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DNA CONCENTRATION AND QUALITY AMONG NIGERIAN SHEEP BREEDS

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ABSTRACT
The effect of breed on DNA concentration and quality as well as the relationship between DNA concentration and quality among Nigerian sheep breeds was assessed using Two hundred and sixty six (266) sheep from each of three main agro-ecological zones of Nigeria. The 266 sheep from each zone is comprised of 90 Balami, 40 Uda, 106 Yankasa and 30 West African Dwarf (WAD) breeds. DNA was extracted from 50µl of whole blood using a ZymoBead^{TM} Genomic DNA Kit according to the manufacturer's recommendations. Quantification of DNA yield was done using the Nanodrop ND-100 Spectrophotometer to determine DNA concentration and quality. The results revealed that there was no significant ($p>0.05$) effect of breed on DNA concentration among the sheep breeds studied. However, the highest mean concentration of 14.23µg/ml was observed in WAD. This was followed by Yankasa, Balami and lastly Uda with values of 12.64µg/ml, 12.56µg/ml and 10.52µg/ml respectively. However, a significant effect of breed on DNA quality was observed among the Nigerian sheep breeds used in this study. While Uda had a significantly ($p<0.05$) higher DNA quality (1.64) when compared to the other of Nigerian sheep breeds, Balami had the lowest quality (1.52). A Pearson correlation analysis revealed a negative, significant ($p<0.001$) but low ($r = -0.209^{**}$) relationship between DNA concentration and quality of the Nigerian sheep breeds. A similar trend was observed for the non parametric correlations. The results of this study have shown that there is no significant breed effect on the concentration of DNA; but a significant breed effect is seen in the quality of DNA across the Nigerian sheep breeds used in this study.

KEYWORDS: DNA concentration, quality, Nigerian, Sheep

INTRODUCTION

Sheep is one of the most important livestock breed in Nigeria for its many uses. More than one-third of the reported mammalian and avian breeds could not be classified because of a lack of information on population size and structure (Rischkowsky*et al*., 2006). This lack of information has led to various molecular researches on the Nigerian livestock breeds (*Adebamboet al*., 2004; Agaviezor*et al*., 2012; Okpeku*et al*., 2011; Abdulmojeed*et al*., 2012). Since, the basis of these researches in the DNA, more information is needed on the concentration and quality of DNA isolated from Nigerian sheep breeds. Thus, this study is directed at examining the variation in DNA concentration and quality among Nigerian sheep breeds.Deoxyribonucleic acid (DNA) quantification is normally performed to determine the average concentrations of DNA present in a mixture, as well as their purity. In DNA analyses, DNA is required in particular amounts and purity for optimum performance. There are several methods to establish the concentration of a solution of DNA. These include spectrophotometric quantification and UV fluorescence in presence of a DNA dye.DNA absorbs ultraviolet light in a specific pattern. In a spectrophotometer, a sample is exposed to ultraviolet light at 260 nm, and a photo-detector measures the light that passes through the sample. The more light absorbed by the sample, the higher the nucleic acid concentration in the sample. Beer Lambert Law can be used to relate the amount of light absorbed to the concentration of the absorbing molecule. At a wavelength of 260 nm, the average extinction coefficient for double-stranded DNA is 0.020 $(\mu g/ml)^{-1}$ cm^{-1}, for single-stranded DNA it is 0.027 $(\mu g/ml)^{-1}$ cm^{-1}, for single-stranded RNA it is 0.025 $(\mu g/ml)^{-1}$ cm^{-1} and for short single-stranded oligonucleotides it is dependent on the length and base composition. Thus, an optical density (or "OD") of 1 corresponds to a concentration of 50 µg/ml for double-stranded DNA. This method of calculation is valid for up to an OD of at least 2 (Sambrook and Russell, 2001). A more accurate extinction coefficient may be needed for oligonucleotides; these can be predicted using the nearest-neighbor model (Tataurov*et al*., 2008). The ratio of the absorbance at 260 and 280nm ($A_{260/280}$) is used to assess the purity of nucleic acids. For pure DNA, $A_{260/280}$ is ~1.8 and for pure RNA $A_{260/280}$is ~2.

MATERIALS & METHODS

Two hundred and sixty six (266) sheep from each of three main agro-ecological zones of Nigeria were used for this study. The 266 sheep from each zone is comprised of 90 Balami, 40 Uda, 106 Yankasa and 30 West African Dwarf (WAD) breeds. The sheep were sampled from selected cities and villages across the country. Seven ml of blood was collected into heparinized tubes from the jugular vein of the sheep, stored on ice before they were transferred to the laboratory for DNA isolation using ZymoBead^{TM} Genomic DNA Kit (Irvine, CA, USA) following the manufacturer's instructions.

