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# Identification of quantitative trait loci for fat percentage in buffaloes

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ABSTRACT

The milk fat percentage records of 2174 daughters belonging to 12 half sib families were analyzed for the identification of QTLs on 8 chromosomes in buffaloes using chromosome scans. The single marker analysis revealed 49 markers to be associated with milk fat percentage in 10 sire families. The interval mapping using R/qtl identified 43 QTLs on 8 chromosomes of buffalo. The meta-QTL analysis was carried out to define consensus QTLs in buffaloes and total 28 meta-QTL regions could be identified for milk fat percentage. Most of the QTLs identified in the experiments have been reported for cattle; however, few new chromosomal locations were also identified to be associated with fat percentage in buffaloes. The additional QTLs identified in buffalo may be due to high level of heterozygosity in buffalo compared to Holstein Friesian and other exotic milk breeds for which QTLs have been reported. Assuming buffalo-cattle synteny, a total of 1118 genes were identified underlying the QTL regions, out of these 45 genes were identified to be associated with lipid metabolism. The interaction among the genes and gene ontology analysis confirmed their association with lipid metabolism. These 45 genes have potential to be candidate genes for milk fat percentage in buffaloes and underlie the QTL regions identified in buffaloes in the present study.

**Key words**: Buffaloes, Candidate genes, Milk fat percentage, QTLs

It is an established fact that milk fat percentage in buffalo is higher than in cattle. The composition differences in milk of cow and buffaloes get reflected in the physico-chemical properties and also the preference of the consumers. In India even the price of the milk is determined on the basis of fat percentage. The premium price of buffalo milk is due to its milk solids content, milk fat, milk viscosity, superior status for dairying manufacturing sector who prepare milk products using traditional methods. The analysis of fat percentage in buffaloes have been shown to exhibit genetic variation with moderate heritability. The fat in milk includes the short and medium chain fatty acids which have moderate to high heritability while the fatty acids derived from the endogenously produced lipids have low to moderate heritability (Krag *et al.* 2013, Stoop *et al.* 2008, Soyuert *et al.* 2007). The short and medium chain fatty acids are synthesised in the mammary gland. There are large differences between cattle and buffaloes in fat percentage and composition of fatty acids. The polymorphisms in DGAT1 and ABCG2 have been reported to be fixed in buffaloes (Tantia *et al.* 2006). However, it is very likely that the same genes are responsible for the biosynthesis of fatty acid profile in buffalo and cattle but the processes involved may be slightly different (Bouwman *et al.* 2014).The purpose of this study was to identify the chromosomal regions associated with the fat percentage in buffaloes. Owing to small sire family size in buffaloes at the organised farms in India (Annual Reports of AICRP on buffaloes 2013–14, 2014–15 and 2015–16), there are no reports of QTLs identification in buffaloes for fat percentage and this is the first attempt to identify them.

## MATERIALS AND METHODS

The reference family of buffaloes (animals of known pedigree and having both genotype and phenotype information) was created for the work (Vijh *et al.* 2013, 2018). The accuracy of the paternity records was authenticated using a set of DNA markers and only the daughters with confirmed paternity were recorded for phenotypes (Vijh *et al.* 2014). The daughters belonging to 12 half sib families were recorded for fat percentage during three stages of lactation. The test day fat record was generated by mixing the milk of morning and evening and then estimating the fat percentage of milk through an automated milk analysis system.The mean fat percentage of the three records was utilised for the analysis. The genotype data on 8 chromosomes of these animals was generated and has been reported (Vijh *et al.* 2013, 2018).

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The single marker analysis of data was carried out using QTL cartographer software. For the identification of QTLs, the software R/qtl was utilised (Broman and Sen 2009). It has been implemented as an add-on package to the general statistical software R. The data generated fits into half sib design and the method used for the analysis of data was backcross which is the simplest possible experimental cross. The QTL data was prepared as per the requirement of the software, i.e. three interrelated data structures: the phenotypes, the genotypes and the marker map. Thus, total 12 data set were created and analysis was carried out family wise. For a single QTL model, the standard interval mapping was utilised which uses maximum likelihood estimation under a mixture model, while other algorithm Haley-Knott regression methods use approximations to the mixture model. The third method utilised was multiple imputation which uses the same mixture model but with multiple imputation in place of maximum likelihood. The LOD score indicated the evidence for the presence of a QTL, with larger LOD scores corresponding to greater evidence. We utilised the permutation test (Churchill and Doerge 1994) with 1000 replicates and calculated p-value as the proportion that meet or exceed a particular observed LOD score. The multiple QTL models as implemented in R/qtl were used as they have increased power to detect QTL, better separation of linked QTLs and defining epistatic interactions. We fitted the full QTL model using the *fitqtl* function of R/qtl package and this also included the interaction among the QTLs. The ANOVA table indicates the overall fit of the model. The LOD score obtained was relative to the null hypothesis of no QTL. The drop one QTL model was utilised to see the effect of each QTL and its related interactions and thus provides support for the individual terms in the model.

QTL meta-analysis was utilised to synthesize QTL information from 12 independent half sib family analysis results and to refine the chromosomal region involved in trait variation control as implemented in Biomercator software v3.0. The QTL meta-analysis algorithm developed by Goffinet and Gerber (2000) was used. We fitted five models, the most likely QTL arrangement, assuming a Gaussian distribution, was determined by means of the maximum likelihood method and an Akaike-type statistical criterion provided information about the best fit amongst five models. For this model, consensus QTL positions were determined as the mean of QTL distribution maximizing the likelihood along with confidence interval.

