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Comparative analysis of milk quantity and quality in native goat breeds of Kerala*

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Abstract

Goat rearing occupies an important place in augmenting the economy of our country. In India there are 23 well defined goat breeds and Kerala has two native breeds namely Malabari and Attappady Black, which vary significantly in milk production. Data on quantity of milk was collected from 100 Malabari and 70 Attappady Black goats. There was a significant influence of breed (p≤0.01) and parity (p≤0.05) on milk production. The average milk yield of Malabari goats was, 558.94 ± 4.21 *ml per day, while for Attappady Black it was* 340.22 ± 4.45 *ml per day. Milk samples were collected from 54 Malabari and 35 Attappady black goats and significant difference was observed in fat per cent between two groups. Double digest restriction associated DNA sequencing (ddRADseq) is a modern reduced representation sequencing technique which is a powerful and inexpensive approach for identification of Single Nucleotide Polymorphisms (SNP) across populations. This technique was applied on two groups of Malabari and Attappady Black goats with differing milk production and the preliminary evaluation of results revealed SNPs in five major genes affecting milk production.*

Keywords: *Milk production*, *quantity of milk, Malabari, Attappady Black, fat per cent, ddRADseq, SNP*

Goats are recognised as important components of livestock farming system and it plays a very useful role in giving a steady income to people of economically backward sections throughout a year. India has a goat population of 128 million, which is about 20 per cent of the total global goat population. India is having 23 well recognized goat breeds and Kerala has two native goat breeds namely, Malabari and Attappady Black. Malabari goats have medium sized body with high milk

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production and are reported well adapted to any climatic condition (Verma *et al*., 2009). Malabari goat breed is as the most popular dual purpose breed of Kerala (Bindhu and Ragavan, 2010). Attappady Black goats were reared by tribal community of hilly region of Attappady, Kerala. This breed is predominantly black in colour. It had long drooping ears with medium sized body (Stephen *et al*., 2005). Attappady black goats had less than 200 mL milk production per day (Mathew *et al*., 2005).

In India, annual milk production from goats was reported to be 15.2 million metric tonnes during 2014-15 as per Basic Animal Husbandry and Fisheries Statistics (2015). Goat milk is recommended for infants and convalescent people (Haenlein, 1992). This milk was an alternative source of animal protein for people with cow milk intolerance, and average diameter of individual fat globules in goat milk (2.76) was smaller than of cow milk (3.51) (Tziboula - Clarke, 2003), thus increasing its digestibility.

In the present study, milk quantity and quality were compared between Malabari and Attappady Black breed. Milk quality was compared between breeds during different lactation stages, namely early, middle and late. Double digest Restriction Associated DNA sequencing (ddRADseq) is modern reduced representation sequencing method for identification of Single Nucleotide Polymorphims (SNP) with low cost and high efficiency (Peterson *et al*., 2012). This technique was applied on Malabari and Attappady Black goats and on preliminary analysis of results five SNPs were identified in five major candidate genes affecting milk quantity and quality traits in goats

Materials and Methods

For milk quantity analysis, milk production records from the date of kidding to the date of drying were collected from 100 Malabari and 70 Attappady Black goats from University Goat and Sheep farm, Mannuthy. Animals were in first or second parity maintained at University Goat and Sheep farm, Mannuthy. Milk production per day was calculated for each goat by dividing the total milk production by number of days in milk yield. Milk Samples were collected from 54 Malabari and 35 Attappady Black goats from University Goat and Sheep farm, Mannuthy randomly. Milk samples were collected during early (28-30thday), middle (58-60thday) and late lactation (88-90th day) stages. The milk samples were cooled to refrigeration temperature. After thorough mixing all milk samples were analysed for fat, protein and lactose content immediately using milk analyser (mro scientific instrument) with frequent standardization using Gerber's method (BIS, 1981) for fat, Kjeldahl method (AOAC, 2012) for protein and Lane-Eynon method (BIS, 1981) for lactose.

In Double digest Restriction Associated DNA sequencing, whole genomic DNA digested with two types of restriction enzymes- rare cutter and common cutter. Ligation was done using adaptors, size selection of the products and PCR amplification was performed followed by Illumina sequencing, and further SNP analysis using bioinformatics tools. This technique was applied on two groups of Malabari and Attappady Black goats with differing milk production and the preliminary evaluation results revealed SNPs in five major candidate genes affecting milk production.

