

Genetic Polymorphism of α -Lactalbumin and Lactoferrin and Their Relationship with Milkfat in West African Dwarf Goat

ABSTRACT

Milk samples of 39 lactating West African Dwarf does in selected households in Akinyele Local Government Area of Oyo State were analyzed to study the genetic polymorphism of α -lactalbumin and lactoferrin and their effect on milk fat content. Genetic variants of milk protein were detected by cellulose acetate electrophoresis; milk samples were processed to remove fat prior to analysis. The interpretation of electrophoretic migrations revealed the presence of two alleles in each locus studied. The two alleles A and B controlled three genotypes: AA, AB and BB. Homozygous genotypes AA and BB at the α -Lactalbumin locus was observed in 17 and 5 individuals representing 42.5% and 12.5% respectively and 11 and 6 individuals representing 27.5% and 15% at the lactoferrin locus. No significant effect on milk fat content was observed for the two loci studied.

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Keywords: Goat, Milk, Lactalbumin, Lactoferrin, Polymorphism

1. INTRODUCTION

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Milk proteins secreted by mammary epithelial cells mainly contain caseins, whey proteins (α -Lactalbumin, lactoglobulin, serum albumin, immunoglobulin, lactoferrin and transferrin) and enzymes [1, 2]. Since the discovery of polymorphism of whey protein and a quantitative distribution of its variants [3] researchers have become interested in genetic polymorphism of milk proteins. These genetic variants occur as a consequence of either substitution or deletion of amino acids within the polypeptide chain [4]. These variants are heritable and differ based on specie and breed in their occurrence and frequency.

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Several interesting relationships between milk protein genetic variants and economically important traits have been reported [5,6,7]. Such association could be exploited commercially by identifying favorable genotypes which could be used as additional selection criterion in the improvement of the nutritional and technological properties of milk.

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Goat has been identified as one of the main contributors of dairy and meat products for rural people, more than any other mammalian farm animal, particularly in developing countries [8,9]. The West African Dwarf (WAD) goat is widely distributed across the rainforest belt of Southern Nigeria. They are short-legged and small-bodied animals, present variable coat colours, ranging from black, brown, gray, red and white, and sometimes combinations of these in a variety of patterns [10]. These goats are found in all of humid Africa and are well adapted to the humid forest zone. They are highly prolific and can be bred all year round with up to three parturitions in two years. [11] About 85% of the smallholder farmers in the Nigerian subhumid zone keep West African Dwarf (WAD) goats [12]. Although goat milk is rarely utilized for human consumption in Nigeria due to social belief it has been found to be useful in the treatment of dyspepsia, peptic ulcer and biliary disorder [13]. The global contribution of goat milk to the nutritional and economic well-being of humanity is enormous. The fat content of goat milk across breeds ranges from 2.45% to 7.76% and has more advantages over cow milk in having higher digestibility of protein and fat.

41 Thus, goat milk provides essential nutrients in human diets and is greatly valued by those who have cow
42 milk allergy and other nutritional diseases [9]
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44 This study was conducted to characterize some whey protein variants in WAD goats and investigate their
45 association with milk fat as a preliminary work to allow a better knowledge of the breed for sustainable
46 genetic improvement and to contribute to the global process initiated by the Food and Agriculture
47 Organization to document the World's Animal Genetic Resource.
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49 2. MATERIALS AND METHODS

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51 Milk samples were collected from 39 WAD ewes of second parity; animals were sampled from 15 different
52 households located in Akinyele Local Government Area of Oyo State. The animals were semi-intensively
53 raised in an agrarian locality; they are left to graze during the day and fed crop residues as supplements.
54 Individual samples were collected in the morning, 5ml was taken from each doe after washing the udder
55 with water and moping with clean napkin. The samples were preserved in a cooler containing ice blocks
56 and transported to the laboratory where the samples were analyzed. Milk samples were centrifuged at
57 3000 x g for 10 minutes to remove the fat fraction. Casein was precipitated by adding drops of acetic acid
58 (10µl to 100µl) to skimmed milk and centrifuged [14]. The whey fraction was examined for α-lactalbumin
59 and lactoferrin genetic types. Genotypes were determined by cellulose acetate electrophoretic analysis of
60 individual milk samples as reported by [15] and [16]

61 Milk protein alleles are co-dominant allele; thus, the genotypes were recognizable based on their relative
62 electrophoretic migration. Electrophoretic analysis revealed three migration zones: fast named A,
63 intermediate labeled AB and slow designated B.
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65 2.1 Statistical analysis

66 Gene and genotypic frequencies at the two milk protein loci were computed by direct counting method.
67 Mean heterozygosity, expected heterozygosity and conformation to Hardy-Weinberg equilibrium (HWE)
68 was tested using TFPGA software [17]

69 Influence of milk protein loci on milkfat content was analyzed by linear model without interaction as
70 follows:

$$71 Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}$$

72 Where Y_{ijk} = the observed value of milk fat; μ was population mean;

73 α_i was the fixed effect of α-lactalbumin genotype;

74 β_j was the fixed effect of lactoferrin genotype;

75 e_{ijk} was random residual effect.

