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Managing genetic diversity in pig populations: implications of optimal contribution selection in the Black Slavonian pig

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ABSTRACT

Implications of optimal contribution selection for litter size in the Black Slavonian pig were analysed using 23,882 litter records from 6764 sows. To obtain population parameters, pedigree analysis was performed for 7367 animals. The average inbreeding coefficient was 2.87%, the average inbreeding rate was 1.96%, and the effective population size was 25.52. The average maximum number of generations traced back was 4.12 and the average number of full generations was 1.17. Selection candidates were chosen using the following criteria: animals had no culling date in the record; animals had sufficient pedigree depth and an estimated breeding value. Using these criteria, 8 boars among 24 and 386 sows among 2028 were defined as selection candidates. Two scenarios were tested. The first scenario was based on the application of the traditional optimal contribution selection with the maximisation of genetic gain and restricting the mean kinship in the offspring accounting also for breeding values. The second scenario was based on minimising inbreeding by restricting the average kinship of the population. In the first scenario, different kinship constraints between candidates resulted in changing the number of selected animals and changed the average breeding values such that the number of selected candidates increased when the kinship constraint was stronger, with a simultaneous decrease in breeding values. The second scenario resulted in increased inbreeding when the additional weight on genetic gain was added. The results of the study showed that the use of optimal contribution selection in Black Slavonian is possible, however, with additional efforts to improve pedigree and data quality.

HIGHLIGHTS

- Genetic diversity analysis and application of optimal contribution selection in the Black Slavonian pig were analysed using pedigree.
- Results indicate a recent loss of genetic variability in the Black Slavonian pig.
- Optimal contribution selection enabled simultaneous genetic improvement and achieving conservation goals.

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Introduction

Sustainable management of autochthonous breeds can be challenging due to conflicting breeding objectives: maintaining genetic diversity and genetically improving economically important traits (Wang et al. 2017). The sustainability of the breed includes the improvement of breed competitiveness with other mostly conventional breeds and the ability of the breed to supply the consumers with products of required characteristics (Wellmann and Bennewitz 2019). Moreover, sustainable pig production has a reduced impact on the environment, and this is highly dependent on production systems. Local breeds of

pigs are adopted to extensive semi-outdoor or outdoor systems, and environmental impacts of these systems may be avoided by controlling amino-acid and crude protein levels in feeds, as these breeds have low nutritional requirements (Monteiro et al. 2019).

Currently, the focus in the management of autochthonous pig breeds is mostly on maintaining genetic diversity, while genetic evaluation is rarely performed with rare exceptions such as the Iberian pig breed (Gourdine et al. 2012; Noguera et al. 2019). Furthermore, the complexity of breeding in such pig populations is increased by the diversity of breeding systems, usually with predominant natural mating.

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Genetic evaluation in such systems can be very challenging when genetic diversity is low and variability in traits of interest is insufficient for effective selection.

Best Linear Unbiased Prediction (BLUP) is a standard method for estimating breeding values in such populations if highly accurate pedigree and data are available and there is a link between the contemporary groups of animals being compared (Kuehn et al. 2007; Škorput et al. 2018). Information on pedigrees of local breeds is sometimes sparse and the use of such information must be taken with caution. In practice, pedigrees occasionally do not meet this requirement and the relatedness between selection candidates might be underestimated due to missing parents resulting in large contributions of candidates with scarce pedigree information. In reality, these candidates might have high genetic relations to the remaining population and contribute to the reduced genetic variability of the population. (Woolliams et al. 2015). The use of molecular information in such a situation might be beneficial. Moreover, the combined use of molecular markers and pedigree is recommended when the available sample size is small and pedigree shallow (Álvarez et al. 2008). However, genotyping capacity in such breeds is usually small and routine genotyping is rarely done.

The use of the BLUP procedure tends to increase the selection of related animals in the population and thus the increased inbreeding compromises long-term genetic improvement. This is particularly important when traits with low heritability, such as fertility, are included in the genetic evaluation (Fernández and Toro 1999). Inbreeding can affect litter size in pigs through reduced conception rates of inbred sows and lower vitality of piglets (Köck et al. 2009).

