

**PEDIGREE ANALYSIS OF THE CROATIAN
AUTOCHTHONOUS CATTLE BREEDS: MANAGEMENT OF
CONSERVATION STRATEGY****A. Ivanković, J. Ramljak, N. Kelava, M. Konjačić****Summary**

Autochthonous Croatian cattle breeds belong to groups of endangered European cattle breeds. Genetic diversity is essential for preservation of adaptive potential of populations and improvement of production potential in the future. Monitoring genetic diversity is the basis for effective conservation programmes. Pedigree analysis is a useful tool in the management of conservation strategy. In the work presented here, we investigate a pedigree information on autochthonous cattle breeds in order to get an insight into population structure and management of the genetic variability. The analysis included 3017 records of Istrian cattle, 578 records of Slavonian-syrmian podolian cattle and 898 records of Busha, registered in the Herd Book from 1994 to 2009. The positive population trends are observable in increased number of animals per breeds and frequent calving recorded in the Herd Book, with a stagnation from 2002 to 2004. High coefficient of inbreeding level and specific relationship with high average relatedness coefficient in the population of Istrian cattle indicates the necessity of changing the breeding management. The main reason is utilisation of sires mostly born in the same or related herd and the interchange of animals with other herds is not frequent. In the population of Slavonian-syrmian podolian cattle a redesign of the mating scheme is needed. Share of the high inbreed animals in the population of Istrian and Slavonian-syrmian podolian cattle is relatively high. The lowest inbreeding and lowest average relatedness coefficient are found in Busha population. The obtained information will be useful in implementing a conservation program in the Croatian autochthonous endangered cattle breeds.

Key words: Pedigree analysis, genetic variability, cattle breeds, conservation strategy.

Introduction

Thousands of years of natural and human selection, migration, mutation, genetic drift and cross-breeding have contributed to today's farm animal genetic diversity.

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Farm animal genetic richness allowed development of sustainable livestock production in different agro-ecological zones and production systems. The trends of globalization and industrialization of farm production during the twentieth century have led to significant erosion of farm genetic resources, especially in economically developed countries. From a total 5775 mammals breeds registered in the FAO DAD-IS 3 database in endangered status are 925 domestic breeds (16.01%) and 645 or 11.17% is extinct (FAO, 2009). The regions of Europe and the Caucasus are with the highest proportion of breeds classified as at risk (29% of mammalian and 50% of avian breeds). FAO report (2009) suggests that from the total number cattle breeds (1351), breeds at risk (218; 16.14%) and extinct breeds (418; 30.94%) have the highest values. Autochthonous Croatian cattle breeds (Istrian cattle, Slavonin-syrmanian podolian cattle, Busha) belong to groups of endangered European cattle breeds. During the second half of the twentieth century due the loss of economic importance, autochthonous cattle became an endangered breed. Their population sizes (\approx 150 to 500 adult animals) make them vulnerable, but we can observe positive trends in structure of this population and rising public interests for their breeding. Beside the public financial support to the breeders of autochthonous cattle, we stimulate the harmonization and development of breeding programs, economic affirmation of autochthonous breeds and their involving in other programs (conservation of protected nature areas, tourism, etc.).

We know that genetic diversity is essential to preserve adaptive potential of populations and to improve production potential of highly selected commercial breeds (fitness, adaptability, resistance to pathogens, and others). Therefore it is important to preserve maximal genetic diversity, between and within populations, avoiding the high adaptive potential of species. In managed populations of domestic animals, genetic diversity can be maximised by selection according to optimal contributions, giving each reproductive animal a specific contribution for the next generations (Sonesson and Meuwissen, 2001; Pong-Wong and Woolliams, 2007). Monitoring and preservation of genetic diversity is the basis for effective selection and/or conservation programmes (Gutiérrez et al., 2003). Closed populations with high level of genetic drift suffer from reduction of genetic diversity leading to higher inbreeding levels, inbreeding depression as well as high incidences of particular heritable (often recessive) diseases (Oliehoek et al., 2009). Small natural populations raise several problems when faced with their conservation: they have lost most of their economic value, they usually show a high inbreeding level which threatens their long term maintenance, and the conservation of biodiversity that they represent makes them unsuitable for the introduction of individuals in other populations (Dunner et al., 1998). Genetic variability can be studied through the estimation of the genetic variance of quantitative traits, analysis of pedigree data and description of visible genes and markers in

