Taq I PCR-RRFLP analysis of Myostatin gene revealed species specific sites in turkey and chicken

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Summary

Myostatin (growth and differentiation factor-8) gene is 8.0 Kb in size comprising of 3 exons and 2 introns and expressed at higher levels in skeletal muscle. Myostatin gene exerts negative regulation on muscle growth by inhibiting myoblast cell proliferation and differentiation and maintaining quiescent status of satellite cells. SNPs of chicken Myostatin gene were found associated with skeletal muscle growth. Myostatin gene therefore was chosen for analysis in turkey and chicken maintained at institute farms. Using specific reverse and forward primers the myostatin gene region (exon1: 23bp + inron1: 2071 bp + exon2 : 373 bp) was amplified as 2517 bp band. PCR reaction mixture (25 μl) contained 100ng of genomic DNA, 1X PCR buffer, 1.0 mM $MgCl_2$, 200 μM of dNTPs, 2.5 mM of each primers, and 1.25 U of Taq DNA polymerase. Optimized PCR conditions were: Initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 40 s, annealing at 62°C for 40 s, extension at 72°C for 90 s and final extension at 72°C for 10 m. The amplified products were column purified and subjected to *Taq I* restriction digestion. Amplification of turkey myostatin gene using primers specific to chicken suggested that the myostatin gene regions are conserved across the two species. PCR-RFLP analysis using *Taq I* showed two sites (113 and 679 bp) with three bands of 1838, 566 and 113 bp in all the turkey individuals thus exhibiting monomorphic pattern. Chicken showed polymorphism with two alleles A and B, and genotype AB and BB. Allele A had cutting sites at 887, 2358 and 2465 nt yielding four bands of 1471, 887, 107 and 52 bp. Allele B had sites at 887 and 2358 nt yielding three bands of 1471, 887 and 169 bp. The sites for *Taq* I in chicken myostatin gene were found different from that of turkey (113 and 679 bp). Therefore, *Taq I* polymorphism may be used as species-specific marker for differentiating turkey and chicken.

Introduction

Poultry stands in 2nd position in the world meat production. Skeletal muscle is the most essential tissue for the poultry meat industry. Muscle growth occurs prenataly by hyperplasia (increase in cell number) and postnataly by hypertrophy (increase in cell size). Many growth factors promote or inhibit cellular function thus growth is a multifactorial phenomenon. Myostatin (nick name - Schwarzenegger gene) is a strong negative regulator of skeletal muscle growth (Joulia *et al.*., 2003). Myostatin (GDF-8) is a cytokine belonging to the transforming growth factor-β (TGF-β) super family of signalling molecule and is expressed at high level in skeletal muscles. The transforming growth factors β are multifunctional cytokines with diverse effects on cell growth, differentiation and function (Piek *et al.*., 1999). Myostatin gene is highly conserved, and homologous genes have been found in the vertebrates examined, including mice, human and avis (McPherron and Lee, 1997; Lee, and McPherron, 2001). Myostatin has also been reported to block adipogenesis by signalling through TGF-β like pathway (Rebbapragada *et al.*. (2003). Since myostatin plays an important role in muscle growth and meat quality therefore chosen as candidate gene for PCR-RFLP analysis in turkey and broiler chicken.

Materials and methods

GERMPLASM

Coloured boiler parent line chicken, which have been selected for higher 5-week body weight and black and white turkey (*Meleagris gallopavo*) maintained at institute farms were used in present investigation.

MANAGEMENT PRACTICES

The chicks were housed at deep litter system under hanging brooder fitted with electric bulb/infrared bulb as light and heat source. Standard management conditions were maintained in brooder house. The feed was provided at libitum from 0-5 weeks. At 4 weeks chicks were given booster for F1 and IBD vaccines. The birds were selected on the basis of high 5 weeks body weight and thereafter they were maintained under restricted feeding schedule up to 20 weeks. At 18-20 weeks of age the pullets were transferred to individual cages. The light schedule was followed thereafter till 40 weeks. Turkey individuals were also maintained at deep litter system under standard management and nutrition.

GENOMIC DNA ISOLATION FROM BLOOD

About 1 ml of venous blood was collected from the jugular vein by a sterile disposable heparinized syringe from each bird and about 200 μl of blood was transferred into 2 ml eppendorf tubes for isolation of DNA. Genomic DNA was isolated from 0.2 ml of venous blood following the method of Kagami *et al.* (1990). The genomic DNA isolated from the blood samples was checked for its quality, purity and concentration. Only the pure and good quality DNA samples were used for the study.

PCR AMPLIFICATION OF MYOSTATIN GENE

The specific forward and reverse primers were designed as per sequence vide EMBL Accession number AF346599 and myostatin gene region (part of exon1+inron1+part of intron2) was amplified as 2517 bp band. The amplified product has 73 bp from exon1, 2071 bp of intron1 (complete intron1) and 373 bp of exon 2.

PCR REACTION MIXTURE

The PCR reaction mixture (25 μl) contained 100ng of genomic DNA, 1X PCR buffer (10mM Tris-HCl, pH 8.3, 50mM KCl, 0.01% Triton X-100), 1.0 mM $MgCl_2$, 200 μM of dNTPs, 2.5 mM of each primers, and 1.25 U of Taq DNA polymerase (Life Tech).

PCR CYCLING CONDITIONS

The optimized PCR conditions were: Initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 40 s, annealing at 62°C for 40 s, extension at 72°C for 90 s and final extension at 72°C for 10 m.

VISUALIZATION OF PCR PRODUCTS

PCR products were visualized by electrophoresis of amplicons on 1.4% agarose gel stained with ethidium bromide. The PCR products were photographed under UV light.