Once the meta-QTL regions with their confidence interval were known, webserver Annot QTL (http: // annotqtl.genouest.org) was utilised for the identification of genes underlying the QTL region. Each meta-QTL region was taken as an input. We considered the Buffalo—Cattle synteny for the work as reported by Amaral *et al.* (2008). This provided a list of genes underlying the identified QTLs in buffaloes as Ensembl IDs and also provided a list of human genes assuming the synteny between cattle and human. The genes were then mapped using Reactome database (https://reactome.org) (Croft *et al.* 2014, Fabregat *et al.* 2016) to identify the genes related to lipid metabolism in cattle. We downloaded the Uniprot IDs associated with the identified genes. The Uniprot ID of these genes were used as input for the webserver STRING (https: //string-db.org) (Szklarczyk *et al.* 2015) to obtain network of predicted association for a particular group of proteins/ genes. The gene ontology terms obtained from STRING web server were enriched for the three components using another web server REViGO (http: //revigo.irb.hr) (Supek *et al.* 2011)

## RESULTS AND DISCUSSION

The fat percentage in buffaloes gives it premium over cattle milk. The fat percentage is a quantitative trait and follows a near normal distribution and controlled by large number of genes. The milk fat consists of large number of fatty acids with carbon chains of different length, but primarily categorized into short chain fatty acids and long chain fatty acids. The study of the genotype based on molecular markers (microsatellites) and the phenotype data and their associations can help determine the number and nature of QTLs controlling the fat percentage in buffaloes. To detect the association between microsatellites and fat percentage, different data analysis approaches can be used which include the single marker analysis as well as interval mapping. As the fat percentage was recorded on daughters of 12 sires, the analysis was also carried out family wise. There were 2,174 fat records with a mean of approximately 182 records per sire family. The number of daughters recorded for each sire, the mean, minimum and maximum fat percentage during the first lactation is given in Table 1.

The single marker analysis is simplest of regression analysis and does not require complicated models/software. It does not require the linkage map to be available for the species (Collard *et al.* 2005). In the present half sib family analysis we tested for linkage of a marker to a QTL and the evidence of linkage was measured by a LOD score representing likelihood ratio. The hypothesis that there is a QTL at the marker as compared to the hypothesis of no

Table 1. Sire wise distribution of daughters with milk fat percentage records, mean, minimum and maximum fat percentage in each sire family.

| Sire | No. of daughters | Mean±SE | Min fat% | Max fat% |
|---|---|---|---|---|
| Sire1 | 292 | 7.85±0.11 | 3.51 | 13.6 |
| Sire2 | 161 | 7.98±0.16 | 3.34 | 13.61 |
| Sire3 | 86 | 7.51±0.17 | 3.31 | 12.36 |
| Sire4 | 207 | 7.94±0.13 | 3 | 12.88 |
| Sire5 | 264 | 7.67±0.12 | 3.49 | 12.85 |
| Sire6 | 71 | 7.61±0.23 | 3.99 | 13.27 |
| Sire7 | 327 | 7.97±0.10 | 3.54 | 13.91 |
| Sire8 | 264 | 7.98±0.11 | 4.12 | 14.89 |
| Sire9 | 219 | 7.84±0.12 | 3.5 | 15.79 |
| Sire10 | 159 | 7.92±0.13 | 4.51 | 12.4 |
| Sire11 | 77 | 7.85±0.20 | 4.32 | 11.55 |
| Sire12 | 47 | 7.53±0.25 | 4.42 | 12.97 |

QTL anywhere in the genome.The analysis of 12 half sib families revealed markers which were significant. The LOD score and $R^2$ values are given in Table 2.

No markers in sire family 6 and sire family 12 were significant. Total 49 markers were significantly associated with the phenotype of fat percentage in rest of 10 sire families. Single marker analysis is considered inferior to two marker analysis by several research workers (Haley and Knott 1992, Knott and Haley 1992, Kao 2000, Rebai *et al.* 1995). However, Coffman *et al.* (2003) reported that intersection tests for single marker QTL analysis can be more powerful than two marker QTL analysis (reviewed in detail by Doerge *et al.* 1997).

Table 2. Significant QTL of fat percentage for 12 half sib families using single marker analysis

