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Short Communication

ASSOCIATION OF B-LACTOGLOBULIN PROTEIN ISOFORMS WITH MILK CONSTITUENTS IN GOAT BREEDS

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ABSTRACT

The composition of milk and its dairy products is strongly influenced by the polymorphic milk proteins especially β-Lactoglubulin (β-LG). Polymorphism of β-LG has been explored in goats due to the unique nutritional value of milk. In order to explore dairy potential of local Beetal and Daera Din Panah (DDP) goats, we aimed to identify β-LG protein isoforms and their association with milk yield and composition. Whey and casein proteins were isolated from raw skim milk by acid precipitation method. Protein and fat contents were estimated through established methods. Polymorphism in β-LG protein was identified through SDS-PAGE. Daily milk yield and total milk fat contents were significantly elevated in Beetal goat. Three β-LG protein isoforms were identified with genotypes; AA, AB and BB. Frequency of A allele was significantly higher in both goat breeds (p=0.0023 and 0.0001) while heterozygous AB genotype was the most prevalent one. In both goat breeds, all three β-LG protein isoforms lacked association with milk yield and composition. Polymorphism exists in β-LG milk protein in Beetal and DDP Pakistani goats with A allele being the dominant one. The β-LG proteins isoforms lacked association with milk yield and its composition.

Key words: Polymorphism, Milk, Proteins, Dairy, Genetics.

INTRODUCTION

Polymorphism in milk proteins of dairy animals has been investigated for more than 40 years since its discovery by Aschaffenburg in 1955 of genetically distinct forms of β-LG from cow milk. It is the most prevalent protein in milk whey of ruminants, comprising 10% of the total milk protein or about 58% of the whey protein (Walstra and Jeness, 1984). Based on protein structure, β-LG belongs to lipocalin protein family comprising small secretory transport proteins with a variety of functions, but the true physiological function of β-LG is still to be resolved (Sawyer *et al*., 1998). In ruminants, native form of β-LG protein is a dimer (MW 36.4 KDa), made-up of 162 amino acids, whereas, in other species it is reported to be predominantly in a monomeric form (Mirella *et al.,* 2003). Regarding genetic architecture of β-LG, bovine, sheep and goat genes share similar positions except one at 6 positions (Strzelec and Ni znikowski 2009; Yahyaoui *et al.,* 2009). Comparison of the amino acid sequence of bovine, ovine, and caprine β-LG shows greater than 95% sequence homology. As compared to extensive polymorphism in cattle, goat whey β-LG protein lacks much of the genetic variability with majority of the variants reported at DNA level without accompanying changes at protein level (Maria *et al.,* 2013). The β-LG protein isoforms have also been reported to affect both properties (Amigo *et al.,* 2000 and Pena *et al.,* 2000) and yield of milk (Ng-Kwai- Hang, 1998). Based on the variations in raw milk composition arising from polymorphisms, β-LG protein isoforms are regarded as useful genetic markers for selection criteria in animal breeding program. However, there still is a large number of goat breeds across the world including Pakistan whose dairy genetic potential still needs to be explored with the aim of finding new β-LG protein variants and their affect on the properties of raw milk.

Goats in Pakistan are mainly reared for meat for both local consumption and export. Beetal is reared throughout Punjab as well as in other parts of country while DDP is specifically found in south Punjab. Both goat breeds are amongst high milk producing animals which could be utilized for the manufacture of export quality dairy products as in Europe. But the dairy potential of indigenous goat breeds is still a neglected or ignored area and so is the exploration of milk protein(s) polymorphism at Molecular level. Keeping in view the importance of milk protein isoforms as potential dairy molecular markers, present study was designed to identify β-LG protein isoforms in Beetal and DDP goat breeds and to explore the associations of identified variants with milk yield and its composition. The information gained will add to the already existing world-wide scientific data on β-LG protein isoforms from this region.

MATERIALS AND METHODS

Samples Collection and Processing of Milk: Two goat breeds, Beetal and DDP, being best dairy animals, were selected for the present study. A total of 400 animals (200 per breed), reared at livestock research stations located in

Khairi moorat (Fateh Jang), Rakh khairy wala (Leiah) and Rakh Ghulaman (Bhakkar), were sampled based on good health and being in mid- lactation period. Approximately 50ml of fresh milk sample from each animal was collected in sterilized bottles during morning milking session. A 20 ml aliquot of each sample was skimmed through centrifugation at 3000rpm (720xg) for followed by refrigeration to remove solidified fat layer. The whey content was separated through acid precipitation of milk casein at its isoelectric point pH 4.5 with 1N HCl (Tsuji and Togamura, 1987) and centrifugation at 3000rpm (720xg).

