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Morphometric Analysis and Associated Genes of Beef Characteristics of Large Ruminants of Pakistan

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ABSTRACT

Pakistan holds various multipurpose large ruminant species well adapted to its climatic conditions. However, lack of a certain breed for beef production to overcome the meat demand. To explore different breeds for beef production, this research was conducted on animals for beef characteristics by using ultrasonography to determine the relationship between live body weight, linear body measurements, and beef parameters associated with beef genes. Different body measurements, growth characteristics, beef parameters, and associated genetic markers of cattle and buffaloes at different age groups were used to analyze the beef potentials of selected breeds. For this purpose, four breeds (02 each for cattle and buffalo) of both genders with different age groups at 12-month intervals were considered. It was found that different body measurements viz. body length, hearth girth, growth characteristics viz. birth weight, body weight, beef parameter significantly increased ($P \leq 0.05$) with the advancement of age but the rate of increment varied for different measurements at different age groups. All the estimates reached the maximum level at the highest age class (above 36 months). In addition, the Sahiwal breed showed higher beef characteristics in terms of growth rate, and development in longissinus muscle areas associated with genetic markers (somatostatin, SST and β-2-Adrenergic receptor, ADRB2) expression as compared to the Dhanni breed while the Kundi buffalo showed comparatively better results than the Azikheli breed. Based on the results, it is concluded that Sahiwal cattle and Azikheli buffalo have higher beef production potentials and could be recommended for beef production.

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Key words
ADRB2, Azi Kheli breed, Beef characteristics, β-2-Adrenergic receptor, Characteristics, Genetic markers, Large ruminants, Kundi breed, Somatostatin

INTRODUCTION

Livestock breeds in Pakistan are known for their multipurpose performances in dynamic climatic conditions and under sub-optimum management and nutrition practices. The wide range of characteristics including milk and meat production and load traction in remote areas, favorable production during drought conditions on average quality nutrition, high immunity, and resistance to tick-borne diseases make these breeds more popular worldwide. In different locations of the country, several breeds of cattle are present which may be milch breed, draft breed, and general utility breed but no specific breed for meat production. Therefore, certain cattle and buffalo breeds of dual characteristics are used for meat production to fulfill the need for protein requirement. The major *Bos indicus* breeds of Pakistan are Red Sindhi, Bhagnari, Lohani, Sahiwal, and Dhani cattle (Khan, 2011) each having distinctive characteristics. Sahiwal is worldwide popular for its physically fleshy body, and tick resistivity, with an average body weight of adult males 400-500 kg and that of females 300-350 kg. Dhani is another dual-purpose medium-weight cattle breed with an average male body weight of 400 kg while females are at 300 kg. Bulls of this breed are known to be having great agility. It is mainly a draught breed and is frequently used for slaughtering due to the desired taste of the meat (Khan, 2011). Similarly, the role of buffalo in the national economy is quite obvious by providing the major commodities. Due to the wide range of characteristics, the Pakistani buffalo is also slaughtered to fulfill the meat requirement. Pakistan has the best buffalo breeds i.e., Neeli Ravi and Kundi (Bilal *et al*., 2006). Kundi breed, the male animal's weight ranges from 500-600kg while

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that of the female ranges from 300-400kg (Bilal *et al*., 2006). The Kundi buffalo has a different genetic makeup as compared to other common breeds, which makes it disease-resistant. Having the heavy weight of Kundi male animals, people like its meat for consumption (Mishra *et al*., 2017). The Azi Kheli breed of buffalo species has an estimated live weight is 350 to 450 kg (Khan, 2011). Their main domestic uses are as draught animals and for the production of milk and meat.

