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IS IT POSSIBLE TO OBTAIN ZERO ESTIMATES OF GENETIC DIVERSITY? A CASE STUDY OF THE NIGERIAN INDIGENEOUS GOAT BREEDS AT THE *β*-LACTOGLOBULIN GENE LOCUS

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Abstract: The current investigation was conducted to appraise the genetic diversity and genetic distance of three goat populations namely; Red Sokoto, Sahel and West African Dwarf (WAD), in Nigeria, making use of blood samples collected from 20, 20 and 20 individual from which blood DNAs were extraction, respectively. The DNAs extracted were used to study polymorphism at the β-lactoglobulin gene locus using RLFP-PCR process. Results revealed that the mean total number of alleles was 1 while the effective number of alleles was also 1. The percentage of polymorphic locus was 0% while Shannon's information index, observed homozygousity, expected heterozygosity, unbiased expected heterozygosity and inbreeding coefficient (F) were all observed to be 0.000. The pairwise Fst was 0.000 between all the breeds of goats. Variation within and between the populations of goats was 0% at p>0.05. The genetic distance between the goat breeds was 0.000. The present study revealed that RLFP-PCR may not be a powerful tool for the study of the β-lactoglobulin gene locus and hence other methodologies should be employed for a broader judgment on the genetic status of the goat population at the locus.

Key words: *β*-lactoglobulin, RLFP-PCR, genetic diversity, Nigerian indigenous goats and DNA.

Introduction

All over the world, indigenous small ruminant breeds such as goats are playing key roles in the lives of people *(Pollot and Wilson, 2009)*. In most economically emergent countries Nigeria inclusive, no serious consideration is given to sheep and goat genetic assets management policies *(Wilson, 1990)*.

Inadequacy or lack of these kinds of policies in most cases, have led to reduced outputs, unsystematic copulation and decline of genetic differences *(Kosgey et al., 2006; Groenevald et al., 2010)*. As a result of the inadequate national breeding programmes, animal genetic diversity records are inadequate in most developing countries *(Guimarães et al., 2007; FAO 2008)*.

The goat is a domesticated subspecies of the wild goat originating from Eastern Europe and southwest Asia (Hirst, 2008). It belongs to the lineage *Bovidae* and is very much interrelated to the sheep as they belong to the subfamily *Caprinae*. Food and Agricultural Organization *(FAOSTAT, 2008)* reported that there are 861.9 million goats worldwide out of which 33.8% are found in Africa *(FAOSTAT, 2008)*. Goats have specific weight ranges for each recognized breed which varies from 20 to 27kg for smaller goat does to over 140kg for males of bigger breeds for instance the Boer *(Taylor and Field, 1999)*. The different strains or bloodlines within the breeds also have diverse established sizes. Naturally, most goats have two horns which come in various sizes and shapes being breed dependent.

β-lactoglobulin is a protein found in mammals with the exception of the humans, rodent and lagomorphs milks. In mature cattle milk having a concentration of 3.2g/l, *β*-lactoglobulin represents roughly 10% of the combined milk proteins, and roughly 50-60% of the combined whey proteins. It is the most copious protein existing in the whey portion of sheep, goat and cattle milk *(Hinz et al., 2012)*. Owing to its great quantity and comparative simplicity of cleansing, bovine *β*-lactoglobulin has served as a brand protein for numerous biophysical studies of folding, stability and self-association. *β*-lactoglobulin is in the lipocalin family of proteins. The earliest reported atomic level resolution composition of *β*-lactoglobulin solved by X-ray crystallography for bovine *β*-lactoglobulin *(Papiz et al., 1986)*, revealed extraordinary resemblance to retinol-binding protein and led to the categorization of *β*-lactoglobulin as a lipocalin.