Estimation of Milk Proteins and Fat Contents: Total milk protein and whey protein contents were estimated using Bradford method (Bradford, 1976) while total fat contents of milk were estimated using Gerber method (Everts *et al.*, 2000).

SDS-PAGE Identification of B-LG Isoforms: To detect polymorphism(s) in β-LG protein, standard SDS-PAGE method (Sambrook and Russell, 2001) was employed with 4% of stacking gel and 15% of separating gel (Bio-Rad system). The finished gels were stained with Coomassie brilliant blue stain and analyzed in a Gel Documentation System under white light. Results were interpreted by visualizing separated protein bands in comparison with purified β-LG of bovine origin as a standard as well as unstained protein ladder (116 KDa to 14.4 KDa (Thermo Scientific, USA), run on gel along with uncharacterized whey samples.

Statistical Analysis of Data: Whey protein isoforms based gene/allele frequencies were estimated and checked for deviation from Hardy Weinberg equilibrium (HWE) using Chi-Square (χ^2) test applied at 5% significance level. Differences in the yield and composition of milk (total protein, total fat and whey contents) among two goat breeds and their association with protein isoforms, was performed using ANOVA available through Statistical Package for Social Sciences software program (SPSS v. 16.). Logistic regression and multinomial logistic regression was applied to explore the relationship/associations between milk yield, its components and BLG protein based genotypes. For this purpose codominent, dominant and recessive models were applied and odds ratios (OD) were estimated with 95% confidence intervals (CI). For analyses SPSS. V. 21 software was used.

RESULTS

The results for the comparison of daily milk yield and composition including, total milk protein, whey protein, caseins and total fats among Beetal and DDP goats are presented in Table 1. The total daily milk yield was significantly high for Beetal goat as compared to DDP goat (p=0.02). With regards to milk composition, there was no significant difference in the contents of total protein (p=0.5), whey protein (p=1.0) and casein protein contents (p=0.4) among both goat breeds. However, total milk fat contents were significantly higher (p<0.001) in Beetal goat milk (4.52±0.41) as compared to DDP (4.18±0.66) goat.

The SDS-PAGE based analysis of whey milk protein revealed existence of polymorphism with three genotypic protein isoforms for β-LG protein (AA, AB, BB) in both Pakistani goat breeds (Figure 1). Homozygous genotypes, AA and BB, were characterized by one major band, while heterozygous genotype was identified as two overlapping, a major and a minor, band. The electrophoretic profile of whey proteins clearly demonstrated that homozygous AA β-LG protein isoforms moved faster as compared to β-LG BB (Figure 1). Gene frequency of heterozygous β- β-LG AB in Beetal and DDP goats was calculated to be about 0.55 and 0.53 (Table 2). Overall, frequency of A allele was significantly higher in both goats (Beetal=0.55, p=0.0023 and DDP=0.59, p=0.0001) as compared to the B allele. Our results clearly demonstrated that the selected populations of both goats were in HWE (χ^2 p values of 0.116 and 0.15 for Beetal and DDP respectively).

The comparison of three β-LG protein isoforms; AA, AB and BB with total daily milk yield (L) and raw milk composition was also carried out (Table 3). The milk protein contents including total protein, whey protein and casein protein as well as total fat contents lacked any significance of difference between two goat breeds (Table 3). In Beetal goats, daily milk yield, total milk protein, whey protein, casein protein, and total fat contents were all raised in heterozygous β-LG AB genotypes as compared to the homozygous AA and BB (Table 3). Whereas in DDP goats daily milk yield and total fat contents were higher in AB and BB genotypes, while all three milk protein fractions were raised in homozygous AA genotype followed by heterozygous AB (Table 3).

Table 4 presents results for the association of β-LG protein isoforms with milk yield and components. Milk yield, total protein and casein protein contents lacked association with any of the BLG genotypes in both goat breeds (p= 0.48, 0.3, 0.14 respectively in beetal and p=0.29, 0.3 and 0.82 in DDP respectively). The heterozygous AB genotype carrier animals had 2.44 times higher whey protein contents (Table 4) in beetal goats. In contrast the 0.58 folds (CI = 0.31-1.08 p=0.03) decrease in whey protein contents was observed in case of heterozygous AB genotype carrier DDP goats. Though of low significance (p=0.045), fat contents were also raised (OR = 1.72 CI = 0.98-3.03) in AB genotype carrier Beetal goats only.

Table 1. Comparison of Milk Parameters among Goat Beetal.

