

A methodology for assessment of the risk status of the breeds in Republic of Bulgaria

Vasil Nikolov^{1*} and Zhivko Duchevev²

¹Agricultural University, 4000 Plovdiv, Bulgaria

²Agricultural Academy, Institute of Animal Science, 2232 Kostinbrod, Bulgaria

*Corresponding author: vsn3480@abv.bg

Abstract

Nikolov, V. & Duchevev, Zh. (2022). A methodology for assessment of the risk status of the breeds in Republic of Bulgaria. *Bulg. J. Agric. Sci.*, 28 (Supplement 1), 5–13

The assessment of the risk of loss of livestock genetic resources is an integral part of their monitoring. Within this study, a first attempt to develop a methodology for estimation of the breeds' risk status in Bulgaria was made. The methodology combines worldwide-accepted measurable criteria – population size and trend, time to double the population, effective population size, with indicators, describing the socio-economic conditions in the country, and subjective indicators like the economic importance of the breed for the region and the country. According to this methodology each local breed is classified as endangered if the number of breeding females or the expected number of females after two generation intervals, or the effective population size are below the respective thresholds for the species.

Keywords: genetic resources; animal breeding; risk

Introduction

The local breeds are part of the global biodiversity and important element in providing nutrition for the population. Their importance for the food security and the sustainable agriculture is widely recognized, also in Goal 2 of the Sustainable Development Goals (United Nations, 2015) of the United Nations (UN), where Indicator 2.5.2, measuring the achievement of Target 2.5 of this goal, is “Proportion of local breeds classified as being at risk of extinction” (United Nations, 2021).

In the recent decades, the risk of loss of breeds was studied by many scientists and organizations, resulting in various criteria for estimation of the risk status.

On the global level the Food and Agriculture Organization of the UN (FAO) uses a simplified criteria, based on three parameters (FAO, 2013):

- Population numerical scarcity:
 - ✓ Number of breeding females;

- ✓ Reproductive capacity;
- ✓ Demographic trend;
- ✓ Percentage of females being bred to males of the same breed.
- Rate of inbreeding:
 - ✓ Total number of breeding males.
- Presence of conservation programmes.

Each breed is assigned to one of the following six categories: “Extinct”, “Cryoconserved only”, “Critical” (including subcategory “Critical-maintained” for breed included in conservation programmes), “Endangered” (including subcategory “Endangered-maintained”), “Vulnerable”, “Not at risk”. In addition, if there is not enough data to estimate the risk status, the breed is assigned to “Unknown”. When applying these criteria, the livestock species are grouped in two classes – with high and low reproductive capacity, and all species within one class are evaluated by the same threshold values.

Lawrence Alderson (Alderson, 2009; 2010) describes the principal factors of the breeds' extinction risk. The first

factor is the numeric scarcity of the breed, expressed by the number of breeding females, or the number of annually registered young females, which, according to Alderson is a better indicator. He also notes that the estimation of the effective population size is more informative than the number of breeding females, however it also requires more data, or the utilization of molecular methods. The thresholds for the various species must be different, as there are differences in the generation interval, the reproductive capacity and the ratio between male and female animals.

The second factor, according to Alderson is the geographical concentration of the animals. The concentration of most of the population in a small area increases the risk of extinction of the breed in case of disaster, e.g. disease outbreak.

The third factor is the genetic erosion, which can be assessed by molecular methods, or by the rate of inbreeding estimated through the effective population size. The introgression of genes is also stressed as a factor, 2.5% introgression per generation is a reason for increased attention, and 12.5% being critical for the breed composition.

In the proposed by Ducheve et al. (2006) early warning system, they describe criteria based on demographic and genetic components. Each component is separately evaluated and the worst of both grades is taken as final score.

