

## HETEROISIS IN REPRODUCTIVE TRAITS OF LANDRACE X LARGE WHITE CROSSBRED SOWS FROM A LOCAL SWINE BREEDING FARM IN THE PHILIPPINES

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### ABSTRACT

Heterosis estimates were determined for reproductive traits using 2,587 litters of 1,256 sows [i.e. 1,088 Landrace (LDR), 1,048 Large White (LRW), 234 F<sub>1</sub> cross (LDR x LRW) and 217 reciprocal R1 cross (LRW x LDR)] produced from January 1, 2017 to June 30, 2018 at a local swine breeding farm. Analysis of litter data showed that reproductive traits in crossbred litters were different than in purebred litters in terms of litter size at birth (LSB, +1.05 piglets), litter size at weaning (LSW, +0.87 piglets), piglet weight at birth (BWt) and at weaning (WWt), both -0.05 kg, number of stillbirths (NSB, -0.22 piglets) and number of mummified piglets (NMP, -0.08 piglets). Heterosis estimates were  $H_{LSB} = 0.33\%$ ,  $H_{LSW} = 9.58\%$ ,  $H_{BWt} = -3.38\%$ ,  $H_{WWt} = -0.57\%$ ,  $H_{NSB} = -48.31\%$  and  $H_{NMP} = -31.06\%$ . Using the repeated measures analysis, crossbred sows had lower age at first farrowing (AFF, -4.5 days) and average farrowing interval (AvFInt, -3.8 days), but higher farrowing index (FI, +0.01 litter), average litter size at weaning (AvLSW, +0.91 piglets) and sow productivity index (SPI, +1.65 piglets per sow per year). Heterosis estimates were  $H_{AFF} = -1.24\%$ ,  $H_{AvFInt} = -2.27\%$ ,  $H_{FI} = 0.61\%$ ,  $H_{AvLSW} = 10.56\%$  and  $H_{SPI} = 8.06\%$ .

Key words: heterosis, reproductive traits, F1 and reciprocal crossbred sows

### INTRODUCTION

Crossbreeding has already been incorporated into most commercial swine breeding programs worldwide for many decades to take advantage of heterosis and breed complementarity (Sellier, 1976), example is to cross lines specialized for different traits (Smith, 1964). In a terminal crossbreeding program, for example, purebred lines or breeds in nucleus breeding herds are selected to produce pigs which are both efficient and lean at market weight, litter size and carcass characteristics. These are used to produce maternal or terminal lines for use in multiplier herds usually associated with one particular purebred breeder or breeding company. The commercial swine producers which contain the vast majority of pigs in the population purchase crossbred or F1 cross females (commonly a two-way cross between Landrace and Large White/Yorkshire) and make the final cross (market hog) between the

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terminal line sires (often a Duroc) and the maternal line gilts and sows. Other breeding schemes had also tried backcrosses (mating F1 to one of the parent breeds), mating of F1's together and results in F2's and composite lines (e.g. 3-breed composite). However, these breeding schemes result in less heterosis than that achieved by an F1.

Heterosis, in which crossbreds outperform the average of the purebred parents, results from the hybrid make-up of the dam (maternal heterosis), the individual or market hog (offspring heterosis) and terminal sire (paternal heterosis). Because of the economic importance of the number of pigs weaned per sow, maternal heterosis is considered the most important as it improves her progenies' chance of conception and survival. Offspring heterosis benefits the individual pig as it affects the pig's growth and survival throughout its life, but mostly after weaning when it is independent of its dam (McLaren *et al.*, 1987). Paternal heterosis may also be important in improving the mating success, such as through libido and conception rate associated with greater testis weight, large ejaculate volume and better semen quality (Buchanan, 1987). Another benefit to this breeding scheme is uniformity of the market hog having the same breed composition (i.e. 25% Landrace, 25% Large White and 50% Duroc).