More than two heritable variants of *β*-lactoglobulin are recognized over the years with the most common variants labelled A and B *(Godovac-Zimmermann et al. 1996)*. These variants have identical amount of amino acids (162), but vary at positions 64 and 118 in two amino acids. At position 64 and 118, variant A has an aspartic acid residue and valine residue in that order whereas variant B has glycine in location 64 and alanine in location 118. The make up of *β*-lactoglobulin is composed of 15% α-helix, 50% β-sheet, and 15-20% reverse turn *(Sawyer and Kontopidis, 2000)*. Under physiological circumstances, *β*-lactoglobulin is in equilibrium amid monomers and non-covalent dimers. Protein concentration, pH, ionic strength, and temperature all have an effect on this equilibrium and as a result, the amount of monomers and non-covalent dimers in solution *(Mercadante et al., 2012)*. In spite of the structural similarities between variants A and B, quite a lot of dissimilarities occur in their physical and chemical properties especially as it relates to isoelectric point *(Yan et al., 2013)*, stability of native dimers *(Mercadante*

et al., 2012), thermal denaturation temperature *(Manderson et al., 1999),* denaturation reaction rate *(O'kennedy et al., 2006),* vulnerability to chemicals *(Bouhallab et al., 2004; Boye et al., 2004),* and attraction for fatty acids *(Loch et al., 2013).*

β-lactoglobulin unlike the other main whey protein of milk; α-lactalbumin, has no clear cut function. However, it is one of the whey proteins that has been most evaluated and is known to connect hydrophobic ligands like fatty acids or vitamins, suggesting a responsibility in the conveyance of retinol from the mother to the neonate in view of the fact that it is homologous with serum retinol-binding protein, and its capacity to bind retinol *in vitro (Puyol et al. 1991). McMeekin et al. (1949), Lišková et al. (2011)* and *Le Maux et al. (2012)* had all reported on the binding of sodium dodecyl sulfate (SDS) to native *β*-lactoglobulin. *β*-lactoglobulin has also been shown to demonstrate an ability to bind other hydrophobic ligands such as cholesterol, curcumin, fatty acid in addition to their derivatives, aromatic compounds, catechin, and cations. *β*-lactoglobulin has been shown to be able to bind iron *(Roth-Walter et al., 2014),* and thus might have a role in combating pathogens. The natural functions of the complicated protein/ligand are still exploratory. Assumed roles could be an enhancement in fatty acid assimilation *(Solène Le et al., 2014),* adjustment of the kinetics of the enzymatic hydrolysis of protein *(Mandalari et al. 2009),* safeguarding of susceptible ligands to counter oxidation or additional stresses *(Solène Le et al., 2014),* and adjustment of the bioaccessibility of the ligands *(Riihimäki-Lampén, 2009).* Furthermore, in food produce, the binding and biological properties of *β*-lactoglobulin /ligand complexes possibly will be affected by the constitution of *β*-lactoglobulin, and/or the occurrence of additional proteins with the capacity of contending with *β*-lactoglobulin for ligand binding.

Ballister et al. (2005) amplified and sequenced the leading six exons containing the whole coding section of the *β*-lactoglobulin gene in eleven goat breeds of Spain, France, Italy, Switzerland, Senegal and Asia in a bid to ascertain the different genetic variants. Recent studies on *β*-lactoglobulin polymorphism have also been carried out on Egyptian goat breeds; Barki, Damascus and their crossbred *(El-Hanafy et al., 2010)* and indigenous Ardi, Habsi and Harri goats of Saudi-Arabia *(El-Hanafy et al., 2015),* Honamli, Hair and Saanen goats of Turkey *(Özgecan et al., 2012),* and the small east African goat breeds; Samburu and Narok *(Lekerpes et al., 2014).* There is however a dearth of literature on similar works using the Nigerian indigenous goat breeds. This may have been brought about by the non-involvement in biotechnologically oriented researches as a result of their high cost, inadequate governmental incentives on research and general lack of interest *(Udeh, 2015).* This work was therefore embarked upon to provide information on the genetic diversity at the *β*-lactoglobulin gene locus of indigenous Nigerian goat breeds using RFLP- PCR methods.

Materials and Methods

Animals used for the experiment and their brief description

Blood samples were collected from 6O goats belonging to three different breeds (20 each from Sahel, Red Sokoto and West African Dwarf). The animals were sampled at two locations in Nigeria namely; Ibadan where the West African Dwarf goats were sampled, and the National Animal Production Research Institute (NAPRI), Shika, Zaria, Nigeria where the other three breeds were sampled. The WAD goat is evolved from the short-legged goats and is well adapted to the humid tropical conditions after many years of adaptation and natural selection *(Leak et al., 2002)*. The goats are predominantly raised under backyard systems. The Sahelian goat derives its name from the Sahel region of Africa and is most suited to desert or semi arid environments; it is intolerant of areas with high humidity. Populations of this goat breed can also be found elsewhere in the world in areas which provide suitable environments such as Australia *(Deneice, 2011)*. The Sokoto Red goat falls within the savannah group of goat with a somewhat diminutive size which infers probable cross breeding with forest or dwarf goats prior to selection in its current locale *(DAGRIS, 2007)*. The Red Sokoto goat is the predominant indigenous breed in the northern part of the country *(Onyenwe et al., 2005)*.

