



## Can Inbreeding Increase Viability? A Case Study on An Infectious Outbreak in An Inbred Pigeon Flock

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### ABSTRACT

Inbreeding increases homozygosity, resulting in a loss of alleles and lower genetic variation than the parental population. In a small population, this low genetic variation and homozygosity can quickly lead to adverse effects. This study was conducted on a flock of pigeons with 32 adult pigeons. A disease characterized by dyspnea, anorexia, vomiting and sudden death has occurred in birds. Mortality and morbidity were recorded during the outbreak. The inbreeding coefficient was determined in the flock, with pedigree records going back 12 years. Although not significant, the inbreeding coefficient of dead birds are lower than that of symptomatic or healthy birds. It is also seen that there is no statistical significance in terms of inbreeding depression in morbidity or mortality status. The result indicated that inbreeding depression in morbidity and mortality was not observed in this particular disease case.

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### Introduction

Hobby pigeon breeders usually have small and confined pigeon flocks and accordingly, inbred matings are often practiced. Pigeon houses in in Türkiye are generally small, poorly ventilated, and have a high population density (Erdem, 2012; Aslan et al., 2015). Therefore, pathogens in the environment are more likely to cause disease.

When fungal infections in pigeons are examined, candidias, aspergillosis, macrorhabdus ornithogaster infections stand out. Aspergillosis, is distinguished from other infections by the symptom of respiratory distress (Rupiper, 1998; Wages, 1987; Pollock, 2003; Hoppes 2024). Aspergillosis is a rare non-contagious fungal infection in birds, caused by *Aspergillus flavus*, *A. niger*, *A. nidulans*, and *A. terreus* but much less frequently than *A. fumigatus* (Friend et al., 1999; Akan et al., 2002; Arné et al., 2011). This respiratory system disease occurs in acute or chronic forms and is an opportunistic infection that worsens with lowered immunity (Beernaert et al., 2008). Aspergillosis particularly affects young birds (Tokarzewski et al., 2007; Arné et al., 2011). Symptoms of

aspergillosis include dyspnea, weakness and fatigue, vomiting, anorexia, excessive water drinking, and sudden death (Shoukat et al., 2018).

Due to inbreeding, homozygosity increases, leading to a loss of alleles and lower genetic variation than the parental population (Shikano et al., 2001). Inbreeding can have negative effects on fitness traits in particular. (Nestor, et al., 1973; Price & Schluter, 1991). These effects are referred to as inbreeding depression. In a small population, the low genetic variation and homozygosity can rapidly result in deleterious effects, known as inbreeding depression, which may be caused by the combination of lethal or semi-lethal recessive alleles. As inbreeding increases, “deterioration” in some traits, particularly viability, in the population is to be expected. (Charlesworth & Willis, 2009; Justinski et al., 2023).

This case study examined the impact of inbreeding on the viability of a pigeon flock afflicted by an emerging infection.

## Materials and Methods

This study was conducted on 32 Baska pigeons (the oldest birds were hatched in 2019) currently in the flock bred by the authors. The pedigree of these 32 pigeons goes back 12 years. Since 12 years, the pigeons were fitted with a numbered foot ring at the age of 1 week and the parentage was recorded. When these records are analysed, it is seen that a total of 198 pigeons constitute the pedigree. Due to the small and closed structure of the population, matings were made with distant relatives as much as possible. For this reason, the inbreeding coefficient for the next generation was calculated before each mating and matings that would cause a high inbreeding coefficient were avoided. From time to time, new birds were added to the flock to prevent an increase in the inbreeding coefficient. This flock of 32 pigeons was raised in a pigeon house of 2×2×1.5 m. This pigeon house had a ventilation window (60×40 cm) and a 150m<sup>3</sup>/h ventilation fan. Baska pigeons are a locally common breed raised in the Aegean and Marmara regions of Türkiye. The Baska pigeon is a diving breed with a short beak and small body. Its primary wing feather and head up to the neck are white (Figure 1).