| Sire No. | Chromosome BTA | Marker name | LOD | $R^2$ | P (F value) |
|---|---|---|---|---|---|
| Sire1 | 1 | BMS4037 | 6.362 | 0.016 | 0.012* |
| | 4 | BMS1788 | 6.559 | 0.018 | 0.011* |
| | 4 | DIK4854 | 6.804 | 0.019 | 0.010** |
| | 14 | BM8215 | 6.286 | 0.024 | 0.013* |
| Sire2 | 1 | BMS2321 | 6.363 | 0.030 | 0.012* |
| | 2 | BMS1987 | 6.052 | 0.036 | 0.015* |
| | 6 | DIK5153 | 3.947 | 0.042 | 0.049* |
| | 7 | BB719 | 3.990 | 0.027 | 0.048* |
| Sire3 | 3 | BM3020 | 4.532 | 0.051 | 0.036* |
| | 6 | DIK5153 | 4.665 | 0.075 | 0.033* |
| | 7 | BB719 | 5.392 | 0.046 | 0.022* |
| | 14 | BM6425 | 4.925 | 0.037 | 0.029* |
| Sire4 | 2 | BMS356 | 5.300 | 0.042 | 0.022* |
| | 4 | TGLA116 | 6.321 | 0.025 | 0.012* |
| | 6 | BM4311 | 4.018 | 0.017 | 0.046* |
| | 7 | DIK2819 | 4.024 | 0.017 | 0.046* |
| | 7 | BMS522 | 6.115 | 0.014 | 0.014* |
| Sire5 | 3 | DIK4116 | 7.022 | 0.016 | 0.008** |
| | 4 | BMS1074 | 5.911 | 0.019 | 0.016* |
| | 7 | DIK4204 | 5.434 | 0.019 | 0.020* |
| | 7 | BMS2258 | 4.813 | 0.008 | 0.029* |
| Sire7 | 1 | BMS4048 | 5.137 | 0.015 | 0.024* |
| | 3 | DIK1057 | 4.674 | 0.016 | 0.031* |
| | 4 | BMS1788 | 8.094 | 0.010 | 0.005** |
| | 7 | BMS522 | 7.219 | 0.026 | 0.007** |
| Sire8 | 1 | BMS4011 | 5.913 | 0.026 | 0.016* |
| | 2 | BMS803 | 6.984 | 0.026 | 0.009** |
| | 3 | BM3020 | 4.493 | 0.021 | 0.035* |
| | 3 | DIK5085 | 5.029 | 0.017 | 0.026* |
| | 4 | BMS1788 | 5.637 | 0.010 | 0.018* |
| | 4 | BMS827 | 11.252 | 0.025 | 0.001*** |
| | 4 | BMS1237 | 13.520 | 0.045 | 0.000*** |
| | 4 | DIK4373 | 4.819 | 0.000 | 0.029* |
| | 6 | DIK5285 | 4.911 | 0.020 | 0.027* |
| | 6 | BM1329 | 5.331 | 0.023 | 0.022* |
| | 9 | DIK2810 | 7.824 | 0.040 | 0.005** |
| | 9 | TGLA261 | 4.010 | 0.010 | 0.046* |
| | 14 | BMS2055 | 18.736 | 0.065 | 0.000**** |
| Sire9 | 3 | DIK2511 | 4.220 | 0.012 | 0.041* |
| | 14 | CSSM066 | 7.101 | 0.025 | 0.008** |
| Sire10 | 1 | TGLA49 | 5.704 | 0.044 | 0.018* |
| | 3 | BM3020 | 4.609 | 0.025 | 0.033* |
| | 6 | MNB-66 | 5.280 | 0.033 | 0.023* |
| Sire11 | 1 | BMS4011 | 7.634 | 0.092 | 0.007** |
| | 1 | DIK2189 | 5.073 | 0.048 | 0.027* |
| | 3 | BM3020 | 5.284 | 0.058 | 0.024* |
| | 9 | BMS2063 | 3.986 | 0.044 | 0.050* |
| | 14 | DIK5082 | 6.062 | 0.073 | 0.015* |
| | 14 | BM1577 | 4.086 | 0.091 | 0.047* |

Signif. codes: ***0.001, **0.01, *0.05.

In the single marker analysis, different markers were identified in different sire families and this can be attributed to heterozygosity of the sire at marker loci. All the sires may not be heterozygous for all the loci and QTLs can only be identified in sires which are segregating. However the primary disadvantage of the single marker analysis is need to omit the individuals with missing marker information. Such a situation was not encountered in present analysis since all the markers in all the individuals of the 12 families had marker genotypes. The second notable shortcoming is that one cannot inspect the position between the markers and one receives only a poor information about the position of QTLs. We thus also applied interval mapping and multiple QTL models for analysis.

The interval mapping as implemented in R/qtl was carried out using the Haley-Knott regression and extended Haley-Knott regression method to fit the multiple QTL model. The chromosome scans of all the 8 chromosomes studied in the experiments revealed QTLs for fat percentage in buffaloes. The details of the sire families, detected QTLs, their LOD score, percentage of the phenotypic variance explained and level of significance is depicted in Table 3. The interaction among the QTLs whereever significant are also mentioned. The representative chromosomal scans are depicted in Fig. 1(a–b).

A total of 43 QTLs were associated with fat percentage in the 12 half sib families analysed. One sire family did not reveal any significant QTL or a probable QTL. One half sib family (sire 11) however revealed only one probable QTL. Seven QTLs were interacting among themselves in these families; out of which, six interactions were statistically significant and one was probably interacting. The interacting QTLs and their locations are also provided (Table 3).

The positions of the QTL regions may differ from family to family and need to be further analysed to find consensus regions. The meta-analysis of the identified QTL regions for fat percentage in buffaloes was carried out on QTLs on 8 chromosomes of buffaloes identified using interval mapping. The selection of the model from the 5 models tested for the analysis was based on Akaike Information Content. The meta-analysis of the QTLs across 12 families of buffaloes are depicted in Table 4. The table depicts the chromosome number, Meta-QTL location and their confidence interval.

Four meta-QTL regions were identified for BTA chromosome equivalents (Amaral *et al.* 2008) in buffalo chromosomes 1, 3, 4 and 6 while 3 meta-QTLs were identified on 4 chromosomes of buffaloes (BBU2q, BBU9,

