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CONSERVATION OF GENETIC RESOURCES OF AUTOCHTHONOUS DOMESTIC LIVESTOCK BREEDS IN BULGARIA. A REVIEW

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Abstract

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The first part of the present paper makes on overview of available literature reflecting the global trends for conservation of genetic resources in livestock husbandry. The second part presents the status and organisation of activities aimed at conservation of autochthonous domestic livestock breeds in Bulgaria. The role of state establishments and non-governmental organisation, the state of populations and conservation approaches, as well as the economic and social significance of Bulgarian autochthonous breeds' conservation are described. A special attention is paid on research devoted to conservation on Bulgarian autochthonous animal breeds in both historical and modern perspective. The analysis of the state of genetic resources demonstrated that the tendencies in Bulgaria were not quite different from European and global tendencies. In conclusion, it is emphasized that there is a need for development, update and implementation of efficient programmes and strategies for conservation of the genetic fund and genetic variability, specific for autochthonous animal breeds. This process requires proper decisions that could be achieved through continuous interaction between state institutions and non-governmental organisations, namely breeding associations and unions, animals' farms and livestock husbandry research centres in Bulgaria.

Key words: animal genetic resources, conservation, Bulgarian autochthonous breeds

Introduction

Livestock genetic resources include a remarkable variety of domestic animal breeds and their populations, which have developed and adapted to the various environmental conditions for centuries. According to the definition of FAO experts, farm animal genetic resources (AnGR) comprise all animal species and their populations, which are used or could be used for production of foods or for agricultural purposes. This group involves also all breeds, populations and selected farm animal lines together with their conserved genetic material. All they are now of economic, scientific and cultural interest to humanity (Henson, 1992), but before being preserved, conservation priorities have to be set (Barker, 2001). The global state of genetic resources in the field of livestock breeding is presented on a periodical basis under the form of World watch list for domestic animal diversity от FAO. Despite the increasing number of populations according to national reports and included in global databases, the last decade has witnessed a trend for increase in threatened and endangered breeds. The number of extinct farm animal breeds is also increasing. Were the global data more accurate, probably this negative tendency would be even more pronounced in the view of FAO experts (FAO, 2011, 2013).

It could be noted that this is especially true for autochthonous and local breeds, most of which are distinguished with low productivity. Thus, their rearing is economically unprofitable and often, farmers are forced to seek different solutions to this problem. They are mostly limited to change or crossbreeding of the autochthonous breed with another, contemporary high-yielding breed or its total eradication (Rege, 1999; Bianchi et al., 2011). Numerous authors outlined other causes for reduction of the number of breeds and populations as disturbances in habitats' environment, intensification and mechanisation of the agriculture, sudden changes in market preferences etc. (Soysal et al., 2003; Tisdell, 2003; Roosen et al., 2005). The need for conservation of breeds and their genetic variability in farm animals is a problem that humanity

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is fully aware of. An evidence for this is the Convention of Biological Diversity ratified by 168 states in 1992. The Convention requires from ratifying states to carry the responsibility for preservation and sustainable development of genetic resources on earth including these relevant to people's sustenance and agriculture (Martyniuk and Plachenaut, 1998). As the conservation of all livestock breeds is not possible, they should be classified according to specific criteria. The latter include divergence among the breeds, extinction threat, breed merits, within-breed differences etc, With this regard, some authors discuss the main arguments for inclusion of a given breed in conservation measures which could be summarised as follows: threat extent, possibility for adaptation to specific environmental conditions, traits of economic significance, uniqueness and cultural historical value of the breed etc. (Oldenbrok, 1999; Caballero et al., 2008). At present, different criteria are used for classification of breeds according to their risk status – number of breeders, degree of inbreeding and effective population size, introduced by the leading international monitoring systems (Nunney, 1993; Hedgecock, 1994; Alderson, 2003; Gandini et al., 2004; Maiwashe and Blackburn, 2004; Gama, 2006; Avdi and Banos, 2008; Fernandez et al., 2011; Hristova et al., 2014).