Most of the meat in Pakistan comes from buffaloes, cattle, goats, sheep, chickens, and fish. In Pakistan, the per capita consumption of meat is 16.42 kg, which is much less as compared to the required quantity (GoP, 2016). Meat production in Pakistan is a sideline hobby, the potential of which has not yet been exploited. Therefore, a huge gap is present between requirement and production. In Pakistan, either very young or older animals are slaughtered for consumption. Animals selected for slaughtering are not properly fed and are usually culled at older age. Therefore, the quality of meat and its production is poor in the indigenous breed animals of Pakistan (GoP, 2016). On the other hand, despite rich livestock diversity with huge potential, still, per capita meat consumption is still far beyond standard requirements. A major concern in this regard is primarily the lack of research on breed improvement for meat purposes (Pakistan Economic Survey, 2019). The current research on the mentioned breeds is an initiative to discover the meat characteristics for better production of beef and well-reputed beef breeds in the country. To narrow the meat production and consumption gap, certain conventional and recently adopted mechanisms required for the development of beef breeds should be considered. For example, several methods have been widely adopted to associate various physical parameters with the estimation of meat production of an animal. In this connection, for the assessment of breed for meat production, the ultrasonic use for studying longissimus muscle for predicting meat production has also gained attention worldwide (Saladin, 2015). Certain studies have indicated a positive correlation between the depth and width of these muscles with other beef production parameters of an animal to predict meat production (Bergen *et al*., 2003, 2005; Ragland *et al*., 2014). In addition, morphometric and growth characteristics are the key points in the estimation of beef quality and breeding strategies of cattle species. The body conformation and their measurements are valuable for estimation and judging the meat quality parameters and are also useful for the development of selection procedures for the improvement of different livestock species especially body length and hearth girth (Islam *et al*., 1991). Weight is also a key feature in beef cattle and numerous studies have estimated birth weights and standard weights of 120, 205, 365, and 550 days to determine the final weight (Amaral *et al*., 2014; Araujo *et al*., 2014; Ferreira *et al*., 2015; Passafaro *et al*., 2016). Birth weight plays a key role in a calf's ability to show its genetic makeup. Phenotypic changes with age can be represented by a function of time. Growth is an excellent example of such characteristics (Kirkpatrick *et al*., 1990).

Genotypes and environmental variables affect the growth and development of cattle. The characteristics of an animal breed are its first approach to the sustainable use of animal genetic resources. In tropical countries, meat production is mainly based on cattle and buffalo species. Genomic development can make value in each sector of the meat business (Eenennaam and Drake, 2012). Somatostatin (SST or somatotropin release-inhibiting factor) is known to have inhibitory effects on both endocrine and exocrine secretions, and it is therefore important in metabolism, tissue differentiation, and development (Zhao *et al*., 2018). Adrenergic receptor beta 2 (ADRB2), stimulation leads to the activation of both stimulatory and inhibitory α-subunits of the guanosine triphosphate-activated protein (Cho *et al*., 2015). Hence, these two genes are highly lined with meat quality and quantity. Therefore, the current study was designed and conducted to unrevealed the genetic, morphometric, and growth characteristics of beef quality and quantity in the indigenous breeds of cattle (Sahiwal, Dhani) and buffalo (Kundi, Azi Kheli). For the exploration of beef breeds, this research was conducted on animals for beef characteristics by using ultrasonography to determine the relationship between live body weight, linear body measurements, and beef parameters and confirmation of these characteristics by relevant gene expression. The breed of cattle and buffalo species having beef characteristics can improve the beef industry of Pakistan and further work will provide a recognized beef breed to the country.

MATERIALS AND METHODS

A total of 192 animals, 96 cattle (48 Sahiwal, 48 Dhani) and 96 buffaloes (48 Kundi, 48 Azi Kheli) were selected. The animals selected from each breed were further divided into four age groups, each of 12 animals viz., yearling age group (upto 12 months), young age group (13 to 24 months), middle age group (25 to 36 months) and mature age group (above 36 months). Each group was comprised of 6 amles and 6 females. The animals were screened for deformity, infection, and any other metabolic or pathological infections. All animals were provided uniform feeding and management practices at the herd. Straw and green fodder were offered to the animals as a basal bulk diet with concentrate feeds and premix mixture according to body weight and production. Animals

were dewormed and vaccinated regularly according to prevailing diseases and parasites.

