PCR BASED GENETIC DIVERSITY IN KAJLI SHEEP BREED IN PUNJAB, PAKISTAN USING MITOCHONDRIAL DNA CYTOCHROME-B

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خلاصہ

پنجاب کے تین اضلاع خوشاب، میانوالی اور سرگودھا جہاں کاجلی بھیڑ بکثرت پائی جاتی ہے میں موجود اس نسل کے سائیڈ کروم بی کا مطالعہ کیا گیا۔ اس تحقیق میں جو حیران کن چیز سامنے آئی وہ ان بھیڑوں کی افزائش نسل کے مختلف طریقے تھے۔ ضلع سرگودھا میں یہ جین مکمل طور پر محفوظ پایا گیا۔ جبکہ میانوالی اور خوشاب میں اس میں خاطر خواہ تبدیلیاں نظر آئیں۔ جسکی وجہ ان علاقوں میں پائی جانے والی بھیڑوں کا دیگر نسلوں کے ساتھ باہمی اختلاط تھا۔

Abstract

Main focus is to study the three districts of Punjab as well as the nearby areas where Kajli breed exists. DNA quality and quantity was tested by 1% agarose gel. DNA and amino acid content was also analyzed from the blood samples. Majority of amino acids of different breeds are similar to each other which showed that their origin is similar from Urial. However, Alanine content in sheep located from Sargodha was higher than those located with other areas. . It is suggested that the minute difference in these breeds is due to different kinds of breeding that is done in different areas of Pakistan. Sequence of the cytochrome b gene was visualized by the use of Chromas software. Position of band in similar region showed that all the breed belongs to similar origin. Populations of Mianwali, Khushab and Sargodha regions are clustered into one group. The cluster patterns were concordant with their geographic localities

Key words; Mitochondrial DNA، Cytochrome-B. Kajli sheep breed، PCR applications، Genetic diversity

Introduction

Pakistan is the country blessed with various sheep breeds which are a rich source of meat milk and wool. People of Pakistan like to eat mutton as compared to other meat types like chicken, camel and beef. There are 28 sheep breeds in Pakistan. In Punjab Pakistan sheep occupy the largest population of domestic animals (Akhtar, 1996). Sheep breeding is an important livestock sector, which contributes significantly to the food security, wool industry and welfare of rural households. Sheep and goats together contribute to 44.7% and 19.9% of national income from ruminants and animal production respectively. Consequently, they contribute to 6.4% of national income from agriculture. Furthermore, meat, and milk from sheep and goats together comprise 45.6 and 33.8% of the output of meat and milk of ruminants, respectively (Ministry of planning, 1991). As far as the various breeds of sheep are concerned, Kajli is the most important one. The basic habitat of Kajli sheep is Sargodha, Khushab, Gujrat and certain areas of Mianwali (Ahmed and Rehman, 1996).

Kajli sheep have strong muscular body longer legs as compared to the other breeds. Its wool is white in color. Black circle around the eyes is the unique feature of this breed .This eye circle is the reason why they are called Kajli. (Kajal is the cosmetic that provide thick dramatic line around the eyes (Economic Survey of Pakistan 2013-14). Sheep have long ears and a Roman nose. The tail is short and thin. It has a large size head. Normally most of them are 85 cm in height and 82 cm in length. Weight of male is 55 kg while that of female is 45 kg. The wool yield per head is 3 Kg. The average milk production is one liter per day. They are mostly use for mutton and wool purposes. Because of their strong physical appearance Kajli is better in sale and purchase as compared to the other breeds. Kajli breed of sheep due to its juicy mutton and better wool quality is found to be a better breed in Sargodha, Khushab and Mianwali regions (Khan *et al.*, 2006). This research would play a vital role in future breeding as well as the improvement in the Kajli breed of Pakistan. Genetic diversity is the difference in the genes among the individuals as well as among the species. Genetic diversity is the number of different alleles of all the genes. To study the genetic diversity among Kajli sheep breed provide, Information for the conservation of this breed. Basic concept of this research is to evaluate the genetic diversity among the Kajli sheep breed in certain areas of Punjab Pakistan. Sargodha Khushab livestock stations are of prime importance for this research. Kajli sheep is also found in certain areas of district Mianwali. Cytochrome *b* is one

