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Polymorphisms of PAD14 Gene in White Fulani and Red Bororo Cattle: Association with Milk Yield and Mastitis Resistance Traits in Nigerian Indigenous Breeds

Mas'ud Abubakar [1, *], Abba Ibrahim Abubakar [1] Sadiya Ibrahim Karaye [1], Muhammad Sani Tukur [2] and Muhammad Salihu Ibrahim [3]

[1] *Department of Animal Health and Production Technology, Federal University of Science and Technology, Kabo, Nigeria.*
[2] *Department of Science Laboratory Technology, Federal University of Science and Technology, Kabo, Nigeria.*
[3] *Department of Pharmaceutical Technology, Federal University of Science and Technology, Kabo, Nigeria.*

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Abstract

Genetic improvement of indigenous cattle breeds through molecular characterization of functional genes has become increasingly important in sustainable livestock production. This study investigated the polymorphisms of the Peptidyl Arginine Deiminase 14 (PAD14) gene in White Fulani and Red Bororo cattle and evaluated their association with milk yield and mastitis resistance traits. A total of 120 lactating cows comprising 60 White Fulani and 60 Red Bororo cattle were sampled from herds in Northern Nigeria. Blood samples were collected for genomic DNA extraction, and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used to identify genetic variants of the PAD14 gene. Three genotypes (AA, AB, and BB) were detected in both breeds. Genotype frequencies in White Fulani cattle were 0.45, 0.40, and 0.15 for AA, AB, and BB respectively, while Red Bororo cattle exhibited frequencies of 0.38, 0.47, and 0.15 respectively. The A allele was more frequent in both breeds. Significant associations ($P < 0.05$) were observed between PAD14 genotypes and milk yield, somatic cell count, and mastitis incidence. Cows possessing the AA genotype produced higher milk yield and exhibited lower somatic cell counts compared to BB genotype carriers. The findings suggest that PAD14 gene polymorphism could serve as a useful genetic marker for improving udder health and dairy productivity in indigenous Nigerian cattle breeds.

Keywords: PAD14 gene; Polymorphism; White Fulani cattle; Red Bororo cattle; Mastitis resistance; Milk yield; PCR-RFLP; Somatic cell count

1 Introduction

Cattle production remains one of the most important agricultural enterprises in Nigeria and contributes significantly to food security, employment generation, and rural livelihoods. Indigenous cattle breeds such as White Fulani and Red Bororo cattle are widely distributed across Northern Nigeria because of their adaptability to harsh environmental conditions, disease tolerance, and ability to survive under low-input management systems. Despite these advantages, productivity in these breeds remains relatively low, especially with regard to milk production and resistance to diseases such as mastitis [1,2].

Mastitis is an inflammatory condition of the mammary gland usually caused by microbial infection and is considered one of the most economically devastating diseases in dairy cattle worldwide. The disease reduces milk yield and milk quality while increasing treatment costs and culling rates [3,4]. In Nigeria, mastitis represents a major challenge to dairy production because most herds are managed under traditional systems with limited veterinary interventions. The occurrence of mastitis not only affects animal health but also diminishes the profitability of dairy enterprises [5,6].

* Corresponding author: Mas'ud Abubakar

Advances in molecular genetics have made it possible to identify genes associated with disease resistance and productive performance in livestock. Candidate gene approaches are increasingly used to identify functional genes that influence economically important traits in cattle. Among these genes, members of the Peptidyl Arginine Deiminase (PAD) gene family have attracted attention because of their involvement in inflammatory and immune responses. PAD enzymes catalyze the conversion of arginine residues into citrulline in proteins, thereby regulating several physiological and pathological processes associated with immunity and inflammation [7].

The PAD14 gene is believed to play a role in innate immune response mechanisms, particularly in tissues exposed to microbial invasion. Variations within immune-response genes may influence an animal's resistance or susceptibility to mastitis and other infectious diseases. Previous studies have reported associations between polymorphisms in immune-related genes and somatic cell count, milk composition, and udder health in dairy cattle [8,9]. However, information regarding PAD14 gene polymorphism in Nigerian indigenous cattle breeds remains scarce.

White Fulani cattle, also known as Bunaji, constitute one of the most important dairy breeds in Nigeria due to their relatively higher milk production compared to other indigenous breeds. Red Bororo cattle are also highly valued for their adaptability, hardiness, and resistance to environmental stressors. Understanding the genetic architecture of these breeds is important for designing sustainable breeding programs aimed at improving productivity while maintaining adaptive traits. Therefore, this study was conducted to investigate the polymorphisms of the PAD14 gene in White Fulani and Red Bororo cattle and determine the association between PAD14 genotypes and economically important traits such as milk yield, somatic cell count, and mastitis incidence.

