

EFFECTS OF THE RN⁻ GENE ON SOME TRAITS OF MUSCLE AND LIVER IN PIGS

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SUMMARY

The present study involved 2 experiments. The pigs under study originated from a population where the presence of the RN⁻ gene has been previously demonstrated. The animals were slaughtered in a commercial abattoir and were classified as normal or RN⁻ carriers on the basis of the Napole yield (Ny) as measured on the *Semimembranosus* muscle (normal : Ny \geq 91 ; RN⁻ carriers : Ny < 91). In experiment 1, the contents of water and protein were measured on the *Semimembranosus* muscle of 90 pigs slaughtered in 4 weekly series. Samples were taken from the *Semimembranosus* muscle around 30 min after slaughter, for determination of Ny and protein and water contents. Muscle from RN⁻ carriers (n = 55) contained less protein (P < 0.01) and slightly more water (P > 0.01) than muscle from normal pigs (n = 35). In experiment 2, samples were taken from the *Semimembranosus* muscle and from the liver of 27 pigs around 30 min after slaughter, for determination of Ny and protein and glycolytic potential levels. Muscle from RN⁻ carriers (n = 17) contained a higher glycolytic potential (P < 0.01) and a lower protein content (P < 0.01) than muscle from normal pigs, while no difference was observed in liver between the 2 groups of animals. It was concluded that the increase in muscle glycolytic potential induced by the RN⁻ gene does not result from a hormonal deficiency, but from a defect inherent in skeletal muscle cells.

INTRODUCTION

The RN⁻ gene decreases the processing yield of cooked cured ham (Naveau, 1986 ; Le Roy *et al.*, 1990). It has been shown to decrease the glycogen content of the white muscle *Longissimus dorsi* and to modify ultrastructural features of the muscle tissue (Fernandez *et al.*, 1989, 1992 ; Estrade *et al.*, 1991). Monin (1989) reported that the RN⁻ gene could also decrease the muscle protein content. However, the effects of the RN⁻ gene on other tissue components or on other tissues have not been investigated so far.

The present article reports the results of 2 experiments. In the first experiment, the influence of the RN⁻ gene on muscle contents of water, protein and neutral lipids was investigated. The second experiment was designed to assess whether the RN⁻ gene affects also the glycogen level in the liver.

MATERIAL AND METHODS

Animals. Pigs from a population known as carrying the RN⁻ gene (composite lines P66 and P77 of the Pen Ar Lan Company) were used in both experiments. The animals were fed *ad libitum* and slaughtered at a liveweight of around 100 kg, in an industrial abattoir. They were classified as rn+rn+ homozygotes (referred to as normal) or as RN⁻ carriers (referred to as RN⁻) on the basis of the Napole yield measured one day after slaughter, *i.e.* normal for a yield equal to or higher than 91 % and RN⁻ for a yield lower than this value.

Sampling.
Experiment 1. Samples weighing about 150 g were taken from the *Semimembranosus* muscle of 100 pig carcasses, in 4 weekly slaughter series, around 30 min after slaughter. These samples were put on ice for transportation to the laboratory, then kept overnight at 4 °C. On the day following slaughter, the Napole yield was assessed from a subsample of 100 g. The rest was freeze-dried for determination of water,

protein and neutral lipid contents.

Experiment 2. About 150 g of *Semimembranosus* muscle and around 50 g of liver were taken from 27 pig carcasses, in 1 slaughter series around 30 min after slaughter. Subsamples of muscle and liver (around 20 g) were dipped in liquid nitrogen and freeze-dried for the determination of glycolytic potential and protein. The rest of the muscle was used for assessment of the Napole yield as described above.

Analytical techniques. Freeze-dried tissues were powdered in a coffee-mill before use for analysis. Nitrogen was determined by the method of Ferrari (1960) after Kjehldal mineralization of 100 mg of freeze-dried tissue, and protein was calculated as $N \times 6.25$. Lipid content was assessed by the Soxhlet method using 2 g of freeze-dried tissue. Water content was determined by weighing around 3 g of muscle before and after drying for 48 h at 104 °C. As the samples of muscle and liver were obtained *post mortem*, glycogen was estimated as glycolytic potential ($2 \times ([\text{glycogen}] + [\text{glucose}] + [\text{glucose-6-P}]) + [\text{lactate}]$) determined from 250 mg of freeze-dried tissue as described by Monin and Sellier (1985).

The Napole yield was determined as described by Naveau *et al.* (1985). Muscle was cut in cubes of around 1 cm³; 100 g were mixed with 20 g of a brine (12 % NaCl and 0.07 % NaNO₂ w/w in water) and left at +4 °C for 24 h. The mixture was incubated in boiling water for 10 min, then the liquid in excess was allowed to drain for 2.5 h. The cooked muscle was weighed, and the Napole yield was calculated as the ratio of cooked muscle to fresh muscle.

Calculations. In experiment 2, water content was determined neither in muscle nor in liver. In order to compare the results relative to muscle in both experiments in a convenient way, the water content of muscle was assumed to be 75.5 % and this value was used to express glycolytic potential and protein relative to fresh tissue.