DNA extraction and RAPD-polymerase chain reaction

DNA was extracted from whole blood using a ZYMOBeadTM Genomic DNA kit (ZYMO Research Corporation). β-Lactoglobulin genotypes were identified as described by *Feligini et al. (1998)* and *Anton et al. (1999)*. In the first step, the 120 bp fragment of the goat β-lactoglobulin gene was amplified using forward primer 5-CAACTCAAGGTCCCTCTCCA-3 and reverse primer 5-CTTCAGCTCC TCCAGGTACA-3. PCR amplifications was performed in a reaction mixtures of 25 μL containing 12.5 μL of 2× PCR Master Mix (ZymoBIOMICS™ PCR PreMix), 0.5 μM of each primer, and 25-75 ng genomic DNA. Amplification was performed in a Biologix Thermal Cycler (TC1000-G), programmed for an initial denaturation at 95° C for 10 minutes, followed by 35 cycles each with denaturing at 93° C for 15 seconds, annealing at 60° C for 30 seconds, extension at 72° C for 30 seconds, and a final extension at 72° C for 10 minutes. In the second step, the 105 bp fragment of the goat β-lactoglobulin gene was amplified using forward primer 5-TCAGGACCCCGGAGGTGGACAAC-3 and reverse primer 5-CCTCCAGCTGGGTCGGGTTGAAG-3. The cycling programme began with an initial denaturation step (1 min at 94° C) followed by 30 cycles consisting of 15 seconds at 94° C, 1 minute at 60° C, 10 seconds at 72° C, and final elongation for 10 minutes at 72° C. The same PCR reaction mixtures used

in the first step was used for amplification. In both cases, PCR products (12 μL) were digested with 8 U of *Ras*a and 10 U of Mspl restriction enzyme in a 20 μL total reaction volume for 2 hours at 37° C. The restriction fragments was directly analyzed by electrophoresis using a 3 % agarose gel in 1× TAE buffer, stained with ethidium bromide, and visualized under Ultra Violet (UV) light to detect amplification.

Statistical analysis

The measurement of genetic diversity including number of alleles (Na), effective number of allele (Ne), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygosity (uHe), Shannon's information index (I), % polymorphic locus, fixation index (F), Analysis of Molecular Variance (AMOVA) and Nei's unbiased genetic distance *(Nei, 1978)* was estimated using GenAlEx 6.2 software *(Peakall and Smouse, 2008)*.

Results

Genetic diversity of β-lactoglobulin gene locus across Sahel, Red Sokoto and West African Dwarf goat

The various parameters of genetic differentiation at the β-lactoglobulin gene locus of indigenous Nigerian goats such as number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygozity (uHe), Shannon's information index (I) and fixation index (F) are presented in Table 1. The entire genetic diversity parameters analyzed resulted in zero (0) values, except the number of alleles (1).

Table 1. Genetic differentiation at the *β*-lactoglobulin Locus of three Nigerian goat breeds

Goat population	N	Na	Ne	I	Ho	He	uHe	F	%P
Sahel	20	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WAD	20	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Red Sokoto	20	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	20	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

SE = standard error, Na = number of alleles, Ne = number of effective alleles, I – Shannon's information index, Ho = observed heterozygosity, He = expected heterozygosity, uHe = unbiased expected heterozygosity, F = fixation index, %P = percentage of polymorphic locus, WAD = West African Dwarf.

The pairwise F-statistics (Fst) values measured for the three breeds of goat are presented in Table 2. The Fst value showed zero level genetic differentiation among the breeds. It was 0.00 between all the breeds of goats.

Table 2. Pairwise Fst values presented for different goat breeds

	Sahel goat	WAD goat	Red Sokoto goat
Sahel goat	0.00		
WAD goat	0.00	0.00	
Red Sokoto goat	0.00	0.00	0.00

Variation within and between populations of goat breeds was estimated using AMOVA (Table 3). The results revealed that no proportion (0 %) of the observed variance (0.00 at $p>0.01$) occurred both within the breeds and between the breeds.

