TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2025, vol. 10, No 2(19): 106–119

WATER BUFFALO: ORIGINS AND GENETIC DIVERSITY ASSOCIATED WITH ECONOMICALLY IMPORTANT TRAITS – A REVIEW

Ivona Dimitrova[1], Nevyana Stancheva[2], Krasimira Genova[3], Yordanka Yordanova[2], Pencho Penchev[2], Radena Nenova[2], Milena Bozhilova-Sakova[4]

[1]*University of Forestry, Faculty of Agronomy, Sofia, Bulgaria*
[2]*Agricultural Academy, Agricultural Institute, Shumen, Bulgaria*
[3]*University of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria*
[4]*Agricultural Academy, Institute of Animal Science, Kostinbrod, Bulgaria*
E-mail: ivonna_dimitrova@yahoo.co.uk
ORCID: 0000-0003-1830-3782 I.D., 0000-0002-5908-6916 N.S., 0000-0002-5599-7931 K.G., 0000-0002-7637-1217 M.B.-S., 0000-0002-2449-9956 Y.Y., 0000-0001-8029-6857 P.P., 0000-0002-0190-5456 R.N.

(Submitted: 6 July 2025; Accepted: 19 September 2025; Published: 27 November 2025)

ABSTRACT

Buffaloes provide humanity with high-quality food products. Due to the growing interest in healthy nutrition, the number of buffaloes in Bulgaria has been increasing since 2016. The Bulgarian Murrah breed is one of the two officially recognized buffalo breeds in Europe, which ranks among the leading dairy breeds in the world, is resistant to stress, has good adaptation abilities. Their breeding is difficult due to their late sexual maturity, summer anestrus, silent estrus and extended inter-calving interval. In order to improve the existing population, it is necessary to apply genetic selection aimed at improving the health and welfare of animals, increasing tolerance to stress factors and increasing reproductive abilities, milk and meat production by applying appropriate molecular markers. This review provides information on the identified candidate genes in buffaloes that could help optimize the production efficiency and productive potential of the Bulgarian Murrah buffaloes.

Key words: water buffalo, Murrah breed, candidate genes.

Origin and meaning

The two main species of buffalo in the world are the Asian water buffalo (*Bubalus bubalis*) and the African wild buffalo (*Syncerus caffer*) (Iamartino *et al.*, 2017; Strillacci *et al.*, 2021). The domestic water buffalo (*Bubalus bubalis*) is a two-hoofed animal with a dark color and powerful horns and is a representative of the family of the horned animals (*Bovidae*), genus *Bubalus* and tribe *Bovini*. The water buffalo was domesticated about 3,000–6,000 years ago and has significant economic importance as a meat, dairy, and draft animal (Rehman *et al.*, 2021). Domestic buffaloes are distributed throughout the world and are currently found in 129 countries (Iamartino *et al.*, 2017). Although buffaloes are classified as terrestrial mammals, they spend much of their lives in moist grasslands, tropical and subtropical forests, swamps, and rivers, which provide them with ample food, water, and shelter. An important adaptive response is wallowing in mud, which not only helps the animals cool down, given their limited sweating, but also protects them from insect bites (Rehman *et al.*, 2021).

It is believed that the domesticated water buffalo (*Bubalus bubalis*) originates from the wild buffalo (*Bos arnee*), discovered in the northeastern region of India (Rehman *et al.*, 2021). The Asian buffalo (*Bubalus bubalis*) is subdivided into two species – swamp buffalo (*Bubalus bubalis carabanesi)* with karyotype 2n = 48 and river buffalo (*Bubalus bubalis bubalis)* with karyotype 2n = 50, which differ morphologically from each other. The one-chromosome pair difference is the result of a fusion between chromosome pairs 4 and 9 in the swamp buffalo genome (Di Bernardino and Iannuzzi, 1981; Tanaka *et al.*, 2000). Swamp buffaloes are common in rice-growing regions of East and Southeast Asia, while river buffaloes are mainly found in India, Pakistan, the Middle East, and Europe and are valued for their high-quality milk with a high fat and dry matter content (Luo *et al.*, 2020). River buffaloes are usually larger than swamp buffaloes and weigh between 450 and 1000 kg, while swamp buffaloes weigh between 325 and 450 kg (Minervino *et al.*, 2020). The river buffalo originated in the Indian subcontinent and spread westward to the Balkans, North Africa, and Europe, while the swamp buffalo is found throughout Southeast Asia, from Assam and Bangladesh westward to the Yangtze River Valley in China. The current geographic ranges of river and swamp buffaloes overlap in eastern India (Assam) and Bangladesh (Zhang *et al.*, 2020). Analysis of molecular markers in river and swamp buffalo populations shows that the subspecies were domesticated independently (Kumar *et al.*, 2007; Lei *et al.*, 2007; Colli *et al.*, 2018; Young *et al.*, 2019).

These animals are raised for their economic value and as sources of labor, meat, milk, hides, and horns (Michelizzi *et al.*, 2010). They have the ability to adapt to harsh climatic conditions, are resistant to local parasites, are capable of converting low-quality feed into valuable milk and meat, and last but not least, have a long productive life (Rehman *et al.*, 2021; Strillacci *et al.*, 2021).

The world population of water buffalo is estimated at 207 million, of which 81.5% are river buffalo and 18.5% are swamp buffalo. It is concentrated primarily in Asia (196 million, 97.0%), with other significant populations in Africa (2%), South America (0.7%), and less than 0.2% in Europe and Australia. Nearly 69% of river buffalo are raised in India, while 63% of swamp buffalo are raised in China. Buffaloes make up just over 11.1% of the global bovine population. Over the past 20 years, there has been an annual increase in their population worldwide of about 2%, while in some countries, such as Iran, their numbers are decreasing (Rahimnaha *et al.*, 2012). River buffalo produces about 13% of the world's milk (Zhang *et al.*, 2020; Noce *et al.*, 2021; Shao *et al.*, 2021).

European buffaloes are a river type (Borghese, 2011). The Balkans (Eastern Europe) are a significant point in the historical migration route of the river buffalo. River buffalo moved into Southwest Asia from the domestication area of India, reaching Egypt and Turkey, and arriving in Eastern Europe and Italy in the seventh century (Noce *et al.*, 2021).

The so-called Mediterranean buffalo represents about 3% of the world's river buffalo population and includes a group of phenotypically similar populations raised in Italy, Bulgaria, Romania, Greece, Turkey, Egypt, Iran, Iraq and Syria. Although these populations share a common ancestry, in Bulgaria, Romania and to a lesser extent in Turkey, Mediterranean-type buffaloes have been crossed with Indo-Pakistani breeds (mainly Murrah and Nili-Ravi) to increase milk production. On the other hand, the Iranian and Italian populations have retained their original genetic makeup (Colli *et al.* 2018; Zhang *et al.* 2020).