2 Materials and Methods

2.1 Study Area

The study was carried out in selected cattle herds located in Kano, Katsina, and Kaduna States, Northern Nigeria. The region lies within the Sudan Savannah ecological zone and is characterized by annual rainfall ranging from 600 to 1000 mm and average temperatures between 25°C and 38°C.

2.2 Experimental Animals

A total of 120 lactating cows comprising 60 White Fulani cattle and 60 Red Bororo cattle aged between 3 and 7 years were used for the study. Animals were selected randomly from semi-intensively managed herds. All animals appeared clinically healthy during sample collection.

2.3 Blood Sample Collection

Approximately 5 mL of blood was collected from the jugular vein of each animal using sterile syringes and transferred into EDTA-containing vacutainer tubes. Samples were transported in ice packs to the molecular genetics laboratory for analysis (African Biosciences Ibadan).

2.4 DNA Extraction

Genomic DNA was extracted using the standard phenol-chloroform extraction method described by Sambrook and Russell [10]. DNA quality and concentration were evaluated using agarose gel electrophoresis and spectrophotometric analysis.

2.5 PCR Amplification of PAD14 Gene

Specific primers were designed based on published bovine PAD14 gene sequences.

2.6 Primer sequences

- Forward primer: 5'-AGCTGCTGACCTTGATGACT-3'
- Reverse primer: 5'-TTGGTAGGCTGGTAGTTGGA-3'

PCR amplification was performed in a 25 µL reaction mixture containing:

- 12.5 µL PCR Master Mix
- µL forward primer
- µL reverse primer

- 2.0 µL genomic DNA template
- 8.5 µL nuclease-free water

PCR conditions included initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes.

2.7 Restriction Fragment Length Polymorphism (RFLP) Analysis

Amplified PCR products were digested using EcoRI restriction enzyme and separated on 3% agarose gel electrophoresis stained with ethidium bromide. Banding patterns were visualized under ultraviolet light to determine genotypes.

2.8 Measurement of Milk Yield

Daily milk yield was recorded for each cow over a 90-day lactation period using calibrated measuring cylinders. The average daily milk yield was calculated for statistical analysis.

2.9 Somatic Cell Count Determination

Milk samples were collected aseptically and somatic cell count (SCC) was determined using direct microscopic counting techniques. SCC values were expressed as $\times 10^5$ cells/mL.

2.10 Mastitis Diagnosis

Clinical mastitis was diagnosed based on visible abnormalities in milk appearance, udder inflammation, and veterinary examination. California Mastitis Test (CMT) was also used to identify subclinical mastitis cases.

2.11 Statistical Analysis

Genotype and allele frequencies were calculated according to standard population genetic procedures. Chi-square analysis was used to test deviation from Hardy-Weinberg equilibrium.

The statistical model used for association analysis was:

$$Y_{ijk} = \mu + B_i + G_j + e_{ijk}$$

Where:
Y_{ijk} = observed trait
μ = overall mean
B_i = effect of breed
G_j = effect of genotype
e_{ijk} = residual error

Data were analyzed using SPSS version 25, and means were separated using Duncan's Multiple Range Test at 5% significance level.

3 Results

3.1 Genotype and Allele Frequencies of PAD14 Gene

Table 1 Genotype frequencies of PAD14 gene in White Fulani and Red Bororo cattle

Breed	AA	AB	BB
White Fulani	0.45	0.40	0.15
Red Bororo	0.38	0.47	0.15

The ^{AA} genotype was the most predominant genotype in White Fulani cattle, whereas ^{AB} genotype occurred more frequently in Red Bororo cattle.

Table 2 Allele frequencies of PAD14 gene

Breed	A	B
White Fulani	0.65	0.35
Red Bororo	0.62	0.38

The ^{A} allele was more frequent in both cattle breeds.

3.2 Association between PAD14 Genotypes and Milk Yield

Table 3 Least square means of milk yield (L/day)

Genotype	White Fulani	Red Bororo
AA	4.85 ± 0.22^{a}	4.42 ± 0.19^{a}
AB	4.31 ± 0.18^{b}	4.10 ± 0.16^{ab}
BB	3.95 ± 0.15^{c}	3.81 ± 0.14^{b}

Different superscripts within columns indicate significant differences ($P < 0.05$).

Cows possessing the ^{AA} genotype exhibited significantly higher milk yield compared to cows with ^{BB} genotype.

3.3 Association between PAD14 Genotypes and Somatic Cell Count

Table 4 Somatic cell count ($\times 10^{5}$ cells/mL)

Genotype	White Fulani	Red Bororo
AA	2.10 ± 0.11^{c}	2.25 ± 0.13^{c}
AB	3.05 ± 0.17^{b}	3.22 ± 0.16^{b}
BB	4.50 ± 0.21^{a}	4.73 ± 0.24^{a}

Different superscripts within columns indicate significant differences ($P < 0.05$).