Variables	Beetal	DDP	p-value
	Mean±SD	Mean±SD	
Milk Yield (L/Day)	4.48±0.46	4.37±0.52	0.02
Total Protein (%)	2.46±0.99	2.52±0.93	0.5
Whey Protein (%)	0.36±0.19	0.36±0.19	1.0
Casein Protein (%)	2.10±0.83	2.16±0.77	0.4
Total Fat (%)	4.52±0.41	4.18±0.66	<0.001

Table 2. Genotype and Allele Frequencies of β-LG Protein Isoforms in Goat Breeds.

Genotype Frequency	Goat Breeds	
	Beetal	DDP
AA	0.28	0.33
AB	0.55	0.53
BB	0.17	0.14
p-value	0.116	0.15
	Allele Frequency	
A	0.55	0.59
B	0.45	0.41
p-value	0.0023	0.0001

Table 3. β-LG Protein Genotypes Based Comparison of Milk Parameters in Beetal and DDP Goat Breeds

Milk Constituents	Beetal (Means ± SD)			p-value	DDP (Means ± SD)			p-value
	AA	AB	BB		AA	AB	BB	
Milk Yield (L/Day)	4.3±0.44	4.4±0.54	4.2±0.51	0.299	4.4±0.48	4.5±0.44	4.5±0.46	0.277
Total Milk Protein (%)	2.29±0.99	2.63±0.84	2.53±0.98	0.089	2.6±1.05	2.3±0.94	2.29±1.09	0.160
Whey Protein (%)	0.32±0.19	0.38±0.18	0.36±0.2	0.106	0.39±0.2	0.35±0.18	0.33±0.21	0.206
Casein Protein (%)	1.9±0.84	2.24±0.71	2.17±0.81	0.110	2.25±0.86	2.04±0.78	1.96±0.92	0.168
Total Milk Fat (%)	4.11±0.67	4.2±0.67	4.11±0.61	0.457	4.46±0.42	4.55±0.40	4.50±0.46	0.2

Table 4. Association Analysis of β-LG Protein Genotypes with Milk Parameters in Beetal and DDP Goat Breeds.

Goat Breeds	Milk Parameters	Associated BLG Genotypes	OR (95% CI)	p-value
Beetal	Milk Yield (L/Day)	B/B	0.72 (0.31-1.69)	0.48
	Total Protein (%)	B/B	1.24 (0.53-2.91)	0.3
	Whey protein (%)	A/B	2.44 (1.24-4.83)	0.032
	Casein protein (%)	A/B	1.92 (0.99-3.70)	0.14
	FAT (%)	A/B	1.72 (0.98-3.03)	0.045
DDP	Milk Yield (L/Day)	A/B	1.36 (0.77-2.41)	0.29
	Total Protein (%)	B/B	0.47 (0.18-1.24)	0.3
	Whey protein (%)	A/B	0.58 (0.31-1.08)	0.03
	Casein protein (%)	A/B	0.94 (0.53-1.65)	0.82
	FAT (%)	A/B	1.47 (0.81-2.65)	0.2