The buffalo in Bulgaria

Nowadays, the only officially recognized breeds in Europe are the Mediterranean Italian Buffalo and the Bulgarian Murrah. Originally, the native buffalo of the Bulgarian buffalo breed were a significant European Mediterranean population, raised for draft, meat and dairy products. Since 1962, intensive crossbreeding of buffaloes from the local Bulgarian buffalo with bulls of the Murrah breed began, mainly with the aim of improving their productivity for milk production, as the Murrah breed is one of the most widespread in the world with high productivity for milk and meat and resistance to stress (Thiruvenkadan *et al.*, 2013). As a result, significant changes occur in the body composition and productivity of animals (Fig. 1) (Ilieva and Peeva, 2007). The developed breeding program for the Bulgarian Murrah breed is aimed at creating dairy animals with a high milk fat content (Borghese and Moioli, 2016). It has a higher resistance than cattle to various diseases, including "mad cow" (Bovine Spongiform Encephalopathy), and has a lower degree of contamination of buffalo milk by radioactive nuclides (Alexiev, 1998). According to the Annual Agrarian Reports of the Bulgarian Ministry of Agriculture and Food (2016 – 2024), the number of buffaloes has been increasing annually since 2016 and the amount of buffalo milk produced has increased accordingly.

Milk productivity

Buffalo milk accounts for 13% of total world milk production, making it one of the best raw materials for dairy product production (El-Khishin *et al.*, 2020). It contains less water, but higher amounts of protein, fat, minerals and lactose compared to cow's milk (Rehman *et al.*, 2021).

There is a significant difference in milk production between the two types of buffalo. Swamp buffaloes typically produce 1–2 kg of milk per day or an average of 350 kg of milk per lactation, while river buffaloes in Italy produce more than 2000 kg of milk in a 270-day lactation with 8.1% fat and 4.6% protein. In India, the Jaffarabadi breed and in Pakistan, the Nili-Ravi breed, produce over 2000 kg in a 319-day lactation with 7.6% fat (Iamartino *et al.*, 2017). It can be generally stated that the relatively low milk yield seriously limits the development of buffalo farming (Du *et al.*, 2019; Ye *et al.*, 2022), although Bulgarian experience in buffalo breeding and research results show that for the profitability of the farm, milk yield is not the most important trait, but the age of the first calving followed by the calving interval (Peeva, 2000; Ilieva & Penchev, 2019).

Buffalo milk production in Bulgaria, presented in Table 1, shows growth from 2016 to 2023 with a slight decline in 2022.

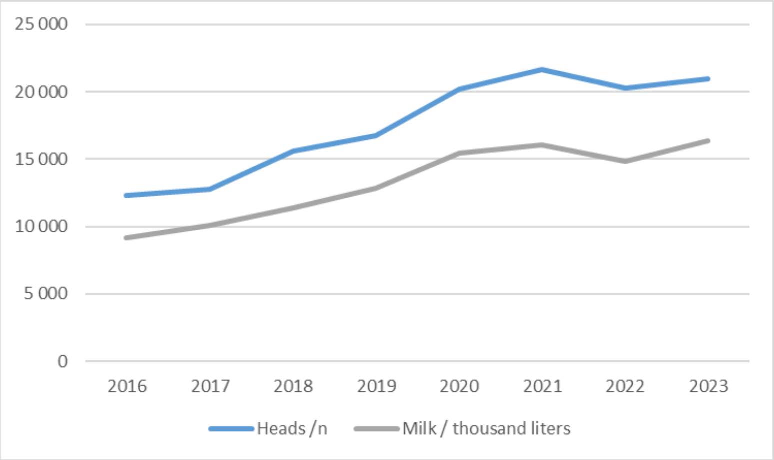

Figure 1: Buffaloes in Bulgaria – number and milk production.

Reproduction

The reproductive efficiency of buffaloes is determined by various genetic and non-genetic factors, this is expressed in a later economic maturity and a correspondingly higher age at first calving, a longer service period and a correspondingly longer calving interval, seasonality of calving, a longer gestation period – 315 days, a high percentage of silent estrus (Penchev *et al.*, 2014). Domestic buffaloes have many breeding disadvantages, most notably lower reproductive performance and higher infertility rates compared to cattle, limited productivity levels, and lower neonatal survival (Michelizzi *et al.*, 2010).

The improvement of buffalo reproductive abilities is slower compared to dairy and beef cattle (Shao *et al.*, 2021). Improving buffalo fertility is important for reducing production costs. Reproductive traits are economically important for sustainable food production, especially in animals such as cattle and buffalo, which are typically pregnant with single offspring. Low reproductive ability or infertility in this type of animal is considered to be a prolonged period between two calvings. Depending on the case, one or more additional inseminations, hormonal treatment, which often leads to changes in the current and subsequent lactations, are applied (Shao *et al.*, 2021).

Despite the great contribution of buffaloes to the livestock sector, their use for dairy production is difficult, mainly due to late sexual maturity, summer anestrus, silent estrus and prolonged calving interval (Haldar, Prakash, 2005; De Rensis and López-Gatius, 2007; Rehman *et al.*, 2021). The age of sexual maturation has a significant impact on the subsequent milk productivity of animals. A buffalo cow that reaches productive maturity earlier will produce more milk over her lifetime. Delayed puberty in water buffalo is a major problem for the dairy industry. Among all factors, body weight at an early age plays the most important role in the reproductive performance of the animals (Gupta *et al.*, 2016).

Another factor is genetics – different breeds reach maturity at different ages – Murrah reaches puberty at an average age of 33 months, Nilli Ravi at 32.5 months, and Surti at 45.5 months (Gupta *et al.*, 2016).

The optimal age of first calving according to Peeva (2000) is the subject of controversial discussions. According to the author, both very early and very late calving have negative consequences, expressed in an insufficiently well-developed organism, lower milk productivity and income realized from buffalo breeding.

According to Kanchev (1988) and Terzano (2010), puberty is the age at which specific endocrine changes occur, leading to estrus and the appearance of the first ovulation and fertilization in the body of the female. This transition usually occurs at a genetically predetermined age, but non-genetic factors such as photoperiod, body weight, season, etc. can change the inception of puberty.

Barile (2005) points out that the difficulty in determining the age of puberty in young female buffaloes is a result of difficulties in detecting the first signs of estrus and most studies have been done retrospectively according to the age of first calving.