Optimal contribution selection (OCS) represents a set of procedures that optimise genetic gain and loss of genetic variability in the population being selected (Meuwissen 1997; Grundy et al. 1998). Several published studies provided the theoretical framework for the development of the procedures, and their practical application has been investigated in dairy cows (Sørensen et al. 2008; Kohl et al. 2020) and horses (Hasler et al. 2011; Solé et al. 2013). The application of OCS in pig populations has been tested through simulations (Gourdine et al. 2012; Howard et al. 2018; Kaplon et al. 1991; Zhao et al. 2021).

The Black Slavonian pig, an autochthonous breed from Croatia, is a conserved population with a growing and stable population in recent years without risk of extinction (Food and Agriculture Organisation 2022), providing the conditions for sustainable

management of the breed. Natural mating is the predominant type of mating in the population, resulting in low inter-herd connectedness, high average intra-herd relatedness and low inter-herd relatedness (Škorput et al. 2018). The current breeding programme has been focussed on preserving genetic diversity and no genetic selection, including OCS, was applied to the population.

The aim of this paper was to show the current status of genetic diversity of the Black Slavonian pig using pedigree data and to describe the effects of implementing OCS on genetic diversity parameters and genetic gain for the number of piglets born alive.

Material and methods

Data

Litter data for 6764 sows of Black Slavonian pig farrowed between January 1998 and October 2021 were provided by the Croatian Agency for Agriculture and Food. The data set contained 23,882 records on the number of piglets born alive (NBA). In addition to basic information (sow identification number and information about NBA), the individual farrowing record contained information on the herd, parity (from 1 to 10), service boar, and mating season (year/month interaction). After data quality control, there were 255 seasons, 471 herd and 737 service boar levels. The basic statistics for NBA can be found in Table 1.

Population parameters

The pedigree data file was created based on animals with phenotypes and contained 7367 animals. The basic pedigree structure (Table 2) was determined using CFC software (Sargolzaei et al. 2006).

The reference population was set for animals born between 2016 and 2021. In order to determine the quality and integrity of the pedigree, ENDOG v4.8

Table 1. Descriptive statistics for number of piglets born alive in Black Slavonian pig.

<i>n</i>	Mean	SD	CV	Min.	Max.
23,822	5.97	2.32	38.97%	0	17

CV: coefficient of variation

Table 2. Pedigree structure for Black Slavonian pig.

Item	<i>n</i>
Sows with records	6764
Total number of animals	7367
Number of inbreds	1991
Founders	1775
Non-founders	5592
Sires in total	456
Dams in total	1689

software (Gutiérrez and Goyache 2005) was used. The calculated pedigree quality was evaluated using the average number of maximum generations traced back and the average number of full generations. The same software was used to estimate parameters that describe the population structure: the individual and mean inbreeding coefficient (F), defined as the probability that an individual has two identical alleles by descent and calculated using Meuwissen and Luo (1992) algorithm; the average relatedness coefficient (AR) (Goyache et al. 2003; Gutiérrez et al. 2003), defined as the probability that an allele randomly chosen from the whole population in the pedigree belongs to the animal; inbreeding rate (ΔF), and the effective population size (N_e), defined as the number of breeding animals that would lead to the actual increase in inbreeding if they contributed equally to the next generation and usually calculated as follows:

$$N_e = \frac{1}{2\Delta F}$$

However, because of the low population size and unfavourable pedigree structure, effective population size was computed here by computing the regression coefficient (b) over the number of full generations traced, over the maximum number of generations traced, and over the equivalent complete generations, where the corresponding regression coefficient is considered to be the increase in inbreeding between two generations. Consequently, the effective population size was calculated as proposed by Gutiérrez and Goyache (2005):

$$N_e = \frac{1}{2b}$$

Genetic evaluation

Genetic parameters for NBA were estimated using the following single trait repeatability model:

$$y_{ijklmno} = \mu + S_i + P_j + B_k + H_l + p_{im} + a_{mn} + e_{ijklmno}$$

where effects of mating season ($S_i = 255$), parity ($P_j = 10$), service boar ($B_k = 737$) and herd ($H_l = 471$) were considered as fixed class effects. Permanent environmental (p_{im}) and direct additive genetic effect (a_{mn}) were included in the model as random effects.

