Is there possible a milk improvement in Romanian buffalo using genetic markers?

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Abstract. The aim of this paper is to argue the necessity of buffalo breeding in Romania using different genetic markers for their selection/guiding mating. The better quality of buffalo milk compared to cow's milk is also discussed, and a possible line of connection between genetic markers already used in cow' selection and those proposed for buffalo selection is drawn. Biotechnologies may be necessary to ensure a reproductive success, considering the claimed difficulties in detecting heat in buffaloes.
Key Words: buffalo, milk production, genetic markers, reproductive biotechnologies.

Introduction. Buffalo breeding was an important activity of Romanian peasants, especialy for draught power and then for milk purpose. Buffaloes are very powerful animals, being used in different works in forests or rough terrains. Buffalo milk production was reported between 800 and 1500 kg, with possibilities to obtain 1600–2000 kg in good breeding conditions. The fat percent in buffalo milk was reported between 7 and 12, with an average of 8.5 which allows the possibility for its use for cream and cheese obtaining (Coroian et al 2011b; Acatincăi 2004; Angelescu et al 1964). The lactose percent was reported between 4.19 and 4.94 and the protein percent in milk between 5.17-5.42 (Coroian et al 2011a). These contents are important for different types of fermentations occuring, and acidic dairy products and cheese obtaining. The buffalo meat is considered shoddy due to its rough and tough fibers (Angelescu et al 1964).

Unfortunately, the buffalo breeding interest gradually decreased over the time; its numerical decline registered all over the world led Food and Agriculture Organization of United Nations (FAO) to include this breed in a list of domestic animals threatened with extinction (Scherf 2000).

In this context, the aim of this paper is to identify several polymorphisms which may form the basis of a marked-assisted selection/marked-assisted guiding mating as a suitable route for improving milk production and the yield of milk processing in buffaloes.

The Romanian buffalo origin and breeding. Worldwide, there are two species of domesticated buffaloes, the river or water buffalo (*Bubalus bubalis,* with 50 chromosomes as diploid garniture) and swamp buffalo (*Bubalus carabanesis,* with 48 chromosomes as diploid garniture) (De Camargo et al 2015), and the wild Tamarao (*Anoa mindorensis*) which is with 46 chromosomes (Pandya & Khan 2006).

In Romania are found Asian Buffaloes of the river type, this species being also distributed in the South East of Europe, including Bulgaria, Hungary, Yugoslavia, but also in Greece, Italy, Turkey, Russia, Pakistan, Iran, Iraq etc. The European Buffalo is usually black or dark brown, with white marks on its head, lower legs and tail tips. Swamp buffalo is found in Thailand and West Malaysia, hybrids derived from its mating with water buffalo, being with an intermediate number of chromosomes (Pandya & Khan 2006).

From cultural point of view, the buffalo breeding in Romania has a tradition of approximately 1,500 years but nowadays is an endangered species in our country. Its

breeding system was based on buffalo keeping in households, both to use its products in human and animal nutrition, and for different kind of agriculture works. In time, its distribution area and number of individuals has changed; for example, if in 1980, the population of buffaloes in our country reached about 200,000 heads, nowadays there are only 14,000 individuals, most of them being exported during the past time to Italy, England, France or Switzerland. The reasons for this dramatic drop in their number refer to young people migration to cities, rural ageing population, and the lack of interest of young people from these areas to work in agriculture field (http://citynews.ro). Romania ranks the second in Europe in terms of buffalo breeding (after Italy). The Romanian buffalo has been approved as a native breed since 1987 due to Professor Constantin Velea, long time before its approval as a domestic breed in Italy (in 2007). In Romania, its area of distribution is Transylvania, but a novelty element is the use of this breed also in Moldavia.

Why buffalo milk? The buffalo is mainly used for milk but also for meat production. Milk, as a nutritionally complete food, contains all macro- and micronutrients which are necessary to sustain the human diet. Buffalo milk consumption is recommended in case of atherosclerosis, milk allergy, anaemia or dental problems due to its superior properties comparative to cows milk. All of its major components are in higher concentrations than in the milk of others species like cow, goat or human. It also has a higher total solids content than milk provided by other species, which gives it higher nutritional and technological properties. Other advantages of buffalo milk are:

- buffalo milk fat has a higher proportion of saturated fatty acids;
- there is a higher percentage of butyric acid in triglycerides in buffalo milk compared to the milk of cows (50% and 37%, respectively), reason why a higher butter yield is obtained from buffaloes milk than from cows milk;
- the cholesterol content of bufallo milk is about 0.65 g L^{-1}, whereas for cow milk is 3.14 g L^{-1} (Park & Haenlein 2013).

