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Full Length Research

RFLP-PCR polymorphism of beta-casein and K-casein gene in White Fulani, Sokoto Gudali and Red Bororo indigenous cattle in Nigeria

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ABSTRACT: One key genetic determinant of milk quality and yield is the casein protein, specifically β-casein and κ-casein, which is associated with important traits such as milk production, protein content, and digestibility. This study investigates the genetic polymorphisms of β-casein and κ-casein genes in three indigenous Nigerian cattle breeds: White Fulani, Sokoto Gudali, and Red Bororo, using the Restriction Fragment Length Polymorphism - Polymerase Chain Reaction technique. A total of 40 blood samples from each breed were collected, and DNA was extracted for Polymerase Chain Reaction amplification and the nucleotides were measured for variations using SNPs genotyping. The alleles identified for the β-casein gene were A1 and A2, with the A1 allele being more prevalent in White Fulani (63%) compared to Sokoto Gudali (54%) and Red Bororo (41%). For κ-casein, the A and B alleles were observed, with the B allele being more frequent in Red Bororo (56%) than in Sokoto Gudali (47%) and White Fulani (36%). Genotype frequencies showed a higher proportion of the A2A2 genotype in Red Bororo (47%) than in White Fulani (14%). The Shannon Index (H') and Effective Number of Alleles (Ne) were highest in Sokoto Gudali for β-casein (H' = 0.65, Ne = 2.15) and κ-casein (H' = 0.60, Ne = 2.03), indicating greater genetic diversity. Genetic distance analysis revealed significant differentiation between the breeds, with White Fulani and Red Bororo being the most genetically distinct. It was concluded that White Fulani is more inclined towards higher milk yields due to its higher A1 allele frequency, while Red Bororo, with its predominant A2 allele and A2A2 genotype, shows potential for producing healthier milk with beneficial protein-to-fat ratios and Sokoto Gudali on the other hand, exhibited the highest genetic diversity and could serve as a critical breed for maintaining genetic resilience and adaptability in future breeding programmes.

Keywords: allele, β-casein polymorphism, gene frequency, genetic diversity, milk yield, Shannon index,

INTRODUCTION

Globally, meat, milk and milk products obtained from cattle constitute the largest proportion of animal protein origin and contribute a significant proportion to the dietary protein intake of humans (Oke *et al*., 2022). The cattle population in the world is estimated at 1.5 billion (FAO, 2022), and Africa alone contributes about 300 million heads of cattle, constituting approximately one-fifth of the world's cattle population (Dessie and Okeyo Mwai, 2019). Bovine (Cattle) are an important part of Nigeria's agricultural system, providing meat, milk, milk products, and labour to farming communities. Indigenous breeds such as White Fulani, Sokoto Gudali, and Red Bororo are known for their resilience and adaptability to local environmental conditions (Dauda and Idi, 2023). Although the production potential of indigenous African cattle is lower than most exotic breeds, they are widely reared by farmers in agro-

pastoral systems, especially where most exotic breeds exhibit poor performance under traditional (extensive) management systems (Kim *et al*., 2017). One key genetic determinant of milk quality and yield is the casein protein, specifically β-casein and κ-casein, which have been associated with important traits such as milk production, protein content, and digestibility (Zepeda-Batista *et al*., 2017).