the population, such as microsatellite markers (Vu Tien Khang, 1983). Pedigree analysis is important tool to describe genetic variability and its evolution across generations (Gutiérrez et al., 2003), providing information about inbreeding level, effective population sizes, generational interval, effective number of founder or important ancestors, reproductive parameters and population structure. Although inbreeding is unavoidable in reference populations, understanding of population structure, utilization of aimed mating or avoiding mating of related individuals, makes possible to reduce the level of loss of genetic variability. Informativeness (lengths) and pedigree completeness level have significant influences on reliability of mentioned estimated parameters.

The objective of this study was to analyse the pedigree information of autochthonous cattle breeds in order to get insight in the population structure and potential danger from the loose of genetic variability. Population structures were analysed in terms of number of founders, generation interval, effective number of herds, pedigree completeness level, inbreeding coefficient and average relatedness.

Materials and methods

Three Croatian cattle breeds were included in analysis: Istrian cattle (IC), Slavonian-syrmian podolian cattle (SSP) and Busha (BS). Pedigree information in the Herd Book register at the Croatian Agricultural Agency (HPA) was analysed. In analysis 3017 records for Istrian cattle, 578 records for Slavonian-syrmian podolian cattle and 898 records for Busha were included. Pedigree data in the Herd Book register were collected between 1994 and 2009. They contained unique ID, information about father, mother, date of birth, sex, breeders and region. According to the breeding area population of Istrian cattle are divided in seven, Slavonian-syrmian podolian cattle in five and Busha in five regions.

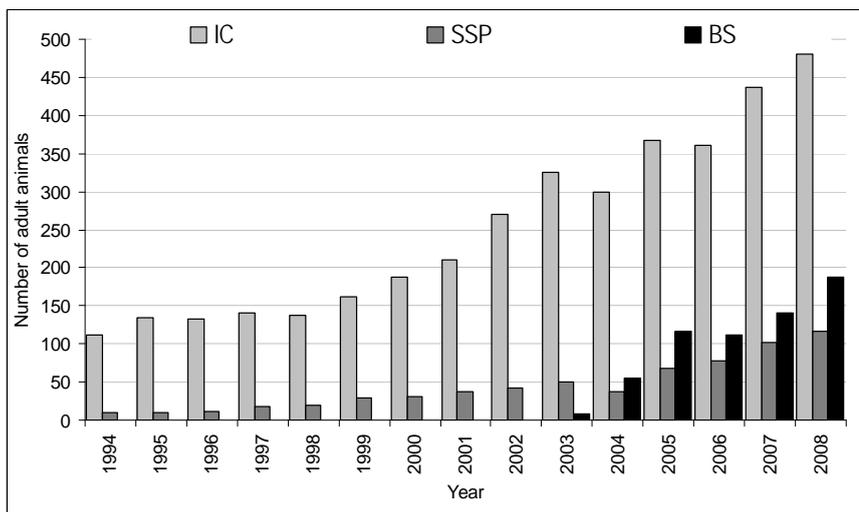
Analyses of pedigree data have been performed using the program ENDOG v4.6 (Gutiérrez and Goyache, 2005b). The effective number of founders (f_e) is the number of equally contributing founders that would be expected to produce the same genetic diversity as in the population under study (Lacy, 1989) and effective number of ancestors (f_a) is the minimum number of ancestors, founders or not, necessary to explain the complete genetic diversity of the population under study (Boichard et al., 1996). Genetic structure of the three cattle population was assessed using pedigree completeness level, coefficient of inbreeding (F), average relatedness coefficient (AR), generation interval (I) and F -statistics. Malécot (1948., cit. Gutiérrez and Goyache, 2005a) was defined individual coefficient of inbreeding (F) as probability that an individual has two identical alleles by descent while

