The Pharma Innovation Journal 2023; SP-12(8): 576-578

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(8): 576-578
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www.thepharmajournal.com
Received: 09-06-2023
Accepted: 19-07-2023

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Genotyping of Myostatin gene in white turkey using PCR- RFLP

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Abstract
Myostatin gene plays an important role in limiting skeletal muscle growth in livestock and poultry species. The purpose of the present study was to identify the polymorphism in Myostatin gene and to associate the genetic variants with growth and feed efficiency traits in White turkey. Genomic DNA was isolated from 83 poults using Phenol: Chloroform extraction method. Polymerase chain reaction (PCR) was used for amplification of 611 bp fragment from intron 1 of Myostatin locus. The amplified products were analyzed for Restriction fragment length polymorphism (RFLP) using *Hinf I* restriction enzyme and the resulted fragment were visualized on 2% agarose gel stained with ethydium bromide under gel documentation system. PCR- RFLP analysis revealed monomorphism. Since no genetic variability for Myostatin gene was observed in the population, it was not possible to perform analysis studies with growth and feed efficiency traits. Lack of variability indicates the conserved nature of the gene.

Keywords: Myostatin, Turkey, Growth, Feed efficiency, PCR- RFLP

1. Introduction

India is the sixth largest producer of poultry meat in the world and poultry meat contributes to 51.44% of total meat production in the country as per 20th livestock census. As per the current census, around 70% of the total poultry population belongs to commercial poultry farms and only 30% belong to backyard poultry. Compared with the 19th census there is 45.79% increase in backyard poultry compared with commercial poultry (BAHS, 2022). Rearing of unconventional poultry species for meat, like turkey (*Meleagris gal1opavo*) is a promising hope for medium and small- scale farmers predominating in Indian agricultural sector. Turkey being adapted for the local agro-climatic conditions and have the ability to utilize natural resources available with the farmers which make it suitable for backyard rearing. In India, turkey production is in infancy and has to take huge leaps in future for competing with broiler chicken industry. The major limiting factors which impede the popularization of turkey rearing for meat, among famers are poor growth rate and feed efficiency compared with broiler chicken.

Growth is primarily attributed by increase in muscle mass, and skeletal muscle is an important tissue in meat industry. Myostatin (MSTN) or Growth and differentiation factors 8 (GDF8) is an important candidate gene which plays an important role in regulating skeletal muscle development in livestock and poultry species (Dilger *et al.*, 2022, Gaina *et al.*, 2022, Ilori *et al.*, 2022, Dimitrova *et al.*, 2016, Alakilli *et al.*, 2012) [6, 9, 10, 7, 1]. Myostatin gene belongs to the super family of Transforming growth factor β (TGF β) is a potent inhibitor of skeletal muscle growth. There are many popular examples of mutations in Myostatin gene leads to an increase in muscle mass among animal species. Myostatin gene knock-out mice showed greater muscle mass compared to normal wild type mice (McPherron *et al.*, 1997) [12]. “Double muscling” in Belgian blue due to muscular hypertrophy as a result of 11bp deletion in Myostatin is another example (Clop *et al.*, 2006) [4].

There have been several studies done in genetic polymorphism of Myostatin gene substantiating its role in growth performance in poultry (Ilori *et al.*, 2022, Fijabi *et al.*, 2020, Dementeva *et al.*, 2017) [10, 8, 5]. Evaluation of genetic potential for growth and meat production is important identification of superior individuals for selection and cross breeding with locally adapted varieties for improving the production. Thus, identification of Myostatin gene variant as a potential genetic marker for selection will favor growth and meat production in turkey. Scanty information is available about the polymorphism for Myostatin gene among different

varieties of turkey available in India. The purpose of this study was to identify the genetic variants of Myostatin gene in white turkey using Polymerase chain reaction (PCR) - Restriction fragment length polymorphism (RFLP) technique and its association with growth and feed efficiency traits.

2. Materials and Methods
2.1 Management of Experimental birds
Eighty three single hatch out unpedigreed CARI-Virat variety of turkey maintained at Experimental turkey farm, Central Avian Research Institute (CARI), Izatnagar (UP) were investigated for the study polymorphism in Myostatin gene. Up to 6-week birds were maintained in battery brooders, thereafter shifted to individual cages and temperature, standard floor space were provided. *Adlibitum* quantity of CARI- formulated feed was provided on alternate days and feed left was measured at the end of two weeks.

2.2 Genomic DNA isolation
Genomic DNA was isolated from 100µl of venous blood of 83 poults using Phenol: Chloroform extraction method (Kagami *et al.,* 1990) [11]. The quantity of DNA was estimated by spectrophotometer/ ND-1000 spectrophotometer (NanoDrop Technologies, Inc, USA), considering one O.D. at 260 nm equal to 50 µg/ml of double stranded DNA. Similarly, the purity of genomic DNA was determined with the help of ratios of OD at 260 nm and 280 nm and samples showing an OD ratio between 1.7 and 1.9 were considered as of satisfactory purity. DNA Quality was assessed through 0.7% horizontal submarine agarose gel electrophoresis

2.3 Optimization of PCR
Forward and reverse primers were designed (Forward: 5'-TGTCAGCAGGCTAGAAGATGG-3', Reverse: 5'-ATCCTGGCATGGAGATACGC -3') for amplification of 611 bp from part of intron 1 of Myostatin gene (NC_015017.2). The PCR reactions were carried in a final volume of 25µl containing 1.5µl of 10X Taq buffer, 0.5µl of dNTP mix (10mM each), 0.5µl each of forward and reverse primer (10pmol/µl), 0.2µl of Taq polymerase (5 U/µl, Dream Taq DNA polymerase), 20.3µl of nuclease-free water for make up the volume and 1µl of template DNA (50ng/ µl). Reactions were done on a programmable thermo cycler under the following conditions in table1.

