Divers of climate change impacts on bird communities

CHAPTER X

Molecular genetics and selection in diary buffaloes: the Italian situation

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Abstract: The swamp and the river type constitute two distinct clades of buffaloes. A long period of isolation and the lack of crossbreeding allowed an evident morpho-functional differentiation of the Mediterranean river type, whose population has increased 19 fold in Italy in the past fifty years. This increase lies in the growing interest in the productive characteristics of this rustic animal, actually breed mainly as dairy purpose animal. Marker assisted selection may represent a possible option for designing a suitable breeding scheme for Italian river buffaloes. Gene polymorphisms significantly associated to milk production traits may provide useful indications for identifying selection candidates with high genetic merit. The literature associated with different aspects of the genetic progress of buffalo is abundant, this chapter is a review of a part of publications dealing with the molecular bases for the improvement of the quali-quantitative characteristics of the Italian dairy buffaloes mainly during the last decade.

Keywords: River buffalo, molecular selection, genetic improvement of dairy traits, casein cluster, milk yield

1. INTRODUCTION

Domestic water buffalo were historically divided into swamp and river subspecies that differ in morphology, behaviour, and chromosome number (2n=48 and 2n=50, respectively). Swamp buffalo is predominantly in Southeast Asia and China, whereas the river type is mainly found in India, Southwest Asia and Mediterranean area [1]. Although their phenotypic differences, there is still a great interest on their time of domestication [2], as well as a debate to consider appropriate their classification in two related subspecies [3]. In fact, molecular evidences based on mitochondrial DNA analysis [4, 5], molecular markers [6, 7] and Y-chromosome genes variations [8] showed that the two types are distinct and the separation of swamp and river type predates domestication. They share several haplotype both at genomic and mitochondrial level, but the swamp and the river buffaloes constitute two distinct clades.

In the last years, several research projects focused on the buffalo genome. In particular 8 different consortium are working to fill the gap with other livestock species. 5 out of 8 projects are related to transcriptome sequencing, 2 focused on the genome sequencing and one is relative to radiation hybrid. The NCBI database (http://www.ncbi.nlm.nih.gov/assembly/GCA_000471725.1/#/st) reports the following state of art for the UMD CASPUR WB 2.0 project updated at 30th September 2013.

UMD CASPUR WB 2.0	
Assembly level:	Scaffold
Genome representation:	Full
Total sequence length	2,836,150,610
Total assembly gap length	74,388,041
Gaps between scaffolds	0
Number of scaffolds	366,982
Scaffold N50	1,412,388
Number of contigs	630,367
Contig N50	21,938
Total number of chromosomes	0

Although the sequencing of buffalo genome is complete, currently the annotation of the sequences is not yet available and the knowledge of nuclear genes with known function is still very limited, representing only 1.47% of the sequences present in the database (Michelizzi et al., 2010 [9]).

A list of sequences is available on the website of NCBI: 16692 nucleotide sequences, 1868 EST and 4797 GSS, almost all (11203) belonging to *Bubalus bubalis* species, followed by *Bubalus bubalis bubalis* (107) and other taxa.

The lack of the gene annotation is reflected also in the very limited information for the genetic variability, which represents the first step of the knowledge for the genetic improvement of the species.
The total number of available reference SNP reported on NCBI data base is only 502 for this species (http://www.ncbi.nlm.nih.gov/snp/?term=bubalus+bubalis) and the validation status of these polymorphisms is in most cases missing. Considering the close distance between *Bos taurus* and *Bubalus bubalis*, Michelizzi et al. (2010b [10]) employed the Illumina Bovine SNP50 BeadChip in buffalo. Although most of SNP were fully scored (41870 *vs* 54001), only 1159 SNP were polymorphic in the species.
The conservation of the SNP sites but not of the polymorphisms between cattle and buffalo indicates that as long as the buffalo genome and its annotation is not complete, the use of already existing tools for the genetic improvement of the species is not useful at all. Therefore, the application of genome wide association studies (GWAS) is still very far to be a reality as for instance happens in cattle, and a classical research approach based on marker assisted selection (MAS) or gene assisted selection (GAS) seems to be still a faster way for the planning and the application of breeding selection schemes.

2. THE ITALIAN SITUATION

The buffalo breed reared in Italy is named the Mediterranean Italian to distinguish it from other European breeds which belong to the same lineage, but they are not at the same genetic level [11]. Buffalo livestock in Italy is a small reality in comparison with the large population numbers of many east Asian countries, however the Italian buffalo population has increased 19 fold in the past fifty years (http://faostat.fao.org), becoming the livestock that has registered the highest increase (together with Brazil) in the world among the years 1961-2011 (table 1).

