growth performance phenotype on *IGF-I* gene polymorphisms. For instance, Kadlec *et al*. (2011) reported that there was a relatedness of *IGF-I* gene polymorphism with growth and slaughter characteristics in two different broiler chicken breeds (Cobb 500 and Ross 308). Promwatee *et al*. (2013) also elicited that there was association between *IGF-I* gene polymorphism and growth and carcass traits in Thai synthetic chickens. On contrary, Wang *et al*. (2004) reported no differences between polymorphisms in *IGF-I* gene and growth traits in six broiler chicken breeds.

In this context, real time PCR was carried out to quantify *IGF-I* mRNA level in the liver of the three studied strains. Our results revealed that, the expression pattern in Avian is significantly higher than both Cobb and Ross strains indicating that Avian is the best strain regarding growth performance. This is shown by significantly lower ΔCT value. Previous studies reported *IGF-I* expression pattern in chicken tissues within the same breed (Abdalhag *et al*. 2016; Xiao *et al*. 2017). Abdalhag *et al*. (2016) indicated a similar *IGF-I* gene expression level between males and females of Jinghai yellow chickens in all tissues except the breast and leg muscles. Xiao *et al*. (2017) also observed decreased *IGF-1* expressions in slow-growing Ross broilers relative to fast-growing broilers. The expression pattern comparisons among different broiler strains and the utilization of the variation in such expression as biomarker for selection of favourable growth performance breed are scarcely reported (Giachetto *et al*. 2004; Pechrkong *et al*. 2017). Giachetto *et al*. (2004) indicated that the changes in liver IGF-I mRNA expression in three broiler chicken strains with different growth rate were independent of chicken strain, but varied depending on chicken age. Pechrkong *et al*. (2017) elicited that IGF-1 expression has no effect on growth performances in Thai indigenous chicken and commercial broilers but might affect with muscle fiber type.

In this study, the findings of *IGF-I* serum level are consistent with the findings of Duclos (2005) and Xiao *et al*. (2017) who cited that, there was a positive

correlation between serum level of *IGF-I* and growth rate. Both serum analysis and gene expression results of *IGF-I* are compatible reflecting that Avian has higher growth rate than Cobb and Ross strains respectively.

Conclusion: PCR-RFLP of fragment of 5′ untranslated region of *IGF-I* gene using *Pst*I restriction enzyme was monomorphic among the studied strains. Therefore, *IGF-I- Pst*I locus could not be used as marker for marker assisted selection for growth performance traits in broiler chicken. However, quantitative real time PCR can analyse variable *IGF-I* gene expression patterns which could be used as a biomarker to follow up and improve growth performance of chickens. This was indicated by higher expression pattern of IGF-I gene in Avian than both Cobb and Aves breeds. Serum analysis of *IGF-I* could be used to confirm gene expression results which in this study revealed that Avian is the best one of all studied strains regarding growth performance. The association of numerous polymorphisms in diverse regions of the *IGF-I* gene and other chicken breeds should be discovered in future studies.

Acknowledgments: The authors acknowledge members of Animal Husbandry and Development of Animal Wealth Biotechnology lab, Faculty of Veterinary Medicine, Mansoura University for their valuable advices and helpful discussions.

REFERENCES

Abdalhag, M., T. Li, L. Duan, T. Zhang, G. Zhang, J. Wang and Y. Wang. (2016). Association analysis of IGF-I gene expression with growth and reproductive traits in Jinghai yellow chickens. Genetics and Molecular Research 15(4). 1-11.

Alberti, C., D. Chevenne, I. Mercat, E. Josserand, P. Armoogum-Boizeau, J. Tichet and J. Léger. (2011). Serum Concentrations of Insulin-like Growth Factor (IGF)-1 and IGF Binding Protein-3 (IGFBP-3), IGF-1/IGFBP-3 Ratio, and Markers of Bone Turnover: Reference Values for French Children and Adolescents and z-Score Comparability with Other References. Clincal Chemistry 57(10). 1424-1435.

Ali, A., K. Javed, M. Akram, M. Dawood andand A. Saleem. (2016). Polymorphism of insulin-like growth factor-1 gene and its association with growth rate in desi chicken of Pakistan. J. Animal and Plant Sciences 26(3). 858-861.

Amills, M., N. Jimenez, D. Villalba, M. Tor, E. Molina, D. Cubilo, C. Marcos, A. Francesch, A. Sanchez and J. Estany. (2003). Identification of three single nucleotide polymorphisms in the chicken insulin-like growth factor 1 and 2 genes and their associations with growth and feeding traits. Poultry science 82(10). 1485-1493.

Boschiero, C., E.C. Jorge, K. Ninov, K. Nones, M.F. do Rosário, L.L. Coutinho, M.C. Ledur, D.W. Burt and A.S.A.M.T. Moura. (2013). Association of IGF1 and KDM5A polymorphisms with performance, fatness and carcass traits in chickens. [journal article]. J. Applied Genetics 54(1). 103-112.

Cheriyedath, S. (2018). Restriction Fragment Length Polymorphism (RFLP) Technique.

Dawson, B. and R. Trapp. (2004). Basic and clinical biostatistics.; 2004. Monterey: McGraw-Hill.

Deeb, N. and S.J. Lamont. (2002). Genetic Architecture of Growth and Body Composition in Unique Chicken Populations. J. Heredity 93(2). 107-118.

