Proceedings of 29th Annual Meeting of DAGENE

Danubian Animal Genetic Resources

Volume 3 (2018)

DAGENE
International Association for the Conservation
of Animal Breeds in the Danube Region
1078 Budapest, István street 2.
Hungary

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“Ecosystems, products, conservation”

Proceedings of 29th Joint Annual Meeting of DAGENE and SAVE
in Kozárd, Hungary
from 24th to 27th of June 2018

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Supporting and advertising are possible at the office.

CONTENT

PROGRAM OF JOINT ANNUAL MEETING OF DAGENE AND SAVE

(Safeguard of Agricultural Varieties in Europe)

Event is held under the patronage of
Dr. István Nagy, Minister of Agriculture and
Dr. Miklós Beer, Bishop of Vác Diocese

The date of 29th Joint Annual Meeting of DAGENE and SAVE is from
24th to 27th of June 2018 (four days).

Place of Conference at the location of Village Centre, Fő út 12. (Main street No. 12)
3053 Kozárd, Hungary, degrees of latitude: 47.9158651 and longitude: 19.6203057.

The program contains is the following:

Sunday **24th June 2018**

- from 12:00 Arrival to Kozárd Village (http://www.kozard.hu)
Contact point and registration: Vadvirág (Wildflower) Restaurant, Fő út 29. (Main street No. 29), Kozárd
Lunch: in Vadvirág (Wildflower) Restaurant (optional)
Afternoon: Preliminary free discussions on various SAVE and DAGENE issues
Accommodation in rural tourism guestrooms in Kozárd and Alsótold

- 13:30 *Local Study Tour*:
- Field visit (Kozard area, 4-6 km): harmonisation of ecosystem services with digitalization in agriculture (new approaches to protect/reinstate agro-bio diversity, creation and maintaining habitats for pollinator insects, bees and small game – land nesting birds e.g. grey partridge)

Free time excursions and leisure programs until 19.30
- Visit Hollókő UNESCO World Heritage Village and Castle (12 km) http://www.holloko.hu/
- Enjoy wellness treatment in Castellum Hotel, Hollókő (12 km) https://hotelholloko.hu/
- Visit Carpathian Hunting Museum and Castle in Hatvan (26 km) http://vadaszatimuzeum.hu/
- Visit Bükkszék Family Spa to enjoy Salvus Spring water to treat worn joints (57 km) http://www.bukkszekfurdo.hu/family_spa_center
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- 19:30 Dinner in Kozárd (Vadvirág (Wildflower) Restaurant)

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$16^{:30}$ - $18^{:30}$ **DAGENE Annual Assembly**
(Village Theatre)

$17^{:00}$ - $18^{:00}$ **Meeting of the SAVE Arca Deli Award Jury**
(Vadvirág (Wildflower) Restaurant 1st Floor Meeting Room)
Kozárd, Fő út 29.

$19^{:00}$ - $22^{:00}$ **Gala Dinner**
(including traditional Hungarian Palinka and Wine Tasting) and
Arca Deli Award celebration
(Vadvirág (Wildflower) Restaurant) Kozárd, Fő út 29.

Tuesday **26th June 2018**

$7^{:00}$ - $8^{:00}$ Breakfast (Vadvirág (Wildflower) Restaurant) Kozárd, Fő út 29.

$08^{:15}$ - **Study tour #1: Travel to Csákvár**
(Transdanubia, 150 km)

$10^{:30}$ - Visit *Pro-Vértes Foundation*: preservation of ecological farming, indigenous breeds (cattle, buffalo, sheep, and donkey) and maintaining rich nature diversity of plants and wild birds (http://provertes.hu/)

$13^{:00}$ - ***COUNTRY-STYLE LUNCH***

$14^{:00}$ - $16^{:00}$ **SAVE Board of Directors meeting**
(closed session)

$14^{:00}$ - $16^{:00}$ Opportunities to see/taste traditional local deer and donkey products from a wild game reserve of *Bőszénfa* (*Kaposvár University*, West Hungary) and
"Puszta tour": Opportunities to drive around on coaches to see the diverse natural landscape.

$16^{:00}$ - Last round of "Puszta tour" for SAVE BoD members

$17^{:30}$ - Travel by Bus to Kozárd via Budapest

$18^{:30}$ - In Budapest: Drive around the Royal Castle, Matthias Church at Buda Hill (http://www.matyas-templom.hu/; 60 km). Cross the historical Chain Bridge, look at the Parliament, then drive along the Andrassy Avenue, pass by Heroes' Square, Budapest

$19^{:30}$ - Drive to Kozárd (80 km)

$20^{:30}$ - $22^{:00}$ **Dinner**
(Vadvirág (Wildflower) Restaurant) Kozárd, Fő út 29.

Wednesday **27th June 2018**

$7^{:00}$ - $8^{:00}$ Breakfast (Vadvirág (Wildflower) Restaurant) Kozárd, Fő út 29.

$08^{:15}$ - **Study tour #2: Travel to Biodiversity Centres**

$9^{:00}$ - Visit the Hungarian Plant Biodiversity Centre (NÖDIK) at Tápiószele (http://www.nodik.hu/; 85 km).

$11^{:00}$ - Visit the Hungarian Domestic Animal Gene Conservation Centre, Gödöllő (http://genmegorzes.hu/) with particular attention to indigenous poultry genetic conservation, freshwater fish gene pool and honey bee genetic collection and preservation sub-centres (60 km).

$13^{:00}$ - ***LOCAL LUNCH***
traditional Hungarian fish dishes

$14^{:00}$ - Departure to Budapest "Liszt Ferenc Airport" (35 km) and farewell to homeland, then/or
return to Kozárd (90 km)

$20^{:30}$ - $22^{:00}$ **Dinner**
(Vadvirág (Wildflower) Restaurant) Kozárd, Fő út 29.

Thursday **28th June 2018**

$7^{:00}$ - $8^{:00}$ Breakfast (Vadvirág (Wildflower) Restaurant) Kozárd, Fő út 29.

Farewell & departure

Sponsors:

Hungarian National Association of Mangalica Breeders
Hungarian Association of Sheep- and Goat Breeders

In Memoriam Prof. Dr. Jean Boyazoglu (1937-2018)

In May 2018, we have learned the sad news and shook us deeply that Prof. Jean Boyazoglu passed away. His former colleagues and friends from all over the world expressed condolences, and we, on behalf of DAGENE community wish to pay tribute here.

Andreas Georgoudis, Greece: *Jean with his high level education and strong personality put a very remarkable stamp on many generations of scientists and practices in animal production and biodiversity conservation and contributed to the creation of structures that operate successfully until today. To those of us who have had the privilege of communicating with him in our native language will be unforgettable as an excellent and enlightened mentor and supporter.*

Pal Hajas, Hungary: *Jean Boyazoglu an excellent, devoted scientist, hardworking and respectful person. It has been my privilege to work with him for global animal husbandry in FAO*

Elzbieta Martyniuk, Poland: *Dear Jean, truly a renaissance man, knowledgeable in many fields and passionate about things he believed in. He has done so much on many fronts to pursue work on animal genetic resources, in the time when many believed that local breeds should be left behind… He was one of those who really paved the way for our work in 21st century. On the personal note, you could always count on him, he was very happy to help, if somebody needed anything. And he was able to find innovative, out of the box solutions for problems that seemed not to be possible to manage. Jean enjoyed life, I will always remember his Zorba dance and many of his jokes and stories. I feel lucky and privileged to know him since 1985 and enjoy his friendship and outstanding personality. He will be missed a lot.*

Keith Ramsay, South Africa: *A friend and colleague - he will be sadly missed.*

Elisabetta Rosetti, Italy: *The best boss I have ever had. A mentor. A great person and a good friend. A big loss in our lives.*

Christina Ligda, Greece: *I feel really lucky that I had the opportunity to meet him and appreciate his exceptional personality. He was an international man, but also an emblematic scientist of the Mediterranean region contributing significantly to the development of sheep and goat sector in the region.*

Sandor Kukovics, Hungary: *I had the luck to receive him as my personal guest in Hungary in several times, and organised him several programmes, what's more, he had hospital treatment under my name in Hungary several years ago. He was to me as my older friend, and I always remember him!*

Mike Roper, United Kingdom: *So sorry to hear this news Andreas. He was a great supporter of the European Focal Point for FAnGR (ERFP) from its inception and I have very positive memories of his persuasive input! He will be missed.*

Eyüp Yüksel, Turkey: *sad news. Wish a calm sleep in lights, with peace. I respect such scientists, researchers, but we have unfortunately no remedy, no cure for such highly valuable scholars, bureaucrats, academicians.*

Oya Akin, Turkey: *I am very sorry for his loss.*

Marija Pontaga, Latvia: *Unfortunately I never had luck to meet him personally, but have heard a lot of very respectful words on him. He definitely made significant footprint on animal breeding and conservation all over the world and became a legend for younger generation.*

Jean Boyazoglu was born 29 November 1937 to Greek parents in Alexandria, Egypt.
After completing his schooling he studied agriculture in Pretoria, South Africa. His first degree in general agriculture and land economy was followed by a Master's degree in the genetics of domestic animals. Further studies in quantitative biology and animal genetics led him to the National Agricultural Research Institute (INRA) in France for 2 years. Then, in 1965, he was awarded a doctorate at the University of Pretoria for his dissertation on populations of Lacaune milk sheep in the Roquefort region, south of France. Jean had a long history of service and made great contributions to the scientific knowledge of sheep and goat production and genetics.
Further academic qualifications include a post-graduate State Diploma in International Trade and Commerce from the Institute of International Trade in Paris in 1973 and a Professorship at the Aristotle University in Thessaloniki, Greece in 1979. In the period from 1960 to 1973 he moved from being Research Assistant to Research Director of the Research Centre for Animal Production and Dairy Science of South Africa in Pretoria.
From 1973 to 1983 he lived in Paris where he was Agricultural Advisor for South African Research Development and Technology for Europe and the Near East as well as being a member of the South African Research Council for Animal Production. From 1983 to 1985 he was Advisor for South African Research Development and Technology for North America living in Los Angeles, USA.
In 1986 he became Secretary-General of EAAP, ICAR and WAAP located in Rome. In 1993 Professor Boyazoglu was appointed Senior Officer in the FAO European Regional Office in Rome with special responsibilities for Research and Higher Education.
He was a member of many scientific committees of these countries and in the USA and Canada. Since 1991 he has contributed as Coordinator of the BAAP Steering Committee on Animal Production for Eastern Europe. Jean served as IGA's President from 1992 to 1996.
Jean was also a member and President of the EU's Scientific Advisory Committee on Appellations of Origin, Geographic Denominations and Special Products. He served from 2003 to 2006 as Coordinator of regional programs at the World Organization of Animal Health (OIE).
About 250 publications in 13 languages testify to Professor Boyazoglu's versatility. He serves on the Editorial Boards often scientific or technical publications and he is Editor of the FAO/UNEP Journal *Animal Genetic Resources Information* (AGRI) and of the EAAP publications series, and as Editor-in-Chief for *Livestock Science* and *Small Ruminant Research* from 2005 to 2012. Professor Boyazoglu has received many professional honours in South Africa, France, Belgium, Gabon, Italy, Romania, Egypt and from EAAP; he was Honorary Member of the German Society for Animal Breeding.

Dr. Jean Boyazoglu died on May 17th. His ashes was spread on the Mediterranean Sea on June 1st at 11:00 am in Menton, France.

(Major source of compilation: J Hodges/Livestock Production Science 42 (1995) 81-103.)

Pedigree analysis of the Hungarian Gidran Horse breeding population

POSTA, János – HARDON, Daniella – MIHÓK, Sándor

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H-4032 Debrecen, Böszörményi str.138, Hungary

Abstract

An effective gene conservation programme requires the knowledge of genetic diversity of the population. The genetic structure of Gidran breed was studied from pedigree records. Herd book data available up to 2014 of registered Gidran horses were analysed. The generation interval varied between 10.28 and 12.42 for the analysed population. The proportion of known parents for the reference population was above 90% in the 7th generation. There were 1.875 and 135 horses covering the total genetic variability of the whole and reference populations. Most important ancestor was the English Thoroughbred St.Simon (3.49%) for the whole population, whereas it was Gidran IV (9.93%) for the reference stock. Inbreeding coefficient for most inbred horses varied between 19.7 and 27.6 for the whole population and between 9.2 and 18 for the reference stock.

Introduction

An effective gene conservation programme requires the knowledge of genetic diversity of the population. All Gidran horses are originated from the stallion Gidran Senior and descendants of his son Gidran II after the mare 74 Tifle (MIHÓK and ERNST, 2015). The Gidran breed is a result of a classical line breeding and was formed in Mezőhegyes Stud. It is often originated from the establishment date (1785) of the stud farm, though the Austrian Ministry of Defence declared this heavy riding horse as a separate breed in 1885. About 200 mares played their roles on the formation of the breed but only 16 of them became family founder. Three genealogical lines have been formed during the years (MIHÓK, 2006, 2012).

The demand to get to know the genetic variability of livestock animals has been continuously increasing (WOOLLIAMS et al., 2002). Analysis of pedigree data gave information about the ancestors and relatives of the animals and there are various measurement variables to describe the population genetic structure and variability (MAIGNEL et al., 1996).

The analysis of pedigree data is most popular and used in horse breeding. Inbreeding, pedigree completeness and generation intervals are estimated for Arabian (CERVANTES et al., 2008; GLAZEWSKA and JEZIERSKI, 2004) as well as for English Thoroughbred (BOKOR et al., 2013; MOUREAUX et al., 1996) horses. Among the Hungarian traditional breeds, the Shagya-Arabian stock of Bábolna (GERSTNER, 2014) and Hucul horses were also evaluated (MIHÓK et al., 2016).

The aim of the research study was to analyse the pedigree information of the registered Hungarian Gidran population.

Material and methods

The basis of the current study was the Hungarian active breeding population of Gidran Horse between 2012 and 2014. The active population was chosen as reference when needed. The base pedigree information was given by the Kisberi and Gidran Horse Breeding Association. The pedigree information of Shagya-Arabian and English Thoroughbred horses were completed using www.shagya-database.ch and www.pedigreequery.com, respectively. There were the pedigree data of 8581 animal in the developed database.

The homozygosity of the population was characterized using the inbreeding coefficient (WRIGHT, 1922). Its precision depends on the length and the completeness of the pedigree (BOICHARD et al., 1997).

Pedigree completeness can be characterized by the values of the number of full generations traced, the maximum number of generations and the equivalent complete generations. The equivalent complete generations is computed as the sum over all known ancestors of the terms computed as the sum of $(1/2)^n$ where n is the number of generations separating the individual to each known ancestor (MAIGNEL et al., 1996). The first is defined as the furthest generation in which all the ancestors are known. Ancestors with no known parent were considered as founders (generation 0). The second is the number of generations separating the individual from its furthest ancestor.

Generation interval shows the average age of parents at the time of their offspring's birth (JAMES, 1977). The value was calculated along four different pathways (sire–daughter, sire–son, dam–daughter and dam–son) on the basis of the recoded individuals' and their parents' birth dates.

All the above described parameters were computed using ENDOG (GUTIÉRREZ and GOYACHE, 2005) and POPREP (GROENEVELD et al., 2009) software.

Results and discussion

The longest generation interval was computed for the sire-to-son pathway (Table 1). The dam-to-son pathway was found to be the shortest generation interval among the different paths. The four pathways were compared pairwise using independent samples t-test. There were significant differences between stallions and mares ($P<0.05$) as stallions were used in breeding two years longer than broodmares.

Table 1. The generation intervals (years) of the Gidran population for the different pathways

Parent-offspring lineages	Number of pathways	Generation interval
Sire-to-son	2092	12.42
Sire-to-daughter	3801	12.30
Dam-to-son	1955	10.28
Dam-to-daughter	3658	10.51
Average	11506	11.41

Our recent findings were lower than found by MOUREAUX et al. (1996) estimations for Anglo-Arabians (11.5). Their results for Selle Francais and French Trotters (11.7 and 11.8) were also greater than our results. BOKOR et al. (2013) found similar values for the Hungarian English Thoroughbred population (11.41) to our estimations for the Gidran horses.

Figure 1 shows the pedigree completeness of the population based on birth years. Pedigree of more than 90% of horses born after mid 1970's was known at least six generations back. The average pedigree completeness was 5.67 for the reference population. Because of the historical storms of the breed as well as some pedigree filling problems, these values were smaller compared to Lipizzaner (15.2; ZECHNER et al., 2002) and English Thoroughbred (15.64; BOKOR et al., 2013) horses. The proportion of known parents for the reference population was above 90% in the 7th generation.

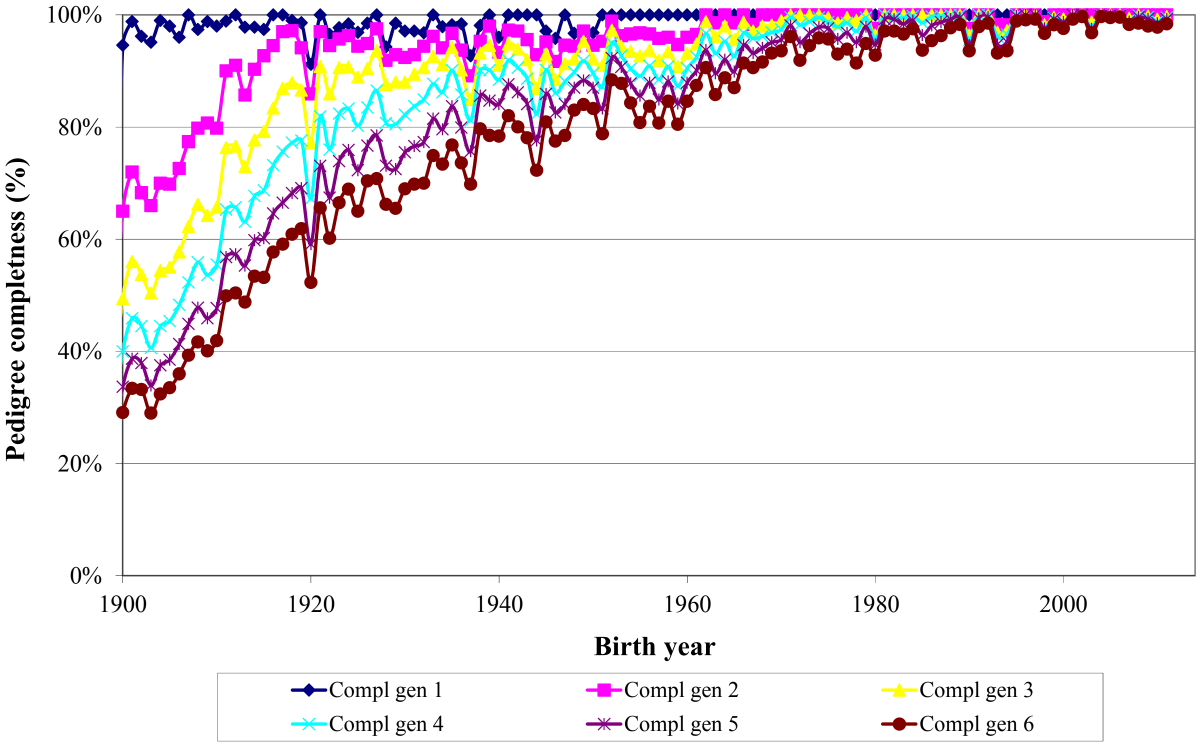

Figure 1. Evaluation of pedigree completeness

Table 2. Concentration of genetic variability

Percentage of genetic variability	Whole population	Reference population
50%	143	10
60%	256	15
70%	444	20
80%	725	29
90%	1116	45
100%	1875	135

Table 2 gives information the genetic variability of the recent stock. Only 143 and 10 horses cover the 50% of the genetic variability for the whole and reference population, respectively. It can be seen easily, that the reference population could be coved with much less ancestors which show reasonable gene loss during the history of the breed.

Ancestors having greatest impact on the total population are detailly described in Table 3. There was only one broodmare among the ten most important ancestors, most of them were English Thoroughbreds. Most important ancestor was St. Simon xx (3.49%) The single mare among these ancestors was 46 Kengyel Gidran-7 (1.44%) from the 'Mezőhegyes 4th' mare family.

Table 3. Most important ancestors of the total population

Name of the horse	Gender	Birth year	Covering genetic variability (%)
St. Simon xx	1	1881	3.49
Newminster xx	1	1848	1.79
Isonomy xx	1	1875	1.52
46 Kengyel Gidran-7	2	1892	1.44
Bend'Or xx	1	1877	1.38
Gidran XXVIII (1857)	1	1857	1.25
Hampton xx	1	1872	1.13
Birdcatcher xx	1	1833	1.04
Buccaneer xx	1	1857	1.00
Algy xx	1	1897	0.93

Table 4. Most important ancestors of the reference population

Name of the horse	Gender	Birth year	Covering genetic variability (%)
Gidran IV	1	1963	9.93
Gidran VI (1972)	1	1972	8.59
Gidran XXIV (XXXIX R)	1	1983	7.40
14 Regöly Gidran (Iram)	1	1968	5.73
Gidran XXII (XXXVIII R)	1	1979	3.89
Gidran VIII (1975)	1	1975	3.75
2394 Déva xx	1	1980	3.72
46 Kengyel Gidran-7	2	1892	2.80
Gidran Razbeg I	1	1980	2.53
82 Szikrázó Gidran-8	2	1981	2.36

Ancestors responsible for the genetic variability of the reference population are shown in Table 4. Gidran IV, born in 1963, covers 9.93% of the total genetic variability of the present stock. The three most important stallions appear in the pedigree of almost every breeding horse in the present breeding stock. This is a huge problem during the development of the annual mating plans to avoid inbreeding. There were two broodmares among the most important ancestors, the above mentioned 46 Kengyel Gidran-7 (2.80%), and 82 Szikrázó Gidran-8 (2.36%) which is from the 'Mezőhegyes 1st' mare family.