The matrix notation of the repeatability model can be written as follows:

$$y = \mathbf{Xb} + \mathbf{Z_p p} + \mathbf{Z_a a} + \mathbf{e}$$

where y is a vector of observations, \mathbf{X} is the incidence matrix, for fixed effects, \mathbf{b} is a vector of unknown parameters for fixed effects, $\mathbf{Z_p}$ and $\mathbf{Z_a}$ are incidence

matrices for permanent environmental and additive genetic effects, \mathbf{p} and \mathbf{a} are the corresponding vectors of parameters for random effects, and vector \mathbf{e} presents residuals. The following covariance structure was assumed:

$$\text{var} \begin{bmatrix} \mathbf{p} \\ \mathbf{a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} I_p \sigma_p^2 & 0 & 0 \\ 0 & \mathbf{A} \sigma_a^2 & 0 \\ 0 & 0 & I_e \sigma_e^2 \end{bmatrix}$$

Where \mathbf{A} is the numerator relationship matrix, while I_p and I_e are identity matrices, and σ_a^2 , σ_p^2 and σ_e^2 are the variances of additive genetic, permanent environmental effects, and residual, respectively. Covariances between random effects were assumed to be zero.

Estimation of the variance components for NBA was based on the residual maximum likelihood method using the VCE-6 software package (Groeneveld et al. 2010). Breeding values for NBA were estimated with the same statistical model.

Selection candidates

To ensure the practicality of the OCS procedures, candidates were selected from the Black Slavonian Pig herdbook based on the following criteria:

- Animals are still alive, as there is no elimination date in the record.
- Animals have sufficient pedigree depth (number of equivalent generations > 2).
- Animals have an estimated breeding value.

The total number of living animals without culling date was 24 boars and 2028 sows.

The OptiSel package (Wellmann 2019), within R programming environment (R Core Team 2020) was used to calculate the completeness of the pedigree in terms of the number of equivalent complete generations (NEG) and mean completeness of the pedigrees of specified individuals within sexes. NEG was calculated as the sum of the proportions of known ancestors of an individual overall traced generations (Maignel et al. 1996) as follows:

$$\sum_{i=1}^{n_i} \frac{1}{2^{g_{ij}}}$$

where n_i is the number of ancestors of an individual j , and g_{ij} is the number of generations between individual j and its ancestor i . In this way, 1/2 is added for each known parent, 1/4 for each known grandparent, 1/8 for each known great grandparent, and so on. The average NEG in the analysed population was 2.02. The