of the proteins related to the mitochondrial oxidative phosphorylation system and is the only gene in this complex encoded by the mitochondrial genome (Irwin *et al.*, 1991). Cytochrome *b* has been a widely utilized genetic marker to estimate phylogenies on various systematic levels in mammals. Not only does DNA contain the instructions for life-enabling metabolic processes, but this molecule also contains the information determining the characteristics unique to each individual species at a molecular level. Sequence analysis allows for better understanding of the function and shape of the protein for which a gene encodes at macroscopic level. Information concerning the phylogeny, phylo geography, and maternal ancestry can also be derived. DNA sequencing is a powerful tool that has made enormous advancements in the last twenty years. Currently more than 260,000 organisms have at least some section of their genome sequenced and available for comparison in on-line gene Databases (Benson *et al.*, 2008). This has made it possible for researchers across the globe to conduct comparative studies fairly easier and has had dynamic impacts in a number of fields, including population ecology and taxonomy. Another application of this technology is the identification of species from tissue samples, as discussed in this paper.

The real boom of utilization of molecular markers in phylo genetic inference was caused by invention of the polymerase chain reaction (Mullis and Faloona 1987), which enables large scale investigation of the direct source of genetic information and the sequences of DNA.

Phylogenetic inferrence is based on a central concept of correspondence of compared characters due to common ancestry. This correspondence is called homology (Fitch, 1970). More specifically, the compared characters should descend directly from a speciation event, in other words, be orthologous (Fitch, 1970). Although, according to the 'tree of life' concept all organisms share a common ancestor (Woese and Fox, 1977) . This issue is particularly obvious in sequences of nucleic acids, which are the most straightforward source of genetic information for estimation of phylogeny (Salemi and Vandamme 2003, Avise, 2004). Each nucleotide position in a DNA sequence is considered a character, which can attain only four character states, i.e. nucleotides adenine, cytosine, guanine and thymine.

Materials and Methods

Animal Selection: In this study Kajli breed of sheep from Sargodha, Khushab and Mianwali districts were used.
Sample collection: Blood samples of 30 animals up to 5 ml were collected in tubes containing EDTA as anticoagulant.
DNA Extraction: DNA from these blood samples were collected with phenol chloroform extraction method. DNA quality and quantity was tested by 1% agarose gel.

Specific primers were designed from flanking regions of cytochrome b gene from complete mitochondrial genome of *Ovis aries*(AF010406) available on NCBI (http:// www. ncbi. nlm. nih.gov).

Primer set 1	**N6RU102 CBL402**	**CCATAACTATACAAAGCAGCAA** **CCTCAGAATGATATTTGTCCTCA**
Primer set 2	**CBU162** **LTHR**	**CAGGACTATTCCTGGCAATACA** **CCCTTTTCTGGTTTACAAGACC**

Thermo cycler was used for primer optimization and PCR amplification of all samples for Cytochrome b gene. The structure of DNA which is double stranded is denatured at temperature of 94°C to convert it into single stranded DNA which is now called template DNA. Primers are allowed to bind with the template DNA. DNA polymerase is the enzyme which adds nucleotide bases to the end of each primer, according to template DNA to extend the primer to generate new double stranded DNA. This process is repeated for many times to make more strands of DNA for the desired genes.

PCR Conditions

Ingredients	Unit Quantity used/ reaction
PCR Water	15.3 µl
$MgCl_2$	1.2 µl
DNAs	4 µl
10X Buffer	2.5 µl
F. Primer	2.0 µl
R. Primer	2.O µl
DNTPs	0.6 µl
Taq Polmearse	0.4 µl
Total	**25 µl**

Table 1. This table show that kajli breed of different region orginate from Urial if we look at the Domain Data of bases among the sheep of Sargodha, Khushab and Mianwali regions