Horses having highest inbreeding coefficient within the reference population are shown in Table 5. The most inbred animal is Gidran XXIV-126 (Sirály) in the present breeding stock. The mare was born from a grandfather-granddaughter mating. Furthermore, 555 Gidran XXIX-32 (Merengő) could be found among the ancestors of her sire and maternal grandmother (Gidran XXXIX-41 (Moldova)) as well. The maternal grandmother was born after maternal half-siblings. The average inbreeding of the reference population was 3.02%. Own results were higher compared to GOLEBIEWSKA et al. (2013) and SIERSZCHULSKI et al. (2005) findings.

Table 5. Animals with highest inbreeding coefficient (IC) within the reference population

Name of the horse	Gender	Birth year	Sire	Dam	IC (%)
Gidran XXIV-126 (Sirály)	2	2007	Gidran XXIV (XXXIX R)	Gidran XIX-63 (Moldika)	18.0
Gidran XXIII-1 (Niké)	2	1999	Gidran XXIII	89 Gidran VI-16 (Nápoly)	16.0
Déva Gidran-18 (Linda)	2	2000	Déva Gidran II	148 Déva Gidran-3 (Kincses)	14.9
Gidran-57 (Durcás)	2	2006	Gidran XXIV (XXXIX R)	Koheilan Gidran-123 (Dorka)	14.7
Gidran XXIII-32 (Sudár)	2	2000	Gidran XXIII	105 Gidran VI-4 (Sellő)	14.6
Gidran XIII-35 (Szigony)	2	1996	Gidran XIII	Gidran IX-2 (Sziszegő)	14.5
Gidran XXIII-29 (Pilla)	2	2000	Gidran XXIII	115 Gidran VI-6 (Pillangó)	14.5
Gidran XVI-16 (Gazella)	2	2002	Gidran XVI	1129 Gidran XIII-35 (Gizella)	9.7
Gidran XIX-23 (Zárda)	2	1997	Gidran XIX	97 Gidran IX-2 (Bálvány)	9.2
Gidran XIX-31 (Zsálya)	2	1998	Gidran XIX	97 Gidran IX-2 (Bálvány)	9.2
Gidran X-22 (Pántlika)	2	2000	Gidran X	1197 Gidran VIII-9 (Pálma)	9.1

Conclusions and recommendations

The generation interval varied between 10.28 and 12.42 for the analysed population. The shortest was estimated for mare-to-son pathway, whereas the longest was sire-to-son pathway. There were 1.875 and 135 horses covering the total genetic variability of the whole and reference populations, respectively. Most important ancestor was the English Thoroughbred St.Simon (3.49%) for the whole population, whereas it was Gidran IV (9.93%) for the reference population. Inbreeding coefficient for most inbred horses varied between 19.7 and 27.6 for the whole population and between 9.2 and 18 for the reference population, respectively.

Acknowledgement

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Genetic diversity determined by Microsatellites and Single Nucleotide Polymorphism Markers: case study of two native cattle breeds

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Abstract

Highly informative genetic markers are essential for efficient management in populations of domestic animals (i.e. individual identification, establishing genetic variability and structure, parentage verification, etc.) and for food traceability. During the last decade, microsatellites were the most used marker system, although replaced graduadly in the last few decades by single nucleotide polymorphisms (SNPs). The aim of the paper was to compare the degree of information provided by microsatellites with those gained by SNPs for native Croatian cattle breeds. Both markers system have been proven as a useful tool for determination of genetic variability in Istrian cattle and Slavonian Syrmian Podolian cattle. The application of both marker systems is advantageous in simultaneously addressing a variety of questions related to breeding and selection. However, decission which method should be used depends on the purpose and objective of the research and available equipment. The chosen method should be, above all, practical and user-friendly.

Introduction

The application of molecular markers has revolutionized management of domestic animals in many segments: from both, individual and population identification, assesing relationship between two or more individuals to parentage verification and food traceability. At the beginig, there were several types of molecular marker systems as a matter of choice (protein polymorphisms, blood group, etc.). In the last two decades, microsatellites or short tandem repeats (STRs) have been used the most. Microsatellite markers are usually di-or three-nucleotide repeat (e.g. CACACA), repeated several times in tandem. They are highly polymorphic, informative and interspersed throughout the entire genome what makes them a good tool for genetic analyses. They do not encode proteins and are thus assumed selectively neutral. However, these markers have disadvatages such as appearance of null-alleles (existing alleles that are not observed using standard assays), they are specific to species and therefore more difficult to compare, time-consuming and expensive to develop (VIGNAL et al., 2002). At the beginning, microsatellite pannel was primarly developed and used for cattle genotyping (with the exception of human and mice genotypng) and now they are available for most livestock species: sheep, goats, horses, donkeys, pigs, and chicken. For easier comparison of research results and to overcome inconsistencies by different laboratories (i.e. allele size calling and errors in size determination) FAO and the ISAG – FAO Advisory

Group on Animal Genetic Diversity proposed panels of 30 microsatellite markers (FAO, 2011).
The most recent tool for studying DNA sequence variation is single nucleotide polymorphism (SNP) which is a single base-pair variation that exists between individuals. SNPs gained high popularity due to greater abundance, small mutation rate (1×10^{-9}), automated analysis and data interpretation (VIGNAL et al., 2002). Genome-wide studies using SNPs have enabled the mapping of quantitative trait loci (QTL) and prediction of animal's genetic merit for the traits of interest in different animal species (SCHMID and BENNEWITZ, 2017). Permanent progress in genomic selection has encouraged the development of high density chips for almost all domestic animal species, i.e. BovineHD 777K with > 777,000 evenly spaced SNPs (Illumina) or Affymetrix Axiom Equine HD array with 670,000 SNPs (Neogen). However, because typically SNP loci are biallelic, heterozygosity cannot exceed 0.5, and such low heterozygosity is disadvantageous for analysis which requires high statistical power (i.e. parentage).

In Croatia, over the last 20 years, attention has been given to protecting autochthonous breeds of domestic animals. Therefore, National Programme with the aim of protection and conservation of livestock was brought in 2010 (NATIONAL PROGRAMME, 2010). This program includes 27 native breeds, of which two, Istrian cattle (IC) and Slavonian Syrmian Podolian cattle (SSP) were the subject of this study. Systematic monitoring and inventarisation for IC started early in 1989 and for SSP latter, in 2008. This comprehensive process included engagement of breeders, breeders associations, State and Public administration bodies as well as scientific and educational institutions. According to the latest ANNUAL REPORT (CAA, 2017) IC counts 866 individuals (823 femaes, 42 males) and SSP counts199 individuals (189 females, 10 males). Although population of IC and SSP increased, estimated effective population size suggests that IC is highly endangered (*Ne* =159.8) and SSP (*Ne* =37.9) critically endangered breed (CAA, 2017).
Important measure of population protection includes the assessment of preserved neutral genetic variability (evident as number of alleles, allelic richness, heterozygosity, etc.) that are mainly accumulated in nonselected native breeds (MEDUGORAC et al., 2009). It was demonstrated that unselected Busha strains show higher alleleic diversity (in terms of total number of alleles, private and rare alleles) than some European breeds. In addition, reduced genetic variation that results from inbreeding and small population size has also been found to correlate with a range of defects, many of which are associated with reproductive traits, fitness and decreased production. This has been demonstrated by GONZÁLEZ-RECIO et al. (2007) using pedigree-based inbreeding of Holstein cattle where pregnancy rate decreased by 1.68% for cows with inbreeding level from 6.25 to 12.5%. PRYCE et al. (2014) found that increase in inbreeding by 1% based either on pedigree or genomic data was associated with a decrease in milk, fat and protein yields of around 0.4 to 0.6% and an increase in calving interval of 0.02 to 0.05% in population of Holstein and Jersey cattle in Australia. Therefore, genetic variations displayed by genetic differences between individuals and populations within a given species are the basis for future livestock management. There is a growing need to maintain animal genetic diversity to be able to facilitate rapid adaptation considering production and environmental demands and challenges in the future. In this respect, it is important to use accessible, feasible and cheap analyses to facilitate comparing results from other surveys with a high percentage of reliability. Therefore, the aim of this paper was to compare genetic diversity results of IC and SSP using two different marker systems from previous researches. In addition, we also want to see which marker system seems more effective and appropriate for some analyses (i.e. determine genetic diversity, parentage verification, etc.).

Material and methods

This survey included data set from previous published research with 105 microsatellites and 51 unrelated animals of Istrian cattle (IC) and Slavonian Syrmian Podolian cattle (SSP) as described in RAMLJAK et al. (2011).

Bovine SNP50 BeadChip (iScan SY101-1001, 189 Illumina) was used for the second research (RAMLJAK et al., in press) and after passing filtering criteria (call rates < 95%, minor allele frequencies < 0.025, and samples with more than 10% of missing genotypes) 45.454 SNPs were used. In order, to reduce ascertainment bias 4-SNP haplotype blocks were defined and considered as multi-allelic markers with their haplotypes as alleles as described in SIMČIČ et al. (2015). These multi-allelic markers and derived allele frequencies were used to infer unbiased allelic diversity and heterozygosity. The number of analysed animals for SNP analyses was 30 for IC and 24 for SSP.

According to NEI (1987) mean number of alleles (*mA*, in second case *mA* per block), observed (H_O) and expected heterozygosity (H_E) were estimated. More information about methodology used in the estimation of genetic diversity is described in SIMČIČ et al. (2015) and RAMLJAK et al. (in press).

Results and discussion

Description of some of genetic diversity parameters based on SNP and microsatellite analysis in two Croatian native cattle breeds, IC and SSP is shown in Table 1. In general, both breeds have similar values for estimated parameters of genetic variability. The average *mA* for SNP showed a higher value for about 1.06 times compared to *mA* for microsatellites. Precisely, for SNP an average *mA* in IC was of 7.19 and in SSP 5.17 while average *mA* for microsatellites was 6.82 and 4.85. The same situation happened conserning observed and expected heterozygosity values. Higher H_O for SNP were 0.713 for IC and 0.675 and SSP compared to H_O for microsatellites of 0.635 and 0.593. Microsatellite-based diversity analysis revealed low values for H_E of 0.677 (IC) and 0.583 (SSP) compared to SNP-based results of 0.705 and 0.636 for IC and SSP, respectively.

Table 1. Average number of alleles (*mA*), observed (H_O) and expected (H_E) heterozygosity for the SNP and microsatellites sets of markers in Istrain cattle (IC) and Slavonian Syrmian Podolian cattle (SSP) cattle. Numbers in bold show the highest value.

Marker type	*mA*		H_O		H_E	
	IC	*SSP*	*IC*	*SSP*	*IC*	*SSP*
SNP*(4-SNP)*	**7.19**	**5.17**	**0.713**	**0.675**	**0.705**	**0.636**
Microsatellites	6.82	4.85	0.635	0.593	0.677	0.583

Almost identical values of *mA,* H_O and H_E for IC and SSP were obtained in SIMČIČ et al. (2015), i.e. H_O and H_E for IC were 0.719 and 0.711 while for the SSP were 0.681 and 0.642. Therefore, parameters of genetic variability are comparable since similar number of SNPs (44 496) were used in SIMČIČ et al. (2015). If we compare diversity measures using SNPs (without being classified into blocks) for IC (H_O = 0.325, H_E = 0.321) and SSP (H_O = 0.306,

H_E = 0.289; RAMLJAK et al., in press) with other research results, both of these are comparable. In such a way, IC and SSP as typical Podolian breeds show lower level of diversity compared to a group of four Podolic cattle breeds (H_E = 0.386; PARISET et al., 2010). Indigenous Croatian SSP populaton had similar genetic diversity values compared to indigenous and locally-developed breeds from South Africa (0.28-0.30 for H_E; MAKINA et al., 2014) or even lower compared to Spanish beef cattle (0.299-0.319 for H_E; CAÑAS-ÁLVAREZ et al., 2015) but expected heterozigosity for indigenous IC was higher. Although clarification of diversity results of Crotian indigenous breeds is not a topic of this paper, it will contribute to a better understanding and results interpretation of used markers. Both, microsatellite and SNPs show lower variability in SSP population as a result of severe bottleneck and genetic drift that were reflected in lower *Ne* and lower heterozygosity (RAMLJAK et al., 2011; RAMLJAK et al., in press). Moreover, a founder effect is occurred as a consequence of the low number of first reproducers (only four bulls) at the beginning the 1990s when revitalization of this breed started. On the other hand, greater diversity in IC reflects centuries of systematic breeding and care implemented in breeding program at the end of the 19th. At the beginning of the 19th century, crossbreeding of IC with the Italian breeds (Maremmana) is abandoned due to lower resistance and worse characteristics as working animals (at that time famous were Istrian oxen for field work) and breeding of Istrian cattle in pure-blood was continued.

Placing in relationship diversity results between microsatelites and SNPs, EDEA et al. (2013) reported lower values for observed (0.382) and expected (0.385) heterozygosity in five native Ethiopian cattle populations based on SNP study than those obtained using microsatellites (H_O = 0.674; H_E = 0.726) in 10 Ethiopian cattle populations by DADI et al. (2008). Althoug, using only six SNPs and 20 microsatellite markers in analysis, CARRUTHERS et al. (2011) obtained similar findings of higher observed and expected heterozygosity values using microsatellite markers than SNPs in nine Angus cattle populations. The difference in the results using these two approaches could be explained due to multi-allelic nature of microsatellites. It is well known that microsatelites have large numbers of alleles per locus (between 3 up to 17 alleles; see cited papers) compared with two allels for each SNP. According to FAO standards, proposed panels of 30 microsatellite markers provide sufficient and comparable population structure results, parentage analysis and identity verification. Microsatellites with core repeats 3 to 5 nucleotides long, probability of exclusion > 0.999 are preferred in parentage analysis while number of microsatellite can vary (VIEIRA et al., 2016). SCHNABEL et al. (2000) demonstrate high efficiency of 12 microsatellite markers (exclusion probabilities of 0.999) for determining parentage in domestic cattle. In populations that might be expected to have a high level of homozygosity due to traditional method of breeding (i.e. horse breeds), the number of markers of 13 show to be sufficient for parentage testing with high efficiency (KHANSHOUR et al., 2013).

However, as stated above, the lower polymorphism of SNP markers does not necessarily mean a weaker result in genetic sturucture analysis. Numerous studies reveald that the 2-2.5 SNPs are sufficient for replacing one polymorphic microsatellite locus. The comparison of the two types of markers showed that about two SNPs were necessary to provide the same statistical power as one microsatellte marker. WERNER et al. (2004) reported that 37 SNPs provided the same power as a commonly used microsatellite set, while HERRÁEZ et al. (2005) found that 2.6 SNPs matched one microsatellite for prentage assessment in Galloway cattle. FISHER et al. (2009) observed that 40 SNPs were equivalent to the 14 microsatellites (ratio 2.5 SNPs to 1 microsatellite) for parentage testing. More recently, FERNÁNDEZ et al. (2013) reported that 24 SNPs were equivalent to the ISAG minimal recommended set of 12 microsatellite markers (match probability10^{-11}) for parentage verification. Nevertheless, there are also cases when one marker system is not good enough to detect the genetic structure. TOKARSKA et al. (2009) reported unsuccessful microsatellite-based paternity and identity analysis compared to a panel of 50–60 bovine SNPs characterized by high heterozygosity and even distribution in the genome in European bison.

Decision which method for genotyping to choose depends on many criteria and several aspects have to be taken into account. HERRÁEZ et al. (2005) mention geneticist’s aspect (i.e. simple and unexpensive procedure due to large amounts of genotypic data) and statistician’s aspect (precision and accuracy of used statistic method). In general, both microsatellite and SNP analysis are similarly suited for cattle genotyping. The choice of the method depends to the purpose and desired objective of the study and the available equipment as well. In the case of parentage verification and individual identification good and reliable result is provided by the microsatellite analysis (e.g. cost ~23€/sample, personal communication GeneControl, Grub). On the other hand, more complex research (selection, QTLs, diseases) requires complex and demanding analysis and data processing, which automatically requires higher financial resources (e.g. cost ~45€/sample for SNP50 BeadChip, personal communication GeneControl, Grub).

The development of both marker systems is advantageous for simultaneously addressing a variety of questions related to breeding and selection. However, practical considerations and commercial purpose should be carefully considered too.

Conclusion

Micrsatellites and SNPs are important and very useful marker system in animal breeding. Approximately twice as many SNP markers were needed to provide the same effectiveness as microsatellites for genetic studies. Beneficiary of the genetic test (private person, association, institution, etc.) has to be aware that each marker system (microsatellites or SNPs) has advantages and disadvantages. No matter whether the genetic markers will be used for practical purpose or for scientific reserch, each of them has to reflect efficiency, applicability, easy data analysis and interpretation. Moreover, the decision concerning which type of marker to use should be carefully considered based on cost and labor time. Only with such an approach they fulfill will their original purpose.

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Research regarding the corporal development of young calves belonging to Grey steppe breed, as viable perspective for orientation of those breed to meat production

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Abstract

Taking into account the fact that in Romania, Grey steppe breed can be found fewer than 150 specimens, according to the international standards, it is considered a cattle breed with a certain risk of extinction, being included in a critical category.

From the productive point of view, this breed no longer responds to the actual requests, but due to its historical, economic and genetic role it could have in the future, as a resource of valuable genes, this breed is still very important, fact which imposes the preservation of this biological source as a main priority due to the situation in which it can be found, an almost extinct breed.

The re-evaluation of breeding cattle with a high risk of extinction for the preservation of the valuable genetic fond and the assurance of the genetic diversity of the animals' populations is a current theme which preoccupies the specialists in the field.

This approach is the major priority, given the conditions in which the extinction process of certain animal populations can irremediably affect the biodiversity of the genetic resources, this being essential for the production of goods of animal origin and the preservation of the planning diversity, as a source of income for the farmers and as an important part of the natural and cultural legacy of a country.

The current state of the problem concerning the cattle preservation of the breeds in danger of extinction determines us to continue these studies and to finding the most modern and efficient preservation methods of these valuable resources in the genetic fond, represented by Grey steppe breed.

Introduction

The current paper aimed to evaluate the youth belonging to Grey steppe breed from the point of view of corporal development and growing gain to be able to establish the potential of those breed for its utilization for meat production in extensive or semi-intensive system.

Romania, being in FAO programme for preservation and management of animal genetic resources, align at reconsideration and preservation policies of genetic fund for breeds in risk of extinction, situation in which is Grey steppe.

Grey steppe breed represent one of the ancient autochthonous breed, which was formed from *Bos Taurus Primigenius*, having a common origin with other European breeds (Andalusia, Romagnola, Zamorana, Salers, Podolica, Ukrainian grey steppe, etc.).

The stem of Grey steppe breed, in which is included Romanian grey steppe breed, has the origin in Ukrainian plains, place from where this animal spread in Balkans and in Mediterranean area, where was formed podolic type breeds.

Till 1850, the cattle herd from Romania, was composed by two indigenous breeds, Grey steppe and Mocănița, first one being spread mainly in steppe areas, being extended till the second half of 19th century on a very large geographical area, totally occupying the Old Kingdom, Central and Western Transylvania, as well as the centre of Basarabia, and the other breed was spread in mountainous regions. After 1892, with the first law regarding the import of animals from improved breeds (Simmental, Schwyz, Pinzgau), autochthonous breeds entered into a process of numerical decreasing. So, in 1935 Grey steppe breed have a rate of almost 57.3% from total.

Material and methods

Research was carried out on a number of 12 calves belonging to Grey Steppe breed at which were effectuated corporal measurements and were calculated different growing and development indexes, from birth till age of 15 months.

The statistical interpretation and processing of obtained data was carried out with the aid of the program SPSS 19 referring to the position and variation estimators (arithmetic mean X, standard deviation of the mean ±s, standard deviation s, variation factor V%) for the studied features.

Results and discussions

At the end of the studies regarding development of youth belonging to Grey steppe breed effectuated on a number of 12 heads were highlighted different mean values for the main corporal measurements function of age.

Height at withers at birth was of 73.1 cm, at 3 months 85.4 cm, at 6 months 94.6 cm, at 9 months 106.0 cm, at 12 months 111.5 cm, at 15 months 117.5cm.

Thoracic perimeter at birth was of 74.3 cm, at 3 months 110.6 cm, at 6 months 129.3 cm, at 9 months 144.4 cm, at 12 months 159.8 cm, at 15 months 171.0 cm.