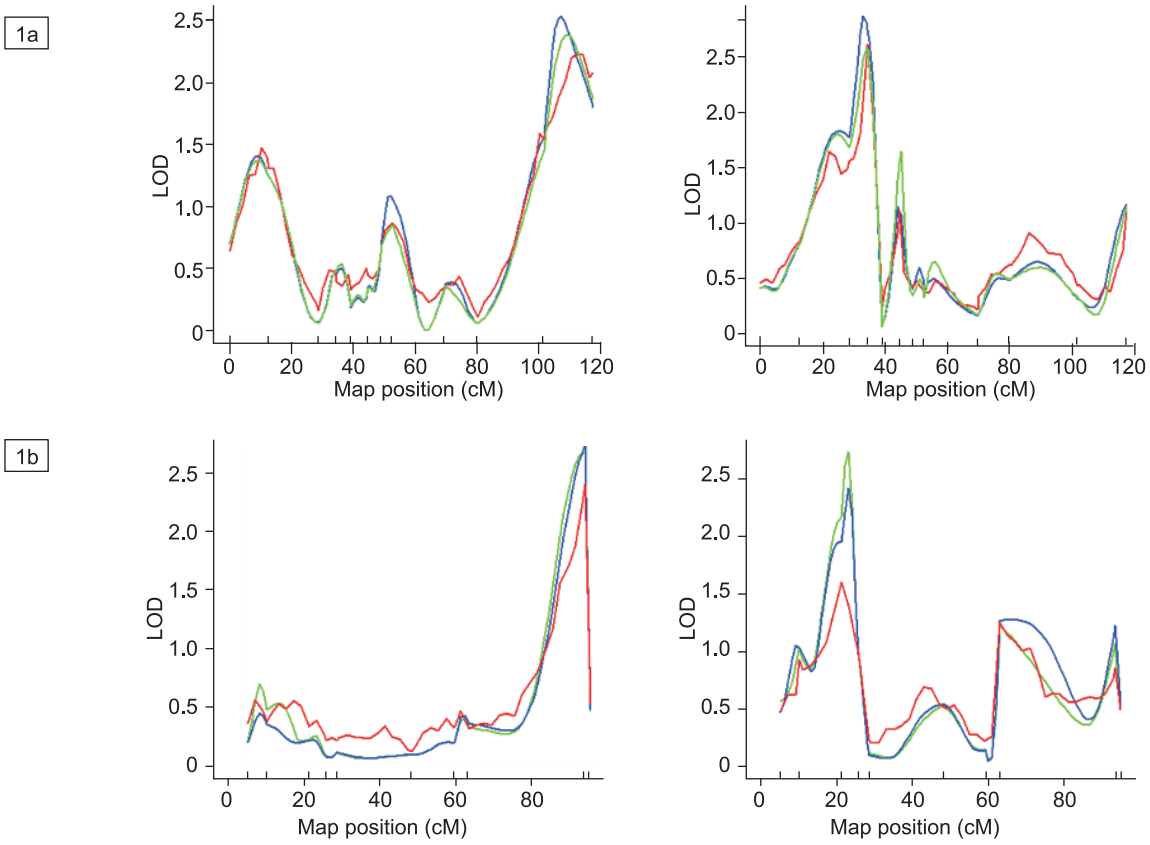


Fig. 1(a–b). (a) Chromosome scans of BTA 4 (BBU8) in two sire families depicting significant QTL locations for fat percentage in buffaloes. (b) Chromosome scans of BTA 14 (BBU15) on two half families showing QTLs for fat percentage in buffaloes.

BBU10 and BBU15). For BBU1q at positions between 6.34–11.61 cM we identified QTL which had already been reported by Nadesalingam *et al.* (2001). The meta-QTL at position 43.11 with confidence interval 36.11–51.11was obtained, the region had been reported to have QTL by Cole *et al.* (2011). The region identified at 103.29 and 115.05 had not been reported earlier in cattle. The meta-QTL region on chromosome BBU2q at position 25.97 cM was supported by reports of Cole *et al.* (2011) and at position 109.37 by Ron *et al.* (2004). For chromosome BBU6 (BTA3), the positions at 10.04 (8.16–11.92), 27.43 (24.37–32.34) and 61.61–65.72 and 81.34 are supported by QTL positions already reported by Cole *et al.* (2011), Rodriguez *et al.* (2002) and Ashwell *et al.* (2004). Similarly, the meta-QTL at position 108.04 (102.62-113.46) of BTA4 (BBU8) was supported by Lindersson *et al.* (1998) and Capomaccio *et al.* (2015). Similarly, the meta-QTL positions estimated on BBU7 (BTA6) had supportive literature available for locations at 6.0 (4.07–7.92) by Schrooten *et al.* (2004), 38 (34.00–44.00) by Nadesalingam *et al.* (2001), Chen *et al.* (2006), Gao *et al.* (2009) and Zhang *et al.* (1998). On BTA9 (BBU10) metaQTL region at position 10.20 had been reported by Kolbehadri *et al.* (2009) and Nayeri *et al.* (2016) and at 95.38 by Cole *et al.* (2011). Similarly, on BTA14 (BBU15) two positions are at 6.26 were supported by Heyen *et al.* (1999), Viitala *et al.* (2003), Boichard *et al.* (2003) and Farnir *et al.* (2002) and at positions 21.29 cM by Heyen *et al.* (1999) and Bennewitz *et al.* (2003). The positions at 27.12 was supported by report of Zhang *et al.* (1998).

We carried out the exercise of analysis family wise (12 half sib families) on 8 chromosomes of buffaloes. The meta-analysis helped in the objective assessment of evidence of QTLs which resolved the disagreement and uncertainty of the location of a QTL in different sire families. The meta-QTL analysis provided us a means to find the precise location of an QTL by generating consensus on the basis of 12 sire families. Thus combining the results across 12 sires provided us the locations which can be further taken up for identification of genes underlying the QTL regions which are related to lipid metabolism.

The results presented above are on the basis of partial genome scans (8 chromosome equivalent of cattle in buffaloes). It can be seen from the analysis that the chromosome scans indicate the chromosome regions that harbour genes related to fat percentage and are identified within a confidence of 2.5 to 3.0 cm. There are usually significant differences on the magnitude of effects of these QTLs. The differences may be there in the same experiment itself and may exist among the families. The total number of genes underlying the QTL regions of 8 BTA chromosomes were 1,118. These genes were mapped on Reactome database and 45 genes associated with fat metabolism were identified (Table 5). The chromosome wise genes identified underlying QTL regions and related

Table 3. Significant QTL locations for fat percentage for 12 half sib families using Interval Mapping (Haley-Knott regression and extended Haley-Knott regression) method of R/qtl.