Table 1: Optimized PCR reaction conditions for amplification of Myostatin gene

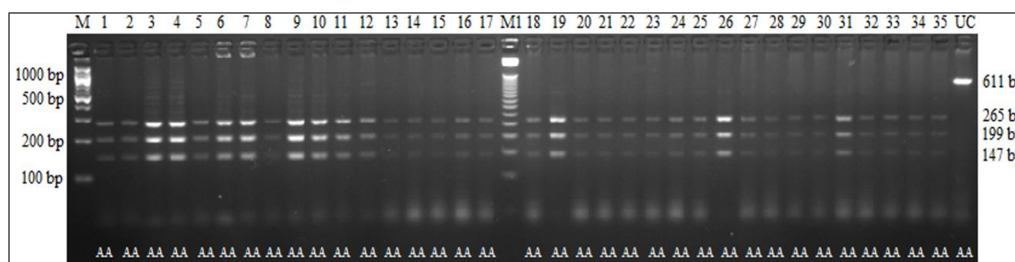
Initial heat inactivation	**94 ºC/ 5 min.**
30 cycles of	
a. Denaturation	94 ºC/ 1 min.
b. Annealing	59 ºC/ 1 min
c. Extension	72 ºC/ 1 min.
Final Extension	72 ºC/ 15 min.

Quality of amplified product was checked using 1% agarose gel with ethidium bromide staining at a constant voltage of 80V for 45 min. Migrated bands were later viewed under UV light and photographed for documentation (Bio- rad Laboratories, USA).

2.4 PCR-RFLP allele based genotyping
PCR- RFLP technique was used to study the genetic variations in Myostatin genes in white turkey. Restriction enzyme (RE) digestion was performed in reaction mixture contain a final volume of 15 µl containing 0.2µl of *Hinf I* RE, 2.5µl of R buffer followed by incubation of reaction mixture at 37 ºC for 15hrs in water bath. The digested products were subjected to 2% agarose gel electrophoresis and visualized in Gel documentation system using Quantity One®(Bio- Rad Laboratories Inc., U. S. A.) software. Sizes of the digested products were compared using 50bp and 100bp DNA markers.

3. Results and Discussion
In our investigation 611 bp fragment from intron 1 of Myostatin gene was amplified, which up on digestion with *Hinf I* RE generated three fragments of sizes 147, 199, 625 bp in all the poults thus showing monomorphic pattern (Figure 1). Allele A was fixed and no others variant observed in the population. RFLP patterns resulted only AA genotype with frequency of A allele as 1.0. Since all locus were monomorphic, it was not possible to analyze the association with growth and feed efficiency traits.

Similar to our study Saxena *et al.* (2002) [13] studied *Taq1* PCR- RFLP polymorphism in exon 1, intron1 and exon 2 regions in Myostatin gene of CARI- Virat White turkey. They could not observe any polymorphism in Myostatin gene. Ilori *et al.* (2022) [10] reported three SNPs (38C>T, 39T>C, 50T>C) in local and two SNPs (38 C>T, 39T>C) in exotic Nicholas white turkey in intron 1 region. Fijabi *et al.* (2020) [8] investigated *Msp*l PCR- RFLP polymorphism in exon 1 region of Nigerian indigenous turkey, revealed three alleles A, B and C forming three genotypes AA, AB and AC, with genotypic frequencies 0.58, 0.40 and 0.02 respectively. The genotypes did not show any significant association with body weights at 4, 8 and 12 weeks of age in the studied population.



Lane 1-35: 1-35 samples of white turkey. M: 100 bp DNA marker, M1: 50 bp DNA marker, UC: Uncut PCR product. All poults are showing AA genotype. Sizes of the digested fragments have been shown on the right side; size of the marker on the left side.

Fig 1: Myostatin/ *Hinf I* PCR-RFLP genotyping

Dementeva *et al.* (2017) [5] studied 2244G>C SNP in exon 1 region of Myostatin gene in G5 line of Cornish chicken breed using PCR-RFLP technique, revealed G_2G_2 genotype had significantly ($p<0.05$) higher body weight than CC and CG_2 genotypes at 7 and 33 days of age. Bhattacharya and Chatterjee (2013) [3] studied SSCP polymorphism of myosatin gene on growth traits in three lines of layers. A total of 13 haplotypes were observed across 3 chicken lines (PB-1 and CB as broiler lines and IWI as the layer line). Myostatin haplogroups had a significant effect on BW at 28, 42, and 49 day of age in the PB-1 line. The significant association of haplogroups was observed with BW at day 14 and 49 in the CB line. In the IWI layer line, the Myostatin gene was polymorphic but had no significant association with growth traits.

4. Conclusion

It may be concluded that Myostatin gene is monomorphic for this population of white turkey. Fixation of allele A in the population was observed and the lack of variability indicating highly conserved nature of the gene. So further investigations are needed with large number of birds to explore the polymorphism in Myostatin gene to be associated with growth and feed efficiency traits for developing a genetic marker for selection.

5. Acknowledgements

The authors are thankful to the Director, ICAR-Central Avian Research Institute, Izatnagar for all the facilities provided.

6. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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