



Investigating expression of IGF-1 candidate gene for growth-associated microsatellite genotypes in a resource population of RIR chicken

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ABSTRACT

Present study was undertaken to analyze relative expression of IGF-1 gene in liver and breast muscle, and to investigate probable association of chicken growth-linked microsatellite genotypes with IGF-1 gene expression in a resource population of RIR chicken. In this experiment, 114 birds of combined sexes were screened for growth-associated microsatellite genotypes and 25 alleles were separated through 3.4% MetaPhore agarose gel electrophoresis with their sizes ranging from 97 bp at MCW0010 to 349 bp at LEI0071. Twelve birds of either sexes comprising equally from these birds were selected at the age of 12 weeks for gene expression study by quantitative reverse transcriptase PCR method. Higher $40-\Delta C_t$ value was considered as higher expression. Least squares analysis of variance revealed that birds' hatch factor had significant effect on relative mRNA expression of IGF-1 gene in liver tissue other than breast muscle. Birds from the second hatch showed higher expression (35.86 ± 1.120) in liver tissue in comparison to the chicks from first hatch (32.58 ± 0.792). The results also evidenced that the liver tissue had higher expression (34.22 ± 0.690) than that in the breast muscle (33.923 ± 0.186) tissue. The males had numerically higher expressions in both the tissues as compared to female birds. The microsatellites could not demonstrate significant genotypic differences in IGF-1 gene expression in liver and breast muscle tissues of the birds. It is suggested to study a large sample to investigate significant variation in IGF-1 gene expression in the birds under different sexes and growth-linked microsatellite genotypes to draw a valid conclusion.

Keywords: Association, IGF-1 gene expression, Liver, Microsatellite genotypes, Muscle tissue, RIR chicken

DNA markers could be used in tracking of inheritance pattern of linked gene, and establishing significant association between marker and the trait of interest. Among various DNA markers, microsatellites are the marker of choice (Debnath *et al.* 2020), because of its ease identification, moderate to high degree of polymorphism and co-dominant inheritance pattern (Rahim *et al.* 2017, Debnath *et al.* 2019). More than 800 highly polymorphic microsatellites are available in the chicken genome, which allows scanning for markers linked to economic traits of interest by using a genome-wide search (Groenen *et al.* 2000). Several microsatellites like ADL0328, LEI0068, LEI0071, LEI0079, LEI0146, MCW0010, MCW0018, MCW0043, MCW0058, MCW0106, etc. have been reported to be associated with growth traits at different weeks of age in several chicken breeds (Crooijmans 1996,

Wardecka *et al.* 2002, Navarro *et al.* 2005, Liu *et al.* 2008, Zhang *et al.* 2008, Nassar *et al.* 2012, Radwan *et al.* 2014).

Chickens with high growth rate show higher circulating levels of insulin like growth factor-1 (IGF-1) and higher hepatic IGF-1 mRNA levels as compared to those with low growth rate (Beccavin *et al.* 2001). The chicken IGF-1 is a non-glycoprotein hormone and structurally homologous to pro-insulin. It acts on an endocrine and a paracrine mode as a general enhancer of lean tissue growth. More recently, with the purpose of improving carcass quality, chickens have been selected for high breast meat yield, low abdominal fat percentage, and high body yield. The IGF-1 gene expression is ubiquitous, so that invalidation of the IGF-1 gene by targeted recombination of null mutant leads to general hypoplasia (Liu *et al.* 1993). Overexpression of the IGF-1 gene can induce a general hyperplasia if circulating levels are increased like in IGF-1 transgenic mice (Mathews *et al.* 1986) or simply a muscular hypertrophy if overexpression is restricted to the muscle without marked modifications of circulating levels (Coleman *et al.* 1995). These are the things of motivation to select IGF-1 as a candidate gene for the present study to analyze mRNA expression of IGF-1 candidate gene for growth-associated microsatellite markers in liver and breast muscle tissues of RIR chicken.

