The impact of inbreeding on birth weight in the Pag sheep breed

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Abstract

The aim was to estimate the impact of inbreeding on the birth weight (BW) in the population of Pag sheep breed undergoing selection for milk yield. All available pedigree records were used in the estimation of the coefficient of inbreeding (F_{PED}), but only lambs born after the year 2010 with number of equivalent generations ≥ 3 were used in the inferential statistical analysis. Impact of F_{PED} on BW was tested under the 5-way ANCOVA statistical model with gender, litter size, parity, season and flock used as categorical, and F_{PED} as numerical predictor. Although statistically insignificant, the estimated inbreeding depression (-0.001 kg for $\Delta F_{\text{PED}} = 1\%$, P>0.05) indicates that inbreeding should be minimised in order to prevent a genetically influenced decrease of the BW. More evidence is needed to generalize this effect in sheep.

Keywords: Pag sheep, selection, inbreeding depression, birth weight

Introduction

The Pag sheep breed has great economical, traditional, cultural, demographic, and touristic importance for the residents of Croatia. In the last couple of decades, the population size has been estimated to be ~30,000 animals. The breed had a historical importance in wool production, but today, majority of breeders raise this breed for milk production due to high demands for extraordinary expensive Pag sheep cheese (~40 €/kg). The breed has also a significant importance in lamb meat production, since all non-replacement male and female lambs are slaughtered before the age of 45 days and sold as delicacy suckling lamb meat (also sold for the extremely high price (12-15 €/kg)). It is important to note that there is no surplus of the products of this sheep breed on the market, evidencing thus its' tremendous cost effectiveness. Due to the favourable situation on the dairy market, many of the dairy orientated Pag sheep breeders would like to permanently increase the milk production in their flocks via selection, so they enrolled the national selection program. Currently there are 34 flocks with ~4,500 animals undergoing selection based on BLUP genetic evaluation system (HAPIH, 2023). Breeding values for several dairy traits have been predicted under the test-day repeatability animal model (Špehar et al., 2020) and regularly provided to the breeders. More recently, a substantial number of animals in the breeding population have been genotyped with 50K ovine SNP bead chip in order to establish a genomic selection under the framework of the single-step genomic BLUP (Legarra et al., 2009). There is also intention to follow the basic principles of optimum contribution selection (OCS), in order to provide selection gain with minimal loss of genetic variability. In addition to preventing genetic erosion of the breed, the purpose OCS is to avoid unwanted and unpredictable consequences of inbreeding such as incidence of genetically influenced defects and inbreeding depression. Inbreeding can impair growth, milk production, health, fertility, and survival. The inbreeding depression has been reported in many sheep populations for different traits (e.g. Ercanbrack and Knight, 1991, Analla et al., 1998, Dario and Bufano, 2003, van Wyk et al., 2009). By taking into account the fact that inbreeding is inevitable in small populations, especially in the populations undergoing selection due to overuse of genetically superior animals that tend to be genetically related, the genomic OCS seems to be the best possible solution. However, in order to justify and setup this novel and comprehensive selection strategy, some scientific and pragmatic questions need to be answered. Among the numerous specific goals in the series of the studies conducted under the scope of the scientific project OPTI-SHEEP (CSF, IP: 2019-04-3559), the aim of this study was to estimate the impact of inbreeding on the birth weight (BW) in the subpopulation of Pag sheep breed to check if inbreeding can severely impair this important trait related to survival and early lamb growth in extensive outdoor sheep breeding system.

