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## Responses of pigs divergently selected for cortisol level or feed efficiency to a challenge diet during growth

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### Summary

Selection for feed efficiency is questioned as reducing the ability of farm animals to face stress and overcome challenges. The hypothalamic-pituitary-adrenocortical (HPA) axis activity, measured by the cortisol level in plasma after ACTH injection, has been proposed as an indicator of robustness. The objectives of this study were to evaluate 1. if a modified cortisol level in pigs alters their feed efficiency and their production performance, 2. if alternative dietary resources would affect these responses. Parallel trials including divergent lines for plasma cortisol 1 hour after ACTH injection and divergent lines for residual feed intake (RFI) were run during growth with a conventional diet and a diet with high fibres, low energy and low protein content. Selection for higher or lower plasma cortisol levels after stimulation of the adrenal gland did not impact growth and feed intake traits, but it had a significant impact on body composition and carcass yield, with improved composition in the high cortisol animals. The two lines had similar responses to an alternative diet, with decreased growth rate and feed intake, and increased feed conversion ratio. On the other hand, lines selected for divergent RFI had different responses to the alternative diet, the more efficient line having a more reduced growth rate with the diet with lower energy and AA contents. However, in terms of FCR it remained more efficient. The initial hypothesis of decreased efficiency associated to increased cortisol was not validated.

*Keywords: pig, dietary fibres, feed efficiency, ACTH, cortisol, genetics*

### Introduction

Selection for more efficiency is often questioned as reducing the ability of farm animals to face stress and overcome challenges (Knap, 2009). The hypothalamic-pituitary-adrenocortical (HPA) axis, via the release of cortisol in blood by the adrenal gland, is one of the main actors in these responses (Mormède & Terenina, 2012). It has thus been proposed as an indicator of robustness. The objective of this study was to evaluate if a genetic difference in HPA axis activity alters feed efficiency and production performance, and to evaluate how these performances would be affected when alternative dietary resources are available. A parallel trial was run under the same conditions on pigs genetically divergent for feed efficiency.

## Material and methods

### Selected lines

From 1999 on, two genetic lines have been divergently selected for residual feed intake during growth (RFI) for 9 generations (G0 to G9) for low (LRFI, more efficient) and high (HRFI, less efficient) RFI, as described in Gilbert et al. (2007). Thirty sires and 30 dams from the French Large White commercial population constituted the base population. In G9, the line difference was 3.84 genetic standard deviations for RFI (Gilbert et al., 2017).

Divergent CORT lines have been selected at INRA for 3 generations (G0 to G3) for low (LCORT) and high (HCORT) plasma cortisol level one hour after a standardized injection of ACTH at 6 weeks of age (Larzul et al., 2018). The selection started from 30 sires and 30 dams from the French Large White commercial population in 2009. After 3 generations, the line difference was about 5 genetic standard deviations for the selection criterion (Larzul et al., 2018).

### Animals and data collection

Sows were inseminated with boars from their own line, 7 to 8 boars per line in the CORT lines and 6 boars per line in the RFI lines. On average seven sows from each line farrowed in two successive farrowing batches. The RFI sows were in two experimental farms. At weaning (28 days of age), all piglets were equally distributed in the two farms for testing: in each farm, the CORT piglets on one hand and the RFI piglets on the other hand were allotted in two post-weaning pens of 24 (12 piglets from each line originating from 6 litters (one female and one castrated male per litter)). At the end of the post-weaning period (10 weeks of age), the post-weaning pens were separated in two growing-finishing pens on the same farm, with 3 females and 3 castrated males from each line, representing all the initial litters. The first half of the pigs was fed a unique conventional diet (**Control**) with 9.7 MJ net energy (NE) and 160 g crude protein (CP)/kg. The second half was fed an alternative unique diet (**Test**) with 10% reduction in net energy and the same ratios of AA/NE and AA/digestible Lysine as the Control diet (8.8 MJ NE and 144 g CP/kg), thanks to the inclusion of feed resources with higher proportions of dietary fibres. Diets came from the same processing batches in the two farms. Animals had free access to feed and water during the test. The heavier half of each pen was slaughtered at 23 weeks of age, the lighter half being slaughtered at 24 weeks of age. Slaughter dates and slaughterhouses were the same for the two farms.

Pigs were weighed at 10 (**BW10w**), 15 (**BW15w**) and 23 (**BW23w**) weeks of age, and 24 weeks of age when slaughtered on the second week. The average daily gain from 10 to 23 weeks (**ADG**) was computed. The growing-finishing pens were equipped with single-place electronic feeders and individual feed intake was available from 13 to 23 weeks for all pigs, the average daily feed intake (**ADFI**) and feed conversion ratio (**FCR**) for this period were evaluated. At 23 weeks of age, ultrasonic backfat measurements were recorded at six locations on the back of the pigs (right and left shoulder, right and left midback, right and left loin) and the average backfat thickness was computed (**BFT**). At slaughter, the lean meat content (**LMC**) of the carcass was computed as a linear combination of muscle and backfat thicknesses (Image meater procedure, Blum *et al.*, 2014), and the carcass weight after 24h of cooling at 4°C was used to compute the carcass yield (**Yield**). A total of 187 pigs were allotted at 10 weeks of age, 181 had records for the growing period, and 171 had records at slaughter.