76 All data were analyzed with [18] software package. Means were separated using Duncan Multiple Range
77 Test (DMRT) of the SAS software.
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79 3. RESULTS

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81 Electrophoretic separation of α-lactalbumin and lactoferrin are shown in Plates 1 and 2. The two loci
82 investigated were found to be polymorphic and interpreted into three genotypes (AA, AB and BB). The
83 genotypic and allelic frequencies of two whey protein locus of WAD goats are presented in Table 1. Two
84 alleles A and B were identified in each locus with allele A occurring at a higher frequency in the loci
85 investigated. Genotype AB was the most predominant representing 45.0% and 57.5% at the α-
86 lactalbumin and lactoferrin locus respectively. The observed and the expected frequencies for the
87 different alleles showed no significant difference in this study. The population sampled conformed to
88 Hardy-Weinberg equilibrium at the loci investigated. Table 2 shows the effect of milk protein loci on the
89 relative percentage of milk fat of the milk of WAD does.
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Plate 1: Electrophoretic separation of α -lactalbumin in WAD goats



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Plate 2: Electrophoretic separation of lactoferrin in WAD goats

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Table 1: Genotypic and allelic frequencies of α -Lactalbumin and lactoferrin variants in WAD goat

| Locus | Observed Genotypes (Expected) | | | Allele frequencies | | Ave Het | P(HW) |
|--------------------|---------------------------------|-------------|-----------|--------------------|--------|------------|-------|
| | AA | AB | BB | A | B | | |
| Lactalbumin | 17(16.5584) | 17(17.8831) | 5(4.5584) | 0.6538 | 0.3462 | 0.4527 | 0.75 |
| Lactoferrin | 11(12.2857) | 22(19.4286) | 6(7.2857) | 0.5641 | 0.4359 | 0.4918 | 0.40 |
| HW- Hardy-Weinberg | Ave Het- Average Heterozygosity | | | | | | |

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110 **Table 2: Means and standard deviation values of the milk fat content for the different genotypes of**
 111 **α -Lactalbumin and Lactoferrin in WAD does.**

| Locus | Genotype | Sample size | Milk fat content (%) |
|-----------------------|----------|-------------|----------------------|
| α -Lactalbumin | AA | 17 | 2.52±0.85 |
| | AB | 17 | 2.88±0.88 |
| | BB | 5 | 2.44±0.70 |
| Lactoferrin | AA | 11 | 2.42±0.81 |
| | AB | 22 | 2.86±0.89 |
| | BB | 6 | 2.41±0.63 |

112 *P*= .05

114 4. DISCUSSION

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 117 Goat milk exceeds cow milk in monounsaturated, polyunsaturated fatty acids and medium chain
 118 triglycerides and is known to be beneficial for human health, especially for cardio-vascular conditions [19].
 119 There has been considerable interest in goat milk protein due to availability of different bioactive peptide
 120 derived from milk proteins, which have pharmaceutical and nutraceutical applications and may have
 121 future commercial importance [20,21]. While α -Lactalbumin and lactoferrin are mainly found in whey, their
 122 association with other milk constituents have been reported [22,23] α -Lactalbumin, alpha (LALBA) plays a
 123 key role in the biosynthesis of lactose by regulating the substrate affinity of the lactose synthase complex
 124 and has also been reported to have a role in induction of cell growth inhibition or apoptosis in tumor cells
 125 and immature cells [24]. However, α -Lactalbumin knockout mice have been reported to produce highly
 126 viscous milk which is occasioned by the absence of lactose in the milk followed by marked elevation of
 127 protein and fat content [23] suggesting its role in regulating the fat content of milk. Lactoferrin is an iron-
 128 binding glycoprotein that is closely related in structure to the iron-transport protein, transferrin. It has been
 129 reported to be an anti-infective agent as it activates the transcription of important immune-related genes
 130 in the small intestine and promotes systemic host immunity [25]

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 132 Studies on protein polymorphism, which have been extensive over the recent years, indicate that milk
 133 proteins can be utilized in both breeding practice and milk processing [26,27]. Allele and genotypic
 134 frequencies observed in this study are similar to those described in literature for other goat breeds [28,
 135 29]. Findings observed at the α -Lactalbumin locus revealed two alleles A and B with allele A being the
 136 most common as previously reported in Barbari and some Indian goat breeds [28, 29]. Monomorphism at
 137 this locus has been reported by [2] and [30] in the Red Sokoto goats and some Indian goat breeds
 138 respectively. The predominance of this allele likely confers certain selective advantage on milk traits in
 139 goats. In this report, lactoferrin locus was found to exhibit polymorphism with allele A occurring at a higher
 140 frequency. However, using a genome wide approach (PCR-SSCP) [31] reported three genotypes: GG,
 141 AA and GA in some dairy goat breeds. Variants of this protein has also been reported in Holstein-Friesian
 142 cattle breeds [32]. Reports on the effect of milk protein types on milk traits in goat breeds are scanty
 143 however several studies on milk protein polymorphism and their association with milk fat and mastitis in
 144 cows have been reported [33,34].

145 There were no significant associations between the genetic variants observed in this population and milk
 146 fat content. This may be due to the sample size of the population used in the present study. However, in a
 147 study on the effect of lactalbumin on milk production traits in two breeds of water buffalo, [35] reported
 148 significant association between genotype BC and total and daily milk yield in Bhadawari breed. In the
 149 Bhadawari, animals with genotype BC had 40% more total lactation yield than the those with AB
 150 genotype.

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 152 The mean heterozygosity values of 0.4527 and 0.4978 obtained at the lactalbumin and lactoferrin locus
 153 respectively suggests significant genetic variation in the population sampled. The population studied was
 154 in Hardy-Weinberg's equilibrium with respect to the two loci investigated. This may be due to the constant
 155 gene flow within the sampled population.

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4. CONCLUSION

The present investigation revealed that α -Lactalbumin and lactoferrin locus showed polymorphism in the milk samples of WAD goats. However further analysis should be performed using molecular tools to investigate the association between the genetic variants and milk quality traits. Such findings can be used for genetic improvement of the breed and to understand the role that each variant can have on milk nutritional and technological properties

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

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