Oblique length of trunk at birth was 69.6 cm, at 3 months 90.6 cm, at 6 months 141.7 cm, at 9 months 124.2 cm, at 12 months 130.8 cm, at 15 months 134.0 cm.

Corporal mass at birth was 28.7 kg, at 3 months 89.8 kg, at 6 months 141.7 kg, at 9 months 209.4 kg, at 12 months 277.0 kg, at 15 months 342.5 kg (Table 1).

Index of corporal format: illustrates the general development level of body, as well as the format in which this is classified; value of that index increase from birth to adult stage and it is higher at meat breeds.

Index of thorax depth: is modified with aging, being higher at meat breeds in comparison with dairy breeds and intermediary at mixed ones, it is higher at bulls than at cows.

At Grey steppe breed this one has a progressive increase, at birth being 95.4%, at 3 months 106.6%, at 6 months 112.4%, at 9 months 117.1%, at 12 months 117.3%, at 15 months 114.0%.

Table 1. Mean values (in cm) and variability estimates for corporal development at young calves (n=12)

Specification	Pos.	0 months	3 months	6 months	9 months	12 months	15 months
Height at withers	1	73.1	85.4	94.6	106.0	111.5	117.5
Height at dorsum	2	69.3	84.6	93.4	104.4	110.8	125.5
Height at rump	3	70.7	85.7	94.4	106.2	111.8	130.5
Height at the tails' base	4	71.6	85.8	94.3	105.0	109.5	124.5
Depth of thorax	6	41.2	47.3	56.5	64.6	65.8	63.5
Height at stern	5	31.9	37.8	38.1	41.8	46.8	54.0
Oblique length of body	7	63.7	84.3	93.7	119.0	123.8	128.0
Horizontal length	12	69.6	90.6	106.5	124.2	130.8	134.0
Length of rump	14	16.7	22.6	25.3	29.8	31.3	45.5
Length of thorax	18	36.7	47.0	54.1	55.6	68.3	63.5
Length of head	19	19.7	24.6	28.0	31.4	62.0	50.0
Perimeter of thorax	11	74.3	110.6	129.3	144.4	159.8	171.0
Perimeter of shin-bone	9	8.7	10.8	12.4	13.4	14.8	16.5
Width of thorax	13	16.1	22.7	26.9	32.2	37.8	47.5
Width of chest	10	18.5	23.1	25.0	29.0	32.0	34.5
Width of rump at hips	15	17.2	22.5	29.0	33.2	32.0	43.5
Width of rump at articulations coxo-femurale	16	18.9	24.1	28.7	35.0	36.5	38.0
Width of rump at ischia	17	12.4	19.2	20.8	30.2	30.3	32.0
Width of head	20	11.8	14.6	16.6	20.8	28.5	27.5
Corporal mass, kg		28.7	89.8	141.7	209.4	277.0	342.5

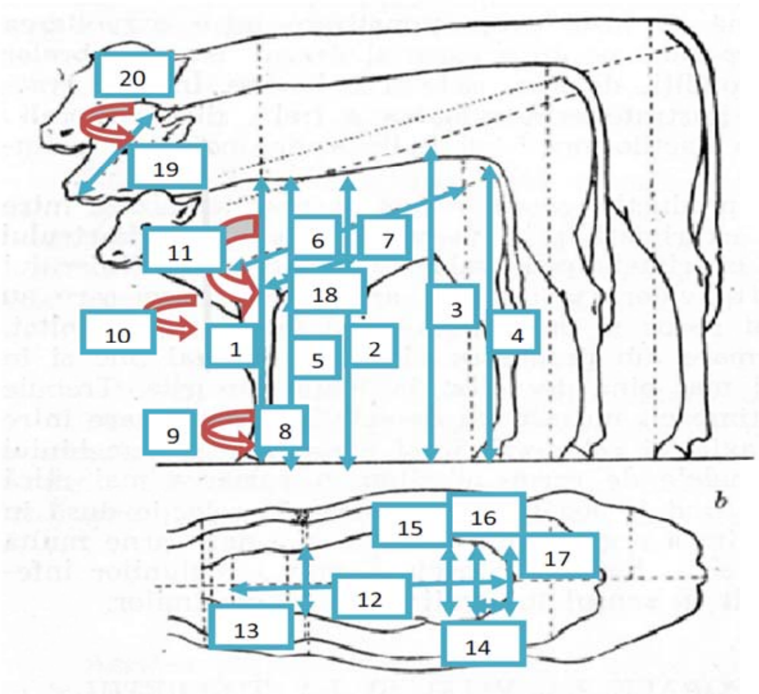

Figure 1. Mean values and variability estimates for corporal development at young calves

Index of thorax depth: is modified with aging, being higher at meat breeds in comparison with dairy breeds and intermediary at mixed ones, it is higher at bulls than at cows.

This index presented insignificant fluctuations with aging, at birth being 56.4%, at 3 months 55.0%, at 6 months 59.4%, at 9 months 60.9%, at 12 months 58.9%, at 15 months 54.0%.

Massiveness index: is a index which illustrate the rate between corporal mass and animal size, its value being higher at meat breeds and also increase from birth to adult age, respectively at birth was 101.9%, at 3 months 129.9%, at 6 months 136.9%, at 9 months 136.3%, at 12 months 143.3%, at 15 months 145.5%.

Index of height difference: is an index which not presents evident differences between morph-productive types. Generally, values lower than 100 for this index are recorded at primitive breeds, which have the posterior train weak developed and at bulls. A high height difference is common to young animals, but which disappear with aging.

At young calves belonging to Grey steppe breed that corporal index presented the following mean values: at birth 96.6%, at 3 months 100.4%, at 6 months 99.8%, at 9 months 100.2%, at 12 months 100.3%, at 15 months 111.1%.

Index of sub-sternum vacuum: is modified with aging, being higher at youths than at adults and it is lower at meat breeds in comparison with dairy ones. In the current case it presented fluctuations, having the following mean values: at birth 43.6%, at 3 months 44.6%, at 6 months 40.6%, at 9 months 39.4%, at 12 months 42.0%, at 15 months 46.0%.

Skeleton index: indicate the general development of skeleton and it is higher at dairy and mixed breeds face to meat ones. At Grey steppe breed it increasing progressively, at birth being 11.9%, at 3 months 12.8%, at 6 months 13.3%, at 9 months 12.6%, at 12 months 13.2%, at 15 months 14.0%.

Compactness index: indicate the general development of the body (its length and depth in relation with length). Generally, its value increases from birth to adult stage, being higher at meat breeds, at birth having the value of 107.3%, at 3 months 122.2%, at 6 months 122.2%, at 9 months 116.5%, at 12 months 121.1%, at 15 months 127.6%.

Dactylo-thoracic index: its value increase, generally, from birth to adult stage, being higher at meat breeds. At Grey steppe breed it decreased, having the following mean values: at birth 11.7%, at 3 months 9.9%, at 6 months 9.7%, at 9 months 9.3%, at 12 months 9.2%, at 15 months 9.7%.

Index of shin-bone charge: its value increases, generally, from birth to adult stage, being higher at meat breeds. At Grey steppe breed it decreased, having the following mean values: at birth 30.3%, at 3 months 12.1%, at 6 months 9.0%, at 9 months 6.4%, at 12 months 5.3%, at 15 months 4.8%.

Index of rump keenness: illustrate the rump development in width and it is important in appreciation of reproduction animals; the more his value is lower, with that much the rump is tight in its posterior part and has a negative influence on calving. The more the value of this index is higher with that much the rump width will be on whole length and calving will took place normally. For Grey steppe breed those indices presented the following mean values: at birth 90.4%, at 3 months 93.2%, at 6 months 102.2%, at 9 months 95.0%, at 12 months 86.0%, at 15 months 114.5% (Table 2).

Growing intensity of character *corporal mass* at youth belonging to Grey steppe indicate the following mean values: at 3 months 679.6 grams, at 6 months 601.1 grams, at 9 months 624.4 grams, at 12 months 730.6 grams, at 15 months 638.9 grams.

Analysis of growing absolute speed for character *height at withers* for young calves belonging to Grey steppe breed indicate the following mean values: at 3 months 13.7 cm, at 6 months 10.0 cm, at 9 months 4.9 cm, at 12 months 5.3 cm, at 15 months 8.9 cm.

Study of growing intensity for character *height at withers* for young calves belonging to Grey steppe breed indicate the following mean values: at 3 months 15.3%, at 6 months 9.9%, at 9 months 4.3%, at 12 months 4.3%, at 15 months 7.0%.

Table 2. Mean values and variability estimates for corporal indexes at young calves (n=12)

AGE		ICF	ITD	IM	IHD	ISV	IS	IC	DTI	ISC	IRK
0 months	Mean	95.4	56.4	101.9	96.6	43.6	11.9	107.3	11.7	30.3	90.4
	SD	7.5	3.9	5.4	13.2	3.9	1.4	8.6	1.5	3.1	12.2
	SEM	2.2	1.1	1.6	3.8	1.1	0.4	2.5	0.4	0.9	3.5
	Var.	56.9	15.3	29.4	174.0	15.3	1.9	74.4	2.3	9.6	147.7
	Min.	84.3	50.0	89.7	55.7	34.3	10.5	87.0	10.3	27.6	68.8
	Max.	113.2	65.7	111.4	108.8	50.0	14.7	123.7	14.9	35.7	121.1
3 months	Mean	106.6	55.0	129.9	100.4	44.6	12.8	122.2	9.9	12.1	93.2
	SD	7.5	6.6	11.4	2.1	6.1	2.2	10.1	1.3	1.5	12.5
	SEM	2.2	1.9	3.3	0.6	1.8	0.6	2.9	0.4	0.4	3.6
	Var.	55.6	43.0	129.3	4.4	37.6	5.0	101.9	1.8	2.3	156.9
	Min.	93.6	43.8	110.1	96.6	37.2	10.2	102.1	8.2	9.8	68.2
	Max.	117.3	62.8	152.0	105.3	56.3	17.3	134.1	11.8	14.4	108.7
6 months	Mean	112.4	59.4	136.9	99.8	40.6	13.3	122.2	9.7	9.0	102.2
	SD	7.4	6.1	4.4	2.2	6.1	2.5	8.1	1.8	2.1	16.6
	SEM	2.4	1.9	1.4	0.7	1.9	0.8	2.6	0.6	0.7	5.3
	Var.	55.4	37.8	19.2	4.9	37.8	6.0	66.3	3.4	4.6	276.1
	Min.	100.0	46.6	133.3	96.9	34.0	9.9	111.2	7.2	7.1	82.6
	Max.	120.2	66.0	146.6	104.1	53.4	16.7	133.3	12.5	12.5	140.9
9 months	Mean	117.1	60.9	136.3	100.2	39.4	12.6	116.5	9.3	6.4	95.0
	SD	3.9	3.0	3.3	1.0	2.6	1.0	6.1	0.8	0.3	5.3
	SEM	1.8	1.3	1.5	0.5	1.2	0.4	2.7	0.3	0.1	2.4
	Var.	15.3	9.1	11.2	1.1	6.8	1.0	36.9	0.6	0.1	28.5
	Min.	111.7	57.5	133.3	99.0	37.0	11.3	111.5	8.5	5.9	89.2
	Max.	122.6	64.2	141.7	100.9	42.5	13.9	127.0	10.4	6.6	103.0
12 months	Mean	117.3	58.9	143.3	100.3	42.0	13.2	122.1	9.2	5.3	86.0
	SD	2.4	5.0	7.8	1.5	4.4	0.9	5.3	0.7	0.4	21.8
	SEM	1.2	2.5	3.9	0.8	2.2	0.4	2.6	0.3	0.2	10.9
	Var.	5.8	25.4	60.6	2.3	19.5	0.8	27.7	0.5	0.2	475.2
	Min.	114.7	52.3	133.3	98.3	37.1	12.6	114.7	8.4	5.0	55.2
	Max.	120.2	62.9	152.3	101.8	47.7	14.5	126.7	10.1	5.9	105.7
15 months	Mean	114.0	54.0	145.5	111.1	46.0	14.0	127.6	9.7	4.8	114.5
	SD	0.7	0.9	0.3	0.1	0.9	0.7	1.1	0.5	0.1	1.9
	SEM	0.5	0.7	0.2	0.0	0.7	0.5	0.7	0.3	0.0	1.3
	Var.	.471	.859	.107	.004	.859	.471	1.114	.243	.003	3.463
	Min.	113.6	53.4	145.3	111.0	45.3	13.6	126.9	9.3	4.8	113.2
	Max.	114.5	54.7	145.8	111.1	46.6	14.5	128.4	10.0	4.9	115.8

ICF – index of corporal format; ITD – index of thorax depth; IM – index of massiveness; IHD – index of height difference; ISV – index of sub-sternum vacuum; IS – index of skeleton; IC – index of compactness; DTI – dactylo-thoracic index; ISC – index of shin-bone charge; IAC – index of rump keenness.

Analysis of growing absolute speed for character *oblique length of trunk* for young calves belonging to Grey steppe breed indicate the following mean values: at 3 months 23.3 cm, at 6 months 18.7 cm, at 9 months 8.0 cm, at 12 months 4.7 cm, at 15 months 3.9 cm.

Study of growing intensity for character *height at withers* for young calves belonging to Grey steppe breed indicate the following mean values: at 3 months 26.3%, at 6 months 16,4%, at 9 months 6.1%, at 12 months 3.3%, at 15 months 2.6% (Table 3).

Table 3. Mean values and variability estimates for growing indexes at young calves (n=12)

AGE		VA HG	VR HG	IC HG	VA LOC	VR LOC	IC LOC	VA GC (DMG)	VR GC	IC GC
M.U		cm	%	%	cm	%	%	g	%	%
3 months	Mean	13.7	16.9	15.3	23.3	30.8	26.3	679.6	213.5	102.8
	SD	7.1	8.9	7.7	7.6	12.8	8.7	95.0	30.3	7.3
	SEM	2.0	2.6	2.2	2.2	3.7	2.5	27.4	8.8	2.1
	Var.	50.1	78.9	58.6	57.9	163.7	75.4	9019.8	920.8	52.8
	Min.	3.3	3.9	3.9	15.6	18.9	17.3	533.3	169.0	91.6
	Max.	22.2	29.4	25.6	44.4	67.8	50.6	811.1	257.1	112.5
6 months	Mean	10.0	10.6	9.9	18.7	18.5	16.4	601.1	61.6	46.5
	SD	5.6	6.2	5.6	12.9	12.8	10.7	180.3	16.7	10.4
	SEM	1.8	1.9	1.8	4.1	4.0	3.4	57.0	5.3	3.3
	Var.	31.8	37.9	31.3	167.6	163.6	114.3	32495.2	278.9	108.0
	Min.	1.1	1.1	1.1	2.2	2.3	2.2	333.3	33.3	28.6
	Max.	16.7	20.0	18.2	35.6	36.4	30.8	811.1	76.8	55.5
9 months	Mean	4.9	4.4	4.3	8.0	6.3	6.1	624.4	36.9	31.0
	SD	1.7	1.6	1.5	5.1	4.3	4.0	120.5	7.4	5.5
	SEM	0.8	0.7	0.7	2.3	1.9	1.8	53.9	3.3	2.5
	Var.	2.8	2.5	2.4	26.2	18.9	16.0	14530.9	54.9	30.2
	Min.	2.2	1.9	1.9	3.3	2.5	2.4	433.3	23.9	21.4
	Max.	6.7	6.2	6.0	16.7	13.6	12.8	722.2	41.9	34.7
12 months	Mean	5.3	4.5	4.3	4.7	3.4	3.3	730.6	31.2	26.8
	SD	4.2	3.6	3.4	1.4	1.0	1.0	170.3	6.9	5.0
	SEM	2.1	1.8	1.7	0.7	0.5	0.5	85.1	3.5	2.5
	Var.	17.6	12.8	11.4	2.0	1.1	1.0	29002.1	48.1	25.5
	Min.	2.2	1.9	1.8	3.3	2.3	2.3	588.9	27.2	23.9
	Max.	11.1	9.4	9.0	6.7	4.8	4.7	977.8	41.5	34.4
15 months	Mean	8.9	7.3	7.0	3.9	2.7	2.6	638.9	20.2	18.3
	SD	1.6	1.3	1.2	0.8	0.6	0.5	39.3	0.7	0.6
	SEM	1.1	0.9	0.9	0.6	0.4	0.4	27.8	0.5	0.4
	Var.	2.5	1.8	1.6	0.6	0.3	0.3	1543.2	0.5	0.4
	Min.	7.8	6.4	6.2	3.3	2.3	2.3	611.1	19.6	17.9
	Max.	10.0	8.3	7.9	4.4	3.1	3.0	666.7	20.7	18.8

Conclusion and recommendations

Grey steppe young calves presented a harmonious corporal development, specific to primitive breeds, with a slow growth (daily mean gain varied between 601.1 grams at age of 6 months and 730.6 grams at age of 12 months), having a progressively growing intensity till the age of 12 months, after that decreasing.

Could be remarked the fact that indexes which appreciate the resistance degree of feet had decreasing values, from birth to 15 months, even if the tendency is for increasing at meat breeds.

Development of rump indicated progressive values, which demonstrate a developed posterior train and a wide rump, fact which allow animals to have parturition without problems.
The morphological values revealed in the current study indicate a rustic, primitive corporal development, but through selection and improving of maintenance conditions could be made the conversion to a meat breed with good aptitudes for meat production, without losing the important role of those breed in keeping the genetic diversity, as well as its social, cultural and historical importance.

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Fattening of lambs of different breeds on extensively grazed grassland – first results of possibilities and limitations

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Abstract

The aim of this study is to examine the potential of extensive grassland for fattening of Merino lambs and purebred Waldschaf lambs under different stocking rates. During the first months of grazing Merino lambs are pastured taking advantage of the high grass growth intensity. The Waldschaf lambs are grazed in the second half of the vegetation season with lower growth intensity of the pasture until the end of the pasture period. Both breeds are grazed in two separated paddocks with different stocking rates and grass growth heights.

Zusammenfassung

Lämmermast verschiedener Rassen auf extensiv beweidetem Grünland – erste Ergebnisse von Möglichkeiten und Grenzen

Ziel dieses Projektes ist es, die Auswirkungen unterschiedlicher Besatzstärken auf der Fläche bzw. der Futter-Aufwuchshöhen auf die Mast- und Schlachtleistung von abgesetzten Mastlämmern bei reiner Weidehaltung zu untersuchen bzw. das Potential einer Magerwiese im Hinblick auf Mastleistungen zu erfassen. Die Merinolandschaflämmer werden in der ersten Weideperiode unter Ausnutzung der hohen Wachstumsintensität der Weide bis zur Schlachtreife geweidet. Die Waldschaflämmer werden anschließend in der zweiten Weidehälfte bei geringeren Wachstumsintensitäten der Weide bis zum Ende der Weideperiode geweidet. Beide Rassen werden auf zwei Koppeln mit unterschiedlichen Besatzstärken bzw. Aufwuchshöhen geweidet.

Introduction

Lamb fattening is roughly distinguished between intensive lamb fattening, extended lamb fattening and fattening on pasture. Intensive lamb fattening is based on concentrate feeding. It starts at a live weight of about 20 kg following a rearing period. With average daily gains of more than 300 g, the final weight of 40-45 kg is reached at the age of 4-5 months. This form of fattening requires an intensive nutrient supply, with ad libitum feeding of concentrates.

Extended fattening of lambs is designed to increase the use of feedstuffs produced on farm. The basic idea is to reduce the amount of concentrates and to use high quality roughage, which is obtained on pastures or in field forage production. In this form of fattening, weights of 45-50 kg are reached within 6-7 months. The higher age of the lambs and the associated mutton flavor is not appreciated by Austrian consumers. This is one important reason why this form of lamb fattening using intensive meat breeds is not common.

Pasture fattening is almost not existent in Austria. However, in the pre-alpine and inner-alpine areas not only of Austria there are enough areas that could be grazed with different animal species including lambs (SCHMID, 2003). In the case of mother bound fattening with Waldschaf and crossbred Waldschaf x Ile de France, good weight gains were achieved (PODSTATZKY and BERGER, 2017). The aim of this trial was to evaluate the possibilities of fattening intensive and extensive breeds on extensively grazed grassland.

Material and methods

The study is carried out over two grazing periods (2017 and 2018).
The extensive pasture was grazed by sheep in the last two years. In April 2017 20 Merino lambs were bought and adapted for pasture over 10 days. Grazing with Merino lambs started on the 24th of April. At the end of June the Merino lambs were slaughtered. On the 4th of August grazing with Waldschaf lambs started. They were slaughtered on the 31st of October.

The lambs of each breed were assigned to two groups. Both groups differ in stocking rate or grass growth heights. The grazing area of group N (normal=reference group) is managed according to the grass growth heights of about 6 cm (measured with Rising Plate Meter). The necessary adaption of the grazing area in the group N (< 5 cm Rising Plate Meter) causes an adaption of the grazing area in group L (long) by the factor 1.2. Growth height in each paddock was measured weekly. Shelter was provided by a pasture tent with straw bedding. Water and a salt lick were always available. Descriptive statistics is used in this report because only results from the first project year are available.

