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Original study

Assessment of the effect of heterosis on semen parameters of two-breed crosses of Duroc, Hampshire and Pietrain boars

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Abstract

The study was performed on 4 609 ejaculates obtained from 32 boars, including 14 pure-bred and 18 two-breed crosses. The ejaculates were collected manually every 4-5 days. The study involved ejaculates collected from each boar from the first day of its insemination use until the day of semen depletion. Each ejaculate was assessed for the following physical parameters: ejaculate volume, sperm concentration, percentage of spermatozoa with correct motility, total sperm count per ejaculate and number of insemination doses obtained from one ejaculate. The effect of heterosis was calculated on the physical parameters of the boar crosses ejaculates in relation to the mean value of a given parameter for the parent breeds. Positive and well pronounced heterosis effects were identified in the majority of the physical ejaculate parameters of the cross boars for all the cross-breeding variants. A negative effect of heterosis was only identified in the ejaculate sperm concentration of the Hampshire × Pietrain crosses. The highest ejaculatory efficiency was identified in the hybrids produced on the basis of the Hampshire breed. These breeders were also found to feature significant heterosis effects on ejaculate volume, total ejaculate sperm count and number of insemination doses prepared from one ejaculate.

Keywords: heterosis, semen, boar hybrid

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Introduction

Cross-breeding is a method of using and adapting extraneous genes in a population that is being improved or in production herds in order to take advantage of inter-breed differences and heterosis for increasing hybrid productivity. Assessments of genetic effects on pig reproduction performance are useful when evaluating breed potential and devising efficient cross-breeding systems (Cassady *et al.* 2002). They also represent the prime method of increasing the economic efficiency of pig breeding (Nehring & Staehr 2001, Gjurgji & Sena 2009). Pig cross-breeding programs take advantage of the effect of individual as well as maternal and paternal heterosis. Heterosis is caused by a non-additive (non-summative) effect of genes and corresponds with higher efficiency of hybrids in comparison with the average efficiency of the parents. Hybrid efficiency is sometimes higher than the level attained by the better parent breed in a given parameter. The effect of heterosis may vary in intensity depending on the type of performance traits (Nwakpu & Ugwu 2009). The effect of cross-breeding is generally more prominent in the case of low-heritable traits, i.e. those related with reproduction performance. In boars, the effect may appear in qualitative and quantitative semen characteristics. Two-breed crosses are readily used for reproduction, as they have intensive sexual urge and easily produce ejaculates, which is of particular importance for insemination. They are also generally tougher and more resistant than pure-bred boars (Czarnecki *et al.* 1999). Additionally, the semen of pure-bred boars is usually more vulnerable during transport and storage than hybrid semen (Sonderman & Luebbe 2008). These observations show that ejaculates obtained from cross-bred boars have better quantitative and qualitative parameters as compared with ejaculates of pure-bred boars (Ciereszko *et al.* 2000, Maćkowski *et al.* 2004, Wysokińska & Kondracki 2004, Kmieć *et al.* 2006, Terman *et al.* 2006, Wysokińska *et al.* 2006, Kawęcka *et al.* 2008). This suggests that they can be more economically and efficiently employed in insemination practice. As it turns out, however, not all cross-breeding variants are equally beneficial as regards qualitative ejaculate characteristics. The effect of cross-breeding largely depends on the accuracy of breed selection and appropriate cross-breeding practice. Hybrids usually provide high-quality ejaculates, as evidenced in the effect of heterosis, showing the advantage of crossbreds over pure-bred males (Smital *et al.* 2004, Wysokińska & Kondracki 2004, Smital 2009, Wolf & Smital 2009). Some hybrids also produce ejaculates whose parameters are less advantageous than those of the pure-bred animals (Falkenberg & Ritter 1994, Wierzbicki *et al.* 2010) or intermediary in relation to the ejaculates of the pure-bred boars (Wysokińska *et al.* 2009).

The present work aimed at evaluating the effect of heterosis on semen parameters in two-breed boar hybrids derived from the Duroc, Hampshire and Pietrain breeds.