The beef characteristics of selected animals were determined through morphological characters (body length, hearth girth) according to Younas *et al*. (2013) and Mamdouth (2014) growth parameters (birth weight, body weight) according to Beef Improvement Federation Guidelines (2002) beef parameters (longissimus muscle area) according to Yokoo *et al*. (2014) and Crews *et al*. (2003) while expression of beef markers (SST, ADRB2) were determined using the procedure described by Kim *et al*. (2012) and Schimpf *et al*. (2001).

Statistical analysis was carried out by using SPSS software, Version 16.00. A T-test was used for comparison between the two species and breed groups. One-way ANOVA was used for comparison between the age groups in each breed with the level of statistical significance set at $P<0.05$. Significant differences among means were detected using the duncan multiple range test. Expression data of the selected genes derived from the samples of longissimus dorsi muscle of the selected breeds of indigenous cattle (Sahiwal, Dhani) and buffalo (Azi Kheli, Kundi), taken from the slaughter houses were processed using RT-PCR and comparative threshold value (Ct) method. Expression data was normalized against the geometric mean of the expression of selected markers (SST and ADRB2). Excel sheet graphs were used to plot the relative expression of mRNA of the selected genes. All experiments were performed in three biological replicates and relative expression values are used as mean ± SD of normalized Ct values.

RESULTS AND DISCUSSION

Table I reveals the comparative beef characteristics of cattle and buffalo calves. During 1-12 months, the longissimus muscle area (LMA) and heart growth (HG)

Table I. Beef morphometric and growth characteristics of cattle and buffalo breeds for different age classes.

Parameters	Dhani (n=48)	Sahiwal (n=48)	Kundi (n=48)	Azi Kheli (n=48)	P value
Age (up to 12 months)					
LMA (cm^2)	19.00±0.80^{d}	25.27±0.38^{b}	30.08±0.30^{a}	21.50±1.10^{c}	0.01
BL (cm)	120.96±5.23^{a}	108.80±1.12^{b}	68.26±2.03^{c}	69.21±1.93^{c}	0.03
HG (cm)	140.51±6.25^{a}	125.32±0.76^{b}	102.37±1.40^{c}	105.57±1.48^{c}	0.01
BRW (kg)	24.45±0.81^{b}	25.41±0.60^{b}	37.78±1.00^{a}	36.58±1.14^{a}	0.02
BWT (kg)	240.99±8.86^{b}	242.75±20.27^{b}	296.75±7.96^{a}	273.58±5.06^{ab}	0.02
Age (13-24 months)					
LMA (cm^2)	34.45±0.79^{c}	42.10±0.53^{b}	44.54±0.42^{a}	36.00±0.54^{c}	0.03
BL (cm)	124.53±4.25^{a}	115.15±1.93^{b}	87.60±1.13^{c}	88.47±1.12^{c}	0.02
HG (cm)	146.87±4.76^{a}	140.56±1.27^{a}	145.19±2.46^{a}	119.70±1.24^{b}	0.02
BRW (kg)	24.34±0.98^{b}	25.33±0.71^{b}	37.33±1.31^{a}	36.33±1.07^{a}	0.01
BW (kg)	285.72±5.96^{b}	287.67±5.90^{b}	319.67±2.70^{a}	297.33±4.98^{b}	0.01
Age (25-36 months)					
LMA (cm^2)	51.12±0.72^{c}	59.87±0.71^{b}	68.54±0.30^{a}	52.25±0.66^{c}	0.01
BL (cm)	133.00±4.32^{a}	127.64±1.68^{a}	109.33±2.25^{b}	98.63±1.24^{c}	0.03
HG (cm)	163.61±4.57^{c}	185.17±1.27^{a}	173.81±2.51^{b}	148.66±1.34^{d}	0.03
BRW (kg)	24.44±0.63^{b}	25.25±0.61^{b}	36.98±1.25^{a}	35.91±0.84^{a}	0.01
BW (kg)	328.00±11.28^{b}	330.00±11.28^{b}	372.50±5.07^{a}	343.58±12.88^{ab}	0.02
Age (Above 36 months)					
LMA (cm^2)	66.85±0.65^{c}	75.32±0.72^{b}	82.13±0.38^{a}	71.70±1.58^{c}	0.01
BL (cm)	142.73±2.94^{a}	142.24±1.25^{a}	142.96±4.55^{a}	124.88±2.74^{b}	0.02
HG (cm)	170.26±4.82^{b}	205.08±2.00^{a}	206.59±5.37^{a}	197.28±1.84^{a}	0.03
BRW (kg)	24.81±0.80^{b}	25.75±0.69^{b}	38.38±1.12^{a}	37.33±0.87^{a}	0.02
BW (kg)	369.17±11.74	371.17±11.74	388.75±15.69	390.92±14.56	0.55