From the point of view of conservation biology, the preservation of a given population depends on its effective size (Taberlet et al., 2008; Groeneveld et al., 2010; Taberlet et al., 2011). According to Mendelsohn (2003) and Boettcher et al. (2010) the breed uniqueness should be of utmost priority as compared to breeds with similar genetic constitution. In the context of genetic diversity preservation of Bulgarian indigenous breeds, Danchev (1994) states that local breeds occupy a strategic place in the natural evolutionary chain between their wild ancestors and high-yielding specialised breeds, so measures aimed at their conservation should be taken. In line with the FAO global strategy for the management of farm animal genetic resources, Hinkovski and Stoykov (2001) affirmed that the fundamental problem of Bulgarian livestock husbandry was the creation of a molecular database for local breeds of domestic animals with respect to their conservation. All reviewed global policies, administrative stipulations and research opinions associated to conservation of genetic resources in livestock husbandry enhanced the interest of Bulgarian scientists and farmers towards the conservation and economic utilisation of genetic resources of autochthonous and local animal breeds in the Republic of Bulgaria.

State policy for conservation and management of genetic resources of autochthonous breeds

The preservation of genetic resources in livestock husbandry is among the main priorities of the Ministry of Agriculture and Food of the Republic of Bulgaria and its structural unit – the Executive Agency on Selection and Reproduction in Animal Breeding (EASRAB). The entire activity in this direction is subordinated to Bulgarian, European and global legal provisions, covenants and requirements which set the guidelines and responsibilities of the national state policy for conservation of genetic resources of Bulgarian livestock breeds. Evidence for this could be found in the latest amendments of the Livestock Act made in 2010. With them, the functions of EASRAB are expanded and "management and conservation of genetic resources" is added to its primary obligations (Nikolov, 2011). In line with that, livestock breeds are classified in a new way including: 1. Local breeds; 2. Locally adapted and newly created breeds; 3. Transborder breeds; 4. Introduced breeds and 5. Synthetic populations.

This allows classification of genetically valuable breeds within the genetic fund according to their risk status as well. For this purpose, Bulgaria has adopted the approach of FAO (2007), to classify breeds into 4 groups according to the risk of extinction – extinct breeds, critical breeds, endangered breeds and breeds not at risk. For constant monitoring of genetic resources and implementation of adequate conservation measures, the EASRAB has created and maintains registers of breeder herds and male and female breeders. The administrative responsibility for management and conservation of AnGR is entrusted to the Executive Director of EASRAB, which fulfills also the functions of a National Coordinator of these activities. The collective management body is the National Council of Livestock Genetic Resources, which included representatives of formal breeder associations in Bulgaria (Nikolov, 2011). Apart the legally regulated organisational and control activities of EASRAB, the state is working to ensure the preservation and increase of animal populations from unique national breeds through financial stimuli for owners.

From the beginning of 2015, a National network for conservation of rare breeds has been founded to the EASRAB with development of internet-based register.

State of genetic resources of autochthonous livestock breeds

Of all livestock breeds, lines and hybrids subject to control from the part of EASRAB, a total of 29 Bulgarian breeds from different species have been declared autochthonous with a different not-at-risk status or endangered with extinction. This group encompasses all old Bulgarian breeds, result from traditional selection in different regions of the country. Information on this topic could be found in the Fifth National Report of the Ministry of Environment and Waters under the Convention of Biological Diversity for the period 2009-2013 (Table 1). The analysis of change in animal populations from

these breeds showed that 14 of them were not at risk for extinction. Another 10 breeds are in the group of non-protected endangered breeds and only one has been assessed as critically endangered. Analysing data with respect to species, it could be affirmed that for this period, the number of cattle from autochthonous breeds has increased twice, while Karakachan horse population – almost seven times, with relatively stable and consistent rates. In goats, a process of initial registration is currently ongoing and that is why the population is rapidly increasing. Such an increase has been detected for other species in the beginning of registration periods. The most inconsistent dynamics was established for sheep breeds. The Central Stara Planina, Karakachan, Duben, Copper Red Shumen, Central Rhodope, Patch-faced Maritza and Sakar sheep breeds exhibit a stable development. The state of White Maritza, Breznik, Koprivshtitsa and Stara Zagora sheep breeds is alarming. The local Karnobat sheep is in a very difficult situation, as it is not protected and critically endangered with extinction. The two hunting dog breeds – Bulgarian Ludogorian hound and Bulgarian course-haired hound (Barak) are also in the group of noon-protected endangered breeds. The most serious risk is incurred by the Black Shumen chicken, whose population is significantly reduced and critically endangered.

