RESEARCH ARTICLE

MOLECULAR GENETICS AND PHENOTYPIC CHARACTERIZATION OF SOME BEEF QUALITY TRAITS IN SUDANESE BAGGARA CATTLE

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ABSTRACT

The genotype frequencies of single nucleotide polymorphisms (SNPs) of the growth hormone1 (GH) locus (rs:4193484), the Insulin-Like Growth Factor Gene (IGF1)(rs:109763947) and association between genotypes and meat quality traits were studied in two subtypes of Baggara cattle (65 Nyalawi and 65 Mesairi bulls) raised under dry land farming in Western Sudan. All Nyalawi and Mesairi bulls were homozygous for the 2141C wildtype allele of GH1 gene. The heterozygote (CT) IGF1 genotype (13% and 14%) and homozygote CC (86 and 85%) frequencies were almost similar in the two genotypes. The mutant homozygotes (TT) existed only in the Mesairi subtype (2%). Difference between Nyalawi and Mesairi subtypes in live weight (459.6 vs 446.1 kg), hot carcass (234.3 vs 208.5 kg) weight, dressing percentage (50.9 vs. 47.3%), meat quality traits, fat percentage, moisture, and ash content were significant (P<0.05). No associations between IGF1 genotypes and weights and meat quality traits were found. No differences between IGF1 genotypes in meat chemical composition (P>0.05) except for fat content (P<0.05). Breed x IGF1 genotypes interactions were not significant (P>0.05). Further research in other regions of the sequence of these genes and other candidate genes affecting meat quality traits is called for in all subtypes of Sudanese Baggara cattle.

INTRODUCTION

Livestock, especially cattle, play a very important role in the economic life of most of the population in Sudan and are mainly kept under dryland pastoral and agropastoral production systems (R. T. Wilson, 2018). Baggara cattle belongs to Northern Sudan zebu cattle and represents about 33% of the national herd of the Sudan (H. H. H. Bashir and I. E. M. Elzubier, 2013). Baggara cattle contributes more than 67.2% of local meat consumption and considerable share to the export trade of meat and livestock (R. T. Wilson, 2018). The homeland of Baggara cattle is the savannah belt of Central Sudan lying about latitudes 10°-16°N (I. M. Hashim and B. Fadlalla, 1989), raised by agropastoral (transhumant) tribes in the plains of Sudan's

Darfur and Kordofan states (H. H. H. Bashir and I. E. M. Elzubier, 2013). Cattle migrate seasonally on defined migration routes to overcome limitations of grazing and water scarcity, and as such subjected to shortage of feed in the dry season each year. This results in delays of sexual development and maturity and lower growth rates (A. A. Ayantunde *et al*, 2005) to reach a marketable slaughter weight. Genetics accounts for 5-30% of the total phenotypic variance in beef quality traits, depending on the trait (R. D. Warner *et al*, 2010).Tenderness is considered the most important meat quality trait; however, other traits such as color, marbling, cut size, amount of intramuscular and subcutaneous fat and palatability are also valued by consumers (J. M. Malheiros *et al*, 2020).The effect of genetics on meat tenderness is clearly observed when meat of *Bos taurus* is compared with meat of *Bos indicus*, the latter type presents tougher meat (M. C. Bressan *et al*, 2011).Insulin-like Growth Factor I (IGF1), being moderately heritable, is known to play an important role in various aspects of muscle growth and development (M. E. Davis and R. C. M. Simmen, 2010).

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IGF1 effects on the hypertrophy of muscle cells might lead to increased muscle fiber diameter and decrease tenderness (H. K. Herring *et al*, 2009). This has led to the suggestion that IGF-I might be used as an indirect selection criterion for improving carcass characteristics of beef cattle (B. J. Wood *et al*, 2004) and for predicting growth and meat quality traits in animal genetic improvement schemes (M. B. B. Machado *et al*, 2003; P. C. Andrade *et al*, 2008).The objectives of this study were to estimate the genotype frequencies of single nucleotide polymorphisms (SNPs) of the growth hormone1 (GH) locus, the Insulin-Like Growth Factor Gene (IGF1) and to test the association between genotypes and meat quality traits of two subtypes of Baggara cattle (Nyalawi and Mesairi).