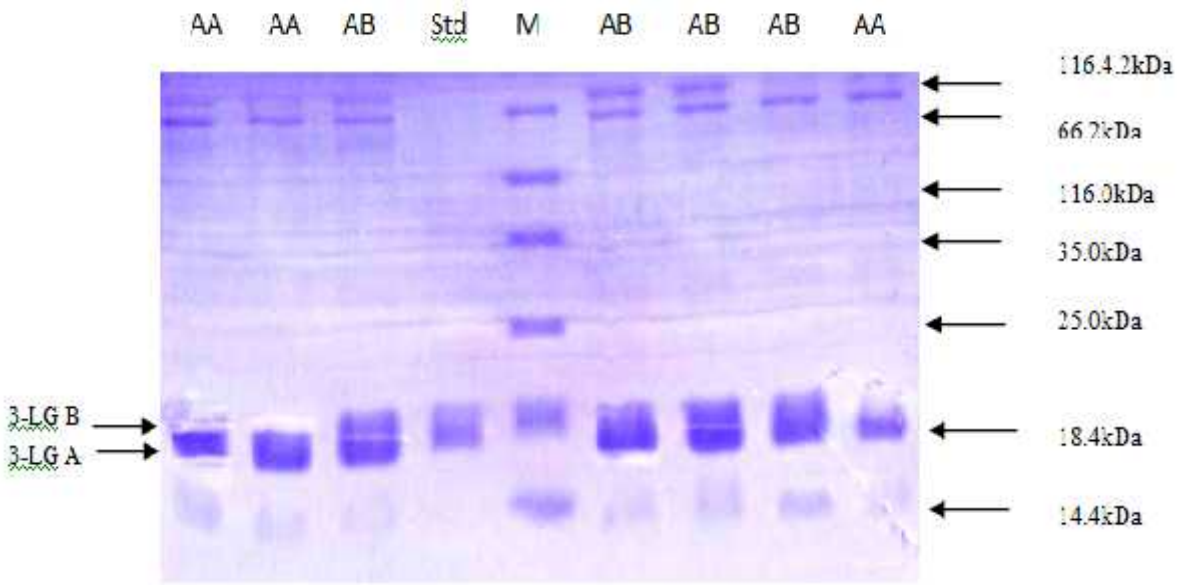

Figure. β-LG Milk Protein Isoforms Resolved on SDS PAGE
Std represents purified β-LG bovine protein (Heterozygous AB) used as a standard
M represent known molecular weight protein markers/protein ladder (116 KDa to 14.4 KDa)
AA symbol represents homozygous AA β-LG protein isoforms with a single lower band
AB symbol represents heterozygous β-LG protein isoforms with two closer bands

DISCUSSION

The study confirmed existence of polymorphism in β-LG milk protein in Beetal and DDP goats of Pakistan. The β-LG milk protein variants, A and B were identified with three genotypes; AA, AB and BB. The frequency of β-LG A allele was significantly high in both goat breeds thus confirming its dominance. All three protein isoforms did not show association with daily milk yield and its composition.

The existence of A and B alleles with three genotypes for β-LG protein have already been reported especially in Alpine and Saanen French goats (Boulanger, 1976) and Jamunapari, Sirohi, Jakhrana and Barbari Indian goats (Kumar *et al*., 2002; Garg *et al*., 2009). Our findings of A allele being the dominant one in Beetal and DDP goats are also in agreement with all the above mentioned studies (Boulanger, 1976; Kumar *et al*., 2002; Mandal *et al*., 2009; Mohanty *et al*, 2013). However, frequency of A allele in our goat breeds was lower (0.55 in Beetal and 0.59 in DDP) in contrast to 0.910 reported for Indian Barbari goats (Garg *et al.,* 2009).

With regards to β-LG protein based, polymorphism several previous studies report homozygous AA genotype being the dominant form in dairy goats (Boulanger, 1976; Kumar *et al*., 2002; Mandal *et al*., 2009; Mohanty *et al*, 2013). In contrast present study reports heterozygous β-LG AB protein isoform being the most frequent genotype in in Beetal and DDP goat breeds. Although high frequency of heterozygous AB genotype in Beetal and DDP could, in part, explain the co-dominance of A and B alleles in our studied animals. However, allele frequency estimations clearly indicate dominance/significantly high frequency ($p= 0.0023$ and $p <0.0001$ respectively) of A allele in both goat breeds (Table 2). Therefore our results are in accordance with previous research on different world goat breeds indicating the dominance of A allele of BLG milk protein in goats (Boulanger, 1976; Kumar *et al*., 2002; Garg *et al*., 2009).

With regards to differences in raw milk parameters based on β-LG isoformsP goats, daily milk yield, milk proteins (Total protein, casein and whey protein), and fat contents were all raised in β-LG AB Beetal goats. While in DDP, daily milk yield rates were higher in both AB and BB β-LG genotyped animals as compared to the homozygous AA. All protein contents were raised in homozygous AA animals while total fat contents were higher in animals heterozygous (AB) for β-LG protein. A higher percentage of total milk proteins could reflect increased casein content in milk with a higher total protein value as compared to the ones with a lesser protein content (Moioli *et al.,* 1998). A positive influence of β-LG AA genotype on milk protein contents and of homozygous BB genotype on the milk fat contents has been featured in a number of past studies (Aleandri *et al*., 1990; Bovenhuis *et al*., 1992). These characteristics strongly influence quality of raw milk and dairy products manufacture as well as its quality (Manfredi *et al*. 2000; Montaldo and Manfredi 2002). Therefore, milk proteins and fat contents are considered as important markers

during animal selection. In present study heterozygous AB BLG protein isoform significantly associated with whey protein contents in both goat breeds ($p<0.05$), however, the effect was opposite. In Beetal goats co-dominance of A and B alleles led to 2.44 times increase in whey protein contents while a decrease of 0.58 times in DDP goats. These contrasting effects of the heterozygous β-LG isoforms have not been reported earlier. Thus findings of present study could be extended to future studies with larger population cohorts, also taking into account the genetic pools of maternal/paternal lineages, to fully explore the dominance patterns of β-LG protein isoforms and their associations with milk production and composition traits. The information gained could be exploited for marker based selection of dairy goats.

Conclusions: Pakistani goats (Beetal and DDP) were found polymorphic for β-LG milk protein locus with abundance of A allele and heterozygous AB genotype. All three β-LG milk protein isoforms lacked statistically significant associations with milk yield and its composition.

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