Significance and role of non-governmental organisations (NGOs)

So far, 47 breeder associations and unions have been registered in Bulgaria, representing the non-governmental organisations in livestock husbandry. The activities of 18 of them are associated to the conservation and economic use of different Bulgarian autochthonous animal breeds. Their foundation is based both on the strive for preservation of historically deep family and regional traditions, as well as on the possibilities for group upholding specific interests and positions to state establishments, referring to organisation of production, subsidisation of activities and realisation of the produce. Also, breeder associations maintain registers of animals and implement respective breeding programmes, organise exhibitions etc. Here, it should be noted that breeder associations and unions, together with EASRAB and the support of various research teams, play an important role for the conservation and management of AnGR of Bulgarian livestock husbandry.

Conservation Methods and Results

The requirements for protection and conservation of AnGR and have imposed two main directions in conservation: in vivo – conservation of live individuals and in vitro – cryoconservation of semen, oocytes, embryos and embryonic cells, somatic cells etc. (Simianer, 2005; Vincent et al., 2014). Practically, the realisation of conservation programmes is performed through two methods: *in situ* – rearing the animals from respective breeds in their natural habitats (Henson, 1992; Rege, 2001; Yakubu et al., 2011) and *ex situ* – rearing of animals out of their natural environment or via genetic plasma cryoconservation (Hiemstra et al., 2005; Lustykova et al., 2008; Vincent et al., 2014). In Bulgaria, EASRAB and breeder associations implement projects and programmes for in situ conservation of genetic resources. EASTAB is the only establishment in the country that performed ex situ, in vitro conservation of genetic material of animal origin. So far, the genetic fund contains 45093 doses if deeply frozen semen from 13 autochthonous livestock breeds – 2 cattle breeds, 10 sheep breeds and one horse breed (Nikolov, 2011).

Evaluation of the economic and social importance

Because of the varying productivity (milk, meat, wool, skin, labour force), in the past Bulgarian autochthonous livestock breeds have been an important prerequisite for the development of works and trade and played an important role in the national economy. Although the development of agriculture and livestock husbandry has created a number of new high-yielding breeds that have almost replaced some autochthonous breeds from their habitats, the interest of farmers to the latter in some regions still exists. The main reasons are some valuable biological traits as high livability and resistance to diseases, high potential for adaptation to local conditions, unpretentiousness to feeds and good quality of produce. Another important reason is the positive attitude of the national Ministry of Agriculture to the conservation of genetic resources of autochthonous animal breeds and more specifically, the provision of funds for financial support of farms rearing animals from these species. Although animals from local breeds are relatively low-yielding, their most valuable trait is the unique genetic fund, which is or both theoretical and practical interest and that should be conserved. That is why in most of these breeds, intensive selection is not carried out, and breeding activities are aimed at conservation of the genetic fund and increase of populations until the extinction threshold is surpassed. Having in mind the high adaptation potential of Bulgarian autochthonous animal breeds to the respective natural and geographical conditions, they could of preserved and maintained not only as a genetic reserve, bit also could be used in organic farming. The conservation of Bulgarian autochthonous animal breeds would also allow for utilisation of their adaptation potential for production in the mountainous and semi-mountainous regions of Bulgaria, a prerequisite for organic production (Dimitrov and Dimitrova, 1994). On the

Table 1
Dynamics of autochthonous animal breeds' populations in Bulgaria for 2009-2013