Bovine milk proteins are generally classified as caseins, which make up about 80% of the milk proteins, consisting of four proteins: Alpha S1 (CSN1S1, 39-46% of total caseins), alpha S2 (CSN1S2, 8-11%), beta (CSN2, 25-35%), and kappa (CSN3, 8-15%) (Eigel *et al*., 1984). Of the total milk protein produced by cattle, 16% is Whey protein and it contains two major proteins, alpha-lactalbumin and beta-lactoglobulin (Abd El-Salam and El-Shibiny, 2015). Genetic polymorphisms in casein proteins have been extensively studied in cattle worldwide, but there is limited research on these genes in indigenous Nigerian cattle (Olanrewaju *et al*., 2020). Since the pioneer work done by Aschaffenburg and Drewry in 1955 who discovered alleles A and B of β lactoglobulin in cattle researchers worldwide became interested in genetic polymorphisms of major milk proteins. The different genetic variants of milk proteins differ from each other by only a few amino acid substitutions or deletions within the polypeptide chain (Eigel *et al*., 1984). Identifying polymorphisms in β-casein and κ-casein genes using molecular techniques such as Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR) and then Single Nucleotide Polymorphisms (SNPs) genotyping offers valuable insights into the genetic variation of these breeds (Zhang *et al*. 2005). Understanding such variation is essential for designing breeding programmes that can improve milk traits and enhance the overall productivity of the cattle population (Caroli *et al*., 2009a). Therefore, this study aims to investigate the polymorphisms of β-casein and κ-casein genes in selected indigenous cattle breeds in Nigeria using the RFLP-PCR technique.

MATERIALS AND METHODS

Data collection site

A total of 120 blood samples were collected from three indigenous cattle breeds in four locations across Ogun State, Nigeria, Ten blood samples per breed were collected from Abeokuta, Ilaro, Ikenne and Ijebu-Ode, all in Ogun State, Nigeria to give a total 40 blood samples for White Fulani, 40 blood samples for Sokoto Gudali and 40 blood samples for Red Bororo. These locations were chosen because they are the recognized four agricultural zones as divided under the Ogun State Agricultural Development Programme (OGADEP).

DNA extraction and amplification

The blood samples were collected from the jugular vein of each animal and stored in EDTA tubes at -20°C until DNA extraction was done. Genomic DNA was extracted from the blood samples using the phenol-chloroform method. The concentration and purity of the extracted DNA were determined using a Nanodrop spectrophotometer, and the integrity of the DNA was assessed by agarose gel electrophoresis.

The β-casein and κ-casein gene fragments were amplified using specific primers designed to target the polymorphic regions of each gene. The sequences of the primers used are as follows:

β-casein forward primer: 5'-AGGGTCCATGTAGATAGGCTTG-3'
β-casein reverse primer: 5'-CAGGTTGTTGTCAGGAGTGG-3'
κ-casein forward primer: 5'-TGGGTGATAGTGGCTCTCAG-3'
κ-casein reverse primer: 5'-GTACATGGCTGGAAGTTGG-3'

PCR reactions were carried out in a total volume of 25 µL containing 50 ng of genomic DNA, 0.2 mM dNTPs, 0.5 µM of each primer, 1X Taq polymerase buffer, and 1 unit of Taq DNA polymerase. The PCR conditions included an 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 30 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 10 minutes. The amplified PCR products were digested with specific restriction enzymes to detect polymorphisms in the β-casein and κ-casein genes. The restriction enzymes used were Hind III for β-casein and HinfI for κ-casein. The digestion reactions were incubated at 37°C for 2 hours, and the resulting fragments were separated by 2% agarose gel electrophoresis. The gels were stained with ethidium bromide and visualized under UV light.

Data analysis

The genotypes of the cattle for each casein gene were determined based on the banding patterns observed on the agarose gels. Allele frequencies were calculated using standard population genetics formulas of Hardy-Weinberg law, and the chi-square test was employed to determine the significance of differences in genotype frequencies among the breeds.

RESULTS AND DISCUSSION

Allele and genotype frequencies

The Genotype frequencies of the β-casein gene in Nigerian indigenous cattle breeds are presented in Table 1. The results from the RFLP-PCR analysis of the β-casein in White Fulani, Sokoto Gudali, and Red Bororo cattle

Table 1. Genotype frequencies of the β-casein gene in Nigerian indigenous cattle breeds.

Breed	A1A1 (%)	A1A2 (%)	A2A2 (%)	BB (%)	BE (%)	EE (%)
White Fulani	25.0	55.0	20.0	30.0	50.0	20.0
Sokoto Gudali	15.0	60.0	25.0	40.0	45.0	15.0
Red Bororo	30.0	50.0	20.0	20.0	60.0	20.0

Table 2. Allele frequencies of the *k*-casein gene in Nigerian indigenous cattle breeds.

