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Association between growth hormone gene and growth traits in three colour type camel populations of Yobe state.

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Abstract

The present and continues change in climate pose a serious challenge to livestock husbandry especially in arid and semi arid environment where heat stress significantly interfere with performance, thus the need for breeding drought tolerant species become imminent. In Nigeria, there is paucity of information on the association between growth hormone gene polymorphism and growth traits in camel population and therefore, the need to identify even within population genotype, that is most adapted to the local environment remain critical for effective breeding of camel for meat production. In literature camel has been identified as the best adapted animal of the desert. This research was conducted to study the association between growth hormone gene and growth traits. Morphometric traits recorded for each animal include body weight (BW); body length (BL); abdominal girth (AG); hearth girth (HG); front limb length (FLL); hind limb length (HLL) and shoulder height (SH) in three distinct color type populations camel. A total of one hundred (100) animals comprising thirty three (33) each of dark brown (JA), Brown black (KR) and Grey White (FR) from Yobe state were sampled for the study. A Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method was used which revealed two alleles (T and C) and three genotypic variants (TT, TC and CC) with varied frequencies. All the camel types were found to be carriers of T allele with frequencies ranging from 0.73 (GW) to 0.40 (KR) respectively. Those for C allele ranged from 0.27 (GW) to 0.60 (KR). Frequencies of TT, TC and CC genotypes in GW were 0.65, 0.15 and 0.20, respectively with corresponding value of 0.39, 0.26 and 0.35 recorded for JA while KR had 0.35, 0.11 and 0.54. The analyses of the morphometric traits revealed that GW camel had significantly higher means for BW (543.35 Kg), AG (160.85 cm), FL (139 cm), and SH (184.60 cm). However, it was observed that Sex had no effect on all the traits studied. The association between genotype and morphometric traits also showed that Camels with homozygous (TT) genotype had highest means for BW (482.48kg), BL (147.26 cm) and SH (179.90 cm) compared (TC) heterozygous 389.50 kg, 131.83 cm and 171.92 cm and 425.50 kg, 139.81 cm and 172.42 cm for (CC) homozygous. It was concluded that GW colour type camels had best growth traits values and animals with TT genotype appears to have better adaptation to the arid environment. Consequently, they could be selected for improve growth rate and meat production in camel population.

Keywords: Camel, GH gene, growth traits, morphometric traits in camel

Association entre le gène de l'hormone de croissance et les traits de croissance dans les populations de chameaux de trois types de couleur de l'état de Yobe.

Résumé

Le changement climatique actuel et continu pose un sérieux défi à l'élevage, en particulier dans les environnements arides et semi-arides où le stress thermique interfère considérablement avec les performances, d'où la nécessité d'élever des espèces tolérantes à la sécheresse. Au Nigeria, il y a peu d'informations sur l'association entre le polymorphisme du gène de l'hormone de croissance et les traits de croissance dans la population de chameaux et, par conséquent, la nécessité d'identifier, même au sein de la population, le génotype le plus adapté à l'environnement local reste essentiel pour un élevage efficace de chameaux pour production de viande. Dans la littérature, le chameau a été identifié comme l'animal le mieux adapté du désert.

Cette recherche a été menée pour étudier l'association entre le gène de l'hormone de croissance et les traits de croissance. Les traits morphométriques enregistrés pour chaque animal comprennent le poids corporel (PC); longueur du corps (LC); circonférence abdominale (CA); circonférence du foyer (CF); longueur du membre avant (LMA); la longueur des membres postérieurs (LMP) et la hauteur des épaules (HE) chez les chameaux de trois populations de couleur distinctes. Un total de cent (100) animaux comprenant trente-trois (33) chacun de couleur brun foncé (JA), brun noir (KR) et gris blanc (FR) de l'état de Yobe ont été échantillonnés pour l'étude. Une méthode de polymorphisme de longueur des fragments de restriction par réaction en chaîne de la polymérase (PCR-RFLP) a été utilisée qui a révélé deux allèles (T et C) et trois variants génotypiques (TT, TC et CC) avec des fréquences variées. Tous les types de chameaux se sont avérés porteurs de l'allèle T avec des fréquences allant de 0,73 (GW) à 0,40 (KR) respectivement. Ceux pour l'allèle C variaient de 0,27 (GW) à 0,60 (KR). Les fréquences des génotypes TT, TC et CC chez GW étaient de 0,65, 0,15 et 0,20, respectivement avec une valeur correspondante de 0,39, 0,26 et 0,35 enregistrée pour JA tandis que KR avait 0,35, 0,11 et 0,54. Les analyses des traits morphométriques ont révélé que le chameau GW avait des moyennes significativement plus élevées pour PC (543,35 kg), CA (160,85 cm), LM (139 cm) et HE (184,60 cm). Cependant, il a été observé que le sexe n'avait aucun effet sur tous les traits étudiés. L'association entre le génotype et les traits morphométriques a également montré que les chameaux de génotype homozygote (TT) avaient les moyennes les plus élevées pour BW (482,48 kg), BL (147,26 cm) et SH (179,90 cm) par rapport aux hétérozygotes (TC) 389,50 kg, 131,83 cm et 171,92 cm et 425,50 kg, 139,81 cm et 172,42 cm pour les (CC) homozygotes. Il a été conclu que les chameaux de type couleur GW avaient les meilleures valeurs de traits de croissance et que les animaux avec le génotype TT semblent avoir une meilleure adaptation à l'environnement aride. Par conséquent, ils pourraient être sélectionnés pour améliorer le taux de croissance et la production de viande dans la population de chameaux.

