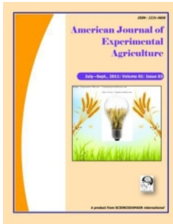

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Prevalence of Coat Colour Phenotypes in West African Dwarf Sheep Reared by Small Holder Farmers in South Western Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. The MNB designed the study, performed part of the statistical analysis and wrote the protocol and the first draft of the manuscript. ASA also managed part of the analysis; other authors listed equally participated in the survey and data collection. All authors participated in literature searches, read and approved the final manuscript.

Research Article

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ABSTRACT

The West African Dwarf (WAD) sheep reared extensively by small holder farmers in South Western Nigeria has not been selected based on preference for coat colour, which varies considerably but without adequate information on the current distribution. The prevalence of different coat colour phenotypes was therefore investigated based on a total randomly sampled size of 9, 195 sheep (5,978 females and 3,217 males) from February to October, 2011. A total of 17 different colour patterns including 3 solid colours (white, black and brown) were identified in the breed. Phenotypes mainly influenced by phaeomelanin pigment, considered to be governed by a dominant allele (A^{wt}) include: predominantly white with black marking, solid white, white and black in approximately equal proportions, spotted white, white and brown in approximately equal proportions, buckskin, tan, badgerface and grey. The remaining phenotypes (black with white marking, brown, black, spotted black, spotted brown, black and brown, brown with white marking and Swiss marking) reflected the presence of eumelanin pigment governed by A^a recessive allele. Based on the two

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pigment types, 71.58% and 28.42% of sheep had coat colour predominated by phaeomelanin and eumelanin pigments, respectively. Result of chi square (X^2) analysis showed that the difference between observed and expected frequencies was significant (*P*=.05) implying that there was a deviation from the expected 3:1 Mendelian ratio. Conscious selection could be embarked upon to investigate and conserve phenotypes that are more genetically superior in terms of productivity and adaptation.

Keywords: Coat colour; distribution; West African Dwarf sheep.

1. INTRODUCTION

The WAD sheep is a trypanotolerant species of small ruminants reared by small holder farmers in South Western Nigeria. The National Population Commission (2004) puts the population of sheep in Nigeria at 29.0 million. The WAD sheep are the predominant sheep breed of the humid tropics. Adult males weigh approximately 37 kg and are horned. Ewes are polled and have mature weight of 25 kg. They can be bred at age of 7 to 8 months. They tend to have a short lambing interval with prolificacy of adult ewes ranging from 1.15 to 1.50 lambs per lambing season (Mason, 1988; Thomas, 1991). Phenotypically, the WAD sheep exhibit great variation in conformation, coat colour, size, height, length and size of tail, presence or absence of horns and their shape and behavior patterns, among others (Ngere, 2002). Qualitative traits have been studied as possible indicators of genetic superiority or adaptability with more emphasis on dwarf goats (Odubote, 1994b; Ebozoje and Ikeobi, 1998; Ozoje and Mgbere, 2002; Adedeji et al., 2011). Early discovery by Odubote (1994b) working with WAD goat indicated that coat colour is very variable and irregular, including black, brown, pied and mixed colours. Basic black colour predominated (53.3%) while basic white and brown goats accounted for 6.8% and 39.9% respectively. Review by Kine (2005) showed that dark-coloured animals of various livestock species grow faster in the tropics and subtropics and survival and growth are less in lighter coloured animals, which seem to have increased milk yield (Kine, 2005). Oke and Ogbonnaya (2011) however reported insignificant differences in live weight and heart girth among different colour groups even though black sheep had the highest values for these traits.

