

Phenotypic and molecular genetic studies on draughtability in Umblachery breed of cattle

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ABSTRACT

A study was conducted to assess the draught potential of Umblachery cattle of Tamil Nadu. Phenotypic traits related to draughtability and their associations with 16 SNPs in six candidate genes (ACE, ADRB2, BDKRB2, GPX-1, IGF-1 and VEGFA) and five microsatellites in two candidate genes (IGF-1 and VEGFA) were studied in 112 Umblachery bullocks aged 2.5 to 12 years in the breeding tract. The mean body length, chest girth and height at withers were 120.21 ± 0.89 , 151.92 ± 1.04 and 123.94 ± 0.72 cm respectively. The estimated average body weight was 265.64 ± 5.88 kg. Draughtability parameters (in 43 pairs of bullocks) viz. stride length, speed of ploughing and horse power generated during ploughing averaged 1.20 ± 0.21 m, 0.95 ± 0.03 m/s and 0.39 ± 0.04 hp (for an average draught load of 27.5 ± 2.05 kg obtained in this study) respectively. Among the draughtability parameters, only stride length had highly significant positive correlation with all the three morphometric traits. Optimum draft load at which Umblachery bullocks could give uniform and maximum power output was found to be 75 to 78 kg. The mean serum creatine kinase and lactate were estimated to be 52.35 ± 5.04 U/L and 52.87 ± 8.22 mg/dL before work; and 35.92 ± 4.17 U/L and 35.98 ± 1.68 mg/dL after work respectively. Microsatellite locus, VEGFA-(ACAT)n was found to be significantly associated with serum creatine kinase and lactate, with 373/373 and 365/365 bp as favourable genotypes. Microsatellite locus, VEGFA-(GA)n was found to be highly significantly associated with serum lactate alone, with 175/177 and 177/177 bp as favorable genotypes. SNPs ACE 2620 (A>G) and BDKRB2 41872 (G>A) were found to be significantly associated with body weight and creatine kinase respectively, with GG genotypes in both SNPs as favorable. Thus, ACE, BDKRB2 and VEGFA genes could be used as potential candidate markers for selecting Umblachery cattle breed with high draught power.

Keywords: Draughtability, molecular marker, Umblachery cattle

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INTRODUCTION

Draught Animal Power (DAP) is listed as one of the 14 renewable resources of energy in UN Conference on New and Renewable Sources of Energy held in Nairobi, 1981. The recent trend for mechanization though accelerates agricultural production, consumes lot of natural gas resources and fossil fuels. Sastry and Thomas (2005) stated that India's petroleum and natural gas resources may last only for 25 to 35 years and coal for 130 to 140 years. India is a rich source of DAP with 25 out of 40 recognized cattle breeds belonging to draught type. The energy for ploughing two-third of cultivable area and two-third of rural transport are coming from animals (Report, 2008). But, there also seems to be a constant decline in the number of male cattle from 101 million

in 1992 to around 83 million in 2007 (Report, 2012). Shisode *et al.* (2010) estimated that DAP saved 20 million tonnes of petroleum per year. Though extensive studies had been carried out on physical characteristics, work performance and biochemical parameters of work bullocks, reports on genetic improvement of draught cattle and molecular markers related to draught power are scanty. Hence a candidate gene study was undertaken to find out the association of molecular markers (SNPs and microsatellites) in six candidate genes with the draughtability traits in Umblachery cattle, a prominent draught breed of South India.

MATERIALS AND METHODS

The principal morphometric traits like body length, chest girth and height at withers; and draughtability

traits namely, stride length, speed and horse power during ploughing were measured in 112 and 86 (43 pairs) Umblachery bullocks respectively sampled from the breeding tract comprising of Thanjavur, Thiruvavur and Nagapattinam districts. The age of the bullocks varied from 2.5 to 12 years. In addition, physiological parameters viz. pulse rate, respiration rate and rectal temperature were also recorded in animals before and after work. Simultaneously, blood samples were collected from 98 bullocks (both in EDTA and serum vacutainers) before and after work, to isolate the genomic DNA and to estimate the biochemical parameters such as serum creatine kinase and lactic acid using commercially available diagnostic kits (Agappe Diagnostics kit-Catalogue No. 11405007, 11405002 and Bio systems-Catalogue No.735-10 respectively).