Animals need to be selected and raised for earlier sexual maturity in conditions that allow them to realize their genetic potential. Early puberty can also be stimulated by providing adequate nutrition (Cardoso et al, 2014), as well as stimulating females who do not reach puberty on time (Oliveira *et al.*, 2009; Choudhary et al, 2022). Another problem is the seasonality in buffalo reproductive behavior, which is attributed to the effect of melatonin related to photoperiod (Gunwant et al, 2018). Modern reproductive technologies, including artificial insemination and embryo transfer, are routinely used in cases where buffaloes are bred to a good standard, with the aim of increasing genetic progress (Iamartino *et al.*, 2017).

In this regard, Nenova (2023) studied the effectiveness of the Ovsynch and Ovsynch+ PRID Delta protocols for optimizing reproduction in female buffaloes and as a result found that the influence of the season was overcome.

Molecular markers to aid buffalo selection

Genetic improvement of existing buffalo breeds requires knowledge of their genome and genetic diversity. The first buffalo genome sequence was published in 2017 (Williams *et al.*, 2017), and the next two in 2019 (Low *et al.*, 2019; Mintoo *et al.*, 2019). The genome size of *Bubalus bubalis bubalis* is estimated to be 2.77 Gb with 24,613 identified genes, and phylogenetic tree analysis indicates that the cattle and water buffalo genera diverged approximately 5.8–9.8 million years ago (Mintoo *et al.*, 2019).

The use of markers for milk yield, other production traits, disease resistance, tolerance, fertility and carcass quality in animals plays a crucial role in improving their health and productivity (Khan *et al.*, 2023). These markers allow for targeted selection and breeding programs, optimizing production and ensuring a reduced risk of infections.

Molecular markers can be a powerful tool for improving livestock performance through breeding strategies (Mishra *et al.*, 2023). In the selection of domestic buffaloes, the inclusion of highly polymorphic core genes as molecular markers is an opportunity to control important economic traits. Inbreeding can also be assessed using SNP data, for example using homozygosity series. Controlling inbreeding is important for maintaining genetic diversity and avoiding the manifestation of recessive defects. This is especially important for small local populations, where buffalo are kept in herds of few animals and inseminate naturally.

Studies of genetic diversity before the advent of high-throughput genomic technologies began with protein-coding loci such as allozymes, followed by analysis of highly variable nuclear DNA loci such as microsatellites, or uniparental markers such as maternally inherited mitochondrial DNA. Discovering the influence of genetic variation on phenotypic traits is a major challenge in genetics. A number of studies have been devoted to investigating the relationship between genotypic variation in buffalo coat color, reproduction, production traits, and diseases. (Zhang *et al.*, 2020).

Genes related to milk production

Du *et al.* (2019) reported a total of 517 candidate genes associated with milk production in different buffalo breeds, and 19 of them contained 47 mutation sites identified using the candidate gene approach. El-Khishin *et al.* (2020) identified a total of 3889 genes associated with milk itself, 1383 genes associated with milk during pregnancy, 3111 genes associated with lactation, 867 genes associated with involution, and 840 genes associated with mastitis.

Among the promising candidate genes in buffalo are DGAT1 (Diacylglycerol O-acyltransferase 1) gene, STAT1 (Signal transducer and activator of transcription 1) gene and others. In buffalo, the gene DGAT1 is located on chromosome 15, has a size of 10,733 bp, and is structurally composed of 19 exons. In animals of the Murra breed (Amaral *et al.*, 2008), an Ala484Val substitution resulting from a change in exon 17: g.11785T>C (AY032689) of the gene was found, which is associated with the protein and fat content of milk (Freitas *et al.*, 2016). Haplotypes of the DGAT1 gene showed significant associations with milk yield, % protein and % fat in buffalo (Liu *et al.*, 2020).

Another important regulator of mammary gland differentiation and cell survival and considered a candidate gene affecting milk production characteristics in buffalo is the STAT1 gene. In crosses involving the Murrah and Nili-Ravi breeds, SNPs in STAT1 have been identified that influence milk yield, fat percentage, and protein (Deng *et al.*, 2016).

The ABCG2 gene belongs to the ATP-binding cassette family of transmembrane transporters, which are also expressed in mammary epithelial cells. The protein encoded by this gene can transport a variety of molecules into milk. Its expression levels increase significantly during lactation, influencing milk yield and composition (Farke *et al.*, 2008; Sharma *et al.*, 2014). *ABCG2 is highly expressed in buffalo mammary glands (higher only in liver and brain, and lower in adipose tissue, heart and kidney) and plays an important role in milk fat synthesis* (Zhou *et al.*, 2023).

The FABP gene family encodes a group of proteins that transport long-chain fatty acids (LCFA) and play a crucial role in milk fat synthesis. FABPs bind fatty acids and transport them to various organelles for lipid metabolism. Relatively little information is available regarding the identification of members, evolutionary background, and functional characteristics of FABP genes in buffalo. In this regard, According to phylogenetic analysis, FABPs can be divided into three groups – the first includes FABP1 and FABP6, the second – FABP3, FABP4, FABP5, FABP7, FABP8, FABP9 and FABP12 (Chmurzynska, 2006), and only FABP2 belongs to the third (Ye *et al.*, 2022). Upregulation and relative mRNA abundance during lactation have been observed for various genes, including FABP3, related to the uptake of fatty acids from the blood into the mammary gland and intracellular trafficking of fatty acids (Bionaz and Loor, 2008).

Ye *et al.* (2022) performed an analysis to identify members of the FABPs in buffalo and found that the family is composed of a total of 17 FABP genes, which are grouped into five groups. These 17 genes are distributed across 13 chromosomes of the buffalo genome. One SNP in LOC102401361 was found to be significantly associated with buffalo milk yield. The expression levels of several genes in buffalo mammary epithelial cells (CRABP1, CRABP3, FABP3, FABP4, FABP5, FABP7 and MP2P) could be regulated by treatment with palmitic and stearic acids, indicating that these genes may be involved in the absorption and metabolism of these two types of fatty acids.

Peroxisome proliferator-activated receptor-γ coactivator-1α (PPARGC1A) is a gene of the PGC-1 family and is located in the middle part of chromosome 7 of the river buffalo, which is homologous to the bovine chromosome 6, known for its association with dairy production traits (Liang and Ward, 2006; Schennink *et al.* 2009). The protein synthesized by the PPARGC1A gene in both river and swamp buffalo is composed of 797 amino acid residues. It plays an important role in the activation of essential hormone receptors and transcription factors (Hosseini *et al.*, 2021).

