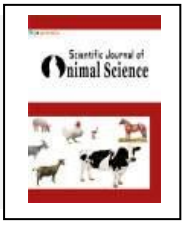
Sjournals
Scientific Journals
Scientific Journal of Animal Science (2013) 2(5) 109-115
ISSN 2322-1704

Contents lists available at Sjournals
Scientific Journal of Animal Science
Journal homepage: www.Sjournals.com



Original article

Polymorphisms of *Tyrosinase* gene (Exon 1) and its impact on coat color and phenotypic measurements of Sudanese Camel Breeds

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ARTICLE INFO

Article history:
Received 06 April 2013
Accepted 16 April 2013
Available online 28 May 2013

Keywords:
Genotyping
Tyrosinase gene
PCR-RFLP
Sudanese camels
Body measurements
Coat colour

ABSTRACT

The objectives of the present study were the detection of allelic variants of *tyrosinase (TYR)* gene (Exon 1) in six Sudanese camel breeds (Kenani, Lahwee, Rashaidi, Anafi, Bishari and Kabbashi) and afford the chance of investigating the possibility of the presence of associations between coat color and body measurements with SNPs of the *TYR* gene (Exon 1). The gene and genotypic frequencies of *TYR* gene in Sudanese camel breeds were studied using PCR-RFLP analyses. Genomic DNA samples were obtained from 181 mature unrelated animals representing the six tested camel breeds. The results showed insignificant differences in the allele frequency between the breeds. Kenani and Anafi camels had slightly higher T allele frequency (0.21 and 0.20) than those of Lahwee, Kabbashi and Bishari camels, while the Rashaidi camel had the lowest T allele frequency (0.08). The genotype frequencies for all breeds obtained were 0.02, 0.25 and 0.72 for TT, TC and CC genotypes respectively. The genotype TT was detected only in Lahwee and Anafi camels. The analysis of variance showed that the age and breed of camel significantly influenced barrel girth, heart girth and body weight, however, the sex of camel significantly affected heart girth, shoulder height and body weight. On the other hand, the *TYR* genotype was significantly affect shoulder height but had no significant influence on the other tested traits. The results also revealed that insignificant

association between camel coat colour and *TYR* genotypes. Further studies with larger numbers of animals are required in the future to investigate or verify these associations.

1. Introduction

Camel population in the world is estimated at 18.5 million heads of which dromedary camels comprise 95% while the remaining 5% are Bactrian camel. Bactrian camels are found mainly in the cold high altitudes of Asia. The Near East, North Africa and the Sahel Region have about 70% (12.6 million) of the world's dromedary camels. Somalia and Sudan together own about half of this number (Kesseba *et al*., 1991). Sudan is rated the second in numbers of camel population in the world. Camels constitute 22% of the animal biomass in Sudan and 26.3% of the numbers of camel in the Arab world (Sakr and Majid, 1998). The numbers of camels in the Sudan were estimated at about 3908 thousand heads (Ministry of Animal Resources, 2005).

Phenotypic and genetic characterization to assess the existing biodiversity and differences among the Sudanese camel ecotypes is an essential prerequisite to facilitate the conservation and utilization programme in an effective and meaningful way. However, the Sudanese camel ecotypes are not well classified or defined, with very limited information available (Ishag *et al.,* 2010). Genetic polymorphisms are playing an increasingly important role as genetic markers in many species of livestock and camels are no exception. Tyrosinase (TYR) is a key enzyme in the metabolic pathway leading to coat colour pigmentation and in hormone production (Shah *et al.,* 2005). Mutations in the *tyrosinase* gene are responsible for the albino phenotype in mammals and chicken (Schmidtz *et al*., 2001) and they are also known to influence quantitative traits in mice. Based on the partial sequences of mouse and human it was possible to design a primer pair which can be used for the amplification of an 820 bp fragment in exon 1 of the camel *tyrosinase* gene (Shah *et al.,* 2005). By sequencing different Pakistani camel breeds, a single nucleotide polymorphism (C>T) on position 200 after the ATG causing an amino acid substitution (Pro>Leu) was detected (Shah *et al.,* 2005; Shah *et al.,* 2008).

The objectives of the present study were the detection of allelic variants of *tyrosinase (TYR)* gene (Exon 1) in six Sudanese camel breeds (Kenani, Lahwee, Rashaidi, Anafi, Bishari and Kabbashi); also afford the chance of investigating the possibility of the presence of correlations between body measurements and coat colour with SNPs of the *TYR* gene (Exon 1).