The speed of ploughing was calculated by measuring the time taken to cover a particular distance and stride length was measured by dividing the distance covered with the number of strides taken. Horse power generated was estimated with a spring balance by using a modified technique given by Maurya and Devadattam (1982b). Spring balance was used to calculate the pull (in kg) directly. Draught was calculated by multiplying the pull in kg by $\cos\theta$, where $\cos\theta$ is the angle the beam of plough makes with the horizontal ground. Thus, horse power is calculated by $\text{Draught} \times \text{Speed (m/s)} / 75$, where 75 is the constant.

Molecular Genetic Marker Identification

Genomic DNA was extracted using standard Phenol-Chloroform extraction procedure (Sambrook *et al.*, 1989) with slight modifications by using DNAzol reagent. A total of 16 SNPs already identified and reported in the exons and promoter regions of Angiotensin I-converting enzyme (ACE), Adrenergic beta 2 receptor (ADRB2), Bradykinin beta 2 receptor (BDKRB2), Glutathione peroxidase 1 (GPX-1), Insulin-like growth factor-1 (IGF-1), Vascular endothelial growth factor alpha (VEGFA) genes and 5 microsatellites in the promoter and intronic regions of IGF-1 and VEGFA genes were screened and genotyped. Out of sixteen SNPs, ten were genotyped using Tetra-Primer ARMS-PCR and six SNPs were

genotyped using Restriction Fragment Length Polymorphism (RFLP). PCR was performed for 96 samples for the five microsatellite regions and the amplicons were genotyped (Eurofins Genomics, Bangalore).

Statistical Analysis

Using SPSS version 20, phenotypic correlations between morphometric and draughtability traits were calculated by Pearson's formula. General Linear Model (GLM) was used to find the effect of microsatellites in IGF-1 and VEGFA genes on all draught related traits. Association of effects of each SNP with physiological, biochemical and draughtability parameters was carried out by Restricted Maximum Likelihood Method (REML), fitting mixed model equation using WOMBAT programme of Meyer (2011). Additive genetic effect was fitted as random effect. SNP effect was fitted as fixed effect, in addition to the significant fixed effects obtained from GLM.

RESULTS AND DISCUSSION

Morphometric Traits

The overall least-squares means of morphometric traits ($n=112$) of body length, chest girth, height at withers and body weight were 120.21 ± 0.89 cm, 151.92 ± 1.04 cm, 123.94 ± 0.72 cm and 273.4 ± 5.08 kg respectively. Body length and chest girth of Umblachery bullocks obtained in this study, concurred with the observations of Rajendran *et al.* (2008) in 383 Umblachery bullocks (118.7 and 150.9 cm respectively). Whereas, height at withers recorded in this study (123.34 ± 0.72 cm) was higher compared to that observed by the same author (116.8 cm). When compared with Kangayam cattle ($n=102$; considered as the ancestral source for Umblachery breed) having the body length, chest girth and height of 154.8, 184.8 and 146.7 cm respectively (Panneerselvam and Kandasamy, 1999), the Umblachery cattle is shorter. In another study made by Ganapathi *et al.* (2013) in Bargur bullocks ($n=444$), another draught breed of Tamil Nadu, these measurements averaged 126.29, 139.44 and 126.33 cm respectively. The mean of body length, height at withers and chest girth measured in other draught breeds like Hallikar ($n=119$) and Malvi ($n=4$),

respectively by Singh *et al.* (2008) and Singh *et al.* (2009), were 138.94, 134.55 and 163.15; and 157.2, 135.2 and 162.7 cm, emphasizing that Umblachery breed is an outcome of selection for short stature.