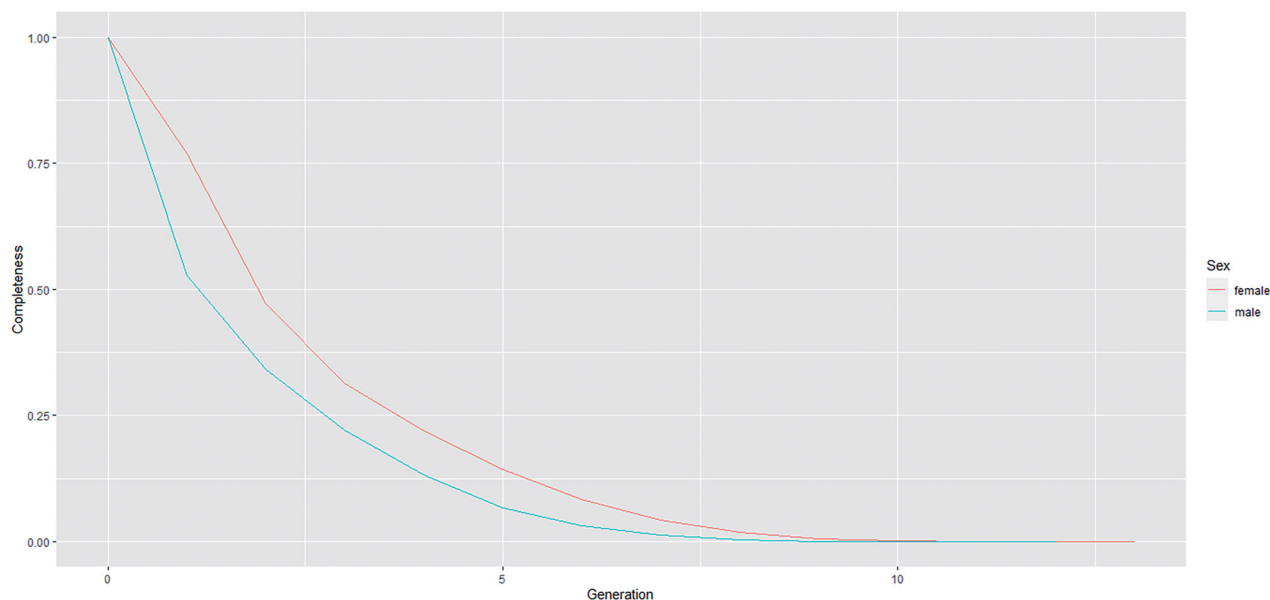


Figure 1. Mean completeness of the pedigrees of specified individuals.

mean completeness of the pedigrees of specified individuals within sexes is shown in Figure 1 and indicated the quick loss of information with every further generation.

Using all criteria, 8 boars among 24 and 386 sows among 2028 were defined as selection candidates. Since gilts and sows of the Black Slavonian pig can have 5–20 piglets per year in one or two parities per year, depending on the intensity of production, the optimisation was performed for both male and female candidates.

Optimal contribution selection

The main goal of the OCS procedure is to obtain individual genetic contributions, which are expressed as vector \mathbf{c} . The genetic contribution of an individual is the proportion of genes originating from this individual to the next generation. Since animals cannot have negative numbers of offspring, the genetic contributions should be positive. The total genetic contribution of each sex should be equal to 0.5 for diploid species (Wang et al. 2017).

Optimal genetic contributions were determined in two different scenarios. The first scenario was to apply traditional optimum contribution selection by maximising the breeding value in the offspring for NBA (Meuwissen 1997) while restricting the mean kinship in the offspring by defining constraints. Constraints were set on the following levels of kinship: 0.01, 0.05 and 0.1. Different levels of constraints on kinship enabled focussing on different breeding goals in the population, balancing between genetic progress and

conservation. The second scenario was to minimise inbreeding by minimising the average kinship of the population accounting also for breeding values since inbreeding arises from the mating of related parents. The optimisation problems were solved using the CCCP solver from the R package OptiSel (Wellmann 2019). The package contains routines for solving cone constrained convex problems using interior-point methods that are partially ported from Python's CVXOPT and based on Nesterov–Todd scaling (Vandenberghe 2010). The solver uses a primal-dual path following algorithms for linear and quadratic cone constrained programming.

Results

Genetic diversity parameters

The average inbreeding coefficient in the population was 2.87% and the average inbreeding rate, which is more useful in predicting the future state of genetic diversity in the population than the coefficient of inbreeding, was 1.96%. The measures of the effective population size of the Black Slavonian pig population estimated from the genealogical data over the number of full generations traced, over the maximum number of generations traced, and over the equivalent complete generations were 85.69, 13.08 and 25.52, respectively (Table 3).

Genetic parameters and breeding values

Genetic parameters are shown in Table 4. Total phenotypic variance as a sum of all estimated variance

Table 3. Genetic diversity in Black Slavonian pig obtained from genealogical information.

Item	Value
Mean maximum number of generations traced back	4.12
Mean number of full generations traced back	1.17
Coefficient of inbreeding (F)	2.87
Average relatedness (AR)	1.81%
Inbreeding rate (ΔF) – NMG	0.58
Inbreeding rate (ΔF) – NCG	3.82
Inbreeding rate (ΔF) – NEG	1.96%
Effective population size (N_e) – NMG	85.69
Effective population size (N_e) – NCG	13.08
Effective population size (N_e) – NEG	25.52

Abbreviations. NMG: number of maximum generation; NCG: number of complete generations; NEG: number of equivalent generations.

Table 4. Genetic parameters for litter size in Black Slavonian pig.