Breed	A1 (%)	A2 (%)	B (%)	E (%)
White Fulani	52.5	47.5	55.0	45.0
Sokoto Gudali	45.0	55.0	62.5	37.5
Red Bororo	55.0	45.0	50.0	50.0

breeds revealed critical insights into the genetic composition of these breeds, with potential implications for important traits such as milk production, milk composition, and overall adaptability. For the β-casein gene, two alleles (A1 and A2) were identified in all three cattle breeds, with the A1 allele being more frequent in White Fulani, while Sokoto Gudali and Red Bororo showed a relatively more balanced distribution of the A1 and A2 alleles (Dogru, 2015). Specifically, the allele frequencies for β-casein in White Fulani were 0.63 (A1) and 0.37 (A2), whereas Sokoto Gudali had frequencies of 0.54 (A1) and 0.46 (A2), and Red Bororo exhibited 0.41 (A1) and 0.59 (A2). The higher frequency of the A1 allele in White Fulani suggests that this breed may have a predisposition for traits associated with the A1 variant of β-casein, which has been linked to increased milk yield but a potential predisposition to adverse health effects in humans (Jenkins *et al*., 2020). In contrast, the A2 allele, more frequent in Red Bororo and moderately balanced in Sokoto Gudali, has been associated with beneficial health effects in humans, such as improved digestion and reduced risk of certain diseases, as well as favourable traits for dairy production (Bell *et al*., 2021). The significance of this result highlights the potential for Red Bororo to be a valuable resource for breeding programs focused on improving the health benefits of milk and dairy products. The genotype frequencies further reinforce this. In White Fulani, the most common genotype was A1A2 (0.52), followed by A1A1 (0.34) and A2A2 (0.14). This high proportion of heterozygotes suggests the presence of some degree of genetic diversity within this breed, although the overall allele distribution still indicates a dominance of the A1 allele. Comparatively, in Red Bororo, the A2A2 genotype (0.47) was the most frequent, followed by A1A2 (0.37) and A1A1 (0.16), reflecting a stronger presence of the A2 allele in this breed. The importance of these allele and genotype frequencies becomes clear when considering the impact on milk production traits. Research has shown that cattle carrying the A2A2 genotype tend to produce milk with a better protein-to-fat ratio, which is desirable for both milk processors and consumers (Villarreal *et al*., 2019). The A1A1 genotype, on the other hand, has been linked to higher milk yields, though potentially lower in protein content and associated with less favourable health outcomes (Xu *et al*., 2017). The A1A2 genotype, present in significant proportions in all breeds, is associated with intermediate traits, providing a balance between milk yield and milk composition quality.

For the κ-casein gene, as shown in Table 2, the A and B alleles were identified, with allele frequencies for the A allele being 0.64 (White Fulani), 0.53 (Sokoto Gudali), and 0.44 (Red Bororo). The B allele was more frequent in Red Bororo (0.56) compared to Sokoto Gudali (0.47) and White Fulani (0.36). The B allele of κ-casein is known to be positively associated with improved milk coagulation properties and higher cheese yield, making it a valuable trait for dairy industries focused on cheese production (Caroli *et al*., 2009b). The genotype frequencies for κ-casein mirrored these allele distributions. In White Fulani, the AA genotype was the most common (0.44), followed by AB (0.40) and BB (0.16). This suggests that White Fulani cattle may not possess the same potential for enhanced cheese production as Sokoto Gudali and Red Bororo, where BB genotypes were more frequent in Red Bororo (0.35) and Sokoto Gudali (0.29). Studies have shown that the BB genotype is favourable for milk destined for cheese production due to its impact on casein micelle size and milk curdling efficiency (Ahmed *et al*., 2017). The allele and genotype frequencies observed in this study are consistent with findings from similar studies on African indigenous cattle breeds. For instance, Mwai *et al*. (2015) reported that many African cattle breeds exhibit significant genetic diversity, particularly in milk-related genes like β-casein and κ-casein, due to their adaptation to diverse environmental conditions and management systems. Duifhuis *et al.* (2014) reported polymorphism in these casein genes in their study and observed little or no influence on milk production. However, our results support

Table 3. Genetic diversity measures for β-casein and κ-casein genes in Nigerian indigenous cattle breeds.