Material and methods

The study was performed on 4 609 ejaculates obtained from 32 boars, including 14 pure-bred ones (4 Duroc, 5 Hampshire and 5 Pietrain boars) and 18 two-breed crosses (9 Duroc × Pietrain, 5 Hampshire × Pietrain and 4 Hampshire × Duroc boars [Table 1]). The ejaculates were collected manually (King & Macpherson 1973) every 4-5 days. The study involved ejaculates

collected from each boar from the first day of its insemination use until the day of semen depletion. Each ejaculate was assessed for the following physical parameters: ejaculate volume, sperm concentration, percentage of spermatozoa with correct motility, total sperm count per ejaculate, and number of insemination doses obtained from one ejaculate.

Table 1
Number of collected ejaculates

Breed	Number of boars	Number of ejaculates
Hampshire	5	675
Duroc	4	582
Pietrain	5	1 018
Hampshire × Pietrain	5	1 330
Hampshire × Duroc	4	471
Duroc × Pietrain	9	533
Total	32	4 609

Following filtration of the gelatinous fraction, the ejaculate volume was determined on the basis of ejaculate weight measured with electronic scales. The sperm concentration in the ejaculates was determined with a photometric method using a spectrophotometer. The method consists in measuring the intensity of light passing through a suspension of spermatozoa in an isotonic solution of sodium chloride or sodium citrate. The percentage of adequately motile spermatozoa was determined microscopically. Using a zoom of approximately 200-fold magnification, the percentage share of correctly motile spermatozoa in the total number of spermatozoa visible within the scope was determined. The total number of spermatozoa with correct motility and the number of insemination doses obtained from one ejaculate were calculated using SYSTEM SUL v. 6.35 (Gogosystem, Warsaw, Poland) computer program.

The results were statistically processed using analysis of variance according to the following mathematical model:

$$Y_{ij} = \mu + a_i + e_{ij} \tag{1}$$

where: Y_{ij} is the trait value, μ is the population mean, a_i is the boar breed effect and e_{ij} is the error.

Significance of between-group differences was verified by means of Tukey's test at $P \leq 0.01$.

The effect of heterosis on the physical parameters of the boar hybrid ejaculates in relation to the mean value of a given parameter for the parent breeds was calculated as follows:

$$VR = \frac{X_{F1} - X_{MP}}{X_{MP}} \times 100 \tag{2}$$

where VR is the effect of heterosis, X_{F1} is the mean value of a given parameter for the boar crosses, X_{MP} is the mean value of a given parameter for the parent breeds.

Results

Table 2 shows the effects of heterosis on the physical parameters of the ejaculates obtained from the Duroc × Pietrain hybrids, calculated relative to the mean value of the parameter for the Duroc and Pietrain breeds, respectively.

The identified effect of heterosis assumed positive values ranging from 0.49 % for the percentage of progressively motile spermatozoa to 7.43 % for the total ejaculate sperm count. The greatest advantage of the Duroc × Pietrain hybrids over the parent breeds was identified for the total ejaculate sperm count. The heterosis factor calculated for this parameter was the highest, at 7.43 %. The mean number of spermatozoa in the hybrid ejaculates was over 6 bn higher than in the ejaculates of the Duroc and Pietrain pure-bred boars ($P \leq 0.01$). The two-breed Duroc × Pietrain hybrid males revealed intermediary values for ejaculate sperm concentration and sperm motility as compared with the parent breeds, Duroc and Pietrain. The effect of heterosis on these parameters was positive. However, it did not exceed 1 %. The cross-breds produced over 30 ml more voluminous ejaculates than the Duroc boars. On the other hand, the hybrid ejaculates had a 15 ml lower volume than the Pietrain ejaculates ($P \leq 0.01$). The ejaculates of the hybrids and pure-bred Pietrain boars served to prepare approximately 24 insemination doses, significantly more than in the case of the Duroc ejaculates ($P \leq 0.01$).

Table 2
The effect of heterosis on the semen parameters of the Duroc × Pietrain hybrids in comparison with the mean value of the parameter for the parent pure-bred boars (VR)

Item	Mean of the trait (means±SD)			Heterosis effect, %
	Duroc × Pietrain	Duroc	Pietrain	VR
Number of ejaculates	533	582	1 018	
Ejaculate volume, ml	$176.06^{A*}\pm51.93$	$145.87^{B}\pm44.78$	$191.67^{C}\pm57.10$	4.32
Sperm concentration, $\times10^3/mm^3$)	$682.49^{A}\pm196.65$	$754.15^{B}\pm186.87$	$600.13^{C}\pm178.43$	0.79
Percentage of spermatozoa with correct motility, %	$76.09^{A}\pm5.07$	$76.32^{A}\pm5.07$	$75.12^{B}\pm5.61$	0.49
Total sperm count per ejaculate, $\times10^9$)	$89.81^{A}\pm32.93$	$83.80^{B}\pm33.22$	$83.41^{B}\pm28.52$	7.43
Number of insemination doses	$23.85^{A}\pm7.20$	$22.69^{B}\pm7.74$	$24.48^{A}\pm7.08$	1.15

*Different superscripts mean significant differences among means within particular rows; upper-case letters: $P \leq 0.01$.