Mot-clé : Chameau, gène GH, traits de croissance, traits morphométriques chez le chameau

Introduction

Dromedary camels (*Camelus dromedarius*) are multi-purpose and versatile livestock domesticated for their adaptation to arid and semi arid climates. They are prominent for their performance due to their disease, parasitic and drought tolerance as well as enhanced utilization and feed conversion efficiency. They are capable of surviving without water for more than two weeks losing up to about 30% body weight through dehydration. This loss can rapidly be replenished by drinking large volumes of water quickly at the next opportunity (Porter *et al*., 2016). This, among others, makes camel an important farm animal in Nigeria predominantly found in the Northern part Borno, Yobe, Jigawa, Kano, Katsina, Kebbi, Sokoto and Zamfara States (James-Ruga and Jidayi 2004; Mohammed and Hoffman, 2006) with a total population of estimate of 289,794 head (FAOSTAT, 2019). About 80% of the population are spread across Borno, Yobe, Katsina, Sokoto and Kano states, which are desert gateways with important camel trade (Timothy *et al*., 2015).

In livestock, growth traits are very important indices used in population studies. They are known widely to be affected by certain factors and are used as tools to assess several characteristics in farm animals. A well and carefully collected morphometric data could provide reliable information on variation within and between animal populations. They can also serve as a basis for molecular studies (Tandoh and Gwaza, 2017). Growth hormone gene has proven to be the major regulator of postnatal growth and metabolism in mammals and thus affect growth rate, body composition, health, milk production and aging by modulating

(regulating) the expression of many genes (Carnicella *et al*., 2003; Ge *et al*., 2003).
The objectives of the study were to determine the association between GH gene polymorphism and morphometric traits in camel populations and to evaluate the effect of color type, sex and genotype on body weight and other body measurements in camels.

Materials and methods

A total of hundred (100) camels of distinct colour types comprising thirty three (33) for each of brown black (KR) and dark brown (JA) and thirty four (34) Grey white (FR) camels from Garin Alkali market in Northeastern Nigeria, were sampled for the study. Coat colour was determined by visual appraisal and categorized according to Abdussamad *et al*. (2015). Hair follicle samples were collected from the camel's tail by plucking them directly from the roots. The samples were placed in to a small transparent labeled container according to colour type, sex and camel number before laboratory analysis. DNA extraction was carried out with Jena Bioscience Blood-Animal-Plant tissue preparation kit following manufacturer's instructions.

PCR was conducted in 25µl reaction mixture consisted of 2.5 µl 10x buffer, 0.5 µl dNTPs, 0.2 µl High fidelity Taq polymerase, 1 µl (10 pmol) each of the forward and reversed primer, 2 µl DNA template and 17.8 µl sterile nuclease free water. The primer (Table 1) used in this study was designed on the basis of DNA sequence of camel GH gene (Ishag *et al*., 2010). The amplification of DNA by PCR consisted of 37 cycles. The first cycle was characterized by pre-PCR denatuaration at 94°C for 2 minutes, annealing at 56°C for 30 seconds,

Table 1: The primer sequences, location and size of the amplified

Name	Annealing (°C)	Product size (bp)	Sequence (5'-3')	GeneBank Accession
KGH1B up	56	508	Cagggaccaattcc	JX891650
KGH1B low			Ccatccctgaggag	

extension at 72°C for 40 seconds. The next 36 cycles involved denaturation at 94°C for 1 minute, annealing at 56°C for 30 seconds and extension at 72°C for 40 seconds and a final extension at 72°C for 10 minutes. Analysis of the amplicons was performed on a 1.5% (wt/vol) agarose gel dissolved in 0.5x Tris-borate buffer, stained with ethidium bromide and visualized under blue led trans-illuminator. The length of the PCR products was 508bp.