	σ_{ph}^2	σ_p^2	σ_a^2	σ_e^2
Variances	4.0240	0.0165	0.1463	3.8430
Ratio of the phenotypic variance		p^2	h^2	e^2
		0.0041	0.0360	0.9593

σ_{ph}^2 : phenotypic variance; σ_p^2 : permanent environmental variance; σ_a^2 : additive genetic variance; σ_e^2 : residual variance; p^2 : ratio for permanent environmental effect; h^2 : heritability; e^2 : residual.

components was 4.24, whereas permanent environmental variance was negligible, and additive genetic variance was 0.14. The estimated heritability for the number of piglets born alive was 0.04. Estimated breeding values (EBVs) for NBA were expressed as absolute values.

Optimal contribution selection

Table 5 reports the average EBVs, constraints on kinship, inbreeding in the offspring generation, distribution of candidates and genetic contribution of male and female animals obtained by running optimisation algorithm in the first approach considered for obtaining an OCS on selection candidates. Different degrees of upper bound kinship between candidates caused changed different numbers of selected candidates such that the number of selected candidates increased as a constraint on kinship was stronger, with a simultaneous decrease of average EBVs.

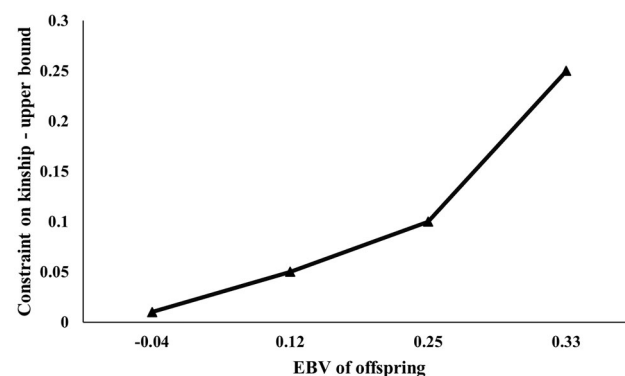
The change in the average EBVs with the change in the upper bound of kinship constraint is shown in Figure 2. Looser constraints on kinship allowed the selection of more related animals with higher EBVs.

Table 6 reports the targeted EBVs, distribution of candidates, inbreeding in the offspring generation and genetic contribution of male and female animals obtained by running optimisation algorithm in the second approach considered for obtaining an OCS on selection candidates. When minimal targeted EBVs were set to 0.00 in order to choose only animals with positive

Table 5. Number of selected breeding pigs, average inbreeding in offspring generation, minimum and maximum male and female contribution under different restrictions on average kinship using traditional OCS.

Upper bound – kinship	No. of selected candidates (M/F)	F	Maximal male contribution	Maximal female contribution
0.01	8 122	0.00	0.09	0.09
0.05	8 35	0.02	0.09	0.05
0.10	6 8	0.02	0.11	0.15
0.25	2 3	0.08	0.12	0.43

OCS: optimal contribution selection.


Figure 2. Relationship between the constraint on kinship (expected inbreeding) and the average EBVs (expected gain) of the next generation for NBA.

EBVs: estimated breeding values.

Table 6. Number of selected breeding pigs, average inbreeding in offspring generation, minimum and maximum male and female contribution under different given EBVs.

EBV	No. of selected candidates (M/F)	F in the offspring generation	Maximal male contribution	Maximal female contribution
0.00	6 104	0.00	0.10	0.02
0.05	8 61	0.01	0.09	0.03
0.10	8 41	0.01	0.09	0.04
0.25	8 8	0.02	0.11	0.15

EBVs: estimated breeding values.

Table 7. Pearson's correlation between EBVs and average kinship among selection candidates.

Number of selected candidates	Pearson correlation
Top 50	0.63
Top 100	0.35

EBVs: estimated breeding values.

breeding values, there was no occurrence of inbreeding in the offspring generation. With the increase of the desired genetic gain, the inbreeding in the offspring increased and the distribution of the candidates changed to select fewer female candidates, but with larger genetic contributions. The number of selected boars increased with the increase of targeted EBVs, with slightly increased maximal genetic contributions.

The correlations between individual EBVs for NBA and the average kinship of an individual with other

individuals in the population were moderate to high, especially for animals with the highest EBVs (Table 7).

