Influence of FASN gene polymorphism on milk production and its composition traits in Murrah buffaloes

Manoj Kumar, Vikas Vohra*, Poonam Ratwan¹, Alka Chopra¹ and A.K. Chakaravarty¹

Received: 08-06-2015 Accepted: 19-08-2015 DOI:10.18805/ijar.7077

ABSTRACT
In present study the effect of genetic polymorphism of Fatty acid synthase (FASN) gene on eight traits related to milk production and its composition in 162 Murrah buffaloes was studied. The traits studied includes 305 days milk yield, lactation fat average (LFA), 305 days fat yield (305LFY), lactation solid not fat average (LSA), 305 days solid not fat yield (305LSY), lactation total solid average (LTSA), 305 days total solid yield (305LTSY) and peak yield (PY). Restriction Fragment Length Polymorphism (RFLP) was used to identify the SNP in a 472 bp PCR amplified product of exon-40 in FASN gene. It was found to be polymorphic with Guanine to Adenine transition and three genotypes namely AA, AG and GG were observed. Allele A was found to be more frequent than G allele. We report for the first time that exon-40 FASN gene is associated with LFA, LTSA and peak yield in Murrah buffaloes. This information can augment future studies to determine the role of bovine FASN gene as a candidate gene marker for a milk fat, total solids and peak milk production.

Key words: FASN gene, Milk fat, Murrah buffalo, PCR-RFLP, SNF, Total solid.

INTRODUCTION
FASN is involved in fat metabolism, and plays a central role in de novo lipogenesis in mammals. Bovine FASN was mapped to chromosome 19 (BTA19) at q22 band (Roy et al., 2001). FASN is a complex homodimeric enzyme that catalyzes the formation of fatty acids of 16 carbon atoms in length from acetyl-CoA and malonyl-CoA in the presence of NADPH (Chakravarty et al., 2004). This synthesis involves a conserved set of chemical reactions for the cyclic step elongation of activated precursors by two carbon units (Smith, 1994). Morris et al. (2007) identified FASN gene as a potential candidate gene for some milk production and polymorphism in various exons of FASN gene were earlier reported to be associated to fat and milk of cattle (Taylor et al., 1998, Biochard et al., 2003, Roy et al., 2006). However, the genetic variations in bubaline species need to be explored.

Thioesterase (TE) domain of FASN gene regulates the termination of fatty acid synthesis (Abe et al., 2009) and thus determine the quality of fat synthesized in milk through early termination of fatty acid chains. TE domain spreads from exon 39-41 within the FASN complex. In recent years, genetic studies have focused on the manipulation of unsaturated fatty acid composition of livestock products which have healthier effects on human metabolism (Taniguchi et al. 2004; Mele et al. 2007; Moioli et al. 2007; Schennink et al. 2008; Kgwatalala et al. 2009). However, scanty literature is available regarding the status of genetic variations in TE domain of FASN gene. Therefore, it is imperative to explore these genetic variations in Murrah buffalo, known for having higher fat than cow milk.

MATERIALS AND METHODS
Population studied and sample size: Sample of Murrah buffaloes were collected from animals maintained at Livestock Farm of ICAR-National Dairy Research Institute, Karnal (Haryana) over a period of 11 years (2004-2014). About 162 random blood samples (approximately 8 to 10 mL) were collected from lactating buffaloes of the Murrah breed.

DNA isolation and Primers: Genomic DNA was isolated from aseptically collected venous blood using the standard phenol/chloroform method with minor modifications (Sambrook and Russel, 2001). Quality check of isolated DNA was done using 0.8% agarose gel electrophoresis and quantification of was done by using Nanodrop. Forward primer P₅’-CTCGCACACCTTCGTAGTG-3’ and reverse primer P₆’-CACGTTGCGGTGGTAGGTAG-3’) having Tₘ of 57.5°C and 57.4°C respectively were used to amplify exon-40 region of FASN.