Body weight and linear measurements were taken using a specially designed tape (WE-BO MALEBAND^{R}) for camel. The tape also gives approximate body weight estimate. Body measurements recorded were;

Abdominal girth (AG) measured as the distance around the abdomen over the highest part of the hump,

Hearth girth (HG) circumference of the body immediately behind the shoulder blades in a vertical plane, perpendicular to the long axis of the body

Shoulder height (SH) height (vertical) from bottom of the front foot to the highest point of the withers,

Anterior limb length (ALL) distance from the ground level to front of sternum

Posterior limb length (PLL) distance from bottom of leg to pin bones

Body length (BL) horizontal distance from point of shoulder to the pin bones and

Body weight (BW) recorded directly from the tape as proportional to heart girth

The genotypic and allelic frequencies were determined by counting method while Hardy Weinberg equilibrium (HWE) was tested using Chi square (X^2). The data recorded for body measurement traits (BL, BW, AG, HG, ALL, PLL and SH) were subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) of the Statistical Analysis Systems (SAS, 2000). The following statistical model was adopted:

$$Y_{ijkl} = \mu + A_i + S_j + G_{k+} e_{ijkl}$$

where Y_{ijk} = is the observation on each trait of the *ijkl*th animal body linear measurement of the ith colour of camel.
μ = general mean of each trait.
A_i = effect of ith colour of camel (i= 1,2,3).

$S_{j=}$ fixed effect of j^{th} sex (j= 1,2)
$G_{k=}$ fixed effect of k^{th} genotype (k= 1,2,3)
e_{ijkl} = Random residual error effect associated to the *ijkl* observation.

Results

The genotypic and allelic frequencies of the three camel populations based on colour types are presented in Table 2. The results indicated the presence of two alleles (T and C) and three genotypes (TT, TC and CC) in all the camel populations studied as shown on plate 1. The Chi square (X^2) test showed that the observed genotypes in both GW and KR populations were not in Hardy-Weinberg equilibrium ($p<0.05$), while JA population was. All camel color types were found to be carriers of the T-alleles. The GW and JA camels had higher frequencies of T alleles (0.73 and 0.52, respectively) compared to the C (0.27 and 0.48). In contrast, KR camels had higher frequency of C alleles (0.60) compared to T (0.40). In this study, the higher frequency of T-alleles observed in GW camels may be attributed to better adaption to semi arid climate. The observation in (GW and JA) is in line with those of EL-Aziem *et al*. (2014) who reported high frequency of T-alleles among the Somali, Falahy and Mowaled breeds of camel reared in Egypt. That of KR is in line with findings of Afifi *et al*. (2014) and Ishag *et al*. (2010) who observed higher frequencies of C allele among the Saudi and Somali camel populations, respectively. With regards to the genotype frequency, GW and JA camels had higher frequency of TT (0.65 and 0.39) compared to TC (0.15 and 0.26) and CC (0.20 and 0.35). The frequency of genotype CC (0.54) was higher for the KR camel compared to TC (0.11) and TT (0.35). Similarly, this is in agreement with the observations made by Ishag *et al*. (2010), who reported higher frequency of CC among the Sudanese camel breeds. Similar observation was also made by Shan (2006) on Pakistani camels. However, Afifi *et al*. (2014) observed higher frequency of heterozygous genotype (TC) in Majaheem, Saheli, Wadda and Homar camel breeds of Saudi Arabia, and the authors concluded that these variations might be due to breed adaptation and environmental differences.

Table 2: Genotype and allele frequencies of different camel population

Colour types	Genotypes			Alleles	
	TT	TC	CC	T	C
KR	0.35	0.11	0.54	0.40	0.60
GW	0.65	0.15	0.20	0.73	0.27
JA	0.39	0.26	0.35	0.52	0.48