The advantages of using crossbred sows for the production of pigs for slaughter have been reported extensively covering a wide variety of breeds, but mostly from experiment stations. The single cross between Landrace and Yorkshire was consistently found to be the most precocious, farrowed and weaned the greatest number of pigs per litter and had the heaviest litters at 3 weeks post-farrowing (Fahmy *et al.*, 1975; Holtmann *et al.*, 1975). In the analysis of heterosis reported worldwide, average maternal heterosis mostly benefits the number and weight of pigs born and weaned, ranging from 2.0 to 5.5% (Bondoc *et al.*, 2001; Bondoc, 2008). However, variation among experiments in observed heterosis for specific crosses was large especially for reproduction and sow productivity traits even between crosses of the same breeds, as a result of different strains of a breed crossed and in the testing environments used (Johnson, 1981). As a consequence, it becomes difficult to accurately predict how much heterosis to expect from a crossbred. The average breed effects for purebreds that make up the cross are also more difficult to predict the degree of heterosis in sow reproductive traits (albeit easier for growth and carcass traits), as information on average breed effects are scarce and may be obtained only in central genetic improvement programs operated by local breed associations or global breeding companies or a few private nucleus breeding farms.

In this regard, the objectives of this study were to evaluate heterosis for various sow reproductive traits and to predict crossbred performance of F1 Landrace x Large White cross and reciprocal R1 Landrace x Large White cross in a local swine breeding farm in the Philippines.

## MATERIALS AND METHODS

Data on 2,587 litters born by 1,256 sows between January 1, 2017 and June 30, 2018 were obtained from the International Farm Corporation (INFARMCO) swine breeding farm in Barangay San Isidro, Cabuyao City, Laguna (approx. 14° 14' 49.69" N, 121° 8' 34.41" E). Only records with at least one pig born alive, not more than 8 parity and litters with complete sire and dam identification were used. Age of sow at farrowing was restricted to a range of 307 to 542 days. Records removed for not meeting the above criteria were

about 3% of total pigs. Means and phenotypic standard deviations showed hardly any change due to these restrictions.

The final data set consisted of 1,088, 1,048, 234 and 217 litters born from Landrace, Large White, F1 Landrace x Large White cross and reciprocal (R1) Large White x Landrace cross, respectively (see Table 1). Traits of interest per farrowing record were litter size at birth (LSB) and at weaning (LSW), average piglet weight at birth (BWt) and at weaning (WWt) and number of stillbirths (NSB), mummified piglets (NMP) and age at first farrowing (AFF).

Individual sow's farrowing index (FI) or the number of litters born by a sow per year and sow productivity index (SPI) or the number of piglets weaned by a sow per year were calculated based on average farrowing interval (AvFI), average litter size at weaning (AvLSW) of all litters produced by each sow in one-and-a-half years, i.e.  $FI = 365 \div AvFI$  and  $SPI = AvLSW \times FI$ .

Simple descriptive statistics were initially determined for the various reproductive records for each litter and for each sow using the MEANS procedure of SAS (2009) and are given in Table 2. Similar range of values was reported by Roehe and Kennedy (1995) who analyzed data obtained from the Quebec Record of Performance sow productivity program. The Pearson product-moment correlation coefficients were then computed to measure linear relationships among the litter traits using the CORR procedure of SAS (2009).

The general least squares procedures for unbalanced data were used to examine the principal sources of variation affecting litter records. The following linear statistical model was used to determine, using an F-test, the appropriate model that would best describe each

Table 1. Number and distribution of litters born by different sow breed groups and different parity from January 1, 2017 and June 30, 2018.

Item	Landrace (LDR)	Large White (LRW)	LDR x LRW F1 Cross	LRW x LDR R1 Cross	Total
No. of litters	1,088	1,048	234	217	2,587
No. of sows	542	484	115	115	1,256
No. of sires of sows					190
No. of dams of sows					691
<b>No. of records per parity</b>					
1	303	187	69	64	623
2	228	169	45	40	482
3	199	175	26	22	422
4	126	158	20	16	320
5	101	144	25	32	302
6	62	97	28	22	209
7	43	71	17	17	148
8	26	47	6	4	83

Table 2. Simple descriptive statistics for various reproductive traits (January 1, 2017 to June 30, 2018).