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MATERIALS AND METHODS

Experimental birds and genetic background: In this experiment, 114 birds of combined sexes were screened for growth-associated microsatellite genotypes (Yadav 2016). Among these birds, twelve birds of either sex were equally selected at the age of 12 weeks for gene expression study. The source of these birds was a resource population of 286 straight-run chicks, progeny of 11 sires and 44 dams in few hatches at the experimental layer farm of ICAR-Central Avian Research Institute, Izatnagar (Debnath *et al.* 2020), generated through molecular breeding of RIR chicken based on the genotypes at ADL0176 microsatellite locus (Debnath *et al.* 2015, Yadav *et al.* 2015) demonstrating strong association with layer economic traits (Das 2013, Debnath *et al.* 2015, Rahim 2015). The base population was from a pure strain of RIR chicken undergoing 30 generations of genetic selection based on 40th week part-period egg production record along with some independent culling levels practiced for egg weight (individual basis) at 28th weeks of age (Das *et al.* 2020). The chicks were maintained at the institute layer farm under standard brooding, housing and *ad lib.* feeding management and vaccination practices (Debnath *et al.* 2015, Yadav *et al.* 2015). All the experimental procedures on animals were carried out according to the recommendations and approval of the Institute Animal Ethics Committee (IAEC) as per the guidelines set forth by the Institutional Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Screening for microsatellite genotypes: Genomic DNA samples were extracted by following Phenol-Chloroform-Isoamyl alcohol method (Kagami *et al.* 1990) from the birds' venous blood and analyzed for quality checking on 0.7% horizontal submarine agarose gel electrophoresis

followed by its purity and quantity determination by using NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). PCR ready DNA samples were prepared at a final concentration of 50 ng/μl from those samples showing intact DNA band and optical density ratio (260 nm : 280 nm) between 1.7 and 1.9. These DNA samples were screened for nine informative microsatellite markers (Yadav 2016) and their primers were synthesized from M/S Xcelris Genomics Labs Ltd. (Ahmadabad). Annealing temperature for each of the primer pairs was optimized (Table 1) and PCR amplifications of the DNA samples were carried out for each microsatellite marker as described by Debnath *et al.* (2017). The PCR-amplified products were checked for their probable sizes through 2% horizontal agarose gel electrophoresis. The microsatellite alleles were then identified by running the PCR-amplified products on horizontal MetaPhore™ agarose gel electrophoresis (3.4% MAGE) along with 50 bp DNA ladder in parallel @ 6-8 V/cm for 2 h 30 min. The molecular sizes of all the alleles at different microsatellite loci were estimated using the Quantity One® software 4.6.8 on Gel Documentation system (Biorad Laboratories Inc., USA) (Debnath *et al.* 2020). Allelic patterns of microsatellites were documented and probable genotype in each sample at each microsatellite loci was recorded.

RNA extraction and cDNA synthesis: The birds were sacrificed by cervical dislocation at 12 weeks of age and two tissues viz. liver and breast muscle weighing approximately 50-100 mg were collected aseptically in ~1.0 ml RNeasy® (Ambion, USA) and cryopreserved at -80°C. Total RNA was extracted from each homogenized (using tissue homogenizer at 20,000 rpm for about 30 sec) tissue sample by using TRIzol® reagent (as described by Hongbao *et al.* 2008), then dissolved into 50 μl of nuclease

Table 1. Panel of growth-linked microsatellite loci along with their primer sequences, optimized annealing temperatures (T_a) and alleles recorded in a resource population of RIR chicken