2. Materials and methods

2.1. DNA extraction

Hair samples were obtained from 181 mature unrelated individuals of Sudanese camels. Thirty one hair samples were obtained from Kenani (KEN) and 30 hair samples were obtained from each of the Rashaidi (RAS), Lahwee (LAH), Anafi (ANA), Bishari (BIS) and Kabbashi (KAB) breeds. Genomic DNA was extracted from hair roots by using Nucleospin® tissue kit (Macherey-Nagel). DNA concentration was measured with a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc).

2.2. DNA amplification and genotyping

The published nucleotide sequence information of the camel *TYR* gene and primer pairs designed by Shah (2006) were used to amplify a fragment of exon 1 of camel *TYR* gene**.** The 181 animals of the six tested Sudanese camel breeds were genotyped for g.200C>T using the PCR-RFLP method. A 474 bp fragment (table 1) covering the sequence containing the mutation site was amplified. PCR was performed in a reaction volume of 25 µl using 100 ng of DNA, 0.2 pmol of each primer, 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP and 0.5 U of GoTaq flexi-DNA polymerase (Promega). The amplification programmes consisted of 37 cycles. The first one was characterised by denaturation at 94 °C for 2 min, annealing with 56 °C for 30 s and an extension step at 72 °C for 40 s. The next 36 cycles involved a denaturation step at 94 °C for 1 min, annealing at 56 °C for 30 s and extension at 72 °C for 40 s with the exception that in the last cycle the extension time was 10 min. The amplicon was digested with *Dde*I

restriction endonuclease (Promega) at 37° C for four hours, to distinguish between the two alleles. For each reaction, 15 µl of PCR product, 2 µl buffer, 2.6 µl H_2O, 0.2 µl BSA and 0.2 µl enzyme containing 5 U of *Dde*I were used. The digested fragments (T allele, unrestricted: 474 bp; C allele, restricted: 339 bp and 135 bp) were analyzed by electrophoresis in 2% agarose gel, stained with ethidium bromide and photographed under UV light (Plate 1).

Table 1
Primer sequences, annealing temperature and size of the amplified fragments

Name	Annealing temperature (°C)	Product size (bp)	Sequence (5'-3')
D-TYRA up	56	474	agcctgtgcctcctccaagaac
D-TYRA low			tgcatccatacaaagaggtcataa

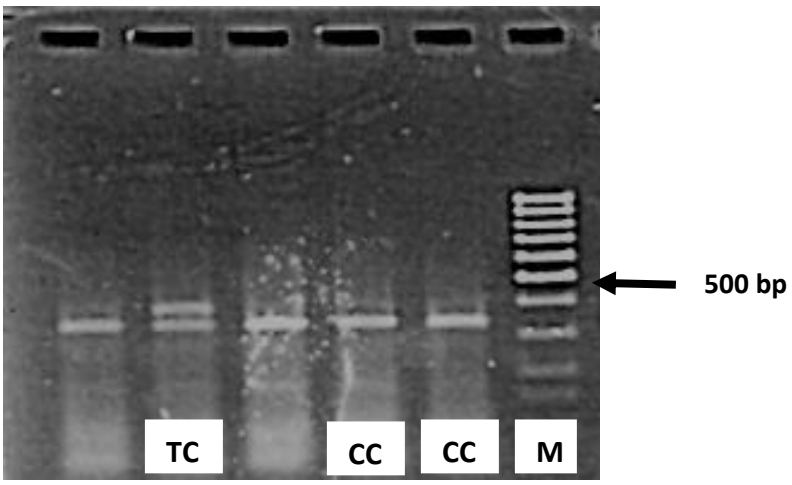

Fig. 1. Different genotypes of DdeI restriction for SNP for all studied camel breeds.

2.3. Phenotypic measurements and coat colour

Heart girth was measured immediately behind the breast pad; barrel girth was determined over the highest part of the hump and the shoulder height was measured for each animal. Weights of animals were then estimated using Boue (1949) formula as follows:

P = 53 TAH

Where P is body weight (kg); T heart girth (m); A barrel girth (m); and H is shoulder height (m). The age and sex of each animal were also recorded. On the other hand; the coat colour of each animal was considered and then classified into five groups *vis:* dark brown, brown, grey, yellowish and white.

2.4. Statistical analysis

Genotype and allele frequencies were determined by gene counting. The Chi-square test was employed to evaluate whether the populations were in Hardy-Weinberg equilibrium. However, Student's t-test was used to determine the significance of differences in gene frequencies between populations. On the other hand, Chi-square contingency table for independence was run to detect association between Tyrosinase genotypes and camel coat color. The data on the estimation of body weights and phenotypic measurements of the different genotypes were subjected to analysis of variance (ANOVA) using the general linear model (GLM) from the Statistical Analysis Software (SAS Institute Inc., 2000). The statistical model used was:

$Y_{ijklm} = \mu + Ai + S_j + B_k + G_l + e_{ijklm}$

Where Y_{ijklm} is the observation on each trait of the *ijklm*th animal, μ is the general mean of each trait, A_i is the fixed effect of *i*th age group, S_j is the fixed effect of *j*th sex, B_k is the fixed effect of the *k*th breed, G_l is the fixed effect of the *l*th genotype and e_{ijklm} is the random error term associated to the *ijkl*th observation.

