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Meat Science 69 (2005) 703–707
MEAT SCIENCE
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Stress hormones, carcass composition and meat quality in Large White × Duroc pigs

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Received 5 August 2004; received in revised form 1 October 2004; accepted 1 November 2004

Abstract

The levels of stress hormones, cortisol and catecholamines (adrenaline and noradrenaline), were measured in urine collected after slaughter from the bladder, in 309 pigs (females and castrated males) from an F2 intercross between the Large White and Duroc breeds to analyze the relationships between stress-responsive neuroendocrine systems, carcass composition and meat quality. Intramuscular fat content was measured from a biopsy sample taken at a live weight of 70 kg from the *longissimus lumborum* muscle, and carcass and meat quality traits were also collected. Carcass fat content was higher and estimated carcass lean meat content was lower with increasing urinary levels of cortisol and adrenaline (that are highly correlated with each other), but was not related to the levels of noradrenaline, showing that adrenal hormones favor the accretion of fat at the expense of muscle proteins, a typical physiological effect of cortisol. On the contrary, intramuscular fat levels were unrelated to either hormone level. Finally, muscle pH measured 24 h after death was positively correlated with catecholamine levels, an effect related to the catabolism of muscle glycogen by catecholamines released by preslaughter stress, which impairs post-mortem acidification of meat. These results show the importance of a control over stress neuroendocrine systems to increase pork production and product quality, and the value of the genetic approach to reach this goal.

Keywords: Cortisol; Catecholamines; Urine; Adiposity; Intramuscular fat; Meat pH

1. Introduction

The two main stress-responsive neuroendocrine systems play a critical role in the regulation of energy fluxes. The hypothalamic–pituitary–adrenocortical (HPA) axis influences feeding behavior, pancreatic hormone secretion, energy expenditure and the protein/lipid balance (Dallman et al., 1993). Altogether, cortisol, the main active hormone of the axis, released by the adrenal cortex, favors the accretion of fat at the expense of proteins (Devenport, Knehans, Sundstrom, & Thomas, 1989). Indeed, pig breeds with a higher carcass content of fat like Meishan (Bidanel, Caritez, Gruand, & Legault, 1993) or Duroc (Smith & Pearson, 1986) also produce more cortisol (Bergeron, Gonyou, & Eurell, 1996; Désautés, Bidanel, & Mormède, 1997; Désautés, Sarrieau, Caritez, & Mormède, 1999; Hay & Mormède, 1998; Mormède et al., 2004; Weiler, Claus, Schnoebelen-Combes, & Louveau, 1998). On the other hand, catecholamines (adrenaline and noradrenaline) released by the sympathetic nervous system (SNS) increase the use of energy stores (glycogen and lipids; Scheurink &

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0309-1740/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2004.11.002

Steffens, 1990) and exert anabolic effects on protein metabolism (Navegantes, Migliorini, & Kettelhut, 2002). Sympathetic activation by stress before slaughter reduces muscle glycogen content and post-mortem acidification, leading to DFD-type meat (dark, firm and dry) (Fernandez & Tornberg, 1991). The present experiment was designed to study the relationships of stress hormones secretion, as measured by their content in urine collected after slaughter, with carcass composition and meat quality in a segregating (F2) cross between two contrasting breeds, the Large White and Duroc pigs.

2. Materials and methods

The animals were reared and slaughtered in compliance with national regulations applied in research and commercial slaughtering.

2.1. Animals

The animals were bred in the experimental research farm of Le Magneraud (Surgères, Charente Maritime, France). We obtained 309 female and castrated male pigs from a F2 intercross between the Large White and Duroc breeds (Sanchez et al., 2002). Pigs were slaughtered at 104 ± 7 kg live weight in a commercial abattoir (SOCOPA, Celles-sur-Belle, Charente Maritime, France).

2.2. Carcass composition and meat quality traits

At a live weight of 70 kg a muscle biopsy sample (approx. 1 g) was taken from the *longissimus lumborum* muscle 7–8 cm behind the 14th rib and around 5 cm from the dorsal line, the penetration depth being 4.5–5 cm (Talmant, Fernandez, Sellier, & Monin, 1989), to measure intramuscular fat (IMF) content by gas chromatography after extraction and transmethylation (Folch, Lees, & Stanley, 1957; Morrison & Smith, 1964).

After slaughter the following traits were recorded:

- G1 (backfat thickness measured 8 cm lateral to the dorsal median line between the last 3rd and 4th lumbar vertebrae) and G2 and M2 (respectively, backfat and muscle thickness measured 6 cm lateral to the dorsal median line between the last 3rd and 4th ribs) with a "FAT-O-METER" probe, in order to compute the estimated carcass lean content (ECLC) according to the following formulae (Daumas, Causeur, Dhorne, & Schollhammer, 1998):

  For females: $ECLC = 61.68 - 0.142 * G1 - 0.449 * G2 + 0.154 * M2$

  For castrated males: $ECLC = 58.15 - 0.198 * G1 - 0.570 * G2 + 0.255 * M2$

- Lightness of the *biceps femoris* (L_BF) and the *gluteus superficialis* (L_GS) muscles measured with a Minolta chromameter CR-200,
- pH measured 24 h after slaughter in the *longissimus lumborum* at the level of the last rib (pH_LL), *biceps femoris* (pH_BF), *gluteus superficialis* (pH_GS) and *adductor femoris* (pH_AF) muscles.