Microsatellite loci	Forward (F) and Reverse (R) Primer Sequences	References	T _a (°C)	Allele code: allele size (bp)
ADL0328	F: 5'-CAC CCA TAG CTG TGA CTT TG-3' R: 5'-AAA ACC GGA ATG TGT AAC TG-3'	Nassar <i>et al.</i> (2012)	49	A:128, B:123, C:122
LEI0068	F: 5'-GTG CAG AAA GAC AAG GCA GTC-3' R: 5'-AGC AGG TAA AGA GGC TAC AGG-3'	http://www.thearkdb.org/	55	A: 200, B: 190, C: 185
LEI0071	F: 5'-TCA GGT TAG TCT GAC CAT TGC-3' R: 5'-TGA GTG TAA GAT TGC TAA TGG A-3'	http://www.thearkdb.org/	55	A: 349, B: 336, C: 311, D: 285
LEI0079	F: 5'-AGG CTC CTG AAT GAA TGC ATC-3' R: 5'-TCA TTA TCC TTG TGT GAA ACT G-3'	Liu <i>et al.</i> (2008)	55	A: 223, B: 214
LEI0146	F: 5'-TCA AGC CAC CAA AGT GCT TGG-3' R: 5'-GAT CAC TCT GCT CAT AGC AGT-3'	Pandya <i>et al.</i> (2005)	55	A:290, B: 270
MCW0010	F:5'-CTG TAG AAT TAC AGA AAT ACA-3' R: 5'-TAG TAC AAG AAT CTA GTG TTA AAA-3'	Nassar <i>et al.</i> (2012)	55	A: 114, B: 103, C: 97
MCW0018	F:5'-GGA ATT TGA ACA CCT GAG ATT TCC-3' R:5'-CAC TAT GTT TAT GGC AAA CTC CTG-3'	Nassar <i>et al.</i> (2012)	55	A: 236, B: 223
MCW0058	F: 5'-GGG CACACA GAGTGG ACACC A-3 R: 5'-TAGCATTTT CTCCAA TGA CTCCGG-3'	http://www.thearkd	55	A: 189, B: 181, C: 164
MCW0106	F: 5'-GGC AAC TAA GTT GTG GAC TG-3' R: 5'-GCA GCA TTC AGT GGG ATA AT-3'	Kong <i>et al.</i> (2006)	50	A: 134, B: 129, C:128

free water and stored at -80°C until further use. All the samples showing an absorbance ratio (260 nm : 280 nm) of $>1.8-2.0$ were considered (Krishnan *et al.* 2022) and the possible trace of genomic DNA was removed from the samples by using DNase I Kit[®] (Amplification Grade, Invitrogen) (Yadav 2016). The samples were stored at -20°C . Concentration of each sample was computed and adjusted to make a uniform concentration ($1\mu\text{g}/\mu\text{l}$). One microlitre of purified RNA from each sample was taken as template and first strand cDNA was prepared by using Verso cDNA synthesis kit[®] (Thermo Fisher Scientific Inc., USA) and random hexamer primers ($400\text{ ng}/\mu\text{l}$) as per the manufacturer’s protocol. Concentration of cDNA of each sample was equalized to $25\text{ ng}/\mu\text{l}$ for use in Real time Quantitative Reverse Transcriptase PCR (qRT-PCR) and stored at -80°C until further use.

Quantitative real-time PCR analysis: Primer pairs of IGF-1 gene (F 5'-GGTGCTGAGC TGGTTGATGC-3'/ R 5'-CGT ACA GAG CGT GCA GAT TTA GGT-3') (Bhanja *et al.* 2014) and β -actin (F 5'-GGAAGT TAC TCG CCT CTG-3'/ R 5'-AAA GAC ACT TGT TGG GTT AC-3') (Higgs *et al.* 2006) reference gene were synthesized and obtained in lyophilized form (M/S Xcelris Genomics Labs Ltd.) A working primer solution of $10\mu\text{M}$ was prepared from the reconstituted stock solution ($100\mu\text{M}$) and used in PCR reaction mix. The PCR conditions for each gene were found optimized at 58°C . Relative quantification of mRNA expression in each tissue samples was done by qRT-PCR method using CFX 96[®]-Real Time PCR detection system (Biorad Laboratories Inc., USA) (Krishnan *et al.* 2022). The qRT-PCR was carried out using DyNAmo ColorFlash SYBR Green qPCR Kit[®] (Thermo Fisher Scientific Inc., USA) in $20\mu\text{l}$ reaction mixture, containing $10\mu\text{l}$ of $2\times$ SYBR Green master mix with blue dye, $0.5\mu\text{l}$ of $10\mu\text{M}$ each primer, $1.5\mu\text{l}$ of cDNA (37.5 ng) into $7.5\mu\text{l}$ of nuclease free water, prepared in 0.2 ml clear thin walled nuclease-free 8-tube strips with optically clear flat lid (Axygen Scientific Inc., USA) strictly avoiding light exposure and sometimes storing at -20°C , whenever needed. The reaction mixture for each sample was prepared in triplicate to avoid pipetting error. A negative control containing all the ingredients except cDNA in triplicate was also set up to check any contamination. The thermal profile consists of initial denaturation at 95°C for 7 min followed by 40 cycles of denaturation at 95°C for 10 sec, annealing at