MATERIALS AND METHODS

Ethics Approval: All experimental protocols used are approved by the University of Khartoum and in accordance with the University of Khartoum Laboratory Authority guidelines.The guidelines and regulations set out by the Sudan Veterinary Council were strictly followed during animal handling and sampling.

Experimental Animals: One hundred and thirty Baggara bulls of two subtypes (Nyalawi and Mesairi) 4-5 years of age were used. The animals were originally raised under dryland farming conditions in western Sudan (Kordofan and Darfur regions), brought to Khartoum and finished on a traditional feedlot for three months. Blood samples were, then, collected from each bull, and slaughtered thereafter in a commercial abattoir. Live weights (kg), hot carcass weights and dressing percentages were determined. Under aseptic conditions and within one-hour post-slaughtering, 130 meat samples were cut from the *Longissmus dorsi thoracis* muscles and kept in an icebox and transferred to a freezer (-20°C) in the meat laboratory, Department of Meat Production, Faculty of Animal Production, University of Khartoum pending meat quality determination.

Blood samples and Extraction of Genomic DNA: Blood samples for DNA genotyping were collected from the jugular vein of each Baggara bull in test tubes containing EDTA and stored in a refrigerator (4°C) pending analysis. DNA was isolated using salting-out method (S. A. Miller *et al*, 1988). The DNA concentration was measured using Nano-Drop (ND) 1000 Spectrophotometer, (Nano-Drop Technologies, Inc)

Genotypic characterization of GH1 gene and IGF1: Sections of the growth hormone (GH1) and IGF1 loci were analysed covering the sequence containing the missense mutation 2141C>G in exon 5 of GH1 gene and the mutation C/T at position -472 in the 5-noncoding region of the IGF1 gene relative to the start of transcription site. The PCR-RFLP methods were carried out to detect the gene polymorphisms according to the methods of J. Yao *et al* (1996) and W. Ge *et al.* (2001) and were digested with Alue1 and SnaB1 restriction endonucleases, respectively.

Meat quality and chemical composition traits: Traits analyzed were meat tenderness attributes which were cooking loss (P. B. Pathare and A. P. Roskilly, 2016), water holding capacity (WHC) (J. M. Lorenzo *et al*, 2015), Myofibril Fragmentation Index (MFI) (R. D. Culler *et al*, 1978) and the proximate chemical composition (protein, moisture, fat, and ash) (AOAC, 2016).

Statistical analysis: Statistical analysis was carried out using least-squares procedures (W. R. Harvey, 1977). Chi square (x^2) was used to test for association between genotypes and levels of traits. The expected genotype frequencies were calculated using the Hardy-Weinberg law (O. Mayo, 2008).

RESULTS AND DISCUSSION

Genotypic Characterization: Genotyping the SNP 2141C>G (Leu/Val substitution in exon 5) showed that all Nyalawi and Mesairi bulls were homozygous for the 2141C allele (Leu variant).

Previously, M. A. Alsiddig *et al*. (2010) mentioned that the genotyping results (using PCR-RFLP) for Nyalawi and Mesairi cattle in exon 5 showed similarity between the subtypes although they were phenotypically different. J. Jakaria and R. R. Noor (2011) also did not find the mutation in position 2141 bp in Bali cattle. The results of PCR-RFLP of the IGF1genotypes of Baggara cattle subtypes showed the existence of the SNP C>T (Table 1). Genotype frequencies were 86% (CC) and 14% (CT) in Nyalawi subtype compared to 85% (CC),13% (CT) and 2% (TT) in Mesairi. Using Chi-square (x^2) test, both Nyalawi and Mesairi subtypes were found to be in Hardy-Weinberg equilibrium (x^2 =0.407and 1.085, respectively) at this locus (Table 1). However, the frequencies of the mutant homozygotes (TT) were very low and existed only in the Mesairi subtype while the heterozygote (CT) genotype existed in the two subtypes. The presence of the C and T alleles in Nyalawi and Mesairi subtypes of Baggara cattle were comparable with the results reported by W. Ge *et al*. (2001) in Angus cattle, who showed that these two alleles are present with allelic frequencies 0.36 and 0.64. Similarly, E. Siadkowska *et al* (2006) found that C and T alleles are present in Polish Holstein-Friesian cattle with frequencies of 0.52 and 0.48, respectively. However, C allele frequencies were higher and T alleles frequencies were very low in Baggara cattle strains (Table 1) compared with frequencies reported for these two alleles in Angus (W. Ge *et al*, 2001) and Polish Holstein-Friesian (E. Siadkowska *et al*, 2006) cattle.