Physiological Parameters

The least-squares means of physiological parameters *viz.* heart rate, pulse rate, respiration rate and rectal temperature obtained before and after work in this study were $77.51 \pm 2.44/\text{min}$, $74.02 \pm 2.15/\text{min}$, $23.56 \pm 0.72/\text{min}$ and $38.4 \pm 0.1^\circ\text{C}$; and $90.01 \pm 2.44/\text{min}$, $86.31 \pm 2.19/\text{min}$, $29.18 \pm 1.06/\text{min}$ and $39.3 \pm 0.1^\circ\text{C}$, respectively. A highly significant ($P < 0.01$) difference was observed in the physiological parameters before and after work. Similar increase in physiological reactions after work was recorded by Maurya and Devadattam (1982a), Bhosrekar and Mangurkar (1989) and Singh (2013) in crossbred bullocks; Kumaravelu *et al.* (1997) in Kangayam; Vinoo *et al.* (2010) in Ongole; and Singh *et al.* (2014) in Malvi bullocks.

Biochemical Parameters

The least-squares means of serum creatine kinase before and after work were 52.35 ± 5.04 and 35.92 ± 4.17 U/L, respectively. Serum levels of creatine kinase before and after work did not differ significantly in Umblachery bullocks. These results are inconsistent with the report of Brancaccio *et al.* (2007) that the significant increase of creatine kinase after exercise was lower in trained subjects. However, the lower level of serum creatine kinase after work and insignificant difference before and after work obtained in the present study indicate the better ability of the bullocks to cope with strenuous exercise without marked muscle damage, which otherwise would result in significant increase in the level of serum creatine kinase after work.

The least-squares means of serum lactate before and after work were 52.87 ± 8.22 and 35.98 ± 1.68 mg/dL respectively. Serum levels of lactate were significantly lower after work than before work. This may be attributed to the reason that, as the samples were collected almost immediately after work (which may not in many case be after exhaustion), excess lactate produced in the muscle as a result of anaerobic glycolysis may be effluxed into plasma

from which it is transported to various tissues like RBCs, liver, heart and other skeletal muscle cells where it acts as an important source of energy, resulting in reduced serum lactate levels (Pösö, 2002). It was also stated that monocarboxylate transporter (MCT), a major transporter of lactate, was higher in RBCs of athletic animal species like horse and dog and identified the higher concentration of lactate in RBCs of horses with best individual performance, but not in serum. This might also be a possible reason for lower serum lactate levels after work. However, RBCs, plasma and whole blood all have different values from each other, as there is a marked plasma to RBC lactate gradient during exercise as well as at rest (Goodwin *et al.*, 2007). Hence, to perform individual-to-individual comparisons, Pösö (2002) suggested the use of whole blood lactate values.

Draughtability Traits

The overall least-squares means of draughtability parameters of stride length, speed of ploughing and horse power generated during ploughing recorded in 86 bullocks were 1.20 ± 0.21 m, 0.95 ± 0.03 m/s and 0.39 ± 0.04 hp respectively (for an average draft load of 27.5 ± 2.05 kg). The speed of Umblachery cattle recorded in this study is almost similar to reports on indigenous and crossbred bullocks, that include 0.99 m/s in a pair of Haryana bullocks (Devadattam and Maurya, 1978) and 0.93 m/s in Jersey $\times$ Red Sindhi crossbreds ($n=2$) (Maurya and Devadattam, 1982b); but lower than 1.59 m/sec in Ongole bullocks ($n=55$) (Vinoo *et al.*, 2010). The speed of Umblachery bullocks was also much lesser when compared to Kangayam cattle, exhibiting 1.24 m/s at ploughing (Sree Kumar and Thomas, 1990) and 1.31 m/s at carting (Gogoi, 2012). The stride length recorded was higher on comparison with Kumaravelu (1995) for Kangayam bullocks ($n=20$) on ploughing (0.88 m) and Gogoi (2012) on carting (0.77 m).