## Statistical analyses

Data from each trial were analysed separately. Linear models for traits recorded during growth included BW10w as covariate, the sex (2 levels), batch within farm of test (4 levels), diet (2 levels), line (2 levels) and line x diet interaction as fixed effects. The same model was applied to BFT, with no covariate or with BW23w as covariate (**BFT wBW**). Linear models for LMC and Yield included the sex (2 levels), slaughter date (4 levels), diet (2 levels), line (2 levels) and line x diet interaction as fixed effects, and the BW at slaughter as covariate for LMC. Effects are reported as significant for  $P < 0.05$ , and as tendencies for  $0.10 > P \geq 0.05$ .

## Results and discussion

### Growth and feed intake (Table 1)

Pigs from all lines had the same BW10w ( $\mu = 26.1$  kg,  $SD = 3.8$  kg,  $P = 0.13$ ). After 5 weeks of test, the Test diet reduced BW in the two sets of lines ( $P < 0.002$ ). No line effect was observed in the CORT lines. In the RFI lines BW15w was lower in the LRFI pigs than in HRFI pigs ( $P = 0.008$ ), with no line x diet interaction. At 23 weeks of age, similar differences were obtained, with a tendency for a line x diet interaction in the RFI lines, the LRFI pigs fed the Test diet being 9.6 kg lighter than when fed the Control diet, whereas the difference was 5.2 kg in the HRFI line. As a result, the Test diet decreased ADG for both sets of lines ( $P < 0.03$ ), with a line effect and a tendency for a line x diet interaction in the RFI lines only.

Table 1. Least square means<sup>1</sup> for line (High and Low) x diet (Control and Test) interaction on traits recorded during the growing period<sup>2</sup> on the CORT and RFI lines<sup>3</sup>.

		P			High line		Low line	
		Line	Diet	Line x Diet	Control	Test	Control	Test
BW15w, kg	CORT	0.99	0.0016	0.79	50.9b	48.5a	50.7b	48.7a
	RFI	0.0082	$4.10^{-7}$	0.36	54.6c	50.9b	53.2c	48.0a
BW23w, kg	CORT	0.74	0.033	0.93	97.0b	94.3a	96.7b	93.7a
	RFI	0.008	$1.10^{-8}$	0.06	101.2c	96.0b	100.2c	90.6a
ADG, g/d	CORT	0.77	0.030	0.97	774b	744a	771b	738a
	RFI	0.011	$1.10^{-8}$	0.06	822c	765b	812c	707a
ADFI, g/d	CORT	0.64	0.011	0.45	2.21a	2.30ab	2.19a	2.36b
	RFI	$3.10^{-13}$	0.75	0.07	2.57b	2.65b	2.21a	2.10a
FCR	CORT	0.63	$6.10^{-7}$	0.58	2.85a	3.09b	2.84a	3.15b
	RFI	$8.10^{-11}$	$2.10^{-6}$	0.38	3.12c	3.49d	2.72a	2.98b

<sup>1</sup> See text for the statistical models. Different letters indicate significant differences within row ( $P < 0.05$ )

<sup>2</sup> See text for trait definitions.

<sup>3</sup> CORT lines: high line = High cortisol line, low line = Low cortisol line; RFI lines: high line = less efficient; low line = more efficient.

The ADFI increased with the Test diet in the CORT lines (+135 g/d,  $P = 0.01$ ), as expected from the reduced energy content of the Test feed. However, this increase was lower than the 10% expected from the energy content change, in relation with increased gut filling and lower transit time due to the fibre content. This effect was not significant in the RFI lines, but a numerical increase in the HRFI pigs (+78 g/d,  $P = 0.28$ ) was observed whereas ADFI was decreased by 110 g/d ( $P = 0.14$ ) in the LRFI pigs fed the Test diet, leading to a tendency for an interaction line  $\times$  diet ( $P = 0.07$ ). When the two pigs with lowest ADFI records ( $< 1.5$  kg/d, LRFI line, one with each diet) were removed from the dataset, the interaction was significant ( $P < 0.05$ ). For ADFI, no line difference was observed in the CORT lines, whereas LRFI pigs ate 435 g/d less than HRFI pigs ( $P < 0.0001$ ). The LRFI pigs, contrary to the other lines, seemed to be unable to increase their voluntary feed intake to face the lower energy and AA content of the Test diet. In addition, it has been shown previously that LRFI pigs have higher AA requirements per kg of feed (Gilbert et al., 2017), resulting in a larger impact on growth of a reduced AA content. On that respect, LRFI were more sensitive to the diet challenge than HRFI pigs.