Table 1 First ten stocks of buffaloes given in thousands of heads and ranked for the incidence of growth rate folds between the years 1961-2011 (FAO, 2013)

Country	Total buffalo population		Growth rate fold
	1961	2011	
Brazil	63000	1278075	19,28
Italy	18000	365086	19,28
Nepal	795000	4993650	5,28
Pakistan	6700000	31726000	3,74
Syrian Arab Republic	1400	5000	2,57
Myanmar	1048523	3096887	1,95
China	8043000	23378000	1,91
Lao People's Democratic Republic	420000	1197000	1,85
Bangladesh	500000	1394000	1,79
Egypt	1501000	3800000	1,53

The reason for this increase lies in the growing interest in the productive characteristics of this rustic animal, actually breed mainly as dairy purpose animal. In Italy, the produced milk is processed almost completely into mozzarella PDO (Protected Denomination of Origin - Reg. EC 510/2006). The increased demand for this product, both on the national and international market (14% of the Italian production is exported to Germany, France, UK, Switzerland, USA and Japan), together with the cow milk quotas imposed by the EU, have favoured the buffalo breeding and productions [9]. The buffalo milk production amounted to 1,924,553 tons in 2012, with an increase of 7,79% compared to 2010 (http://www.aia.it). Also milk composition has been improved, with the average protein and fat content moving from 4,65 and 8,10% in 2003 to 4,70 and 8,30% in 2012, respectively (http://www.anasb.it/home.htm).
The Italian buffalo Herd book has 56075 registered buffaloes. Most of them are involved in a dairy recording program. However, the implementation of a conventional breeding program for dairy traits based on progeny testing and EBV calculation is hampered by the poor efficiency of AI in this species. In fact, although about 11000 semen doses have been used for AI only in 2011 within the herd-book, the natural mating is still the most widely used technique of reproduction. This happens mainly for the difficulties in the buffalo oestrus detection and variability of its length which are among the main causes of AI failure [12]. Moreover, small values of estimated genetic parameters have been ascribed to inaccurate identification of true paternity [13]. Problems related to paternity in buffalo have always existed and even today they are not easy to solve due to logistic and financial constraints, therefore the genetic importance of buffalo females is still much higher than in dairy cattle. Italian breeders association (ANASB) is working hard to increase the reliability of genetic merit predictions, including the evaluation of the genetic merit of natural mating bulls, which are strongly perceived by the mangers of larger farms.

3. MOLECULAR SELECTION IN ITALIAN RIVER BUFFALO

Marker assisted selection may represent a possible option for designing a suitable breeding scheme for Italian river buffaloes. Gene polymorphisms significantly associated to milk production traits may provide useful indications for identifying selection candidates with high genetic merit. Recently, in this direction the Italian government financed a research project named SelMol (currently updated with the Innovagen project) with the aim to start a partnership programme which connects breeders and researchers in order to improve the productive performances of dairy buffaloes with the support of the information of molecular genetics. Since almost all the buffalo milk produced in Italy is used to produce Mozzarella cheese, the most important breeding goal for Italian buffalo is the estimated mozzarella yield per lactation (PKM), a trait calculated in a single trait animal model according with the following formula:

$$PKM = Milk\ (kg) * \{[(3.5 * protein\ \% + 1.23 * fat\ \%) - 0.88] / 100\}$$

It is quite clear that the improvement of each of the aforementioned milk components results in higher values of the PKM. Therefore, several candidate genes were chosen for the improvement of quali-quantitative characteristics of buffalo milk. In particular, the following loci *OXT*, *OXTR*, *PRL*, etc... were studied for the milk yield; the casein cluster (*CSN1S1*, *CSN1S2*, *CSN2*, *CSN3*) for the protein content; *DGAT1*, *FASN*, *LEP*, etc… for the fat content, whereas *SCD*, *ACACA*, *LPL*, etc… for the quality of fatty acids. Examples of molecular genetics progresses are reported below for some of these genes.

3.1 Oxytocin gene (OXT)

The oxytocin (*OXT*) is a candidate gene for improving milk yield and milkability, due to the role of the oxytocin hormone in alveolar milk ejection and in milk flow rate. In fact, a successful milking requires a complete milk removal from both cisternal and alveolar compartments of the mammary gland. For a complete milk ejection, oxytocin must be released from the pituitary gland and transported to the udder where it acts on myoepithelial cells to promote contraction [14]. Milk ejection in response to suckling or milking is achieved via a classical neuro-endocrine process, known as the milk ejection reflex [15]. This process is of particular importance for buffaloes, in which alveolar milk represents about 95% of total milk due to the absence or small size of the udder cistern [16, 17].

The *OXT* gene is 912 bp long and it codes for 106 amino acids of the oxytocin complex. Three SNPs have been discovered in Italian river buffalo: two transitions in the promoter 5'flanking region (AM234538: g.28C>T and g.204A>G) and a non-synonymous transversion (g.1627G>T) at the 170th nucleotide of the second exon. The latter SNP is responsible for the Arg^{97}→Leu amino acid substitution in the mature protein, which yields two alleles respectively named A (EMBL Acc. No AM234538) and B (EMBL Acc. No AM234539) [18].

Recently, Pauciullo et al. [19] reported an association between these SNPs at the OXT locus and daily milk yield. Although the results refer to a single herd and should be tested population-wide, it is of great importance as one of the first indications of association between a trait of economic importance and a candidate locus in this species. The superiority in milk yield showed by TT buffaloes (transversion g.1627G>T) over the heterozygous genotype (more than 1,7 kg/d) is relevant compared to the relatively low level of production of this species. An interesting feature of such a difference is that it remains quite constant throughout the whole lactation (Figure 1a). This feature has been observed also for other candidate genes found to affect dairy traits in cattle as *DGAT1* [20], where the effect can be observed after 40 days in milk.