Results and discussion

The growth heights and the dry matter (DM) yield of harvested grass were higher in the group L. The energy content of the grass was highest in spring, showed a slight decrease during the vegetation period and reached nearly the same content in autumn as in spring (Table 1). In June 2017 a drought period decreased the DM yield dramatically.

Table 1. Grass growth height (cm), Dry matter yield (kg/ha) and energy content (ME MJ/kg MD) during the pasture period

	20.04.17	29.05.17	21.06.17	18.08.17	29.09.17	31.10.17
Grass growth height (cm)	N	6.2	2.1	5.9	3.9	2.9
	L	7.6	5.4	10.6	5.3	5.2
DM-yield (kg/ha)	N	1997	220	1017	1771	311
	L	2153	560	1849	2062	484
Energy content (ME MJ/kg DM)	12.0	10.66		10.21	10.49	11.20

The botanical composition of the grazed sward is seen in Table 2. At the end of June the proportions of legumes and herbs reduced dramatically due to the water stress. The proportion of grass was stable and rose towards the end of the grazing period.

Table 2. Plant inventory (area percent)

	4.5.17	29.5.17	21.6.17	10.8.17	20.9.17	31.10.17
Gaps	0	3	70	0	15	8
Grass	18	17	20	40	15	42
Legumes	70	60	5	25	30	20
Herbs	12	20	5	35	40	30
Total	100	100	100	100	100	100

Table 3. Body weight (BW, kg), weight increase (WI, kg) and daily weight gain (DWG, g) of Merino and Waldschaf

		BW (kg)		WI (kg)		DWG (g)	
		N	L	N	L	N	L
Merino	Beginning	24.9	26.1	14.8	14.0	220.28	215.14
	Slaughter	39.8	40.1				
Waldschaf	Beginning	17.0	16.9	11.2	14.9	113.48	166.97
	Slaughter	28.3	31.8				

In Merino slightly better daily weight gains were found in the group N. But in Waldschaf much better daily weight gain was found in the group L (Table 3). The average slaughter weight in Merinos was nearly at the desirable level for the market. The endangered breed Waldschaf had less daily weight gain, especially in the group N (Table 3).

Table 4. Life weight, carcass weight and dressing percentage per breed and group

Group	Breed	Life weight (kg)	Carcass weight. cold (kg)	Dressing percentage. cold (%)
Merino	N	39.8	17.8	44.5
	L	40.1	18.1	44.9
Waldschaf	N	28.3	12.8	45.0
	L	31.8	14.7	46.3

Between the N and L group the dressing percentage in Merino sheep was similar but different in Waldschaf sheep (Table 4), with higher values in the L group.

Fattening of Merino sheep on pasture with carcass weight nearly meeting the demands of the market was possible within 5 months. The endangered breed Waldschaf is no fattening breed. Weaning took place at an age of about 4 month and the fattening period on pasture lasted about 2.5 month until the end of the grazing season (Table 5). Life age of the Merino lambs at the time of slaughter was similar to data by KOCAK et al. (2013) with 145 and 144 days, respectively.

Table 5. Average age (days and months) of Merinos and Waldschaf sheep at the beginning of pasture and at slaughter

		Days		Months	
Group	Breed	Beginning	Slaughter	Beginning	Slaughter
Merino	N	74	144	2.5	4.81
	L	78	145	2.6	4.8
Waldschaf	N	123	204	4.1	6.8
	L	120	201	4.0	6.7

The effectiveness of the utilisation of grassland can be measured in the production of meat per area. With the Merino sheep more carcass weight could be produced by intensive pasture conditions even on extensive grassland pasture than with the Waldschaf, but great carcass weight differences could be seen between the Waldschaf lambs (Table 6). Examinations with other breeds in Switzerland showed the influence of different types of pasture on daily weight gain and carcass weight. The Swiss examinations started very late with about 36 kg body weight and 26 week of age (WILLEMS et al., 2013). Mother bound fattening of lambs on pasture was tried by PODSTATZKY and BERGER (2017) with daily weight gains in Waldschaf lambs of 150 – 200 grams.

Table 6. Meat production (kg) per hectare with Merino and Waldschaf lambs

Group	Breed	ha (Ø)	Weight increase (kg)	Weight increase (kg)/ha	Carcass weight (kg/ha)
Merino	N	0.274	14.8	54.0	64.9
	L	0.327	14.0	42.8	55.4
Waldschaf	N	0.247	11.2	40.9	46.7
	L	0.275	14.9	45.6	53.5

More results will be available after finishing the second grazing period in 2018 and final evaluation of all data. Especially the influence of intensive grazing on the botanical composition of the extensive grassland will be monitored.

Conclusion and recommendation

Good fattening performance can be achieved even on extensive grassland pasture. The quality of grazed grass and breed influence the weight gain. Beside these first results other open questions are waiting i.e. as sheep show a selective feeding strategy (SCHMID, 2003) on pasture. Changes of botanical composition are expected especially with intensive grazing of extensive grassland.

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The role of ecosystem service in conservation of autochthonous sheep breeds exposed to tick infections in Serbia

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Abstract

Sheep breeding represents one of the most perspective branches of the livestock production in Serbia. The specific climate and the unic habitat include a high variability of flora and fauna of grasslands, pastures and meadows throughout the country, ensuring high biodiversity of the ecosystem. Autochthonous breeds are most adapted to the local environment. The present study was performed on 143 autochthonous sheep breed flocks in Serbia during the grazing season from March to October 2016 and 2017, and included a total of 1069 adult sheep. Tick infection was detected in all tested flocks, affecting 49.02% of examined sheep. The most dominant was *Ixodes ricinus* (44.71%), followed by *Dermacentor marginatus* (30.40%), *Rhipicephalus bursa* (15.15%), *Ripicephalus sanguineus* (8.70%), *Hyalomma savignyi* (3.18%), *Haemaphysalis punctata* (2.81%) and *Dermacentor recticulatus* (2.62%). In tested years the recorded tick infections showed two peaks, in spring (April-May) and in autumn (September-October). The considerable difference between detected spring and autumn tick populations can be attributed mainly to climatic and environmental conditions of the ecosystem where ticks and sheep cohabitat.

Introduction

Sheep production has an old tradition in Serbia. Today it represents one of the most perspective branches of the livestock production of the country. The National programme of agricultural and rural development for the period of 2014-2024 puts important efforts on the improvement of sustainable agriculture and resource conservation. The climate and geographical conditions in Serbia are highly variable, resulting in high heterogeneity of ecosystems where sheep are raring (MILUTINOVIĆ et al., 1996). In central, southern, eastern and western regions, where mountain regions with abundant grasslands, pastures and meadows predominate, the extensive low input sheep production is practiced. On the north, in regions of flatlands, the intensive crop production with semi-intensive sheep management is applied. As all mentioned regions are geographically different, their ecosystems are also unique and diverse (JANKOVIĆ et al., 1973, 1984). The rational use of pastures in the period April-October makes the sheep production sustainable. The specific climate and the unique habitat include a high variability of flora and fauna of grasslands, pastures, meadows and fields of Serbia, ensuring high biodiversity of ecosystems. Tick populations are inevitable elements of specific biotop (MILUTINOVIĆ, 1992). A diverse tick fauna mainly influences the health status of grazing ruminants (DIMITRIĆ et al., 1999; PAVLOVIĆ et al., 2009). Ticks are known as vectors for different diseases, some with zoonotic potential. However, as sheep and ticks share the same habitat, it is very difficult to avoid infections with different types of ticks that are contaminants of green areas. Tick infections are common, especially during late spring and autumn months (PETROVIĆ et al., 1996; MILUTINUVIĆ et al., 1998; ANDERSON and MAGNARELLI, 2008; PAVLOVIĆ et al., 2009).

This research was carried out in various ecosystems of Serbia, included 136 sheep flocks with a total of 1069 adult sheep, taking into account also the epidemiological importance of tick infections.

Material and methods

The present study was performed on 136 flocks of autochthonous sheep breeds in Serbia. The prevalence and biodiversity of ticks were analysed during the grazing season, in the period of March to October, in 2016 and 2017. A total of 1069 adult sheep were observed for the presence of tick infection. The following parameters were considered: biodiversity, relative abundance and sex ratio of tick populations of the observed ecosystems enhabited by sheep, taking into account the seasonal climate changes.

Ticks were collected by removing them from sheep with tweezers and were placed in vials with 70% ethanol. Determination of species and sex of the collected ticks was done using standard morphometric characterization given by POMERANCEV (1950), KAPUSTIN (1955) and KOLONIN (2009).

Results and discussion

Ticks were present in all 136 tested sheep flocks in different geographical regions and ecosystems of Serbia. Infection was detected in 496 (49.02%) out of 1069 examined sheep. A total of 4080 tick specimens were collected. Seven tick species were determined, all from the Ixodidae family. Sex ratio was as follows: 2151 females and 1929 males. As it is shown in the Figure 1, the most frequent species was *Ixodes ricinus* (44.71%), followed by *Dermacentor marginatus* (30.40%), *Rhipicephalus bursa* (15.15%), *Ripicephalus sanguineus* (8.70%), *Hyalomma savignyi* (*Hyaloma marginatum*) (3.18%), *Haemaphysalis punctata* (2.81%) and *Dermacentor recticulatus* (2.62%).

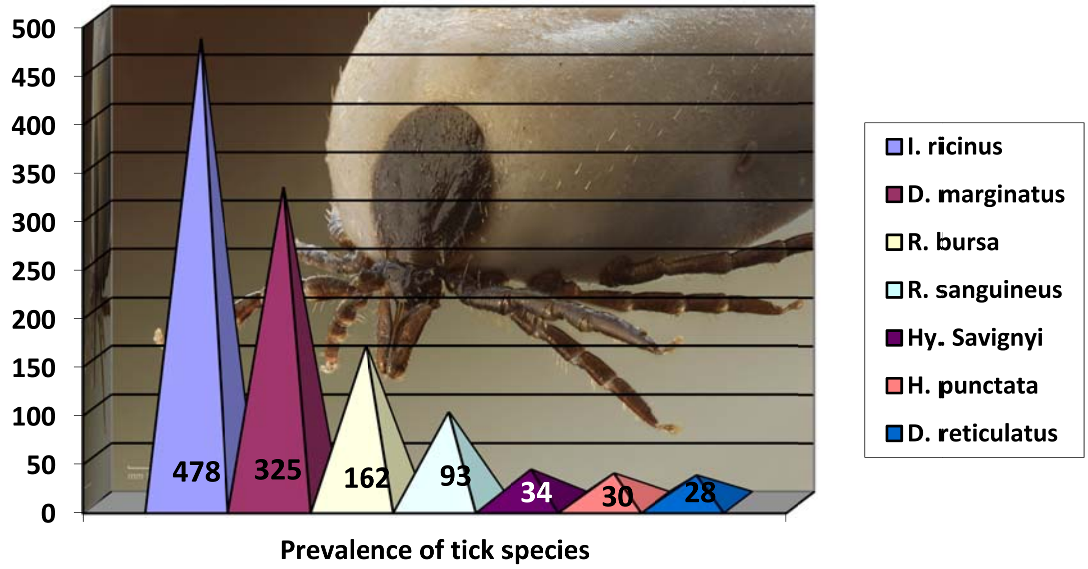

Figure 1. Biodiversity and prevalence of ticks detected

Sex ratio of detected tick species showed a higher number of females in four species (*I. ricinus, H. punctata, R. sanguineus* and *D. marginatus),* while higher number of males were detected in two species *(R. bursa* and *Hy. savignyi*), and an equal number of ticks in *D. recticulatus.*

Comparison of the obtained results with findings of previous similar surveys of tick diversity in Serbia, indicated that there is a great similarity in the established results. Examination performed in sheep on eastern, north-eastern, western and southern parts of Serbia (MIŠČEVIĆ et al., 1995; PAVLOVIĆ et al., 1995; MILUTINOVIĆ et al., 1996; PAVLOVIĆ et al., 2013, 2014; BECSKEI et al., 2015; PAVLOVIĆ et al., 2016) pointed to the presence of almost the same tick species, including *I. ricinus, Hy. savignyi*, *H. inermis, Boophilus calcaratus* and *Ornythonisus lachorenis.* Similar results were established in Belgrade area and north-west parts of Serbia (Mačva district) where *I. ricinus. R. sanguineus, D. pictus* and *D. marginatus* were detected as most abundant species in small ruminants (MILUTINOVIĆ et al., 1987, 1996; PAVLOVIĆ et al., 2013, 2016; BECSKEI et al., 2015).

It is well known that climate conditions have the major influence on the seasonal population dynamics of ticks. The most important are temperature, relative humidity and rainfall (PETROVIĆ et al., 1996; MILUTINOVIĆ et al., 1998). In the present study, the population dynamics of tick infections was monitored from March to October. They showed two annual peaks, in spring (April-May) and in autumn (September-October) in the tested years. March was a period when the grazing season started and it overlapped with the occurrence of *I. ricinus, H. punctata* and *D. marginatus.* During April the following ticks species were found, presented in order to their prevalence: *I. ricinus, R. sanguineus, D. marginatus* and *Ha. punctata.* In May, *I. ricinus, D. recticulatus, R. bursa* and *Hy. savignyi* were observed. In June, a population peak was observed for *R. sanguineus*, which is the most common species both in July and August. Autumn population peak were observed in September in two species of ticks: *I. ricinus* and *D. marginatus*, while in October the most abundant tick populations observed were the following: *H. punctata, R. sanguineus* and *R. bursa.* In October *D. marginatus* and *D. recticulatus* were rarely detected. The considerable seasonal changes of tick populations detected in spring and autumn can be attributed mainly to climatic and environmental conditions in all ecosystems where ticks and sheep cohabitat. Biodiversity and seasonal distribution of ticks detected in this survey correlates with results of other authors in the region of the Western Balkans, the Mediterranean region and Central Europe (RIVOSECCHI et al., 1980; L'HOSTIS et al., 1995; PAPAZAHARIADOU et al., 2003; HORNOK, 2009; ESTRADA-PENA et al., 2011; OMERAGIĆ, 2011; MIHALCA et al., 2012).

These findings are of valuable epidemiological importance because it is wellknown that tick populations are potential vectors for infective agents such as *Borrelia burgdorferi, Erlicihia spp., Anaplasma spp.,* and some viral vector-borne diseases and zoonoses such are tick-borne encephalitis, haemorrhagic fewer, etc. (NUTTALLl and LABUDA, 2008).

Results of the present study also necessitate further investigation of the characteristics of health, tolerance and resistance to tick infections, considering breed and individual genetic variations in sheep in various regions of Serbia. Resistance or tolerance to tick infections, and to a lesser extent to tick-borne diseases, are well documented, especially in cattle breeds (SAMISH, 2008; BISHOP et al., 2010). Autochthonous breeds adapted to local ecosystems are much more tolerant to parasite infections than exotic breeds. Results of DIMITRIJEVIĆ et al. (2012) showed higher susceptibility for parasitic infections of exotic sheep breeds compared to autochthonous Zackel sheep types.

Conclusion and recommendation

Sheep production is an example of a sustainable production, fully integrated in local rural development. One of the main threats on the outdoor breeding of sheep is parasitism. Based on the obtained results, it can be considered that tick infections represent a significant epizootiological issue of sheep production in Serbia. The method of sheep rearing allows permanent tick infections in sheep, and climatic conditions favor their development and maintenance on grazing surfaces of various ecosistems. Fauna, seasonal dynamics and sex ratio of ticks do not differ much from results obtained in the neighbouring regions. In tested years the population dynamics of recorded tick infections showed two annual peaks, in spring (April-May) and in autumn (September-October). The considerable differences between detected spring and autumn tick populations can be attributed mainly to transition of climatic and environmental conditions of ecosystems where ticks and sheep cohabitat. As tick infections have epizootiological and epidemiological importance and causes direct and indirect economic losses, it is necessary to undertake more detailed strategies for antiparasitic management of ecosystems where sheep are reared.

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Preserving and educating of traditional animal husbandry and our genetic heritage

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Abstract

According to the practical experiences of Imre Bodó, technological elements can be categorized into three actors (genetics, welfare, and farming) with relation to animal husbandry and production. Farming is the summary of rules, measures and facilities necessary to profitable production. Breeding includes genetic background and production activities.

Organic animal husbandry is an alternative husbandry system, which equals to natural livestock farming and excludes animal welfare objections. All animal species and breeds kept and bred in Hungary are suitable for the production of organic produces, however, traditional, historical species and breeds are preferred. The criteria of organic animal breeding are animal welfare, ethology and grazing. Animal welfare is closely linked to the relation of humans and animals. Keeping domesticated animals necessarily relates to certain protective activities for housing, defending, and breeding of them.

Movement is an essential requirement in organic husbandry. In case of extensification generally applied in organic husbandry the limitation of animals is minimal, and the environment is ecologically and socially rich. According to general experiences, the reduction of group size and organic criteria develops production results to a certain extent, and ensures the exclusion of abnormal behavioural problems.

The organic labelling of agricultural raw materials and foodstuffs is possible, when the production is in accordance with the related legislation and monitored by a certification body. Only those farms can be involved into organic husbandry, where the regulations of environmental protection is followed. Species and breeds to be kept should be suitable for organic husbandry. Breeds bred for intensive housing are inappropriate due to their susceptibility for certain diseases. Local landraces should be preferred. The main criterion of organic husbandry is to ensure the harmony between the animals and their environment. An important aspect of prevention is to keep the resistance of animals in a high level: general resistance (appropriate breed, optimal environment, fitness, and stress), specific resistance, passive immunity (natural and artificial), and active immunity. When therapeutic intervention is needed, alternative medicine should be preferred and natural-based curative drugs are recommended. A general rule in organic farming is provide protection only against severely pathogenic agents; in case of facultative pathogenic agents the aim is to reach an acceptable balance between the pathogen and the livestock.

The organically produced meat makes a difference in chemical-free foraging, antibiotics- and performance enhancer-free medicine; in addition, the use of traditional breeds and outside grazing turns it into a premium product.

Verarbeitung der Abstammungsdaten des vollen Zuchtbuches bei der gefährdeten Schafrasse Cikta

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Zusammenfassung

Die Auswertung der Population von Cikta Schafbestand erstreckt sich von 2000 bis 2014. Es wurde dabei Folgendes ausgewertet: die Anzahl mütterlicher Linien, der Inzuchtkoeffizient, die Tiere, die für die genetische Variabilität verantwortlich sind und das Generationsintervall zählen zu den wichtigsten Faktoren.

Das Zuchtbuch umfasst insgesamt eine Population von 3648 Tieren. Von diesen hatten 3176 Cikta-Schafe bekannte Eltern und 472 unbekannte Eltern (Widder 27 und Mutterschafe 445). Demnach liegt die Anzahl der Familien (Mutterlinien) im Herdbuch bei 445. Der durchschnittliche Inzuchtkoeffizient des Herdbuchbestandes ist sehr gering (1-2%). Für die genetische Variabilität des Bestandes sind, im Vergleich zum Gesamtbestand von 3648 Tieren, deutlich weniger Tiere (476) verantwortlich. Das Generationsintervall beträgt in dem Cikta Bestand durchschnittlich 4 Jahre, aber die Spanne zwischen Vätern und Nachkommen ist signifikant kürzer ($P < 0,05$) als die Spanne zwischen Müttern und Nachkommen.

Diese Schlussfolgerungen, vor Allem die Kenntnisse der mütterlichen Linien sind sehr wichtig, da diese bei der Zuchtauswahl innerhalb der Familie vorteilhaft sind. Außerdem können die Züchter die tauglichsten Nachkommen für die weitere Zucht behalten.

Abstract

Processing of genealogical data of the full herd-book at endangered sheep breed Cikta

The analysis of population structure in Cikta was ranged between 2000 and 2014. The following parameters were estimated: number of maternal lineages, coefficient of inbreeding, and number of responsible individuals in genetic variability, and generation interval.

The processing made it clear that the pedigree of Cikta breed is composed of 3648 individuals. From these, 3176 and 472 (altogether 27 rams and 445 ewes) animals were registered with known and with unknown parents, respectively. Accordingly, the number of families (maternal lineages) found in the herd-book is 445. The average inbreeding coefficient was very low, between 1 and 2%. It can be clearly seen that the genetic variability of the herd-

booked population is given by 476 individuals which are much less than the factual population number of 3648. The average generation interval was four years. Longer generation interval is typical between ewes and their progenies than rams and their progenies ($P<0.05$). This is because of the usually longer use of females in breeding.
The results, especially the knowledge of maternal lineages are very important. These are helpful in a within-family selection, and for choosing the appropriate individuals for a preserving breeding.

Einleitung

Die offiziellen, hochmodernen Zuchtprogramme für Nutztiere werden von Züchtervereinigungen in jedem Land geführt. Zu diesem Zweck dient die Führung des Herdbuches, in das die Zuchtdaten - über Identität, Abstammungskontrolle, Leistungsprüfung, Zuchtwertschätzung und Paarungsplan - eingetragen werden. Das Herdbuch der einheimischen Rassen ist meist geschlossen, nur die Nachkommenschaft der bereits eingetragenen Eltern sollten eingetragen werden. Ein organisiertes, institutionelles System ist zur Erhaltung gefährdeter Haustierarten gegründet worden. Eine Reihe nationaler Vereine, z.B. Öngene - Österreichische Nationalvereinigung für Genreserven Landwirtschaftlicher Nutztiere, GEH - Gesellschaft zur Erhaltung alter und gefährdeter Haustierrassen e.V., TRBA - Transsylvanien Rare Breeds Association und internationalen Organisationen, z.B. RBI - Rare Breeds International, SAVE - Sicherung der landwirtschaftlichen Arten Vielfalt in Europa, DAGENE - Internationale Vereinigung für die Erhaltung der Tierrassen in der Donauregion, sind dafür verantwortlich und nehmen an der Operation als NGO teil.