Breed	Gene	Shannon index (H')	Effective alleles (Ne)	Observed heterozygosity (Ho)	Expected heterozygosity (He)
White Fulani	β-Casein	0.61	2.02	0.58	0.50
	κ-Casein	0.58	1.95	0.50	0.48
Sokoto Gudali	β-Casein	0.65	2.15	0.60	0.53
	κ-Casein	0.60	2.03	0.55	0.50
Red Bororo	β-Casein	0.63	2.08	0.55	0.52
	κ-Casein	0.62	2.10	0.57	0.51

this conclusion, with all three Nigerian breeds showing a range of alleles and genotypes for both casein genes. The higher frequency of the A1 allele in White Fulani, observed in this study, has been reported in other African breeds, where it is often linked to higher milk production levels but potentially reduced milk quality in terms of protein content (Lollike *et al*., 2022). In contrast, the relatively higher frequency of the A2 allele in Red Bororo aligns with findings from Bell *et al*. (2021), who reported that indigenous cattle breeds that adapted to harsher environments tend to carry the A2 allele more frequently, which is beneficial for both milk quality and health attributes. The presence of the B allele of κ-casein, particularly in Red Bororo and Sokoto Gudali, further confirms the adaptability of these breeds to dairy production systems. Similar findings were reported by Caroli *et al*. (2009b), where the B allele was found to be prevalent in cattle populations selected for dairy production, particularly in those producing milk for cheese manufacturing.

The results from the allele and genotype frequency analyses have important implications for the development of breeding programs in Nigeria. The higher frequency of the A2 allele and A2A2 genotype in Red Bororo suggests that this breed could be a key resource for breeding programs aimed at producing healthier and higher-quality milk (Heck *et al*., 2009). On the other hand, the higher frequency of the A1 allele in White Fulani suggests that this breed may be more suited for regions where milk yield is prioritized over milk composition. For the κ-casein gene, the higher frequency of the B allele and BB genotype in Sokoto Gudali and Red Bororo makes these breeds particularly suitable for the dairy industry, especially in regions focusing on cheese production. This is consistent with the findings of Ahmed *et al.* (2017), who highlighted the economic benefits of breeding cattle with the B allele for improving cheese yield and quality. Overall, these findings underscore the genetic potential of Nigerian indigenous cattle breeds for targeted breeding strategies. The presence of significant genetic diversity, as indicated by the range of alleles and genotypes for both β-casein and κ-casein genes, provides a strong foundation for the sustainable development of the dairy sector in Nigeria.

Genetic diversity measures in cattle

The genetic diversity indices such as Shannon's Index (H'), Effective Number of Alleles (Ne), Observed Heterozygosity (Ho) and Expected Heterozygosity (He) are shown in Table 3. This further underscores the differences in genetic variation among the three breeds. For the β-casein gene, the highest Shannon's Index (0.65) and Effective Number of Alleles (2.15) were observed in Sokoto Gudali cattle, indicating that this breed has the greatest genetic diversity. This is likely due to more extensive crossbreeding and less genetic isolation compared to the other breeds as observed by Gustavsson *et al.* (2014) among three breeds of dairy. In contrast, White Fulani had the lowest Shannon's Index and Effective Number of Alleles, suggesting a reduced level of genetic diversity. This could be due to selective breeding pressures favouring specific traits in the White Fulani population, as observed in other studies (Mohammadi *et al*., 2020). For the κ-casein gene, similar trends were observed, with Sokoto Gudali having the highest genetic diversity indices. The higher genetic diversity within the Sokoto Gudali and Red Bororo breeds for both β-casein and κ-casein genes suggests that these breeds may be better suited to survive under changing environmental conditions or disease pressures. Their diverse genetic makeup could confer greater resilience, which is essential for sustainable breeding programs in Nigeria (Olijira *et al*., 2022).