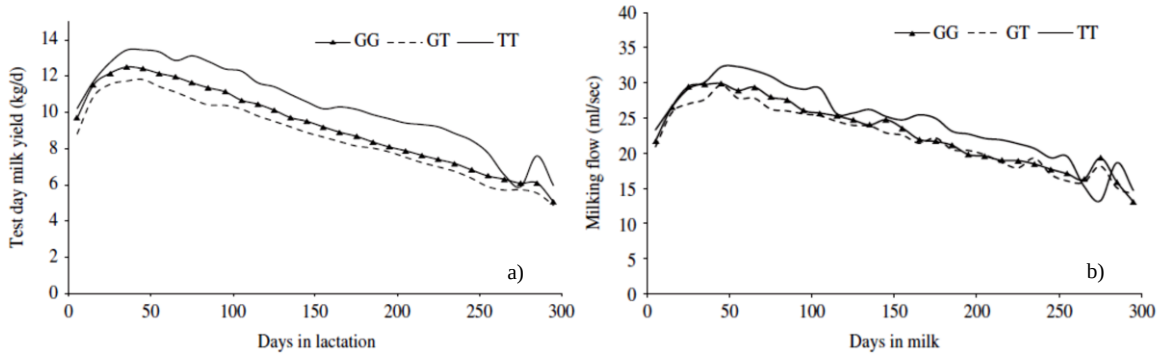

Figure 1. Lactation curves of different OXT genotypes for: a) milk yield (kg/d) and b) milking flow (ml/s). (Modified from Pauciullo et al. [19])

Results on milk flow are of a lower magnitude as far the effect of the gene is concerned (Figure 1b). Its contribution to the total phenotypic variance in milk flow is markedly lower than in the case of milk. Such result is quite unexpected, considering the physiological role of the oxytocin and the correlation between milk yield and ejection. However, it should be remembered that previous studies carried out in buffalo and cow highlighted that is the increase of the oxytocin level over a threshold that affects milk ejection and not its absolute concentration [21, 22].

3.2 The casein cluster

Buffalo milk is characterized by the presence of all four casein fractions (αs1, β, αs2 and κ) encoded by the four tightly linked autosomal genes (*CSN1S1*, *CSN2*, *CSN1S2* and *CSN3*, respectively) mapped on chromosome 7 [23]. The buffalo casein aminoacidic complete sequences [24] are available, as well as the complete sequence and the relative regulatory regions of genes encoding β [25] and k casein [26], the 5' UTR, exon 1 and partial cDNA of *CSN1S1* gene (EMBL No. GU593719, AF529305, AY948385, AJ005430) and the sequences related to the cDNA as well as short intronic sequences of the *CSN1S2* gene [27]. Feligini et al. [28] developed a method for the quantization of αs1, β, αs2 and κ-caseins in water buffalo milk using reverse phase high-performance liquid chromatography, whereas a recent investigation by Cosenza et al. [29] reported, for the first time, a quantitative characterization of the buffalo casein transcripts and showed that the four genes are not transcribed and translated with the same efficiency. The findings of this research are very important to explain the different technological properties of buffalo milk compared to the milk of other domestic ruminants (cattle, sheep and goat).

In particular, the analysis of individual milk samples of Mediterranean river buffaloes showed that the most abundant casein fractions are β (53.45%, SD 6.63) and αs1 (20.61%, SD 4.29), followed by αs2 and k (14.28%, SD 4.88, and 11.66%, SD 2.26, respectively). These results are quite different from those obtained for cattle, sheep and goat which show a percentage distribution of β and αs1 casein fractions of about 38% each [30]. The observed differences in the percentage distributions of the four casein fractions, and particularly of the β fraction, in the buffalo milk compared to those characterizing the milk of other ruminant species could account for its peculiar technological properties.

Quantization of the corresponding mRNAs shows that the percentage of transcripts of the four caseins was about 16.48 (SD 4.99), 23.18 (SD 5.41), 55.87 (SD 8.22) and 4.47 (SD 0.96) for αs1, β, αs2 and k respectively. These values were significantly different from those characterizing the transcripts of the same genes in cattle, sheep and goats. In fact, for these species each casein transcript represents nearly 25% of the whole casein transcript population [30].

The ratio between the percentage of single milk protein fractions and the percentage of transcripts produced in the mammary gland allowed the evaluation of the translation efficiency of the buffalo casein cluster gene transcripts. A low translation efficiency (0.25, SD 0.07) was showed for the *CSN1S2* transcripts, whereas for the *CSN3*, *CSN2* and *CSN1S1* the efficiency was higher, as follows: 2.69 (SD 0.74), 2.39 (SD 0.49) and 1.31 (SD 0.30), respectively.