Die Anzahl eines Tierbestandes wird als kritisch angesehen, wenn dieser weniger als 100 weibliche Tiere oder nur 5 männliche Tiere hat; Tiere, in denen keine fortpflanzungsfähigen Tiere vorkommen, können als ausgestorben angesehen werden. (FRÖLICH und KOPTE, 2014).
Lediglich die ländliche Rassen wurden wegen ihrer niedrigeren Leistungen (MAIJALA, 1970), bzw. weil sie den Anforderungen der industriellen und kommerziellen Standardisierung nicht entsprechen (SAMBRAUS, 2016), aus der Zucht ausgeschlossen.

Ein wichtiges Element ist die exakte Unterbringung in den Genbanken von Tieren. Ergänzend zu diesen, sorgen die Satellitenstationen (und andere kleinere Einheiten) für Erhaltung und für Vermehrung der meisten Bestände, eventuell für Gebrauchstier-Herstellung von integrierter Kreuzung. Dies beinhaltet, laut ungarischen Begriffes, die Konzeption und Realisierung von Genschutz und Generhaltung (SZALAY, 2017). Die Verteilung von Tieren reduziert das potenzielle Katastrophen- oder Krankheitsrisiko erheblich. Diese können ergänzt werden durch "lebendige Museen" und Rettungsstationen, die für Bildungszwecke in Verbindung mit Naturschutz konzipiert sind. Hinzu kommen Möglichkeiten der genetischen Forschung, wodurch auch die kleinen Populationen erhalten bleiben können (in vivo Form; BODÓ, 1985, 1991), sowie moderne Techniken, wie gefrorene Samen- oder Embryo Produktion, die in der sogenannten in-vitro Form sehr hilfreich sind (BREM und Mitarb., 1990).

Gründe um für die Erhaltung der gefährdeten Rassen entgegenzuwirken, sind Folgende:
1. Eine mögliche Existenz von Eigenschaften kann nicht definiert werden, da diese einen positiven Einfluss auf die veränderten Rahmenbedingungen und Marktbedingungen haben werden.

2. Die genetische Verengung von intensiven Beständen kann vermieden, oder durch ihre bessere Anpassung zur verändernden Umwelt, durch Einkreuzung mit gefährdeten Rassen erleichtert werde.
3. Die alten Rassen geben die historischen und kulturellen Werte der ländlichen Umgebung an die neu entstanden Rassen weiter.

Das wertvolle Landschaf, das Cikta, wurde nach dem Zweiten Weltkrieg fast vollständig von der Zucht ausgeschlossen. Im Jahr 1974 begann OTÁF (Landesanstalt für Tierernährung und Tierzucht) den Bestand von angesammelten 40 Mutterschafen und 3 Widdern nachhaltig zu retten. Diese Zahl wuchs in 10 Jahren auf 200 Tiere an. Dies ist die einzige Herde, die den Kern des heutigen wachsenden Zuchtbestands um 600 Tiere bildet. Das aktuelle Herdenbuch der nationalen Cikta-Bestände ist bis zu dem Jahr 2000 zurückzuführen.

Das Ziel dieser Ausarbeitung ist es, erweiterte populationsgenetische Analysen, basierend auf den Zuchtbuchdaten des Cikta Schafbestandes (geführt vom Zuchtverband der ungarischen Schaf- und Ziegenzüchter, MJKSZ), zu ermitteln.

Material und Methoden

Um die Bestandsdatei im Herdbuch zu bearbeiten, hat uns die MJKSZ die nationale Datenbank, als Excel-Dateiformat, zur Verfügung gestellt. Diese enthält die Daten der hier ausgeführten Darlegungen zwischen den Jahren 2000 bis Ende 2014. In dieser Erhebung wurden die Anzahl mütterlicher Linien, der Inzuchtgrad, die Anzahl der Tiere, auf welche die volle genetische Varianz zurückgeführt werden kann und das Generationsintervall überprüft.
Inzucht impliziert die Fortpflanzung zweier blutsverwandter Elterntiere. Der Inzuchtkoeffizient ist die Wahrscheinlichkeit, dass zwei Allele hinsichtlich derselben Herkunft identisch sind. Ein Tier kann zwei wirklich identische Allele haben, wenn seine Eltern mindestens einen gemeinsamen Vorfahren haben. Der Inzuchtkoeffizient ist eine absolute Zahl zwischen 0 und 1. Dann ist 0 der Inzuchtkoeffizient, wenn die Jungtiere aus der Paarung nicht verwandter Eltern geboren wurden. Je größer ist die Verwandtschaft zwischen den Eltern, desto mehr wird der Inzuchtgrad den Wert 1 (100%) nähern. Der Wert des Inzuchtkoeffizienten wird von der Länge und Vollständigkeit des Stammbaumes beeinflusst. Die kurzen Stammbäume verfälschen die Größe des Inzuchtkoeffizienten.
Gemäß der Fachliteratur ist das Generationsintervall gleich dem Durchschnittsalter der Eltern bei der Geburt von ihnen Jungtieren, die bei der Entstehung der nächsten Generation beteiligt sind. Für genetisch gefährdete Rassen ist es von Nutzen, wenn das Generationsintervall lang ist, da dies zur geringsten genetischen Diversitätsreduktion führt (GÁSPÁRDY u. Mitarb., 2003). Je kleiner die Anzahl einer Rasse und je ärmer ihre genetische Vielfalt ist, desto notwendiger ist es, das Generationsintervall zu verlängern.
Während dieser statistischen Verarbeitung und Bewertung Software Pedigree Viewer (KINGHORN und KINGHORN, 2010), Endog (GUTIÉRREZ und GOYACHE, 2005) und Poprep (GROENEVELD u. Mitarb., 2009) wurden angewandt.

Ergebnisse und Auswertung

Das Cikta Zuchtbuch (in der Zeit zwischen 2000 und 2014) umfasst 3648 Tiere. Von diesen hatten 3176 Schafe bekannte Eltern und 472 unbekannte Eltern. In der ersten Gruppe gibt es Zuchttieranzahl von 50 Zuchtböcken und 657 Mutterschafen. Die zweite Gruppe besteht aus der Gründertieren und rassekonforme Tieren, die im Laufe der Zeit, aber mit unbekannten Hintergrund, im Stammbuch eingetragen wurden (insgesamt 27 Widder und 445 Mütter). Demnach liegt die Anzahl der Familien (Mutterlinien) im Herdbuch bei 445. Diese Zahl ist beachtenswert, da es bei der Familienauswahl in jeder Familie nötig ist, weibliche Nachkommen für die Weiterzucht zu behalten. Die größte Familie, die auf einen Mutterschaf zurückgeführt werden kann, hat insgesamt 128 Nachkommen (durchschnittlich 6-7). Aufgrund der gründlichen Führung des Stammbuches verbessert sich die Pedigree-Vollständigkeit mit fortschreitender Zeit. Die Pedigree-Vollständigkeit erreicht fast 50% bei Tieren mit 6 Ahnenlinien. Die jüngsten Tiere sind bereits in der 7. und 8. Generation.

Der durchschnittliche Inzuchtgrad betrug bei der Gesamtpopulation 1%. Die Inzucht pro Generation ist in den Daten von Tabelle 1 veranschaulicht. Die Gründertiere und die darauffolgende Generation, die ursprünglich von unbekannten Eltern stammten, waren keinen berechneten Inzuchtkoeffizienten. Eine Paarung der verwandten Tiere vollzog sich erst ab der dritten Generation, so dass die Inzucht auf 1-2% geschätzt werden kann. Bezüglich der größten Inzuchtkoeffizienten des Gesamtbestandes, beträgt der Inzuchtgrad der ersten 10 Tiere 37,5%. Die Meisten dieser Tiere waren Mutterschafe und wurden kurz nach der Neuauflage des Zuchtbuches geboren. Dies ist zwar ungünstig, jedoch zu dieser Zeit notwendig gewesen.

Tabelle 1: Entwicklung des Inzuchtkoeffizienten (%) von Cikta-Generationen

Generationen	Anzahl der Tiere	Inzuchtkoeffizient
1	373	-
2	742	-
3	568	2,0026
4	723	1,1022
5	716	1,3508
6	487	1,3905
7	37	1,9373
8	2	0,0000
insgesamt/durchschn.	3176	1,0007

Tabelle 2 legt die genetische Struktur des Cikta Schafbestandes dar. Es ist deutlich zu erkennen, dass die gesamte genetische Variabilität der Herdbuch-Population ist mit wesentlich weniger Zahl der Tiere als der tatsächlichen Bestandsgröße angegeben; das heißt, dass 476 Tiere für die gesamte genetische Vielfalt verantwortlich sind, im Herdbuch ist jedoch eine Anzahl von 3648 Tieren beschrieben.

Tabelle 2: Anzahl der Tiere, die die genetische Vielfalt der Herde beeinflussen (weibliche und männliche Tiere sind zusammen aufarbeitet)

Rate der genetischen Vielfalt	Anzahl der verantwortlichen Tiere
50%	17
60%	26
70%	42
80%	78
90%	174
100%	476

Der Einfluss, den die Vorfahren auf die genetische Vielfalt der Herde haben, ist in Tabelle 3 dargestellt. Nach der Tabelle beträgt der Anteil von sieben Zuchtböcken (gleichzeitig Gründerväter), die am meisten die Variabilität des Tierbestandes besitzen, einzeln jeweils 3-8%, was als ausgewogen betrachtet werden kann, insgesamt aber 35%. Dies ist jedoch relativ gering ist, somit tragen auch andere Zuchttiere zur Diversität bei.

Tabelle 3: Vorfahren, die zur höchsten Rate der genetischen Variabilität (%) in der Herde beitragen

Kennzeichnungsnummer	Geschlecht	Geburtsdatum	Rate der genetischen Vielfalt
HU1323083201	1	-	7,7220
HU100032324	1	02.03.2002.	6,2421
HU9901001787	1	-	5,9981
HU12160692	1	-	4,3490
HU9901004339	1	-	3,8994
HU9901004016	1	-	3,7040
HU9901001310	1	-	3,2824

Das Generationsintervall kann durch die vier Abstammungspfade: Vater-Sohn-, Vater-Töchter-, Mutter-Sohn- und Mutter-Tochter, unter Verwendung des Geburtsdatums der Tiere und ihrer Eltern, berechnet werden. Das längste Generationsintervall zeigen die Mütter, die die Bocklämmer produzieren. 4,26 Jahre ist das Durchschnittsalter einer Mutter, wenn ihre männlichen Nachkommen geboren werden, was man Tabelle 4 entnehmen kann. Der kürzeste Zeitabstand zwischen aufeinanderfolgenden Generationen konnte in den Zuchtböcke-Söhne Pfad (3,80 Jahre) errechnet werden. Die entsprechenden Generationsintervall waren für die zwei vereinigt betrachteten Väter-Nachkommen und Mütter-Nachkommen Pfade statistisch signifikant ($P < 0,05$) unterschiedlich. Der Zweistichproben-t-Test ergab jedoch keinen Unterschied ($P > 0,05$) nach Geschlecht der Jungtiere pro Paare.

Tabelle 4: Generationsintervall (Jahr) der Cikta in den vier Eltern-Nachkommen Abstammungspfaden

Abstammungspfade	Anzahl der Nachkommen	Generationsintervall	Standardfehler des Mittelwerts
Vater-Sohn	799	$3,80^{a}$	0,056
Vater-Tochter	823	$3,85^{a}$	0,056
Mutter-Sohn	754	$4,26^{b}$	0,069
Mutter-Tochter	800	$4,09^{b}$	0,065
Eltern-Nachkommen	3176	3,99	0,031

$^{a,\ b}$ - die unterschiedliche Buchstaben drücken die statistisch bewiesene Abweichung ($P < 0,05$) aus

Schlussfolgerungen und Empfehlungen

Das Zuchtbuch der Rasse Cikta verzeichnete in fast anderthalb Jahrzehnten bis zu viertausend Tiere. Es enthält nun, infolge der notwendigen Zugeständnisse, einen bedeutenden Anteil von Gründervorfahren unbekannter Herkunft.

Die Anzahl der weiblichen Gründertiere, wie Familien (Mutterlinien), ist jedoch trotzdem bedeutsam. Außerdem ist es wichtig, dass diese Daten an Züchter weitergeleitet werden, um die Selektion innerhalb der Familien zu erleichtern.

Der durchschnittliche Inzuchtkoeffizient des Herdbuchbestandes ist sehr gering (1-2%) und ändert sich von Generation zur Generation nicht, dieser ist als günstig zu bewerten.

In Zukunft sollte darauf geachtet werden, die Paarung der zu nahen Verwandten zu vermeiden, da bereits 37,5% Inzuchtgrade vorhanden sind. Diese sollten, um die Diversität zu erhalten, nicht als Zuchttiere zugelassen werden.

Es wurde festgestellt, dass die genetische Variabilität der Herdbuch-Population nur von wenigen Tiere gebildet wird. Die Differenz eines Verhältnisses von etwa 1:8 zeigt einen signifikanten Genverlust, durch welche sich zum Einen die unsorgfältige Auswahl der Zuchttiere zum Teil erklären lässt, zum Andern aber auch durch breitere Geschlechterverhältnis und die Verluste von väterlichen und mütterlichen Linien.

Die Anzahl der Vorfahren, die für die genetische Vielfalt der Herde verantwortlich ist, sollte relativ hoch, aber deren Gesamtbeitrag klein gehalten werden. Das bedeutet, dass der 3-8% Anteil der sieben Zuchtböcke jeweils als ausgewogen betrachtet werden kann.

Das Generationsintervall beträgt in dem Cikta Bestand durchschnittlich 4 Jahre. Die Spanne zwischen Väter und Nachkommen ist kürzer als die Spanne zwischen Mütter und Nachkommen. Diese Situation spiegelt den traditionellen Natursprung wider, die weitgestehend erhalten bleiben sollte. Der Unterschied ist jedoch nicht so groß (etwa ein halbes Jahr), dass die Beiträge der Eltern zur nächsten Generation zu überproportional wären, vorausgesetzt, dass das Verhältnis der Geschlechter relativ niedrig ist.

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Genetic variation based on pedigree data in Slovenian Alpine goat, Slovenian Saanen goat and Drežnica goat

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Abstract

The objective of this study was to evaluate the population structure and genetic diversity in Slovenian local goat breeds based on pedigree information of animals registered in the herd book. Pedigree data records were analysed for Slovenian Alpine goat (8,594), Slovenian Saanen goat (3,660) and Drežnica goat (1,886). Animals born in years 2012 – 2016 were considered as reference population for the analysis. The pedigree included 22.0% base animals in Slovenian Alpine goat, 23.9% in Slovenian Saanen goat and 25.0% in Drežnica goat. The number of equivalent complete generations of ancestors was higher in does in all breeds in comparison to bucks and was from 3.10 in Drežnica goat to 4.36 in Slovenian Alpine goat. The average inbreeding coefficient for inbred animals was 7.36%, 8.22% and 9.15% in Slovenian Saanen goat, Slovenian Alpine goat and Drežnica goat, respectively. An incomplete pedigree leads to underestimation of inbreeding.

Introduction

The conservation of genetic diversity is an essential objective in the management of animal populations, both in breeding and conservation programs (FERNANDEZ et al., 2005). A complete pedigree is essential for the evaluation of inbreeding, effective population size, generation interval, genetic diversity, and several other important population parameters (MARTINEZ et al., 2008). Lack of information, incomplete pedigree, and the recent introduction of animals into the population can lead to underestimation or overestimation of inbreeding coefficient and other population parameters (GOYACHE et al., 2003).

Altogether, Slovenian goat population accounts close to 25,000 animals. The most widely used dairy goat breeds are local Slovenian Alpine goat (the number of the purebred does is around 4,000) and the local Slovenian Saanen goat with the number of the purebred does around 2,500. Both dairy breeds are widespread throughout the whole territory of Slovenia and they are considered being endangered according to their reproduction capacity, population trend and proportion of purebred mating. The third local goat breed named Drežnica goat is the only Slovenian autochthonous goat breed. Due to the purpose of rearing and its original location two types of Drežnica goat were developed: dairy and meat type. The population of Drežnica goat consists of about 720 animals and it is listed as a critically endangered breed mainly due to the concentration of a major part (90%) of the total population in a restricted geographical area, which means within a radius of less than 30 km (ŽAN LOTRIČ et al., 2013).

The objective of this study was to evaluate the genetic diversity and population structure of Slovenian Alpine goat, Slovenian Saanen goat and Drežnica goat based on pedigree information. The results could help to make recommendations regarding the genetic management of the flocks of Slovenian local goat breeds, for possible implementation of a breeding program aimed to conservation of their genetic diversity.

Material and methods

The data included 8.594, 3.660 and 1.886 records for Slovenian Alpine (SAl) goat, Slovenian Saanen (SSa) goat (both in the period 1985 - 2015) and Drežnica (DR) goat (in the period 2000 - 2015), respectively. However, there were 1889 (SAl), 873 (SSa) and 471 (DR) animals with both parents unknown. The data were obtained from the Slovenian national database for small ruminants, which collects records according to the ICAR standards. The Herdbooks for Slovenian Alpine goat and Slovenian Saanen goat were established in the eighties of the last century, while for Drežnica goat in the year 2000. The population size of all three goat breeds differs and is the lowest in Drežnica goat. Animals born in the period 2012 – 2016 were used as reference population for the analysis (1.402 SAl, 727 SSa and 518 DR). The individual animal records included identification number, breed, gender, identification numbers of sire and dam, birth date, breeder and origin.

Population structure based on pedigree data is described by two groups of parameters: demographic and genetic. Demographic analysis characterizes the structure and dynamics of populations considered as a group of renewed individuals whereas the genetic analysis is interested in the evolution of a population's gene pool. One of criteria to describe pedigree completeness is the number of complete generation equivalent, defined as the sum of the proportion of known ancestors over all traced generations (MAINGEL et al., 1996). LACY (1989) introduced the concept of the effective number of founders which measures the overall founder representation in a population accounting for the loss of genetic variability from unequal founder contributions. DUNNER et al. (1998) proposed mating strategies based on average relatedness coefficient, which defined as the probability that an allele randomly chosen from the whole population in the pedigree belongs to a given animal.

Coefficients of inbreeding and relationship, equivalent complete generations, the effective number of founders, ancestors and equivalent number of founder genomes were computed using the PEDIG package (BOICHARD, 2002). Others parameters were calculated using statistical package SAS (SAS Inst. Inc., 2012).

Results and discussion

Generation interval

Generation intervals were computed for the bucks and does based on the average age of bucks and does at birth of their offspring and for the four paths of selection (sire - son, sire - daughter, dam - son and dam – daughter; Table 1). Female offspring tend to have shorter generation interval than male offspring in all three breeds. Sires in Slovenian Alpine goat and sires in Drežnica goat were younger when their offspring were born in comparison to dams in both breeds. Moreover, bucks in Drežnica goat produce offspring very young (male offspring at the age under 2.6 years and female offspring at the age of 2.4 years). Does are kept in reproduction for a longer period. In Slovenian Alpine goat and Slovenian Saanen goat, there were almost no difference in the generation interval between bucks and does, whereas does in Drežnica goat had offspring mostly one year later in comparison to bucks.

Table 1. Mean generation interval (GI) for all born kids and for the four paths of selection in Slovenian local goat breeds

Path	No. of parents	No. of offspring	GI (yr)	No. of parents	No. of offspring	GI (yr)	No. of parents	No. of offspring	GI (yr)
	Slovenian Alpine goat			Slovenian Saanen goat			Drežnica goat		
Sire-son	105	171	3.59	46	76	5.17	29	46	2.65
Sire-daughter	200	2057	3.54	92	712	3.95	63	301	2.51
Dam –son	169	177	4.36	69	78	4.95	59	62	4.81
Dam-daughter	1357	2113	4.06	516	786	3.98	350	490	4.68

Pedigree completeness

The degree of pedigree completeness was evaluated by calculating the equivalent number of complete generations known per animal. The pedigree showed not more than 8 to 12 complete known generations of ancestors for animals born in the period from 2012 to 2016. The equivalent complete generations of ancestors increased over the years in all goat breeds (Figure 1).