Discussion

Population parameters

Pedigree analysis showed reduced values of genetic diversity in the Black Slavonian pig population. The average inbreeding coefficient in the population of 2.87% appeared to be generally high, especially when low pedigree completeness was considered. However, a more meaningful measure of the loss of genetic variability in the population is the inbreeding rate. The values obtained for the inbreeding rate in this study were higher (1.96% over NEG) than the 1% proposed by the Food and Agriculture Organisation of the United Nations (Food and Agriculture Organisation 2000), and the effective population size was lower than 50, what puts the breed at risk in the context of genetic variability of the population and the occurrence of inbreeding depression, as has been reported for similar breeds, such as rare strains of Iberian pig (Saura et al. 2015). Since the pedigree completeness of analysed population indicates a lack of information, the effective population size was calculated by three additional measures, as suggested by Gutiérrez and Goyache (2005). In this way, the state of genetic diversity was characterised by considering the pedigree depth and provided a more reliable estimation of the effective population size by providing upper and lower limits of N_e . This enabled low genetic diversity is characteristic for the majority of local breeds despite conservation efforts, especially those where sustainable utilisation of breed has not yet been achieved (Muñoz et al. 2019). Sustainable utilisation of breeds requires well-established breeding programmes that could help the breed to compete with other breeds by emphasising recognisable traits. Local pig breeds are usually characterised by optimal meat quality and lower productive and reproductive traits (Čandek-Potokar et al. 2019). Thus, establishing breeding programmes with a genetic evaluation of important traits could be helpful in improving productive and reproductive traits. The choice of method for genetic evaluation depends on the availability of resources and the commitment of breeders and their associations to providing reliable data for evaluation. The BLUP procedure might be an appropriate tool for genetic evaluation in such populations, provided that reliable and accurate data are available. However, selection by BLUP tends to select animals with high EBVs at the expense of higher inbreeding rates (Woolliams et al.

2015). The solution to overcoming this problem is to use the procedures within the OCS frame (Meuwissen 1997; Grundy et al. 1998; Woolliams et al. 2015) that control the contribution of selection candidates (Woolliams and Thompson 1994).

Genetic parameters and estimated breeding values

Estimated variance components for Black Slavonian pigs were lower than in similar breeds, such as Iberian pigs (Noguera et al. 2019). Although the reduced phenotypic variance is a common characteristic in local breeds of pigs (Kaplon et al. 1991), a higher phenotypic variance was observed in the analysed population compared to the previous analysis by Škorput et al. (2014), resulting in lower heritability.

Optimal contribution selection

Information on the application of OCS pig selection on field data is scarce. Gourdine et al. (2012) found that the application of OCS is possible for quality traits through simulation data, while Howard et al. (2018) evaluated the application of OCS in commercial pig herds in the combination with genomic information. Zhao et al. (2021) compared the long-term effects of applying conventional conservation and OCS methods on genetic diversity and genetic gain in local pig breeds using stochastic simulations and showed that applying OCS methods resulted in higher genetic gains than conventional methods, especially when combined with genetic information.

To our knowledge, the application of OCS procedures on the field data in Black Slavonian pigs is the first attempt to balance genetic improvement and maintenance of genetic diversity in the local pig population in a practical environment. Practical problems arising from the low pedigree completeness and data accuracy had to be overcome by incorporating criteria for selecting candidates with sufficient genealogical information, as the optimisation process is highly dependent on pedigree quality (Woolliams et al. 2015). Because this study was conducted on a living population with very different organisational and technological levels of development between herds, we encountered some practical problems related to data recording and there is a possibility that some male animals might not be recorded in the pedigree file. To obtain reliable estimates of relationships in the population, which is key information for generating future inbreeding, only animals meeting

pedigree depth criteria were considered. In populations with low pedigree quality, this problem might be overcome by the use of molecular methods (Goyache et al. 2003; Wellmann 2019). This requires genotyping of selection candidates, which might lead to higher expenses for breeders and breeding associations.