KR- Brown black, GW- Grey White, and JA- Dark brown

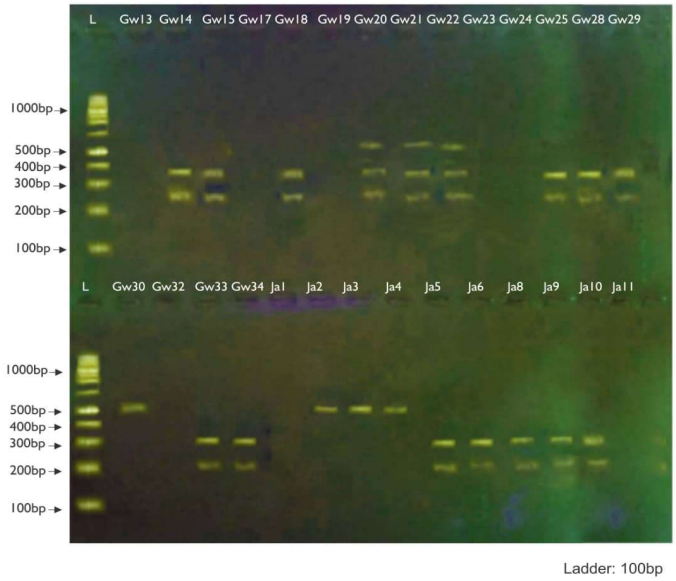

Plate 1. Different genotypes of *Mspl* restriction

The effects of colour type, sex and genotype on body weight and other measurement are presented in Table 3. The results showed that colour type had significant ($P<0.05$) effect on most of the traits studied. Grey white (GW) colour camels recorded significantly higher means for BW, BL, HLL AG and SH, while the JA and KR did not differ significantly ($P<0.05$) for most of the traits. Similarly, body weight of GW (543.35kg) was higher than 425.09 and 386.54 kg for JA and KR, respectively. GW camels were therefore not only heavier but also better than any of the other two (2) colour types for most body measurements.

However, it was observed that sex had no significant effect on all the traits studied. This may probably be attributed to age and sample size. This is similar to the observation made by Ishag *et al*. (2010) who reported no significant sex differences in the least squares means for abdominal girth among the Sudanese camel populations. However, this finding is contrary to earlier reports by some authors (Mehari *et al*., 2007; Abdallah and Bernard, 2012; Afifi *et al*., 2014 and Tandoh and gwaza (2017) who divulged the existence of variations due to sex on body weight (462, 432 kg in males and females, respectively) and other morphological traits in camels.

Table 3 Effects of color type, sex and genotype on body weight and measurements of camels in Yobe state

Parameters	BW	BL	FL	HL	AG	HG	SH
Color	*	*	NS	*	*	NS	*
Brown Black(Kr)	386.54^{b}±14	137.35^{b}±19.1	134.00±8.88	137.31^{b}±6.06	143.85^{b}±23.43	167.04±18.84	169.12^{b}±12.88
Grey White (Fari)	543.35^{a}±10	146.35^{a}±19.6	139.95±7.32	149.40^{a}±9.54	160.85^{a}± 22.42	191.05±18.36	184.60^{a}±10.90
Dark Brown (Ja)	425.09^{b}±90	140.17^{ab}±172	135.35±13.58	139.17^{b}±8.33	151.00^{ab}±20.61	217.57±21.14	175.39^{b}±9.59
Sex	NS	NS	NS	NS	NS	NS	NS
Female	438.59±10.59	143.41±16.9	134.79±7.6	140.93±6.8	149.76±19.0	175.93±16.6	176.59±10.0
Male	449.38±14.03	140.58±21.9	137.18±17	141.80±13	152.18±25.2	201.65±16.2	175.05±14.4
Genotype	*	*	NS	NS	NS	NS	*
TT	482.48^{a}±11.9	147.26^{a}±16.1	138.58±16	144.00±8.6	158.48±20.6	182.39±17.3	179.90^{a}±16
TC	389.50^{b}±90.1	131.83^{b}±15.8	132.08±9.2	139.33±12	144.83±18.9	168.33±13.3	171.92^{b}±14
CC	425.50^{ab}±13.4	139.81^{ab}±29	135.19±10	139.35±93	145.35±24.5	211.31±19.4	172.42^{b}±10

a',b means a subset within a column with different superscripts are significantly (P<0.05) different, ns =not significant BW=body weight, BL= body length, HG= heart girth, AG= abdominal girth, SH= shoulder height, HL=hind limb and FL=front limb length

The analysis of the morphometric traits revealed a significant (P<0.05) effect of genotype. The results showed that camels carrying homozygous (TT) had higher means for BW, BL and SH than either those of heterozygous (TC) or homozygous (CC). The body weight (482.48 kg), body length (147.26 cm) and shoulder height (179.90 cm) of the camels with TT genotype were higher than those (389.50 kg, 131.83 cm and 171.92, respectively) recorded for TC and those (425.50 kg, 139.81 cm, and 172.42 cm, respectively) recorded for CC. The heterozygous TC had the least values.

Conclusion

In other to harness the huge potential of camels as the best adapted animal of the desert, it is important to identify genotypes that are superior in growth trait indices, and based genetic improvement decisions. This study therefore showed that there is an association between growth hormone gene polymorphism

and growth traits. Farmers or scientists interested in camel husbandry should use Grey White color type camel because of its better adaptation and higher performance. There is also the need for further research using genome wide association studies (GWAS) to better understand the growth traits characteristics in camel population of Nigeria.

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