The demographic component is based on the expected, after two generation intervals, number of females in reproductive age ($N^{2GI,f}$). The breeds are classified in three categories:

- Not at risk – $N^{2GI,f} > 1000$
- Endangered – $100 < N^{2GI,f} \leq 1000$
- Critical – $N^{2GI,f} \leq 100$

The genetic component is based on the effective population size, as indicator for the inbreeding rate. For compliance with criteria used by the European Federation of Animal Science (EAAP), the thresholds used are the same as the ones described by Simon (1999).

Verrier et al. (2015), propose a multi-indicator method for assessment of the risk status, based on six indicators. For each breed, the overall score was calculated as the average of the separate indicator scores. The first indicator is the number of the breeding females in the population. When defining the thresholds by species, the reproductive capacity of the respective species is taken into account, using the concept of the minimum time to double the population (DT). This time (DTs) is calculated for each species and then, based on international consensus that a cattle breed is considered endangered when its number of breeding females is under 7500, a threshold for each of the other species was calculated according the following the equation:

$$DT_{females} = 7500 \times \frac{DT_{species}}{DT_{cattle}}$$

The second indicator, used in the estimation of the risk status, is the change in the number of breeding females over the last 5 years (mammals) or generation (poultry). Breeds with decreasing population sizes were allocated positive, species independent, scores for risk.

The third indicator is the percentage of crossbreeding in the population, which causes exclusion of young animals from future breeding of purebred animals. This indicator gets the highest score for risk when the number of crosses is so high, that there are no enough young purebred animals to be used as a replacement of old females.

The effective population size is the five indicator. When there are enough genealogical data (i.e. sufficiently great pedigree depth), the effective population size is estimated by the pedigree data. If this is not the case, the classic equation is used:

$$\frac{1}{N_e} = \frac{1}{4N_m} + \frac{1}{4N_f}$$

This indicator is also uniform across the species, with maximum threshold of 245, representing a loss of 10% genetic diversity in 50 generations, due to genetic drift.

The last two indicators describe the breeders' organization and the socio-economic context. For assessing of these indicators expert evaluation of a system of sub-indicators are used, e.g. presence of breeders' association, in situ management program, stock in cryobank, presence of technical support, cohesion and collective dynamics of breeders, young livestock farmers start off raising the breed, availability of the breed for sale, markets for products and services, labels used to distinguish products, financial support.

In the European Union, Regulation (EC) 2016/1012 from 08 June 2016, defines "endangered breed" as a "local breed, recognised by a Member State to be endangered, genetically adapted to one or more traditional production systems or environments in that Member State and where the endangered status is scientifically established by a body possessing the necessary skills and knowledge in the area of endangered breeds"

In France, a simplified version of the proposed by Verrier et al. (2015) method for recognition of endangered breeds is used. In this version, for each species the thresholds are based on the first indicator only – the number of breeding females (Table 1). The other five indicators are used to characterize other aspects of breed condition, and if the conditions are considered adverse, the thresholds are increased by 20 percent.

Table 1. Breeding females thresholds for classification of endangered breeds in France

Species	Horse and Donkey	Cattle	Sheep and Goat	Pig	Poultry
Number of breeding females	10000	7500	6000	1000	500

Spain uses national system (National Advisory Committee in Zootechnics, 2011), based on two main criteria: demographic, genetic, and additional parameters for analysing the values close to the thresholds. The demographic criteria includes three parameters number of breeding females, number of breeding males and number of young females registered in herdbooks in the last three years. For each parameter, thresholds are set, below which the breed is considered endangered (Table 2).

The genetic criteria is based on the annual rate of inbreeding computed from the effective population size and the generation interval of the respective species. The threshold of endangerment is set to 1% inbreeding rate and this criteria is used to classify breeds which are not already classified as endangered by the demographic criteria.

If the demographic thresholds are exceeded by no more than 15%, and the breed is not classified as endangered by the genetic criteria, additional parameters for the risk estimation are applied – geographic distribution, population growth trend, number of farms, and presence of enough reproductive material in gene banks. Each of these parameters is graded from 0 to 2 points, and if the total score is at least 4 (i.e. unfavourable environment) the breed is also considered endangered.