The genetic basis of coat colour inheritance has been unraveled by several studies (Odubote, 1984b; Adalsteinsson et al., 1994; Sponenberg, 1995, Ozoje, 1998; Saldaña-Muñoz et al., 2004; Nadeau et al., 2007; Norris and Whan, 2008; Fontanesi et al., 2010; Ren et al., 2011). According to Adalsteinsson et al. (1994) and Sponenberg (1995) colour is due to melanin deposits in the hair which comes in two basic types-eumelanin and phaeomelanin. Eumelanin is usually black, but sometimes brown. It is the pigment responsible for black and brown areas on goats, or rarely for dusky blue colour. Black wool has long been known to be due a recessive gene, with heterozygotes for the recessive allele indistinguishable from the dominant homozygotes. Norris and Whan (2008) identified a 190-kb tandem duplication encompassing the ovine agouti signaling protein (ASIP), the AHCY coding regions and the ICTH promoter region as the genetic cause of the dominant white/tan (A^{wt}) agouti sheep. Alternative splicing of ASIP transcripts showed expression in multiple tissues of white sheep and lack expression of single copy allele in black sheep. Tyrosinase-related protein 1 (TYRP1) has also been shown to have a role in pigmentation in birds (Nadeau et al., 2007), pig (Fontanesi et al., 2010) and sheep (Ren et al., 2011). Mutation screens of sequence variants in the coding region of TYRP1 by the latter authors revealed a strong candidate causative mutation, the protein-altering deletion showing complete association with the brown colouration across Chinese-Tibetan, Kele and Dahe breeds by occurring exclusively in brown pigs and lacking in all non-brown-coated pigs from 27 different breeds. The findings provided the compelling evidence that brown colours in Chinese indigenous pigs are caused by the same ancestral mutation in TYRP1. Fontanesi et al. (2010) further revealed that two coat colour types segregate in Massese sheep: black and grey. Mutations in the agouti signaling protein and melanocortin 1 receptor (MC1R) genes are associated with these two coat colour types in this breed, even if other genetic factors could be involved.

The WAD sheep has been characterized for most qualitative and quantitative traits but more current information on prevalence of various colour types in the population is not available, hence the need for this study.

2. MATERIALS AND METHODS

2.1 Location of Study and Management of Animals

Data obtained for this study was randomly sampled from five Local Government Areas (LGAs) of Ogun State in South Western Nigeria. They are Abeokuta North (30 villages), Abeokuta South (15 villages), Odeda (22 villages), Ewekoro (18 villages) and Obafemi-Owode (31 villages). The number of villages randomly sampled was proportional to the total number of villages present in each Local Government Area. The region is about 288 meters above sea level and falls within latitudes 6º54' - 7º54' N and longitudes 3º11' - 3º53'E. The climate is humid and located in the Derived Savanna vegetation zone of South Western Nigeria. It receives a mean annual precipitation of 1,037 mm distributed through late February to October, with a mean annual temperature of 34.7ºC. Relative humidity averages 82% throughout the year thereby favoring growth of lush vegetation. Most of the villages under study are inhabited by subsistence farmers working under the supervision of the Ogun State Agricultural and Rural Development Programme (OGADEP) extension officers. Animals were commonly maintained on free range and minimal housing (open shed or holding area with roof) provided to serve as shelter. Sheep were fed with kitchen wastes, cassava peels or corn chaff in the morning before being left to roam the surroundings to forage for grasses and browse plants. Based on information obtained from the farmers, they do not have preference for specific coat colours thereby lowering the chance of artificial selection.