Traditional OCS based on maximising breeding values was conducted by imposing constraints on the kinship of selection candidates. As the constraints on the inbreeding rate (lower upper bounds on the kinship) were higher, the proportion of selection candidates available for mating showed higher dispersion of selection candidates. The main reason for this result is a stronger relationship between candidates with high breeding values, which is to be expected since BLUP tends to select more related animals with higher EBVs (Woolliams et al. 1999). Looser constraints on the inbreeding rate (higher upper limits on kinship) allowed a greater number of selected candidates with higher breeding values to be selected and a lower total number of selected candidates. This relationship between EBVs and kinship was confirmed by analysing the correlations between individual breeding values and the average relationship of the individual with other individuals in the population. Thus, the increase in EBVs in offspring can be achieved at the cost of increased inbreeding in the next generation. The maximum contributions of selection candidates increased when kinship constraints were relaxed, which is to be expected because fewer animals were selected in such a scenario.

The second scenario was based on minimising inbreeding by minimising kinship between candidates. Such a system is suitable for breeding schemes where the main breeding goal is to preserve genetic diversity and the importance of genetic gain is the secondary goal (Wellmann 2019). When the desired average EBV in the next generation was omitted and the only goal was to minimise inbreeding, the wider dispersion of candidates with lower contributions was observed. By including the desired EBV in the optimisation, the number of selected candidates decreased, but with higher contributions resulting in an increased average inbreeding coefficient in the next generation. This relationship between genetic gain and contributions was formally described by Woolliams et al. (1999).

The application of OCS for litter size in the Black Slavonian pig population has shown that there is a possibility to balance genetic gain with loss of genetic variability as a cause of improvement. The balance between breeding goals becomes more important

because genetic improvement tends to increase inbreeding in the population as BLUP forces to choose related animals and leads to the occurrence of inbreeding depression, especially in the case of strong selection intensity (Muir 2000). This is important for breed management and optimisation process and decision holders should be focussed on maintaining and increasing effective population size in order to prevent the decline of economically important traits due to inbreeding depression.

In the first analysed scenario, breeding values were maximised with respect to the level of kinship, considering genetic progress as the main breeding goal, while the second scenario was based on minimisation of inbreeding by minimising the average kinship of the population. Since the strong increase in litter size in the Black Slavonian pig is not the main breeding goal in the population, optimisation process can be conducted with higher restrictions on inbreeding rate (kinship), choosing animals with positive breeding values from litter size at the same time. Thus, optimisation process similar to the second scenario might be adequate for the Black Slavonian pig. Several practical problems arise that should be overcome in order to obtain a selection response. As mentioned earlier, the quality of pedigrees should be improved to obtain reliable information on genetic relationships within the population. Moreover, this problem could be alleviated by integrating molecular information which increases the reliability of selection (Zhao et al. 2021). Another problem related to selection using BLUP is that cross-herd comparison is reliable only if there is a link between current herds. Škorput et al. (2018) analysed the connectedness between contemporary units in the population of Black Slavonian pigs, stating that across herd comparison is reliable just for half of the herds. Therefore, genetic evaluation and, in addition, the introduction of the OCS procedure into the evaluation should consider the connectedness between herds. Selection becomes more complex because natural mating is prevalent in the population under study. One way to increase connectedness is to establish artificial insemination centres or to intensify the exchange of live animals.

Conclusions

The results showed that the introduction of OCS for NBA in the Black Slavonian pig population is possible and that optimisation between genetic gain for litter size and preservation of genetic diversity can be achieved in both applied scenarios depending on the

constraint levels (0.01, 0.05, 0.1 on average kinship in the first scenario; targeted EBV from 0.00 to 0.25 in the second scenario). Since the strong increase of NBA in the Black Slavonian pig is not the main breeding goal for the breed, an optimisation process can be conducted with higher restrictions on the inbreeding rate (kinship) simultaneously choosing individuals with positive breeding values.

However, practical problems arising from low pedigree completeness and data accuracy must be overcome by improving pedigree and data quality, as these are key factors for reliable estimation of breeding values and relationship between selection candidates and consequently for controlling inbreeding in the population. Future research will examine the occurrence of inbreeding depression in the Black Slavonian pig population for economically important traits, such as litter size.

Ethical approval

This study did not require manipulation or modification of the usual handling of the animals, since we have worked directly with the records obtained and provided by the Croatian Agency for Agriculture and Food.

Disclosure statement

The authors declare that there is no conflict of interest associated with the paper.

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Data availability statement

All data analysed during this study are included in this published article. The data reported in this study are available from the corresponding author, Dubravko Škorput, Ph.D.

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