Means in same row with different superscript are significantly different at α=0.05. LMA, Longissimus muscle area; BL, body length; HG, hearth girth; BRW, birth weight; BW, body weight.

in the Sahiwal breed were found significantly (P<0.05) higher as compared to the Dhani breed while body length (BL) was found inversely. In buffalo, the LMA parameter was higher in the Kundi breed as compared to the Azi Kheli breed in the yearling age group. The same results represent a strong and positive direct additive genetic association between weight and age which suggests that gains in one feature indicate improvements in others. The estimates of the direct additive genetic relationships were greater than 0.70 at most ages, which is according to previous research in Brazil and Africa (Ndofor *et al*., 2012). Birth weight (BRW) and body weight (BW) in the breeds of cattle species and BL, HG, BRW, and BW in the breeds of buffalo species have insignificant (P>0.05) effects in the yearling age group. According to Wasike *et al*. (2009) growth traits are influenced by both direct additive genetic influences and maternal effects. Due to data limitations, maternal effects are often disregarded during genetic studies in tropical breeds. When these effects are significant but are ignored, the genetic parameters are biased upwards, reducing selection efficiency. During 13-24 months, the LMA was (P<0.05) higher in the Sahiwal cattle as compared to Dhani while BL and HG were vice versa. In buffalo, LMA, HG, and BW parameters were higher in Kundi as compared to Azi Kheli. Costa *et al*. (2011) examined the same parameters in Nellore, Canchim, and Angus breeds, and observed greater h^2 for weaning and yearling weights using a multi-trait model. When selecting weights at the four analyzed ages, moderate and similar responses to selection are expected because h^2 were similar and of average magnitude. BRW and BWT in the breeds of cattle and BL, and BRW in the breeds of buffalo have insignificant (P>0.05) effects in the young age group. According to Rosales *et al*. (2004) the average weights for W120, W240, W365, and W450 were 121.6, 183.8, 215.5, and 248.5 kg, respectively. The average weight gains before and after WWG were 0.645 kg (0.124) and 0.288 kg (0.162), respectively. Body weight is now the selection criterion in Brazilian breeding programs, however, if average weight gain is taken into account, animals with higher W120 and W240 values would be preferred. During 24-36 months, the LMA and HG were (P<0.05) higher in the Sahiwal cattle as compared to Dhani. While BL is inverse. In buffalo, LMA, BL, and BW parameters were higher in Kundi as compared to the Azi Kheli breed. Similar research was conducted by Ferreira *et al*. (2015) who reported that estimates of maternal heritability were low for all ages studied. Even at extreme ages, the genetic correlations were modest to high. BRW and BWT in the breeds of cattle and HG, and BRW in the breeds of buffalo have insignificant (P>0.05) effects in the middle-age group. In the opinion of Souza *et al*. (2011) negative correlation values could suggest antagonism between the impacts of genes associated with growth and maternal capacity, which is an essential influencing assignment for optimal calf development. Negative correlations between direct additive genetic effects and maternal effects. During the above 36 months, the LMA was (P<0.05) higher in the Sahiwal cattle as compared to Dhani. While HG was vice versa. In buffalo, LMA and HG were higher in Kundi as compared to Azi Kheli. According to Stelzleni *et al*. (2002) ultrasound provides the capacity to quickly and economically evaluate carcass characteristics of live animals for eventual use in either breeding or terminal programs. BL, BRW, and BW in the breeds of cattle, and BL, BRW, and BWT in the breeds of buffalo have insignificant (P>0.05) effects in the mature age group. Cho *et al*. (2007) explored the relationship between subcutaneous fat thickness in beef cattle and gene polymorphisms; they assessed the prevalence of low A alleles and low AA genotypes. It was stated that some genes were not connected with carcass weight and back fat thickness to establish the relationship between SNP in the FABP3 gene and carcass weight and back fat thickness.