The criteria used in Portugal (Carolino et al., 2013) is similar, here the parameters for risk estimation are number of breeding males, number of breeding females, decrease in number of breeding females in the last five years, effective population size, number of varieties/ecotypes, presence of enough reproductive material in gene banks. For each species, thresholds for male and female breeding animals are determined (Table 3).

The estimation of the risk status is a two steps process. If the number of the breeding animals is below the threshold

(Table 3), the breed is considered endangered. When this is not the case, the other four parameters are evaluated at step 2. When two out of the four parameters are not positive for the breed, it is also considered endangered.

There are many local – autochthonous (Hinkovski et al., 1984) and modern breeds, in Bulgaria, most of which are considered endangered (Nikolov, 2013; 2015). Until 2019, various criteria for estimation of the risk status of the breeds were used, based on the existing European or global systems. The current study is the first attempt to develop a national methodology for assessment of the risk status in Bulgaria.

Material and Methods

In the development of the methodology data about the local autochthonous and commercial breeds considered endangered and included for support in Measure 10: “Agroecology and Climate” of the Rural Development Programme (Ministry of Agriculture and Food, 2015): **Cattle**- Bulgarian Grey Cattle (with population size, at the time when the methodology was developed – 3315 breeding females), Iskar Cattle (2321), Rhodope Shorthorn Cattle (5031), Bulgarian Rhodope Cattle (3129), Bulgarian Red Cattle (10), Bulgarian Brown Cattle (899); **Buffalo** – Bulgarian Murrah (9748); **Pig** – East Balkan Swine (1089), Danube White (790); **Horse** – Karakachan Horse (4452), Eastbulgarian Horse (190), Danubian Horse (366), Plevan Horse (76), Bulgarian Heavy Draft Horse(2311); **Sheep** – Karakachan Sheep (11349), Replyana Sheep (5343), Copper-red Shumen Sheep (12642), Local Karnobat Sheep (1232), Breznik Sheep (2732), ElinPelin Sheep (Sofia Sheep)(5474), Kotel Sheep (8224), Duben Sheep (12208), Teteven Sheep (4374), Koprivshitsa Sheep (2790), Patch-Faced Maritza Sheep (7055), White Maritza

Table 2. Thresholds for endangered breeds in Spain

Parameter	Risk status	Horse	Cattle	Sheep, Goat	Pig	Poultry
Number of breeding females		5000	7500	10000	15000	25000
Number of breeding males		100	150	200	300	500
Average annual number purebred females registered in herdbooks in the last 3 years	Critical	45	75	90	105	200

Table 3. Thresholds for endangered breeds in Portugal

Parameter	Cattle	Sheep and Goat	Pig	Horse	Poultry
Number of breeding females	7500	10000	15000	5000	25000
Number of breeding males	150	200	300	100	500

Sheep (642), Local Stara Zagora Sheep (758), Central Stara Planina Sheep (9804), West Stara Planina Sheep (3992), Central Rhodope Sheep (8217), Sakar Sheep (1905), Stara Planina Tsigai (9676), Rhodope Tsigai (3580), North-East Bulgarian Merino Sheep (3789), Thracian Fine-Wool Sheep (192), Karnobat Fine-Wool Sheep (234); **Goat** – Kalofer Long-Haired Goat (4088), Bulgarian Screw-Horn Longhair Goat (2949), Local Longhair Goat (Malashev Type) (1939), Bulgarian White Dairy Goat (9152). Several breeds, which are included in the Regulation № 7 of 2015 – Svishtov Sheep, Strandzha Sheep and Bulgarian Simmental Cattle, are not included in this study as there are no data for existing population, and the Bulgarian Red Cattle is only represented by 10 animals in one farm. Two poultry breeds were also included – Black Shumen Chicken (200) and Stara Zagora Red chicken (300).