Reproductive Trait	N Litters	Ave. $\pm$ S.D.	Min	Max
Litter size at birth	2,587	10.28 $\pm$ 3.10	1	19
No. of stillbirths	2,587	0.40 $\pm$ 0.82	0	7
No. of mummified piglets	2,587	0.26 $\pm$ 0.74	0	9
Piglet birth weight, kg	2,539	1.50 $\pm$ 0.30	0.46	3.27
Litter size at weaning	2,546	9.21 $\pm$ 2.89	1	18
Piglet weight at weaning, kg	2,465	8.54 $\pm$ 1.59	3.50	16.10
Age at first farrowing, days	623	365.70 $\pm$ 31.2	307	542
	N Sows	Ave. $\pm$ S.D.	Min	Max
Ave. litter size at weaning	1,234	9.08 $\pm$ 2.46	1	16
Ave. farrowing interval, days	978	167.20 $\pm$ 34.9	125	459
Farrowing index	978	2.24 $\pm$ 0.31	0.80	2.92
Sow productivity index	867	20.73 $\pm$ 5.74	2.48	35.78

reproductive trait:  $y_{ijkl} = \mu + B_i + P_j(B_k) + Q_k + e_{ijkl}$ , where  $y_{ijkl}$  represents the litter record (i.e. litter size at birth and at weaning, average piglet weight at birth and at weaning, number of stillbirths and mummified piglets),  $B_i$  represents the effect of the  $i^{\text{th}}$  breed groups (i.e. Landrace, Large White, Landrace x Large White F1 cross and Large White x Landrace R1 reciprocal cross),  $P_j(B_k)$  represents the effect of the  $j^{\text{th}}$  parity nested within the  $i^{\text{th}}$  breed,  $Q_k$  represents the effect of the  $k^{\text{th}}$  date (quarter) of farrowing year (i.e. 1st Quarter - January to March, 2nd Quarter - April to June, 3rd Quarter - July to September and 4th Quarter - October to December 2017, 1st and 2nd Quarter 2018) and  $e_{ijkl}$  is the random error. Age at first farrowing was analyzed using the same statistical model but without the effect of parity nested within a breed.

The repeated measures analysis was used to evaluate 1,256 sows born from 190 sires and 691 dams using general mixed models (SAS Proc GLM) based on the following statistical model:  $y_{ijkl} = \mu + B_i + S_j(B_i) + P_k + (B \times P)_{ik} + e_{ijkl}$ , where  $y_{ijkl}$  represents the litter record (i.e. AvLSW, AvFInt, FI and SPI),  $B_i$  is effect of the  $i^{\text{th}}$  breed group,  $S_j(B_i)$  is effect of the  $j^{\text{th}}$  sow nested within the  $i^{\text{th}}$  breed,  $P_k$  is effect of the  $k^{\text{th}}$  parity,  $(B \times P)_{ik}$  is interaction effect between the  $i^{\text{th}}$  breed and  $k^{\text{th}}$  parity and  $e_{ijkl}$  is the random error. In the repeated measurements analysis, the main interest was in “between sows” and “within sow” effects. “Between sows” effects are those whose values change only from sow to sow and remain the same for all observations on a single sow, for example, breed ( $B_i$ ). “Within sow” effects are those whose values may differ from measurement to measurement, for example, by parity – i.e.  $S_j(B_i)$ . Also, “between sows” and “within sow” interaction – i.e.  $(B \times P)_{ik}$  was determined. Tests of hypothesis for the effects of breed, date (quarter) of farrowing and parity within breeds on the reproductive traits used the type III mean squares for the sow within breeds as the error term.

Heterosis (H) was estimated as the mean crossbred deviation expressed in percentage of mid-parent performance, where crossbred average =  $(\overline{F1} + \overline{R1}) \div 2$  and purebred

average =  $(\overline{\text{LDR}} + \overline{\text{LRW}}) \div 2$ .

Reciprocal or maternal effects were computed as the difference in average F1 and R1 performance, representing differences in the ability of purebred dams to provide an environment for her crossbred offspring to survive and grow, mainly by the dam's milk production and mothering ability. In this study, the advantage of using a Large White or Landrace dam in the production of crossbred sows is equal to  $F1 - R1$  and  $R1 - F1$ , respectively.

## RESULTS AND DISCUSSION

Table 3 shows that LSB is positively correlated with LSW ( $r = +0.88$ ,  $P < 0.01$ ). BWt is also positively associated with WWt ( $r = +0.32$ ,  $P < 0.01$ ). Litter size is negatively correlated ( $P < 0.01$ ) with the weight of the piglet at birth ( $r = -0.28$ ) and at weaning ( $r = -0.29$ ). The number of stillbirths is positively correlated with the number of mummified piglets ( $r = +0.12$ ,  $P < 0.01$ ). NSB is positively correlated with LSB ( $r = 0.11$ ,  $P < 0.01$ ) but negatively correlated with BWt ( $r = -0.10$ ,  $P < 0.01$ ). NMP is negatively correlated ( $P < 0.01$ ) with both LSB ( $r = -0.07$ ) and BWt ( $r = -0.11$ ).