The average draft obtained in this study was 27.5 ± 2.05 kg, for which the mean horse power developed by Umblachery bullocks was 0.39 ± 0.04 hp. This was lesser when compared to HF $\times$ Haryana crossbreds (0.75 hp; Gattewar *et al.*, 1989), Kangayam bullocks

Table 1. Phenotypic correlations of morphometric traits with draughtability parameters

Traits	Body length	Chest girth	Height at withers	Stride length	Draft	Speed
Chest girth	0.562**					
Height at withers	0.434**	0.815**				
Stride length	0.316**	0.333**	0.399**			
Draft	0.043	0.116	0.103	0.459**		
Speed	0.137	0.123	0.115	0.437**	0.321**	
Horse power	0.086	0.158	0.133	0.491**	0.974**	0.484**

** -Highly Significant ($P < 0.01$)

(0.73 hp; Kumaravelu, 1997) and Ongole bullocks (0.57 hp; Vinoo *et al.*, 2010). However, power output of an animal is a complex trait that depends on various external factors like draft, speed, duration of work, climate, moisture of the soil, type of agricultural operation, as opined by Bhattacharya and Singh (1987). In addition, human factor plays a major role in exhibition of draught power in bullocks. The lesser horse power in Umblachery cattle obtained in this study could be mainly attributed to the heterogenous environment that existed at field level during recording of draught performance; whereas in the other studies cited above, the draught performances were recorded in organised farms under uniform test conditions with lesser number of samples (less than 10).

Phenotypic Correlations

The phenotypic correlations between morphometric and draughtability traits are given in Table 1. The phenotypic correlations of morphometric parameters revealed highly significant ($P < 0.01$) positive correlations among them. Of all the draught parameters, only stride length had highly significant ($P < 0.01$) positive correlation with all the three morphometric traits. Draught parameters were also highly significantly and positively correlated with each other. Highly significant ($P < 0.01$) positive correlations were also detected between draught

parameters and draught load applied.

Association of SNPs with Biochemical Parameters and Draughtability Traits

Of the 16 SNPs genotyped, only 13 were polymorphic and three were monomorphic. The effects of 13 SNPs on physiological, biochemical and draughtability parameters were analyzed. It was found that only two SNPs had association with draughtability traits (Table 2), one at ACE 2620 (A>G) for body weight and the other at BDKRB2 41872 (G>A) for serum creatine kinase before and after work. This supports our hypothesis that ACE and BDKRB2 genes are associated with draughtability in *Bos indicus* cattle. However, SNPs previously identified in IGF-1 (Gogoi, 2012) as associated with stride length and serum creatine kinase, did not exhibit any association with draught power in Umblachery bullocks, suggesting requirement of a detailed study with more number of samples. This study also indicates that serum creatine kinase can be used as one of the biomarkers to assess the endurance power.

Draughtability of an animal greatly depends on its body weight as it is evident that the draughtability parameter is being expressed in terms of per cent body weight. For the same draft, heavier bullocks moved faster and consequently produced more power compared to bullocks with lesser weight (Premi and Singh, 1987). They demonstrated a linear

Table 2. Association of SNPs with serum creatine kinase and body weight Umblachery bullocks

SNP locus	Serum creatine kinase (U/L)						Body weight		
	Before work			After work					
	Estimate	d.f.	t-value	Estimate	d.f.	t-value	Estimate	d.f.	t-value
ACE A-2620G	8.31 ± 7.54	72	1.101NS	8.49 ± 8.86	46	0.958NS	-9.79±7.22	79	-1.36*
BDKRB2 G-41872A	14.89 ± 5.83		2.556*	15.65 ± 7.18		2.180*	-15.99±6.15		-2.60NS

d.f. – degrees of freedom; * – Significant ($P < 0.05$); NS – Not significant; 'Estimates' refers to partial regression coefficient.