The population size and trends are traced for a period of 5 years. For each breed, the average values of the main reproductive traits – age at first parturition, parturition interval, prolificacy, number of weaned offspring number, and annual number of animals removed from the herd are computed for this period.

Data are collected from the respective breeding societies, along with their annual reports, submitted to the Executive Agency Selection and Reproduction in Animal Breeding (EASRAB) – national competent authority within the meaning of Regulation (EC) 2016/1012 of 8 June 2016. When there was data provided by several breeding societies for a single breed, these data was averaged.

Results and Discussion

Methodology proposal

Based on the analysis of the state of national genetic resources, the international experience and taking into account the specificities of conservation of local breeds in Bulgaria, we propose the following methodology for assessment of the risk status of the breeds per species in our country:

Parameters

Accepted worldwide, objective, and measurable criteria:

- Population size, expressed as number of breeding females in reproductive age;
- Trend of the population size;
- Effective population size.

Reference value:

For definition of the thresholds per species, we use a reference value of 7500 breeding females for cattle, adopted by the policy makers in consensus with the scientific com-

munity as the threshold value, below which a cattle breed is considered endangered.

Time to double the population size (DT) – objective criteria, based on the reproductive capacity of the species and the breed. When estimating the DT, the following indicators are taken into account:

- Number of offspring per female per year (Nr) – defined as the number of suckled offspring per breeding female per year (mammals), number of live hatched chicks (poultry). This also accounts for gestation length, parturition interval, prolificacy of the animals;
- Annual number of removed females (%) – percentage of females removed from the herd per year;
- Age at first parturition (years).

The time, needed to double the population size is computed based on the change in number of breeding females presuming most favourable demographic conditions, in the same manner this was done in the study of Verrier et al. (2015). The number of females in reproductive age for each following year is computer by the equation:

$$Nf_{t+1} = \left(1 - \frac{Rf}{100} + \frac{No}{2}\right) \times Nf_t,$$

where Nf_t is the number of females in reproductive age in year t , Nf_{t+1} is the number of females in reproductive age in the next year ($t+1$), Rf – percentage annually removed females, and No – number of offspring per female per year. Assuming a 1:1 ratio between the male and female offspring gives the number of suckled female offspring. In the next year, the number of females is reduced by the number of removed females and increased with the number of female offspring. The percentage of annually removed females and the number of female offspring is considered constant through the years.

With steady trend of increasing females, after k years the population will double in size:

$$Nf_{t+k} \geq 2 \times Nf_t$$

This is the time to double the population DT, i.e. $DT = k$.

When applying this equation, one should take into account that a time is needed for the young females to reach reproductive age, i.e. the equation is not valid in the first years. Thus, the equation should be applied with a delay of number of years equal to the age at first parturition. An example for computing of DT will be demonstrated later in this article, when estimating the DT for the populations in Bulgaria.

Taking into account, that the aim is to define thresholds per species, we propose to count for each indicator the worst value within the species, regardless of the breeds for which they are recorded. This accounts for the reproductive capacity of the most vulnerable breed.

Indicators of the specific situation in the country:

- Geographical concentration;
- Number of farms;
- Average farm size;
- Cryo-conserved reproductive material in the National Genetic Reserve;
- Market for products and services linked with the breed;
- Economic importance for the country;
- Economic importance for the region.

The indicators in this group are scored with 0, 1 or 2 points, 2 points given in most unfavourable situation. Similar to the scoring of the indicators from the previous group, and taking into account that the methodology is for assessing of the risk status per species, for each indicator we use the value of the less-favoured breed. For example, if there is a breed of this species, for which there are no cryo-conserved reproductive material, then the respective species gets 2 points for this indicator. The conditions for scoring the indicators are given in Table 4.

Calculation of basic thresholds

The basic thresholds for the number of females are calculated following Verrier et al. (2015) :

$$N_{females} = 7500 \times \frac{DT_{species}}{DT_{cattle}}$$

where 7500 – reference value for cattle, $DT_{species}$ – time to double the population (females) by the respective species, DT_{cattle} – time to double the population (females) by cattle.