58°C for 20 sec and extension at 72°C for 20 sec followed by detection of fluorescent signal by the real time detection system to generate amplification curve. After completion of 40 cycles, each sample was subjected to $60-95^{\circ}\text{C}$ @ $+0.5^{\circ}\text{C}$ increment for 10 sec to generate dissociation curve or melt curve to ensure specific amplification. After the completion of qRT-PCR, the threshold cycle (C_t) value and melting point temperature of each tube was carefully retrieved and reviewed for its corresponding amplification and dissociation curve to ensure appropriateness of specific amplifications (Kumar *et al.* 2019). Subsequently, the data were imported into MS-Excel file and saved for further statistical analysis.

Determination of $40-\Delta C_t$ values: Among triplicate C_t values of the target gene, minimum two C_t values which showed high resemblance were chosen and averaged for subsequent analysis. ΔC_t value for each sample was calculated after subtracting average C_t value of housekeeping gene from average C_t value of target gene. For each sample, the ΔC_t value was subtracted from 40 (total cycle number) so as to obtain $40-\Delta C_t$ (Krishnan *et al.* 2022) whose higher value was considered as higher expression (MacKinnon *et al.* 2009).

Statistical analysis: Differential expression of the target gene in two tissues was analyzed by least squares analysis of variance using JMP 9.0.0 statistical program package (SAS 2010) (SAS Institute, Cary, NC). Hatch and sex were taken as fixed effects in the linear model.

$$Y_{ijk} = \mu + S_i + H_j + e_{ijk}$$

where Y_{ijk} , $40-\Delta C_t$ value of IGF-1-mRNA expression in k^{th} tissue of an individual bird of i^{th} sex under j^{th} hatch; μ , a constant representing the overall mean; S_i , a constant representing the fixed effect of i^{th} sex; H_j , a constant representing the fixed effect of j^{th} hatch; and e_{ijk} , an error term assumed to be normally distributed with mean '0' and variance σ^2 .

Genotypes at particular microsatellite locus (independent) were considered as fixed effect in the linear model as well to assess valid expression variation in genotypic levels.

RESULTS AND DISCUSSION

The Real Time qRT-PCR results of each sample showed clear amplification plot and dissociation curve without any primer dimmer formation, and its multi-component plot

Table 2. Least squares analysis of variances and associated means (\pm SE) of adjusted C_t values of mRNA expression of IGF-1 gene in liver and breast muscle of RIR chicken of a resource population

Source of variation	df	Factors	Liver			Breast muscle		
			MSS	P value	Means (\pm SE)	MSS	P value	Means (\pm SE)
		Overall			34.22 \pm 0.690			33.923 \pm 0.186
Hatch	1	Hatch-1	28.755	0.0403*	32.58 ^a \pm 0.792	0.006	0.9015	33.947 \pm 0.215
		Hatch-2			35.86 ^b \pm 1.120			33.900 \pm 0.305
Sex	1	Male	8.535	0.2246	35.06 \pm 0.94	1.442	0.0802	34.270 \pm 0.256
		Female			33.38 \pm 0.94			33.577 \pm 0.256
Remainder	9		45.180			3.340		

df denotes degrees of freedom; MSS, mean sum of squares; *P<0.05; Means with different superscripts differ significantly (P<0.05).