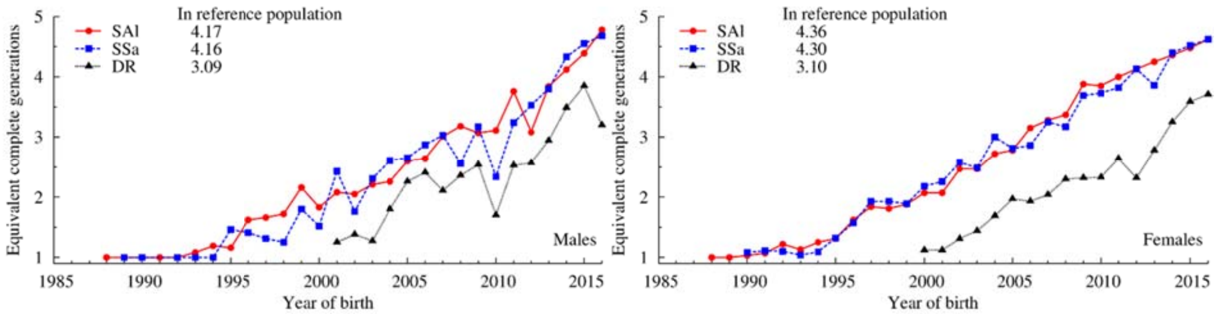

Figure 1. The equivalent of the complete generations of ancestors by age according to gender and breed (SAI – Slovenian Alpine goat, SSa – Slovenian Saanen goat, DR – Drežnica goat)

Inbreeding and relatedness

The total number of inbred animals in the population was 1.629, 693 and 243 in Slovenian Alpine goat, Slovenian Saanen goat and Drežnica goat, respectively (Table 2). The proportion of inbred animals in the whole population of each breed was 19.0% in Slovenian Alpine goat, 18.9% in Slovenian Saanen goat and 12.9% in Drežnica goat. The mean coefficient of inbreeding in reference population, for entire pedigree analysed and for inbred animals were 7.36% for Slovenian Saanen goat, 8.22% for Slovenian Alpine goat and 9.15% for Drežnica goat. The highest inbreeding coefficient of an individual animal among all breeds was similar (from 34.67 to 38.67%).

Table 2. Inbreeding coefficient by breeds

Class for inbreeding coefficient (%)	Slovenian Alpine goat		Slovenian Saanen goat		Drežnica goat	
	Number	%	Number	%	Number	%
0<x≤5	918	56.4	389	56.1	95	39.1
5<x≤10	213	13.1	123	17.7	59	24.3
10<x≤15	158	9.7	61	8.8	47	19.3
15<x≤20	37	2.3	37	5.3	10	4.1
20<x≤25	11	0.7	8	1.2	13	1.2
25<x≤30	273	16.8	69	10.0	27	11.1
30<x≤35	7	0.4	6	0.9	1	0.4
35<x≤40	12	0.7			1	0.4
Total	1629[1]	19.0[2]	693	18.9	243	12.9
Average[3] (%)	8.22		7.36		9.15	
Max (%)	38.67		34.67		37.50	

[1]Number of inbred animals, [2] % of inbred animals in the population, [3] average inbreeding coefficient in inbred animals

Average relatedness

The average relatedness (AR) is a parameter computed for each individual that enables mattings between sires and dams from which offspring will have lower inbreeding coefficients (DUNNER et al., 1998). The AR was computed for animals in the reference population and represents mean value of coefficients in a row from the numerator relationship matrix, which belongs to particular animal. Range of values of AR in the reference population in Slovenian Alpine goat was between 0.001 and 0.075 with the mean 0.035 and the median 0.039 (Table 3). The AR in Slovenian Alpine goat decreased, probably due to the introduction of new animals in the population. The same trend for recent years was observed for Slovenian Saanen goat where a range of AR was from 0.001 to 0.094 with average 0.046 and median 0.039. The lowest median (0.020) and average of AR (0.029) were in Drežnica goat with a range similar to other two breeds (0.002 – 0.087).

Table 3. Average relatedness (Avg.) in the reference population by breeds

Breed	Number	Avg.	SD[1]	Min	Max	Me[2]	KA[3]
Slovenian Alpine goat	1402	0.035	0.018	0.001	0.075	0.039	-0.61
Slovenian Saanen goat	726	0.046	0.025	0.001	0.094	0.039	0.19
Drežnica goat	518	0.029	0.025	0.002	0.087	0.020	0.53

[1] Standard deviation, [2] median, [3] skewness

However, the AR values for the majority of animals in Drežnica goat was under 0.01 probably due to incomplete pedigrees (Figure 2).

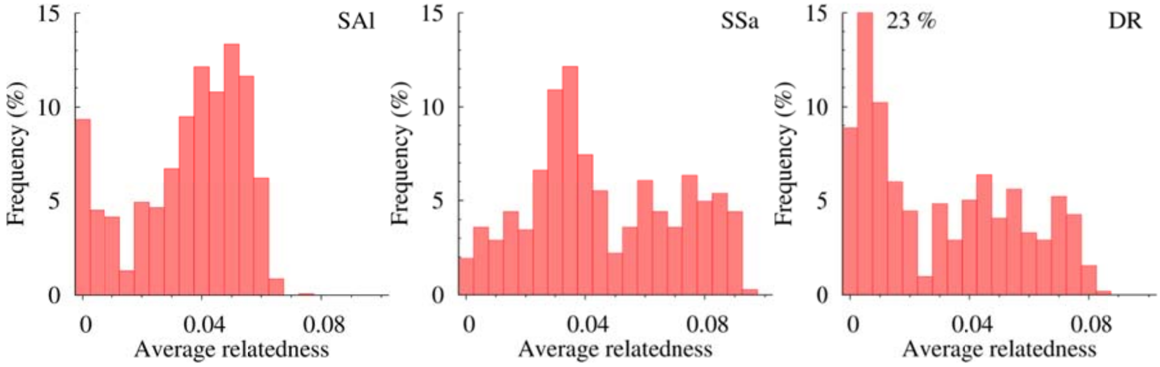

Figure 2. The distribution of average relatedness by breeds in reference population (SAI – Slovenian Alpine goat, SSa – Slovenian Saanen goat, DR – Drežnica goat)

Contribution of ancestors and an effective number of ancestors

Ancestors with unknown parents are considered as founders. Number of founders in the reference population for bucks was between 111 (Drežnica goat) and 328 (Slovenian Alpine goat) while for does, the number of founders was from 188 in Drežnica goat to 595 in Slovenian Alpine goat (Table 4). In closed populations, it is not expected that effective number of founders increases with the time, which was found in Slovenian Alpine goat and Slovenian Saanen goat (Figure 3). Such a period was between 2005 and 2010 in Drežnica goat. The increase could also reflect the introduction of animals from other populations. Differences in the effective number of founders in both genders within breeds were observed from 35.0 (Drežnica goat) to 102.4 (Slovenian Saanen goat) in bucks, and between 30.8 (Drežnica goat) and 93.6 (Slovenian Saanen goat) in does (Table 4).

In recent years, the effective number of founders in bucks in Drežnica goat decreased, whereas in does it has been increasing (Figure 3).
The effective number of ancestors (which are or are not founders) in bucks was between 26.1 (Drežnica goat) and 40.7 (Slovenian Alpine goat) and between 23.7 (Drežnica goat) and 38.2 (Slovenian Alpine goat) in does (Table 4). Introduction of animals from other populations is more noticeable in the effective number of founders in comparison to the effective number of ancestors (Figure 3).

The effective number of ancestors was smaller than the effective number of founders in all breeds as was generally proved by BOICHARD et al. (1997). Furthermore, the effective number of ancestors, which accounts for bottlenecks in the population life, provides an intermediate estimate. The effective number of founder genomes remaining in the reference population is the smallest estimate due to additionally accounts for random genetic drift (MACCLUER et al., 1986). The differences between effective number of founders and effective number of ancestors showed uneven contribution of gens from ancestors, especially sires, while the lowest number of founder genomes in comparison to the effective number of ancestors is due to genetic drift.

Table 4. Representation of founders, ancestors and founder`s genome in bucks and does in the reference population

Parameter	Bucks	Does	Bucks	Does	Bucks	Does
	Slovenian Alpine goat		Slovenian Saanen goat		Drežnica goat	
Number of founder animals	328	595	197	314	111	188
Effective number of founders (f_e)	100.9	85.0	102.4	93.6	35.0	30.8
Effective number of ancestors (f_a)	40.7	38.2	35.8	27.4	26.1	23.7
Eff. no. of founder genomes (N_g)	20.4	24.0	16.8	20.6	12.4	14.0

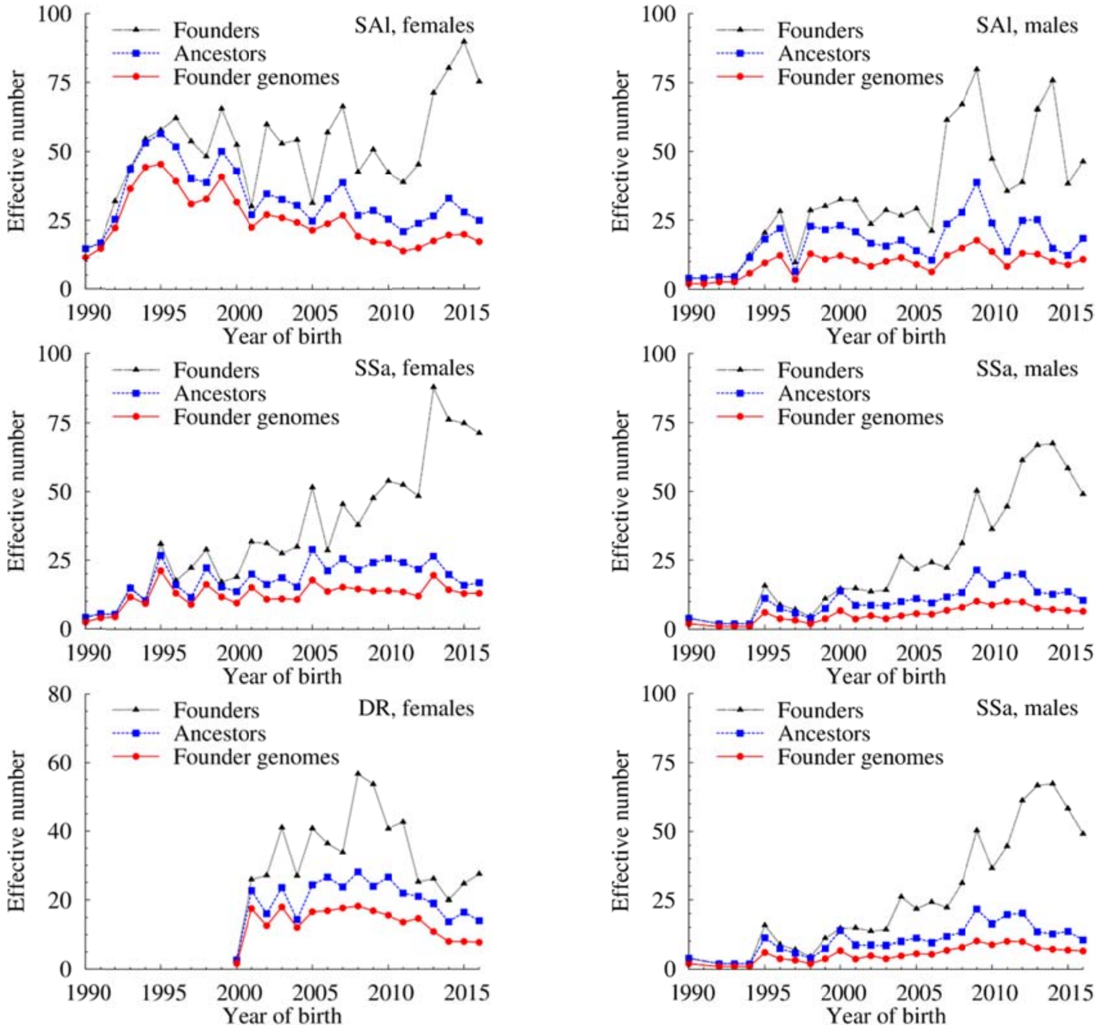

Figure 3. Effective number of founders, ancestors and founder genomes over years by breed and gender (SAI – Slovenian Alpine goat, SSa – Slovenian Saanen goat, DR – Drežnica goat)

Conclusion and recommendation

Pedigree information in three goat populations - Slovenian Alpine goat, Slovenian Saanen goat and Drežnica goat - is incomplete and are known for some generations only. Mostly Herd books have been opened, consequently new herds, as well as new animals were introduced. Likewise, the pedigree recording was introduced in all three breeds relatively recently. Less complete pedigrees resulted in underestimated coefficients of inbreeding and relatedness.

Low values of the effective number of ancestors and the effective population size were caused by the unbalanced contribution of sires in all local goat breeds.

The effective number of ancestors and founder genomes differed among breeds as well as between genders within breeds. However, the assumption that animals with unknown ancestors are unrelated may increase the probability that these values are overestimated.

Nevertheless, it is essential to conserve the genetic diversity, which is necessary for the conservation as well as for breeding program in Slovenian local goat breeds.

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A possible variant of preserving in vivo the local pigs breeds in Romania

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Abstract

The crossbreeding of the modern swine breeds with the traditional ones, as well as the important changes that occurred in their breeding and feeding, led to hybrids with an excellent meat quality. It is also desirable to use the hoop structures for swine like alternative for pig management system. This system respond positively to the new challenges of climate change. In year 2017 is recorded in Romania 662 sows Mangalitza (Red, Blonde and Swallow-bellied) with origin cards and 47 sows Bazna with origin (the main section, which is known ancestors from two generations). The Black of Strei was been identified few specimens pure breed and hybrid (35 specimens) in Hunedoara County. The loss of local pig breeds means the loss of important rustic genes. The hoop structures offer one solution for pig conservation in vivo to preserved local breed. The hoops have the advantages of low initial cost, versatility and simplicity. Hoops offer an alternative to conventional confinement housing systems.

Introduction

Breeds and traditional population of swine such as Bazna, Black of Strei, Mangalitza and White of Banat have developed in areas such as Transylvania and Banat. All this type of swine is pleased for its rusticity, resistance to diseases and for the quality of its meat (MATIUTI, 2017).

The crossbreeding of the modern swine breeds with the traditional ones, as well as the important changes that occurred in their breeding and feeding, led to hybrids with an excellent meat quality. Their resistance to disease and meteorological changes has increased significantly (MATIUTI and MATIUTI, 2012).

As there is no legislation in Romania for livestock farming on free-range and extensively, we propose an alternative for pig management system.

Material and methods

The aim of this paper is to show the hoop structure for swine and the performance data from this type of structure.

This scientific paper is based on information about local breeds of pigs existing in the Data Base of Transylvanian Rare Breeds Association. It is also desirable to use the hoop structures for swine like alternative for pig management system. This system responds positively to the new challenges of climate change.

Results and discussion

From numerical point of view, the swine breeds and populations are poorly represented.
In 2017, there are recorded 662 sows Mangalitza (Red, Blonde and Swallow-bellied) with origin cards and 47 sows Bazna with origin (the main section, which is known ancestors from two generations) in Romania. The population size of the Bazna breed is decreasing. It is necessary, urgently measures for preserved this breed. In the same situation is the pig population Black of Strei and White of Banat. In 2017, conformable of information for Database of Transylvanian Rare Breeds Association, the Black of Strei was been identified few specimens pure breed and hybrid (35 specimens) in Hunedoara County.
In Dabuleni areas (in south of Romania), near of borders of Danube was registered five animals brought from Bulgaria, because in this county exist owners who imported these pigs to meat quality, which resembles the consistency of the boar.
Its bacon is very dry, and before smoking the ham it is sliced according, to taste, in the area the traditional preference being for medium thick ham (MATIUTI et al., 2011).
The mating of the Black of Strei sows with wild boar is practised quite frequently for the same reasons as in the cases of Bazna and Mangalitza in the Zarand region.

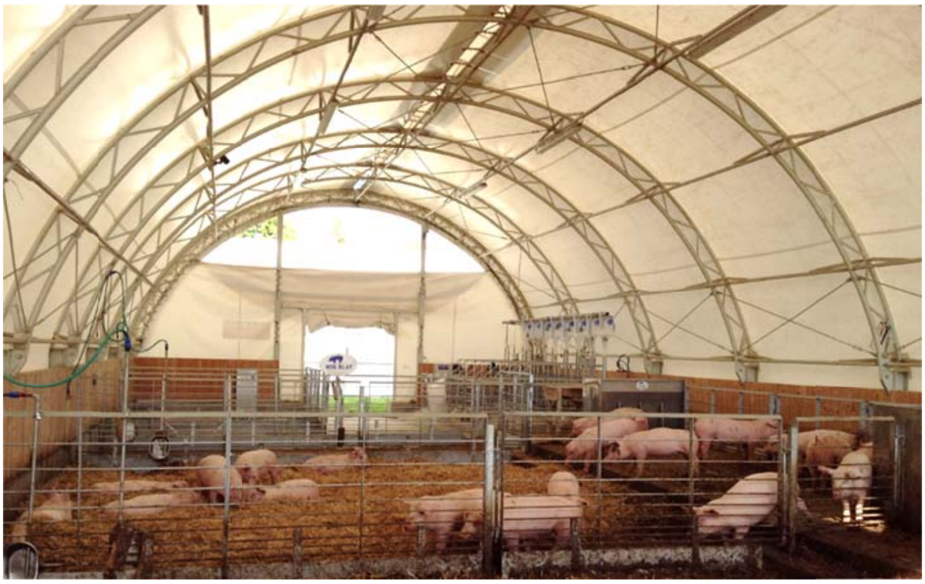
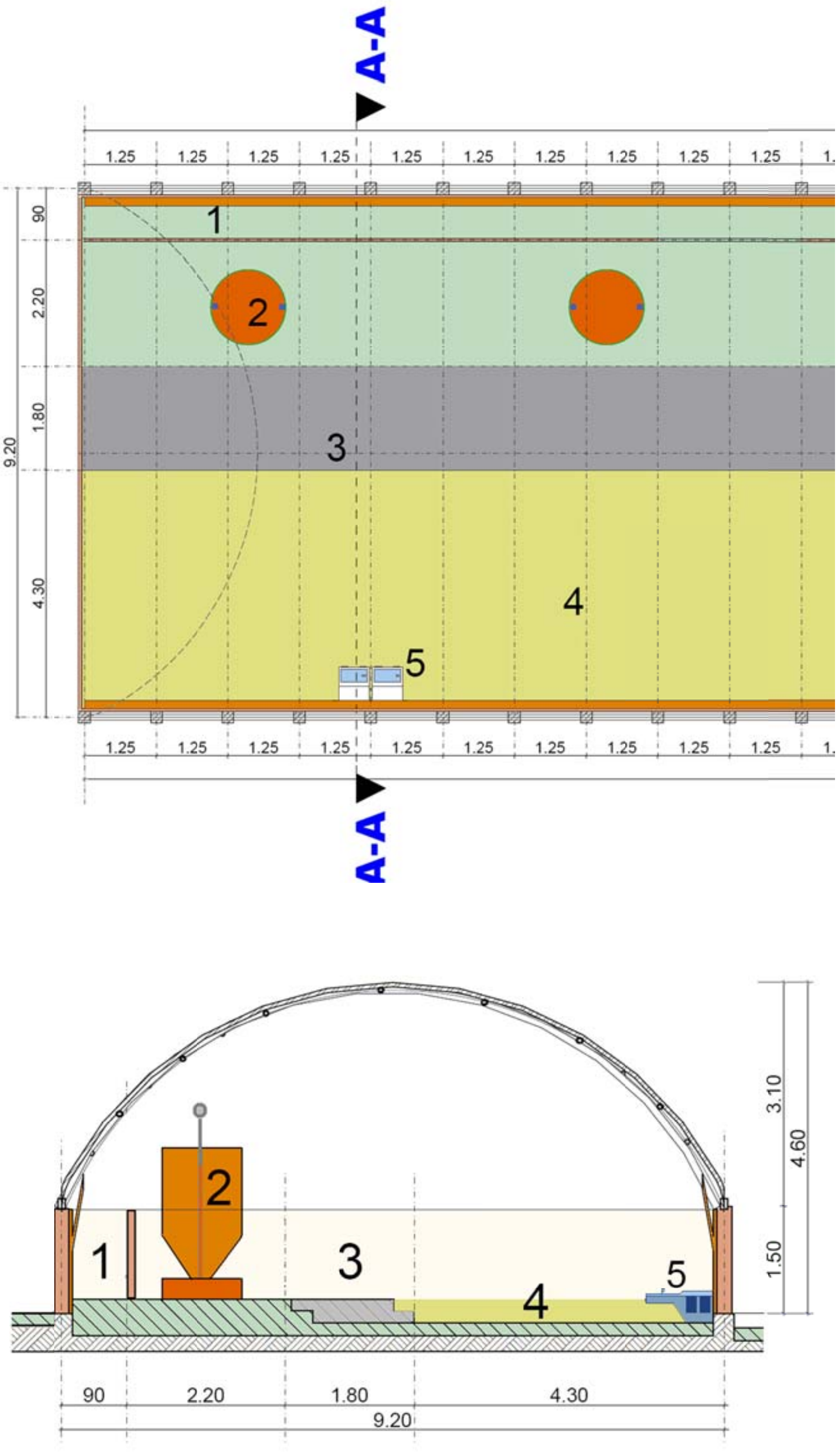


Figure 1. Swine Research Unit during a summer trial period

The population of White Pig of Banat is probably extinct in 2017, because there was not identified any of them.
Since 2017, the Romanian Ministry of Agriculture has initiated a program for the numerical growth of pigs from the Mangalitza and Bazna breeds. Who wants to grow Mangalitza receives 27 piglets for free. In order to support breeders for these breeds, we propose that there is also a new possibility in Romania as preserving in vivo these hoop structures for swine.
The hoop structures offer one solution for pig conservation in vivo to preserved local breed as they are low cost and easy to build it (Figure 1).