PCR amplification and genotyping conditions: Amplification of the exon 40 of FASN gene was optimized to get the best possible amplification of the 472 bp product. PCR was carried out in 25 µl reaction volume consisting of 200µM of each dNTP, 5pM of each primer, 1.5mM MgCl₂ and 1.0U Taq polymerase. Amplification was performed using MASTERCYCLER EP with an initial denaturation at 95°C for 4 min followed by 30 cycles of 94°C for 60 sec, annealing

*Corresponding author’s e-mail: vohravikas@gmail.com; ¹ICAR-National Dairy Research Institute, Karnal-132 001, India.
temperature 61°C for 60 sec and 72°C for 60 sec, with a final extension for 10 min at 72°C. All the buffaloes were screened for the presence of FASN gene polymorphism using PCR-RFLP technique. Genotyping was carried out using MluCl restriction enzyme at 37°C for 6 to 8 hrs. Genotyping was evaluated by running a small aliquot of PCR-RFLP product on 2.5% agarose gel. Genotype and allele frequencies were calculated by gene computing method (Falconer and Mackay, 1996).

**Data and statistical analysis:** Data on 305 days milk yield, lactation fat average, 305 days fat yield, lactation solid not fat average, 305 days solid not fat yield, lactation total solid average, 305 days total solid yield and peak yield were recorded and adjusted for non-genetic factors viz. season of calving, period of calving and age group at first calving. Least-squares analysis of fitting constants (Harvey 1990) was used to overcome the non-orthogonality of effects due to unequal and disproportionate sub-class frequencies, model considered was as follows:

$$Y_{ijkl} = \mu + S_i + P_j + b_i AG + e_{ijkl}$$

Where, $Y_{ijkl}$ observation of the $l$th buffalo calved in $k$th age group, $j$th period and $i$th season; $\mu$, overall mean of the traits; $S_i$, fixed effect of $i$th season of calving (winter, summer, rainy and autumn); $P_j$, fixed effect of $j$th period of calving (1-4) i.e. 2004-2006, 2007-2009, 2010-2012 and 2013-2014, respectively; $b_i$ is the linear regression coefficient for $k$th age group of first calving; and $e_{ijkl}$, random error which is normally and independently distributed with zero mean and unit variance [NID (0, $\sigma_e^2$)]. The effects of non-genetic factors viz. season and period of calving and age group of first calving on production and composition traits were adjusted through least squares analysis using significant effects as shown by analysis of variance.

To study the effect of FASN genotypes on adjusted 305DMY, LFA, 305LFY, LSA, 305LSY, LTSA, 305LTSY and PY fixed linear model with SNP genotypes as fixed effects was used. The model considered was as follows:

$$Y_{i} = \mu + G_i + e_i$$

Where, $Y_{i}$ for 305DMY, LFA, 305LFY, LSA, 305LSY, LTSA, 305LTSY and PY of $i$th genotype; $\mu$, overall mean; $G_i$, fixed effect of $i$th genotype (i = 1 to 3 i.e. AA, AG and GG, respectively) and $e_i$, random error is NID (0, $\sigma_e^2$). The differences of least square means of lactation milk yield between subclasses of FASN genotype were tested for significance using Duncan’s Multiple Range Test (DMRT) as modified by Kramer (1957).

**RESULTS AND DISCUSSION**

A 472 bp fragment of exon 40 of FASN gene was successfully amplified in Murrah buffalo (Fig. 1). RFLP test using MluCl restriction enzyme indicated that exon-40 region of FASN gene is highly polymorphic in Murrah breed of buffalo, with the presence of three genotypes namely, AA, AG & GG as shown in figure 1. Heterozygous AG was found to be most frequent with highest genotype frequency of 0.56 and GG allele was found to be rare with least frequency of 0.10 in Murrah buffalo.