Figure 1. Baska pigeons

In the pigeon house, under normal conditions of care and feeding, a disease suspected to be a bacterial infection of the upper respiratory tract occurred. Dyspnea, anorexia, vomiting, and sudden death were observed in the pigeons. Initially, the birds were treated with antibiotics for 5 days (Gentadur® 10%, solution for injection, Bavet), but no results were obtained. Later, it was determined that the symptoms were similar to the case report by Aslan et al. (2015) and overlapped with aspergillosis. Therefore, a medication containing nystatin (Fungostatin®, oral suspension 100,000 IU/ml, Nobel) was used for treatment according to the recommendations of Aslan et al. (2015). After treatment, symptoms improved in the flock.

During the outbreak, mortality and morbidity were recorded. In the flock, whose pedigree records go back 12 years, the inbreeding coefficient was determined (Figure 2) with the SAS INBREED procedure (SAS,1999). The coefficient of relationship shown below, as introduced by Wright (1922), is determined between two individuals by adding the values for each line connecting them to a common ancestor.

$$R_{ij} = \frac{\sum_{i=1}^1 \left[ \frac{1}{2} \right]^{n_i+n_j} \cdot [1+F_A]}{\sqrt{[1+F_i][1+F_j]}} \quad (1)$$

Where  $R_{ij}$  is the relationship coefficient between individual  $i$  and  $j$ ,  $n_i$  is the generation line from individual  $i$  to the common ancestor,  $n_j$  is the generation line from individual  $j$  to the common ancestor and  $F_A$ ,  $F_i$  and  $F_j$  are the inbreeding coefficients of ancestor, individual  $i$  and  $j$ , respectively.

The estimated inbreeding coefficient for an individual in this relationship is half of the relationship coefficient between the parents,

$$F = \sum_{i=1}^1 \left[ \frac{1}{2} \right]^{n_1+n_2+1} \cdot [1 + F_A].$$

This probabilistic linear Mendelian approach provides the proportion of shared genes between two related individuals who have common ancestors.

Inbreeding coefficients were analyzed for morbidity and mortality using the following linear model (Equation 2 and 3):

$$Y_{ijk} = s_i + a_j + s_i * a_j + e_{ijk} \quad (2)$$

$$Y_{ijk} = s_i + b_j + s_i * b_j + e_{ijk} \quad (3)$$

$Y$  is the inbreeding coefficient,  $s$  is sex,  $a$  is morbidity (symptomatic, healthy),  $b$  is mortality (dead, alive) and  $e$  is error due to chance. A further analysis was conducted using the generalized estimating equation method under the binomial distribution with the categorization of inbreeding coefficients ( $<12.5$  or  $\geq 12.5$ ). In this analysis, the model (Equation 4) used separately for morbidity and mortality ( $Y_{ijk}$ ) included sex ( $s$ ) and the categorized inbreeding coefficient ( $c$ ).

$$Y_{ijk} = s_i + c_j + s_i * c_j + e_{ijk} \quad (4)$$

The odds ratios ( $\Psi=e^b$ ) were calculated using Euler's number ( $e$ ) and estimated values ( $b$ ) obtained Eq.3. The first and second equations were used to determine whether the inbreeding coefficient caused a difference in terms of ill – not ill and death - alive. The third equation was used to explain how the low or high inbreeding coefficient affects mortality and morbidity.

## Results

In the flock subject of the study, 13 out of 32 pigeons showed symptoms and 7 of them died. Table 1 summarizes the mean, maximum, and minimum values of the inbreeding coefficient in terms of disease status. It is seen that the average inbreeding coefficient for the 12-year pedigree of 198 pigeons increases over the years (Figure 2). Inbreeding coefficients vary from 0% to 25% within the flock (Table 1). Pigeons with a 0% inbreeding coefficient are birds (three birds) that are included to add blood refreshment to the flock. Excluding these three birds, the lowest inbreeding coefficient was 6.25%.