Is there a place for genetic markers use? From scientific point of view, the amount of milk depends on the genetic characteristics of individuals. Therefore, a mating guiding using genetic bases for the purpose of milk production increasing could lead to encouraging the breed spreeding in Romania, knowing that the production of buffalo milk is lower compared to cow's milk. The production performances in the case of cow's milk were obtained as a result of intensive research, so it is necessary to follow the same path in the case of buffalo milk in order to widely benefit from its extraordinary nutritional value. Buffalo milk is predominantly used to obtain yoghurt but especially cheese, thus being fully used its compositional particularities.

The yield of cheese is mainly influenced by milk proteins. Milk proteins are classified, according to their degree of solubility, in caseins (αS1-, β-, αS2- and κ-) and soluble proteins (whey proteins, mainly α lactalbumin and β lactoglobulin). As any other protein from the organism, their synthesis is based on the genetic information encoded in segments of DNA named „genes". On homologous chromosomes, each gene may be found in different variants which are named „alleles", occured during the time as a result of mutations. At least in cattle, up to now were performed many assocations between different types of alleles and different productive traits, such as milk production or protein production in milk. Such of researches are rarely on buffaloes due to the fact that just about 60% of their genomes was mapped. However, association studies performed in cows between different allelic variants and milk production yield were used in assessing of their effectiveness in buffalo. Some of alleles in cows, such as B allele of κ-casein and A^1 allele of β-casein were often associated with higher fat and protein content in the obtained milk (Hanusová et al 2010; Çardak 2005; Buchberger & Dovč 2000). The B allele of β-lactoglobulin and the BB genotype were reported to be associated with the fat percentage and fat yield (Jõudu et al 2009; Kübarsepp et al 2005; Lundén et al 1997; Aleandri et al 1990).

Although genes of interest in cows and buffaloes have different locations on chromosomes, their nucleotide sequences may be similar by the fact that, in molecular

biology determinations, some primers (sequences of nucleotides that marks the beginning and end of the gene of interest) are similar for the two species. This can be based on the fact that cows and buffaloes are species related to each other, the different number of chromosomes (60 vs 50) being due to some evolutionary processes such as the union of two acrocentric chromosomes in cows to form submetacentric chromosomes of buffaloes, but keeping the genetic information contained herein.

In buffalo, all four casein fractions are encoded by four autosomal genes closely mapped on chromosome 7, and the β-lactoglobulin gene is mapped on chromosome 12. Some reports identified only the existence of allele B of κ-casein and β-lactoglobulin genes in water buffaloes (Barłowska et al 2012).

Other genetic markers that were reported to influence the quantity and quality of milk, directly or indirectly, are:

- the bovin leptin gene (LEP) was mapped to fourth chromosome in bovine, encoding a protein with 167 aminoacids. The identified polymorphism in the bovine leptin gene locus was associated with serum leptin concentration, dry matter intake, feed intake and milk energy output. The leptin hormone is locally produced by the mammary gland and appears to play an important role in mammary gland development and lactogenesis (Rahimnahal et al 2012);

- the prolactin gene is 10-kb long, being mapped to chromosome 23 in bovine. The prolactin hormone has important functions in mammalian bodies such as the development of mammary gland, indirectly affecting milk yield and composition. Different polymorphisms on prolactin locus were associated with milk Somatic Cell Score, daily milk yield and lactose content, protein and dry matter contents (Othman et al 2011).

Reproductive biotechnologies are required…sometimes. Buffaloes are animals that reach late their sexual maturity. Moreover, many farmers complain about the difficulty in detecting heat in buffaloes. Performing hormonal treatments the artificial insemination will be possible in given times in order to obtain high conception rates. Applying reproductive biotechnologies in individuals carrying of some favorable alleles should be a part of any program of buffalo selection/guiding mating conducted on organized farms.

Conclusions. Buffaloes are primitive animals with a valuable reservoir of genes for resistance to various diseases. Their breeding could be now justified by milk and dairy products obtaining with high nutritional and economical values. Moreover, the buffalo breeding is a part of the cultural heritage in our country, many rural locations owning such animals to the delight of tourists. Like other primitive species of cattle, buffalo number significantly decreased but nowadays, using reproductive biotechnologies and basing on favorable alleles for some characters, its breeding and selection may be performed more efficient.

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