Nasr *et al.* (2016) reported three genotypes (AA, AG, and GG) at the leptin locus, and animals with the AA genotype had the highest milk yield and fat content in Egyptian buffaloes.

Genes related to reproduction

Buffaloes, raised for their high-quality meat and high-fat milk, have a longer lifespan than cattle, exceeding 30 years, while maintaining reproductive capacity until 18–25 years of age

(Minervino *et al.*, 2020). Reproduction directly affects buffalo productivity (Barile 2005), making it of interest to identify candidate variants associated with fertility in both sexes.

Although studies in this area are limited, the genes FSHR (follicle-stimulating hormone receptor gene), OPN (osteopontin gene), and others have been shown to have significant effects on superovulation responses in buffalo (Shao *et al.*, 2021). Follicle-stimulating hormone (FSH) influences the influence of follicles, and pituitary luteinizing hormone (LH) regulates the function of the gonads. The action of LH is mediated by specific receptors that are located in the plasma membrane of specific target cells in the ovaries and testes (Shao *et al.*, 2021). Research has shown that the genes DGAT1 and LEP are also associated with reproduction (Demeter *et al.*, 2009; Nasr *et al.*, 2016). Another gene considered as a candidate marker for reproductive and functional traits is the estrogen receptor-α (ERα) gene – which influences mammary gland development, cell growth and differentiation. Murrah buffaloes show uniformity in this gene, while Egyptian buffaloes show diversity (Othman and Abd-el Samad, 2013; Kathiravan *et al.*, 2018).

In river buffaloes, different genotypes of the melatonin receptor 1A gene (MTNR1A) affect fertility rates (Pandey *et al.*, 2019), with carriers of the TT genotype at position 72 of the 812 bp fragment of exon 2 of the gene showing higher conception rates and earlier active estrus upon melatonin treatment (Gunwant et al, 2018; Zhang et al, 2020). The peak mating activity period of buffaloes with the CC genotype is from November to December, while those with the TT genotype mate mainly between May and July, i.e. the polymorphism in MTNR1 A can be considered as a genetic marker to identify reproduction of Murrah females during or outside the typical breeding season (Gunwant et al, 2018).

Genes associated with disease resistance

The immune system in animals is divided into innate and adaptive (acquired) systems, which actively cooperate with each other. Innate immunity is essential in the initial phase of pathogen invasion and any tissue damage in the first seconds and minutes, while adaptive immunity takes several days to organize a response, i.e. innate immunity is designed for a broad range while the latter is personalized and more specific (Ciliberti et al, 2025). Innate immunity is a result of natural selection and plays an important role against natural pathogens in livestock. Buffaloes are less susceptible to bacterial, viral and parasitic diseases and in general, cattle breed of Indian origin is well adapted to tropical environments due to their ability to survive under extreme nutritional stress, disease resistance, and potential for heat tolerance (Pal *et al.*, 2011). Genetic polymorphism of innate immune genes plays an important role in disease resistance in different buffalo breeds (Patel *et al.*, 2015).

In general, buffaloes have the ability to better tolerate the adverse effects of global climate change on meat and milk production (Mousbah *et al.*, 2023). They are relatively hardy and frugal animals, but they are also vulnerable to the same diseases and parasites that affect cattle, including tuberculosis, piroplasmosis, brucellosis, trypanosomiasis, and rinderpest. The extent to which they are affected varies dramatically depending on the country, region, and production system. As a result of their habit of wallowing in mud, buffaloes are less susceptible to ectoparasites, such as ticks, which cause diseases such as babesiosis, anaplasmosis, and theileriosis. Buffaloes are most commonly infected with the louse Haematopinus tuberculatus, which is specific to them. Water buffaloes are involved in the transmission of schistosomiasis to humans. Buffaloes play a known role in the zoonotic infection Q fever, which is transmitted globally, as Coxiella burnetii has also been detected in buffalo milk (Khademi *et al.*, 2019; Minervino *et al.*, 2020; El-Alfy *et al.*, 2023).

The role of genes in protection against bacterial infections has been demonstrated in water buffalo (Capparelli *et al.*, 2007) and prompted the search for polymorphisms conferring resistance to mycobacterial infection in this species. Toll-like receptors play a key role in innate immunity by recognizing pathogens and activating appropriate defense responses. Such genes are TLR2, TLR4 and TLR9 which have several mutations with a certain association with Mycobacterium bovis the infectious agent of tuberculosis (Alfano *et al.*, 2014). Toll-Like Receptor gene 4, TLR4, has the ability to recognize endotoxins associated with Gram-negative bacterial infections (Alfano *et al.*, 2014).

In mastitis infection, buffaloes upregulate the expression of immune genes related to pathogen recognition (TRL-2, TRL-4) and the cytokine network (TNF-α, IL-1β and IL-8), leading to the secretion of pro-inflammatory cytokines. Endometritis is characterized by high expression of CD14, TLR4, IL-1α, IL1-β, IL-6, IL-8 and TNF-α genes in uterine tissue and cervical mucus. Heat stress induced by climate change causes immune depression, as measured by a reduced lymphoproliferative response; in addition, high levels of HSP70 and a complex crosstalk between pro- and anti-inflammatory cytokines and oxidative stress have been reported (Ciliberti et al, 2025).

Disease resistance genes also positively influence milk production in buffaloes. Immediate protection against viral infection is provided by interferon-stimulated genes, which help increase overall productivity, such as increasing milk yield or efficient feed conversion (Mishra *et al.*, 2023).

The innate immune gene beta defensin DEFB1 is a member of a family of cationic antibacterial peptides with a broad spectrum of potent antimicrobial activity and therefore effector molecules of innate immunity in many animal species. In river buffalo, the DEFB1 gene is located on chromosome 27, and 14 SNPs have been identified in this gene in the Murrah breed (Patel *et al.*, 2015).

In addition to the role of innate immunity, the adaptive immune system – involving antigen-specific responses by T and B lymphocytes – is essential for long-term resistance and vaccination efficacy. Several genes beyond TLRs, such as NRAMP1, BoLA, and LTF, have been implicated in resistance to intracellular pathogens and mastitis. With advances in genomics, marker-assisted selection (MAS) offers opportunities for breeding disease-resistant buffaloes, while epigenetic regulation and environmental factors such as heat and nutrition also modulate immune gene expression and disease outcomes

Genes associated with heat stress

Environmental temperature regulates the physiological mechanisms of the body of living beings. Its abrupt change can culminate in heat stress. High environmental temperature reduces the activity of the thyroid gland, which leads to reduced reproductive efficiency in buffaloes (Singh *et al.*, 2013), and combined with high humidity affects fertility (Marai *et al.*, 2009) through increased production of free radicals and cortisol and reduced production of antioxidants.