RESULTS

Experiment 1. Fifty five pigs were considered as RN- and 35 pigs as normal on the basis of the Napole yield. The protein content was lower (-5 %, $P < 0.01$) and the water content was slightly, but significantly, higher in muscle from RN- animals than in muscle from normal animals (Table 1). This resulted in a much higher water to protein ratio in the RN- pigs as compared to the normal pigs. There was no significant difference in lipid content between the two phenotypes. Muscle from P66 pigs contained more protein and less water than muscle from P77 pigs (results not detailed).

pigs (n)	significance of effects (1)			mean \pm s.d. (2)	
	line (3)	m type	line x type	normal	RN-
water % fresh tissue	*	**	*	75.5 \pm 0.7	76.0 \pm 0.8
protein % fresh tissue	**	**	ns	22.0 \pm 1.3	21.0 \pm 1.0
water/protein	**	**	ns	3.44 \pm 0.17	3.63 \pm 0.21
lipids % fresh tissue	ns	ns	ns	1.44 \pm 1.0	1.38 \pm 0.73

Table 1. Compositional traits of muscle from RN- pigs and normal pigs.

(1) ns : not significant ; * $P < 0.05$; ** $P < 0.01$; (2) water and protein were determined in 35 normal pigs and 55 RN- pigs ; lipids were determined in 11 normal pigs and 12 RN- pigs ; (3) lines P66 and P77

Experiment 2. The protein content was lower (- 6 %, $P < 0.01$) and the glycolytic potential was higher (+ 60 %, $P < 0.01$) in muscle from RN- pigs than in muscle from normal pigs (Table 2). This resulted in a much higher glycolytic potential to protein ratio in the RN- animals. In contrast, no significant difference was observed between both phenotypes for liver tissue.

pigs (n)	muscle		liver	
	normal (10)	RN- (17)	normal (10)	RN- (17)
GP $\mu\text{mol/g}$ (1)	111 \pm 27	174 \pm 45	1372 \pm 548	1244 \pm 484
protein %	21.6 \pm 0.8	20.3 \pm 0.8	71.7 \pm 3.6	73.0 \pm 3.6
GP/protein	0.51 \pm 0.13	0.87 \pm 0.24	1.95 \pm 0.86	1.74 \pm 0.77

Table 2. Effect of the RN- gene on glycolytic potential and protein content in muscle and liver of pigs.

(1) GP : glycolytic potential ; results expressed relative to fresh tissue in muscle and relative to dry matter in liver.

In muscle, all differences between RN phenotypes were significant at the $P < 0.01$ level ; in liver, no difference was significant.

DISCUSSION

Undoubtedly, the values given in table 2 for glycolytic potential and protein in muscle are slightly erroneous, since they were calculated by a theoretical calculation assuming a uniform water content of 75.5 %. But this error is negligible (of the order of 1% or less), and the significance of the differences between the values relative to RN- and normal pigs is not modified whether the results are expressed in this way or relative to dry matter.

Regarding the glycogen content of muscle, the results of the present study agree with the observations of Fernandez *et al.* (1992). These authors reported an increase of about 60 % in the glycolytic potential of the white muscle *Longissimus dorsi* by the RN- gene.

The fact that the RN- gene affects the glycogen content in muscle but not in liver indicates that probably this gene does not act through a metabolic deficiency. In fact, hormones affecting the glycogen level in muscle also influence this level in liver. So it appears that the metabolic defect induced by the RN- gene is probably not due to some changes in the levels of circulating hormones. It is likely to be located in muscle cells, and as far as we know specifically in the muscle fibres.

Naveau (1986) and Sellier (1987) put forward the hypothesis that the RN- gene would have been brought into the French pig lines here from the Hampshire breed. The observations of Fernandez *et al.* (1992) enforced this assumption. Again the present results confirm this hypothesis. Indeed, the differences between the RN- pigs and the normal pigs - *i.e.* higher levels of protein and lower levels of glycogen in the latter - closely resemble the differences reported by Sellier and Monin (1985) and Monin *et al.* (1986) between Hampshire and Pietrain or Large White pigs, and by Monin *et al.* (1987) between Hampshire crosses (P66, also called Panshire, a line with 50 % Hampshire blood) and Large White, Pietrain or Belgian Landrace pigs. Recently, Wassmuth *et al.* (1991) showed in the Hampshire breed the presence of a dominant gene decreasing the ultimate pH and the protein content of muscle ; they called this gene HF- (HF for Hampshirerefaktor). The close similarities between the HF- and RN- genes indicate that these genes are very probably identical.

CONCLUSION

The results of this study confirm that the RN- gene increases the glycogen level and decreases the protein level in muscle, but has no influence on these traits in liver. This indicates that the excess of glycogen in muscle is not secondary to deficiencies in circulating hormones, but rather to a defect located in the muscle cell. Water content of muscle is increased by the RN- gene, but this effect is too small to have an influence *per se* on the technological ability of the meat.

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