The context of the translation initiation codon (AUG) plays important roles in determining the translation rate [31, 32], therefore a possible explanation of a such difference in the translation level of buffalo casein cluster genes is given by the comparison of nucleotide sequences with the Kozak consensus sequence. In general, higher the sequence around the initiation codon is homologous to the Kozak sequence ("strong" consensus), higher is the efficiency of mRNA translation [33]. The sequence comparison of the 4 transcripts in river buffalo (Table 2) shows for the *CSN1S1*, *CSN2* and *CSN3* mRNAs the highest homology with the Kozak sequence. In particular, for *CSN1S1* and *CSN2* three residues directly upstream of the initiation are consecutive (-3, -2 and -1), while *CSN3* is characterized only by two consecutive nucleotide (-3 and -2).

Table 2. Comparison of start codon flanking sequences of the four casein transcripts in the Mediterranean river buffalo. In the first line is reported the optimal context for initiation of translation in mammals (Kozak consensus sequence). The start codon in the four casein transcripts (AUG) is underlining. Conserved nucleotides are shown in shade. (Modified from Cosenza et al. [29])

-6	-5	-4	-3	-2	-1	+1	+2	+3	+4	
G	C	C	R	C	C	A	U	G	G	Kozak consensus sequence
A	G	A	G	C	C	A	U	G	A	*CSN2*
G	U	A	A	A	C	A	U	G	A	*CSN1S2*
A	C	A	A	C	C	A	U	G	A	*CSN1S1*
G	G	U	A	C	A	A	U	G	A	*CSN3*

On the contrary, *CSN1S2* shows the worst combination, because despite having three nucleotides matching with the consensus sequence, these are not consecutive (-6, -3 e -1) (Table 2) and, therefore, can be considered having a "weak" context.

Concerning the higher translation efficiency of the k-casein transcript compared to what was observed by Bevilacqua et al. [30] for cattle, sheep and goat, an explanation was found through the comparison with the sequence of the homologous messenger among these species. The *CSN3* mRNA in buffalo, cattle, sheep, goat, mouse, rabbit and pig, has two consecutive AUG and with the exception of buffalo, in all these species, the first start codon shows a guanine in position -3 (Table 3).

Table 3. Comparison of start codon flanking sequences of the k casein transcripts in different species. The first of the two consecutive AUG codons in ruminants, pig, rabbit and rat is in shade and underlining. (Modified from Cosenza et al. [29])

-6	-5	-4	-3	-2	-1	+1	+2	+3	+4	+5	+6	+7		EMBL
G	G	U	A	C	A	A	U	G	A	U	G	A	buffalo	AM900443
G	G	U	G	C	A	A	U	G	A	U	G	A	cattle	AY380229
G	G	U	G	C	A	A	U	G	A	U	G	A	sheep	NM_001009378
G	G	U	G	C	A	A	U	G	A	U	G	A	goat	X60763
G	G	U	G	C	A	A	U	G	A	U	G	A	pig	NM_001004026
G	G	U	G	C	A	A	U	G	A	U	G	A	rabbit	Z18243
G	G	U	G	C	A	A	U	G	A	U	G	A	rat	NM_007786

It was proven [33] that a start codon flanked by A in position -3 compared with G works considerably better; and therefore it is characterized by higher translational activity. Therefore, the presence of A in position -3 in the first start codon could represent an optimal situation to ensure a more correct and efficient translation of the buffalo *CSN3* transcript compared to the other ruminants.

Compared to the other domestic ruminants, the genetic polymorphism detected in the buffalo casein cluster is quite poor. So far, no polymorphic sites have been detected for the *CSN2* gene (β-casein). Cosenza et al. [27] characterized the *CSN1S2* and found a transversion (g.773G>C) responsible for the inactivation of the intron 7 splice donor site (B allele). This polymorphism resulted in the allele-specific splicing out of the complete exon 7 (27 bp) corresponding to 9 amino acids. Although a shorter αs-2 protein should affect the total protein content, this was never verified. However, excluding the aforementioned case, currently no other quantitative alleles have been detected.

Two alternative forms of αs1-casein were described by Ferranti et al.[34]. The analysis of *CSN1S1* exon 17 showed the occurrence of a transversion (c.578C>T) which results in the amino acid substitution Leu^{178}(A) → Ser(B) of the mature polypeptide chain.

Similar situation for the k-casein where Feligini et al. [28] detected a polymorphism through RP-HPLC. Sequencing of CSN3 exon 4 demonstrated, in agreement with Mitra et al. [35], a c.467T>C transversion in the complete coding sequence, where the presence of T corresponds to the allele X1, whereas C corresponds to the allele X2. This nucleotide substitution results in the amino acid change Ile^{135}(X1)→Thr(X2) of the mature polypeptide chain.