Animals carrying the ^{BB} genotype showed significantly higher somatic cell counts than ^{AA} genotype carriers.

3.4 Mastitis Incidence among PAD14 Genotypes

Table 5 Mastitis incidence (%)

Genotype	White Fulani	Red Bororo
AA	8.3	9.1
AB	16.7	18.2
BB	27.5	29.4

The ^{BB} genotype recorded the highest mastitis incidence in both breeds.

4 Discussion

The present study demonstrated the existence of genetic polymorphism within the PAD14 gene in White Fulani and Red Bororo cattle populations (table 1). The identification of three genotypes (AA, AB, and BB) suggests substantial genetic variability within these indigenous cattle breeds. Genetic variability is important in livestock improvement because it provides the basis for selection and adaptation to environmental challenges.

The predominance of the A allele in both cattle breeds (table 2) may indicate positive selective advantages associated with this allele under tropical environmental conditions. Indigenous cattle breeds in Nigeria are constantly exposed to disease challenges, heat stress, and nutritional deficiencies; therefore, animals possessing favorable immune-response

genes may have higher survival and productivity rates. Similar observations have been reported by Beecher *et al*. [3], who found significant associations between immune-related gene polymorphisms and udder health traits in dairy cattle.

The significant association between PAD14 genotypes and milk yield observed in this study indicates that the gene may influence productive performance either directly or indirectly through immune regulation. Cows carrying the AA genotype produced significantly higher milk yield than BB genotype animals (table 3). This finding agrees with earlier reports suggesting that healthier udders and lower disease burden contribute to improved milk synthesis and secretion [8,11].

Somatic cell count is widely recognized as one of the most reliable indicators of mastitis infection and udder inflammation in dairy cattle. In the present study, cows with BB genotype exhibited significantly higher somatic cell counts than AA genotype carriers (table 4). Elevated SCC values are usually associated with increased leukocyte infiltration into the mammary gland following bacterial infection. The lower SCC observed in AA genotype animals (table 4) suggests enhanced resistance to mastitis infection and better udder health status.

The higher incidence of mastitis among BB genotype animals (table 5) further supports the role of PAD14 gene polymorphism in immune response regulation. During mastitis infection, inflammatory pathways are activated to combat invading pathogens. Since PAD enzymes are involved in protein citrullination during inflammatory reactions, variations in PAD14 gene structure may alter the efficiency of immune defense mechanisms. Khan *et al*. [7] reported that polymorphisms in immune-associated genes significantly influence susceptibility and resistance to bovine mastitis.

The findings of this study have important implications for cattle breeding programs in Nigeria. Indigenous breeds possess valuable adaptive traits that allow them to survive under harsh tropical conditions. However, productivity enhancement remains necessary to meet increasing demands for animal protein and dairy products. Marker-assisted selection using favorable PAD14 alleles could improve milk production and mastitis resistance simultaneously without compromising adaptability.

Furthermore, the study contributes to the limited molecular genetic information available for Nigerian indigenous cattle breeds. Most previous genetic studies in cattle have focused mainly on exotic breeds, while indigenous African breeds remain under-characterized. Molecular characterization of local breeds is essential for conservation, sustainable utilization, and future genomic improvement programs.

5 Conclusion

The present study revealed significant polymorphism in the PAD14 gene among White Fulani and Red Bororo cattle populations. Three genotypes (AA, AB, and BB) were identified, with the A allele occurring more frequently in both breeds. Significant associations were observed between PAD14 genotypes and milk yield, somatic cell count, and mastitis incidence.

Animals carrying the AA genotype demonstrated superior milk yield and lower somatic cell counts, indicating improved mastitis resistance compared to BB genotype animals. These findings suggest that PAD14 gene polymorphism may serve as a useful molecular marker for genetic improvement programs aimed at enhancing udder health and dairy productivity in indigenous Nigerian cattle breeds.

Recommendations

- Larger population studies should be conducted to validate the associations identified in this study.
- Sequencing analysis should be performed to identify specific nucleotide variations within the PAD14 gene.
- Marker-assisted selection programs should incorporate favorable PAD14 alleles for improving mastitis resistance.
- Conservation of indigenous cattle genetic resources should be strengthened to preserve adaptive traits.
- Further studies should investigate the interaction between PAD14 gene polymorphism and environmental factors affecting dairy productivity.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest related to this study.

Statement of ethical approval

This research was conducted in accordance with ethical guidelines governing the use of animals and genetic materials in scientific research as stipulated by the Ethical Committee of Department of Animal Health and Production Technology, School of Science and Technology, Federal University of Science and Technology Kabo, which is in line with Animal health care constitution.

Statement of informed consent

Authors has given an approval and consent for the article to be submitted for publication.

Author declaration

All the authors have declared that this article is original article submitted for publication which has not been published anywhere in the world.

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