individual average relatedness coefficient (AR) is defined as the probability that an allele randomly chosen from the whole population in the pedigree belongs to the animal (Goyache et al., 2003; Gutiérrez et al., 2003). The average inbreeding coefficient of a population is frequently used to measure level of homozygosity and average relatedness coefficient indicates the percentage of representation of each animal in a whole pedigree (Gutiérrez et al., 2003). Parameters of F -statistics (F_{IT} , F_{ST} , F_{IS}) were computed following Caballero and Toro (2000, 2002). Effective number of herds (H_s , H_{SS} , H_{SSS}) are defined as the effective number of herds supplying sires, grand sires, great-grand-sires, and so on (Gutiérrez et al., 2003).

Results and discussion

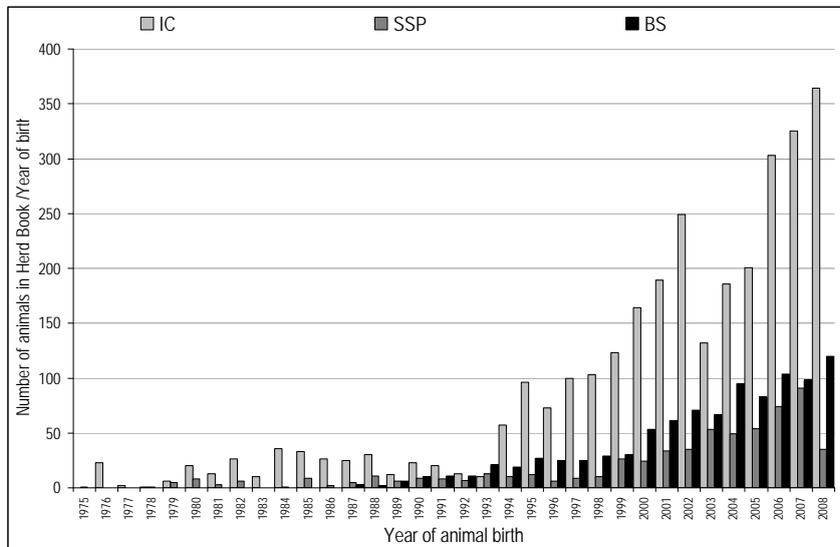
Graph 1 and Graph 2 represent demographic parameters in the analyzed autochthonous breeds: the number of adult animals registered in the Herd Book (Graph 1), and the number of registered animals in regards of year of birth (Graph 2). The Herd Book for Istrian cattle was established in 1994, in 1997 was established Herd Book for Slavonian-syrmian podolian cattle and at the beginning of twenty one century (2003) Herd Book for Busha cattle. In the years that followed after establishment of the Herd Book, some animals (considered to be acceptable to breeds standard after performed inventory) have been additional included.

Graph 1 – NUMBER OF ADULT ANIMALS REGISTERED IN THE HERD BOOK IN PERIOD 1994 TO 2008 (sources: HPA).



The positive trend is noticeable considering the total size of autochthonous population, with the stagnation during 2003 year as a consequence of subsidies changes to the breeders (Graph 1). The positive population trends are observable in the number of animals per breeds and year of birth included in Herd Book, with detectable stagnation in period from 2002 to 2004 (Graph 2). We can notice positive movements in period from 2005 to 2009 considering total and effective population size, distribution of breeding herds and design of programs for economic use. The main approach of conservation of autochthonous cattle breeds combined the strategy of maximum diversity and maximum utility raising the level of their self sustainability.

Graph 2 – NUMBER OF ANIMALS OF AUTOCHTHONOUS CATTLE BREEDS AND THE YEAR OF BIRTH INCLUDED IN HERD BOOK (sources: HPA).



In IC population the observed population trends trough the total number of adult breeding animals and calves, which were annually introduced in the Herd Book registers were the highest. Although SSP population displayed noticeable lower number of calves registered during the last 2008, a moderate increase in number of adult animals in Herd Book is presented. We assume that dispersion of the central nucleus herds into smaller herds contribute to perceived decrease the number of calves.

