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Pierre Mormède, Elena Terenina, Yvon Billon, Stéphane Ferchaud, Laure Gress, Hervé Rémignon, Hélène H. Manse, Catherine Larzul

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Divergent selection on adrenocortical activity in Large White pigs: study of responses to breeding challenges

P. Mormede[1], E. Terenina[1], Y. Billon[2], S. Ferchaud[3], L. Gress[1], H. Remignon[1], H. Manse[1] & C. Larzul[1]

[1] *GenPhySE, INRA, INPT, ENSAT, Université de Toulouse, 31326 Castanet-Tolosan, France*
pierre.mormede@inra.fr (Corresponding Author)
[2] *GenESI, INRA, Le Magneraud, 17700 Surgères, France*
[3] *GenESI, INRA, La Gouvanière, Venours, 86480 Rouillé, France*

Summary

This experiment had the objective to analyze responses to various breeding challenges in the third generation of selection of two lines of pigs genetically selected on the basis of plasma cortisol levels measured one hour after injection of ACTH. This test allows to objectify the activity level of the hypothalamic-pituitary-adrenal (HPA) axis, a major neuroendocrine system of the stress response. The results presented here show that the post-weaning growth rate was significantly lower in the high line, although the two lines had the same overall growth rate throughout their productive lives. The effects of a prolonged exposure to high temperature (30°C) and of group mixing stress prior to slaughter did not differ between the lines. These results show that the responses to different challenges are not affected in the same way by the HPA axis activity. These results, together with production data, will allow to evaluate the relevance of this criterion of selection to increase the robustness of animals.

Keywords: genetic selection, cortisol, stress, robustness, heat stress, weaning stress, mixing stress, pig

Introduction

Improvement of the sustainability of farming and animal welfare requires implementation of new selection criteria in relation to the robustness of the animals. The HPA axis plays a major role in metabolic regulation and adaptation processes and its activity is strongly influenced by genetic factors (Mormede *et al*., 2011a; Mormede & Terenina, 2012). It is therefore a potential lever for the selection of more robust animals (Mormede *et al.*, 2011b). To explore this strategy, a divergent selection was carried out in the Large White pig breed based on the plasma cortisol level measured one hour after injection of ACTH (Larzul *et al.*, 2015). In the 3rd generation of selection, the post-ACTH plasma cortisol level was 2.16 times higher in the high line than in the low line (Larzul *et al.*, 2018). Responses to different breeding challenges were analyzed in these animals: weaning, heat stress at the end of the fattening period, and finally pre-slaughter stress through the mixture of animals.

Material and methods

Experimental procedures

The experimental protocol was accepted by the ethics Committee in animal experimentation Poitou-Charentes (decision of 21/01/2013). The selection experiment and the breeding conditions are presented in Larzul et al. (2018). Briefly, two divergent lines were selected on the plasma cortisol level one hour after ACTH injection at 6 weeks of age, 2 weeks after weaning. The animals of the 3rd generation of selection were studied (N = 643, from 75 litters produced in 3 successive batches). The post-ACTH plasma cortisol level was 2.16 times higher in the high line than in the low line, the line difference being about 5 genetic standard deviations for the selection criterion. Half of the males were castrated at one day. All animals were weighed at birth, at weaning (4 wk), at the ACTH test (6 wk), at the end of the weaning period (9 wk) and at the end of the fattening control period (22 wk).

To measure the response to the stress of weaning, the weight data for all the animals tested with ACTH were used.

The heat stress experiment occurred in the fall. Animals from a single batch (N = 71) were transferred for the end of the fattening period (133 d-158 d) in a room conditioned at 29.9°C, 66.6% relative humidity (RH). The matched controls (N = 69) remained in their breeding rooms in standard conditions (26.1°C, 59.7% RH). Animals were weighed at the beginning (133 d) and at the end of the experimental period (158 d).

To study the effects of pre-slaughter stress, a group of animals (N = 64), entire males and females, was slaughtered at 159 d on average. Before transport (of about 1-h duration) and on arrival at the slaughterhouse, one half of the animals was mixed between groups. After at least 2-h rest, animals were slaughtered and blood was collected at sticking and urine was collected in the bladder for the determination of cortisol. The pH was measured at 24 h in the *longissimus dorsi* and *semi-membranosus* muscles. The number of skin lesions, indicative of aggressive interactions, was counted on the carcasses.

Statistical analyses

For weaning stress, the ADG were calculated for each interval and analyzed according to a linear model including the line (high or low cortisol) and sex (3 levels) as fixed effects, and the weight at the beginning of each period (birth, weaning, and beginning of fattening) as a covariate. For heat stress, the ADG calculated over this period was analyzed according to a linear model including the line (2 levels), the experimental condition (heat and control), sex (3 levels) and the line x condition interaction as fixed effects. For the pre-slaughter stress, data were analyzed according to a linear model including the line (2 levels), sex (2 levels), stress (with or without mixing) as well as the line x stress interaction as fixed effects. All statistical analyses were carried out with the R software.