Calculation of maximum (corrected) thresholds

The maximum corrected thresholds for each species are defined as:

$$N_{max} = N_{females} \times \frac{1 - S_{species}}{14},$$

where $S_{species}$ is the total sum per species of all scores of the indicators in the group IV, 14 being the maximal possible sum.

Final maximum thresholds

The values of the maximum thresholds are rounded up to the nearest 500.

National thresholds for risk status of the breeds, by species

Applying the proposed methodology to the breeds' data, the maximum thresholds for the risk status of breeds of the main farm animal species in the Republic of Bulgaria were determined.

- **Calculation of time to double the population:**

The minimal values of the parameters – age at first parturition, number of suckled offspring per female, annually removed females, by species, and calculated on their basis, annual increase of number of females and the minimal time to double the population size (DT) are shown in Table 5.

The calculation of DT can be illustrated by the following examples:

Table 4. Conditions for scoring by indicator

Indicator	Score		
	0 points	1 point	2 points
1.Geographical concentration – 75% of the population within a circle with radius	Above 50 km	Between 25 and 50 km	Below 25 km
2.Number of farms keeping the breed	More than 50	Between 10 and 50	Less than 10
3.Average farm size (number of females in reproductive age)	Less than 15	Between 15 and 150	More than 150
4.Cryo-conserved reproductive material in long-term storage gene banks, (according to FAO criteria)	Enough	Not enough	None
5.Market for products and services linked with the breed	Large demand of specific products and services linked with the breed	Supply of non-specific products and services to the common livestock market	No demand
6.Economic importance of the breed for the country	Can be replaced in most of the country	Can be replaced in small part of the country	Cannot be replaced
7. Economic importance of the breed for the region	Can be replaced in most of the region	Can be replaced in small part of the region	Cannot be replaced

Table 5. Parameters for calculation of the time to double the population

Species	Age at first parturition, years	Suckled offspring (mammals) or born alive (poultry), number per female per year	Annually removed females, %	Annual growth rate of number of females	Minimal time to double the population size (DT, years)
Cattle	2.25	0.75	15	1.225	5
Buffalo	3	0.60	12	1.180	7
Sheep	2	0.95	17	1.305	4
Goat	2	1.10	15	1.400	4
Horse	4	0.70	10	1.250	7
Pig	1.5	4.00	25	2.750	2
Poultry	0.5	40.00	100	20.000	0.5

In cattle, the annual growth rate of number of females is 22.5% (1.225):

$$\left(\frac{15}{100} + \frac{0.75}{2} \right) = 1.225$$

We start to apply the equation for estimating the number of females at the second year after the offspring was born (age at first parturition – 2.25 years), and for the number of females in the first year we use the initial number of females, i.e.): ($Nf_{t+1} = Nf_t$)

$$Nf_{t+2} = 1.225 \times Nf_{t+1} = 1.225 \times Nf_t$$

At the third year the number of females will reach:

$$Nf_{t+3} = 1.225 \times Nf_{t+2} = 1.225 \times 1.225 \times Nf_t = 1.5006 \times Nf_t$$

At the fourth year, the population size will be 1.8383 times the initial one, and on the fifth – 2.25. Thus, the doubling of the population of females in reproductive age will happen at the fifth year.

In the case of horse species, where the annual growth rate of the females is similar, the doubling of the female population will happen two years later, due to the greater age at first parturition.