Duclos, M.J. (2005). Insulin-like growth factor-I (IGF-1) mRNA levels and chicken muscle growth. J. physiology and pharmacology: an official J. of the Polish Physiological Society 56 Suppl 3. 25-35.

El-Shafaey, E.-S., A. Ateya, H. Ramadan, R. Saleh, Y. Elseady, E. Abo El Fadl and S. El-Khodery. (2017). Single Nucleotide Polymorphisms in IL8 and TLR4 Genes as Candidates for Digital Dermatitis Resistance/Susceptibility in Holstein Cattle. Animal Biotechnology 28(2). 131-137.

Giachetto, P.F., E.C. Riedel, J.E. Gabriel, M.I.T. Ferro, S.M.Z. Di Mauro, M. Macari and J.A. Ferro. (2004). Hepatic mRNA expression and plasma levels of insulin-like growth factor-I (IGF-I) in broiler chickens selected for different growth rates %J Genetics and Molecular Biology. 27(1). 39-44.

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

Kaya, M. and M.A. Yıldız. (2008). Genetic Diversity Among Turkish Native Chickens, Denizli and Gerze, Estimated by Microsatellite Markers. [journal article]. Biochemical Genetics 46(7). 480-491.

Leveque, G., V. Forgetta, S. Morroll, A.L. Smith, N. Bumstead, P. Barrow, J.C. Loredo-Osti, K. Morgan and D. Malo. (2003). Allelic variation in TLR4 is linked to susceptibility to Salmonella enterica serovar Typhimurium infection in chickens. Infection and Immunity 71(3). 1116-1124.

Livak, K.J. and T.D. Schmittgen. (2001). Analysis of Relative Gene Expression Data Using Real-

Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25(4). 402-408.

Nicholson, D. (1998). Research: is it the broiler industry's partner into the new millennium? World's Poultry Science J. 54(3). 271-278.

Pechrkong, T., R. Charoensook, W. Kaewkong and T. Srikanchai. (2017). Comparative study of IGF-1 gene expression and growth performance traits in Thai indigenous chicken (Chee) and commercial broiler.

Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Research 29(9). e45-e45.

Promwatee, N., B. Laopaiboon, T. Vongpralub, Y. Phasuk, S. Kunhareang, W. Boonkum and M. Duangjinda. (2013). Insulin-like growth factor I gene polymorphism associated with growth and carcass traits in Thai synthetic chickens. Genetics and Molecular Research 12(4). 4332-4341.

Scanes, C.G. (2009). Perspectives on the endocrinology of poultry growth and metabolism. General and Comparative Endocrinology 163(1). 24-32.

Scanes, C.G., E.A. Dunnington, F.C. Buonomo, D.J. Donoghue and P.B. Siegel. (1989). Plasma concentrations of insulin like growth factors (IGF-)I and IGF-II in dwarf and normal chickens of high and low weight selected lines. Growth, development, and aging: GDA 53(4). 151-157.

Tavárez, M. A. and F.S.D.L. Santos. (2016). Impact of genetics and breeding on broiler production performance: a look into the past, present, and future of the industry. Animal Frontiers: 6 (4), 37–41.

Wang, T. and M.J. Brown. (1999). mRNA Quantification by Real Time TaqMan Polymerase Chain Reaction: Validation and Comparison with RNase Protection. Analytical Biochemistry 269(1). 198-201.

Wang, W., K. Ouyang, J. Ouyang, H. Li, S. Lin and H. Sun. (2004). Polymorphism of Insulin-like Growth Factor I Gene in Six Chicken Breeds and Its Relationship with Growth Traits. Asian-Australas J Anim Sci 17(3). 301-304.

Weis, J.H., S.S. Tan, B.K. Martin and C.T. Wittwer. (1992). Detection of rare mRNAs via quantitative RT-PCR. Trends in Genetics 8(8). 263-264.

Wen, C., P. Wu, Y. Chen, T. Wang and Y. Zhou. (2014). Methionine improves the performance and breast muscle growth of broilers with lower hatching weight by altering the expression of genes associated with the insulin-like growth factor-I signalling pathway. British J. Nutrition 111(2). 201-206Windhorst, H., W. (2006). Changes in poultry production and trade worldwide. World's Poultry Science J. 62(04).585 – 602.

Xiao, Y., C. Wu, K. Li, G. Gui, G. Zhang and H. Yang. (2017). Association of growth rate with hormone levels and myogenic gene expression profile in broilers. J. Anim. Science and Biotechnology 8(1). 43.

Yuan, J.M., Y.M. Guo, Y. Yang and Z.H. Wang. (2007). Characterization of Fatty Acid Digestion of Beijing Fatty and Arbor Acres Chickens. Asian-Australasian J. Animal Sciences 20(8). 1222-1228.

Zanou, N. and P. Gailly. (2013). Skeletal muscle hypertrophy and regeneration: interplay between the myogenic regulatory factors (MRFs) and insulin-like growth factors (IGFs) pathways. [journal article]. Cellular Molecular Life Sciences 70(21). 4117-4130.

Zhang, C., W. Zhang, H. Luo, W. Yue, M. Gao and Z. Jia. (2008). A New Single Nucleotide Polymorphism in the IGF-I Gene and Its Association with Growth Traits in the Nanjiang Huang Goat. Asian-Australasian J. Anim. Sciences 21(8). 1073-1079.