2.2 Survey of Animals and Data Collection

Population survey of WAD sheep was carried out from March to October, 2011. Information on a total number of 9,195 sheep consisting of 5,978 females and 3, 217 males were obtained from randomly sampled villages. Within the traditional setting, more females than males are retained for reproductive purpose while more males are sold for the meat, hence the wide variation in number. Geographical Positioning System (GPS) navigator was used to obtain the coordinates of the villages and communities sampled during the survey. Prior to the survey, pre- visit arrangements were made through the OGADEP extension officers to sensitize farmers on the visits so that they could get the animals confined at various locations. Various households were visited to undertake a head count of the animals and identify various coat colours with the aid of a coat colour chart (Waller, 2006). The WAD sheep has close similarity with the WAD goats in terms of coat colour variation. According to the coat colours, males and females were classified as predominantly white with black marking, solid white (undiluted), white and black in approximately equal proportions, predominantly black with white marking, spotted white, solid black (undiluted), brown (light to dark brown), white and brown in approximately equal proportions, spotted brown (had mostly white spots on brown surface in any proportion), spotted black (had mostly white spots on black surface in any proportion), black and brown in approximately equal proportions, predominantly brown with white marking, buckskin, tan, Swiss marking, badgerface and grey. The sheep were further classified into two groups based on phaeomelanic and eumelanic pigmentation as described by Machado et al. (2000). This method was adopted for the purpose of clarity and simplicity, since it has been observed that the underlying genetics of pigmentation is complex, exhibiting quantitative as well as qualitative features (Klungland and Vage, 2000). In the “Agouti (A) series” in WAD goats and sheep, the most dominant allele produces a sheep with only phaeomelanin pigment and no eumelanin. Such a goat or sheep, depending on the rest of its genetic makeup, is entirely white, red, yellow, tan or cream (with or without white spots), the most recessive allele produces a goat or sheep with only eumelanin pigment and no phaeomelanin. Such a goat or sheep, depending on the rest of its genetic constitution is entirely black or chocolate brown and blue-gray (with or without white spots). The B or brown gene only affects eumelanic (dark-pigmented) areas of the animal that are black or chocolate-brown. It does not affect phaeomelanic areas. The brown colouration is only found in areas of the coat that would otherwise be black and the brown is chocolate-brown, not yellow or red-brown (Waller, 2006).

2.3 Statistical Analysis

Microsoft excel was used as the database to summarize the data and obtain prevalence of various phenotypes expressed as a percentage of the total population under consideration. The frequency of the recessive alleles (A^a) was estimated based on Hardy-Weinberg principle (Falconer and Mackay, 1996) as shown below:

$$q = \sqrt{\frac{m}{M}}$$

Where,
q = frequency of the recessive gene
m = observed number of animals exhibiting the recessive trait
M = total number of animals sampled

The frequency of the dominant allele (A^{wt}), p was obtained by subtracting q from one. The observed frequencies were tested against the expected Mendelian ratio of 3:1 corresponding to values of 0.75 for the dominant allele and 0.25 for the recessive allele using Pearson’s chi-square test. The null hypothesis (H_O) stated was that the population is in Mendelian proportions while the alternative hypothesis (H_A) that the population is not in Mendelian proportions. The equation used to obtain the chi-square test for goodness of fit was

$$X^2 = \Sigma \frac{(\text{observed - expected})^2}{\text{Expected}}$$

The level of significance of the test was examined at *P*=.05 and one degree of freedom.

3. RESULTS AND DISCUSSION

The summary of actual number of animals based on coat colour phenotype and sex is presented in Table 1. Coat colour distribution in order of decreasing frequencies (Fig. 1) were: predominantly white with black marking (21.35%), solid white (20.90%), white and black in approximately equal proportions (15.92%), predominantly black with white markings (6.75%), spotted white (6.37%), solid black (5.64%), solid brown (5.61), white and brown in approximately equal proportions (5.08), spotted brown (2.64%), spotted black (2.46%), black and brown in approximately equal proportions (2.45%), predominantly brown with white marking (1.45%), buckskin (1.21%), tan (0.96%), wiss marking (0.83%), badgerface (0.37%) and grey (0.01%). The three solid colours identified were white (Fig. 2A), black (Fig. 2B) and brown (Fig. 2D). They are the basis for fourteen different colour combinations accounting for 67.85% in the breed. Among the different phenotypes, those mainly influenced by phaeomelanin pigment, considered to be governed by a dominant allele (A^{wt}) include: predominantly white with black marking (Fig. 2C), solid white, white and black in approximately equal proportions, spotted white, white and brown (Fig. 2G), buckskin, tan, badgerface (Fig. 2F) and grey.