In Table 1 are present the analyses of founders, obtained from average relatedness coefficients. From the total of 3017 Istrian cattle in Herd Book data,

80 animals were recognized as founders and 479 as half founders. Effective population size of founders was 26.02, and expected inbreeding by unbalancing of founders contribution were 1.92%. In Herd Book data for Slavonian-syrmian podolian cattle 18 founders and 94 half founders' animals were detected. From 991 data in Herd Book for Busha, 72 individuals were recognized as founders and 637 individuals as half founders.

Table 1 – NUMBER OF FOUNDER ANIMALS IN POPULATION OF THREE CROATIAN AUTOCHTHONOUS CATTLE BREEDS.

	IC	SSP	BS
Number of herd book data	3 017	629	991
Number of founders	80	18	72
Number of half - founders	479	94	637
Effective number of founders (f_e)	19	15	36
Effective number of ancestors (f_a)	18	9	33
Expected inbreeding of founders (%)	1.92	2.59	0.19

In accordance with the share (number) of the founders and half – founders, genetic variability was confirmed on molecular level (allelic richness; Ramljak *et al.*, 2009). In SSP population the smallest number of founder and half – founders Ramljak *et al.* (2009) based on microsatellite markers were observed as well as the lowest allelic richness value. The largest allelic richness was observed in Busha population, what is expected considering the largest number of founders and half – founders and widest geographical distribution of population. Small effective number of founders in population of Istrian cattle (19) relative to the actual number of founders present in the Herd Book (80) indicates an excessive use of some individuals as parents, so favourable morphological selection has probably increased the representation of some individuals in the whole pedigree (Goyache *et al.*, 2003). The effective number of ancestors is useful to detect recent significant changes in the breeding strategy. Boichard *et al.* (1997) recently defined an effective number of ancestors as a measure for detecting the potential bottlenecks that could have occurred in the pedigree.

An estimated generation intervals of autochthonous cattle breeds are presented in Table 2. The lowest generation interval was found in Busha (5.44 years) and the highest values in Istrian cattle (6.93 years). Larger populations usually have a longer generation interval in relation to smaller populations in which replacements of breeding animals are faster. Similar observations has Gutiérrez *et al.* (2003), noting that the ratio of length of father-offspring pathway in relation to dam-offspring pathway is a consequence of applied reproduction techniques (natural mating, artificial insemination). They indicate

that the shorter father-offspring pathway compared to dam-offspring pathway is result of dominant application of artificial insemination.

Generation interval in population of Istrian cattle is shorter through maternal in relation to paternal pathway. In populations of Slavonian-syrmian podolian cattle and Busha paternal pathways were shorter than maternal pathway. The shortest generation length corresponds to the lower father-offspring pathway because sires were replaced earlier, and used dominant reproduction technique is artificial insemination in regard to natural mating. This observation can be explained with smaller number of herds and specific mating scheme. Generation interval reported for some Spanish and Italian beef cattle breeds were smaller (Gutiérrez et al., 2003; Bozzi et al., 2005) than those reported for autochthonous Croatian cattle breeds. Also, generation intervals for father-offspring pathway in Istrian and Slavonian-syrmian podolian cattle population are comparable with generation interval found in Spanish cattle breeds (Gutiérrez et al., 2003).

Table 2 – GENERATION INTERVALS IN THREE AUTOCHTHONOUS CROATIAN CATTLE BREEDS (years; mean \pm se).