| Sire No. | Chromosome (BTA) | Position | LOD score | $R^2$ or %variance | F value | P value (F) | Significance |
|---|---|---|---|---|---|---|---|
| Sire1 | 4 | 114 | 3.215 | 4.681 | 2.348 | 0.032 | * |
| | 1 | 39.1 | 1.199 | 1.701 | 2.585 | 0.077 | P |
| | 4 | 110 | 3.995 | 5.797 | 2.936 | 0.009 | ** |
| | 14 | 19.1 | 1.339 | 1.902 | 2.890 | 0.057 | P |
| | 3@74.9: 4@110.0 | | 2.071 | 2.959 | 2.248 | 0.064 | P |
| Sire2 | 6 | 4 | 1.770 | 4.389 | 3.620 | 0.029 | * |
| | 3 | 59.3 | 3.172 | 7.849 | 2.208 | 0.046 | * |
| | 4 | 20 | 2.730 | 6.711 | 1.888 | 0.087 | P |
| | 6 | 4 | 1.850 | 4.489 | 3.789 | 0.025 | * |
| | 3@59.3: 4@20.0 | | 2.451 | 6.000 | 2.532 | 0.043 | * |
| Sire4 | 2 | 10.8 | 1.250 | 2.483 | 2.676 | 0.072 | P |
| | 7 | 0 | 2.234 | 4.488 | 4.836 | 0.009 | ** |
| | 2 | 10.8 | 4.587 | 8.843 | 3.327 | 0.004 | ** |
| | 3 | 29.3 | 4.202 | 8.065 | 3.034 | 0.007 | ** |
| | 6 | 35.4 | 1.180 | 2.189 | 2.471 | 0.087 | P |
| | 2@10.8: 3@29.3 | | 3.653 | 6.968 | 3.933 | 0.004 | ** |
| Sire5 | 4 | 14 | 1.643 | 2.614 | 3.526 | 0.031 | * |
| | 6 | 90 | 1.299 | 2.060 | 2.779 | 0.064 | P |
| | 9 | 50.2 | 1.271 | 2.014 | 2.718 | 0.068 | P |
| | 4 | 20 | 2.634 | 4.222 | 1.901 | 0.082 | P |
| | 9 | 50.2 | 1.544 | 2.451 | 3.311 | 0.038 | * |
| Sire6 | 2 | 104.8 | 1.767 | 8.798 | 3.254 | 0.047 | * |
| | 7 | 80 | 1.347 | 6.613 | 2.446 | 0.097 | P |
| | 2 | 104.8 | 4.954 | 21.860 | 3.135 | 0.011 | * |
| | 14 | 5.1 | 4.436 | 19.224 | 2.757 | 0.022 | * |
| | 2@104.8: 14@5.1 | | 3.041 | 12.551 | 2.700 | 0.042 | * |
| Sire7 | 3 | 13.3 | 1.283 | 1.684 | 2.784 | 0.063 | P |
| | 4 | 10 | 1.665 | 2.192 | 3.624 | 0.028 | * |
| | 9 | 10.2 | 2.538 | 3.362 | 1.853 | 0.089 | P |
| | 4 | 4 | 4.203 | 5.519 | 3.107 | 0.006 | ** |
| | 7 | 120 | 4.663 | 6.143 | 3.458 | 0.003 | ** |
| | 4@4.0: 7@120.0 | | 3.233 | 4.214 | 3.559 | 0.007 | ** |
| Sire8 | 1 | 115.1 | 1.507 | 2.371 | 3.233 | 0.041 | * |
| | 4 | 24 | 1.950 | 3.080 | 4.198 | 0.016 | * |
| | 1 | 129.1 | 3.827 | 6.064 | 2.793 | 0.012 | * |
| | 3 | 64.9 | 3.702 | 5.860 | 2.699 | 0.015 | * |
| | 14 | 15.1 | 1.235 | 1.912 | 2.642 | 0.073 | P |
| | 1@129.1: 3@64.9 | | 3.419 | 5.399 | 3.730 | 0.006 | ** |
| Sire9 | 2 | 30.8 | 5.268 | 9.293 | 3.866 | 0.001 | ** |
| | 3 | 9.3 | 4.770 | 8.369 | 3.482 | 0.003 | ** |
| | 9 | 95.4 | 1.501 | 2.544 | 3.175 | 0.044 | * |
| | 14 | 29.1 | 1.772 | 3.012 | 3.758 | 0.025 | * |
| | 2 | 34.8 | 1.483 | 2.730 | 3.104 | 0.047 | * |
| | 2@30.8: 3@9.3 | | 3.710 | 6.437 | 4.017 | 0.004 | ** |
| Sire10 | 1 | 9.1 | 1.350 | 3.387 | 2.832 | 0.062 | P |
| | 1 | 5.1 | 1.681 | 4.090 | 3.442 | 0.035 | * |
| Sire11 | 3 | 84.8 | 3.091 | 12.135 | 1.896 | 0.098 | P |
| Sire12 | 1 | 105.1 | 3.924 | 17.608 | 2.032 | 0.097 | P |
| | 6 | 60 | 2.257 | 9.296 | 3.218 | 0.056 | P |
| | 1 | 100.1 | 4.253 | 21.790 | 2.240 | 0.071 | P |

Signif. codes: ***0.001, **0.01, *0.05, P (0.1)

to lipid metabolism are depicted in Fig. 3.

Further analysis revealed 19 pathways were significantly enriched which included the metabolic pathways, pathways associated with fatty acid metabolism, ABC transporter pathways and PPAR signalling pathways. The interaction among the 45 genes using STRING web server is given in Fig. 4. These 45 genes reflect enrichment for 61 gene ontology terms for biological processes, 15 for molecular functions and 20 for cellular components using the webserver (Fig. 4).