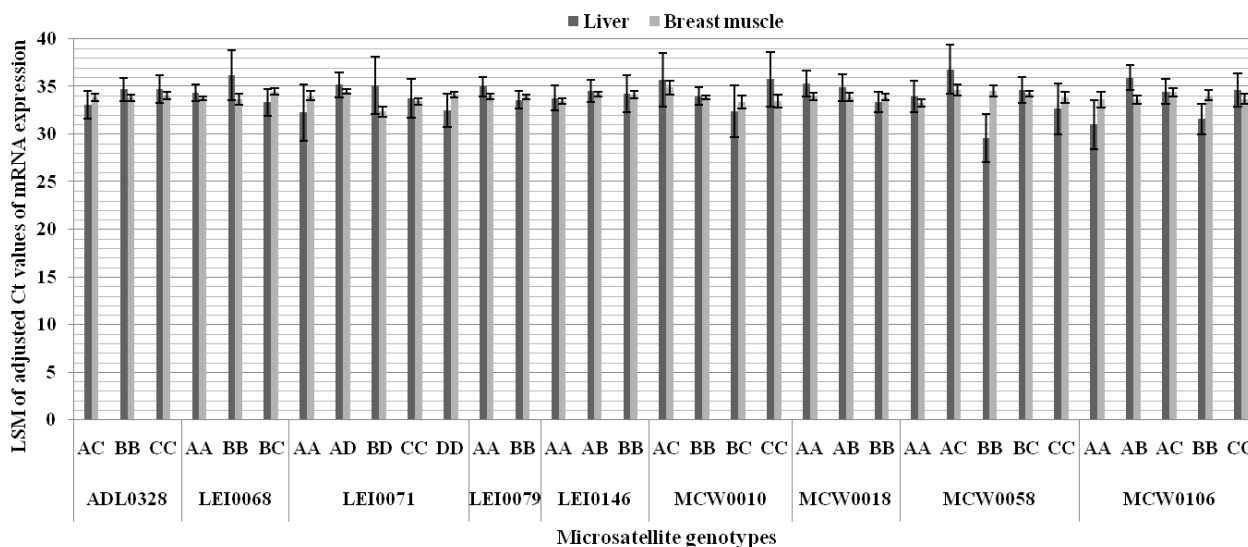


Fig. 1. Column diagram representing least squares means (\pm SE) of adjusted C_t values of mRNA expression of IGF-1 gene in liver and breast muscle under different microsatellite genotypes in a resource population of RIR chicken.

was examined to ensure that the reaction was properly set up and that the reaction mix had not evaporated during the roughly 90-min PCR amplification (Kumar *et al.* 2019). The lack of amplification in the negative control, as evidenced by the non-increased curves, supports the validity of the test.

The results of the least squares analysis of variance of the $40-\Delta C_t$ values of IGF-1-mRNA expression in liver and breast muscle tissues of the RIR birds are presented in Table 2. The analysis revealed that hatch had significant effect on relative mRNA expression of IGF-1 gene in liver tissue other than breast muscle in the RIR birds. Birds from the second hatch demonstrated higher expression (35.86 ± 1.120) in liver tissue in comparison to the chicks from first hatch (32.58 ± 0.792). The mRNA expressions (33.923 ± 0.186 and 33.947 ± 0.215) in the breast muscle tissue could not differ significantly between the birds from two hatches. The results also evidenced that the liver tissue had higher expression (34.22 ± 0.690) than that (33.923 ± 0.186) in the breast muscle tissue. In the line of present findings, Pandey *et al.* (2013) also observed higher expression in hepatic tissue when analyzed the expression of seven genes (IGF1, IGF2, IGF1R, GHR, IGFBP2, MSTN and TGF β 2) in liver and breast muscle of broiler birds. Bhanja *et al.* (2014) reported that arginine and threonine enhanced the expression of growth related genes, viz. IGF-1, IGF-2 and mucin gene in broiler chicken during pre- and post-hatch periods. In the present investigation, the male and female birds had statistically non-significant variation in mRNA expressions in their liver and breast muscle tissues, though the males had numerically higher expressions in both the tissues.