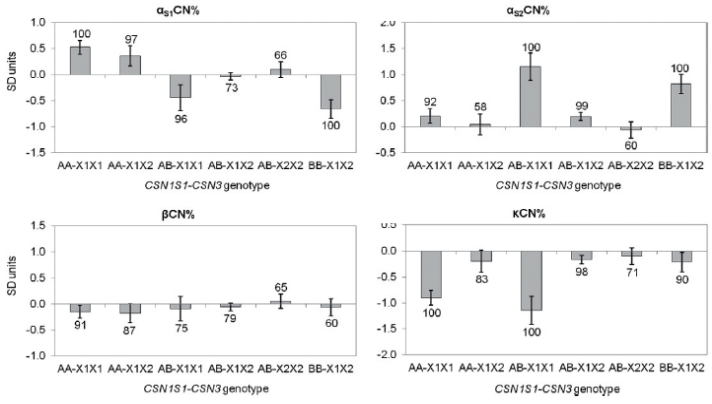

Figure 2. Effect (mean ± SD of the marginal posterior density) of CSN1S1-CSN3 composite genotype, relative to genotype BB-X2X2 on casein content. Numbers at the top of the bars are marginal posterior probabilities of the effect being greater than 0 (if positive). Effect were measured in standard deviation unit of the trait. Caseins were measured as percentage of total casein content. (Modified from Bonfatti et al., [37])

Recently the effect of composite CSN1S1-CSN3 genotype on milk protein composition, milk production traits and milk coagulation properties was evaluated by Bonfatti et al. [36, 37]. Regarding the milk protein composition, an increased proportions of αS1-CN in total casein (TCN) was associated with genotypes carrying the A allele. Genotypes associated with a marked decrease of the proportion of αS1-casein in TCN (composite genotypes AB-X1X1 and BB-X1X2) were associated with marked increases in the proportion of αS2-casein. Composite genotypes carrying the X1 allele at CSN3

were associated with a greater proportion of αS2-casein in TCN compared to those carrying CSN3 X2. The decreased content of glycosylated κ-CN associated with CSN3 X1 was responsible for the overall lower content of total κ-CN in milk of X1-carrying animals.

Regarding the milk production traits the composite genotype AB-X2X2 was associated with decreased test day milk yield (−0.21 standard deviation units of the trait) relative to genotype BB-X2X2. The genotypes did not affect milk protein content, but the genotype AB-X1X1 was associated with increased fat content compared with the genotype BB-X2X2 (+0.28 SD units of the trait) and AB-X1X1 (+0.43 SD units of the trait). For milk coagulation properties, measured as: rennet clotting time (RCT), curd firming time (K_{20}), and curd firmness (A_{30}), the largest difference (+1.91 min; i.e., 0.61 SD units of the trait) was observed between genotype AA-X1X2 and AB-X1X1 for rennet clotting time parameter. The maximum variation in K_{20} due to genotype effects (between AA-X1X2 and AB-X1X1 genotypes) was almost 0.9 SD units of the trait. Magnitude of genotype effects was smaller for A_{30} than for RCT and K_{20}, with a maximum difference of 0.5 SD units of the trait between genotype AA-X1X2 and AA-X1X1. The B allele at *CSN1S1* was associated with increased RCT and K_{20} and with weaker curds compared with allele A. Allele X2 at *CSN3* exerted opposite effects on milk coagulation properties relative to *CSN1S1* B. Because of linkage disequilibrium, allele B at *CSN1S1* and allele X2 at *CSN3* tend to be associated and this likely makes their effects cancel each other.

From these two studies, it is quite evident that increasing the frequency of specific genotypes might be an effective way to alter milk protein composition, which can also play an important role in the variation of milk coagulation properties and technological characteristics of buffalo milk.

3.3 The steraroly CoA desaturase gene (SCD)

Stearoyl-CoA desaturase is a microsomal enzyme which plays a key role in fatty acid metabolism. It is also known as delta-9-desaturase because it catalyzes the introduction of the first cis-double bond in the Δ9 position in a large spectrum of fatty acyl-CoA substrates [38]. The stearoyl-CoA desaturase (SCD) locus has been suggested as a candidate gene affecting milk fatty acid (FA) profile [39].

Figure 3. Key transcription factors binding sites found in the promoter region of the river buffalo SCD gene. The conserved PUFA response region including the sterol response element (SREBP), CCAAT-box (C/EBP), nuclear factor (NF)-1 and stimulator protein 1 (SP1) binding site are shown. TATA motifs and peroxisome proliferator activated receptor-γ (PPAR-γ) are also shown proximal to the transcription start site. SNP g.133A>C is indicated with M nucleotide according to international nomenclature. (Modified from Pauciullo et al. [41])

In river buffalo, the SCD gene and its promoter region were characterized by Pauciullo et al. [40, 41]. Most of the consensus sequences regulating the lipid metabolism were found in a very closed DNA fragment of about 130 bp (nucleotide -382/-250), suggesting that this region could have an essential function in the gene expression. 15 SNPs were detected. Among these, the transversion g.133A>C at position -461 of the promoter falls between two SP1 binding sites, thus creating a new consensus site for this transcription factor. As a consequence, the carriers of the C allele are characterized by three consecutive SP1 binding sites.

SP1 binding sites are well-known enhancer elements for gene expression and occur frequently in clusters generated by the promoter VNTR (Variable Number Tandem Repeats) [42]. Mutation analysis of SP1-binding sites showed that the number of SP1-binding sites within a cluster could determine the transcription rate of the respective gene [43]. Therefore, the variability found in the buffalo SCD SP1 cluster could be responsible for the variation in SCD expression and consequently SCD activity.