2.3. Neuroendocrine excretion profile

Urine was collected from the bladder and frozen after addition of EDTA (tetrasodium salt, 10% solution in saline, 0.2 mL in 10 mL of urine) as a preservative. Cortisol (F) was measured by HPLC with UV detection after extraction on reverse phase columns (Hay & Mormède, 1997a). Adrenaline (AD) and noradrenaline (NA) were measured by HPLC with electrochemical detection after extraction on cationic columns (Hay & Mormède, 1997b). Creatinine was measured by a colorimetric method (Sigma diagnostics) to correct for urine dilution.

2.4. Statistical analysis

Data were analyzed using the Statistical Analysis System software (SAS Institute, 1992). Phenotypic data were adjusted for sex and systematic environmental effects (slaughter date and weight, and farrowing batch) using the General Linear Model analysis of variance before calculation of Pearson correlation coefficients. Due to the limited number of animals, no reliable evaluation of genetic correlations could be obtained. *P* values were not corrected for multiple comparisons.

3. Results and discussion

Means and standard deviations of the traits analyzed are presented in Table 1. As expected, sex influenced carcass composition, but not the intramuscular fat content that was only marginally higher in castrated males than in females ($P = 0.081$). The estimated carcass lean content was higher in females. None of the neuroendocrine traits differed between sexes.

Pearson residual correlations between urinary hormone levels and the different measures are given in Table 2. NA and AD levels were highly correlated. Indeed both catecholamines are synthesized via a common pathway, except the ultimate transformation of noradrenaline into adrenaline by the enzyme phenylethanolamine *N*-methyl transferase (PNMT) in the adrenal medulla, and the activity of the enzymes involved in catecholamine synthesis have been shown to be influenced by genetic factors (see Mormède et al., 2002, for review). Cortisol and AD levels were highly correlated, much more than cortisol and NA levels. One mechanism to ex-

Table 1
Least square means and standard deviations of the traits analyzed

Traits	Least square Mean min-max	Standard deviation	Fixed between-subjects effects (F values)				
			Sex		Slaughter date	Slaughter weight	Farrowing batch
Estimated carcass lean content (%)	59.14 45–65	3.24	72.48*****	F > M	-	7.63***	2.55**
Intramuscular fat content (%)	2.12 0.8–7.2	0.77	3.06**		–	–	5.26*****
Lightness of the *biceps femoris* (scale 0–100)	51.41 39.47–61.95	4.10	–		3.49*****	–	–
Lightness of the *gluteus superficialis* (scale 0–100)	48.71 39.14–56.96	3.46	–		3.02*****	7.68***	–
pH 24 *adductor femoris*	5.99 5.25–6.92	0.27	3.71**		4.81*****	–	–
pH 24 *biceps femoris*	5.75 4.62–6.37	0.18	1.28*		3.28*****	–	–
pH 24 *gluteus superficialis*	5.68 5.41–6.92	0.18	13.56****	F < M	4.55*****	–	–
pH 24 *longissimus lumborum*	5.76 5.41–6.59	0.19	–		5.34*****	3.81**	–
Cortisol (ng/mg creatinine) (N = 302)	57.84 1.70–300.9	50.08	2.16*		7.56*****	–	–
Noradrenaline (ng/mg creatinine) (N = 289)	31.31 7.94–188.9	20.93	–		3.98*****	17.68*****	–
Adrenaline (ng/mg creatinine) (N = 289)	20.72 2.66–130.1	14.85	–		4.69*****	–	–

N = 309, unless indicated.
*P < 0.30, **P < 0.10, ***P < 0.01, ****P < 0.001, *****P < 0.0001.
F, female; M, male.

Table 2
Pearson residual correlation between the different measures

Traits	Cortisol	Noradrenaline	Adrenaline
Cortisol (ng/mg creatinine)		0.15*	0.42****
Noradrenaline (ng/mg creatinine)	0.15*		0.55****
Estimated carcass lean content (%)	−0.24****	−0.07	−0.29****
Intramuscular fat content (%)	0.02	−0.07	0.07
Lightness of the *biceps femoris* (scale 0–100)	−0.12*	0.01	−0.08
Lightness of the *gluteus superficialis* (scale 0–100)	0.01	−0.06	−0.13*
pH 24 *adductor femoris*	0.04	0.24****	0.27****
pH 24 *biceps femoris*	−0.03	0.16**	0.20***
pH 24 *gluteus superficialis*	0.10	0.08	0.22***
pH 24 *longissimus lumborum*	0.02	0.16**	0.17**

* P < 0.05.
** P < 0.01.
*** P < 0.001.
**** P < 0.0001.

plain this relationship is the regulation by cortisol of the enzyme PNMT, that catalyses the methylation of NA into AD (Ciaranello, 1978). It is also possible that the adrenal cortex (cortisol) and medulla (AD) are somehow co-activated, but that the HPA axis (cortisol) and the sympathetic nervous system (NA) are largely independent. Further experiments should sort out the respective influence of these mechanisms.

