Meat speciation in Chabro, Aseel, Vanraja and Kadaknath indigenous chicken

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Journal of Livestock Science (ISSN online 2277-6214) 16: 633-637
Received on 17/5/25; Accepted on 18/10/25; Published on 24/10/25
doi. 10.33259/JLivestSci.2024.633-637

Abstract

A molecular analysis based on Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) of a conserved region in the mitochondrial (mt) cytochrome b (cyt b) gene alongwith BL-β gene was tried for speciation of meat of indigenous chicken breeds (Chabro, Aseel, Vanraja and Kadaknath) in contrast to Cobb-400. The genomic DNA of known identities of meats were extracted and subjected to PCR amplification targeting the mt cyt b gene with restriction enzyme*, RsaI, Hae III and MspI at* approximately 359 bp while BL-β-II gene of approximately 277 bp with *MspI* as well as Taq I. Identification of indigenous chicken breeds and Cobb-400 was determined by digesting the amplicons with different types of restriction endonucleases. RsaI and HaeIII digestion gave monomorphic pattern in all breeds/strain while MspI showed wild zygote with chebro, mutant homozygotes in Cobb-400, Kadaknath and Vanraja. However, Aseel was showing heterozygotic condition on digestion of PCR product of cyt-b with MspI. PCR-RFLP of BL-β gene with TaqI and MspI did not shown any difference among the studied chicken breeds and strains. Therefore, it is concluded that Chabro and Aseel meat can be differentiated with other meat species using PCR-RFLP with Cyt-b and MspI.

Keywords: PCR-RFLP; Cyt-b gene; BL-β gene; Chabro; Aseel; Vanraja; Kadaknath

Introduction

The present-day consumer's awareness is increasing and specificity and quality has become top priority. Consumers are now demanding quality products with honest labeling in order to assure meat safety and fair pricing (Monga, 2025). Meat authentication is utmost important in food chain to safeguard consumers interest (Azad et al., 2023; Vishnuraj et al., 2024; Conter, 2025). It is also important to prevent the malpractices in food commodities (Singh et al., 2014; Onyeaka et al., 2024). Native breeds of chicken are now considered as prime food commodity and possibilities of cheating is more due to its high demand and lower availability. The possibility is yet again due to trend of using ready to eat and readymade availability of the mix. Accurate identification of the origin of meat presents a considerable challenge for food inspectors, game enforcement authorities and individuals seeking to comply with regulations. Therefore, there has been a need for a fast and routinely applicable meat species identification system. Traditionally, species identification has been established through one of three approaches: molecular biology-based methods, enzymatic immunological methods or chromatographic methods. Though the studies on species specifications are available in abundant quantities but limited study is available showing the differentiation in meat of these native chicken breeds. So a study was planned for molecular detection of meat of native chicken breeds like Vanaraja, Asheel and Kadaknath in contrast with a broiler strain Cobb-400.

Materials and Methods

Samples

Fresh meat samples from indigenous chicken breeds (Chabro, Vanraja, Aseel and Kadaknath) and Cobb-400 reared in Instructional Poultry farm of DUVASU, Mathura, U.P. India were collected after slaughter of chicken in Department of Livestock Products Technology. All the samples were stored at -20ºC before DNA extraction to prevent enzymatic degradation of DNA.

DNA Extraction and quality assessment

Genomic DNA extraction was performed using the procedure of Charge Switch ® g DNA Mini Tissue Kit Catalog no. CS 11204 (Invitrogen Pvt Ltd). Quality of DNA was assessed through 0.7% horizontal submarine agarose gel electrophoresis. The quality of genomic DNA was further assessed with Nano Drop of Central Instrumental Laboratory, DUVASU, Mathura.

PCR - RFLP Assay

Polymerase Chain Reaction (PCR)

To study the nucleotide polymorphism of the gene, a simple and reliable technique PCR - RFLP was used in nucleotide sequences in genes. It involved designing of a set of primers for the locus of interest which were used for PCR amplification of that segment of DNA, followed by restriction enzyme (RE) digestion of the PCR product and visualization of restriction fragments in gel.

Primer preparation

The Primer sequence used in study was in reference to the studies conducted by Wong et al. (2010) on Cyt-b and BL-β II gene by Ahmed et al. (2008). Primers were vortexed for 10 seconds followed by centrifugation for 10 seconds. Then they were kept in upright position for some time. After addition of 51.5 µl nuclease free water, primers were vertexed for 10 seconds and then centrifuged for 10 seconds. Prepared solutions were kept overnight at 4^0C.
The forward and reverse primers used to amplify Cyt-b gene was expected to yield a 359 bp product. Similarly, another primer set used to amplify BL-β II gene was expected to yield 277 bp products.

PCR reaction mixture

The PCR was carried out in a total reaction volume of 25µl. The components were optimized using variable concentrations of different components.