Table 3. Summary of AMOVA table showing variation within and between goat populations

Source of variation	df	SS	MS	Estimate of variance component	% variation	P-value
Among population	2	1.172	0.00	0.00	0.00	0.00
Among individual	57	16.144	0.00	0.00	0.00	
Within animal	60	19.000	0.00	0.00	0.00	0.00
Total	119	36.316		0.00	0.00	

Genetic distance and identity of the three goat populations studied

The genetic distance of the indigenous goats is shown in Table 4. The result revealed zero (0) values for genetic distance between all the three goats examined. The genetic distance between the goat breeds indicated no genetic similarity or differences between the goats breeds studied. No cluster analysis based on Nei's standard distance matrix was obtained due to the monomorphic nature of alleles.

Table 4. Pairwise population matrix of Nei genetic identity and Nei genetic distance of the three goat breeds

	Sahel goat	WAD goat	Red Sokoto goat
Sahel goat	1	0.00	0.00
WAD goat	0.00	1	0.00
Red Sokoto goat	0.00	0.00	1

Discussion

The observed number of alleles (Na) at a locus and the genetic distance values (Table 1) indicate genetic differences at that locus and this will suggest the suitability of the locus to be used for the genetic analysis of diversity in goats. The observed number of alleles in this study suggests that the locus is not suitable for the genetic analysis of the three Nigerian goats; or, the methodology used might not have been appropriate particularly at the *β*-lactoglobulin gene locus. *Barker et al. (2001)* had opined that for studies of genetic diversity and genetic distance inside and between populations, microsatellite markers ought to have at least four alleles which might assist in reducing errors of estimation. This was not the situation in the present study and probably explains the results obtained. The effective number of alleles (Ne) was 1. The locus used to analyse the diversity in the Nigerian indigenous goats was not greatly enlightening making it not to be effective in genetic diversity studies. This is attributable to the actuality that if a locus has a PIC estimate < 0.5, that locus is said to be not polymorphic; a locus with PIC estimates ranging from 0.25 to 0.5 is said to be an average polymorphic marker *(Vanhala et al., 1998)*. In the present study, the locus was not polymorphic at all.

The observed mean heterozygosity value (0.00) was similar to the expected mean heterozygosity for all the investigated goats. This is in total disagreement with earlier reports on the domestic goat breeds *(Barker et al., 2001; Behl et al., 2003; Kumar et al., 2005; Aggarwal et al., 2007; Dixit et al., 2008; Kumar et al., 2009; Hassen et al., 2012; Hassen et al., 2016)*. Clearly, extensive bio-ecological selection for acclimatization to the different climatic conditions of the country and the existence of interbreeding due to free movement of animals for grazing and other purposes have not contributed to a large extent to the genetic diversity of the goats at the studied locus.

The population of goats used in this study was observed to be genetically similar (absence of genetic diversity) at the *β*-lactoglobulin gene locus (He = 0). This might be due to the monomorphic nature of the *β*-lactoglobulin gene allele at the gene locus. The high degree of similarity (no genetic distance) at the *β*-lactoglobulin locus encourages complete panmixis; that is, the three populations are interbreeding freely (complete sharing of genetic material). Genetic diversity is a measure of the degree of heterozygosity and allelic variation in a population. A low level of heterozygosity as observed in the present study may lead to genetic similarity (loss of genetic diversity). This zero level of heterozygosity can be caused by the occurrence of null allele which is the allele that fails to proliferate during polymerase chain reaction with a given genetic marker primers site *(Pemberton et al., 1995)*. When the sample size is not large enough, the Wahlund effect (the incidence of lesser quantity of heterozygote in the population than is expected because of subdivision of the population), and inbreeding *(Kumar et al.,*

2006) could also lead to this situation. Low degree of heterozygosity might also be expected if species are isolated with consequent deficit of unexploited genetic capability, while a high level of mean heterozygosity at a locus possibly will be expected to associate with high levels of genetic variation at locus with significant value for adaptive response to environmental changes *(Kotzé and Muller, 1994)*.