Pathway	IC	SSP	BS
Father – son	7.24 \pm 0.601	5.38 \pm 0.445	4.23 \pm 0.848
Father – daughter	7.34 \pm 0.243	5.94 \pm 0.112	4.37 \pm 0.264
Mother – son	6.37 \pm 0.357	5.76 \pm 0.612	6.61 \pm 1.443
Mother – daughter	6.59 \pm 0.183	6.34 \pm 0.173	6.32 \pm 0.492
Average	6.93 \pm 0.139	6.12 \pm 0.101	5.44 \pm 0.337

Estimated values of average inbreeding, average relatedness and equivalent generations in three Croatian autochthonous cattle breeds are presented in Table 3. The average inbreeding value was low, ranging from 0.25% to 3.53%, so it can be considered far from dangerous values. The lowest average coefficient of inbreeding in the whole pedigree was found in Busha (0.25%) and highest values in Istrian cattle (3.53%; Table 3). Inbreeding coefficient is a relative value that greatly depends on pedigree completeness level as regards complete generation equivalents (Gutiérrez et al., 2003), and relationship between coefficient of inbreeding in population of Istrian cattle and Busha can be explained with this statement. According to Gutiérrez et al., (2003) pedigree completeness level is higher in the dam pathway considering recent generations, but considering distant generations, the sire pathway seems more appropriate for pedigree completeness. The highest average relatedness coefficient was found in Slavonian-syrmian podolian cattle (9.11%) indicating on problem in maintenance of genetic variability. That is the reason why further

conservation strategy for Slavonian-syrmian podolian cattle must accept more carefully mating scheme. The lowest AR coefficient was found in population of Busha (0.44%). When average F is higher than half mean AR it pointing out that mating is mainly carried out between relatives (Cervantes et al., 2008). Dunner et al. (1998) proposed the use of AR as a good criterion to keep genetic variability by maintaining the balance of the representation of the founder ancestors using the whole pedigree, permitting us to identify and use animals with the lowest AR coefficient. High value of inbreeding coefficient and a relatively low value for AR coefficient in population of Istrian cattle is results of specific mating management which implies interchange of mating sires among small number of herds. The higher inbreeding coefficient and relatively low AR coefficient reveal breeding management in which utilised sires are usually born in the same herd and the interchange of animals with other herds is not frequently carried out (Gutiérrez et al., 2003). Inbreeding increase equivalent generation in population of Istrian cattle (1.88%), suggesting the needs for careful planning of mating methods, such as factorial and compensatory mating (Caballero et al., 1996) or minimum coancestry mating (Sonesson and Meuwissen, 2002). Gutiérrez et al. (2003) observe that in populations with low AR, the level of inbreeding would dramatically decrease if migration among subpopulations took place. Smaller rate of inbreeding increased by equivalent generation were observed in population of Busha and Slavonian-syrmian podolian cattle, 0.82% and 0.68%, respectively. Boichard et al. (1997) have shown when pedigree information is incomplete, computed inbreeding is biased downwards and the actual effective size is overestimated.

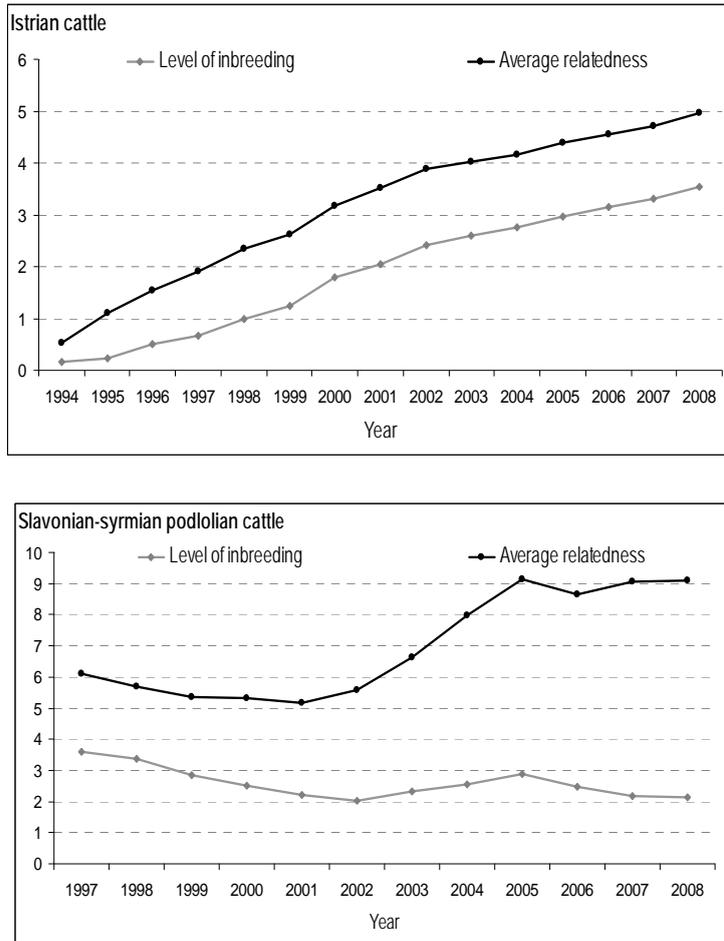
Table 3 – ESTIMATES OF INBREEDING AND AVERAGE RELATEDNESS COEFFICIENTS IN THREE CROATIAN AUTOCHTHONOUS CATTLE BREEDS (%).

