

**Genetic Polymorphism of  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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.

*Keywords: Goat, Milk, Lactalbumin, Lactoferrin, Polymorphism*

**1. INTRODUCTION**

Milk proteins secreted by mammary epithelial cells mainly contain caseins, whey proteins ( $\alpha$ -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.

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.

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]. Goat milk provide essential nutrients in human diets and is greatly valued by those who have cow milk allergy and other nutritional diseases [9]

This study was conducted to characterize some whey protein variants in WAD goats and investigate their association with milk fat as a preliminary work to allow a better knowledge of the breed for sustainable

42 genetic improvement and to contribute to the global process initiated by the Food and Agriculture  
 43 Organization to document the World's Animal Genetic Resource.

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 45 **2. MATERIALS AND METHODS**

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

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

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 60 **2.1 Statistical analysis**

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

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

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

67 Where  $Y_{ijk}$ =the observed value of milk fat;  $\mu$  was population mean;

68  $\alpha_i$  was the fixed effect of α-lactalbumin genotype;

69  $\beta_j$  was the fixed effect of Lactoferrin genotype;

70  $e_{ijk}$  was random residual effect.

71 All data were analyzed with [18] software package. Means were separated using Duncan Multiple Range  
 72 Test (DMRT) of the SAS software.

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 74 **3. RESULTS**

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 76 In this preliminary investigation, the two loci were found to be polymorphic. The genotypic and allelic  
 77 frequencies of two whey protein locus of WAD goats are presented in Table 1. Two alleles A and B were  
 78 identified in each locus with allele A occurring at a higher frequency in the loci investigated. Genotype AB  
 79 was the most predominant representing 45.0% and 57.5% at the α-Lactalbumin and lactoferrin locus  
 80 respectively. The observed and the expected frequencies for the different alleles showed no significant  
 81 difference in this study. The population sampled conformed to Hardy-Weinberg equilibrium at the loci  
 82 investigated. Table 2 shows the effect of milk protein loci on the relative percentage of milk fat of the milk  
 83 of WAD does.

84  
 85 **Table 1: Genotypic and allelic frequencies of α-Lactalbumin and lactoferrin variants in WAD goat**

Locus	Observed Genotypes (Expected)			Allele frequencies		Ave	P(HW)
	AA	AB	BB	A	B	Het	
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

86 HW- Hardy-Weinberg Ave Het- Average Heterozygosity

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90 **Table 2: Means and standard deviation values of the milk fat content for the different genotypes of**  
 91  **$\alpha$ -Lactalbumin and Lactoferrin in WAD does.**

Locus	Genotype	Sample size	Milk fat content (%)
$\alpha$ -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

92 *P*= .05

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#### 95 4. DISCUSSION

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

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112 Studies on protein polymorphism, which have been extensive over the recent years, indicate that milk  
 113 proteins can be utilized in both breeding practice and milk processing [26,27]. Allele and genotypic  
 114 frequencies observed in this study are similar to those described in literature for other goat breeds [28,  
 115 29]. Findings observed at the  $\alpha$ -Lactalbumin locus revealed two alleles A and B with allele A being the  
 116 most common as previously reported in Barbari and some Indian goat breeds [28, 29]. Monomorphism at  
 117 this locus has been reported by [2] and [30] in the Red Sokoto goats and some Indian goat breeds  
 118 respectively. The predominance of this allele likely confers certain selective advantage on milk traits in  
 119 goats. In this report, lactoferrin locus was found to exhibit polymorphism with allele A occurring at a higher  
 120 frequency. However, using a genome wide approach (PCR-SSCP) [30Guo et al 2010] reported three  
 121 genotypes: GG, AA and GA in some dairy goat breeds. Variants of this protein has also been reported in  
 122 Holstein-Friesian cattle breeds [32]. Reports on the effect of milk protein types on milk traits in goat  
 123 breeds are scanty however several studies on milk protein polymorphism and their association with milk  
 124 fat and mastitis in cows have been reported [Hristov33, Li et al 2004 34].

125 There were no significant associations between the genetic variants observed in this population and milk  
 126 fat content. This may be due to the sample size of the population used in the present study. However, in a  
 127 study on the effect of lactalbumin on milk production traits in two breeds of water buffalo, Dayal et al 2006  
 128 reported significant association between genotype BC and total and daily milk yield in Bhadawari breed.  
 129 In the Bhadawari, animals with genotype BC had 40% more total lactation yield than the those with AB  
 130 genotype.

131 The mean heterozygosity values of 0.4527 and 0.4978 obtained at the lactalbumin and lactoferrin locus  
 132 respectively suggests significant genetic variation in the population sampled. The population under study  
 133 is in Hardy-Weinberg's equilibrium with respect to the two loci investigated. This may be due to the  
 134 constant gene flow within the sampled population

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#### 4. CONCLUSION

The present investigation revealed that  $\alpha$ -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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