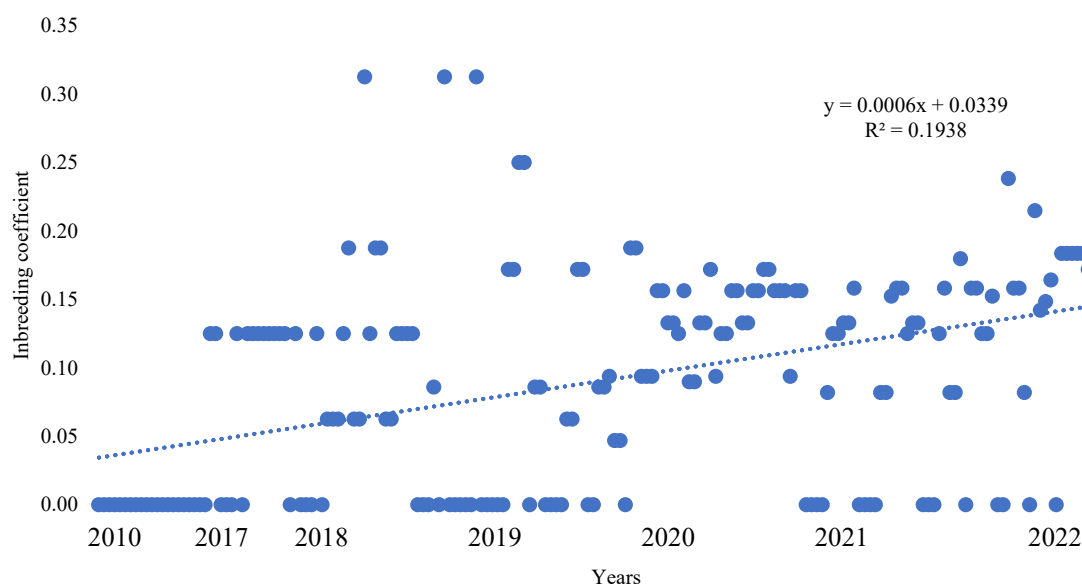


Figure 2. Inbreeding coefficients all of the birds beginning with the first recorded bird

Table 1. In the current flock, means (X), standard deviation (SD), minimum and maximum values of inbreeding coefficient by morbidity and mortality status, (%)

	N	X	SD	Minimum	Maximum
S <sup>-</sup>	19	12.24	6.47	0	25
S <sup>+</sup> D <sup>-</sup>	6	11.39	3.52	8.2	15.63
D <sup>+</sup>	7	8.62	6.86	0	15.82
Flock	32	11.22	6.18	0	25

S<sup>-</sup>: no symptoms; S<sup>+</sup>D<sup>-</sup>: birds that have symptoms but alive; D<sup>+</sup>: death.

Table 2. Least square means ( $\bar{X}$ ), standard error (SE) and P values of inbreeding coefficient by morbidity and mortality status, (%)

	N	$\bar{X}$	SE	P
S <sup>-</sup>	19	12.35	1.459	Sex
S <sup>+</sup>	13	9.90	1.790	Morbidity
				Sex X Morbidity
D <sup>-</sup>	25	12.17	1.195	Sex
D <sup>+</sup>	7	7.27	2.482	Mortality
				Sex X Mortality

S<sup>-</sup>: no symptoms; S<sup>+</sup>: have symptoms, D<sup>-</sup>: alive; D<sup>+</sup>: death

Table 3. Estimates (b), their standard errors (SE), odds ratios ( $\Psi$ ), and P values of categorized inbreeding coefficient by morbidity and mortality status

	Factor	b	SE	$\Psi$	P
For morbidity	F<12.5	0.83	0.76	2.29	Sex
	F≥12.5	0	0	1	Inbreeding coefficient (F)
For mortality	F<12.5	0.69	0.89	2.00	Sex
	F≥12.5	0	0	1	Inbreeding coefficient (F)

The inbreeding coefficients are observed to vary mainly in the range of 10% to 20% (Figure 2). In Table 2, the least square means and significance levels are presented for morbidity and mortality. Although not significant, the average inbreeding coefficient of dead birds (7.27%) is lower than that of symptomatic (9.90%) or healthy birds (12.35%). However, the P-value of the mortality effect is remarkable (P=0.0859).

The risk of inbreeding coefficient in morbidity and mortality are presented in Table 3. P values indicate no statistically significant influence in terms of morbidity and mortality. However, looking only at the odds ratios, an

inbreeding coefficient of less than 12.5% increases the risk of disease or death by a factor of about two compared to an inbreeding coefficient higher than 12.5%.