A total of 25 alleles ranging from one to four at different loci with their sizes ranged from 97 bp at MCW0010 to 349 bp at LEI0071 were resolved at nine microsatellite loci (Table 1) and all the samples were genotyped for each microsatellite locus. Least squares analysis of variance

revealed that any of the growth-linked polymorphic microsatellites could not demonstrate significant genotypic differences in mRNA expression of IGF-1 gene in liver and breast muscle tissues of the birds studied, though some genotypes, for example, ADL0328-CC, LEI0068-BB, LEI0071-AD/BD, LEI0079-AA, LEI0146-AB, MCW0010-AC/CC, MCW0018-AA/AB, MCW0058-AC/BC, MCW0106-AB had numerically higher expression levels in hepatic tissue. Similarly, LEI0071-AD, LEI0079-AA, LEI0146-AB, MCW0010-AC, MCW0058-AC genotypes also had higher expressions in breast muscle tissue (Fig. 1). However, probable association of growth-linked microsatellite genotypes with IGF-1 gene expression in birds could not be confirmed and reports in this regard were not available to compare or contrast the present findings. Although, Yun *et al.* (2005) observed that the circulating IGF-1 concentration gradually increased during growth period, and the breast muscle IGF-1 concentration was mainly correlated with body growth at 1 and 5 week while investigating the expression of IGF system during post-hatch growth in KNOC birds. Song *et al.* (2013) also observed significant age-related change in mRNA expression of IGF-1, IGF-2, and type 1 and type 2 IGF receptors in breast, leg and myocardium muscle tissues in ducks. Developmental expression of IGF-1 and IGF-2 was highest in breast muscle during week 1 but decreased quickly and maintained a relatively lower level. The mRNA expressions of IGF-1 and IGF-2 genes were highest in leg muscle at 3 week of age. Expression level of IGF-1R and IGF-2R genes exhibited a 'rise-decline' developmental trend in myocardial tissues. But it is unfortunate not to show any influence of the growth-linked microsatellite genotypes on the IGF-1 gene expression in the birds might be due to very small sample size investigated in the present study.

It is concluded that the hatch had significant effect on the mRNA expression of IGF-1 gene in hepatic tissue of

RIR birds and the hepatic tissue had more expression than the breast muscle. It is suggested to study a large sample to investigate significant variation in IGF-1 gene expression in the birds under different sexes and growth-linked microsatellite genotypes to draw a valid conclusion.