Breed	Years				
	2009	2010	2011	2012	2013
Cattle					
Rhodope shorthorn cattle *	361	514	698	1067	1320
Bulgarian gray cattle	1156	1411	1630	1908	2149
Iskar cattle	605		604	723	958
Sheep					
Local Stara Zagora sheep *	649	704	680	643	750
White Maritza sheep *	860		757	711	646
Patch-faced Maritza sheep	1887	3115	2343	2974	3299
Central Stara Planina sheep	7847	9078	10788	10221	9289
Duben sheep	3532	6601	4100	4272	5941
Central Rhodope sheep		4856	3356	5783	5574
Teteven sheep *	1606	625	592	636	1846
Koprivshtitsa sheep *	1625	538	1069	1142	385
Karakachan sheep	5732	4509	5200	5722	6394
Local Karnobat sheep **	155	255	160	150	250
West Balkan Mountains sheep		919	984	1893	1692
Replyan sheep	1871	1349	1696	1905	1682
Sakar sheep			1686	3157	3452
Sofia (Elin Pelin) sheep		1646	1543	1054	1110
Breznik sheep*		1239	951	953	490
Copper-red Shumen sheep	3436	4465	4505	4789	5836
Goats					
Kalofer long-haired goat *			727	1368	1989
Local long-haired twisted-horned goat*					515
Horses					
Karakachan horse *	264	1234	1704	1870	1842
Pigs					
East Balkan pig *	595	1269	1277	1176	1314
Dogs					
Karakachan dog*			280		
Bulgarian shepherd dog			4800		
Bulgarian Ludogorian hound **			471		
Bulgarian coarse-haired hound (Barak) **			358		
Poultry					
Black Shumen chicken***			350		
Bees					
Bulgarian honey bee			11625 bee families		

*- protected, endangered;**- endangered;***- critically endangered

(*Source*: Executive Agency on Selection and Reproduction in Animal Breeding, Fifth National Report of the Ministry of Environment and Waters under the Convention of Biological Diversity for the period 2009-2013)

other hand, the issue has a certain social significance. The utilisation of large low-yielding pastures in the plain, arid regions of the country would provide livelihood of sparsely populated territories. The preservation of specificity of a given breed would also preserve the diversity among breeds in accordance with EC and global directives for genetic diversity protection at a worldwide scale. The decline of biodiversity in the animal breeding field is an important topic in a global plan requiring the creation and implementation of conservation programmes to preserve the genetic resources of livestock breeds. The priorities of breeds are evaluated according to a complex of criteria and this determines which of them could be of theoretical and applied interest for animal husbandry at a global scale. From this point of view, multifactor analysis on the basis of linear programming, simulation and dynamic models of complex evaluation including all elements of biological, economical and social relevance of a breed are particularly important. These models and analyses for instance, allow for the assessment of the so-called 'non-market values' of a breed (Mendelsohn, 2003; Fadlaoui et al., 2005; Roosen et al., 2005; Ruto et al., 2008; Yakubu and Ibrahim, 2011).

Research – Historical Development, Present and Future

Immediately after the Liberation of Bulgaria from the Ottoman yoke in 1878, Bulgarian autochthonous livestock breeds are the principal source of food from animal origin and labour force in agriculture. A large of part of first reports on them from this period are mainly of descriptive and popular nature, providing guidelines and advices for farmers and promoting the results from animal exhibitions (Bichev, 1892, 1894, 1896; Hasekiev, 1899; Dechov, 1900; Zlatarov, 1903; Ganushev, 1907; Kostov, 1912; Hlebarov, 1915; Anchev, 1919).

The research on various species and breeds of livestock, including autochthonous ones in Bulgaria has started with the foundation of a Faculty of Agriculture (1921) and Faculty of Veterinary Medicine (1923) to the Sofia University. In this early period, studies were mainly aimed at investigation of certain biological and production traits of various breeds of cattle, buffaloes, goats, horses, pigs and birds (Ganchev, 1926a, 1926b, 1927, 1929; Ganchev and Platikanov, 1930; Hlebarov, 1921, 1923, 1926, 1930, 1933, 1937; Platikanov, 1930; Bichev, 1930; Geroff, 1931; Gerov, 1938; Ishirkov, 1931; Petkov, 1939; Petroff, 1941; Ivanov, 1942). Thus, investigations on Bulgarian autochthonous breeds continued until 1945. During the next almost 45-year period, i.e. the time of socialist development of Bulgaria, an intensive creation of new breeds has occurred. For improving the production traits of Bulgarian autochthonous breeds, male breeders and semen from high-producing foreign breeds are imported.