Table 6. Scores of the indicators in the group IV by species

Indicator	Species						
	Cattle	Buffalo	Sheep	Goat	Horse	Pig	Poultry
1 Geographical concentration – 75% of the population within a circle with radius	2	0	2	2	0	1	2
2 Number of farms keeping the breed	2	0	2	1	1	2	2
3 Average farm size (number of females in reproductive age)	1	1	2	1	0	2	2
4 Cryo-conserved reproductive material in long-term storage gene banks, (according to FAO criteria)	1	1	2	2	2	2	2
5 Market for products and services linked with the breed	1	1	1	2	0	2	2
6 Economic importance of the breed for the country	0	2	0	0	0	2	0
7 Economic importance of the breed for the region	2	0	2	1	0	2	0
Total (correcting) score	9	5	11	9	3	13	10

• Scores of the indicators in the group IV:

The scores for each indicator in the group IV, by species are shown in Table 6.

The scores of the indicators 1 to 4 are objectively determined. In case of cattle, for assigning a score, the Rhodope Shorthorn Cattle and the Bulgarian Brown Cattle breeds were used. In sheep, the model breeds were Local Stara Zagora Sheep, White Maritza Sheep, Local Karnobat Sheep, the fine-wool breeds, however by some indicators most of the breeds are in unfavourable situation. The autochthonous goat breeds are geographically concentrated, most of the population in all three breeds located in one region of South-West Bulgaria. The Danube White is kept in two farms, relative far apart. The population of the East Balkan Swine is also concentrated in two separate regions. The chickens of the both autochthonous breeds are controlled only in flocks by the institutes of the Agricultural academy. The national gene bank and the national genetic reserve contain mostly frozen semen from commercial cattle breeds.

The fifth indicator is a subjective one, heavily dependent on the market situation. Currently, there is a significant supply of pig and poultry products with no real demand. The

products of the other species are offered at the common market in competition with the highly productive commercial breeds. This can be changed by development of unique products from local breeds, geographical indications and traditional specialities (Nikolov, 2015). Taking into account that the autochthonous breeds are reared with less use of cereal feeds, their products are expected to be of higher biological value for humans (Daley et al., 2010; Stanton et al., 2021; Butler et al., 2021), resulting in increased demand.

The last two indicators are related to the economic importance of a given breed for the country or the region, where it is raised. The criteria here is if the breed can be replaced by a similar one, e.g. in case of extinction. Without neglecting the importance of our local breeds and the negative impact their loss will have, we have distinguished two breeds with exceptional importance for our country – Bulgarian Murrah and East Balkan Swine. Bulgarian Murrah is the only preserved from our buffalo breeds, and the only one that is kept in Bulgaria. Its extinction will lead to the closing of the whole buffalo breeding industry in our country, buffalo industry being very promising worldwide. This unique, selected in Bulgaria, breed has its historical value, and it was used as a base for many Latin America populations.

The East Balkan Swine is the only autochthonous pig breed in Bulgaria. With its biological qualities, it is closer to the wild boars than to domesticated breeds and its extinction will be loss not only for the national, but also for the world genetic heritage in the pig breeding.

Many of the local breeds are irreplaceable in the regions, where they are reared and their loss will lead to closing of the sector in the respective region. Looking back at the history, the only breed that can be reared in East Rhodope is Rhodope Shorthorn Cattle; all attempts to rear commercial breeds in the Rhodope Mountain, except Bulgarian Rhodope Cattle failed.

- **Calculation of the thresholds**

The calculated values for the thresholds – basic, corrected and final are shown in Table 7.

The application of thresholds by species, based on a “virtual” breed, combining all the worst cases for the species, simplifies the regular use of the methodology, as there is no need to compute individual thresholds for each breed, although the methodology provides such option. A downside of such approach is the overestimation of the risk status of breeds with higher reproductive capacity, or reared in better conditions.

The chosen criteria for assessment of the risk status gives some priority to the species of economic importance for the country. This is also observed in the systems used in other countries. Thus, in the listed above thresholds for Portugal and Spain, the threshold for pigs is extremely high, taking into account the high reproductive capacity of this species and the potential to double the population in a short time. The threshold used in France for pigs (1000 animals) is 15 times lower than the values in Spain and Portugal. The situation by the horses is reversed, the value in France (10000 animals) is twice as large as the respective values in Spain and Portugal.