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REFERENCES

- Beccavin C, Chevalier B, Cogburn L A, Simmon J and Duclos M J. 2001. Insulin-like growth factors and body growth in chickens divergently selected for high and low growth rate. *Journal of Endocrinology* **168**(2): 297–306.
- Bhanja S K, Sudhagar M, Goel A, Pandey N, Mehra M, Agarwal S K and Mandal A. 2014. Differential expression of growth and immunity related genes influenced by *in ovo* supplementation of amino acids in broiler chickens. *Czech Journal of Animal Science* **59**(9): 399–408.
- Coleman M E, DeMayo F, Yin K C, Lee H M, Geske R, Montgomery C and Schwartz R J. 1995. Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice. *Journal of Biological Chemistry* **270**(20): 12109–16.
- Crooijmans R P, van Oers P A, Strijk J A, van der Poel J J and Groenen M A. 1996. Preliminary linkage map of the chicken (*Gallus domesticus*) genome based on microsatellite markers: 77 new markers mapped. *Poultry Science* **75**(6): 746–54.
- Das A K. 2013. Microsatellite polymorphism, immunocompetence profile and performance evaluation of Rhode Island Red chicken and its crosses. Ph.D. Thesis, submitted to the ICAR-Indian Veterinary Research Institute (Deemed University), Izatnagar, Uttar Pradesh, India.
- Das A K, Kumar S, Rahim A, Debnath J and Kokate L S. 2020. Investigating genetic heterogeneity using microsatellite markers after long term selection for egg production in Rhode Island Red chicken. *Indian Journal of Animal Sciences* **90**(10): 1387–91.
- Debnath J, Kumar S, Yadav R and Rahim A. 2015. Microsatellite genotypes of sire influence early economic traits and mortality in Rhode Island Red Chicken. *Indian Journal of Poultry Science* **50**(3): 248–53.
- Debnath J, Kumar S, Rahim A and Yadav R. 2017. Genetic variability in egg production-associated microsatellites in Rhode Island Red chicken. *Journal of Animal Sciences* **87**(11): 1379–84.
- Debnath J, Kumar S, Das A K and Rahim A. 2019. Association of microsatellites with pre-housing body weights and age at sexual maturity of Rhode Island Red Chicken. *Indian Journal of Animal Sciences* **89**(10): 1118–22.
- Debnath J, Kumar S, Das A K and Rahim A. 2020. Impact of microsatellite based selection on grower and layer economic traits in Rhode Island Red chicken. *Indian Journal of Animal Sciences* **90**(9): 1316–20.
- Groenen M A, Cheng H H, Bumstead N, Benkel B F, Briles W E, Burke T, Burt D W, Crittenden L B, Dodgson J, Hillel J, Lamont S, Ponce de Leon A, Soller M, Takahashi H and Vignal A. 2000. A consensus linkage map of the chicken genome. *Genome Research* **10**(1): 137–47.
- Higgs R, Cormican P, Cahalane S, Allan B, Lloyd A T, Meade K, James T, Lynn D J, Babiuk L A and O'Farrelly C. 2006. Induction of a novel chicken toll-like receptor following *Salmonella enterica* serovar Typhimurium infection. *Infection and Immunity* **74**(3): 1692–98.
- Hongbao M, Young J and Shen C. 2008. RNA, DNA and protein isolation using TRIzol reagent. *Nature and Science* **6**(3): 66–75.
- Kagami H, Nakamura H and Tomita T. 1990. Sex identification in chickens by means of the presence of the W chromosome-specific repetitive DNA units. *Japanese Poultry Science* **27**(5): 379–84.
- Kong H S, Oh J D, Lee J H, Jo K J, Sang B D, Choi C H, Kim S D, Lee S J, Yeon S H, Jeon G J and Lee H K. 2006. Genetic variation and relationships of Korean native chickens and foreign breeds using 15 microsatellite markers. *Asian Australasian Journal of Animal Sciences* **19**(11): 1546–50.
- Krishnan J, Kumar S, Rahim A, Yadav A S and Das A K. 2022. Investigating whether microsatellites could influence the relative expression level of TLR4 gene in bursa of Rhode Island Red chicken. *Indian Journal of Animal Sciences* **92**(8): 1020–23.
- Kumar B, Sahoo A K, Dayal S, Das A K, Taraphder S, Batabyal S, Ray P K and Kumari R. 2019. Genetic profiling of Hsp70 gene in Muarrah buffalo (*Bubalus bubalis*) under sub-tropical climate of India. *Cell Stress Chaperones* **24**(6):1187–95.
- Liu J P, Baker J, Perkins A S, Robertson E J and Efstratiadis A. 1993. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* **75**(1): 59–72.
- Liu X, Zhang H, Li H, Li N, Zhang Y, Zhang Q, Wang S, Wang Q and Wang H. 2008. Fine-mapping quantitative trait loci for body weight and abdominal fat traits: Effects of marker density and sample size. *Poultry Science* **87**(7): 1314–19.
- MacKinnon K M, He H, Nerren J R, Swaggerty C L, Genovese K J and Kogut M H. 2009. Expression profile of toll-like receptors within the gastrointestinal tract of 2-day-old *Salmonella enteritidis*-infected broiler chickens. *Veterinary Microbiology* **137**(3–4): 313–19.
- Mathews L S, Norstedt G and Palmiter R D. 1986. Regulation of insulin like growth factor I gene expression by growth hormone. *Proceedings of the National Academy of Sciences of the United States of America* **83**(24): 9343–47.
- Nassar F S, Moghaieb R E A, Abdou A M and Stino F K E. 2012. Microsatellite markers associated with body and carcass weights in broiler breeders. *African Journal of Biotechnology* **11**(15): 3514–21.
- Navarro P, Visscher P M, Knott S A, Burt D W, Hocking P M and Haley C S. 2005. Mapping of quantitative trait loci affecting organ weights and blood variables in a broiler layer cross. *British Poultry Science* **46**(4): 430–42.
- Pandey N K, Singh R P, Saxena V K, Shit N, Singh R, Sharma R K and Sastry K V H. 2013. Effect of IGF1 gene polymorphism and expression levels on growth factors in Indian coloured broilers. *Livestock Science* **155**(2-3): 157–64.
- Pandya G M, Singh S, Joshi C G, Rank D N, Khanna K, Barot V N and Solanki J V. 2005. Association of LEI-146 and MCW-43 microsatellites with body weights in bantam, bantamised White Leghorn and White Leghorn chicken. *Indian Journal of Poultry Science* **40**(1): 9–15.
- Radwan L M, El-Dlebhany A E and EL-Denary M E. 2014. Microsatellite genetic differentiation analysis and organic matrix of eggshell in the 16th generation of chickens selected for egg production traits. *Egyptian Journal of Animal*