Through various crossing and breeding methods, the authorised research teams creased a number of Bulgarian breeds with substantially better production traits (Semkov, 2013). These breeds, as well as the import of entire herds from some high-yielding foreign breeds are the reason for the gradual replacement, reduction and even extinction of some old national autochthonous livestock breeds. Nevertheless, in some state agricultural farms, research institutes and isolated geographical regions of the country, several herds of different aborigenous animal species are conserved and studied (Alexieva, 1979; Dzhurbineva, 1984; Odzhakova, 1994; Kafedgiev, 1997; Stefanova et al., 1991; Dimov and Mikhailova, 1999).

From scientific point of view, the interest of Bulgarian researchers towards the conservation of livestock genetic resources has increased after 1980 година (Hinkovski and Aleksiev, 1981; Hinkovski et al., 1984; Danchev, 1994; Krastanov, 2003; Gelev, 2004; Nicolov, 2011). These publications are mainly reviews of theoretical nature. On the basis of normative stipulations under the supervision of EASRAB, purposeful activities for conservation of genetic resources of Bulgarian livestock breeds have started after the year 2000. In some instances, these activities are accompanied with research analyses evaluating the real state of breeds and outline the prospects for their conservation and practical use. Some of studies are aimed at determination of population numbers and risk of extinction, investigation on specific breed and production traits and development of breeding programmes for genetic resources protection (Boykovski et al., 2005; Boykovski et al., 2008; Dimov, 2011; Gorinov, 2004; Gorinov, 2011; Iliev, 2012; Kolev et al., 2014; Nakev et al., 2011; Nikolov, 2008, 2009; Nikolov and Gadjev, 2008; Nikolov, 2012; Pamayotiv et al., 2003; Palova and Marchev, 2009; Palova and Marchev, 2011; Stoykov et al., 2007; Staykova, 2005; Vuchkov and Dimov,.2005; Vuchkov et al., 2011).

A strong impetus to the development of research on AnGR conservation at a global scale is provided by new DNA technologies (Todorovska, 2010; Hristova et al., 2012a; Stoykova et al., 2009). They allowed the identification of a large number of DNA polymorphisms and their use as DNA markers (RFLPs, AFLPs, AAD, SSRs, ISSRs, SNPs) for assessment of the genetic base of observed phenotype diversity. From this point of view, molecular DNA markers are an indirect approach to the characterisation of genetic resources in the field of livestock farming. So far, by means of various DNA markers (RFLPs, SSRs, SNPs) two Bulgarian autochthonous cattle breeds and seven autochthonous sheep breeds have been studied. The investigations on Rhodope shortcorned cattle and Bulgarian gray cattle (Teneva et al., 2005; Teneva et al., 2007; Zlatarev et al., 2008; Dalvit et al., 2010; Hristov

et al., 2012; Yordanova et al., 2014) demonstrated the presence of genetic variability comparable to that of other European cattle breeds. The two autochthonous breeds possess a certain genetic uniqueness, although the observed heterosis deficiency with respect to some of studied loci. Analysing the results, the authors suggested measures for their management and conservation as important genetic resources for livestock husbandry.

Microsatellite DNA markers are also employed for investigation on genetic diversity and determination of genetic distances in different Bulgarian sheep breeds. On the basis of microsatellite DNA markers Kusza et al. (2010) evaluated the genetic structure of 5 sheep breeds – White Maritza, Patch-faced Maritza, Pleven Blackhead, Stara Planina Tsigai and Rhodope Tsigai. Despite the high deficiency of heterozygous individuals and the minimum number of population – specific alleles for the Pleven Blackhead population, this breed was outlined by authors as genetically unique.

On the basis of microsatellite DNA markers several teams (Georgieva et al., 2013; Hristova, 2013; Hristova et al., 2014b) investigated the level of genetic diversity and genetic similarity in 7 autochthonous sheep breeds: Breznik, Sofia, Copper-red Shumen, Karakachan, Local Karnobat sheep, Pleven Blackhead and Stara Zagora sheep. In studied populations, the authors have detected polymorphism with respect to all studied microsatellite loci and established high heterozygocity levels, as well as genetic distances that confirmed the genetic uniqueness of the different breeds. In three of studied microsatellite loci, 7 rare alleles have been identified, which are a relevant index for increased allele diversity as a part of the overall genetic variability of populations. On the basis of obtained results, the authors concluded that the high level of genetic diversity in the studied sheep populations could be effectively used in the conservation of the available genetic fund.