Table 4 shows that litter size at birth and at weaning, number of stillbirths, average litter size at weaning and SPI are significantly different between breed groups ( $P < 0.01$ ). On the other hand, WWt, NMP, AFF, AvFIInt and FI are not significantly different between breed groups ( $P > 0.01$ ). Parity had significant effects ( $P < 0.01$ ) on LSB, LSW, BWt and WWt. The date (quarter) of farrowing also had a significant (environmental) effect ( $P < 0.01$ ) on LSW, BWt, WWt, NSB and NMP.

Landrace litters had slightly bigger LSB and LSW, heavier WWt and significantly ( $P < 0.05$ ) heavier BWt and lesser NSB than Large White litters (Table 5). However, WWt and NMP were not significantly different between Landrace and Large White litters

Table 3. Pearson correlation coefficients among litter traits

	Litter Size at Weaning (LSW)	Piglet Birth Weight (BWt)	Piglet Weaning Weight (WWt)	No. of Stillbirths (NSB)	No. of Mummified Piglets (NMP)
Litter size at birth (LSB)	0.88**	-0.46**	-0.28**	0.11**	-0.07**
Litter size at weaning (LSW)		-0.35**	-0.29**	-0.09**	-0.10**
Piglet birth weight (BWt)			0.32**	-0.10**	-0.11**
Piglet weaning weight (WWt)				ns	ns
No. of stillbirths (NSB)					0.12**

ns - correlation coefficient ( $r$ ) is not significantly different from zero at  $P > 0.05$

\*\* $r$  is significantly different from zero at  $P < 0.01$

Table 4. Mean square F tests for the fixed effects of breed group, parity nested within a breed and quarter of farrowing on various litter and individual sow traits.

Reproductive Traits	Breed Groups	Parity Within a Breed	Quarter of Farrowing	CV (%)
Litter size at birth (LSB)	**	**	ns	29.67
Litter size at weaning (LSW)	**	**	**	30.85
Piglet birth weight, kg (BWt)	**	**	**	18.78
Piglet weight at weaning, kg (WWt)	ns	**	**	17.70
No. of stillbirths (NSB)	**	ns	**	>100
No. of mummified piglets (NMP)	ns	ns	**	>100
Age at first farrowing, days (AFF)	ns	-	ns	8.52
Ave. litter size at weaning (AvLSW)	**	-	-	27.01
Ave. farrowing interval, days (AvFInt)	ns	-	-	20.90
Farrowing index (FI)	ns	-	-	13.68
Sow productivity index (SPI)	**	-	-	27.53

ns - litter and individual sow trait is not significantly affected by the fixed effect independent variable at  $P>0.05$ .

\*\*litter and individual sow trait is highly significantly affected by the fixed effect independent variable at  $P<0.01$ .

( $P>0.05$ ), suggesting the important roles of proper management and environmental factors affecting these traits in purebred sows.

Crossbred (i.e. F1 and R1 crosses) litters had higher LSB and LSW but lower BWt and WWt and lesser NSB and NMP than purebred (i.e. Landrace and Large White) litters. This resulted in higher heterosis values for LSB ( $H_{LSB} = 10.33\%$ ) and LSW ( $H_{LSW} = 9.58\%$ ) and negative heterosis values for BWt ( $H_{BWt} = -3.38\%$ ), WWt ( $H_{WWt} = -0.57\%$ ), NSB ( $H_{NSB} = -48.31\%$ ) and NMP ( $H_{NMP} = -31.06\%$ ). This implies improved performance in crossbred sows of 1.05 and 0.87 more piglets at birth and at weaning, respectively. Piglets from crossbred sows are however lighter by 0.05 kg at birth or at weaning. Crossbred sows had 0.22 fewer stillbirths and 0.08 fewer mummified piglets than purebred sows. Similar results showing the superiority of crossbred animals to purebred animals for heterosis particularly in number of live piglets, vigor of the animals at birth, survival from birth to weaning and litter weight at weaning were summarized by Okoro and Mbajorgu (2017).