Table 1. Coat colour distribution in West African Dwarf sheep

Coat colour	Predominant pigment	Hypothesized genotypes	Females	Males	Total
White with black Marking	Phaeomelanin	$A^{wt}A^{wt}$	1358	605	1963
White	Phaeomelanin	$A^{wt}A^{wt}$	1155	713	1868
White and black	Phaeomelanin	$A^{wt}A^{a}$	955	509	1464
Black with white Marking	Eumelanin	$A^{a}A^{a}$	486	189	675
Spotted white	Phaeomelanin	$A^{wt}A^{wt}$	398	188	586
Brown	Eumelanin	$A^{a}A^{a}$	332	184	516
Black	Eumelanin	$A^{a}A^{a}$	310	209	519
White and brown	Phaeomelanin	$A^{wt}A^{a}$	230	237	467
Spotted black	Eumelanin	$A^{a}A^{a}$	154	72	226
Spotted brown	Eumelanin	$A^{a}A^{a}$	151	92	243
Black and brown	Eumelanin	$A^{a}A^{a}$	145	80	225
Brown with white marking	Eumelanin	$A^{a}A^{a}$	93	40	133
Buckskin	Phaeomelanin	$A^{wt}A^{wt}$	81	30	111
Tan	Phaeomelanin	$A^{wt}A^{wt}$	64	24	88
Swiss marking	Eumelanin	$A^{a}A^{a}$	45	31	76
Badgerface	Phaeomelanin	$A^{b}A^{b}$	21	13	34
Grey	Phaeomelanin	$A^{wt}A^{wt}$	0	1	1
Total			5978	3217	9195

The remaining phenotypes reflected the presence of eumelanin pigment being governed by A^{a} allele which is recessive. Based on the two pigment types, 71.58% and 28.42% of sheep had coat colour dominated by phaeomelanin and eumelanin pigment respectively. Even though there has been no detailed report of this nature on the species under consideration, early discovery by Odubote (1994b) working with the WAD goat within the same region indicated that coat colour is very variable and irregular, including black, brown, pied and mixed colours. Three solid colours (white, black and brown) were similarly reported for the species. The striking difference with the current observation is that solid white dwarf sheep outnumbered the proportion (6.8%) reported for dwarf goats by Odubote (1994b) while the latter species had higher estimate (52.5%) for black compared with sheep. These differences could be associated with adaptive value of the phenotypes in the species which needs to be further investigated.

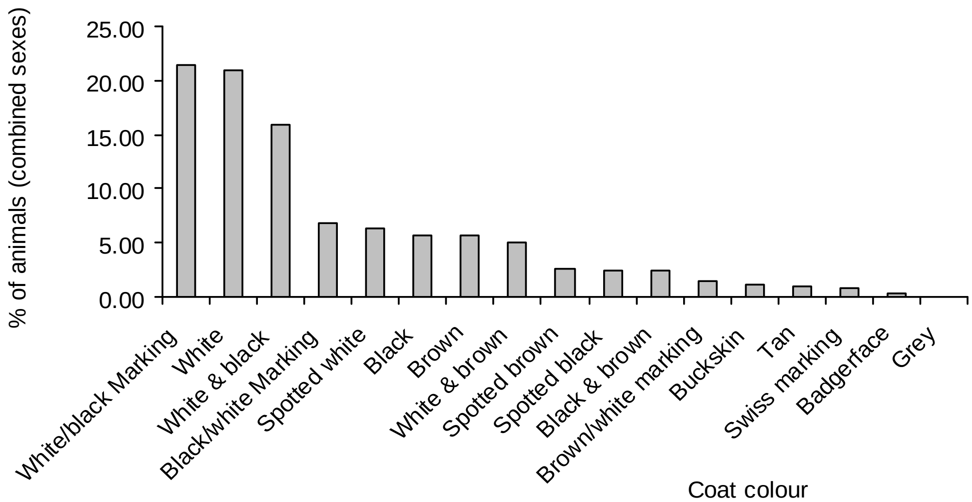

Fig. 1. Coat colour distribution in West African Dwarf sheep (combined sexes)