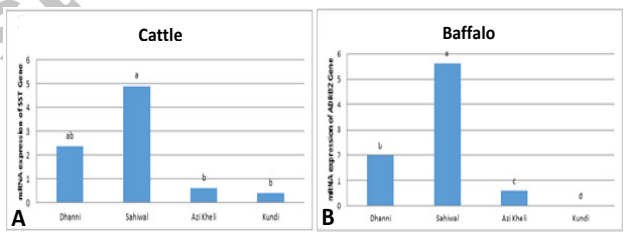

Fig. 1. Expression pattern of SST and ADRB2 gene in the selected breeds of cattle (Sahiwal, Dhani) and Buffalo (Azi Kheli, Kundi).

Figure 1A reveals the expression pattern of *SST* gene for beef characteristics of cattle and buffalo calves. Among breeds, a high level of the *SST* gene expression was noted in the Sahiwal breed followed by the Dhani, Azi Kheli and Kundi breeds respectively. SST has inhibitory effects on both endocrine and exocrine secretions. It is therefore important in metabolism, tissue differentiation, and development (Sheridan *et al*., 2000; Weckbecker *et al*., 2003). The gene is conserved structurally and functionally across mammalian species (Debus *et al*., 2001). The sheep and goat SSTR1 nucleotide sequences reported in GenBank are highly similar, and most of the sequence differences are found in the 3′ -UTR (Zhao *et al*., 2018). Figure 1B reveals the expression pattern (β-2-adrenergic receptor (*ADRB2* gene) for beef characteristics of cattle and buffalo calves. Among breeds, the expression level of the *ADRB2* gene was highly significant (P<0.05)

in the breed Sahiwal followed by Dhani and Azi Kheli. While the Kundi breed shows insignificance expression. A similar research study by Cho *et al*. (2015) that adrenergic receptors play an important role in the physiological adaptation of mammalian cells. Catecholamine interacts with adrenergic receptors (α and β receptors), which stimulate the sympathetic nervous system. These receptors belong to the G-protein-coupled receptor family (McGraw and Liggett, 2005). Activation of G-protein causes the intracellular concentration of the secondary messenger cyclic adenosine monophosphate to increase, resulting in heart muscle contraction, smooth muscle relaxation, and glycogenolysis (Rang *et al*., 2003).

CONCLUSION

It was assumed that in cattle, the Sahiwal breed has the potential for beef characteristics followed by the Dhani breed while in buffalo species, the Kundi breed has beef characteristics followed by the Azi Kheli breed. Based upon satisfactory molecular expression of the Azi Kheli breed, it is recommended to further explore its meat production potential.

DECLARATIONS

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IRB approval

The experimental work was approved by the Advanced Studies and Research Board (No. 744/LM, BandG//UoA) dated 01/07/2022, The University of Agriculture Peshawar, KP, Pakistan.

Ethical statement

The study was approved by the Ethical Committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, before the practical execution of this experiment.

Statement of conflict of interest

The authors have declared no conflict of interest.

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