PCR amplification Program

PCR amplification was carried out in a thermal cycler (PTC-200, MJ Research, USA) and (Bio Rad, USA gradient PCR) using cyclic conditions for Cyt-b and BL-β II gene.

Documentation of PCR products by Agarose Gel electrophoresis

Approximately, 8 µl of PCR product was added with 2 µl distilled water and 2 µl bromophenol blue dye for loading in a 1.4% agarose gel containing ethidium bromide (0.5 µg/ml). The electrophoresis was done at 2-5 V/cm. 100 bp DNA ladder and Low Range DNA ruler (Bangalore Genei, India) was used as molecular size marker for identification of the desired product. The amplified product was examined under UV illumination and photographed for documentation.

Restriction enzyme (RE) digestion

To identify the restriction fragment length polymorphism, restriction enzymes were used for each amplified product. The RE digestion was carried out in 15µl as per the manufacturer's instruction. Restriction Enzyme*, RsaI, Hae III and MspI* were employed for Cyt-b gene and *MspI* as well as Taq I for BL-β II gene. The *RsaI and HaeIII* digestion was carried out for overnight at 37ºC in a water bath. After digestion, the digested products were kept in refrigerator at 4ºC till further study. The pattern of digestion was visualized using Agarose Gel electrophoresis as mentioned earlier. The *MspI* digestion was carried out for overnight at 37ºC in a water bath. After digestion, the digested products were kept in refrigerator at 4ºC till further study. The pattern of digestion was visualized using Agarose Gel electrophoresis as mentioned earlier. The *TaqI* digestion was carried out for overnight at 65ºC in a water bath. After digestion, the digested products were kept in refrigerator at 4ºC till further study. The pattern of digestion was visualized using Agarose Gel electrophoresis as mentioned earlier. The *MspI* digestion was carried out for overnight at 37ºC in a water bath. After digestion, the digested products were kept in refrigerator at 4ºC till further study. The pattern of digestion was visualized using Agarose Gel electrophoresis as mentioned earlier.

Results

For molecular characterization of chicken breeds/strain, the techniques of Polymerase Chain Reaction (PCR) –Restriction Fragment Length Polymorphism (RFLP) was standardized to determine the identities of meats of Cobb-400, Chabro, Vanraja, Aseel and Kadaknath. Raw meat of these breeds was analyzed by targeting their cytochrome-b-gene and BL-β gene. The amplified fragment of Cytochrome-b gene was approximately 359 and BL-β-II gene of approximately 277 bp.

Cytochrome-b gene

By using the primers as discussed in materials and methods, Cyt-b amplified a fragment of the cytochrome –b-gene of approximately 359 bp while BL-β-II gene of approximately 277 bp. The cytochrome-b fragment displayed monomorphic pattern in RFLP profile when digested separately with restriction endonuclease RsaI and HaeIII. The digestion of PCR product of Cyt-b gene with RsaI quantified the products on 149, 210 bp in all the chicken meat products while digestion of same gene PCR product with HaeIII yielded the fragments of 74, 126 and 159 bp for all the chicken meat. However, RFLP profile of cytochrome –b gene fragment digested with MspI showed polymorphic pattern with Aseel, monomorphic pattern with Vanraja, Kadaknath and Cobb-400.

The molecular characterization by Polymerase Chain Reaction- Restriction Fragment Length Polymorphism (PCR-RFLP) for meat of various chicken breeds (Cobb-400, Chabro, Vanraja, Aseel and Kadaknath) was performed using Cytochrome-b (Cyt-b) gene with universal primer. The PCR products of 359-bp were obtained for meat of all chicken breeds. The amplified PCR product of 359-bp when subjected to RFLP with RsaI enzyme resulted into two fragments of 149-bp and 210-bp for all meat sample irrespective of breed.

The PCR product of 359-bp obtained with Cyt-b gene and universal primer when subjected to RFLP with HaeIII enzyme revealed three distinct fragments of 74-bp, 126-bp and 159-bp for meat of all chicken breeds.

The PCR-RFLP profile of Cyt–b gene resultant PCR product of 359-bp when digested with MspI enzyme showed polymorphic pattern with Aseel, monomorphic pattern with Vanraja, Kadaknath and Cobb-400. The specific pattern of Aseel meat revealed the unique pattern than all other meat type. The pattern of chabro meat can also be helpful in differentiating the its meat with other meat breeds/strains studied.

BL-β-II gene

The BL-β-II gene amplification gave the PCR product of approximately 277 bp. On digestion of PCR product with TaqI restriction enzymes yielded the fragments of 116 and 161 bp while its digestion with MspI resulted into three fragments namely 57,100 and 120 bp in all chicken breeds. The BL-β-II gene fragment displayed monomorphic pattern in RFLP profile when digested separately with restriction endonuclease TaqI and MspI. So no significant difference was observed by PCR-RFLP for studied chicken breeds/strain.