Results and discussion

Response to weaning

The results (Table 1) show that the high line animals had a much lower post-weaning ADG (-28 % during the 4-6 wk period, -13 % during the 6-9 wk period). This difference is specific

of the post-weaning period since the ADG did not differ between the two lines during lactation and was higher in the high line during fattening, so that the lifetime ADG did not differ between lines.

Table 1. Average daily gain (ADG) at successive breeding periods.

Period	Low line		High line		RSE[2]	P
	Mean (g/day)	N[1]	Mean (g/day)	N		
Lactation		330		313		
0-4 wk	259		252		42	0.068
Post-weaning		330		313		
4-6 wk	199		143		70	$< 2.10^{-16}$
6-9 wk	479		437		126	$< 2.10^{-3}$
4-9 wk	372		327		84	$< 6.10^{-9}$
Fattening		285		275		
9-22 wk	844		861		75	0.027
Whole life		285		275		
0-22 wk	694		700		73	0.115

[1] N = Number of animals
[2] RSE = Residual standard error of the model

Response to heat

In this batch, the growth at the beginning of fattening in control conditions (62 d-133 d) was not different between the lines (846 g/j, P = 0.15). During the period of exposure to heat (113 d-158 d), growth was significantly higher in animals maintained in control conditions (997 vs 863 g/j, P = 3.10^{-5}) and in the high line (1029 *vs* 842 g/j, P = 9.10^{-8}), without significant interaction between these two factors (P = 0.11). This result shows that exposure to heat at the end of fattening slows down the growth of animals, but to the same extent in both selected lines. It further suggests that the ADG is higher in the high line at the end of fattening only, a result to be confirmed with more frequent weight recordings in standard breeding conditions.
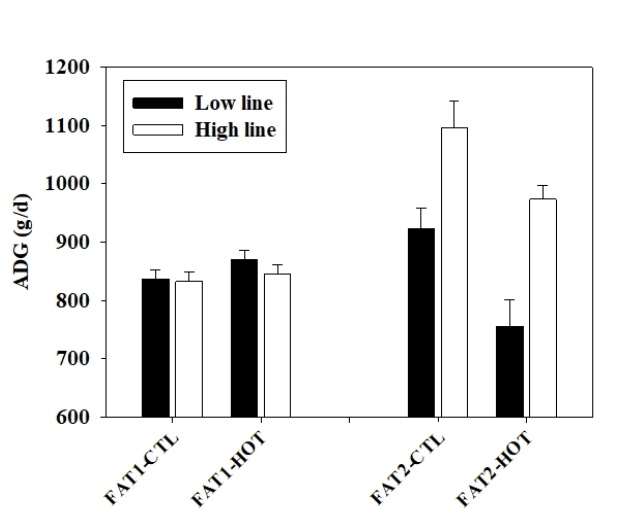

Figure 1. Average daily gain (ADG) of pigs submitted to heat stress. The first part of the X axis labels indicates the experimental step (FAT1 = first phase of the fattening period, 62 d-

133 d, all animals being in the standard conditions; FAT2 = second phase of the fattening period, 133 d-158 d). The second part of the X axis labels indicates the environmental condition during the second phase of the fattening period; CTL = control, HOT = heat exposure).

Response to pre-slaughter stress

Plasma cortisol concentration at slaughter was higher in animals of the high line (70 vs 24 ng/ml, $P < 10^{-7}$), without influence of the pre-slaughter stress. However the total urinary cortisol concentration was higher in the high line (99 vs 62 ng/mg creatinine, $P < 0.01$) and in the animals under stress (99 vs 68 ng/mg creatinine, $P < 0.05$), without interaction. The total number of skin lesions was higher in animals having undergone pre-slaughter mixing (95 vs 28, $P = 2.10^{-6}$), with no effect of line or sex. These endocrine and behavioral data show the importance of social stress.

The line did not influence the pH measured at 24 hours. Pre-slaughter stress increased significantly the 24-h pH of the *longissimus dorsi* (5.95 vs 5.86, $P = 0.01$) and *semi-membranosus* (5.83 vs 5.72, $P = 0.035$) muscles as expected, but without interaction with the line.

Conclusion

This study aimed to reveal responses to various breeding challenges in the third generation of selection of two lines of pigs genetically selected on the basis of the cortisol levels measured one hour after injection of ACTH. The data presented here show that the post-weaning ADG is significantly lower in the high line, suggesting that the high adrenocortical axis activity makes it more sensitive to weaning stress. However, this growth slowdown after weaning is compensated for during fattening, so that the two lines have the same lifetime ADG. On the other hand, the effects on production traits of exposure to a high ambient temperature (30.7°C) or to a mixing stress prior to slaughter do not differ between the lines. We showed previously that the high line is less sensitive to social stress-induced immune changes (Bacou *et al.*, 2017), showing that the responses to various breeding challenges are not affected similarly by genetic differences in adrenocortical axis activity. These results, associated with production data, will allow to evaluate the relevance of this selection criterion to increase robustness in farm animals.

Acknowledgements

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