Cortisol levels were negatively correlated with the estimated carcass lean content (ECLC). This correlation reflects the general metabolic effects of cortisol that favors the accretion of lipids in fat at the expense of

proteins from muscle and other tissues (Devenport et al., 1989). A similar correlation between backfat thickness and plasma cortisol levels was previously found in an intercross between Large White and Meishan pigs (Désautés, 1996) and we demonstrated recently that polymorphisms of corticosteroid binding globulin, the carrier protein of cortisol in plasma, could be involved in this relationship between cortisol and adiposity (Désautés et al., 2002; Ousova et al., 2004).

AD levels were also correlated with ECLC, unlike NA. Activation of β-adrenergic receptors increase mobilization of fat and reduce protein catabolism, mostly via β_2-type receptors (Mersmann, 1998; Navegantes et al., 2002). A direct action of adrenaline, a potent β_2 agonist, in these processes should therefore reduce the fat content of the carcass and increase the yield of muscle. The reverse result was found here. Therefore, in the population studied here, the correlation between AD levels and carcass composition most probably reflects the covariation between adrenal cortex and medulla, as previously noted.

No correlation was found between urine hormone levels and intramuscular fat content, showing that this metabolic compartment has a different hormonal regulation than other fat compartments. Indeed, the correlation coefficient between IMF (measured at 70 kg live weight) and ECLC (measured at 104 kg live weight), although significant, was rather low ($r = 0.16$, $P < 0.01$).

Catecholamine levels were positively correlated with meat pH measured 24 h after slaughter. Indeed, sympathetic activation before slaughter increases muscle glycogenolysis and therefore reduces lactic acid production post-mortem and meat acidification (Fernandez & Tornberg, 1991). It is worth noting that this relationship varies to a large extend among muscles, depending on their metabolic properties and their sensitivity to catecholamines (Larzul, Le Roy, Monin, & Sellier, 1998; Terlouw, Rybarczyk, Fernandez, Blinet, & Talmant, 1997).

Altogether these data show interesting relationships between urinary levels of stress hormones (cortisol and catecholamines) and carcass composition and muscle quality, in a way that can be explained by the action of these hormones or neurotransmitter on energy and protein metabolism. However, several questions are open to further investigation, such as the mechanism(s) of the link between cortisol and adrenaline, or adrenaline and carcass composition. One important question is to know whether the variations found in urinary levels of hormones result from differences in basal secretion or in the intensity of the response to preslaughter stress or in both. At first, it is worth noting that urinary levels of cortisol and catecholamines should not be influenced by the slaughtering procedure itself, since a delay is necessary between hormone secretion and excretion in urine, as we showed for cortisol in sows (Hay, Meunier-Salaün, Brulaud, Monnier, & Mormède, 2000). Furthermore, the relationships between cortisol and adrenaline levels on one hand and structural measures like muscle yield on the other hand demonstrate that stable differences in the HPA axis and SNS activity are probably involved. In the comparison of five genetic lines, we recently found a high correlation between basal urinary cortisol level (urine collected in the farm) and post-stress level measured after transportation (Mormède et al., 2004), suggesting that the levels measured at slaughter in the present experiment may indeed reflect basal HPA axis activity. Further experiments should compare basal levels measured when urine is collected in the farm and levels measured after slaughter.

Finally, since raw data were corrected for major environmental factors, most of these correlations are probably of genetic origin, although we could not obtain reliable direct estimates of genetic correlations, due to the limited number of animals. Indeed, large differences in neuroendocrine measures, carcass composition and meat quality were found between Large White and Duroc pigs in a large multibreed comparison (Mormède et al., 2004). It is now well established that genetic factors influence individual variations in stress behavioural and neuroendocrine responses (Mormède et al., 2002). Genetic selection on stress reactivity traits could improve both animal welfare and product quality. Mapping of chromosome regions involved in genetic variations opens the way to the identification of the molecular mechanisms involved in individual differences in stress responses (Désautés et al., 2002; Ousova et al., 2004) and to the selection of animals with different stress reactivity with molecular genetic markers.

4. Implications

The present data show that stress hormones from the adrenal cortex (cortisol) and the sympathetic nervous system (catecholamines) influence several traits important for pork meat production. It is now well established that genetic factors influence individual variations in stress behavioral and neuroendocrine responses. Genetic selection on stress reactivity traits could improve both animal welfare and product quality. Furthermore, QTL related to these traits have been recently mapped and open the way to the identification of the molecular mechanisms involved in individual differences in stress responses and to the selection of animals with different stress reactivity with molecular genetic markers.

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