Other studies using single nucleotide polymorphism (SNP) restriction analysis (Hristova, 2011; Hristova et al., 2012b) showed presence of genetic polymorphism of alpha S1-casein gene (αS1CN) in three sheep breeds – Karakachan, Local Karnobat and Copper-red Shumen. The polymorphism of melatonin-receptor gene (MTNR1A) was also investigated in four autochthonous sheep breeds – Sofia, Breznik, Stara Zagora and Local Karnobat sheep. In two of them (Sofia and Breznik), the detected genetic diversity level was higher compared to the other two, which are in the group of breeds at higher risk for extinction. In order to evaluate the significance of the Welsh allele in αS1-CN gene for the technological properties of milk and the economic efficiency of breeding animals, Kalaydhziev et al. (2014) analyzed 7 Bulgarian autochthonous sheep breeds. The authors reported low allele frequencies of the D-allele in the studied sheep population and the results corresponded with those from the commonest local breeds in France, Spain and Italy.

The first study based on the methods of molecular genetics dedicated to East Balkan Swine is of special interest. In Bulgaria, the only aboriginal domesticated pig breed is the East Balkan swine inhabiting the western coast of the Black Sea in the country. To reveal the breed's genetic characteristics, Hirata et al. (2015) analysed mitochondrial DNA and Y chromosomal DNA sequences in 54 animals of this breed. Nucleotide diversity of the mtDNA control region, including two newly found haplotypes, was higher compared to that of European and Asian domestic pigs and wild boars. The median-joining network based on the mtDNA control region showed that the East Balkan swine and wild boars in Bulgaria comprised mainly two major mtDNA clades - European (E1) and Asian clade (A). The authors concluded that coexistence of two mtDNA clades in East Balkan swine may be the relict of historical pig translocation. Among the Bulgarian colonies of this breed, the geographical differences in distribution of two mtDNA clades (E1 and A) could be attributed to the source pig populations and/or historical crossbreeding with imported pigs. In addition, analysis of the Y chromosomal DNA sequences in this research for East Balkan swine revealed that all of animals had haplotype HY1, which is dominant in European domestic pigs.

Conclusion

The review of literature and the analysis of the state of Bulgarian livestock genetic resources demonstrated that existing tendencies were not much different from European and world trends. There is a necessity for development and implementation of effective programmes and strategies for preservation of the genetic fund and genetic variability specific for autochthonous domestic animal breeds. This process requires proper decision-making that could be attained through a constant co-operation between state institutions, non-governmental organisations represented by breeder associations and unions, animal farms and last but not least, animal scientific and research centers in the country. This would allow solving the following essential issues, problems and tasks:

- Creation of molecular database of genetic traits and genetic variability of breeds through detailed investigations using DNA markers, compliant with requirements of European and world organisations involved in biodiversity protection. Inclusion of all Bulgarian autochthonous domestic animal breeds in the molecular database) so far, only 2 autochthonous cattle and 7 sheep breeds are investigated via DNA markers).

- Identification of Bulgarian autochthonous breeds possessing unique genes in their genetic fund. The uniqueness of these breeds is of interest for the national, European and world genetic science and for livestock management practice.

- Assessment of the adverse effect of inbreeding and inbreeding depression in small populations and constant monitoring of their size and risk for extinction.

- Development of science-based and efficient breeding programmes in line with the biological specifics and the habitat of respective autochthonous animal populations and breeds.

- Quest for possibilities with respect to the rational use of autochthonous breeds despite their lower productivity by their inclusion in programmes for organic agriculture and organic farming.

- Development of economic models for optimisation of subsidy and other forms of financial support of farms on the basis of prospective use of the genetic fund of a given breed and evaluation of non-material value of its genetic and biological characteristics.

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