Statistical Analysis

Effect of breed and parity on average milk yield was analysed using fixed General Linear Model (GLM) of SPSS V.24. Fixed model used for milk production is given below

$$Y_{ijkl} = \mu + b_i + p_j + h_k + e_{ijkl}$$

Where,

Y_{ijkl} is the milk production measured on $ijkl^{th}$ animal

μ is the population mean

b_i is the fixed effect associated with i^{th} breed (i = 1,2)

p_j is the fixed effect associated with j^{th} breed - parity (j = 1 or 2)

h_k is the fixed effect associated with k^{th} parity-breed (k = 1 or 2)

e_{ijkl} is the random error

Milk quality was analysed using fixed GLM of SPSS V.24.

$$Y_{ij} = \mu + b_i + e_{ij}$$

Where,

Y_{ij} is the milk production measured on ij^{th} animal

μ is the population mean

b_i is the fixed effect associated with i^{th} breed (i = 1, 2)

e_{ij} is the random error

Results and Discussion

Milk quantity analysis

Comparison of milk yield between Malabari and Attappady Black goats revealed that average milk yield of 100 Malabari goats in this study was, 558.94 ± 4.21 mL per day, while for 70 Attappady Black it was 340.22 ± 4.45 mL per day. The least square means with standard error for breed and parity are presented in the Table 1. The results showed statistically significant difference between these breeds in milk production (p≤0.01). Milk production is influenced by genetic and environmental reasons. In the current study, Attappady black goat is having lower milk yield and the reason might be genetic makeup of the animal. Peacock *et al*. (1999) reported that there are various factors affecting milk production including age, breed, body size, udder shape and size, litter size, nutrition, season of kidding, temperature and disease condition. According to their study, non- dairy goat breeds in the tropics had daily milk yield up to 0.5 L, whereas dairy breed of goats, such as Saanen, Alpine, Nubian and Toggenburg produced two-four litres per day. The result obtained in the present study was in accordance with the findings of Verma *et al*. (2009) who stated that the milk yield varied from 0.5 to 1.5 L/day in Malabari goats.

Table 1. Least square means of daily milk yield with standard errors

Factor	Daily Average milk yield (mL)
Breed	**
Malabari (100)	558.94^{b} ± 4.21
Attappady Black (70)	340.22^{a} ± 4.45
p-value	≤0.01

Figures in parenthesis shows number of observations

Means with different superscripts in same column differ significantly (**p-value≤0.01)

In dual purpose goat breed Malabari, breed - parity interaction had significant influence on milk yield (p≤0.05). The milk yield in first parity was 540.740 ± 4.03 mL per day in Malabari and it gradually increased in second parity to 577.130 ± 7.37 mL per day. Crepaldi *et al*. (1999) reported the effect of parity on milk yield and stated that milk yield gradually increased from first to fourth lactation. Similar findings were reported by Idowa and Adewani (2017). The above results in the present study with regard to effect of parity on milk yield were in accordance with findings of Crepaldi *et al*. (1999) and Idowa and Adewani (2017). But in Attappady Black goats, breed – parity interaction had no significant influence on milk yield. The milk yield in first parity was 342.97 ± 5.21 mL per day in Attappady Black, and it decreased in second parity to 337.45 ± 7.22 mL per day. This result disagreed with the results of Crepaldi *et al*. (1999) and Idowa and Adewani (2017). Since Attappady Black is a meat type breed with low milk production, it milk yield did not increase with progress in parity, which might be the reason for no significance breed – parity interaction. In both breeds, parity – breed interaction had significant influence on milk yield ((p≤0.05). The milk yield in first parity was 540.740 ± 4.03 mL per day in Malabari, and 342.978 ± 5.21 mL per day in Attappady Black, which was significantly different. The milk yield in second parity was 577.130 ± 7.37 mL per day in Malabari, and 337.458 ± 7.22 mL per day in Attappady Black, which also shows significantly different. This clearly indicated that Malabari goats had high milk production than Attappady Black goats, in first and second parities, and hence parity – breed interaction had significant effect.