1. run alley; 2. feeder and drinker; 3. feeding concreted area; 4. deep litter bedding; 5. anti-freeze device drinker, useful in fattening barns especially over winter.

Figure 2. Floor plan (upper) and cross-section A-A (lower) of a grow to finish unit with feeding area on one side (HUTU and ONAN, 2008a)

Deep-bedded hoop structures as a management system for grow-finish swine allow for growth performance equivalent to that of standard industry confinement facilities (Figure 2). Furthermore, during periods of potential heat stress, swine in hoop structures continue to perform well. The relative performance of barrows and gilts in hoop structures exhibit

differences similar to those obtained in typical confinement facilities. Therefore, it can be recommended that hoop structures are a viable, indeed desirable, option for low-input swine operations in Romania.
The hoops have the advantages of low initial cost, versatility and simplicity. Hoops offer an alternative to conventional confinement housing systems.

Hoops are a sustainable alternative for producing pigs because they are:
1) environment-friendly with solid manure that can be composted,
2) farmer-friendly with low capital cost and competitive cost of production (HUTU and ONAN 2008b).
The Romanian family farmer can compete and be a viable entity in the Romanian swine industry

Conclusion and recommendation

- The loss of local pig breeds means the loss of important rustic genes. The animal genetic industry places great emphasis on rusticity and meat quality.
- The hoop structures offer one solution for pig conservation in vivo to preserved local breed.

Acknowledgments

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New Slovak animal breeds

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Abstract

In the last fifty years there was a significant decrease in the animal number in the Slovak Republic, and in some species an original (native), autochthonous breeds completely disappeared. From the last update statuses of animal breeds in the Slovak Republic (at the end of 2011) it is obvious that three breeds of cattle and two breeds of pig are subjected to extinction. The situation with animal genetic resources in the Slovak Republic is not satisfactory due to the fact that semen doses, stored in the gene bank, are originated only from several but not from all Slovakian breeds. On the other hand, two new animal breeds at last two years were produced. In 2016, the Strba gepard rex (rabbit) and in 2017 – the Slovak dairy sheep were bred and recognized.

Introduction

Protection of the animal genetic resources has an impact on the present and also future life quality and important effect on the food safety. Ratification of the Convention on the Biodiversity obliges SR to protect biodiversity, to guarantee sustainable use of its components (also animal genetic resources), and fair and equal access to benefit sharing from genetic resources. Protection of the animal genetic resources also emerge from the European Biodiversity Action Plan for Agriculture, The Global Strategy for the Management of Farm Animal Genetic Resources FAO OSN, Council Regulation (EC) No 870/2004 establishing a Community programme on the conservation, characterization, collection and utilization of genetic resources in agriculture; Act No. 194/1998 Coll. on breeding and reproduction of farm animals. It is really necessary to conserve and maintain animal genetic resources as an insurance against climatic changes, occurrence of diseases, social changes, genetic problems, selective failures and unexpected catastrophic events that can markedly affect biodiversity (CHRENEK et al., 2017).

Animal genetic resources can be protected under *in situ* and *ex situ* conditions. In the field of modern biotechnology, preservation strategies are focused mainly on *in vitro ex situ* diversity conservation. This form of protection is represented by long-term storage of biological material (spermatozoa, embryos or stem cells) at temperatures well below freezing point (KULIKOVA et al., 2015; KOVAC et al., 2016).

In situ conditions were defined in the Convention on Biological Diversity as „conditions and natural habitats, and, in the case of domesticated or cultivated species, in the surroundings where they have developed their Distinctive properties“(CBD, 1992). *In situ* conservation is undertaken in the traditional production system of the conserved AnGR. However, *in situ* conservation is not without risks. For example, a population maintained *in situ* may be struck by a disease outbreak or other disaster or may be affected by inbreeding, genetic drift or introgression from another breed.

Ex situ conservation decreases these risks by providing a backup that can be drawn upon if required. However, if the population is also maintained *in situ*, regularly collecting and conserving new samples *in vitro* can help to maintain the potential for future adaptation. As described above, *ex situ* conservation can be undertaken either *in vivo* or *in vitro*. While in many circumstances maintaining a live *ex situ* population adds little to a conservation strategy that already includes *in situ* and *in vitro* components, it can have some advantages.

The situation with animal genetic resources in the Slovak Republic is not satisfactory due to the fact that semen doses, stored in the gene bank, are originated only from several but not from all Slovakian breeds. On the other hand, a same new animal breed was bred.

Slovak dairy sheep

From 1990s, the programme formation of new population of the Slovak Dairy sheep (Figure 1) was started. Some flocks were involved into performance testing; the establishment of the breed was divided into two periods: first, crosses of local breeds (Improved Valachian, Tsigai and Merino) with improving breeds (Lacaune, East Friesian) of various proportions were formed; second, a close (inter se) breeding scheme within the crossbred population was applied. Parents of the next generation were chosen on the basis of breeding values for milk yield and litter size. At present, five thousands female Slovak Dairy sheep (SD) individuals are included in the performance testing; 35 % yearling females enter the breeding scheme each year; a total, 50 thousands Slovak Dairy sheep ewes are kept in commercial flocks. Milk and reproduction traits of the Slovak Dairy sheep were analyzed using mixed model methodology that included fixed and random effects. In similar way growth traits were analyzed. Milk trait analyses included 20,511 milk records belonging to 11,026 ewes kept in 52 flocks during the period between 1995 and 2015; reproduction trait analyses included 30,034 litter size records belonging to 9,671 ewes kept in 26 flocks during the period between 1997 and 2015. The standardized milk yield (SMY) and average daily milk yield (ADMY) increased more than twice: 79.8 ± 5.36 l (1995) vs. 164.0 ± 2.04 l (2015) and 495.1 ± 33.5 ml (1995) vs. 1035.3 ± 12.8 ml (2015), respectively. The population of SD was recognized as a new breed in 2017 (MARGETIN et al., 2017).


Figure 1. Slovak dairy sheep (photo: M. Dukes, SlovChov, 2017)

Strba gepard rex

The Strba gepard rex (Figure 2) was cultivated by breeders from the village of Strba and Letanovce in the North of Slovakia. The breed was recognized at the 2016 at the national competition in Nitra. The genotype is: AA bkbk CC DD gg Kk rexrex – black-yellow colour. It belongs to a medium-sized breed, from 3.5 to 4.5 kg of the body weight.


Figure 2. Strba gepard rex (www.google.sk/search?q=Štrbský+gepardí+rex)

Conclusion and recommendation

The situation with animal genetic resources in the Slovak Republic is not satisfactory, in some species an original (native) autochthonous breed completely disappeared. On the other hand, two new animal breeds - Strba gepard rex (rabbit) and the Slovak dairy sheep at last two years were bred and recognized in Slovakia.

Acknowledgements

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Sweet whey as feedstock for production of silage inoculants

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Abstract

Possible application of sweet whey is as substrate for lactic acid bacteria (LAB) growth to obtain LAB biomass as starter culture or inoculants. We have studied the capacity of some lactic bacteria to grow in whey as culture medium, the productivity of the bioprocess using sweet whey as culture medium and the effect of the LAB inoculants obtained in whey as only substrate on the preservation conditions in ensiled forages. High-quality silage was obtained. The whey produced in the dairy factory can be used as feedstock to produce enough inoculant to preserve up to 5 million tons of forage per year. The silage inoculant consisting of *Lactobacillus plantarum* cultivated in whey was tested to preserve different types of forages: alfalfa and sorghum. Sweet whey is recommended as culture medium to produce lactic bacteria inoculant, to initiate lactic fermentation in silage.

Introduction

Cheese whey resulted from dairy processing in most of the industrial applications is regarded as residue. Whey, together with other organic residues generated in dairy industry represent a major source of water pollution because of high BOD (biological oxygen demand). In some cases, whey proteins are extracted to produce food additives or niche products used in health care and sports as proteinic additives (FOEGEDING et al., 2002). The main difference between sweet whey and acidic (sour) whey is the presence of lactose in the first one and the lack of lactose and presence of organic acids (mainly lactic) in the last one. From biotechnological point of view, sweet whey is more important, as lactose can be fermented by many types of microorganisms, hence this sugar can be used as nutrient in fermentation technologies to produce a wide range of biochemicals and bioproducts, such as ethanol, butanol, methane, hydrogen etc. (LUO and ANGELIDAKI, 2013; BECERRA et al., 2015; POPA et al., 2015; DAS et al., 2016). Another possible application of sweet whey is as substrate for lactic acid bacteria (LAB) growth to obtain LAB biomass as starter culture or inoculants. The silage inoculants consist mainly of viable LAB able to transform low molecular mass sugars from plant substrate in lactic acid. The pH is decreasing in ensiled forages as a result of lactic acid accumulation, together with the absence of oxygen as a result of compression of forage, lead to the inhibition of undesired microflora. This way, the degrading processes are stopped and the nutritive value of the silage will be preserved. The aim of this work is to verify the capacity of some lactic bacteria from the Collection of Industrial Microorganisms of Animal Science and Biotechnology Faculty from Timisoara to grow in whey as culture medium, the productivity of the bioprocess using sweet whey as culture medium and the effect of the LAB inoculants obtained in whey as only substrate on the preservation conditions in ensiled forages.

Materials and Methods

Substrate

Sweet whey containing 4.9 % lactose was collected from a dairy processor in Timis county. The dairy factory is processing around 100 tons of sheep milk per day in peak season and between 6000 and 8000 tons of sheep milk per season (from spring to autumn). Around 75% from the milk is found at the end of the curding process as whey. The dairy factory generates 4000-5000 tons of whey per season, which can be translated into around 200 tons of lactose per season. This enormous quantity of sugar is regarded as industrial residue and is lost in the present, needs additional costs to discard and raises environmental issues if accidentally is discarded in water or on the fields. We have used sweet whey as substrate for growing LAB in laboratory scale.

As control for LAB growth we have used MRS standard medium (CORRY et al., 2003).

Bacterial strains

Three lactic bacteria strains were used to produce silage inoculants: *Lactobacillus plantarum CMIT2, Lactobacillus acidophilus CMIT3* and *Enterococcus faecium CMIT4.* The microorganisms are preserved in the collection of industrial microorganisms of Timisoara (CMIT), belonging to the Banat University of Agricultural Science and Veterinary Medicine „*King Michael I of Romania*” from Timisoara.

Culture conditions

The microorganisms are preserved at -70°C in glycerol solution and before using, fresh cultures were obtained in MRS+$CaCO_3$ 1%. The LAB was cultivated in different conditions:

- different temperatures;
- in MRS (control) and in sweet whey.

The effect of lactic bacteria in laboratory silage

Two types of forages were inoculated with lactic bacteria:

- alfalfa, forage difficult to ensilage, due to the low content in low molecular mass sugar and high content in proteins, with pH buffer effect;
- and sorghum, forage easy to ensilage, due to the high content in low molecular mass sugars in stems (higher then sugar in corn stems), and low content in proteins.

Forages were inoculated with 5 x 10^5 lactic bacteria/ gram forage (fresh mass). After inoculation, the forages were packed and ensiled in 5 kg vacuum bags, closed using a vacuum pump. The bags were air-tight sealed. Silages were preserved at 20°C until opening. The biomass was treated with additives before ensiling, except untreated controls. In the control for alfalfa 4% molasses was added, to increase the content in sugar. Two flasks (inoculated and not inoculated) were opened at 2, 4, 7, and 14 days of storage. The evolution of lactic bacteria, pH value and acidity was determined. The number of lactic bacteria was determined by counting the colonies developed in MRS plates under microaerophilic conditions. Acidity in silage was determined by titration of water extract with NaOH 0.1N, considering 1ml NaOH = 0.009008 g lactic acid.

Results and Discussions

Lactic bacteria strains were cultivated in MRS and whey and evolution of turbidity (Figure 1) and pH (Figure 2) was determined. Since the turbidity in whey is due to protein particles in suspension, the evolution of turbidity couldn't be used as a proof parameter for bacterial growth. The pH drop was considered as an evidence of lactic bacteria activity. Also, after 20 hours of culture in whey, the number of lactic bacteria was determined using the colony plate count method (Table 1).

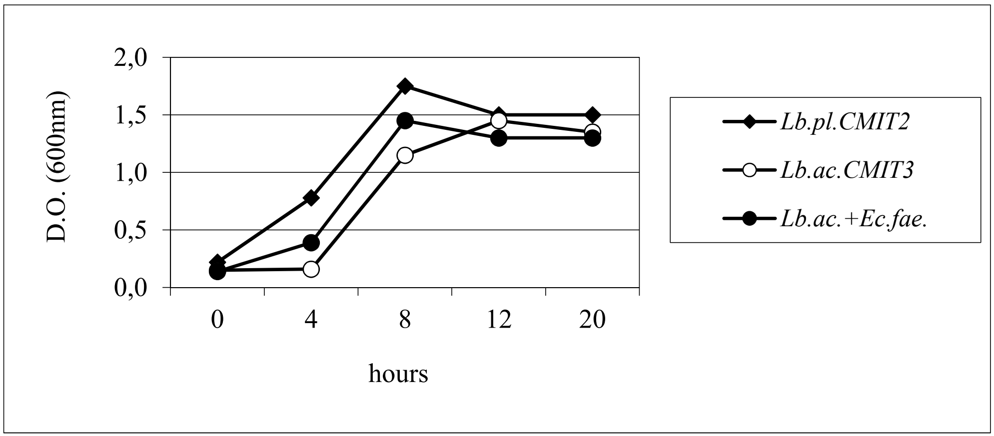

Figure 1. Evolution of turbidity in lactic bacteria cultures in MRS

Figure 1 indicate that after 8 hours of incubation, the tested LAB reaches the end of logarithmic growth phase and enters the plateau phase if MRS medium is used as growth substrate. In other culture media, where the nutrients are not perfect balanced as in MRS standard medium, the growth curve might have another pattern. This hypothesis is confirmed by the pH pattern in Figure 2, where the pH decrease (indicating bacterial growth) is prolonged to 20 hours of incubation.

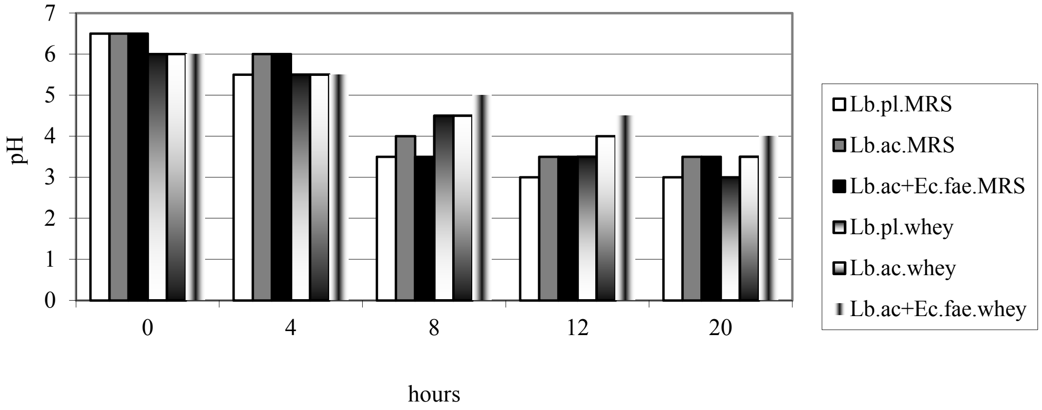

Figure 2. Evolution of pH in lactic bacteria cultures in MRS and whey

Decreasing of pH value indicate accumulation of organic acids (especially or exclusively lactic acid), which is the main factor demonstrating metabolic activity and growth of LAB inoculated in each batch. All three batches containing whey inoculated with LAB indicate decreasing of pH values, the lowest value after 20 hours of incubation is observed in *Lactobacillus plantarum* (pH 3) and the highest (pH 4) is observed in mixed culture of *Lactobacillus acidophilus* and *Enterococcus faecium* in whey as growth substrate.

Table 1. The number of lactic bacteria cultivated in whey

Strain	CFU after 20 hours of incubation
Lb. plantarum CMIT2	4.95×10^8
Lb. acidophilus CMIT3	2.2×10^8
Lb. acidophilus + Ec. faecium	4.2×10^8

The results presented in Figures 1-2 and in Table 1 indicates that *Lactobacillus plantarum CMIT2* is able to grow in whey as good as in MRS. In order to demonstrate that the pH decrease in whey is not determined by preexistent microflora, the whey was sterilized and concomitant cultures was carried out as in table 2.

Table 2. Culture of *Lb. plantarum CMIT2* in sterilized and not sterilized whey

Incubation, hours	MRS+*Lb.pl. CMIT2*		Unsterile whey + *Lb.pl. CMIT2*		Sterilized whey+ *Lb.pl. CMIT2*		Unsterile whey, no inoculation	
	pH	O.D.*	pH	O.D.*	pH	O.D.*	pH	O.D.*
0	6	0.05	6	0.05	6	0.21	6	0.05
24	4.5	0.12	4.5	0.4	5	0.29	6	0.17

*O.D. = optical density at 600 nm.

Data obtained in laboratory scale batches and presented in table 2 demonstrate that *Lb. plantarum CMIT2* can be cultivated in septic conditions, in unsterilized whey, even better as in MRS. The nutritive components were degraded during the sterilization of whey and LAB growth was affected. Also, it is important to observe that the preexistent microflora in un-inoculated whey had a poor growth and the pH value remains unchanged after 24 hours of incubation. This means that microflora present in unsterile whey is not acidophilic, therefore not LAB.

The most propitious temperature for *Lb. plantarum CMIT2* was determined (table 3). The tested strain shows a good growth at high temperatures, this quality can be used to inhibit the contaminants in culture media.

Table 3. Growth parameters of *Lb. plantarum CMIT2* at different temperatures

Temperature(^{o}C)	37	42	44	46
pH	3.5	3.5	4	4
D.O.1 – D.O.2*	1.74	2.31	1.70	0.67
Acidity (g%)	1.27	0.89	0.74	0.44

* difference between optical density at 600 nm measured at the insemination and optical density at 600 nm after 12 hours of incubation in MRS

Effect of lactic bacteria in laboratory scale silages

Results obtained in alfalfa silage

Alfalfa laboratory silage bags prepared as in *material and methods* were opened after 2, 4, 7 and 14 days of storage and pH, CFU, and acidity was verified. The results are shown in Figures 3, 4, and 5. The control consists in alfalfa mixed with molasses 4%, and the test batches consists in alfalfa inoculated with *Lb. plantarum CMIT2*. The maximum accumulation of lactic bacteria (Figure 3) in inoculated batches and in control as well is after four days of ensilage. The number of lactic bacteria in inoculated batches is 90% higher than in control.

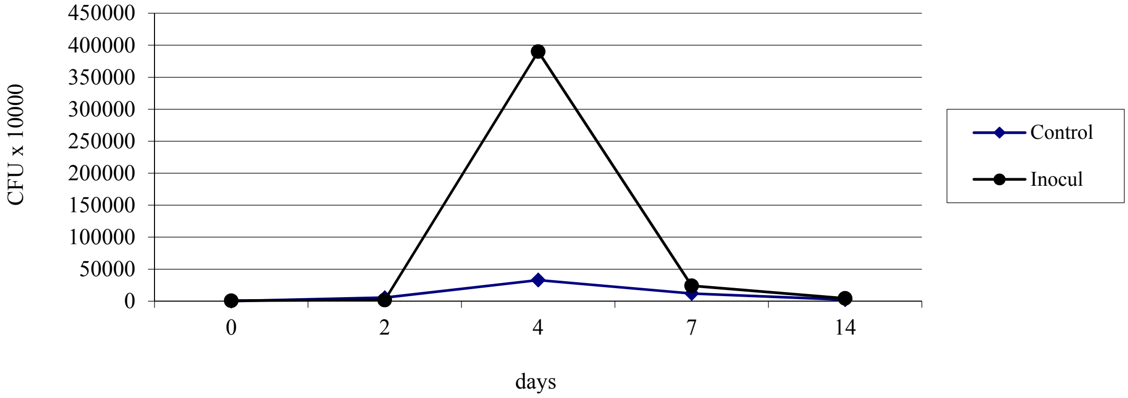

Figure 3. Evolution of lactic bacteria in alfalfa laboratory silage

The pH value decreases with a higher rate in control batches due to the higher content of sugar from added molasses (Figure 4). However, the pH reached the same values (4.4) after two weeks of preservation of silages in anaerobic conditions. This value is normal in alfalfa, where the high content of proteins functions as a buffer for organic acids produced by LAB. This is the main reason why alfalfa raises difficulties in ensiling, even sugar sources are added (such as molasses).