	Domain: Data																			
	T(U)	C	A	G	Total	T-1	C-1	A-1	G-1	Pos#1	T-2	C-2	A-2	G-2	Pos #2	T-3	C-3	A-3	G-3	Pos #3
Ovis vignei breed Urial (NC 026064.1)	27.7	27.9	31.7	12.7	1140.0	21	25.3	31.6	21.8	380.0	42	23.7	20.3	13.7	380.0	19	34.7	43.2	2.6	380.0
Sgd12	27.3	28.3	31.6	12.8	1140.0	21	25.5	31.8	21.6	380.0	42	23.9	20.3	13.7	380.0	19	35.5	42.6	3.2	380.0
Sgd11	27.1	28.5	31.4	13.0	1140.0	21	25.3	31.6	21.8	380.0	42	23.9	20.0	13.9	380.0	18	36.3	42.6	3.2	380.0
Sgd10	27.3	28.3	31.6	12.8	1140.0	21	25.5	31.8	21.6	380.0	42	23.9	20.3	13.7	380.0	19	35.5	42.6	3.2	380.0
Sgd9	27.3	28.3	31.6	12.8	1140.0	21	25.5	31.8	21.6	380.0	42	23.9	20.3	13.7	380.0	19	35.5	42.6	3.2	380.0
Sgd8	27.3	28.3	31.6	12.8	1140.0	21	25.5	31.8	21.6	380.0	42	23.9	20.3	13.7	380.0	19	35.5	42.6	3.2	380.0
Sgd7	27.1	28.5	31.4	13.0	1140.0	21	25.3	31.6	21.8	380.0	42	23.9	20.0	13.9	380.0	18	36.3	42.6	3.2	380.0
Sgd6	27.3	28.3	31.6	12.8	1140.0	21	25.5	31.8	21.6	380.0	42	23.9	20.3	13.7	380.0	19	35.5	42.6	3.2	380.0
Sgd5	27.3	28.3	31.6	12.8	1140.0	21	25.5	31.8	21.6	380.0	42	23.9	20.3	13.7	380.0	19	35.5	42.6	3.2	380.0
Sgd3	27.3	28.3	31.6	12.8	1140.0	21	25.5	31.8	21.6	380.0	42	23.9	20.3	13.7	380.0	19	35.5	42.6	3.2	380.0
Sgd4	27.1	28.5	31.4	13.0	1140.0	21	25.3	31.6	21.8	380.0	42	23.9	20.0	13.9	380.0	18	36.3	42.6	3.2	380.0
Sgd2	27.1	28.5	31.4	13.0	1140.0	21	25.3	31.6	21.8	380.0	42	23.9	20.0	13.9	380.0	18	36.3	42.6	3.2	380.0
Sgd1	27.1	28.5	31.4	13.0	1140.0	21	25.3	31.6	21.8	380.0	42	23.9	20.0	13.9	380.0	18	36.3	42.6	3.2	380.0
Naemorhedus griseus (KF500173.1)	26.7	28.9	31.4	13.0	1140.0	22	25.5	30.8	21.8	380.0	41	25.0	19.7	14.2	380.0	17	36.3	43.7	2.9	380.0
Mianwali10	27.3	28.3	31.3	13.1	1140.0	22	25.3	31.1	22.1	380.0	42	24.2	20.3	13.7	380.0	18	35.5	42.6	3.4	380.0