Since SCD is known to be the key enzyme controlling the desaturation rate of FA, the level of SCD activity can be assumed to have a direct effect on desaturated fat content in several tissues. A preliminary association study with

the milk fatty acid content confirmed that the C allele significantly affects the total desaturation index (P<0.01) [40].

The same SNP was significantly associated with milk yield (*P*=0·02). In particular, buffalo cows with heterozygous genotype AC at the promoter of *SCD* locus showed the highest daily milk yield, with more than 2 kg/d compared with CC buffaloes. Such a difference accounts for about 28% more milk per day. On the contrary homozygous AA were slightly lower than AC. The behavior of the three genotypes tended to remain constant throughout the whole lactation. The allele substitution effect of the adenine into cytosine was about -1 kg/d (P<0·01), and the contribution of the SCD polymorphism to the total phenotypic variance was 12% (Table 3).

Table 3. Mean ± SE of milk yield (kg/d) for the substitution effect g.133A>C in the promoter region of the river buffalo SCD gene and contribution of the such polymorphism to the phenotypic variance. (Modified from Pauciullo et al. [41])

Trait	α	P	d	P	σ^2_{SCD}	σ^2_c	σ^2_e	r^2_c	r^2_{SCD}
Milk yield	-1.01±0.38	0.007	1.22±0.49	0.013	0.93	4.21	2.72	0.61	0.12

α: Substitution effect
d: dominance effect
σ^2: variance components associated to the genotype (SCD); to the individual buffalo cow (c), to residuals (e)
r^2: contributions of genotype (SCD) and of individual buffalo cow (c) to the total phenotypic variance

The large effect of dominance on milk yield observed in the present work, more than 1.2 kg (P<0·02), is also very interesting (Table 3). This offers a possible explanation of the over-dominance effect of the heterozygous (AC) on the best homozygous phenotype (AA). Often such effect is not detected or considered non relevant because numerically much lower than the additive effect, however it might also have an impact on allele substitution effect. It is also well-known that the lipid metabolism and the biosynthesis of de novo fatty acids are complex pathways and they are energetically very expensive. As suggested for dairy cattle [44, 45], in cows with greater desaturase activity, fewer nutrients are directed toward milk yield. In river buffalo, the CC genotype at SCD locus showed an increased Δ9-desaturase activity and higher milk monounsaturated FA content [40], but it is also is characterized by less milk yield. Therefore, these findings confirmed this relationship.

4. CONCLUSION

In recent years, a great deal of work has been done on Italian buffalo farms to improve recording, health, feeding and livestock systems. On the other hand, little has been done in terms of genetic improvement in this species.

In this chapter, without being exhaustive, we reviewed some of the papers dealing with the molecular bases of genetic selection in river buffalo. Several recent studies showed novel genotyping information and haplotype structure of key genes involved in the improvement of quali-quantitavive characteristics of milk.

These findings open the way for a future application of marker-assisted selection which may represent a possible option for designing a suitable breeding scheme for Italian river buffaloes. Gene polymorphisms significantly associated with milk production traits may provide useful indications for identifying selection candidates with high genetic merit. Increases in average milk yield and, consequently, in mozzarella PDO production is of great economic interest for the buffalo dairy industry.

5. REFERENCES

[1] Ajmone-Masan P., Marco Milanesi M.,, Bombal., Colli L., Bacciu N., Ramunno L., Cosenza G., Coletta A, Zicarelli L., Fritz E, Koltes J., Reecy J.M., Williams J., Iamartino D., Sonstegard T. S., Van Tassell C. P., The International Buffalo Consortium (2013). Biodiversity among Buffalo genomes. International Plant and Animal Genome Conference Future Dates, XXI Conference, January 12-16, - San Diego.

[2] Kierstein G. Vallinoto M., Silva A., Schneider M.P., Iannuzzi L., Brenig B. (2004). Analysis of mitocondrial D-loop region casts new light on domestic water buffalo (Bubalus bubalis) phylogeny. Molecular phylogenetics and evolution, 30 (2): 308-324.

[3] Lau C.H., Drinkwater R.D., Yusoff K., Tan S.G., Hetzel D.J., Barker J.S. (1998) Genetic diversity of Asian water buffalo (Bubalus bubalis): mitochondrial DNA D-loop and cytochrome b sequence variation. Animal genetics, 29(4): 253-264.

[4] Kumar S., Nagarajan M., Sandhu J.S., Kumar N., Behl V., Nishanth G. (2007) Mitochondrial DNA analysis of Indian water buffaloe support a distinct genetic origin of river and swamp buffalo. Animal genetics 38(3):227-232.