	Comp. equiv. generat. (years)	Average F in whole population	Average relatedness	Inbred animals	Average F of inbred animals
IC	2.14	3.53	4.96	16.71	13.01
SSP	1.72	2.12	9.11	16.12	22.84
BS	0.42	0.25	0.44	4.06	22.73

Proportion of the inbred animals in population of Istrian cattle was high (16.71%), as well as an average inbreeding coefficient ($F=13.01\%$). Slavonian-syrmian podolian cattle with reported 16.12% percentage of inbred animals were similar to the values reported for Istrian cattle, although, SSP displayed very high level of average inbreeding coefficient (22.84%). Gutiérrez et al. (2003) notify that the inbreeding coefficient of inbred animals decreases while

the percentage of inbred animals and the inbreeding in the whole population increase.

Graph 3 - EVOLUTION OF INBREEDING AND AVERAGE RELATEDNESS OF ISTRIAN AND SLAVONIAN-SYRMIAN PODLIAN CATTLE.



Estimated Wrights coefficient (F -statistics) for three autochthonous cattle breeds divided according to breeding area are presented in Table 4. The total genetic variability of populations (F_{IT}) are base for separating the variability between (F_{ST}) and within subpopulations (F_{IS}).

Table 4 – WRIGHTS F -STATISTICS IN THREE AUTOCHTHONOUS CROATIAN CATTLE BREEDS (IC - 7 BREEDING AREA; SSP- 5 BREEDING AREA; BS - 5 BREEDING AREA).

	IC	SSP	BS
Mean coancestry within subpopulations	0.0318	0.0532	0.0093
Nei-distance between subpopulations	0.0070	0.0077	0.0071
F_{IS}	0.0035	-0.0338	-0.0068
F_{ST}	0.0072	0.0081	0.0071
F_{IT}	0.0108	-0.0255	0.0003

The highest mean coancestry within subpopulations is observed in SSP populations (0.053), which is the result of a small basic (founder) population and small number of nucleus herds. Busha population is in the best position regarding to mean coancestry within subpopulations value of 0.009, as consequence of wide geographical distribution of subpopulations. Small value of F_{ST} coefficients among breeds indicates that observed differences between subpopulations were not significant. Negative value of F_{IS} coefficient in population of Slavonian-syrmian podolian cattle suggest on presence of bottleneck effect in the recent past.

The effective number of herds was smaller than the actual ones (Table 5). Small actual and effective number of herds in population of Slavonian-syrmian podolian cattle and Busha emphasizes their vulnerability. This situation can indicate an unbalanced contribution of herds to the gene pool, since a small number of herds behave as a nucleus supplying the rest of the population with sires (Gutiérrez et al., 2003).

Table 5 – NUMBER OF HERDS SUPPLYING FATHERS, GRANDFATHERS, ETC., AND EFFECTIVE NUMBER OF HERDS SUPPLYING FATHERS, GRANDFATHERS, ETC., IN THREE CROATIAN AUTOCHTHONOUS CATTLE BREEDS.