### Discussion

The average inbreeding coefficient increases in the offspring over the years and the highest value was 0.31 (Figure 2). However, this value was not observed again in the previous and following years. In the current flock, the average inbreeding coefficient was 0.11 and the maximum value was 0.25.

As widely recognized, inbreeding results in an increased homozygosity rate. In highly inbred populations, allelic losses relative to the parental population are observed (Shikano et al., 2001). Inbreeding can lead to negative effects by the combination of recessive semi-lethal or lethal alleles present in the population. This is particularly noteworthy for small populations, where the average inbreeding coefficient can rise rapidly. In particular, traits with low heritability, e.g. reproduction, viability and resistance to diseases and parasites, are more susceptible to inbreeding depression. (Sittmann et al., 1966; Calleri et al., 2006; Norberg & Sørensen 2007; Selvaggi et al., 2010; Patiabadi et al., 2016; Smallbone et al., 2016). However, in the case presented here, it is observed that there was no inbreeding depression in terms of susceptibility to the infection (Table 2 and 3). On the other hand, in rare instances known as "purging," the viability and reproductive features may improve due to the removal of semi-lethal and lethal recessive (or dominant) alleles in the inbreeding population (Gulisija & Crow, 2007; Savaş, 2009). It is therefore possible that a purging-like phenomenon may have developed in this case. It may be the case that pigeons with low viability and low immune system capability may have died at the embryo or growth stage.

On the other hand, statistical evaluations based on the phenotype, which is classified as dead or not dead as a result of the individual's viability, should be approached with caution. Both the observation methods and the statistical methods used can affect the results. Especially, data collected from uncontrolled environments, such as field observations, may not adequately account for variation because they are based on point-in-time observations. Furuya et al. (2014) addressed this issue in the following manner: in a study investigating the effectiveness of a vaccine (X) administered for a viral disease, no statistical difference in survival was observed between the vaccinated and non-vaccinated groups as a result of the data obtained from daily observations. When the same statistical analysis was performed on hourly observation data, a statistical difference between the groups in terms of survival was observed. However, all animals died on the 10th day. In another experiment under the same conditions, another vaccine (Y) was used for the same disease and all vaccinated mice survived. The difference between vaccinated and unvaccinated groups was statistically significant in terms of mortality. As can be seen, even if there is a statistical difference in the mentioned first experiment (where data is evaluated hourly), it does not make a biological sense. In the present study, although the records obtained during the disease as "died" and "survived" are categorical, the viability is actually a quantitative character that shows continuity. In addition, the results obtained should be approached with caution due to the small sample size. Especially in cases resulting in death, the biological meaning of the results obtained is important. Even if there is a difference in life span, this result holds no biological significance because death has occurred.

As seen in this study, the increase in the inbreeding coefficient did not have a negative effect on the population in terms of the traits considered (Table 2 and 3). Therefore, it is possible to say that inbreeding depression was not observed for the traits considered in this study. However, it

is questionable what the situation is for other traits, such as embryo losses. Considering that inbreeding depression is particularly evident in fitness traits such as reproduction and viability (Charlesworth & Charlesworth, 1987; Sewalem et al., 1999; Gallardo et al., 2004; Trask et al., 2021), it is important to consider whether there has been genetic purging due to problems in reproduction (embryo losses) and viability (juvenile mortality) as a result of inbreeding depression. As reported by Erdem and Savaş (2002), although inbreeding had a positive effect on hatching weight in quails, its negative effects on embryo losses were seen. Therefore, it is thought that inbreeding depression may have had a negative effect on the embryo stage and these pigeons may have been eliminated from the population.

## Conclusion

Although the inbreeding coefficient for mortality and morbidity is not statistically significant in the results of the study, the observed differences may still be of biological significance. It should be noted that the study population is limited, which may affect the P-value given the dependence on the sample size. Consequently, no inbreeding depression is observed with respect to the analysed traits. Nevertheless, the possibility that inbreeding may lead to genetic purification of certain traits should be considered.

## Declarations

### Author Contribution Statement

*Hakan Erdem:* Data collection, concept, design, statistical analyses, literature search, writing, review and editing.

*Türker Savaş:* Concept, design, statistical analyses, literature search, writing, review and editing.

### Conflict of Interest

The authors declare no conflict of interest

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