Genetic distance analysis

The pairwise genetic distances between the cattle breeds help to infer the evolutionary relationships between the breeds. The genetic distance analysis between the breeds (Table 4) showed that White Fulani and Red Bororo were more genetically distant from each other compared to their respective distances from Sokoto Gudali. The genetic distance between White Fulani and Sokoto Gudali was 0.105 for β-casein and 0.112 for κ-casein, indicating moderate differentiation. The smaller genetic distance between Sokoto Gudali and Red Bororo (0.080 and 0.090

Table 4. Pairwise genetic distance between Nigerian indigenous cattle breeds.

Breed pair	β-casein	κ-casein
White Fulani & Sokoto Gudali	0.105	0.112
White Fulani & Red Bororo	0.120	0.125
Sokoto Gudali & Red Bororo	0.080	0.090

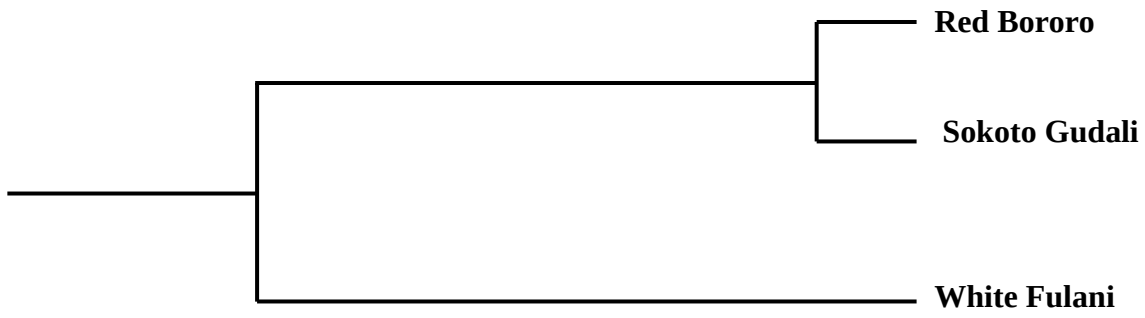

Figure 1. Dendogram of Nigerian indigenous cattle.

for β-casein and κ-casein, respectively) suggests that these breeds are more closely related. This could reflect historical gene flow between these populations due to migration or shared ancestry. The phylogenetic tree based on the genetic distance data, as shown in Figure 1, highlights the close relationship between Sokoto Gudali and Red Bororo, with both clustering together, while White Fulani branches off as a separate group. This clustering pattern supports previous findings of genetic relatedness among these breeds, with the White Fulani being genetically distinct, potentially due to its adaptation to different ecological zones and selective breeding (Mwai *et al*., 2015). The phylogenetic relationships observed in this study align with the broader understanding of African cattle genetics, which suggests that local breeds have undergone significant divergence, yet still retain substantial genetic overlap due to interbreeding and shared historical origins (Gebrehiwot *et al*., 2020). This genetic differentiation is crucial for identifying breeds with unique characteristics that could be incorporated into breeding programmes to improve milk production, disease resistance, and adaptation to local environmental conditions (Biscarini *et al*., 2015).

Conclusion

The results of this study indicate that White Fulani is more inclined towards higher milk yields due to its higher A1 allele frequency, while Red Bororo, with its predominant A2 allele and A2A2 genotype, shows potential for producing healthier milk with beneficial protein-to-fat ratios. Sokoto Gudali on the other hand, exhibited the highest genetic diversity and could serve as a critical breed for maintaining genetic resilience and adaptability in future breeding programs. Additionally, the higher frequency of the B allele and BB genotype in Red Bororo and Sokoto Gudali suggests their suitability for cheese production. This genetic variability among the breeds offers opportunities for selective breeding to enhance dairy production traits, improve milk quality, and promote the sustainability of Nigeria's cattle population. Future breeding efforts should focus on leveraging the genetic strengths of these indigenous breeds to maximize their productivity and economic potential in the dairy and cheese industries.

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