- Production* **51**(1): 41–55.
- Rahim A. 2015. Microsatellite, immunocompetence and candidate gene expression profiling of Rhode Island Red chicken and association of microsatellite alleles and immunocompetence traits with layer economic traits. Ph.D. Thesis, submitted to the ICAR-Indian Veterinary Research Institute (Deemed University), Izatnagar, Uttar Pradesh, India.
- Rahim A, Kumar S, Yadav R, Debnath J and Krishnan J. 2017. Genetic variability determination in a long-term selected Rhode Island Red chicken strain using microsatellite markers. *Veterinarski Arhiv* **87**(4): 511–22.
- Song C L, Liu H H, Kou J, Lv L, Li L, Wang W X and Wang J W. 2013. Expression profile of insulin-like growth factor system genes in muscle tissues during the postnatal development growth stage in ducks. *Genetics and Molecular Research* **12**(4): 4500–14.
- Wardecka B, Olszewski R, Jaszczak K, Zieba G, Pierzchala M and Wicinska K. 2002. Relationship between microsatellite marker alleles on chromosomes 1–5 originating from the Rhode Island Red and Green-legged Partridge breeds and egg production and quality traits in F(2) mapping population. *Journal of Applied Genetics* **43**(3): 319–29.
- Yadav R, Rahim A, Kumar S, Debnath J and Bhanja S K. 2015. Influence of sire's microsatellite genotype on pre-housing body weights and mortality in Rhode Island Red chicken. *Indian Journal of Poultry Science* **50**(3): 239–43.
- Yadav R. 2016. Microsatellites allelic profiling, candidate gene expression and their association with growth in Rhode Island Red chicken. Ph.D Thesis, submitted to the ICAR-Indian Veterinary Research Institute (Deemed University), Izatnagar, Uttar Pradesh, India.
- Yun J S, Seo D S, Kim W K and Ko Y. 2005. Expression and relationship of the insulin-like growth factor system with posthatch growth in the Korean native Ogot chicken. *Poultry Science* **84**(1): 83–90.
- Zhang H, Wang S, Li H, Yu X, Li N, Zhang Q, Liu X, Wang Q, Hu X, Wang Y and Tang Z 2008. Microsatellite markers linked to quantitative trait loci affecting fatness in divergently selected chicken lines for abdominal fat. *Asian Australasian Journal of Animal Sciences* **21**(10): 1389–94.