In the proposed by us system for calculation of the thresholds, the indicators for the specific conditions in the country have a significant weight, which allows for up to 100% increase of the basic thresholds in the most unfavourable situation. In the methods applied by Spain and France, the additional factors allow for increase of the thresholds with 15-20%, whereas in Portugal a breed might be considered as endangered based on only two of these additional factors.

Most of the used by us indicators are measurable, e.g. geographical concentration, number of farms and are used in other countries. We have chosen the conditions for scoring these indicators, based on international consensus levels, and taking into account the specific situation in Bulgaria. For example, the geographical concentration of a breed is scored based on the aggregation of at least 75% of the population in a circle with radius 50 km (“vulnerable”) and 25 km (“endangered”), as by FAO criteria from 2013. We have added also indicators for economic importance, accounting for the specific value of the breeds and species for the vari-

Table 7. Thresholds for number of females in reproductive age by species

Species	DT _{species}	Basic threshold	Correcting score	Corrected threshold	Final threshold
Cattle	5	7500	9	12321	12500
Buffalo	7	10500	5	14250	14500
Sheep	4	6000	11	10714	11000
Goat	4	6000	9	9857	10000
Horse	7	10500	3	12750	13000
Pig	2	3000	13	5786	6000
Poultry	0.5	750	10	1286	1500

ous regions and the whole country. The main criteria are the possibility to replace the breed (species) in the region or the country, and the negative impact the breed extinction will have on the economy.

Methodology for assessing the risk

The methodology for assessing the risk is based on a 3-step process. A breed is considered endangered if fulfils the requirements of at least one step.

Step 1: Check if the number of breeding females from the breed is below the *Final threshold* for the species.

Step 2: Compute the expected number of breeding females after 2 generation intervals (based on the current number of breeding animals and the population trend in the last 5 years) and check if this number is below the *Basic threshold* for the species.

Step 3: Compute the effective population size (when possible based on pedigree data) and check if it is below 245 (for all species).

These three steps address three sources of threat for the breed. The first step is based on the current state of the population in relation to the chosen thresholds. The second step is intended for capturing a negative trend in the population size, which will lead to numerical scarcity. The application of this step allows for earlier reaction and starting of conservation measures. The third step is aimed at the loss of genetic diversity in otherwise numerous populations, which are threatened by inbreeding depression.

There is a certain overlap between these three steps, e.g. a small breed of 1000 females and 50 males with high inbreeding will be classified as endangered both by Steps 1 and 3. More important for Steps 2 and 3 are the cases specific only for them, e.g. a constantly declining cattle breed of 15000 animals, which, presuming the same trend will count only 5000 animals after two generation intervals.

The steps are ordered by the amount of data, needed for their application. Step 1 requires only data about the number of breeding females in a year, information annually collected by the EASRAB. For Step 2 in addition to the current state, a historic data about the development of the population in the previous years are needed. In Bulgaria, these data are also available in the registry of EASRAB. The required by Step 3 pedigree data is kept in the herdbooks of the breeding societies, however it is not always in electronic format suitable for computation. In such cases, the number of the breeding males and breeding females can be used in the classical Wright's equation:

$$\frac{1}{N_e} = \frac{1}{4N_m} + \frac{1}{4N_f}$$

Conclusion

The proposed methodology combines objective criteria, accepted worldwide with subjective indicators, reflecting the specifics of the country. In a simplified version (Step 1 only) it can be applied with minimum required data – number of breeding females of the breed in a single year, which makes it suitable for regular use.

The methodology is developed by the authors and proposed for approval to the working group, appointed with Regulation №ПД 09-858/14.09.2018. Following the approval and the discussion by the breeding societies, the Methodology was adopted for application with the letter №13-2171/20.06.2019, of the Deputy Minister of the Agriculture, Food and Forestry of Republic of Bulgaria to the Executive Director of EASRAB.