The molecular characterization by PCR-RFLP for meat of various chicken breeds (Cobb-400, Vanraja, Aseel and Kadaknath) was performed using BL-β gene with the primer suggested by Ahmed *et al.* (2008). The PCR products of 277-bp were obtained for meat of all chicken breeds.

The resulted PCR product of 277 bp when subjected to RFLP digestion with TaqI enzyme yielded the fragments of 116 and 161 bp for all chicken breeds.

The amplified PCR product of 277-bp with BL-β gene when digested with MspI for RFLP pattern resulted into three distinct fragments of 57-bp,100-bp and 120 bp. The pattern of digestion into fragments was monomorphic for all chicken breeds which could be due to the study at breed level and equal cutting site presents in meat of all chicken breeds. On PCR-RFLP with MspI Aseel was showing heterozygote with Cyt-b while other RE was showing monomorphic patterns

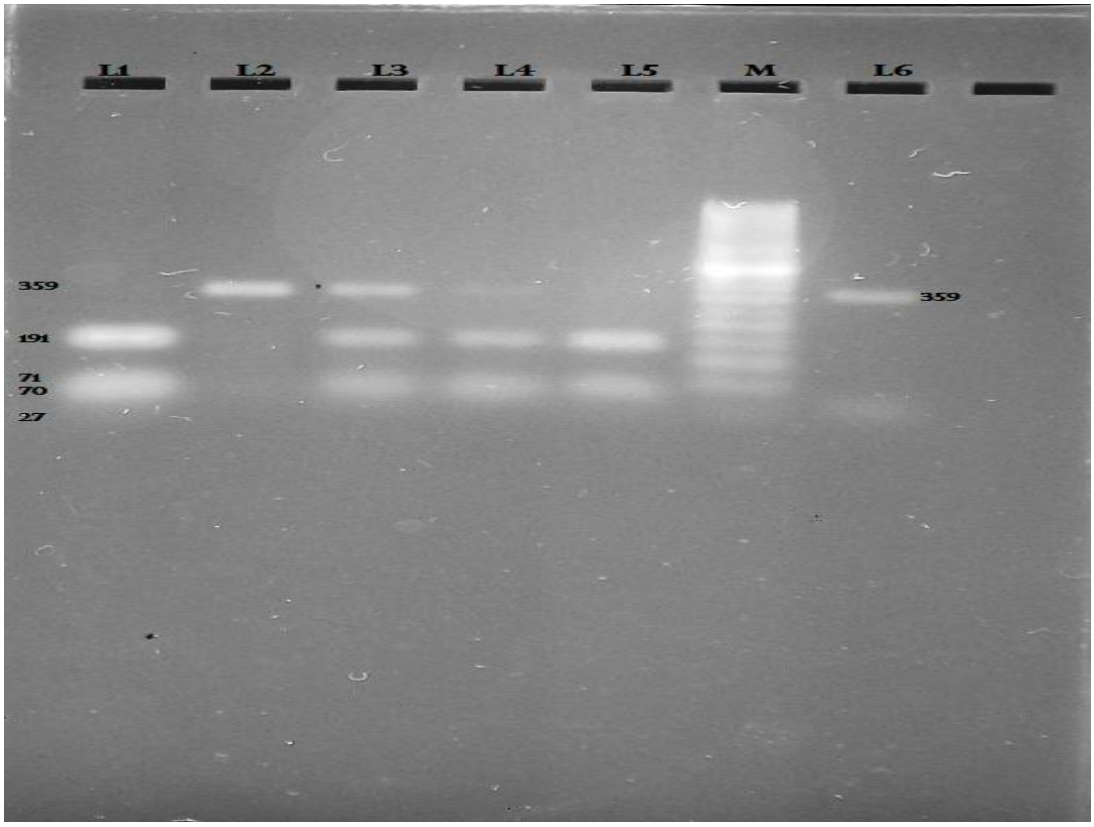

Figure 1. PCR-RFLP with Cyt-b and MspI. Where: L1-Cobb-400, L2-Chebro, L3-Aseel, L4-Kadaknath, L5-Vanraja, M- Marker, L6-PCR product

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Discussion

PCR-RFLP studies using Cytochrome-b gene and BL-β-II gene were conducted to different meat of different animals and birds. But no typical study on native chicken breeds i.e. Chabro, Vanraja, Aseel and Kadaknath with respect to Cobb-400 were conducted. Gargouri et al. (2021) used 359 bp fragment of the cyt b gene amplified by PCR using universal primers. Then they used three enzymatic digestions to distinguish dromedary, rabbit, goat, turkey, rat, donkey and pork. Khan et al. (2018) amplified a very short fragment (359-bp) of cytochrome b gene alongwith restriction endonucleases (Tas1, Hinf) the for authentication of animal species.