3. Results and discussion

3.1. Gene and genotypes frequencies

Table 2 shows the genotype and allele frequencies of the investigated SNP in *TYR* gene in Sudanese camels. The results showed insignificant differences in the allele frequency between the breeds. While Shah *et al.* (2008) explained significant differences among Pakistani camel breeds. KEN and ANA camels had slightly higher T allele frequency (0.21 and 0.20) than those of LAH, KAB and BIS camels, while the RAS camel had the lowest T allele frequency (0.08). The similar T allele frequency in KEN and ANA camels could be due to the fact that these two breeds are often raised alone together in mixed herds. In Sinnar and Blue Nile states ANA camels are raised with KEN camels resulting in inter-mating between the two breeds. The genotypic frequencies overall were 0.02, 0.25 and 0.72 for TT, TC and CC genotypes, respectively. The genotype TT was detected only in LAH and ANA camels. The highest TT, TC and CC genotypic frequencies were found in ANA (0.10), KEN (0.42) and RAS (0.83) camels respectively. The Chi-square test between *TYR* genotypes revealed that the breeds were in Hardy-Weinberg equilibrium (HWE) (P>0.05). Shah *et al*. (2005) and Shah *et al.* (2008) found also a low frequency of TT genotypes but demonstrated significant differences in genotype frequencies between the Pakistani camel breeds. The low frequency of TT genotypes in LAH and ANA camels and its absence in other breeds may reflect active selection against it resulting from local preferences. On the other hand the detection of this genotype (TT) only in LAH and ANA camels is a result of the fact that these two breeds are reared together in Gadaref state and cross mating may occur.

Table 2
Genotype and allele frequencies of g.200C>T in *TYR* gene in Sudanese camel breeds.

Breed	Genotypes			Alleles	
	TT	TC	CC	T	C
KEN	0.00	0.42	0.58	0.21 [a]	0.79
RAS	0.00	0.17	0.83	0.08 [a]	0.92
LAH	0.03	0.23	0.73	0.15 [a]	0.85
ANA	0.10	0.20	0.70	0.20 [a]	0.80
BIS	0.00	0.23	0.77	0.12 [a]	0.88
KAB	0.00	0.27	0.73	0.13 [a]	0.87
Overall	0.02	0.25	0.72	0.15	0.85

Allele frequencies having the same superscript are not significantly different (P > 0.05).

3.2. Phenotypic measurements and coat colour

Table 3 presents the effect of age, sex, breed and TYR genotype on barrel girth, heart girth, shoulder height and body weight. The estimated means for barrel girth, heart girth, height at shoulder and body weight are 2.42 m, 1.97 m, 1.86 m and 439.05 kg, respectively. The breed of camel was found had significant influence on the all studied traits. The significant influence of camel breed on body measurements was obtained by (Ishag *et al.,* 2010; Ishag *et al.,* 2011[a]; Ishag *et al.,* 2011[b]). Also the results revealed that the KEN camel had highest values of heart girth, shoulder height and body weight, then flowed by those of KAB and LAH, while ANA and BIS recorded lowest values. Also the results showed that RAS breed had significantly greater value of barrel girth and lower value of shoulder height and heart girth compared to other breeds. The KEN, KAB and LAH camels generally have more or less similar morphological features (large size, heavily built animals with a developed hump) except for the RAS, ANA and BIS, which are classified as Sudanese pack (heavy) camels and called Arabi camels (Khouri, 2000). The RAS camel is also classified as a pack camel but it has different phenotypic characteristics (light weight and short at shoulders) compared to the other Sudanese pack camels. However; ANA and BIS breeds have similar features (white, yellowish colour and light weight) and are classified as riding camels in Sudan (Khouri, 2000; Wardeh, 2004; Ishag *et al.*, 2010).

The results revealed that sex of animal had a significant (P<0.001) effect on the all studied traits except barrel girth. Moreover; the mean separation results showed that male camels had a greater heart girth, shoulder height and heavier body weight than the corresponding traits of female camels significantly (P<0.05). This finding is similar to that reported by Dioli *et al.* (1992) and Mehari *et al*. (2007) who stated that there is quite distinctive sexual dimorphism in camels, i.e. the male camels is usually taller and of heavier weight than the females. These

differences in tested traits between male and female camels may reflect differences in the hormonal secretions and their activities in the two sexes. The analysis of variance results showed that age of animal significantly (P<0.01) influenced barrel girth, heart girth and body weight; but it insignificantly affected shoulder height. The studied traits had increasing trend from the 1st age group (4 - 5 years) to 3rd age group (8 - 9 years) and then declined. This means that camels reach maturity (growth peak) within 8 to 9 years of age; after which the different measurements decrease. This trend is reflected in the growth curve of the Sudanese camels. Similar results and trends were also reported by Ishag *et al.,* 2010 and Ishag *et al.* 2011[a].