	IC		SSP		BS	
	Actual	Effective	Actual	Effective	Actual	Effective
H_s	54	15.73	12	2.71	15	4.09
H_{SS}	19	5.39	5	1.88	1	
H_{SSS}	9	3.66	1	1.00		

The actual and effective number of herds confirmed earlier preconditions about the population status (number of nucleus herds) at the beginning of implementation of conservation program for autochthonous cattle breeds. It is essential; therefore, to carefully implement conservation program for autochthonous cattle breeds in order to protect maximum diversity through application of optimal mating scheme.

Conclusion

Pedigree information analysis of autochthonous cattle breeds showed significant level of inbreeding in population of Istrian cattle and genetic bottleneck in population of Slavonian-syrmian podolian cattle in the recent past. Genetic status of Busha cattle according to population parameters are in favorable position relative to other two populations. Observed population parameters, especially in Slavonian-syrmian podolian cattle and Istrian cattle indicate necessity for optimization of mating schemes towards more effective conservation of genetic diversity. Results from this survey and earlier experiences indicate using appropriate methods of monitoring and implementation of conservation programs in pedigree. Multiple purposes approach for monitoring populations in conservation programs (pedigree monitoring, DNA markers, validation of parenting) will decrease doubts and errors. Analyses based on DNA levels revealed an underestimated inbreeding level observed through pedigree analysis, but both methods support the conclusions concerning the level of preserved genetic variability (BS>IC>SSP). Population parameters from this research based on pedigree analysis are usable in future conservation programs of autochthonous cattle breeds.

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ANALIZA PEDIGREA AUTOHTONIH HRVATSKIH PASMINA GOVEDA: UPRAVLJANJE KONZERVACIJSKOM STRATEGIJOM

Sažetak

Hrvatske autohtone pasmine goveda dio su skupine ugroženih europskih pasmina domaćih životinja. Genetska različitost je esencijalna za očuvanje adaptivnog potencijala pasmina i unapređenja njihovog proizvodnog potencijala u bližoj i daljnjoj budućnosti. Nadzor genetske različitosti je temelj učinkovitih konzervacijskih programa. Analiza rodoslovlja je korisna u upravljanju konzervacijskom strategijom. U iznesenom radu istražili smo odnose rodoslovnih informacija autohtonih pasmina goveda u cilju sjecanja uvida u populacijsku strukturu i način upravljanja genetskom varijabilnošću. Analizom je obuhvaćeno 3017 rodoslovnih zapisa istarskog goveda, 578 rodoslovnih zapisa slavonsko-srijemskog podolca i 898 rodoslovnih zapisa buše, registriranih u središnjoj matičnoj knjizi u razdoblju od 1994. do 2009. Pozitivni populacijski trendovi u svim autohtonim pasminama uočljivi su kroz povećanje broja uzgojno valjanih jedinki i teladi godišnje registriranih u središnji matični registar, uz uočljivu stagnaciju u razdoblju od 2002. do 2004. Viši koeficijent uzgoja u srodstvu i vrijednost koeficijenta prosječne srodnosti jedinki u populaciji istarskog goveda ukazuju na potrebu redizajna konzervacijske strategije. Glavni razlog tomu je korištenje bikova uglavnom rođenih u istom ili srodnom stadu, odnosno nedovoljna razmjena životinja između stada. U populaciji slavonsko-srijemskog podolca uočeno je 'usko grlo', te se ukazuje potreba usklađivanja sheme sparivanja uzgojno valjanih jedinki. Udio visoko srodnih jedinki u populacijama istarskog goveda i slavonsko-srijemskog podolca je značajan. Najmanja razina uzgoja u srodstvu i najmanji koeficijent prosječne srodnosti jedinki u populaciji zapaženi su u populaciji buše. Uočene informacije mogu biti vrlo korisne u provedbi konzervacijskih programa očuvanja hrvatskih autohtonih ugroženih pasmina goveda.

Ključne riječi: analiza pedigrea, genetska varijabilnost, pasmine goveda, konzervacijska strategija.

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