relationship between work output at maximal load and body weight. Kaushik *et al.* (1987), while discussing structural and functional relationship with draught power stated that draught power generated by the bullocks is largely dependent on their body weight especially the amount distributed over their forelimbs (higher chest girth and larger forelimbs). In the current study, in ACE 2620 (A>G), a non-synonymous mutation, was found to be associated ($P<0.05$) with the variation in body weight. Similar studies in human athletic populations revealed an insertion/deletion polymorphism in ACE gene associated with endurance power. Myerson *et al.* (1999) found that athletes with DD genotype have a greater percentage of type II fibres which was associated with power performance. Insertion allele was associated with increase in type I (slow-twitch fibres) muscle fibres responsible for endurance. Association of insertion allele with endurance was demonstrated by Montgomery *et al.* (1998) in mountaineers climbing beyond 7000 m; Myerson *et al.* (1999) in long distance runners (>5000 m) and Grenda (2014) in long distant swimmers.

Association of Microsatellites with Biochemical Parameters and Draughtability Traits

A strong association ($P<0.01$) was detected between VEGFA (GA)_n repeats (found in 13736 to 13755 bp region of VEGFA gene) and serum lactate levels (Table 3). VEGFA (ACAT)_n repeats (found between 13736 and 13755 bp) was identified to have a significant ($P<0.05$) association with both serum lactate and serum creatine kinase levels before work. A similar conclusion as in this study, (association of

VEGFA genes with serum lactate level) was arrived by Ahematov *et al.* (2008) while studying the G-634C polymorphism in human VEGFA gene as they identified the association of 'C' allele with increased maximal oxygen consumption (VO₂max) and decreased lactate level. The possible mechanism of association of serum levels of creatine kinase with VEGFA could be attributed to strenuous exercise damaging the skeletal muscle cell structure at the level of sarcolemma and Z-disks which results in leakage of creatine kinase into the interstitial fluid (Brancaccio *et al.*, 2007). During strenuous exercise, VEGFA level increases as a physiological counteracting mechanism during inflammation to maintain an intact endothelial cell layer (Jee and Jin, 2012). This indicates the involvement of VEGFA gene in increased serum creatine kinase levels during intense exercise.

In the light of above discussion, it could be inferred that the SNPs at ACE A-2620G have a potential influence on body weight; BDKRB2 G-41872A on serum creatine kinase; polymorphisms in VEGFA (GA)_n microsatellite locus on serum lactate; and polymorphisms in VEGFA (ACAT)_n locus on serum creatine kinase and lactate levels, thereby exerting a cumulative effect on draught power in *Bos indicus* cattle. These loci influencing various parameters pertaining to draught power in bovines could be used as candidate markers for selecting a better genotype of Umblachery cattle for agricultural operations in marshy fields of Cauveri Delta regions of Tamil Nadu. This would also strengthen the conservation efforts of Umblachery cattle at stakeholders level, through their expanding utility value.

Table 3. Least-squares analysis of variance for the effects of genotypes of VEGFA (GA)_n and VEGFA (ACAT)_n on biochemical parameters (creatinine kinase and serum lactate) before and after ploughing

Biochemical parameters		VEGFA (GA) _n			VEGFA (ACAT) _n		
		MSS	d.f.	F value	MSS	d.f.	F value
Creatine kinase	Before work	3204.89	2	2.58 ^{NS}	1325.94	5	2.45*
	After work	57.83	2	0.07 ^{NS}	293.56	4	0.39 ^{NS}
Serum lactate	Before work	37550.60	2	11.41**	10416.90	5	2.53*
	After work	22.14	2	0.12 ^{NS}	304.81	5	1.91 ^{NS}

MSS – Mean sum of squares; d.f.- degrees of freedom; ** - Highly significant ($P<0.01$); * - Significant ($P<0.05$); ^{NS} – Not significant

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