Table 3
Effect of age, sex, breed and *TYR* genotype on barrel girth, chest girth, shoulder height and body weight

Parameter	No.	Barrel girth (m)	Heart girth (m)	Shoulder height (m)	Body weight (kg)
Age groups		**	***	NS	***
4 - 5 years	41	2.24 [b]	1.90 [b]	1.85 [a]	384.42 [b]
6 - 7 years	41	2.45 [a]	1.99 [a]	1.87 [a]	450.53 [a]
8 - 9 years	34	2.52 [a]	2.01 [a]	1.87 [a]	469.80 [a]
10 - 11 years	34	2.50 [a]	2.00 [a]	1.85 [a]	456.74 [a]
≥ 12 years	31	2.43 [a]	1.98 [a]	1.85 [a]	443.00 [a]
Sex		NS	***	***	***
Male	51	2.38 [a]	2.02 [a]	1.93 [a]	463.79 [a]
Female	130	2.44 [a]	1.96 [b]	1.83 [b]	429.35 [b]
Breed		***	***	***	***
KEN	31	2.48 [ab]	2.07 [a]	1.95 [a]	492.99 [a]
RAS	30	2.56 [a]	1.94 [b]	1.75 [d]	422.91 [bc]
LAH	30	2.51 [a]	1.98 [b]	1.84 [c]	444.08 [b]
ANA	30	2.39 [bc]	1.95 [b]	1.84 [c]	425.68 [bc]
BIS	30	2.37 [c]	1.94 [b]	1.84 [c]	409.84 [c]
KAB	30	2.22 [d]	1.97 [b]	1.91 [b]	447.03 [b]
TYR genotype		NS	NS	*	NS
TT	4	2.47 [a]	1.93 [a]	1.82 [b]	419.84 [a]
TC	46	2.40 [a]	1.99 [a]	1.89 [a]	446.26 [a]
CC	131	2.43 [a]	1.97 [a]	1.85 [ab]	437.11 [a]
Overall mean	181	2.42	1.97	1.86	439.05
SEM		0.02	0.01	0.01	4.41

*; **; *** and NS Significant at P<0.05; P<0.01; P<0.001 and not significant P>0.05.
Means without a common superscript differ significantly at P<0.05.
SEM Standard error of means.

Table 4
Distribution of camel coat colour among different *TYR* genotypes.

Color	***TYR* genotypes**		
	TT	**TC**	**CC**
		%	
Dark brown	0.0	38.5	61.5
Brown	0.0	21.4	78.6
Grey	3.6	16.1	80.4
Yellowish	0.0	20.0	80.0
White	3.7	37.0	59.3
Overall	2.2	25.4	72.4

The results also indicated that the *TYR* genotype had no significant effects on all tested measurements except shoulder height was found significantly (P<0.05) affected by *TYR* genotype. The results showed that the TT

homozygous genotype was significantly (P<0.05) shorter at shoulder which in compared with TC heterozygote; while it was insignificantly different from CC genotypes. However; the same genotype CC had insignificantly (P>0.05) lowest values of heart girth and body weight; and had highest value of barrel girth. On the other hand, the heterozygous genotypes TC recoded insignificantly (P>0.05) higher values of heart girth and body weight; while had significantly (P<0.05) greater value of height at shoulders.

Camels of Sudan were characterized by various coat colours. Generally, the back camels (KEN, KAB, RAS and LAH) were described by dark brown, brown, reddish, grey and yellowish coat colour; while the riding camels (ANA and BIS) were characterized by white and yellowish colour (Ishag *et al.,* 2010 and Ishag *et al.* 2011[b]). Table 4 shows ddistribution of coat colour of camels among different *TYR* genotypes. The results of chi-square test for independence revealed insignificant (χ^2=11.15; P>0.05) association between camel coat colour and *TYR* genotypes. This means that the studied SNP in *TYR* gene (Exon 1) had no influence on coat colour of Sudanese camels.

4. Conclusion

This study concludes that insignificant differences were found in allele frequencies of *Tyrosinase* gene (Exon 1) among camel breeds. The study also concludes that insignificant associations were found between *Tyrosinase* genotypes and body measurements except shoulder height. The study shows insignificant association between coat colour and *Tyrosinase* genotypes. Further studies with larger numbers of animals are required in the future to investigate or verify these associations.

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