Mianwali9	27.0	28.6	31.3	13.1	1140.0	21	25.5	31.6	21.8	380.0	42	24.2	20.3	13.7	380.0	18	36.1	42.1	3.7	380.0
Mianwali8	27.0	28.6	31.3	13.1	1140.0	21	25.5	31.6	21.8	380.0	42	24.2	20.3	13.7	380.0	18	36.1	42.1	3.7	380.0
Mianwali7	27.3	28.3	31.3	13.1	1140.0	22	25.3	31.1	22.1	380.0	42	24.2	20.3	13.7	380.0	18	35.5	42.6	3.4	380.0
Mianwali6	27.0	28.6	31.3	13.1	1140.0	21	25.5	31.6	21.8	380.0	42	24.2	20.3	13.7	380.0	18	36.1	42.1	3.7	380.0
Mianwali9	27.3	28.3	31.3	13.1	1140.0	22	25.3	31.1	22.1	380.0	42	24.2	20.3	13.7	380.0	18	35.5	42.6	3.4	380.0
Mianwali4	27.3	28.3	31.3	13.1	1140.0	22	25.3	31.1	22.1	380.0	42	24.2	20.3	13.7	380.0	18	35.5	42.6	3.4	380.0
Mianwali3	27.0	28.6	31.3	13.1	1140.0	21	25.5	31.6	21.8	380.0	42	24.2	20.3	13.7	380.0	18	36.1	42.1	3.7	380.0
Mianwali2	27.3	28.3	31.3	13.1	1140.0	22	25.3	31.1	22.1	380.0	42	24.2	20.3	13.7	380.0	18	35.5	42.6	3.4	380.0
Mianwali1	27.0	28.6	31.3	13.1	1140.0	21	25.5	31.6	21.8	380.0	42	24.2	20.3	13.7	380.0	18	36.1	42.1	3.7	380.0
Khush10	27.1	28.5	31.5	12.9	1140.0	21	25.5	31.6	21.8	380.0	42	23.9	20.3	13.7	380.0	18	36.1	42.6	3.2	380.0
Khush9	27.2	28.4	31.5	12.9	1140.0	21	25.5	31.6	21.8	380.0	42	23.9	20.3	13.7	380.0	18	35.8	42.6	3.2	380.0
Khush8	27.2	28.4	31.5	12.9	1140.0	21	25.5	31.6	21.8	380.0	42	23.9	20.3	13.7	380.0	18	35.8	42.6	3.2	380.0
Khush6	27.2	28.4	31.5	12.9	1140.0	21	25.5	31.6	21.8	380.0	42	23.9	20.3	13.7	380.0	18	35.8	42.6	3.2	380.0
Khush7	27.2	28.4	31.5	12.9	1140.0	21	25.5	31.6	21.8	380.0	42	23.9	20.3	13.7	380.0	18	35.8	42.6	3.2	380.0
Khush5	27.1	28.5	31.5	12.9	1140.0	21	25.5	31.6	21.8	380.0	42	23.9	20.3	13.7	380.0	18	36.1	42.6	3.2	380.0
Khush4	27.1	28.5	31.5	12.9	1140.0	21	25.5	31.6	21.8	380.0	42	23.9	20.3	13.7	380.0	18	36.1	42.6	3.2	380.0