PURIFICATION OF PCR PRODUCT AND RE DIGESTION

After PCR the products were run on agarose gel (1.4%) to check the amplification. A single band of 2517 bp confirmed the amplification of myostatin gene in chicken and turkey samples. The purification of PCR products was done using PCR purification columns. Purified PCR products were subjected for restriction digestion. Gene tool software was used to select the restriction endonuclease enzyme for PCR-RFLP analysis. The digestion was performed with *Taq* I enzyme as per the recommendations of manufacturer. The digestion was performed using 5 U of enzyme at 37 °C for overnight.

POLYACRYLAMIDE GEL ELECTROPHORESIS (NATIVE)

The digested PCR products were run on 6% native Polyacrylamide gel (PAGE) at constant voltage of 100 v for about 1 hour. The DNA markers of suitable size(s) were also run simultaneously parallel to the samples for molecular size determination. After running, the gel was stained by silver staining and reprographic records were obtained. The genotypes/alleles were recorded directly from gel.

Results and discussion

The visualization of amplified product on agarose gel revealed a band of 2517 bp size which confirmed the amplification of myostatin gene (Figure1). Baron *et al.* (2002) also reported amplification of myostatin gene product of 2517 bp using similar primers. Amplification of turkey myostatin gene using primers specific to chicken suggested that the myostatin gene regions are conserved across the two species. Similar findings have also been reported by McPherron and Lee (1997). In turkey individuals, PCR-RFLP analysis using *Taq I* showed two sites (113 and 679 bp) with three bands of 1838, 566 and 113 bp in all the individuals thus exhibiting monomorphic pattern (Figure 2). However, chicken showed polymorphism with two alleles A and B and genotypes AB and BB. Allele A had cutting sites at 887, 2358 and 2465 nt yielding four bands of 1471, 887, 107 and 52 bp. Allele B had sites at 887 and 2358 nt yielding three bands of 1471, 887 and 169 bp. The band pattern for AB genotype was1471, 887, 107, 52 / 1471, 887, 169 bp and for BB genotype the band pattern was 1471, 887,169 / 1471, 887, 169. Baron *et al.*. (2002) also reported high degree of polymorphism in chicken Myostatin sequences within same region. Gu *et al.* (2002) reported SNPs in Myostatin gene and their association with skeletal muscle growth. The sites for *Taq* I in chicken myostatin gene were found different from that of turkey (113 and 679 bp). In chicken, cutting sites of *Taq* 1 were found both in intron 1 (2 sites) and exon 1 (one site) regions but in turkey the sites of *Taq* 1 were present only in intron 1 region and none of the exons had site for Taq1 enzyme. This could be species-specific difference/pattern revealed by *Taq*1 PCR-RFLP of myostatin gene region and thus can be used as marker for differentiation between two species. However, further work with more number of samples is warranted. Due to non-availability of literature on this aspect, the findings could not be compared.

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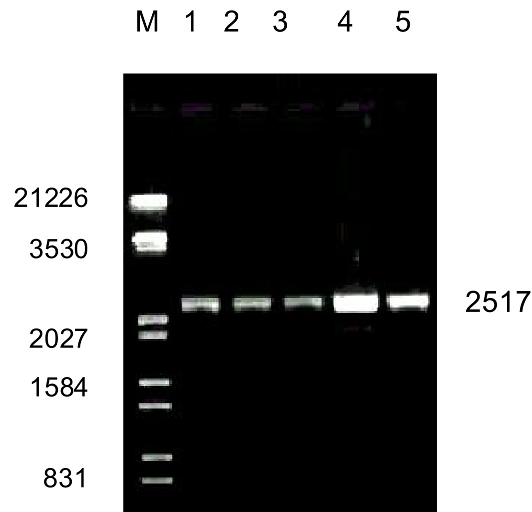

Figure 1 Myostatin gene amplified product (2517bp) in turkey and chicken.
M=Hind III/EcoRI double digest λ DNA; Lane1-3= chicken; Lane 4-5 turkey

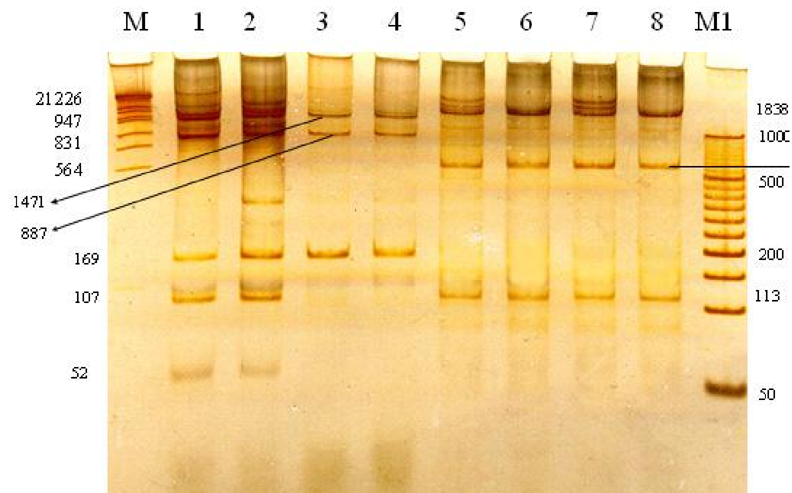

Figure 2 *Taq*1 PCR-RFLP of myostatin gene in chicken and turkey individuals.
M= l DNA Hind III/Eco RI double digest; Lane 1-2= Chicken (AB genotype); 3-4 chicken (BB genotype); 5-6=Turkey white; 7-8 Turkey black; M2= 50 bp ladder