

Original Research Article**Preliminary Analysis on the Genetic Variants of Some Whey Proteins and Their Relationship with Milkfat in West African Dwarf Goat****ABSTRACT**

Milk samples of 40 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.

Keywords: Goat, Milk, Lactalbumin, Lactoferrin, Polymorphism

1. INTRODUCTION

Milk proteins secreted by mammary epithelial cells contain mainly caseins, whey proteins (α -Lactalbumin, lactoglobulin, serum albumin, immunoglobulin, lactoferrin, transferrin) and enzymes [1, 2]. Since the initial 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. 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 [8]. 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 [9] About 85% of the smallholder farmers in the Nigerian subhumid zone keep West African Dwarf (WAD) goats [10]. 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 [11]. Goat milk provide essential nutrients in human diets and is greatly valued by those who have cow milk allergy and other nutritional diseases [12]

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. MATERIAL AND METHODS

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47 Milk samples were collected from 40 WAD ewes of second parity; animals were sampled from 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 [13]. 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 [14] and [15]

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.

59

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 [16]

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; μ was population mean;

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

69 β_j was the fixed effect of Lactoferrin genotype;

70 e_{ijk} was random residual effect.

71 All data were analyzed with [17] software package. Means were separated using Duncan Multiple Range
72 Test (DMRT) of the same software.

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74 3. RESULTS

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

84

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

Locus	Genotypic frequency			Allelic frequency	
	AA	AB	BB	A	B
α-Lactalbumin	0.425	0.450	0.125	0.650	0.350
Lactoferrin	0.275	0.575	0.150	0.563	0.437

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89 **Table 2: Means and standard error values of the milk fat percentage for the different genotypes of**
 90 **α -Lactalbumin and Lactoferrin in WAD does.**

Locus	Genotype	Sample size	Milk fat content
α -Lactalbumin	AA	17	2.52±0.85 ^a
	AB	18	2.88±0.88 ^a
	BB	5	2.44±0.70 ^a
Lactoferrin	AA	11	2.42±0.81 ^a
	AB	23	2.86±0.89 ^a
	BB	6	2.41±0.63 ^a

91 *P*= .05

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94 4. DISCUSSION

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96 Goat milk exceeds cow milk in monounsaturated, polyunsaturated fatty acids and medium chain
 97 triglycerides and is known to be beneficial for human health, especially for cardio-vascular conditions [18].
 98 There has been considerable interest in goat milk protein due to availability of different bioactive peptide
 99 derived from milk proteins, which have pharmaceutical and nutraceutical applications and may have
 100 future commercial importance [19,20]. While α -Lactalbumin and lactoferrin are mainly found in the whey,
 101 their association with/influence on other milk constituents have been reported [21,22] α -Lactalbumin,
 102 alpha (LALBA) plays a key role in the biosynthesis of lactose by regulating the substrate affinity of the
 103 lactose synthase complex and has also been reported to have a role in induction of cell growth inhibition
 104 or apoptosis in tumor cells and immature cells [23]. However, α -Lactalbumin knockout mice have been
 105 reported to produce highly viscous milk this is occasioned by the absence of lactose in the milk followed
 106 by marked elevation of protein and fat content [22]. Lactoferrin is an iron-binding glycoprotein that is
 107 closely related in structure to the iron-transport protein, transferrin. It has been reported to be an anti-
 108 infective agent as it activates the transcription of important immune-related genes in the small intestine
 109 and promotes systemic host immunity [24]

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111 The studies on protein polymorphism, which have been extensive over the recent years, indicate that milk
 112 proteins can be utilized in both breeding practice and milk processing [25,26]. Allele and genotypic
 113 frequencies observed in this study are similar to those described in literature for other goat breeds.
 114 Findings observed at the α -Lactalbumin locus revealed two alleles A and B with allele A being the most
 115 common as previously reported in Barbari and some Indian goat breeds [27, 28]. Monomorphism at this
 116 locus has been reported by [2] and [29] on some Indian goat breeds and in the Red Sokoto goats
 117 respectively. The predominance of this allele likely confer certain selective advantage on milk traits in
 118 goats. Reports on lactoferrin protein variants in goat breeds are scanty. In this report the locus was found
 119 to exhibit polymorphism with allele A occurring at a higher frequency. Variants of this protein has also
 120 been reported in Holstein-Friesian cattle breeds [30]. Reports on the effect of milk protein types on milk
 121 traits in goat breeds are scanty however several studies on milk protein polymorphism and their
 122 association with milk fat in cows have been reported.

123 There were no significant associations between the genetic variants observed in this population and milk
 124 fat content. This is consistent with the findings of [31] and [32] that milk protein polymorphism had no
 125 significant effect on milk production traits.

126 The mean heterozygosity of the population was 0.4786 suggesting significant genetic variation in the
 127 population sampled. The population under study is in Hardy-Weinberg's equilibrium with respect to the
 128 two loci investigated. This may be due to the constant gene flow within the population since the animals
 129 sampled from one location.

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133 **4. CONCLUSION**

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

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141 **ETHICAL APPROVAL (WHERE EVER APPLICABLE)**

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143 All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised
144 1985) were followed, as well as specific national laws where applicable. All experiments have been
145 examined and approved by the appropriate ethics committee"

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