Litters produced by R1 (Large White x Landrace) crossbred sows with Landrace dams are predicted to have 0.70 and 0.77 more piglets at birth and at weaning, respectively

Table 5. Estimates of heterosis and reciprocal (maternal) effects for various litter traits.

	Litter Size at Birth (LSB)	Litter Size at Weaning (LSW)	Piglet Birth Weight (BWt), kg	Piglet Weight at Weaning (WWt), kg	No. of Stillbirths (NSB)	No. of Mummified Piglets (NMP)
Landrace (LDR)	10.16 ± 0.13 <sup>b</sup>	9.14 ± 0.51 <sup>bc</sup>	1.61 ± 0.01 <sup>a</sup>	9.33 ± 0.06 <sup>a</sup>	0.42 ± 0.03 <sup>b</sup>	0.26 ± 0.03 <sup>a</sup>
Large White (LRW)	10.08 ± 0.10 <sup>b</sup>	8.99 ± 0.51 <sup>c</sup>	1.46 ± 0.01 <sup>b</sup>	8.54 ± 0.05 <sup>a</sup>	0.50 ± 0.03 <sup>a</sup>	0.23 ± 0.02 <sup>a</sup>
F1 50LDR-50LRW	10.99 ± 0.25 <sup>a</sup>	9.75 ± 0.55 <sup>ab</sup>	1.48 ± 0.02 <sup>b</sup>	8.60 ± 0.13 <sup>a</sup>	0.18 ± 0.07 <sup>c</sup>	0.10 ± 0.06 <sup>a</sup>
R1 50LRW-50LDR	11.34 ± 0.28 <sup>a</sup>	10.13 ± 0.57 <sup>a</sup>	1.49 ± 0.03 <sup>b</sup>	8.47 ± 0.14 <sup>a</sup>	0.30 ± 0.07 <sup>b</sup>	0.24 ± 0.07 <sup>a</sup>
Purebred average	10.12	9.07	1.54	8.59	0.46	0.25
Crossbred average	11.17	9.94	1.49	8.54	0.24	0.17
Heterosis, %	10.33%	9.58%	-3.38%	-0.57%	-48.31%	-31.06%
Maternal effect using LRW dam	-0.35	-0.38	+0.01	+0.13	-0.12	-0.13
Maternal effect using LDR dam	+0.35	+0.38	-0.01	-0.13	+0.12	-0.13
<b>Improvement due to Heterosis (plus reciprocal effect)</b>						
F <sub>1</sub> cross (50-50)	1.05	0.87	-0.05	-0.05	-0.22	-0.08
F <sub>1</sub> 50%LDR x 50%LRW	+0.70	+0.49	+0.06	+0.08	-0.34	-0.21
R1 50%LRW x 50%LDR	+1.40	+1.25	-0.04	-0.17	-0.10	+0.05
<b>Predicted crossbred performance</b>						
F <sub>1</sub> cross (50-50)	11.17	9.94	1.49	8.54	0.24	0.17
F <sub>1</sub> 50%LDR x 50%LRW	10.82	9.55	1.48	8.66	0.12	0.04
R1 50%LRW x 50%LDR	11.52	10.32	1.50	8.41	0.36	0.30

Least square means (and standard error) in the same column with different letter superscripts are significantly different from one another,  $P < 0.01$ .

than litters of F1 (Landrace x Large White) crossbred sows with Large White dams. Litters of R1 crossbred sows also are predicted to have 0.02 kg heavier piglets at birth and 0.25 kg lighter piglets at weaning than litters produced by F1 crossbred sows with Large White dams. However, litters from R1 crossbred sows are predicted to have 0.24 more stillbirths and 0.26 more mummified piglets than litters from F1 crossbred sows.

Table 6 shows that Landrace sows were slightly younger at first farrowing, had similar AvFInt and FI, but higher AvLSW and therefore higher SPI than Large White sows. However, the differences in AFF and the repeated traits between Landrace and Large White litters were not significant ( $P>0.05$ ).

Crossbred sows were slightly younger at first farrowing by 4.5 days, shorter AvFint by 3.8 days, higher FI by 0.01 litter, more AvLSW by 0.91 piglet and higher SPI by 1.65 piglet than purebred sows. This resulted to low negative heterosis values for AFF ( $H_{AFF} = -1.24\%$ ) and AvFInt ( $H_{AvFInt} = -2.27\%$ ) but higher positive heterosis values for FI ( $H_{FI} = 0.61\%$ ), AvLSW ( $H_{AvLSW} = 10.56\%$ ) and SPI ( $H_{SPI} = 8.06\%$ ).