References

- Alderson, L.** (2009). Breeds at risk: Definition and measurement of the factors which determine endangerment. *Livestock Science*, 123 (1), 23-27.
- Alderson, L.** (2010). Breeds at risk: criteria and classification. *Joint ERFP/RBI/RBST workshop summary report*, London, 16–17 February 2010.
- Butler, G., Ali, A.M., Oladokun, S., Wang, J. & Davis, H.** (2021). Forage-fed cattle point the way forward for beef? *Future Foods*, 3, 100012, <https://doi.org/10.1016/j.fufo.2021.100012>
- Carolino, N., Afonso, F. & Calção, S.** (2013). Assessment of the Risk of Extinction of Portuguese Autochthonous Breeds. Office of Planning and Policy. PDR2020: Lisbon, Portugal (Pt).
- Daley, C. A., Abbott, A., Doyle, P. S., Nader, G. A. & Larson, S.** (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal*, 9, 10. <https://doi.org/10.1186/1475-2891-9-10>
- Ducheve, Z., Distl, O. & Groeneveld, E.** (2006). Early warning system for loss of diversity in European livestock breeds. *Arch. Tierz. Dummerstorf*, 49(6), 521-531.
- FAO** (2013). *In vivo* conservation of animal genetic resources. FAO Animal Production and Health Guidelines. No 14, 243. Rome. <https://www.fao.org/3/i3327e/i3327e.pdf>
- Hinkovski, Ts., Makaveev, Ts. & Danchev, Y.** (1984). Local Forms Domestic Animals. *Zemizdat*, Sofia, 154 (Bg).
- Ministry of Agriculture and Food** (2015). Regulation № 7 of 24.02.2015 г. on the implementation of Measure 10: “Agroecology and Climate” of the Rural Development Programme 2014–2020, State Gazette, 16, 27.02.2015 (Bg).
- National Advisory Committee in Zootechnics** (2011). Criteria for the categorization as endangered breed in the Spanish Official Breed Catalogue.
- Nikolov, V. (Ed.)** (2013). Livestock breeds in the Republic of Bulgaria. *EASRAB*, Sofia, 213 (Bg).
- Nikolov, V.** (2015). Review of the specific measures for support of

- the autochthonous breeds in Bulgaria. *Journal of Central European Agriculture*, 16 (2), 38-46.
- Patin, S. & Sabbagh, M.** (2015). Assessing the risk status of livestock breeds: a multi-indicator method applied to 178 French local breeds belonging to ten species. *Anim. Genet. Resour.*, 57, 105–118.
- Simon, D. L.** (1999). European approaches to conservation of farm animal genetic resources. *Animal Genetic Resources Information*, 25, 79–99.
- Stanton, C., Mills, S., Ryan, A., Di Gioia, D. & Ross, R. P.** (2021). Influence of pasture feeding on milk and meat products in terms of human health and product quality. *Irish Journal of Agricultural and Food Research*, 59(2), 292-302.
- United Nations** (2015). Transforming our world: the 2030 agenda for sustainable development. http://www.un.org/ga/search/view_doc.asp?symbol=A/RES/70/1&Lang=E
- United Nations** (2021). Global indicator Framework for the Sustainable Development Goals and targets of the 2030 Agenda for Sustainable Development https://unstats.un.org/sdgs/indicators/Global%20Indicator%20Framework%20after%202021%20refinement_Eng.pdf
- Verrier, E., Audiot, A., Bertrand, C., Chapuis, H., Charvolin, E., Danchin-Burge, C., Danv, S., Gourdine, J. L., Gaultier, P., Guémené, D., Laloë, D., Lenoir, H., Leroy, G., Naves, M., Patin, S. & Sabbagh, M.** (2015). Assessing the risk status of livestock breeds: a multi-indicator method applied to 178 French local breeds belonging to ten species. *Anim. Genet. Resour.*, 57, 105–118.