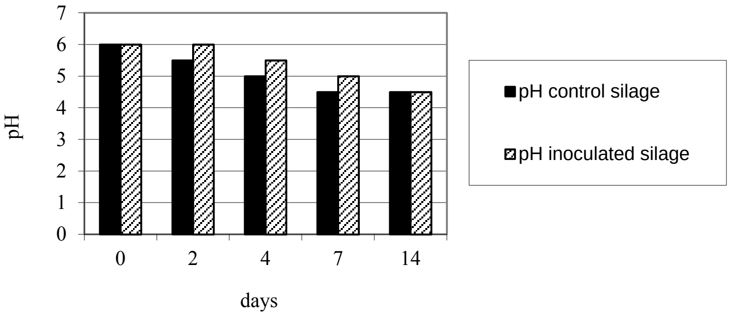

Figure 4. Evolution of pH in alfalfa laboratory silage

Nevertheless, laboratory data shown in Figure 5, indicates higher acidity in inoculated batches then in control (alfalfa and molasses). These findings confirm the buffer capacity of alfalfa: even the organic acids are present (Figure 5), the pH remain high (Figure 4).

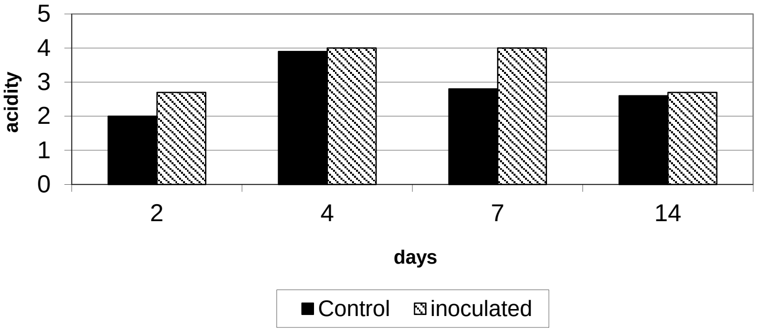

Figure 5. Evolution of acidity in alfalfa laboratory silage

Knowing that alfalfa is a forage that raises difficulties at the ensilage due to the low content in sugars, the results obtained in this experiment are positives. Although the inoculated batches contains less sugar then control because molasses was not added in those batches, observing the parameters of ensilage, the followings can be concluded:

- the growth curve of lactic bacteria is approximately the same and the number is always higher in inoculated batches;
- pH value follows a parallel evolution, after 14 days of storage pH values are equal (4,5);
- acidity is higher in inoculated batches, therefore the nutritive qualities of forage are preserved better than in control.

Results obtained in laboratory sorghum silage

In the second part of our study, the experimental batches are represented by chopped sorghum inoculated with *Lb. plantarum CMIT2* and the control consists in chopped sorghum. Both inoculated batches and control were preserved in anaerobic conditions as described in *material and methods*. The results show that in inoculated batches the number of lactic bacteria is 10% higher than in control (Figure 6).

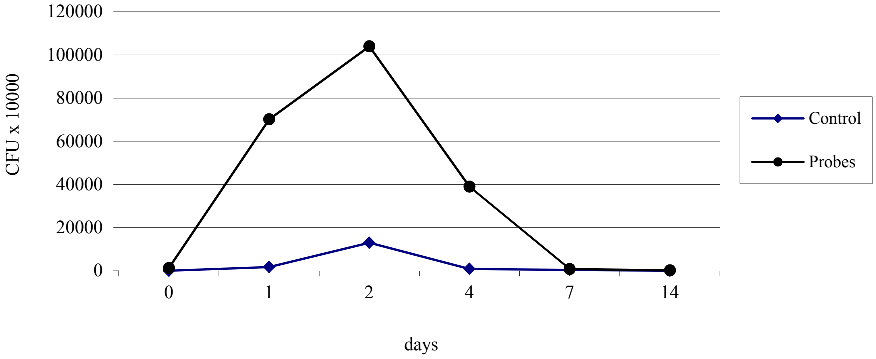

Figure 6. Evolution of lactic bacteria in sorghum laboratory silage.

The evolution of pH show lower values in first four days in inoculated batches (gray columns) and equal values after 14 days of ensilage (Figure 7).

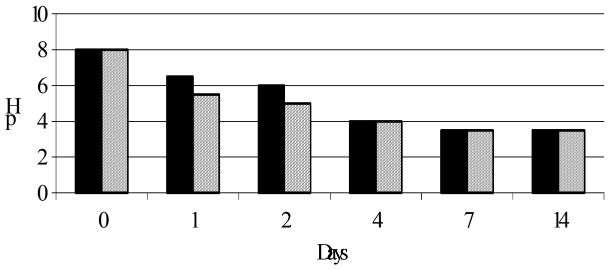

Figure 7. Evolution of pH in sorghum laboratory silage

Regarding the evolution of acidity (Figure 8) in sorghum silage, results leads to the following conclusions:

- the acidity in control increases, reaching the highest value after 14 days;
- the acidity in inoculated batches increases in the first four days and remains constant until the 14th day;
- comparing the evolution of acidity and the lactic bacteria growth curve, we can conclude that a higher number of lactic bacteria (day two) leads to a higher concentration of volatile acids (day four), which leads to the death of lactic bacteria (days 4, 7, 14).

Concluding, a lower pH in the first part of ensilage and a higher acidity leads to a better preservation of nutritive qualities in inoculated silage.

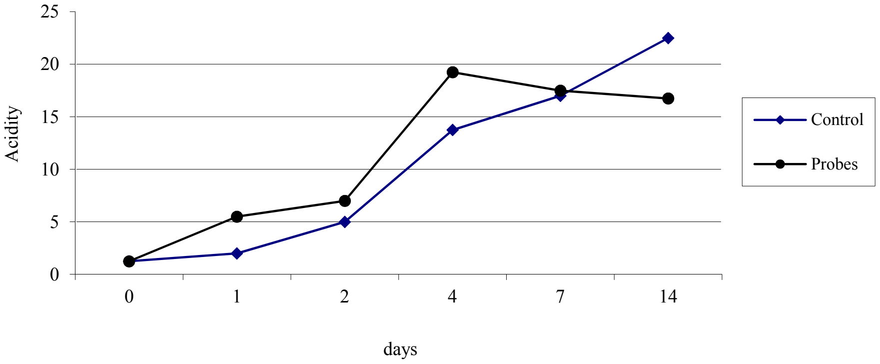

Figure 8. Evolution of acidity in sorghum laboratory silage

The inoculation of the silage promoted proper dynamics of fermentation, at low temperatures, eliminating the risk of caramelizing, phenomenon often met at the ensilage of this kind of forage. At the maturation of the silage, a good fermented forage was obtained, with organoleptic qualities typical to a high-quality silage: pleasant sour odor and flavor, yellow-green color.

Evaluation of the potential

Using data provided by the dairy factory, regarding the quantity of whey available in one year and data obtained in this study, we can calculate the potential production capacity of silage inoculants using sweet whey as feedstock. Data in table 1 indicate that the concentration of lactic bacteria (*Lb. plantarum CMIT2*) in cultures using whey as substrate can reach around 5 * 10^8 cells / ml. Knowing that good inoculating rates applied in large scale silages are around 5 * 10^5 lactic bacteria/ gram forage (fresh mass; SEALE, 1986; BOLSEN et al., 2000; VINTILA et al., 2004; TRULEA et al., 2013), we can calculate that the quantity of LAB culture on whey to be used as silage inoculant is 1 ml of culture for 1 kg forage. We know that the dairy factory generates 4-5 million liters of whey per season. As we can use 1 liter of inoculant to inoculate 1 tone of forage, it can be easily concluded that the whey produced in the dairy factory can be used as feedstock to produce enough inoculant to preserve up to 5 million tons of forage per year.

Conclusions

Sweet whey resulted after milk curding is a valuable substrate to grow LAB and to produce silage inoculants.

The lactic bacteria from the Collection of Industrial Microorganisms Timisoara can be cultivated in affordable and available substrate (whey).

The silage inoculant consisting of *Lb. plantarum CMIT2* cultivated in whey was tested to preserve different types of forages: alfalfa (raises difficulties at the ensilage) and sorghum (high content of sugars).

The obtained inoculant can be applied with positive results for the improvement of preserving conditions in the forages and the improvement of nutritive qualities of ensiled forage.

Sweet whey is recommended as culture medium to produce lactic bacteria inoculant, to initiate lactic fermentation in silage. This method can be applied even in small farms, where milk curdling is made in place, or whey can be obtained for free from dairy processors.

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Influence of various approaches in the use of dry pastures on conservation of biodiversity

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Abstract

Dry Mediterranean karst pastures and hay meadows were created as a consequence of the thousands of years of man's activities, and as such represent an economic, biological and cultural value. The preservation of dry Mediterranean karst pastures and meadows is rendered more difficult primarily due to the reduced intensity of livestock production, insufficient grazing pressure, abandonment of the traditional methods of cattle breeding and cessation of harvesting. In the preservation programme for the biodiversity of dry Mediterranean karst pastures, it is necessary to use a polyvalent approach to grazing and harvesting. The use of animals in the preservation of the biodiversity of dry Mediterranean karst pastures is indispensable. It is desirable to use different species of domestic animals alongside optimal grazing pressure. Considering the nature and feeding potential of karst pastures, sheep, goat and cattle should be given preference in the maintenance of pastures. Autochthonous breeds are more efficient in the use and maintenance of biodiversity of dry Mediterranean karst pastures. Therefore, in selecting of breeds to be used in the maintenance of Mediterranean pastures, preference should be given to autochthonous breeds.

Introduction

Around the world, approximately 3.5 billion ha, which is more than double the size of the cultivable land, are covered in grassland. On the European continent, however, there are fewer grasslands (approx. 230 million ha) and more cultivable land (approx. 300 million ha). In the EU-27, over the past twenty years, around 4 million ha of grasslands have been converted into arable land, mostly to grow maize (CARLIER et al., 2009). Apart from their life sustenance value, grasslands undoubtedly have an agricultural value and purpose as the primary source of feed for domesticated and wild herbivores.

The pastoral vegetation of coastal karst has developed partly in the form of dry Mediterranean grasslands – hay meadows, and partly in the form of expansive rocky pastures. The Slovenian and Istrian karst plateau is a peculiar coastal region. The karst ecosystem is very vulnerable and the management of these resources requires a lot of attention. Since the karst grasslands were used, mainly by manual mowing, for hay and extensive livestock farming for a very long time, the result was the survival of dry grasslands, as well as significant depletion of soil throughout the region. The awareness that the old resource management system is not feasible any more due to lack of adequate labour, from the point of view of environmental protection lead to the development of strategies for sustainable conservation of this region by sheep, goat and cattle farming aimed at conserving the diversity of vegetation and the cultural features of the landscape. The most important functions of grasslands could be summarized into four points: 1) fodder crops as the basis of livestock farming, 2) preservation and conservation of land and water resources; 3) wild plant and animal habitat; and 4) increasing the landscape attraction value. Grasslands can sequester twice as much carbon in the soil in comparison with arable crops (GUO and GIFFORD, 2002; MESTDAGH, 2003). By filtering fertilizers and pesticides, grasslands make an important contribution to the suppression of erosion and regulation of water regime. Finally, grasslands also have an aesthetic role and recreational function, providing humans with an access that is not permitted by other agricultures.

There are several factors, such as mowing, grazing, nutritional ingredient content, influencing the biological diversity of grasslands. Hay mowing is probably the only way to reign in the degradation of grasslands, and if it is not done before the mid-summer, the majority of the meadow plants will grow seed. Later mowing is convenient for some insect species which are food for birds because it provides them with a shelter and possibility to complete their reproductive cycle.

Grazing is the second important factor that affects the biodiversity. The animals with their irregular bite open space for the new plants to sprout, whereas their stomping presses the seed into the ground, improves the soil structure and enhances sprouting. There are great differences among individual animal species when it comes to grazing, so the cattle non-selectively bite off bigger grass sods, while sheep have smaller and more selective bites. Owing to their built, goats can browse on trees and bushes. Differences in the faeces of different animal species also have an impact on the biodiversity and structure of grasslands, so for instance, cattle dislike grazing in the vicinity of their dung, which is large and localized, whereas sheep drop their faeces all over the pasture and do not avoid the surrounding ground. The parts of the grassland around the dung are not tasty to the cattle, and they do not graze there, which is convenient for certain more nitrophilic and other species, to complete their reproductive cycle. Unlike the mowing, in certain types of grasslands, shrubs and woody plants make a significant contribution to biodiversity. Interestingly, the total biodiversity of grasslands that are subject to degradation process is even increased up to a certain extent, due to the resulting share of transitional forms of ecosystem or ecotone. However, once the shrubs and thickets close the meadow, the number of species declines.

Domestic animals on dry karst pastures

There are many reasons why dry karst pastures have been neglected recently, but declining populations of grazing domestic animals have certainly been a significant contributor. The preservation of the dry karst Mediterranean pastures without the adequate domestic animal species and populations, or of the grazing and hay mowing models, is expensive and practically unsustainable. Domestic animals on pastures are the key factor in preservation of the feed value and biodiversity of the habitats of dry karst pastures, as well as in the overall biological diversification of land. In addition, domestic animals on karst pastures represent part of the traditional culture of life and quality of landscape. In order to preserve the landscape quality of karst pastures, they have to be adequately managed, taking into account the tradition and specific features of the climate, and with mandatory presence of domestic animals.

A review of the dry karst grassland management reveals advanced succession process at marginal areas of the grassland in several sites. The necessity of introducing a polyvalent approach in their maintenance (*grazing by different animal species, mowing, clearance of shrub-like and woody plant species from the marginal areas of pastures*), is evident. One of the greatest problems is uncontrolled (free) grazing, where not enough attention is paid to pasture load, optimal start and end of grazing.

Domestic animals are present in the karst pastures of Istria and Primorska region of Slovenia, however, their numbers and composition only partly satisfy the need for prevention of succession and destruction of pastoral areas. Animal management is mainly satisfactory, although rarely there are examples of insufficient care for the animal welfare. In those situations, a mismatch between the environment (*capacity and qualities of the pasture*), animal species and breeds (*mismatch between the animal needs and available feed*) and the farmers' motivation or knowledge to engage in this activity, has been observed.

Generally, we can say that the existing management and animal farming models do not significantly threaten the biodiversity status, and that some forms of human activity practice, more or less favourable to the preservation of biodiversity, have been observed. Such low-intensity management forms are very similar to the traditional forms of management, which preserved the overall biodiversity of this geographical unit in the first place.

Although the majority of the grasslands are to a mild degree subject to progressive succession, which from the point of view of conservation of biodiversity, is a good trend, it should be mentioned that the recent incentive to stimulate the cattle production on the grasslands lead to the clearance of part of the overgrown grasslands, abandoned in the past, signalling the tendency to revitalize the dry rocky grasslands of Istria and the Slovenian region of Primorska, which is very important because by increasing the surface area of such ecosystems, we mitigate the harm and protect their biodiversity.

Improving the feed value of the pasture

Neglecting the pasture leads to its gradual, but continuous degradation. In the Mediterranean karst area, when dry rocky pastures or grasslands are left without anthropogenic influence for longer periods of time, through the process of succession they turn into thickets (*garrigue, maquis*), and eventually into a holm oak forest. In order to prevent succession processes and improve the neglected pastures one needs to: a) implement the optimal utilization method; b) control undesirable and worthless fodder plant species (*by cutting or by use - grazing by different animal species*); c) reseed pastures with good quality fodder plant species where possible (*on flatter, non-skeletal grasslands*); d) improve soil fertility by fertilization (*stable manure*) on less skeletal grasslands.

The optimal way of using littoral dry pastures is a fundamental prerequisite for the implementation of any other method of improving their primary productivity and fodder value. The implementation of only the optimal method of utilizing a certain pasture in order to improve its productivity or fodder value requires a longer period of time.

In order to use dry grasslands optimally, one should take into account: a) the use of an adequate number and species of domestic animals, in order to use the existing pasture vegetation as rationally as possible. Recommended grazing is by two or more domestic animal species. Joint grazing by domestic animals can be implemented at the same time or in a different time period, if one domestic animal species does not endanger the other; b) the distribution of domestic animals on the whole area of the pasture should be as even as possible for all parts of the pasture to have equal grazing pressure, which is achieved through different grazing systems, a more even distribution of watering places, locations for animal salt and additional fodder, placement of additional fences etc.; c) the grazing period, and especially the beginning of grazing, is a very important factor in optimal pasture utilization.

Thus if grazing takes place on moist soil in early spring, at a time when the sward is most sensitive, it can cause significant damage to the pasture. Moreover, if full grazing capacity is applied in the spring, when the vegetation growth has not yet been completed, there will be certain damage to the pasture, and its productivity and nutritional value will not match the nutritional needs of domestic animals. Hence grazing on the pasture should by all means be avoided before the sward has reached its full development.

With regard to species, in preservation models for Mediterranean dry karst pasture and forest habitats it is advisable to use several species of domestic animals (*sheep, goats, cattle, asses, horses*). Sheep and goats have traditionally been the most numerous domestic animal species in the area of the karst Mediterranean zone. This is primarily due to the fact that goats and sheep can use more efficiently the feeding potential of karst pasture and forest plant communities. ROGOŠIĆ (2000) suggests that goats are more successful in controlling the dynamics of growth of ground vegetation than sheep and cattle, and are more successful in consuming woody species. He states that a goat's annual ration consists of 60% shrub species, 30% grasses and 10% herbaceous species. The share of grasses in the feed of goats can on occasion be increased to over 80% of the ration, however the share of herbaceous species very rarely accounts for more than 20% of the ration. The same author suggests that goats prefer grasses during the spring and shrubs in other seasons.

By using the appropriate ratio of the species and number of domestic animals in the herd, it is definitely possible to maintain a favourable botanical composition of the pasture, with a high share of good quality (desirable) pasture species. The number of domestic animals grazing should be equal to or even slightly less than the grazing capacity. Exceeding the grazing capacity could cause damage to the plant cover and soil structure, eventually resulting in reduced pasture productivity from year to year. Therefore, it is especially important during more arid years to reduce the number of domestic animals per pasture area unit, or to feed domestic animals additionally with feed produced in the field. In this way, the degradation of natural pastures can be avoided to a large extent.

The appropriate management and use of pastures preserve the diversity of plant communities and quality of sward, and the vegetation potential is used in line with its growth dynamics. If the standards of adequate management are deviated from, cases of excessive or insufficient use of pasture occur. Pastures with excessive grazing bear clearly visible traces of low-grazed sward that is sometimes completely bare, and frequently the weeds that animals normally avoid have been used to a significant extent. This reduces the pasture capacity, its biological vigor, and causes the loss of desirable plant communities of grasses, which give way to undesirable weed plant communities, and occasionally also causes soil erosion. Domestic animals that graze on such pastures are generally of poor condition, especially if they are breeds that have greater nutritional needs (breeds of larger frame, dairy animals). As opposed to excessive grazing, there is also insufficient pasture grazing, which is visible by the merely superficially used pasture sward, especially of more palatable species. ROGOŠIĆ (2000) states that for optimal utilization of pasture areas, the recommended height of pasture sward is 30 to 35 cm for tall grasses, 15 to 20 cm for medium-tall grasses, and 5 to 8 cm for low grasses.

Autochthonous breeds can greatly contribute to the management of the landscape and of the environment at large. The wish to preserve the environment is in line with the aspiration to preserve the cultural and historical aspects of rural life, including traditional livestock farming, and breeds adapted to the local environment are suitable for grazing in harsh and rough grass surfaces, moors and other marginal agricultural areas. Experience and observations suggest that autochthonous breeds make better use of vegetation than allochthonous ones, precisely due to their high level of adaptability.

Conclusions and recommendations

Dry Mediterranean karst pastures and hay meadows were created as a consequence of the thousands of years of man's activities, and as such represent an economic, biological and cultural value. Therefore, they need to be preserved in their original form as an economic potential in the production of food, and as a unique value of the region's landscape.

It is necessary to publicly affirm livestock breeding production in these areas, support farmers in practicing traditional and introducing new methods of grazing, engage in a marketing promotion of Mediterranean production systems and of the products themselves as foodstuffs of unique nutritional and gastronomic value.

In the preservation programme for the biodiversity of dry Mediterranean karst pastures, it is necessary to use a polyvalent approach to grazing and harvesting (*of areas in which harvesting is possible*). Harvesting, no less than twice a year, reduces significantly or prevents the process of pasture succession.

The use of domestic animals in the preservation of the biodiversity of dry Mediterranean karst pastures is indispensable. It is desirable to use different species of domestic animals alongside optimal grazing pressure. Considering the nature and feeding potential of karst pastures, sheep, goat and cattle should be given preference in the maintenance of pastures.

Autochthonous breeds of domestic animals are more efficient in the use and maintenance of biodiversity of dry Mediterranean karst pastures. This is due to the adaptability of breeds to the area from which they originated. Therefore, in selecting domestic animal breeds to be used in the maintenance of Mediterranean pastures, preference should be given to autochthonous breeds of domestic animals

Dry Mediterranean karst pastures and meadows are habitats to numerous fauna species (*birds, reptiles and insects*), some of which are rare and endangered. The maintenance of their habitats, dry Mediterranean karst pastures, provides them with a living space for survival. Hence, dry Mediterranean karst pastures should be preserved in order to preserve the unique fauna of the area.

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