Taha (2022) reported that amplified PCR products in RFLP with HinfI and RsaI play an important role in detection of animal meat species. He advocated that it is a fast, simple and easily handle method for identification of animal species. Cassier (2013) study of PCR-RFLP on 359-bp with Cyt-b gene, universal primer and HaeIII enzyme resulted into similar fragments for protected animals as per the current study.

A study conducted by Abdel-Rahman et al. (2015) on Cyt-b gene and PCR product of 371-bp for chicken resulted into RFLP fragments of 65-bp, 85-bp and 221-bp which was a species level speciation in which they differentiated the meat of chicken, duck, quail, rabbit and turkey. However, no such study was conducted on native chicken breeds.

Akpan et al. (2019) concluded that PCR product of 277 bp when subjected to RFLP digestion with TaqI enzyme exhibited monomorphic pattern with genotypes AA and at frequency 1.0 in the divergently selected groups in Nigerian locally adapted chicken. This research was used to know the histocompatibility complex in different poultry breeds.

Conclusions

It is evident from the above discussion that RsaI and HaeIII digestion gave monomorphic pattern in all breeds/strain while MspI showed wild zygote with chebro, mutant homozygotes in Cobb-400, Kadaknath and Vanraja. However, Aseel was showing heterozygotic condition on digestion of PCR product of cyt-b with MspI. PCR-RFLP of BL-β gene with TaqI and MspI did not shown any difference among the studied chicken breeds and strains. Therefore, it is concluded that Chebro and Aseel meat can be differentiated with other meat species using PCR-RFLP with Cyt-b and MspI.

Acknowledgements

The authors are grateful to the Hon'ble Vice-Chancellor, U.P. Pt. Deen Dayal upadhyay Veterinary University and Go Anusandhan Sansthan, Mathura, U.P. 281001 India for their financial and infrastructural support of this study.

References

1) Abdel-Rahman, S.M., Elmaghraby, A.M., Haggag, AA., 2015. Identification and differentiation among chicken;s, duck's, quail's, rabbit's and turkey's meat using PCR-RFLP technique. Biotechnology in Animal Husbandry, 31 (**1**), 101-108.
2) Akpan, U., Adenaike, A., Takeet, M.I., Ibiyemi, A.B., 2019. PCR-rflp characterization of major histocompatibility complex (MHC) b-lβII gene in Nigerian locally adapted chickens. Pertanika Journal of Tropical Agricultural Science, 42(1):277-284
3) Azad, M.A.K., Dey, M., Khanam F., Biswas B., Akhter S., 2005. Authentication of meat and meat products using molecular assays: A review. Journal of Agriculture and Food Research, 12, 100586.
4) Cassier, R., 2013. Identifying meat species using RFLP-PCR and the QIAxcel advanced system. Cited from www.giagen.com
5) Conter, M., 2025. Recent advancements in meat traceability, authenticity verification, and voluntary certification systems. Italian Journal of Food Safety, 14:12971.
6) Gargouri, H., Moalla, N., Kacem, H. H., 2021. PCR–RFLP and species-specific PCR efficiency for the identification of adulteries in meat and meat products. European Food Research and Technology, 247, 2183-2192.
7) Khan, W.A., Mustafa, H., Amir-u-Din, U., Yousaf, M., Ajmal, A., Mehmood, K. and Imran, M., 2018. Identification of species-specific molecular markers in different farm animals by PCRRFLP analysis. Pure Applied Biology, 7, 338-342.
8) Monga, S., 2025. Consumer awareness and perceptions of food safety labels in packaged foods. Indiana Journal of Humanities and Social Sciences, 6(3), 14-17.
9) Muflihah, Hardianto, A., Kusumaningtyas, P., Prabowo, S., Hartati, Y.W., 2023. DNA-based detection of pork content in food. Heliyon, 8;9(3): e14418.
10) Onyeaka, H., Ghosh, S., Obileke, K., Miri,T., Olumide Odeyemi, O.A., Nwaiwu O., Tamasiga, P., 2024. Preventing chemical contaminants in food: Challenges and prospects for safe and sustainable food production. Food Control, 155, 110040.
11) Singh V.P., Pathak V., Nayak N.K., Akhilesh, Verma K. and Umaraw P. 2014. Recent Developments in Meat Species Speciation-a review. Journal of Livestock Science 5:49-64
12) Taha, K.M., 2022. Molecular detection of raw meat for some animal species using RFLP-PCR technique. Mesopotamia Journal of Agriculture, 50(3):50-58.
13) Vishnuraj, M.R., Vaithiyanathan, S., Reddy, P.B., Kumar, N.A., Barbuddhe, S. B. 2024. Authentication of traditional meat products (Haleem) sold in India: a first report confirming species mislabeling. Journal of Food Science and Technology, 61(5), 1003-1012.