Material and methods

All data used in the analysis were provided by the Croatian Ministry of Agriculture. The original dataset included a total of 281760 animals (rams, ewes, and lambs) born between 1981 and 2019. Number of equivalent generations (NEG) that was used as criterion for retaining animals in the inferential statistical analysis was calculated as the sum of the proportions of known ancestors of an individual over all traced generations as follows: . where was the number of ancestors of individual, and was the number of generations between individual and its ancestor i (Maignel et al., 1996). In this way, 1/2 was added for each known parent, 1/4 for each known grandparent, 1/8 for each known great-grandparent and so on. The coefficient of inbreeding (F_{PED}) , representing the probability that two alleles chosen at random from the maternal and paternal haplotypes are identical by descent was estimated for all animals in the pedigree, but only subset of the animals with NEG \geq 3 and born after year 2010 (n=17,355) were used in the analysis of $F_{\rm PFD}$. This subset of the animals was additionally reduced to 4470 animals with reliably recorded phenotypes (flocks under selection) for purpose of the analysis of the impact of F_{PED} on BW. The pedigree analysis was conducted in the R programming environment (R Core Team, 2020) using package "optiSel" (Wellmann, 2021.). Preparation of phenotypic data, descriptive statistical analysis, and plotting of the results in preliminary analysis were carried out using several R packages such as "data.table" (Dowle and Srinivasan, 2021), "tidyverse" (Wickham et al., 2019), "pastecs" (Grosjean and Ibanez, 2018), "descriptr" (Hebbali, 2020) and "ggplot2" (Wickham, 2016). The inferential statistical analysis was conducted under the 5-way ANCOVA statistical model with gender, litter size, parity, season and flock used as categorical predictors, and F_{PED} as numerical predictor. Linearity of the data (i.e. presence of linear relationship between the predictor and the outcome in the model) and assumption that residual errors had a mean value of zero were tested graphically (Residual vs Fitted plot). The assumption that residual errors had constant variance were tested graphically (Scale-Location plot) and additionally using the Non-Constant Error Variance Test (p = 0.14833). All assumptions were met. Marginal means (a.k.a. LSM) from the above statistical model were estimated using the "emmeans" package (Lenth, 2022) and predictor effect plots using the "effects" package (Fox and Weisberg, 2018).

Results and discussion

The average F_{PED} in this population was estimated to be 6%. Majority of these animals were slaughtered before sexual maturity which (they did not enter the breeding population). The F_{PED} of breeding animals, i.e. animals recorded as sires and dams in the pedigree, was a bit lower ($F_{\text{PED}} = 5\%$) but still above the reports of F_{PED} for Spanish, French and some Italian dairy sheep breeds. The F_{PED} for Latxa Cara Negra from Euskadi, Latxa Cara Rubia, and Latxa Cara Negra from Navare was estimated at approximately 2% for all three breeds (Granado-Tajada et al., 2020). The estimates of F_{PED} for Basco-Béarnaise, Manech Tête Noire, Manech Tête Rousse, and Lacaune Confederation ranged from 2% to 3% (Rodríguez-Ramilo et al., 2019). The F_{PED} for Comisana, Massese, Delle Langhe, and Sarda was estimated at 1.8%, 3.9%, 6.0%, and 9.2%, respectively (Cortellari et al., 2022). The estimated F_{PED} for all categories in the population of Pag sheep falls below 6.25% (acceptable inbreeding level in small populations undergoing selection). The results of inferential statistical analysis obtained with ANCOVA statistical model are summarised in Table 1, Figure 1 and Figure 2. All categorical predictors had statistically significant impact on BW (P<0.001), except the season (P>0.05). The BW increased up to 4th parity, males were heavier than females, and singletons were heavier than twins. The estimated differences between classes of fixed predictors were in general agreement with many previous findings on those effects in sheep (e.g Držaić et al., 2021 in regard to parity, Babar et al., 2004 in regard to litter size and gender).

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Class	Mean	Standard error	Lower confidence interval	Upper confidence interval
Parity (P<0.001)				
1	2.95	0.03	2.90	3.01
2	3.12	0.03	3.07	3.17
3	3.22	0.03	3.17	3.27
4	3.28	0.03	3.22	3.33
5	3.27	0.03	3.22	3.33
6	3.27	0.03	3.21	3.33
7	3.27	0.04	3.20	3.34
8	3.26	0.04	3.18	3.33
9	3.21	0.05	3.12	3.30
Litter size (P<0.001)				
Singletons	3.46	0.02	3.43	3.49
Twins	2.95	0.04	2.88	3.03
Gender (P<0.001)				
Females	3.17	0.02	3.12	3.21
Males	3.25	0.02	3.20	3.29
Season (P>0.05)				
November	3.18	0.03	3.12	3.24
December	3.20	0.02	3.16	3.24
January	3.22	0.02	3.17	3.27
February	3.23	0.04	3.16	3.30

Table 1. Marginal means of the categorical predictors obtained with ANCOVA statistical model

Due to too many classes, the effect of flock on BW was presented graphically (Figure 1.). It can be seen that BW varied quite a lot between the flocks. These huge discrepancies between the flocks were partially influenced by genotype but most probably, by considerably different nutrition (due to different natural vegetation between different areas of the Pag Island and supplemental nutrition in the last third of gestation provided by more conscious breeders).