Breed	Parity 1	Parity 2
Malabari (100)	540.740^{aA} ± 4.033	577.130^{bA} ± 7.379
Attappady (70)	342.978^{aB} ± 5.218	337.458^{aB} ± 7.224

Means with different superscript (a - b in rows, A – B in columns) differ significantly in 5% level.

Milk quality analysis

Milk samples were collected from 54 Malabari and 35 Attappady black goats in early (28-30th day), middle (58-60th day) and late (88- 90th day) lactation stages. Significant difference were observed in fat per cent (middle and late stages of lactation), lactose per cent (early, middle and late stages of lactation) and protein per cent (late stage of lactation) between Malabari and Attappady Black goats and the results are given in Tables 2, 3 and 4 respectively. The means of fat, protein and lactose contents in milk of Malabari, Attappady Black goats were within the range of recorded value for dairy goats. Both breeds showed significant variation in fat per cent in early, middle and late lactation. Consistent increase in fat content was observed in Malabari and Attappady Black goats, towards late lactation. Usually a general inverse relationship existed between milk yield and fat test.

Fat

The Malabari goats had 2.07 ± 0.10, 3.82 ± 0.09 and 4.16 ± 0.17 per cent of milk fat content in early (28-30th), middle (58-60th) and late (88-90th) stages of lactation, respectively and the Attappady Black goats had 2.17 ± 0.10, 4.17 ± 0.09 and 4.54 ± 0.09 per cent of fat content. In the present study, the milk fat content differed ($p \leq 0.05$) significantly between the breeds, during different stages of lactation. Anfantakis and Kandarakis (1980) reported higher fat values during early and late lactation, but lower in the middle stage of lactation. Milk fat per cent during early stage of lactation showed no significant difference between breeds, the reason might be comparatively more milk production by Attappady Black goats during early stage when compared to middle and late stages of lactation. Fat per cent showed significant ($p \leq 0.05$) breed differences during middle and late lactation. Current study revealed that Attappady Black breed of goats having lower milk yield, produced higher levels of fat content when compared with Malabari goats and the reason may be attributed to negative genetic correlation between milk yield and milk fat content.

Protein

In the current study, protein per cent in milk varied from 2.52 ± 0.06 to 3.08 ± 0.09 per cent in Malabari and 2.65 ± 0.06 to 3.15 ± 0.09 per cent in Attappady black goats in different stages of lactation. Significant difference in protein content was observed between breeds in late lactation but no significant change was seen in early and middle lactation. Singh and Singh (1980) analysed protein per cent in goat milk and reported an average of 2.9 per cent in early lactation, 3.2 per cent mid lactation and 3.8 per cent in late lactation. According to Kandarakis *et al*. (2001) protein and fat content in goat milk are high during colostrum stage, in early lactation, lower thereafter and rise again markedly at the end of lactation, when production is low. Generally, average per cent of goat milk components was influenced by feed, season, stage of lactation and genetic variation. In the present study, values observed for protein were within the expected range. Since both types of breeds were grown under similar farm condition, genetic variation may be the reason for the observed change.

Lactose

Lactose content showed significant variation between breeds in different stages of lactation. However, the values were within range. Qureshi *et al*. (1981) observed that the protein and lactose per cent of Jamunapari goat milk was 3.8 and 3.9 respectively. Boros (1986) and Simos *et al*. (1991) reported that protein and lactose were fairly constant over the different stages of lactation. According to researchers, lactose content usually remained constant under normal farm conditions.

ddRADseq results

Pooled DNA samples of Malabari and Attappady black goats were outsourced for ddRAD sequencing. Whole genome was digested with restriction enzymes, and after size selection of products, Illumina sequencing was performed. On further analysis using bioinformatics tools, SNPs were identified by tool Venny 3 and preliminary evaluation of results revealed five SNPs in five major candidate genes affecting milk quality and quantity.