DNA was extracted from 50µl of whole blood using a ZymoBead^{TM} Genomic DNA Kit according to the

manufacturer's recommendations. Quantification of DNA yield was done using the Nanodrop ND-100 Spectrophotometer to determine DNA concentration and quality. The ratio of the absorbance at 260 and 280nm ($A_{260/280}$) was used to assess the purity of the DNA. For pure DNA, $A_{260/280}$ is ~1.8.

RESULTS & DISCUSSION

The effect of breed on the DNA concentration and quality of Nigerian sheep breeds is shown in Table 1. There was no significant ($p>0.05$) effect of breed on DNA concentration and among the sheep breeds studied. However, the highest mean concentration of 14.23µg/mlwas observed in WAD. This was followed by Yankasa, Balami and lastly Uda with values of 12.64µg/ml, 12.56µg/ml and 10.52µg/ml respectively. Most variations in the concentration of DNA have been attributed to contamination and not breed effect. Sambrook and Russell (2001) reported variation in DNA concentration as a result in contamination with phenol. The widest range of concentration values was observed in Yankasa with values ranging from 1.00µg/ml to 93.00µg/ml and the least seen in Uda with values ranging from 6.10µg/ml to 20.80µg/ml. The trends in standard deviation and standard error are also represented in Table 1.

TABLE 1: Descriptive statistics of the effect of breed on DNA concentration and quality

		N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
DNA concentration	Balami	90	12.56	9.39	0.99	4.10	86.20
	Uda	40	10.52	3.00	0.47	6.10	20.80
	WAD	30	14.23	4.31	0.79	6.90	25.60
	Yankasa	106	12.64	10.55	1.02	1.00	93.00
	Total	266	12.47	8.83	0.54	1.00	93.00
DNA quality	Balami	90	1.52^{b}	0.24	0.02	0.68	2.06
	Uda	40	1.64^{a}	0.31	0.04	1.21	2.82
	WAD	30	1.56^{ab}	0.21	0.04	1.18	2.20
	Yankasa	106	1.57^{ab}	0.24	0.02	0.90	2.61
	Total	266	1.56	0.25	0.02	0.68	2.82

However, a significant effect of breed on DNA quality was observed among the Nigerian sheep breeds used in this research (Table 1). While Uda had a significantly ($p<0.05$) higher DNA quality (1.64) when compared to the of Nigerian sheep breeds, Balami had the lowest quality (1.52). However, there was no significant differences ($p>0.05$) in the quality of WAD and Yankasa with DNA quality values of 1.56 and 1.57 respectively. The ratio of absorptions at 260 nm vs 280 nm is commonly used to assess DNA contamination of protein solutions, since proteins (in particular, the aromatic amino acids) absorb light at 280 nm (Sambrook and Russell, 2001). The reverse, however, is not true since it takes a relatively large amount of protein contamination to significantly affect the 260:280 ratio in a nucleic acid solution (Glasel, 1995). Hence, most of the variation could be attributed to breed effect. The significant breed effect on DNA quality could also be attributed to the genetic variability among this breeds as reported by Adebambo*et al*. 2004).Results of correlation between DNA concentration and quality using Pearson, Kendall's tau_b and Spearman's rho are shown in Tables 2 and 3.

TABLE 2: Pearson correlations between DNA concentration and quality

	DNA concentration	DNA quality
DNA concentration	1	
DNA quality	-0.209^{**}	1

**Correlation is significant at the 0.01 level (2-tailed).

TABLE 3: Nonparametric correlations between DNA concentration and quality

		Concentration	Ratio
Kendall's tau_b	DNA concentration	1	
	DNA quality	-0.050	1
Spearman's rho	DNA concentration	1	
	DNA quality	-0.071	1

In Pearson correlation, a negative, significant ($p<0.001$) but low (r = -0.209**) relationship was observed between DNA concentration and quality of the Nigerian sheep breeds used in this study. A similar trend was observed for the non parametric correlations. For Kendall's tau_b, a negative but not significant ($p>0.05$) low (r = -0.050) relationship was observed between DNA concentration and quality among the Nigerian sheep breeds. The same

trend was seen for Spearman's rho model except with a different correlation value (r) of -0.071. The low values observed in the correlation between DNA concentration and quality buttresses the explanation of Sambrook and Russell (2001) who emphasized that variation in these values are due to contamination.

CONCLUSION

The results of this study have shown that there is no significant breed effect on the concentration of DNA among Nigerian sheep breeds but a significant breed effect is seen in the quality of DNA across the Nigerian sheep breeds used in this study. A low relationship is observed between DNA concentration and quality.

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