Table 3 contains efficiency indices for the cross-breeding of the Hampshire and Pietrain boars relating to the physical parameters of the ejaculates collected from the hybrids. The data in Table 3 show a significant advantage of the Hampshire × Pietrain hybrids over the parent breeds in most of the semen parameters. A very high and positive heterosis effect was identified for ejaculate volume, total ejaculate sperm count and number of insemination doses obtained from an ejaculate. The two-breed hybrids were found to have over 70 ml more voluminous ejaculates than the Hampshire boars and even over 110 ml greater ejaculate volumes than the Pietrain boars ($P \leq 0.01$). Additionally, the cross-bred boars had much better values of the total ejaculate sperm count and number of insemination doses than the parent breeds. The ejaculate sperm concentration of the Hampshire × Pietrain hybrids was more similar to that of the Hampshire boars, as opposed to the Pietrain males, which suggests a stronger influence of the Hampshire breed on the level of this parameter in the hybrids. The hybrid ejaculates were found to have a greater sperm concentration than the Hampshire ejaculates – roughly by 21 thousand/mm^3. On the other hand, the sperm concentration of the hybrid ejaculates was lower than that of the Pietrain boars by approximately 87 thousand/mm^3 ($P \leq 0.01$). The heterosis effect for this parameter was negative, at −6.42 %.

Table 3
The effect of heterosis on the semen parameters of the Hampshire × Pietrain hybrids in comparison with the mean value of the parameter for the parent pure-bred boars (VR)

Item	Mean of the trait (means±SD)			Heterosis effect, %
	Hampshire × Pietrain	Hampshire	Pietrain	VR
Number of ejaculates	1 330	675	1 018	
Ejaculate volume, ml	303.90^{A*}±125.55	229.24^{B}±80.80	191.67^{C}±57.10	44.40
Sperm concentration, ×10^3/mm^3)	513.21^{A}±193.56	492.24^{B}±136.72	600.13^{C}±178.43	−6.42
Percentage of spermatozoa with correct motility, %	74.43^{A}±4.98	71.36^{B}±4.10	75.12^{C}±5.61	1.60
Total sperm count per ejaculate, ×10^9)	105.05^{A}±37.43	77.66^{B}±27.02	83.41^{C}±28.52	30.45
Number of insemination doses	29.14^{A}±8.32	23.02^{B}±6.97	24.48^{C}±7.08	22.69

*Different superscripts mean significant differences among means within particular rows; upper-case letters: $P \leq 0.01$.

Table 4 shows a juxtaposition of the effect of heterosis on the semen parameters of the Hampshire × Duroc hybrids in relation to the mean value of this parameter for the parent breeds. The greatest advantage of the Hampshire × Duroc hybrids over the parent breeds

was found for the total ejaculate sperm count and the number of insemination doses prepared from one ejaculate. The heterosis indices for these parameters were high, 36.11 % and 24.90 % respectively. Positive and significant heterosis effects were also identified for ejaculate volume and ejaculate sperm concentration.

Table 4
The effect of heterosis on the semen parameters of the Hampshire × Duroc hybrids in comparison with the mean value of the parameter for the parent pure-bred boars (VR)

Item	Mean of the trait (means±SD)			Heterosis effect, %
	Hampshire × Duroc	Hampshire	Duroc	VR
Number of ejaculates	471	675	582	
Ejaculate volume, ml	216.73^{A*}±74.78	229.24^{B}±80.80	145.87^{C}±44.78	15.56
Sperm concentration, $\times 10^3/mm^3$)	675.64^{A}±156.26	492.24^{B}±136.72	754.15C	8.42
Percentage of spermatozoa with correct motility, %	76.94^{A}±4.61	71.36^{B}±4.10	76.32^{C}±5.07	4.20
Total sperm count per ejaculate, $\times 10^9$)	109.88^{A}±40.04	77.66^{B}±27.02	83.80^{C}±33.22	36.11
Number of insemination doses	28.54^{A}±8.46	23.02^{B}±6.97	22.69^{B}±7.74	24.90

*Different superscripts mean significant differences among means within particular rows; upper-case letters: $P \leq 0.01$.