As a result, FCR was increased by about 300 g feed per kg BW gain with the Test diet, in the two sets of lines. No significant line effect was observed in the CORT lines, with extremely similar FCR for both lines fed the same diet. When FCR was expressed in MJ NE/kg BW gain, no diet difference remained. Thus, selection for plasma cortisol in the CORT lines did not affect the feed efficiency of the pigs, nor ADG or ADFI, when fed a conventional or an alternative diet. This result is contradictory with the initial hypothesis. Analyses of larger datasets with growth records showed similar responses (Mormède et al., 2018), in accordance with the genetic correlation of  $0.01 \pm 0.08$  estimated between ADG and the selection criterion in these lines (Larzul et al., 2018). A significant RFI line effect was found, as expected from previous results in these lines (Gilbert et al., 2017), with an advantage of -400 g feed/kg BW gain in the LRFI line. No line  $\times$  diet interaction was found, suggesting that the ability of the animals to cope with the dietary challenge was not affected by selection for plasma cortisol on one hand, nor by selection for RFI on the other hand.

## Body and carcass composition (Table 2)

A CORT line effect was found for all body and carcass composition traits, despite the absence of a line effect on growth and feed intake. The HCORT line was leaner than the LCORT line, as indicated by line differences for BFT and LMC. This result is not consistent with the hypothesis of decreased cortisol levels in leaner pigs associated with better production efficiency. In the RFI lines, the LRFI pigs were leaner in terms of BFT than the HRFI pigs, as previously reported (Gilbert et al., 2017). Line differences for LMC were not significant in the present study. A diet effect was observed in the RFI lines but not in the CORT lines. The significant reduction of BFT observed in LRFI pigs fed the Test diet was no longer significant when the pig BW was included in the model. This result suggests a secondary effect of the reduced BW of the LRFI animals rather than an increased leanness due to the feed restriction imposed by the dietary fibre content of the Test diet.

Finally, line and diet effects were observed for both pairs of lines on Yield. The Test diet led to a reduced carcass yield that could be due to the increased development of the gut tract in response to the dietary fibre content of the feed (Montagne et al., 2014). The HCORT line had an increased carcass yield compared to the LCORT line, certainly related to their

higher leanness. The LRFI line had a higher carcass yield than the HRFI line, which was not found in Montagne et al. (2014) at 10 weeks of age.

Table 2. Least square means<sup>1</sup> for line (High and Low) x diet (Control and Test) interaction on body and carcass composition<sup>2</sup> on the CORT and RFI lines<sup>3</sup>

		P			High line		Low line	
		Line	Diet	Line x Diet	Control	Test	Control	Test
BFT, mm	CORT	2.10 <sup>-4</sup>	0.27	0.76	15.69a	14.86a	17.63b	17.19b
	RFI	3.10 <sup>-5</sup>	0.02	0.04	20.67b	20.51b	19.20b	16.35a
BFT wBW, mm	CORT	9.10 <sup>-5</sup>	0.61	0.61	15.78a	15.23a	17.43b	17.41b
	RFI	1.10 <sup>-3</sup>	0.20	0.12	19.94b	20.14b	18.82ab	17.29a
LMC, %	CORT	1.10 <sup>-3</sup>	0.39	0.70	59.10b	58.87b	57.63a	57.05a
	RFI	0.66	0.31	0.57	56.57	56.75	56.00	56.63
Yield, %	CORT	2.10 <sup>-3</sup>	6.10 <sup>-5</sup>	0.26	76.30c	74.74ab	75.03b	74.09a
	RFI	5.10 <sup>-3</sup>	1.10 <sup>-4</sup>	0.98	74.83b	73.60a	75.74c	74.56b

<sup>1</sup> See text for the statistical models. Different letters indicate significant differences within row (P < 0.05)

<sup>2</sup> See text for trait definitions.

<sup>3</sup> CORT lines: high line = High cortisol line, low line = Low cortisol line; RFI lines: high line = less efficient; low line = more efficient.

## Conclusion

Selection for increased or decreased plasma cortisol levels after stimulation of the adrenal gland did not impact growth and feed intake traits, but had a significant impact on body composition and carcass yield, with improved traits in the high cortisol animals. The two lines had similar responses to an alternative diet. On the other hand, lines selected for divergent RFI had different responses to the alternative diet, the more efficient line having a more reduced growth rate with the diet with lower energy and AA contents. However, in terms of FCR this line remained more efficient. To conclude, selection for lower RFI may have reduced the ability of pigs to increase their voluntary feed intake to face dietary fibres diets, whereas selection for increased cortisol levels did not improve the pigs' robustness to the diet challenge.

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