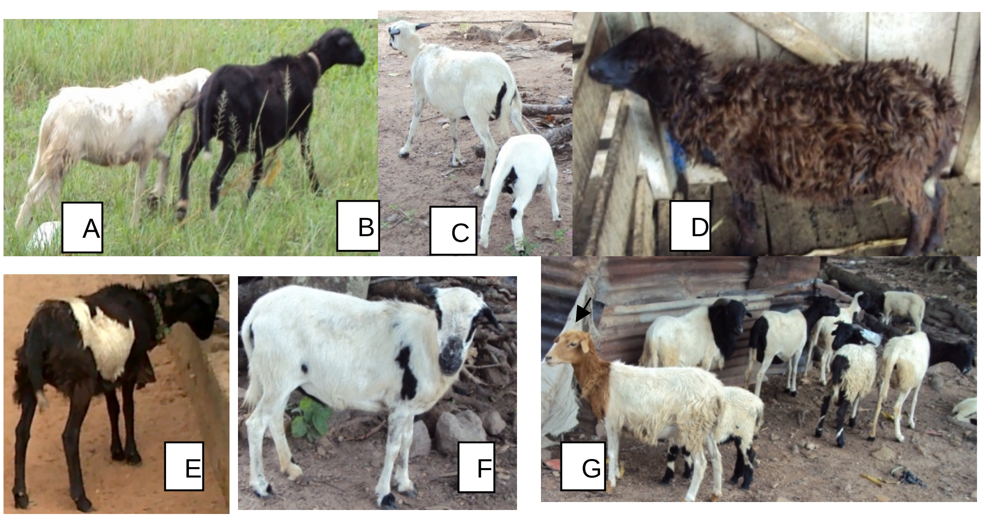

Fig. 2. Illustration of some coat colour patterns in sheep

A - dominant white ($A^{wt}A^{wt}$); B - black ($A^{a}A^{a}$); C - predominantly white with black marking ($A^{wt}A^{wt}$); D - brown ($A^{a}A^{a}$); E - predominantly black with white marking ($A^{a}A^{a}$); F – badgerface ($A^{b}A^{b}$); G - brown and white ($A^{a}A^{a}$)

Saldaña-Muñoz et al. (2004) working on coat colour inheritance in a commercial flock of Pelibuey (70%) and Blackbelly sheep (20%) as well as crosses among them classified the population as white, brown, spotted, blackbelly and black based on coat colours. Using brown Pelibuey rams to mate the ewes in the study, the results suggested that white and brown are co-dominant and both dominant to the other colours (similar to reports by Ponzoni (1992)); white and spotted are the same variable; blackbelly behaves as a heterozygote while black is a possible homozygote recessive and independent to white. These observations could in part provide a possible explanation for the highest frequencies recorded for white and predominantly white with black marking colour groups in this study. Moreover, the emergence of more colour combinations in the species could be attributed to the fact that farmers do not have preference for a specific coat colour leading to uncontrolled mating. The solid colours identified are in agreement with the colours (black, white and brown) recently identified by Oke and Ogbonnaya (2011) in the same breed and also agreeing with the solid and mixed colours (black, white, brown, badgerface, benzoar, Swiss markings, black with white marking and brown with white marking) recently identified by Adedeji et al. (2011) in goats. It is obvious that more colour combinations are possible with a larger sample size as reported in the current study.