[5] Lei C.Z., Zhang W., Chen H., Lu F., Liu R.Y., Yang X.Y., Zhang H.C. et al. (2007). Indipendent maternal origin of Chinese swamp buffalo (Bubalus bubalis). Animal genetics 38 (2): 97-102.
[6] Barker J.S.F., Moore S.S., Hetzel D.J.F., Evans D., Tan S.G., Byrne K. (1997). Genetic diversity of Asian water buffalo (Bubalus bubalis): microsatellite variation and a comparison with protei-coding loci. Animal genetics 28(2): 103-115.
[7] Zhang Y., Sun D., Yu Y., Zhang Y (2008). Genetic variation and divergence among swamp buffalo, river buffalo and cattle: a microsatellite survey on five population in China. Asian-Aust. J. Anim. Sci. 21(9): 1238-1243.
[8] Yindee M., Vlamings B.H. Wajjwalku W., Techakumphu M., Lohachit C., Sirivaidyapong S., Thitaram C., et al. (2010) Y-chromosomal variation confirms independent domestications of swamp and river buffalo. Animal genetics 41(4):433-435.
[9] Michelizzi V.N., Dodson M.V., Pan Z., Amaral M.E.J., Michael J.J., McLean D.J., Womack J.E., Jiang Z. (2010). Water buffalo genome science comes of age. Int. J. Biol. Sci. 6(4): 333-349.
[10] Michelizzi V.N., Wu X., Dodson M.V., Michael J.J., Zambrano-Varon J., McLean D.J., Jiang Z. (2010). A global view of 54,001 single nucleotide polymorphisms (SNPs) on the Illumina BovineSNP50 BeadChip and their transferability to water buffaloes. Int. J. Biol. Sci. 7(1): 18-27.
[11] Borghese A. Buffalo Cheese and Milk Industry. In: Buffalo Production and Research, FAO Regional Office for Europe; 2005. Chapter X.
[12] Barile V.L. (2005). Improving reproductive efficiency in female buffaloes. Livestock Production Science 92: 183-194.
[13] Rosati A., Van Vleck L.D. (2002). Estimation of genetic parameters for milk, fat, protein and mozzarella cheese production for the Italian river buffalo (Bubalus bubalis) population. Livestock Production Science 74: 185-190.
[14] Bruckmaier R.M., Blum J.W. (1998). Oxytocin release and milk removal in ruminants. Journal of Dairy Science 81: 939-949.
[15] Wakerley J.B., Clarke G., Summerlee A.J.S. (1994). Milk ejection and its control. In: The Physiology of Reproduction. Knobil E & Neill JD, Raven Press, New York pp 1131-1177.
[16] Thomas C.S., Svennersten-Sjaunja K., Bhosrekar M.R., Bruckmaier R.M. (2004). Mammary cisternal size, cisternal milk and milk ejection in Murrah buffaloes. Journal of Dairy Research 71: 162-168.
[17] Caria M., Murgia L., Pazzona A. (2011). Effects of the working vacuum level on mechanical milking of buffalo. Journal of Dairy Science 94: 1755-1761.
[18] Cosenza G., Pauciullo A., Mancusi A., Nicodemo D., Di Palo R., Zicarelli L., Di Berardino D., Ramunno L. (2007). Mediterranean river buffalo oxytocin-neurophysin I (OXT) gene: structure, promoter analysis and allele detection. Italian Journal of Animal Science 6: 303-306.
[19] Pauciullo A., Cosenza G., Steri R., Coletta A., Jemma L., Feligini M., Di Berardino D., Macciotta N.P.P., Ramunno L. (2012). An association analysis between OXT genotype and milk yield and flow in Italian Mediterranean river buffalo. Journal of Dairy Research 79(2): 150-156.
[20] Strucken E.M., de Koning D.J., Rahmatalla S.A., Brockmann G.A. (2011). Lactation curve models for estimating gene effects over a timeline. Journal of Dairy Science 94: 442-449.
[21] Thomas C.S., Bruckmaier R.M., Östensson K., Svennersten-Sjaunja K. (2005). Effect of different milking routines on milking-related release of the hormones oxytocin, prolactin and cortisol, and on milk yield and milking performance in Murrah buffaloes. Journal of Dairy Research 72: 10-18.
[22] Schams D., Mayer H., Prokopp A., Worstorff H. (1984). Oxytocin secretion during milking in dairy cows with regards to the variation and importance of a threshold level for milk removal. Journal of Endocrinology 102: 337–343
[23] Iannuzzi L., Di Meo G.P., Perucatti A., Schibler L., Incarnato D., Gallagher D., Eggen A., Ferretti L., Cribiu E.P. and Womack J. (2003). The river buffalo (Bubalus bubalis, 2n = 50) cytogenetic map: assignment of 64 loci by fluorescence in situ hybridization and R-banding. Cytogenet. Genome Res. 102: 65–75.
[24] D'Ambrosio C., Arena S., Salzano A.M., Renzone G., Ledda L., Scaloni A. (2008). A proteomic characterization of water buffalo milk fractions describing PTM of major species and the identification of minor components involved in nutrient delivery and defense against pathogens. Proteomics 8(17): 3657–3666.
[25] Cosenza G., Feligini M., Mancusi A., D'Avino A., Coletta A., Di Berardino D. and Ramunno L. (2009). Italian Mediterranean river buffalo CSN2 gene structure and promoter analysis. Ital. J. Anim. Sci. 8: 57–59.
[26] Masina P., Rando A., Di Gregorio P., Cosenza G., Mancusi A. (2007). Water buffalo kappa-casein gene sequence. Ital. J. Anim. Sci., 6 (Suppl.2): 353–355.