Figure 1. Estimates of flock effect on BW in the population of Pag sheep breed

The estimated slope of regression of BW on F_{PED} was negative, but it did not significantly differ from the 0 (β_1 = -0.001, P>0.05). According to the obtained results, for every 10% increase in the F_{PED} , it is expected decrease of 0.01 kg in the BW (Figure 2). The obtained results are in general agreement (depression) with some previous reports on this phenomenon in other sheep populations, but they differ significantly in the estimated magnitude of the effect. The inbreeding depression of -0.006 kg, -0.007 kg, and -0.013 kg for $\Delta F_{\text{PED}} = 1\%$ was determined in Elsenburg Dormer sheep breed (van Wyk et al., 2009), Polish Olkuska breed (Drobik and Martyniuk, 2016), and Merino sheep breed (Analla et al., 1998), respectively. As it can be seen from the Figure 2, the confidence interval of the estimated regression slope was pretty wide, especially in the domain of the high values of F_{PFD} suggesting that the estimated line could take any other position in the shaded area. Based on this constellation of data, it is possible that inbreeding depression in this population was underestimated due to the small number of highly inbred animals (76% of animals had F_{PED} below 9%). Some experts in this scientific field argue that negative impact of inbreeding became evident only when F_{PED} exceeds 10%, but those claims have not been supported by firm scientific evidences and practically remain only on the spoken words. However, despite the fact that prevalence of lowly inbred animals makes our study inconclusive to some extent, we are glad that in this Croatian indigenous sheep population do not prevail highly inbred animals. This is a kind of evidence that majority of the breeders in this population take care to avoid very close inbreeding in their flocks, but relatively high overall inbreeding ($F_{PED} = 5-6\%$) still calls for attention. The results from this study related to inbreeding depression on BW support previous findings on this issue in sheep to some extent, but in order to be able to generalize this effect in sheep, more research need to be done. Inclusion of genomic data in the future studies in this population is expected to provide more reliable estimates of the overall inbreeding and inbreeding depression. The genomic information (SNP markers across the whole genome) will cancel out pedigree errors (common in outdoor raised and bred sheep populations), and provide realised (genomic based) relationship between animals under concern. Based on these results, we can argue that inbreeding in this population needs to be minimised at least in order to prevent genetically influenced decline in BW. In addition, other unpredictable unwanted side effects connected with inbreeding are additional reason to lower inbreeding in this small population undergoing selection on milk yield. In order to keep inbreeding as low as possible, more systematic approach in future breeding activities needs to be applied. According to the opinion of the experts in this scientific field, the most appropriate strategy would be the OCS (Meuwissen, 2009), preferably under the framework of genomic selection.



Figure 2. Estimate of inbreeding depression in the population of Pag sheep breed (blue areas below and above the regression line represent confidence interval)

Conclusion

These are first insights into direction and magnitude of impact of inbreeding on birth weight in the Pag sheep breed, which implicate presence of inbreeding depression, but more evidence is needed to generalise this effect. Future analysis on this phenomenon should include genome-wide genetic markers (SNPs) which will cancel out potential pedigree errors and should be conducted under the framework of animal genetic model, preferably using the single step GBLUP. In order to keep inbreeding as low as possible, and yet provide selection gain on milk yield, the optimum contribution selection should be implemented in practice. This selection strategy should guarantee selection gain with minimal loss of genetic variability, which is very important for long term viability of the breed (preservation of the "plasticity" of the genome which can respond to unpredictable changes of the environmental effects in the future).

Note

This study is extension of the study conducted by Bartol Smutni (final thesis, Bachelor's degree)

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