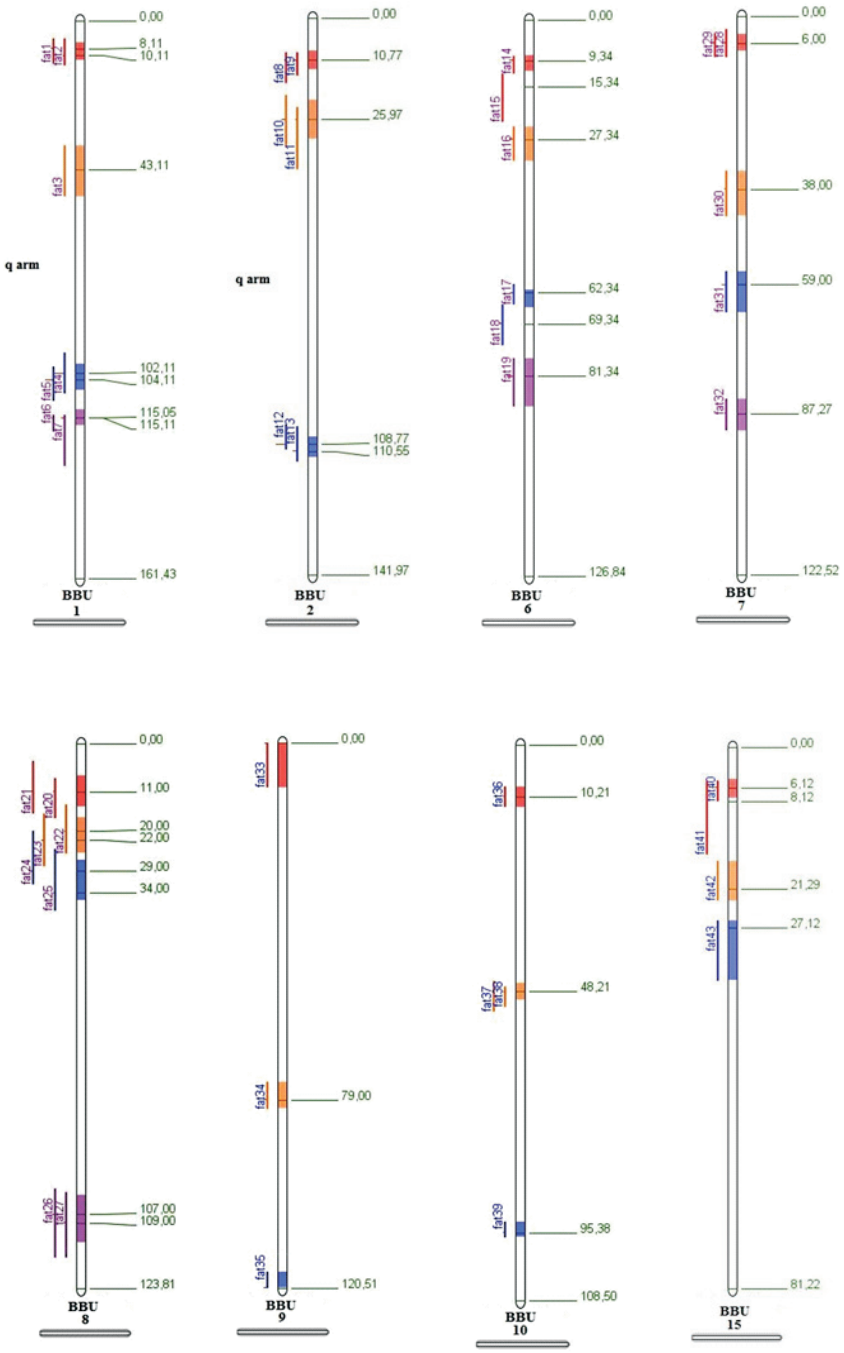


Fig. 2. Meta-QTL positions on 8 chromosomes for buffalo.

The figure of REViGO related to biological processes (Fig. 5a) relates to regulation of phospholipid metabolism, Vitamin D metabolism, cholesterol catabolism, long chain fatty acid metabolism and response to nutrient levels etc. Most of the biological processes are in confirmation with the lipid metabolism. Similarly, for the cellular components (Fig. 5b) pertains to membrane part and extra-cellular regional part, peroxisome and intercellular canaliculus. The molecular functions part relate to several enzyme activities related to lipid metabolism (Fig. 5c). Thus we identified a set of 45 genes underlying the meta-QTL regions which are directly related to the lipid metabolism. These 45 genes are the potential candidate genes for milk fat percentage in buffaloes.

There is no published study on the identification of QTLs in buffaloes. We compared the published literature of QTLs for fat percentage of cattle with the present study assuming large synteny between the cattle and buffalo chromosomes. We did find a lot of similarity between the reports of cattle with our present study. Indeed we found few extra QTL regions in buffaloes which were not reported for cattle. This was because of large amount of heterozygosity in Indian buffaloes. The most important QTL for fat percentage in cattle found on the terminal end of Chromosome BTA14 was not detected in any of the 12 sire families in the current experiment. The gene underlying the QTL was DGAT1 and

Table 4. Chromosomal locations of meta-QTL regions for fat percentage along with their confidence interval.

| BTA Chr | BBU Chr | AIC value | Mean position (Confidence interval) | Mean position (Confidence interval) | Mean position (Confidence interval) | Mean position (Confidence interval) |
|---|---|---|---|---|---|---|
| 1 | BBU1q | 46.37 | 8.98 (6.34–11.61) | 43.11 (36.11–51.11) | 103.29 (99.45–107.13) | 115.05 (112.68–117.42) |
| 2 | BBU2q | 34.89 | 10.77 (8.37–13.17) | 25.97 (20.92–31.01) | 109.37 (106.74–112.0) | – |
| 3 | BBU6 | 46.85 | 10.04 (8.16–11.92) | 27.34 (24.34–32.34) | 63.66 (61.61–65.72) | 81.34 (77.34–88.34) |
| 4 | BBU8 | 57.67 | 11.0 (7.4–14.6) | 20.91 (16.85–24.96) | 31.11 (26.56–35.67) | 108.04 (102.62–113.46) |
| 6 | BBU7 | 29.62 | 6.0 (4.07–7.92) | 38.0 (34.0–44.0) | 59.0 (56.0–65.0) | 87.26 (84.0–91.0) |
| 7 | BBU9 | 14.93 | 0.0 (0.0–10.0) | 79.0 (75.0–81.0) | 120.5 (117.0–121.0) | – |
| 9 | BBU10 | 16.62 | 10.2 (8.2–12.2) | 48.2 (46.54–49.87) | 95.38 (93.2–96.2) | – |
| 14 | BBU15 | 20.74 | 6.26 (4.81–7.71) | 21.29 (17.12–23.12) | 27.12 (26.12–35.12) | – |