[27] Cosenza G., Pauciullo A., Feligini M., Coletta A., Colimoro L., Di Berardino D., Ramunno L. (2009). A point mutation in the splice donor site of intron 7 in the αs2-casein encoding gene of the Mediterranean River buffalo results in an allele-specific exon skipping. Anim. Genet. 40 (5):791.
[28] Feligini M., I. Bonizzi, J.N. Buffoni, G. Cosenza and L. Ramunno. 2009. Identification and Quantification of alpha(S1), alpha(S2), beta, and kappa-Caseins in Water Buffalo Milk by Reverse Phase-High Performance Liquid Chromatography and Mass Spectrometry. J. Agr. Food. Chem. 57:2988–2992
[29] Cosenza G., Pauciullo A., Coletta A., Di Francia A., Feligini M., Gallo D., Di Berardino D., Ramunno L. (2011). Short communication: Translational efficiency of casein transcripts in Mediterranean river buffalo. Journal of Dairy Science 94: 5691-5694.
[30] Bevilacqua, C., Helbling J. C., Miranda G., Martin P. (2006). Translational efficiency of casein transcripts in the mammary tissue of lactating ruminants. Reprod. Nutr. Dev. 5: 567–578.
[31] Kozak M. (1991). Effects of long 5′ leader sequences on initiation by eukaryotic ribosomes in vitro. Gene Expression 1: 117–125.
[32] Kozak M. (1991). Structural features in eukaryotic mRNAs that modulate the initiation of translation. J. Biol. Chem. 266: 19867– 19870.
[33] Kozak M. (1984). Point mutations close to the AUG initiator codon affect the efficiency of translation of rat preproinsulin in vivo. Nature 308 (5956): 241–246.
[34] Ferranti, P., Scaloni A., Caira S., Chianese L., Malorni A., Addeo F. (1998). The primary structure of water buffalo alpha(s1)- and beta-casein identification of phosphorylation sites and characterization of a novel beta-casein variant. J. Protein Chem. 17: 835-844.
[35] Mitra, A., Schlee P., Krause I., Blusch J., Werner T., Balakrishnan C. R., Pirchner F. (1998). Kappa-casein polymorphisms in Indian dairy cattle and buffalo: A new genetic variant in buffalo. Anim. Biotechnol. 9: 81-87.
[36] Bonfatti V., Giantin M., Gervaso M., Coletta A., Dacasto M., Carnier P. (2012). Effect of CSN1S1-CSN3 (αs1-k-casein) composite genotypes on milk production traits and milk coagulation properties in Mediterranean water buffalo. J. Dairy Sci. 95: 3435-3443
[37] Bonfatti V., Giantin M., Gervaso M., Rostellato R., Coletta A., Dacasto M., Carnier P. (2012). Short communication: CSN1S1-CSN3 (αs1-k-casein) composite genotypes affect detailed milk protein composition of Mediterranean water buffalo. J. Dairy Sci. 95: 6801-6805.
[38] Ntambi J.M. (1999). Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. The Journal of Lipid Research 40: 1549-58
[39] Gautier M., Barcelona R.R., Fritz S., Grohs C., Druet T., Boichard D., Eggen A., Meuwissen T.H.E. (2006). Fine mapping and physical characterization of two linked quantitative trait loci affecting milk fat yield on BTA26. Genetics 172: 425–436.
[40] Pauciullo A., Cosenza G., D'Avino A., Colimoro L., Nicodemo D., Coletta A., Feligini M., Marchitelli C., Di Berardino D., Ramunno L. (2010). Sequence analysis and genetic variability of stearoyl CoA desaturase (SCD) gene in the Italian Mediterranean river buffalo. Molecular & Cellular Probes 24: 407-410.
[41] Pauciullo A., Cosenza G., Steri R., Coletta A., La Battaglia A., Di Berardino D., Macciotta N.P.P., Ramunno L. (2012). A single nucleotide polymorphism in the promoter region of river buffalo stearoyl CoA desaturase gene (SCD) is associated with milk yield. Journal of Dairy Reearch 79(4): 429-435.
[42] Yang X., Fyodorov D., Deneris E.S. (1995). Transcriptional analysis of acetylcholine receptor a3 gene promoter motifs that bind SP1 and AP2. J Biol Chem 270: 8514e20.
[43] Simsek S., Bleeker P.M., van der Schoot C.E., von dem Borne A.E. (1994). Association of a variable number of tandem repeats (VNTR) in glycoprotein Ib alpha and HPA-2 alloantigens. Thromb Haemost 72: 757e61.
[44] Macciotta N.P.P., Mele M., Conte G., Serra A., Cassandro M., Dal Zotto R., Cappio Borlino A., Pagnacco G., Secchiari P. (2008). Association between a polymorphism at the Stearoyl CoA Desaturase locus and milk production traits in Italian Holsteins. Journal of Dairy Science 91: 3184–3189
[45] Kay J.K., Weber W.J., Moore C.E., Bauman D.E., Hansen L.B., Chester-Jones H., Crooker B.A., Baumgard L.H. (2005). Effect of week of lactation and genetic selection for milk yield on milk fatty acid composition in Holstein cows. Journal of Dairy Science 88: 3886-3893.