Phenotypic Characterization: Nyalawi and Mesairi subtypes of Baggara cattle seemed to be different in most of the phenotypic traits investigated, with Nyalawi subtype being superior (Tables 2 and 3). Nyalawi subtype had higher liveweight (P<0.05) and higher (P<0.01) hot carcass weight and dressing percentage in comparison to Mesairi subtype (Table 2). M. A. Alsiddig *et al*. (2010) found that the Nyalawi subtype was heavier (P<0.01) and of larger frame size than the Mesairi one. They stated that Baggara cattle have variable size; it can reach large size and heavy weight that ranged between 400 and 600 kg, with Nyalawi ecotype being heavier and of large body conformation at all age growth stages compared with other subtypes. They attributed these differences to nutritional factors. However, differences in weight traits among the three genotypes were not significant (P>0.05), with CT genotype recording comparatively heavier liveweights and higher hot carcass weight (Table 2). On the contrary, M. Szewczuk *et al* (2013) observed that the CC genotype was associated with higher ADG (+ 52 g; P ≤ 0.05) in comparison with individuals with the TT genotype. Least-squares means for Nyalawi and Mesairi subtypes of Baggara cattle and their IGF1 genotypes meat quality traits (Table 3) reflected breed differences. Meat from Nyalawi subtype was of better-quality attributes in comparison with Mesairi subtype. Nyalawi subtype meat had higher (P<0.01) MFI, lower (P<0.01) WHC and lower (P<0.01) cooking losses compared with Mesairi subtype. This indicated that meat from Nyalawi subtype is more tender than that from Mesairi one. The lower cooking loss of Nyalawi is a reflection of the lower and better water holding capacity (H. A. Elbukhary *et al*, 2020). D. M. Fergusson (2004) stated that the variation in beef tenderness may be attributed to the genotype of the animal and carcass composition. No differences (P>0.05) were found among the three genotypes (Table 3), but bulls of CT genotype of both subtypes had comparatively (P>0.05) higher live weight at slaughter, hot carcass weight, fat percentage, WHC and cooking loss compared to the homozygous CC and TT genotypes. It worth mentioning that the genotype CC scored slightly the highest mean MFI in Nyalawi, while in Mesairi comparatively highest mean MFI was recorded by genotype TT.

Table 1. Genotypic and allelic frequencies, χ2 test values for IGF1 genotypes of Baggara cattle

Baggara subtype (N)	Genotype	N.	GF	Allele	AF	χ2/HWE	He
Nyalawi (65)	CC	56	0.86	C	0.93	0.407ns	0.145
	CT	9	0.14	T	0.07		
	TT	--					
Mesairi (65)	CC	56	0.85	C	0.92	1.085ns	0.146
	CT	7	0.13	T	0.08		
	TT	2	0.02				

*GF: Genotype Frequency, AF: Allelic Frequency, χ2/HWE: Hardy-Weinberg equilibrium χ2 value, Hardy–Weinberg equilibrium ($P>0.05$), He: Gene heterozygosity. ns = not significant ($P>0.05$)

Table 2. Least-squares means for Nyalawi and Mesairi subtypes of Baggara cattle and their IGF1 genotypes weights (kg), hot carcass weight, dressing percentage, and Chi-Square test ($χ^2$) for association between IGF1 genotypes and all traits

Factor	N	Liveweight (kg)	Hot carcass weight (kg)	Dressing %
Overall mean	130	452.9	221.4	49.1
Overall SE±		2.64	1.66	0.17
Breed:				
Nyalawi	65	459.6	234.3	50.9
Mesairi	65	446.3	208.5	47.3
SE±		3.74*	2.35**	0.25**
Genotype:				
CC	112	450.7	220.2	49.2
TT	2	425.0	196.0	46.0
CT	16	463.1	227.0	48.9
SE±		22.77ns	14.32ns	1.51ns
Breed x Genotype Interaction:				
SE±		26.77ns	16.83ns	1.77ns
Association with IGF1				
χ2 (df=2)		3.307ns	3.423ns	0.963ns

ns = not significant ($P>0.05$), * = significant ($P<0.05$), ** = highly significant ($P<0.01$)