Table 2. Least square means of milk fat per cent with standard errors

Group	Fat % Mean ± SE		
	Early	Middle	Late
Malabari (N=54)	2.07^{a} ± 0.10	3.82^{a} ± 0.09	4.16^{a} ± 0.17
Attappady Black (N=35)	2.17^{b} ± 0.10	4.17^{b} ± 0.09	4.54^{b} ± 0.09
(p-value)	≥0.05	≤0.05	≤0.05
Breed	NS	*	*

Table 3. Least square means of lactose per cent with standard errors

Group	Lactose % Mean± SE		
	Early	Middle	Late
Malabari (N=54)	4.41^{b} ± 0.09	4.21^{a} ± 0.05	4.94^{b} ± 0.06
Attappady (N=35)	4.09^{a} ± 0.09	4.35^{b} ± 0.05	4.63^{a} ± 0.06
p-value	≤0.05	≤0.05	≤0.05
Breed	*	*	*

Table 4 Least square means of protein per cent with standard errors

Group	Protein % Mean ± SE		
	Early	Middle	Late
Malabari (N=54)	3.08 ± 0.09	2.56 ± 0.05	2.52^{a} ± 0.06
Attappady Black (N=35)	3.15 ± 0.09	2.65 ± 0.06	2.76^{b} ± 0.07
(p-value)	≥0.05	≥0.05	≤0.01
Breed	NS	NS	*

Figures in parenthesis shows number of observations
Means with different superscripts in same column differ significantly (* p-value<0.05), NS- Non-significant

Major candidate genes affecting milk production from ddradseq

In the initial phase of study, screening of five major candidate genes for SNPs from ddRADseq results was performed.

1. Oxidized Low Density Lipoprotein 1 gene (OLR1)

Downstream variant of this gene c.*4533T>C was observed by ddRAD seq. Oxidized Low Density Lipoprotein 1 played as important role in lipid metabolism. This gene was reported to be involved in fattyacid transport during lactation cycle (Khatib *et al.,* 2006). OLR1 was a major protein, which affected milk production traits in cattle (Komisarek *et al*., 2009). Micro RNA (miRNA) binds with 3'UTR of OLR1 gene and altered expression of gene. Reports indicated that this variation of 3'UTR region was associated with milk production traits in cattle (Wang *et al.,* 2013).

2. Acetyl- Coenzyme A Carboxylase α gene (ACACA)

Intronic variant c.154+8944C>T was discovered by ddRAD seq. Acetyl- Coenzyme A Carboxylase α, the important regulator enzyme for biosynthesis of fatty acids, catalysed the conversion of acetyl-CoA to malonyl-CoA. Gene *ACACA* was found to be markedly up regulated during lactation in cattle (Bionaz and Loor, 2008).

3. Lipoprotein lipase gene (LPL)

An intronic variant c.243+1082C>T was identified in sixth intron of *LPL*, by ddRADseq. Lipoprotein lipase was an enzyme in milk which was responsible for enzymatic lipolysis, *i.e*. the hydrolysis of fatty acids from triglycerides and phospholipids in milk. (Barber *et al*., 1997).

4. GLI Family Zinc Finger 2 gene (GLI2)

Exonic variant c.64T>G of this gene was obtained by ddRADseq. Candidate gene *GLI2* was related with udder, and this gene was responsible for mammary gland development, mammary gland duct morphogenesis, mammary gland alveolus development and epithelial tube morphogenesis. (Marete *et al*., 2018).

5. Butyrophilin gene (BTN1A1)

Upstream gene variant c.-11528G>A of this gene was observed by ddRAD seq. Butyrophilin is a transmembrane glycoprotein, specifically expressed on the apical surface of the mammary epithelial cells during lactation. This protein was mainly associated with milk fat droplets and was assumed to be involved in secretion of fat globules, in mammary epithelial cells (Jack and Marther, 1990).

One SNP each was identified on five major candidate genes affecting milk production in goats, of which one was an exonic variant (*GLI2*), One downstream gene variant (*OLR1*), one upstream gene variant (*BTN1A1*) and two were intronic variants (*LPL, ACACA*).

Conclusion

Comparsion of milk production between Malabari and Attappady Black goats, revealed that average milk yield of Malabari goats was higher (558 mL per day) compared with Attappady Black goats (340 mL per day). Regarding milk quality analysis, milk fat content significantly differed between the breeds, during middle and late stages of lactation. ddRADseq technique was applied on two groups of Malabari and Attappady Black goats with differing milk production and the preliminary evaluation of results revealed SNPs in five major genes affecting milk production.

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