Khush3	27.1	28.5	31.5	12.9	1140.0	21	25.5	31.6	21.8	380.0	42	23.9	20.3	13.7	380.0	18	36.1	42.6	3.2	380.0
Khush2	27.1	28.5	31.5	12.9	1140.0	21	25.5	31.6	21.8	380.0	42	23.9	20.3	13.7	380.0	18	36.1	42.6	3.2	380.0
Khush1	27.2	28.4	31.5	12.9	1140.0	21	25.5	31.6	21.8	380.0	42	23.9	20.3	13.7	380.0	18	35.8	42.6	3.2	380.0
Pseudois schaeferi (NC 016689.1)	24.7	30.4	31.3	13.5	1140.0	21	25.8	31.3	21.8	380.0	41	24.5	20.3	13.9	380.0	12	41.1	42.4	4.7	380.0
Avg.	27.1	28.5	31.4	13.0	1140.0	21	25.5	31.5	21.8	380.0	42	24.1	20.2	13.7	380.0	18	36.0	42.6	3.3	380.0

TABLE 2

Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution

	A	T	C	G
A	-	*1.23*	*1.29*	**10.07**
T	*1.42*	-	**28.93**	*0.59*
C	*1.42*	**27.51**	-	*0.59*
G	**24.44**	*1.23*	*1.29*	-

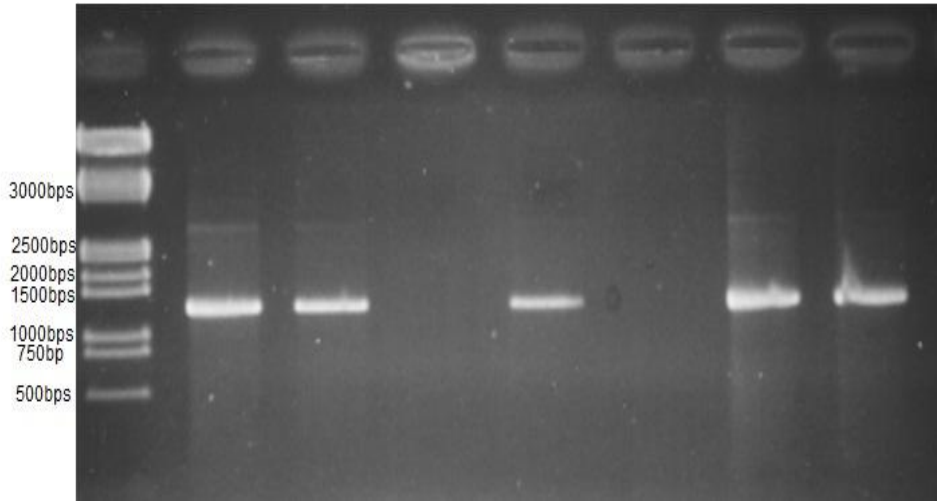

Fig.1. POSITION OF BANDS

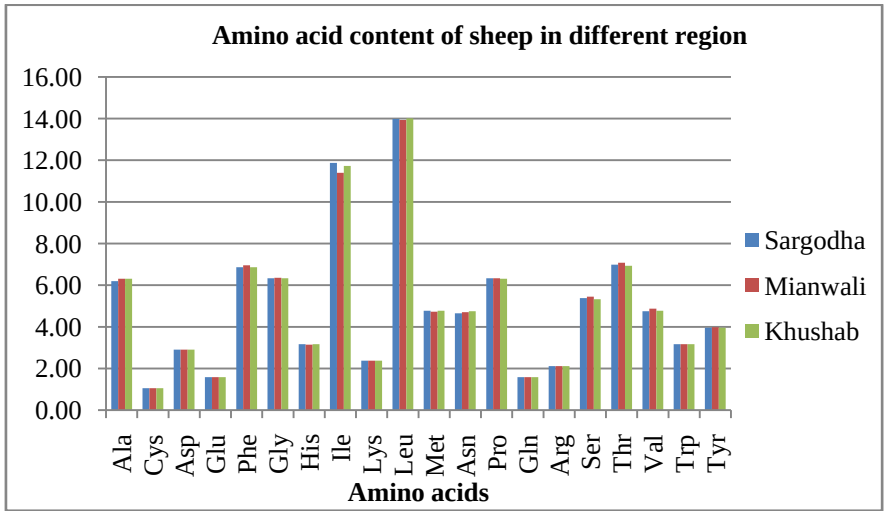

Fig.2

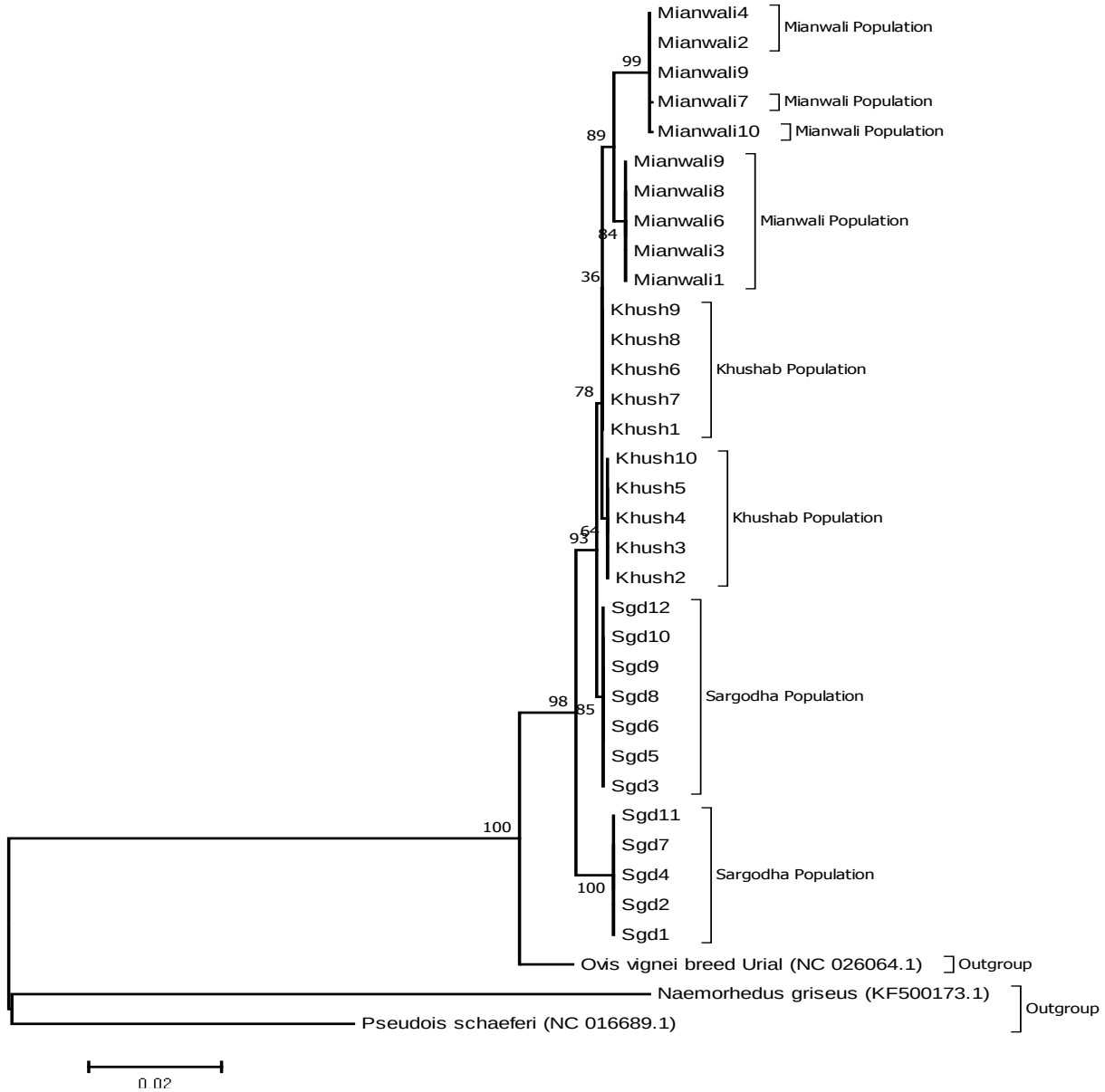

Fig.3

Mianwali show maximum changes due to random mating .

The amplification products as a result of PCR were examined by gel electrophoresis (Labnet. International, Inc. USA) on 1% of agarose gel. Finally the bands were examined using Alpha Innotech gel documentation system, Taiwan. The photograph of the gel was saved and then this PCR product of about 600 bp was sequenced. The PCR products were sent to DNA Core Facility at Centre for Applied Molecular Biology, Ministry of science and Technology, 87-West Canal Bank, Thokar Naiz Baig, Lahore for sequencing where three types of the facilities are provided; DNA Sequencing, DNA Genotyping and DNA Synthesis. Sequences of various species were obtained from Gen bank using NCBI Blast tool. The Geneious software computer package was used to perform the alignment of DN**A** sequences in this region of similarity between species and the regular primers from the literature.