Table 3. Least-squares means for Nyalawi and Mesairi subtypes of Baggara cattle, their IGF1 genotypes meat quality traits, and Chi-Square test ($χ^2$) for association between IGF1 genotypes and all traits

Factor	N	MFI (%)	WHC (%)	Cooking loss (%)
Overall mean	130	28.9	2.95	38.0
Overall SE±		0.34	0.05	0.30
Breed:				
Nyalawi	65	31.4	2.6	36.5
Mesairi	65	26.5	3.3	39.6
SE±		0.47**	0.08**	0.38**
Genotype:				
CC	112	28.8	2.9	38.0
TT	2	30.0	3.0	34.7
CT	16	29.4	2.9	34.4
SE±		2.90ns	0.47ns	2.32ns
Breed x Genotype Interaction:				
SE±		3.41ns	0.55ns	2.72ns
Association with IGF1:				
χ2 (df=2)		0.745ns	1.297ns	1.966ns

ns = not significant ($P>0.05$), * = significant ($P<0.05$), ** = highly significant ($P<0.01$)

Table 4. Least-squares means for Nyalawi and Mesairi subtypes of Baggara cattle and their IGF1 genotypes meat chemical composition (%DM-basis)

Factor	N	Moisture (%)	Protein (%)	Fat %	Ash (%)
Overall mean	130	74.1	20.5	1.8	1.0
Overall SE±		0.37	0.17	0.05	0.01
Breed:					
Nyalawi	65	73.4	20.4	2.0	1.0
Mesairi	65	74.9	20.5	1.6	1.1
SE±		0.17*	0.35ns	0.09**	0.02*
Genotype:					
CC	112	74.6	20.2	1.8	1.0
TT	2	75.0	20.0	2.0	1.0
CT	16	74.1	20.5	2.1	1.0
SE±		0.75ns	1.43ns	0.36ns	0.10ns
Breed x Genotype Interaction:					
SE±		0.89ns	1.77ns	0.44ns	0.12ns

ns = not significant ($P>0.05$), * = significant ($P<0.05$), ** = highly significant ($P<0.01$)

However, no subtype x genotype interaction (P>0.0%) was detected on weight and meat quality parameters. No statistically significant (P>0.05) association was found between the IGF1/SnaBI polymorphisms and growth traits (Table 2) or meat quality traits (Table 3). X. F. De la Rosa Reyna *et al* (2010) studying Polymorphisms in IGF-1 gene and its effect on growth traits in Mexican beef cattle found that the SNP IGF1/SnaBI had shown inconsistencies in validation tests to probe its association with growth traits. Nyalawi meat had higher fat (P<0.01), lower (P<0.05) water and ash, and slightly lower (P>0.05) protein contents compared to meat samples from Mesairi subtype (Table 4). However, meat composition found in this study (Table 4) were comparable to those reported by H. A. Elbukhary *et al* (2020) when comparing meat quality and palatability from Western Sudan Baggara Heifers finished on different dietary energy levels. In contrast, I. M. Sharaf Eldin *et al*. (2013) found that bull's meat had higher moisture and significantly higher protein and ash and had significantly lower fat content compared with heifer's meat.

CONCLUSION

Different factors may affect the association of SNPs with traits. The phenotypic data in our study showed that the Baggara breed subtypes Nyalawi and Mesairi are different. Although these sub-types are phenotypically different, the genotypic characterization based on genes under study showed high genetic similarity in SNP at position 2141C>G of the GH1 gene and a significant difference between the two subtypes in SNP C>T of the IGF1 gene. There were non-significant (P>0.05) associations between genotypes and the traits under study. The effect of the mutations in exon five of GH1 and 5- flanking region of IGF1 should be investigated in all subtypes of Sudanese Baggara cattle while also evaluating their interactions with other functional SNPs. More effort in this field is necessary to improve beef quality in Sudanese cattle.

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