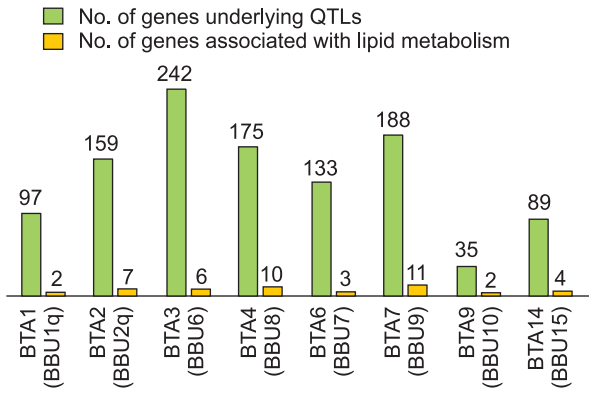


Fig. 3. Graph showing total number of genes underlying QTL regions and genes associated with fat metabolism.

the causative identified mutation had been found to be fixed in Indian cattle as well as buffaloes leading to its non-detection in the current set of experiments in buffaloes. The fat percentage in cattle and buffalo had been reported to have negative genetic correlation with milk yield. Thus the increase in fat percentage might result in decrease in the milk yield. However, the identified QTLs for fat percentage in buffaloes promises a higher potential for buffalo breeding especially in terms of farmers income as he get paid for fat percentage in buffaloes and the total milk. It is pertinent to mention that the application of QTLs are restricted to the families in which they are identified.Their application requires exact estimates of QTL effects and position to select for both for milk yield and fat percentage thus not adversely

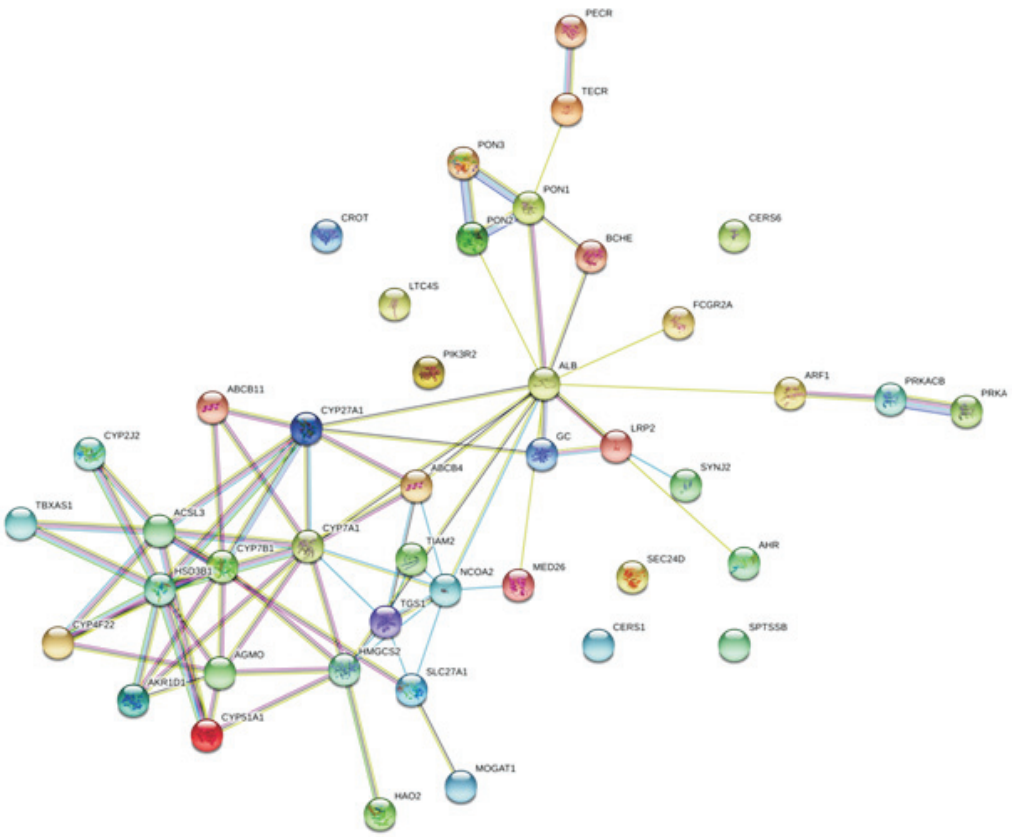


Fig. 4. Protein-protein interaction of the genes responsible for lipid metabolism. The names depicted on the nodes are common names of the genes.