Heat stress is one of the most important factors limiting ruminant production, and studies on thermotolerance in ruminants are of great importance for identifying prospective biomarkers for thermal stress. Heat stress impairs the growth, development, production and reproduction of livestock worldwide. Animals respond to heat stress through various mechanisms such as behavioral, physiological, biochemical, endocrine and molecular mechanisms. Among the aforementioned mechanisms, molecular mechanism plays a crucial role in achieving thermotolerance through the expression of a highly conserved family of proteins known as heat shock proteins (HSPs) across animal species. 382 candidate genes with sites of positive selection were identified that may play a role in the evolution of climatic adaptability in water buffalo in different environments (Mintoo *et al.*, 2019)

HSPs serve as molecular chaperones to alleviate the threat of heat stress in animals, which are critical for protein maturation, refolding, and degradation, and in farm animals, including buffalo, they not only help develop thermotolerance but also act as potential biological markers to measure the degree of heat stress in animals (Mishra, 2022). Under stress conditions, HSPs are crucial for survival, protein homeostasis, and cellular responses. HSPs are classified into 7 gene families, with a total of 64 HSP genes identified in buffalo, members of the HSP40 (39), HSP90 (4), HSPB (8), HSP70 (10), HSPH1 (1), HSP10 (1), and HSPD (1) families (Rehman *et al.*, 2020). Under thermal stress conditions, the expression of HSPs is significantly increased to enhance thermotolerance ability and serve as a first line of defense against heat shock to protect cells and tissues (Rehman *et al.*, 2021).

Conclusion

Genetic improvement of existing buffalo breeds requires knowledge of their genome and genetic diversity. Molecular markers can be a powerful tool for improving the productivity of livestock included in breeding strategies.

Buffaloes in Bulgaria are a genetic resource whose population can achieve faster progress through selection based on the application of molecular markers in terms of the main breeding traits: reproduction, milk production, disease resistance, which would help to optimize the reproductive efficiency and productive potential of buffaloes of the Bulgarian Murrah breed.

References

1. *Annual Agrarian Reports of the Bulgarian Ministry of Agriculture and Food*. (2016 – 2023).
2. Alexiev, A. (1998). *The Water Buffalo*. Bulgaria: St. Kliment Ohridski University Press.
3. Alfano, F., Peletto, S., Lucibelli, M.G., *et al.* (2014). *Identification of single nucleotide polymorphisms in Toll-like receptor candidate genes associated with tuberculosis infection in water buffalo (Bubalus bubalis)*. BMC Genetics, 15:139.
4. Amaral, M., Grant, J., Riggs, P., Stafuzza, N., Filho, E., Goldammer, T., *et al.* (2008) *A first generation whole genome RH map of the river buffalo with comparison to domestic cattle*. BMC Genomics. 9:631. doi: 10.1186/1471-2164-9-631.
5. Barile, V. (2005) *Improving reproductive efficiency in female buffaloes*. Animal Science, 92, 183–94.
6. Bionaz, M., & Loor, J. (2008). *Gene networks governing bovine milk fat synthesis during the lactation cycle*. BMC Genomics 9, 366. https://doi.org/10.1186/1471-2164-9-366.
7. Borghese, A. (2011). *Situation and perspectives of buffalo in the World. Europe and Macedonia*. Mac. J. Anim. Sci. 1, 281–296.
8. Borghese, A., & Moioli, B. (2016). *Buffalo: Mediterranean Region*. Reference Module in Food Science, B978-0-08-100596-5.21232-8. https://doi.org/10.1016/B978-0-08-100596-5.21232-8.

9. Capparelli, R., Alfano, F., Amoroso, M. G., Borriello, G., Fenizia, D., Bianco, A., Roperto, S., Roperto, F., & Iannelli, D. (2007). *Protective effect of the Nramp1 BB genotype against Brucella abortus in the water buffalo (Bubalus bubalis)*. Infection and immunity, 75(2), 988–996. https://doi.org/10.1128/IAI.00948-06

10. Cardoso, R. C., Alves, B. R., Prezotto, L. D., Thorson, J. F., Tedeschi, L. O., Keisler, D. H., Park, C. S., Amstalden, M., & Williams, G. L. (2014). *Use of a stair-step compensatory gain nutritional regimen to program the onset of puberty in beef heifers*. Journal of animal science, 92(7), 2942–2949. https://doi.org/10.2527/jas.2014-7713.

11. Ciliberti, M. G., Santillo, A., Caroprese, M., & Albenzio, M. (2025). *Buffalo Immune Competence Under Infectious and Non-Infectious Stressors*. Animals, 15(2), 163. https://doi.org/10.3390/ani15020163.

12. Chmurzynska, A. (2006). *The multigene family of fatty acid-binding proteins (FABPs): Function, structure and polymorphism*. Journal of Applied Genetics, 47, 39–48.

13. Colli, L., Milanesi, M., Vajana, E., Iamartino, D., Bomba, L., & Puglisi, F. (2018). *New insights on water buffalo genomic diversity and post-domestication migration routes from medium density SNP chip data*. Frontiers in Genetics. 9, 53. doi: 10.3389/fgene.2018.00053.

14. Choudhary, S., Kamboj, M. L., Sahu, D., Dutt, S., Magotra, A., Singh, P., Kumar, N., Ungerfeld, R., & Kotresh Prasad, C. (2022). *Effect of biostimulation on growth rate and reproductive development of Bos indicus dairy heifers*. Tropical animal health and production, 54(2), 138. https://doi.org/10.1007/s11250-022-03129-3.

15. Demeter, R. M., Schopen, G. C., Lansink, A. G., Meuwissen, M. P., & van Arendonk, J. A. (2009). *Effects of milk fat composition, DGAT1, and SCD1 on fertility traits in Dutch Holstein cattle*. Journal of dairy science, 92(11), 5720–5729. https://doi.org/10.3168/jds.2009-2069.

16. De Rensis, F., & López-Gatius, F. (2007). *Protocols for synchronizing estrus and ovulation in buffalo (Bubalus bubalis): a review*. Theriogenology, 67(2), 209–216. https://doi.org/10.1016/j.theriogenology.2006.09.039.