![Fig-1: MluCl digested PCR product (472 bp) in Murrah buffalo](image)

Least squares technique was used to adjust the non-orthogonality of the production data. Means, standard errors, coefficients of variation (C.V.) and range of normalized unadjusted data of first lactation 305DMY (kg), LFA (%), 305LFY (kg), LSA (%), 305LSY (kg), LTSA (%), 305LTSY (kg) and PY (kg) traits of Murrah buffaloes are presented in Table 1 and there least-square means along with standard errors are presented in Table 2. The coefficient of variation varied from 2.6% (LSA) to 26.6% (305 LFY) however, majority of the production traits showed moderate CV this could be due to the selection operating in the herd. The LSA, LTSA and LFA showed higher consistency. Season of calving had no effect on the studied traits, whereas period of calving significantly affected the LFA and LTSA at p<0.01 and remaining traits showed non-significant effect of period. Age

**Table-1:** Means, standard errors, coefficients of variation (CV) and range of normalized unadjusted production traits in Murrah buffaloes (N=162)

<table>
<thead>
<tr>
<th>Traits</th>
<th>Mean± S.E.</th>
<th>Range</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>305 DMY</td>
<td>2065.76±41.29</td>
<td>2905</td>
<td>25.57</td>
</tr>
<tr>
<td>LFA</td>
<td>8.21±0.04</td>
<td>3.23</td>
<td>6.36</td>
</tr>
<tr>
<td>305 LFY</td>
<td>169.87±3.54</td>
<td>253.59</td>
<td>26.55</td>
</tr>
<tr>
<td>LSA</td>
<td>9.74±0.02</td>
<td>1.97</td>
<td>2.15</td>
</tr>
<tr>
<td>305 LSY</td>
<td>201.06±4.00</td>
<td>291.67</td>
<td>25.45</td>
</tr>
<tr>
<td>LTSA</td>
<td>17.69±0.04</td>
<td>2.99</td>
<td>2.54</td>
</tr>
<tr>
<td>305 LTSY</td>
<td>365.39±7.33</td>
<td>514.95</td>
<td>25.65</td>
</tr>
<tr>
<td>PY</td>
<td>11.29±0.20</td>
<td>16</td>
<td>22.45</td>
</tr>
</tbody>
</table>

305DMY (kg) = lactation milk yield, LFA (%) = lactation fat average, 305LFY (kg) lactation fat yield, LSA (%) = lactation average solid not fats, 305LSY (kg) = lactation solid not fat yield, LTSA (%) = lactation total solid average, 305LTSY (kg) = lactation total solid yield, PY (kg) = peak milk yield.
Genotypes of exon-40 of FASN gene significantly influenced the lactation fat average (LFA) at p<0.01, lactation total solid average (LTSA) at p<0.01, peak yield (PY) at p<0.05 and others traits are unaffected by effect of genotype in studied population of Murrah buffaloes (Table 2).

Therefore, the genetic variation present in the coding region of FASN gene had guanine to adenine transition. In Murrah breed FASN genotypes were found to be associated with peak milk production, lactation milk fat average and total solids. This indicates that TE domain not only affect the quality of fatty acids but also affect the milk production and its composition traits. This is the first report of SNP in TE domain of FASN gene and its association with production traits in buffaloes.

**CONCLUSION**

Results suggest that exon 40 (TE domain) of FASN gene is highly polymorphic in Murrah buffaloes. Significant association of FASN gene was observed with lactation fat average, lactation total solid average and peak milk yield. Moreover, sufficient genetic variability is present in this gene in contrast to other well-known candidate genes in Murrah buffaloes for fat percentage like DGAT which is reported to be fixed in Murrah buffalo. This G to A transition can be used further as SNP markers, which could be helpful to breeders for carrying out future association studies and selecting superior Murrah buffaloes for higher fat percentage.

**ACKNOWLEDGEMENTS**

Authors thank the Director cum Vice Chancellor NDRI and Director, NBAGR, Karnal (Haryana) for providing the necessary facilities and Education division of ICAR for providing financial support. Thanks to In-charge LRC, NDRI for their help. Authors thanks to Dr. R.S. Kataria and Dr. S.K. Niranjan (BG lab, NBAGR) for providing primers in this study.

**REFERENCES**