Figure 1 shows the effects of heterosis on the ejaculate parameters of the hybrid boars produced in three cross-breeding variants, measured relative to the mean value of a given parameter for the parent breeds. Positive and well pronounced heterosis effects were identified in the majority of the physical ejaculate parameters of the hybrid boars for all the cross-breeding variants. A negative effect of heterosis was only identified in the ejaculate sperm concentration of the Hampshire × Pietrain hybrids. The highest ejaculatory efficiency was identified in the hybrids produced on the basis of the Hampshire breed. These breeders were also found to feature significant heterosis effects on ejaculate volume, total ejaculate sperm count and number of insemination doses prepared from one ejaculate. It seems, therefore, that the Hampshire breed may be particularly apt as a component in two-breed boar hybridisation. Hampshire pigs generally have a high meat content. They are, however, disadvantaged in having the RN-gene that deteriorates the processing value of meat. Therefore, it is more advantageous to use hybrids with a share of this blood, rather than pure-bred Hampshire males.

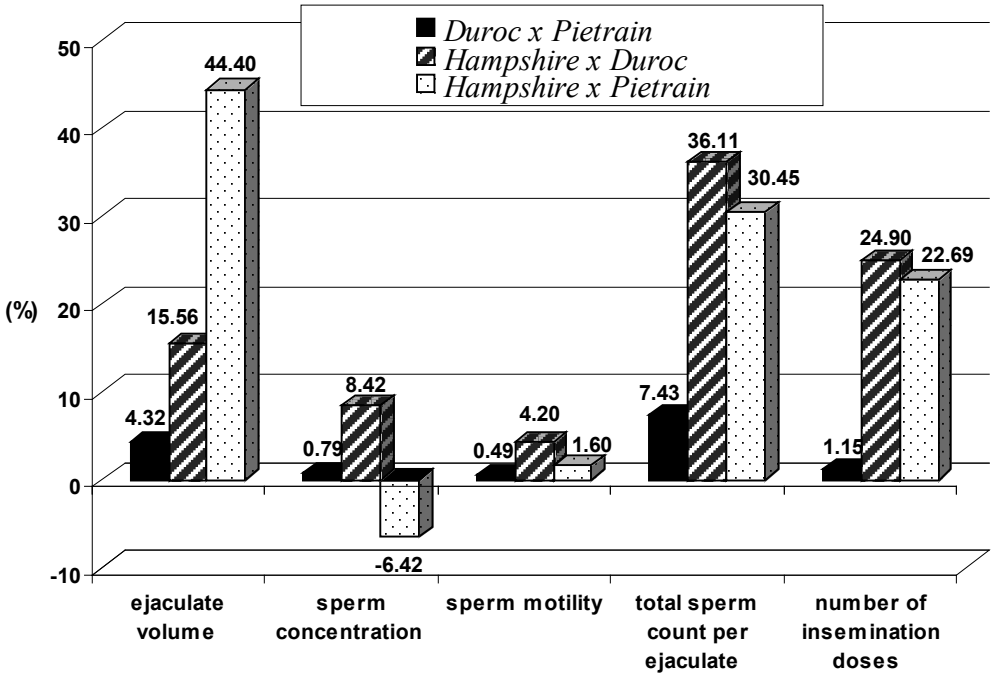

Figure 1
The effect of heterosis on the physical ejaculate parameters of the Duroc × Pietrain, Hampshire × Duroc and Hampshire × Pietrain hybrids

Discussion

The data presented in this study show that hybrids produced with the participation of the Hampshire breed provide ejaculates with the best quantitative and qualitative parameters. Such ejaculates usually contain the highest number of progressively motile spermatozoa. This characteristic is economically crucial, as it is used to determine the number of insemination doses prepared from an ejaculate. One ejaculate of the Hampshire × Duroc and Hampshire × Pietrain hybrids provided an average of roughly 29 insemination doses. The heterosis index for this parameter was high, over 22 % for the hybrids in both the cross-breeding variants. A study by Kondracki *et al.* (2006) shows that one ejaculate of two-breed Hampshire × Pietrain hybrids produces an average of over 5 insemination doses more than an ejaculate of Duroc × Pietrain or Hampshire × Duroc boars. The present study shows that Hampshire × Pietrain hybrids produce the most voluminous ejaculates, as evidenced in the heterosis effect on ejaculate volume, amounting to 44.4 %. Knecht *et al.* (2004) also identified the greatest ejaculate volume in Hampshire × Pietrain hybrids. These crosses, as well as Duroc × Pietrain breeders, were additionally found to be the most efficacious in insemination.