F1 (Landrace x Large White) crossbred sows with Large White dams are predicted to be 11.6 days younger at first farrowing, similar average farrowing interval and 0.06 more litters produced per year than R1 (Large White x Landrace) crossbred sows with Landrace dams. Although F1 crossbred sows are predicted to wean 0.27 less piglets per litter, they are predicted to produce 0.32 more piglets per sow per year than R1 crossbred sows. For a swine breeding farm engaged in the local production of crossbred sows, the small difference in SPI implies equal preference for a Landrace or Large White dam.

In conclusion, heterosis response in crossbred sows was superior to one parent breed only (e.g. BWt, WWt, NMP, FI, SPI) or to both parents (e.g., LSB, LSW, AvLSW, NSB, AFF, AvFInt). The degrees of heterosis reported here may be used as a guide to the choice of breeds for local systematic crossbreeding programs. Recommendations here are not meant to eliminate those breeds which have not been adequately compared under similar farm conditions. Similar on-farm research results (including performance test traits), however, should be updated regularly because the genetic composition of breeds and the frequency of desirable gene combinations do change over time, although the process is quite slow. On the other hand, the choice of breeding system depends on management, pig health and cost considerations, such as the maintenance of own purebreds or the purchase of first cross-breeders and required level of recording.

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Table 6. Estimates of heterosis and reciprocal (maternal) effects for age at first farrowing and repeated reproductive traits of individual sows.

	Age at 1st Farrowing (AFF), days	Ave. Farrowing Interval (AvFint), days	Farrowing Index (FI)	Ave. Litter Size at Weaning (AvLSW)	Sow Productivity Index (SPI)
Landrace (LDR)	365.3 ± 1.8 <sup>a</sup>	167.4 ± 1.7 <sup>a</sup>	2.24 ± 0.02 <sup>a</sup>	9.16 ± 0.11 <sup>ab</sup>	20.77 ± 0.28 <sup>ab</sup>
Large White (LRW)	368.8 ± 2.3 <sup>a</sup>	168.2 ± 1.7 <sup>a</sup>	2.24 ± 0.02 <sup>a</sup>	8.80 ± 0.11 <sup>b</sup>	20.14 ± 0.29 <sup>b</sup>
F1 50LDR-50LRW	359.6 ± 3.8 <sup>a</sup>	164.1 ± 3.8 <sup>a</sup>	2.27 ± 0.04 <sup>a</sup>	9.42 ± 0.23 <sup>a</sup>	22.19 ± 0.63 <sup>a</sup>
R1 50LRW-50LDR	365.4 ± 3.9 <sup>a</sup>	163.8 ± 3.9 <sup>a</sup>	2.27 ± 0.03 <sup>a</sup>	9.56 ± 0.23 <sup>a</sup>	22.03 ± 0.64 <sup>a</sup>
Purebred average	367.0	167.8	2.24	8.58	20.46
Crossbred average	362.5	164.0	2.25	9.49	22.11
Heterosis, %	-1.24%	-2.27%	0.61%	10.56%	8.06%
Maternal effect, using LRW dam	-5.8	+0.4	+0.03	-0.13	0.16
Maternal effect, using LDR dam	+5.8	-0.4	-0.03	+0.13	-0.16
<b>Improvement due to Heterosis (plus reciprocal effect)</b>					
F <sub>1</sub> cross (50-50)	-4.5	-3.8	-0.01	0.91	+1.65
F1 50%LDR x 50%LRW	-10.3	-3.4	+0.04	+0.77	+1.81
R1 50%LRW x 50%LDR	+1.2	-4.2	-0.02	+1.04	+1.49
<b>Predicted crossbred performance</b>					
F <sub>1</sub> cross (50-50)	362.5	164.0	2.25	9.49	22.11
F1 50%LDR x 50%LRW	356.7	164.3	2.28	9.36	22.27
R1 50%LRW x 50%LDR	368.3	163.6	2.22	9.63	21.95

Least square means (and standard error) in the same column with different letter superscripts are significantly different from one another,  $P < 0.01$ .

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