17. Deng, T. X., Pang, C. Y., Lu, X. R., Zhu, P., Duan, A. Q., & Liang, X. W. (2016). *Associations between polymorphisms of the gene and milk production traits in water buffaloes*. Journal of animal science, 94(3), 927–935. https://doi.org/10.2527/jas.2015-0139.

18. Di Berardino, D., & Iannuzzi, L. (1981). *Chromosome banding homologies in Swamp and Murrah buffalo*. The Journal of heredity, 72(3), 183–188. https://doi.org/10.1093/oxfordjournals.jhered.a109469.

19. Du, C., Deng, T., Zhou, Y., Ye, T., Zhou, Z., Zhang, S., Shao, B., Wei, P., Sun, H., Khan, F. A., Yang, L., & Hua, G. (2019). *Systematic analyses for candidate genes of milk production traits in water buffalo (Bubalus Bubalis)*. Animal genetics, 50(3), 207–216. https://doi.org/10.1111/age.12739

20. El-Alfy, E. S., Abbas, I., Elseadawy, R., Saleh, S., Elmishmishy, B., El-Sayed, S. A. E., & Rizk, M. A. (2023). *Global prevalence and species diversity of tick-borne pathogens in buffaloes worldwide: a systematic review and meta-analysis*. Parasites & vectors, 16(1), 115. https://doi.org/10.1186/s13071-023-05727-y.

21. El-Khishin, D. A., Ageez, A., Saad, M. E., Ibrahim, A., Shokrof, M., Hassan, L. R., & Abouelhoda, M. I. (2020). *Sequencing and assembly of the Egyptian buffalo genome*. PloS one, 15(8), e0237087. https://doi.org/10.1371/journal.pone.0237087.

22. Farke, C., Meyer, H. H., Bruckmaier, R. M., & Albrecht, C. (2008). *Differential expression of ABC transporters and their regulatory genes during lactation and dry period in bovine mammary tissue*. The Journal of dairy research, 75(4), 406–414. https://doi.org/10.1017/S002202990800335X.

23. Freitas, A. C., de Camargo, G. M., Stafuzza, N. B., Aspilcueta-Borquis, R. R., Venturini, G. C., Dias, M. M., Cardoso, D. F., & Tonhati, H. (2016). *Genetic association between SNPs in the DGAT1 gene and milk production traits in Murrah buffaloes*. Tropical animal health and production, 48(7), 1421–1426. https://doi.org/10.1007/s11250-016-1110-x.
24. Gunwant, P., Pandey, A. K., Kumar, A., Singh, I., Kumar, S., Phogat, J. B., Kumar, V., Patil, C. S., Tomar, P., Kumar, S., & Magotra, A. (2018). *Polymorphism of melatonin receptor (MTNR1A) gene and its association with seasonal reproduction in water buffalo (Bubalus bubalis)*. Animal reproduction science, 199, 51–59. https://doi.org/10.1016/j.anireprosci.2018.10.006.
25. Gupta, S., Singh, P., Shinde, K., Lone, S., Kumar, N., & Kumar, A. (2016). *Strategies for attaining early puberty in cattle and buffalo: A review*. Agricultural Reviews, 37 (2): 160–167.
26. Haldar, A., & Prakash, B. S. (2005). *Peripheral patterns of growth hormone, luteinizing hormone, and progesterone before, at, and after puberty in buffalo heifer*. Endocrine research, 31(4), 295–306. https://doi.org/10.1080/07435800500422608.
27. Hosseini, S. M., Tingzhu, Y., Pasandideh, M., Liang, A., Hua, G., Farmanullah, Schreurs, N. M., Raza, S. H. A., Salzano, A., Campanile, G., Gasparrini, B., & Yang, L. (2021). *Genetic Association of PPARGC1A Gene Single Nucleotide Polymorphism with Milk Production Traits in Italian Mediterranean Buffalo*. BioMed research international, 2021, 3653157. https://doi.org/10.1155/2021/3653157.
28. Iamartino, D., Nicolazzi, E. L., Van Tassell, C. P., Reecy, J. M., Fritz-Waters, E. R., Koltes, J. E., Biffani, S., Sonstegard, T. S., Schroeder, S. G., Ajmone-Marsan, P., Negrini, R., Pasquariello, R., Ramelli, P., Coletta, A., Garcia, J. F., Ali, A., Ramunno, L., Cosenza, G., de Oliveira, D. A. A., Drummond, M. G., … Williams, J. L. (2017). *Design and validation of a 90K SNP genotyping assay for the water buffalo (Bubalus bubalis)*. PloS one, 12(10), e0185220. https://doi.org/10.1371/journal.pone.0185220.
29. Ilieva, Y., & Peeva, T. (2007). *Productive life in buffalo cows and effect of some factors on it*. Italian Journal of Animal Science, 6: supl 2, 375–377, doi: 10.4081/ijas.2007.s2.375.
30. Ilieva, Y., & Penchev, P. (2019). *Breeding efficiency and related traits in buffalo cows under the conditions of Nature Park "Rusenski Lom"*. Bulgarian Journal of Agricultural Science, 25(2), 380–384.
31. Kanchev, L. (1988). *Radioimmune method for the diagnosis of early pregnancy and the functional activity of the ovaries*. Veterinary Collection, 86(3): 6–16 (Bg).
32. Kathiravan, RS, R. Chitra, N. Murali & Arthanarieswaran, M. (2018). *Genetic polymorphism of estrogen hormone receptor gene (Exon C) in buffalo*. International Journal of Current Microbiology and Applied Sciences, 7(2): 2718–2721. doi.org/10.20546/ijcmas.2018.702.330.
33. Khademi, P., Ownagh, A., Mardani, K., & Khalili, M. (2019). *Prevalence of Coxiella burnetii in milk collected from buffalo (water buffalo) and cattle dairy farms in Northwest of Iran*. Comparative immunology, microbiology and infectious diseases, 67, 101368. https://doi.org/10.1016/j.cimid.2019.101368.
34. Khan, I. M., Khan, A., Liu, H., & Khan, M. Z. (2023). *Editorial: Genetic markers identification for animal production and disease resistance*. Frontiers in genetics, 14, 1243793. https://doi.org/10.3389/ fgene.2023.1243793.
35. Kumar, S., Nagarajan, M., Sandhu, J. S., Kumar, N., Behl, V., and Nishanth, G. (2007). *Mitochondrial DNA analyses of Indian water buffalo support a distinct genetic origin of river and swamp buffalo*. Anim. Genet. 38 (3), 227–232. doi: 10.1111/j.1365-2052.2007.01602.x.