Two-breed Duroc × Pietrain crosses usually produce ejaculates of better quality, i.e. with better sperm motility and a higher total sperm count than pure-bred Duroc and Pietrain boars (Kawęcka *et al.* 2008). Different results were obtained by Wierzbicki *et al.* (2010). The researchers found that pure-bred Duroc, Hampshire and Pietrain breeders had

greater ejaculate volumes and total ejaculate sperm counts than hybrid boars. Additionally, ejaculates of pure-bred males, especially those of Duroc boars, contained spermatozoa with better mobility in comparison with ejaculates of hybrid boars. The present study showed high sperm motility in hybrid boars derived from the Duroc breed as well as in pure-bred Duroc males. Sperm motility is a very important sperm parameter that reflects sperm quality and has a significant effect on egg cell fertilisation. A positive correlation was found between sperm motility and the number of piglets delivered at birth (Falkenberg & Ritter 1994, Popwell & Flowers 2000, Gadea *et al.* 2004, Ruiz-Sanchez *et al.* 2006, Vyt *et al.* 2008). Wysokińska & Kondracki 2004 and Kondracki *et al.* 2003, 2006 and found the percentage of progressively motile spermatozoa in the semen of hybrids to be considerably higher than in the semen of pure-bred boars.

Sonderman & Luebbe (2008), who analysed Duroc × Hampshire crosses and pure-bred boars, found that cross-bred boars were tougher and more resistant to diseases than pure-bred boars. That is why hybrid boars are used longer and their ejaculatory performance is better. According to the above authors, this fact may be somewhat misleading, causing the impression that the hybrids produce more ejaculates than Duroc boars, whereas in fact it is the difference in the period of utilisation that is crucial for ejaculatory efficiency.

The present study identified a positive influence of cross-breeding on the ejaculate parameters of the two-breed boar crosses, as evidenced in the analysed heterosis effects. Positive and very prominent effects of heterosis were determined for the most important ejaculate parameters in the case of all the analysed cross-breeding variants. There are few studies available that present heterosis indices for semen parameters. Smital *et al.* (2004) found positive and prominent effects of heterosis in Hampshire × Pietrain crosses on ejaculate volume (30.6 %) and ejaculate sperm count (18.24 %). Significantly lower but still positive heterosis effects were identified in Duroc × Pietrain hybrids. Slightly different observations were made by Czarnecki *et al.* (1999) as to the effect of Duroc and Pietrain cross-breeding. The scientists identified a positive effect of heterosis on ejaculate volume and a negative heterosis effect on the total ejaculate sperm count (–12.72 %). The results of the present study show that Duroc × Pietrain hybrids produce ejaculates with intermediary values of most of the physical ejaculate parameters in comparison with pure-bred Duroc and Pietrain boars. This is evidenced in the modest, though positive, effects of heterosis. Ejaculates of Duroc boars usually produce fewer insemination doses than ejaculates of other breeds. This is connected with the predisposition of Duroc boars to produce ejaculates with low volumes and high sperm concentration, as confirmed by results of numerous studies (Leidinger *et al.* 1998, Park & Yi 2002, Kondracki 2003, Smital *et al.* 2004, Kondracki *et al.* 2011). Therefore, it is more advantageous to use two-breed reproduction males derived from the Duroc breed, rather than pure-bred Duroc boars.

Summing up, it has to be stated that the heterosis indices revealed a clear advantage of the crossing boars over the pure-bred reproduction males in the case of the most important semen parameters. The greatest advantage of the hybrids over the parent-breed boars was observed for the ejaculates collected from the hybrids derived from the Hampshire breed. The Hampshire × Pietrain and Hampshire × Duroc hybrids displayed high and positive effects of heterosis on ejaculate volume, total ejaculate sperm count and number of insemination doses prepared from one ejaculate.

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