Sponenberg (1995) also reported three basic colours (black, dark brown/mahogany and medium brown) in the pigmy goats. As solid colours, these are fairly rare in the breed, but they are the underlying base for the common caramel and agouti patterns, giving rise to basically nine colours within the Pygmy goat breed. These nine combinations are complicated by the addition of white spotting in some goats, which results in eighteen basic types, one spotted and one non-spotted. These observations corroborate most aspects of the current findings with the exception that white was the additional basic colour (besides black and brown) identified in the WAD goats. The inheritance pattern revealed by Sponenberg (1995) is such that most black-to-black matings produce black offspring. The author further noted that one pattern that can be superimposed over the basic colour is the agouti pattern which is the mixture of white hairs into the base coat colour. Caramel is the second major pattern called badger face after a similar sheep colour pattern consisting of a tan or cream body with dark belly, dark legs, dark marks on the face and a dark stripe down the back. The caramel pattern can be superimposed over the basic colours, and the result is caramels with black marks, dark brown marks, or medium brown marks. The mode of inheritance of coat colour is very complex as agreed by most authors (Ryder, 1980; Odubote, 1994b; Sponenberg, 1995; Saldaña-Muñoz et al., 2004). About four loci (A or agouti, B or brown, S or spotting and C or albino) have been identified in sheep (Ryder, 1980; Sponenberg, 1990) which interact to produce various phenotypes. More facts are emerging at the molecular level that a tandem duplication of a 190-kb portion of the ovine genome is responsible for the dominant white coat colour allele of domestic sheep (Norris and Whan, 2008). The authors further demonstrated that a single copy of ASIP with a silenced agouti signaling promoter occurs in recessive black sheep. The dominant white or tan (A^{wt}) ASIP allele is responsible for the phaeomelanic phenotype in modern sheep breeds, while the most recessive allele, non-agouti (A^{a}) results in eumelanic (black/brown) phenotypes as reviewed by the foregoing authors.

The result of chi square (X^2) analysis showed that the difference between observed and expected phenotypic frequencies was significant (P<.05) as shown in Table 2. The preponderant coat colour in WAD sheep tended towards phaeomelanic standard pigmentation. Different shades of colours are also attributed to variation in size, density and distribution of pigment granules (phaeomelanic, eumelanic and intermediate pigmentation) described by Machado et al. (2000). Based on the two pigment types, the result of chi square (X^2) analysis clearly indicated that there was a significant departure of the observed ratios from the expected 3:1 Mendelian ratio. Klungland and Vage (2000) noted that the underlying genetics of pigmentation is complex, exhibiting quantitative as well as qualitative features. It is possible that deviation from the expected ratio could also be due to epistatic effects of some loci which need to be verified. Independent loci controlling colour directly and structural features of the hair/wool coat also modify the phaeomelanic colours (Norris and Whan, 2008). The "A^{wt}" symbol was adopted for tan sheep because they are genetically white. It is possible that homozygotes (sheep possessing two copies of the A^{wt} gene) are white, while heterozygotes (sheep possessing one copy of the A^{wt} gene) are tan.

Table 2. Gene frequencies of coat colours in West African Dwarf sheep

Coat colour	Allele involved	Observed frequency	Expected Frequency	Gene frequency	X^2 test
Phaeomelanic standard pigmentation	A^{wt}	6582	6896.5	0.47	*
Eumelanic standard pigmentation	A^{a}	2613	2298.75	0.53	

**P < .05*

The preponderance of white could be a form of adaptive mechanism as white colouration is an advantage in an intense radiant environment due to its reflectance property. The apparent wide variation in coat colour is an indication that the WAD sheep populations have not been purified through impeccable selective breeding, therefore, great opportunities exist for their improvement.

4. CONCLUSION

This study derived its significance from the fact that it utilized a copious data set compared with previous studies to provide more detailed information on the current distribution of coat colours in West African Dwarf sheep arising from uncontrolled breeding over many years. Three basic coat colours were identified in the West African Dwarf sheep namely white, black and brown accounting for 20.90%, 5.64% and 5.61% respectively. They probably constituted the underlying base for the badgerface, agouti and spotting patterns, giving rise to fourteen colour combinations which accounted for the remaining 67.85% in the breed. There are practical implications of these for sheep breeders. Conscious selection could be embarked upon to investigate and conserve the phenotypes that are more genetically superior in terms of productivity and adaptation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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