36. Lei, C. Z., Zhang, W., Chen, H., Lu, F., Liu, R. Y., Yang, X. Y., Zhang, H. C., Liu, Z. G., Yao, L. B., Lu, Z. F., & Zhao, Z. L. (2007). *Independent maternal origin of Chinese swamp buffalo (Bubalus bubalis)*. Animal genetics, 38(2), 97–102. https://doi.org/10.1111/j.1365-2052.2007.01567.x.

37. Liang, H., & Ward, W. F. (2006). *PGC-1alpha: a key regulator of energy metabolism*. Advances in physiology education, 30(4), 145–151. https://doi.org/10.1152/advan.00052.2006.

38. Liu, J., Wang, Z., Li, J., Li, H., & Yang, L. (2020). *Genome-wide identification of Diacylglycerol Acyltransferases (DGAT) family genes influencing Milk production in Buffalo*. BMC genetics, 21(1), 26. https://doi.org/10.1186/s12863-020-0832-y.

39. Liu, H., Khan, IM, Khan, MZ, Khan, A., eds. (2023*). Identification of genetic markers for animal production and disease resistance*. Lausanne: Frontiers Media SA.doi: 10.3389/978-2-8325-3096-2.

40. Low, W. Y., Tearle, R., Bickhart, D. M., Rosen, B. D., Kingan, S. B., Swale, T., Thibaud-Nissen, F., Murphy, T. D., Young, R., Lefevre, L., Hume, D. A., Collins, A., Ajmone-Marsan, P., Smith, T. P. L., & Williams, J. L. (2019). *Chromosome-level assembly of the water buffalo genome surpasses human and goat genomes in sequence contiguity*. Nature communications, 10(1), 260. https://doi.org/10.1038/s41467-018-08260-0.

41. Luo, X., Zhou, Y., Zhang, B., Zhang, Y., Wang, X., Feng, T., Li, Z., Cui, K., Wang, Z., Luo, C., Li, H., Deng, Y., Lu, F., Han, J., Miao, Y., Mao, H., Yi, X., Ai, C., Wu, S., Li, A., … Liu, Q. (2020). *Understanding divergent domestication traits from the whole-genome sequencing of swamp- and river-buffalo populations*. National science review, 7(3), 686–701. https://doi.org/10.1093/nsr/nwaa024.

42. Marai, I.F.M., & Habeeb, A.A.M. (2009). *Buffalo's biological function as affected byheat stress: A review*. Livestoc Science, 127(2-3):89–109.

43. Michelizzi, V. N., Dodson, M. V., Pan, Z., Amaral, M. E., Michal, J. J., McLean, D. J., Womack, J. E., & Jiang, Z. (2010). *Water buffalo genome science comes of age*. International journal of biological sciences, 6(4), 333–349. https://doi.org/10.7150/ijbs.6.333.

44. Minervino, A. H. H., Zava, M., Vecchio, D., & Borghese, A. (2020). *Bubalus bubalis: A Short Story*. Frontiers in veterinary science, 7, 570413. https://doi.org/10.3389/fvets.2020.570413.

45. Mintoo, A. A., Zhang, H., Chen, C., Moniruzzaman, M., Deng, T., Anam, M., Emdadul Huque, Q. M., Guang, X., Wang, P., Zhong, Z., Han, P., Khatun, A., Awal, T. M., Gao, Q., & Liang, X. (2019). *Draft genome of the river water buffalo*. Ecology and evolution, 9(6), 3378–3388. https://doi.org/10.1002/ece3.4965.

46. Mishra, D. C., Bhati, J., Yadav, S., Avashthi, H., Sikka, P., Jerome, A., Balhara, A. K., Singh, I., Rai, A., & Chaturvedi, K. K. (2023). *Comparative expression analysis of water buffalo (Bubalus bubalis) to identify genes associated with economically important traits*. Frontiers in veterinary science, 10, 1160486. https://doi.org/10.3389/fvets.2023.1160486.

47. Mishra S. R. (2022). *Significance of molecular chaperones and micro RNAs in acquisition of thermo-tolerance in dairy cattle*. Animal biotechnology, 33(4), 765–775. https://doi.org/10.1080/10495398.2020.1830788.

48. Mousbah AM, Abdullah, HM, Mohammed, WS, El-Refy, AM., & Helmy, M. (2023). *Buffalo Genome Projects: Current Situation and Future Perspective in Improving Breeding Programs*. https://www.researchgate.net/publication/369946197.

49. Nasr, M., Awad, A., & El Araby, I. E. (2016). *Associations of Leptin and Pituitary-Specific Transcription Factor Genes' Polymorphisms with Reproduction and Production Traits in Dairy Buffalo*. Reproduction in domestic animals = Zuchthygiene, 51(4), 597–603. https://doi.org/10.1111/rda.12726.