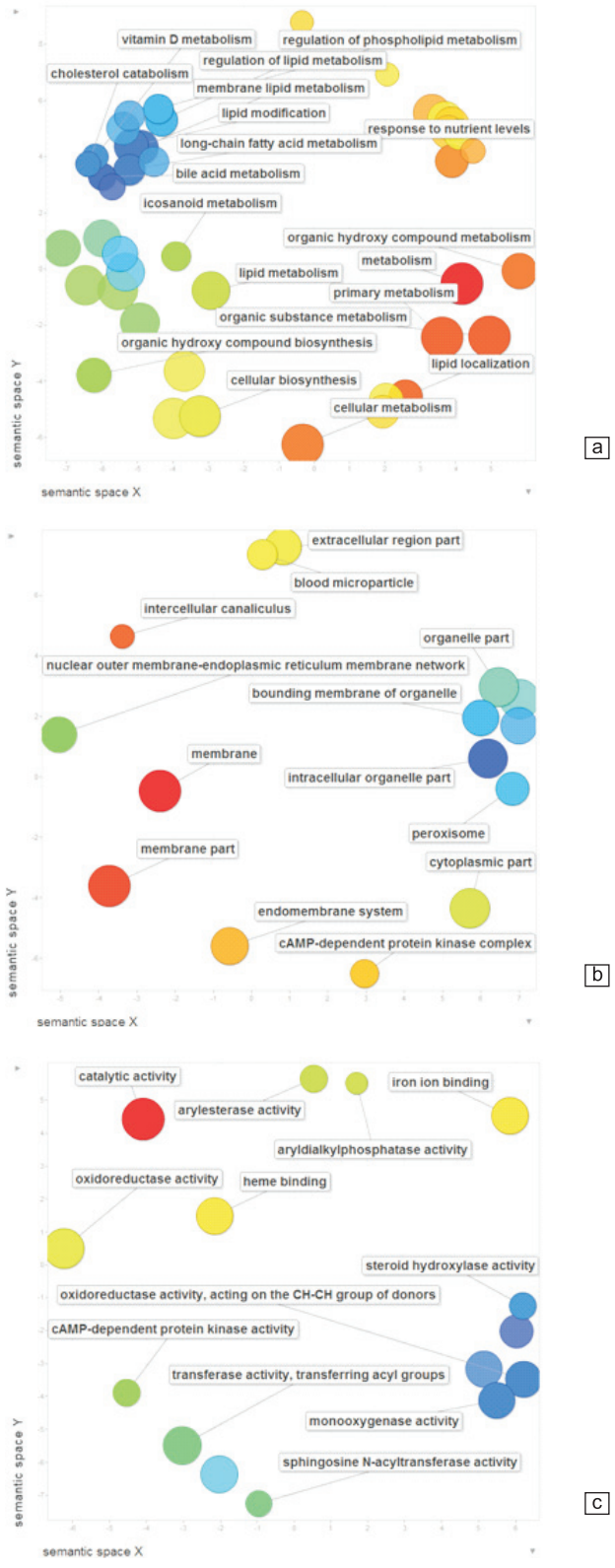


Fig. 5. Scatter graph showing interactions among genes in (a) Biological processes, (b) Cellular components and (c) Molecular functions.

affecting the milk yield. The genes identified under the QTL region can assist in the selection of animals for the fat percentage in synergy with the genes for milk production in buffaloes.

Table 5. Genes underlying the QTL regions of the 8 BTA chromosomes and associated with lipid metabolism.

| Chromosome | Entities associated with fat metabolism | Mapped entity in reactome database | HGNC names of genes |
|---|---|---|---|
| BTA1 | ENSBTAG00000011139 | Q8NFR3 | SPTSSB |
| (BBU1q) | ENSBTAG00000047250 | P06276 | BCHE |
| BTA2 | ENSBTAG00000013489 | P98164 | LRP2 |
| (BBU2q) | ENSBTAG00000006821 | Q6ZMG9 | CERS6 |
| | ENSBTAG00000004555 | Q96PD6 | MOGAT1 |
| | ENSBTAG00000044179 | Q02318 | CYP27A1 |
| | ENSBTAG00000008431 | O95573 | ACSL3 |
| | ENSBTAG00000017258 | O95342 | ABCB11 |
| | ENSBTAG00000026885 | Q9BY49 | PECR |
| BTA3 | ENSBTAG00000003898 | Q9NYQ3 | HAO2 |
| (BBU6) | ENSBTAG00000000123 | P14060 | HSD3B1 |
| | ENSBTAG00000011953 | P22694 | PRKACB |
| | ENSBTAG00000006769 | P54868 | HMGCS2 |
| | ENSBTAG00000010033 | P51589 | CYP2J2 |
| | ENSBTAG00000021842 | P12318 | FCGR2A |
| BTA4 | ENSBTAG00000008361 | P21439 | ABCB4 |
| (BBU8) | ENSBTAG00000034154 | P27169 | PON1 |
| | ENSBTAG00000001658 | P35869 | AHR |
| | ENSBTAG00000007746 | Q9UKG9 | CROT |
| | ENSBTAG00000021535 | Q6ZNB7 | AGMO |
| | ENSBTAG00000005653 | Q15165 | PON2 |
| | ENSBTAG00000034645 | P24557 | TBXAS1 |
| | ENSBTAG00000020225 | Q16850 | CYP51A1 |
| | ENSBTAG00000001992 | P51857 | AKR1D1 |
| | ENSBTAG00000015654 | Q15166 | PON3 |
| BTA6 | ENSBTAG00000003619 | O94855 | SEC24D |
| (BBU7) | ENSBTAG00000013718 | P02768 | ALB |
| | ENSBTAG00000017121 | P02774 | GC |
| BTA7 | ENSBTAG00000002350 | O00459 | PIK3R2 |
| (BBU9) | ENSBTAG00000015735 | O00459 | PIK3R2 |
| | ENSBTAG00000012632 | P98187 | N/A |
| | ENSBTAG00000020923 | O95402 | MED26 |
| | ENSBTAG00000007725 | P17612 | PRKACA |
| | ENSBTAG00000009737 | P84077 | ARF1 |
| | ENSBTAG00000005312 | Q9NZ01 | TECR |
| | ENSBTAG00000002350 | Q16873 | LTC4S |
| | ENSBTAG00000006642 | Q6PCB7 | SLC27A1 |
| | ENSBTAG00000047933 | Q6NT55 | CYP4F22 |
| | ENSBTAG00000016775 | P27544 | CERS1 |
| BTA9 | ENSBTAG00000010047 | Q8IVF5 | TIAM2 |
| (BBU10) | ENSBTAG00000000817 | O15056 | SYNJ2 |
| BTA14 | ENSBTAG00000005898 | Q96RS0 | TGS1 |
| (BBU15) | ENSBTAG00000020312 | P22680 | CYP7A1 |
| | ENSBTAG00000001299 | O75881 | CYP7B1 |
| | ENSBTAG00000005287 | Q15596 | NCOA2 |

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