The Shannon information index revealed no genetic diversity across the populations. The Shannon information index of the Nigerian goat breeds is much lower than the 3.5 set for high species evenness and richness *(Krebs, 1989)*. This means that the goats have very low species richness and evenness at the *β*-lactoglobulin gene locus. This low species richness could be associated with very high level of heterozygote deficiency among the goats population sampled and this could be ascribed to the system of management in use by the goats farmers *(Mukesh et al., 2006)*. The mean Shannon's index (I) of 0.00 is an indication that equitability in the genetic distribution of the Nigerian indigenous goat breeds has been seriously disturbed and eroded hence exhibiting very low genetic diversity. Low amount of genetic diversity has been reported to increase susceptibility of populations to devastating situations like disease and pest outbreaks, and also the expression of negative and disadvantageous alleles or even, loss of over-dominance *(Bizhan et al., 2010)*.

The populations' genetic differentiation was studied base on fixation indice (F). The within breed deficit or excess in heterozygosity value was assessed by the inbreeding coefficients which was 0.00. The locus having positive values (even though zero), indicates that the goat population is just at equilibrium with there being no more than expected number of homozygotes or heterozygotes in the goat breeds studied. The average F revealed that the majority of the overall genetic variation did not correspond to any difference between individuals within goat populations. Elevated F values means that there is significant extent of inbreeding and genetic differentiation between goat populations. The goat populations studied are almost slipping into that. According to *Wang (1996)*, estimates of inbreeding of less than 0.5 or nearer to zero may have occurred because of the absence of mating among close relations and/or within individuals. The values obtained in this study at the locus studied reflect the presence of close relative matings. The results therefore disagrees with those observed for Asian goats *(Barker et al., 2001)*, Indian goats *(Kumar et al., 2005; Aggarwal et al., 2007; Dixit et al., 2008; Dixit et al., 2010)* and indigenous goats of Albanian *(Hoda et al., 2011)*.

The analysis of molecular variance revealed that 0% of all the dissimilarity was present amongst and within the goat populations making the result to be at variance with the partitioning of variance reported *(Vahidi et al., 2014)*. Although the Nigerian goat population's genetic dissimilarity was computed using molecular data, no genetic distance was really observed between the three goats at the locus studied. This made it impossible to generate or create a phylogenetic tree.

Conclusion

The result of this study probably describes the first endeavour to evaluate the molecular genetic differences of the Nigerian indigenous goat populations at the *β*-lactoglobulin locus. The results reveal the presence of very low or no genetic difference among the goat population at the *β*-lactoglobulin locus and such low or zero variation within breed will not provide an excellent basis for designing genetic improvement programme.

Da li je moguće dobiti nultu procenu genetičke raznovrsnosti? Studija slučaja nigerijskih autohtonih rasa koza na lokusu gena β-laktoglobulinskog

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Rezime

Ispitivanje je sprovedeno radi procene genetičke raznovrsnosti i genetske udaljenosti kod tri populacije koza: Red Sokoto, Sahel i zapadno-afričke patuljaste rase (West African Dvarf WAD), u Nigeriji, koristeći uzorke krvi prikupljene od 20, 20 i 20 pojedinačnih grla iz kojih su krvne DNK ekstrahovane, respektivno. DNK ekstrahovana korišćena je za ispitivanje polimorfizma na lokusu β-laktoglobulinskog gena koristeći RLFP-PCR proces. Rezultati pokazuju da je srednji ukupan broj alela bio 1, dok je efektivni broj alela takođe bio 1. Procenat polimorfnog lokusa bio je 0%, dok je Shannonov indeks informacija registrovao homozigotnost, očekivana heterozigotnost, nepristrasnu očekivanu heterozigotnost i koeficijent inbridinga (F), svi na 0.000. Parni Fst je bio 0.000 između svih vrsta koza. Varijacija unutar i između populacije koza iznosila je 0% kod p> 0,05. Genetska udaljenost između rasa koza iznosila je 0.000. Ova studija je otkrila da RLFP-PCR možda nije moćan alat za proučavanje lokusa β-laktoglobulinskog gena i stoga bi trebalo koristiti druge metodologije za širu procenu genetičkog statusa populacije koza na lokusu.

Ključne reči: β-laktoglobulin, RLFP-PCR, genetička raznovrsnost, nigerijske autohtone koze, DNK.

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