Results and Discussion

The PCR of the, extracted genomic DNA of sheep breed was carried out in order to amplify the desired region of cytochrome b. The results are shown in the fig 1 and 2 position of the bands in similar regions showed that they belong to similar origin.

To demonstrate genetic variability within Kajli populations Mega 6 is used for analysis;

The second neighbor-joining tree shows that these different indigenous populations could be roughly divided into two major groups (Fig. 3). Populations of Mianwali, Khushab and Sargodha regions are clustered into one group. The cluster patterns were concordant with their geographic localities and were also in agreement with our previous studies using microsatellite markers (Li *et al.,* 2002).

Amino acid content was also analyzed from the blood samples to determine either there is any difference among sheep belonging to different areas. Majority of amino acids of different breads are similar with each other which showed that their origin is similar from Urial. However, alanine content in sheep located from Sargodha was higher than those located with other areas. Further, valine and isolucoine content of breed located from Mianwali was higher than those located in other areas.

Conclusion

It is concluded from the analysis that all breeds of Kajli sheep are similar. They might be having similar origin from Urial and were spread all over the Pakistan. The minute difference in these breeds is due to different kind of breeding that is done in different areas of Pakistan. In the areas of Noorpoor, Thal and Quaidabad random mating is common while interbreeding is done in different regions of Mianwali which cause slightly difference in the phenotype of the Kajli sheep.

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