50. Nenova, R., Ilieva, Y., Penchev, P., Vasilev, N., Fasulkov, I., Nikolova, G., Karamalakova, Y., & Gadjeva, V. (2023). *Pregnancy Rates Associated with Oxidative Stress after Estrus Synchronization of Bulgarian Murrah Buffaloes in Breeding and Non-Breeding Season*. Journal of Buffalo Science, 12, 38–43. https://doi.org/10.6000/1927-520X.2023.12.05.
51. Noce, A., Qanbari, S., González-Prendes, R., Brenmoehl, J., Luigi-Sierra, M. G., Theerkorn, M., Fiege, M. A., Pilz, H., Bota, A., Vidu, L., Horwath, C., Haraszthy, L., Penchev, P., Ilieva, Y., Peeva, T., Lüpcke, W., Krawczynski, R., Wimmers, K., Thiele, M., & Hoeflich, A. (2021). *Genetic Diversity of Bubalus bubalis in Germany and Global Relations of Its Genetic Background*. Frontiers in genetics, 11, 610353. https://doi.org/10.3389/fgene.2020.610353.
52. Oliveira, C. M., Oliveira Filho, B. D., Gambarini, M. L., Viu, M. A., Lopes, D. T., & Sousa, A. P. (2009). *Effects of biostimulation and nutritional supplementation on pubertal age and pregnancy rates of Nelore heifers (Bos indicus) in a tropical environment*. Animal reproduction science, 113(1-4), 38–43. https://doi.org/10.1016/j.anireprosci.2008.08.006.
53. Othman OE, & Abd-el Samad, MF. (2013). *RFL polymorphism of three fertility genes in Egyptian buffalo*. Journal of Applied Biological Sciences, 7(2): 94–101.
54. Pal, A., Sharma, A., Bhattacharya, T. K., Chatterjee, P. N., & Chakravarty, A. K. (2011). *Molecular Characterization and SNP Detection of CD14 Gene of Crossbred Cattle*. Molecular biology international, 2011, 507346. https://doi.org/10.4061/2011/507346.
55. Pandey, A. K., Gunwant, P., Soni, N., Kavita, Kumar, S., Kumar, A., Magotra, A., Singh, I., Phogat, J. B., Sharma, R. K., Bangar, Y., Ghuman, S. P. S., & Sahu, S. S. (2019). *Genotype of MTNR1A gene regulates the conception rate following melatonin treatment in water buffalo*. Theriogenology, 128, 1–7. https://doi.org/10.1016/j.theriogenology.2019.01.018.
56. Patel, S. M., Koringa, P. G., Nathani, N. M., Patel, N. V., Shah, T. M., & Joshi, C. G. (2015). *Exploring genetic polymorphism in innate immune genes in Indian cattle (Bos indicus) and buffalo (Bubalus bubalis) using next generation sequencing technology*. Meta gene, 3, 50–58. https://doi.org/10.1016/j.mgene.2015.01.002.
57. Peeva, Tz. (2000). *Optimized methods of selection in buffaloes*. Doc. Agric. Sci. Thesis, Sofia, pp. 320.
58. Penchev, P., Ilieva, Y., & Dimov, K. (2014). *Effect of season of birth on season of calving and age at first calving in buffalo heifers*. Bulg. J. Agric. Sci., 20: 447–451.
59. Rahimnaha, S., Keshavarz, V., Sadr, A., & Moosavi, M. (2012). *Investigation of leptin polymorphism by PCR-RFLP in Iranian buffalo*. International Research Journal of Applied and Basic Sciences, 3(8), 1658–1661.
60. Rehman, S. U., Nadeem, A., Javed, M., Hassan, F. U., Luo, X., Khalid, R. B., & Liu, Q. (2020). *Genomic Identification, Evolution and Sequence Analysis of the Heat-Shock Protein Gene Family in Buffalo*. Genes, 11(11), 1388. https://doi.org/10.3390/genes11111388.
61. Rehman, S. U., Hassan, F. U., Luo, X., Li, Z., & Liu, Q. (2021). *Whole-Genome Sequencing and Characterization of Buffalo Genetic Resources: Recent Advances and Future Challenges*. Animals: an open access journal from MDPI, 11(3), 904. https://doi.org/10.3390/ani11030904.
62. Schennink, A., Bovenhuis, H., Léon-Kloosterziel, K. M., van Arendonk, J. A., & Visker, M. H. (2009). *Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition*. Animal genetics, 40(6), 909–916. https://doi.org/10.1111/j.1365-2052.2009.01940.x.
63. Singh, M., Chaudhari, B.K., Singh, J., Singh, A., & Maurya, P. (2013). *Effect of thermal load on buffalo reproductive performance during summerseason*. Journal Biology Science, 1(1):1–8.

64. Sharma, A., Aggarwal, J., Sodhi, M., Kishore, A., Mishra, B. P., Mohanty, A. K., Kataria, R. S., Kaushik, J. K., & Mukesh, M. (2014). *Stage specific expression of ATP-binding cassette and solute carrier superfamily of transporter genes in mammary gland of riverine buffalo (Bubalus bubalis)*. Animal biotechnology, 25(3), 200–209. https://doi.org/10.1080/10495398.2013.839949.
65. Shao, B., Sun, H., Ahmad, M. J., Ghanem, N., Abdel-Shafy, H., Du, C., Deng, T., Mansoor, S., Zhou, Y., Yang, Y., Zhang, S., Yang, L., & Hua, G. (2021). *Genetic Features of Reproductive Traits in Bovine and Buffalo: Lessons from Bovine to Buffalo*. Frontiers in genetics, 12, 617128. https://doi.org/10.3389/fgene.2021.617128.
66. Strillacci, M. G., Moradi-Shahrbabak, H., Davoudi, P., Ghoreishifar, S. M., Mokhber, M., Masroure, A. J., & Bagnato, A. (2021). *A genome-wide scan of copy number variants in three Iranian indigenous river buffaloes*. BMC genomics, 22(1), 305. https://doi.org/10.1186/s12864-021-07604-3.
67. Tanaka, K., Matsuda, Y., Masangkay, J. S., Solis, C. D., Anunciado, R. V., Kuro-o, M., & Namikawa, T. (2000). *Cytogenetic analysis of the tamaraw (Bubalus mindorensis): a comparison of R-banded karyotype and chromosomal distribution of centromeric satellite DNAs, telomeric sequence, and 18S-28S rRNA genes with domestic water buffaloes*. The Journal of heredity, 91(2), 117–121. https://doi.org/10.1093/jhered/91.2.117.
68. Terzano, G.M, Mazzi, M., d'Elisi, M.G., Cuscunà, F.P., Borghese, A., Martiniello, P., & Pacelli, C. (2010). *Effect of intensive or extensive systems on buffalo heifer performances: Body measurements and respective indices*. Italian Journal of Animal Science, 6.
69. Thiruvenkadan, A.K., Rajendran, R., & Muralidharan, J. (2013). *Buffalo genetics resources of India and their conservation*. Buffalo Bull., 32, 227–235.
70. Ye, T., Shaukat, A., Yang, L., Chen, C., Zhou, Y., & Yang, L. (2022). *Evolutionary and Association Analysis of Buffalo FABP Family Genes Reveal Their Potential Role in Milk Performance*. Genes, 13(4), 600. https://doi.org/10.3390/genes13040600.
71. Young, R., Lefevre, L., Bush, S. J., Joshi, A., Singh, S. H., Jadhav, S. K., Dhanikachalam, V., Lisowski, Z. M., Iamartino, D., Summers, K. M., Williams, J. L., Archibald, A. L., Gokhale, S., Kumar, S., & Hume, D. A. (2019). *A Gene Expression Atlas of the Domestic Water Buffalo (Bubalus bubalis)*. Frontiers in genetics, 10, 668. https://doi.org/10.3389/fgene.2019.00668.
72. Zhang, Y., Colli, L., & Barker, J. S. F. (2020). *Asian water buffalo: domestication, history and gene*tics. Animal genetics, 51(2), 177–191. https://doi.org/10.1111/age.12911.
73. Zhou, F., Fan, X., Xu, X., Li, Z., Qiu, L., & Miao, Y. (2023). *Molecular Characteristics and Polymorphisms of Buffalo (Bubalus bubalis) ABCG2 Gene and Its Role in Milk Fat Synthesis*. Animals: an open access journal from MDPI, 13(19), 3156. https://doi.org/10.3390/ani13193156.