VARIATIONS IN EXPRESSION OF CYP2E1 AND COUP-TF1 PROTEINS BETWEEN LARGE WHITE AND DUROC CROSS-BREEDS

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Introduction

An excessive accumulation of skatole in adipose tissue is related to development of one of the major pigmeat quality defects, boar taint. One of the reasons for high skatole accumulation in the fat of some entire male pigs is a low rate of hepatic skatole metabolism via CYP2E1 (Babol et al., 1998). A number of publications have established a negative correlation between skatole level in backfat and CYP2E1 protein expression in liver (Squires and Lundstrom, 1997; Doran et al., 2002). Our previous research has demonstrated that the transcription factor COUP-TF1 plays a key role in regulating CYP2E1 expression in pig liver (Tambyrajah et al., 2004). COUP-TF1 is generally known as a co-repressor of gene expression (Leng et al., 1996). However, in the case of porcine CYP2E1, COUP-TF1 acts as an activator of gene expression (Tambyrajah et al., 2004). To the best of our knowledge, expression of COUP-TF1 protein in pig has not been studied. It also remains unknown whether a defective expression of CYP2E1 proteins in liver of some pigs is related to a low level of COUP-TF1.

This paper aimed (i) to investigate expression of COUP-TF1 protein in liver of pigs of two commercial crossbreeds, and (ii) to elucidate the relationship between COUP-TF1 and CYP2E1 expression in pig liver.

Materials and Methods

The study was conducted on pigs of two commercial cross-breeds: (i) 75% Large White x 25% Landrace (n = 7) and (ii) 75% Duroc x 12.5% Large White x 12.5% Landrace (n =7), further referred as LW and D respectively. The pigs were reared under commercial conditions, fed the same standard diet and had a hot carcass weight of 89.3 ± 0.61 kg for LW and 98.7 ± 0.97 kg for D. Samples of liver were collected immediately after slaughter, snap frozen in liquid N_2 and stored at -80°C until required. Preparation of nuclear extracts was carried out using the method of Andrews and Faller (1991). Microsomes were isolated by differential centrifugation (Schenkman and Cinti, 1978).

Expression of CYP2E1 and COUP-TF1 proteins was estimated by Western blotting in isolated pig liver microsomes and nuclear extracts respectively. Proteins were separated on a SDS gel, electro-blotted on to nitrocellulose membrane, and probed with the appropriate primary and secondary antibody as described previously (Doran et al., 2006). The blots were developed using ECL reagent (Amesham, Bucks, UK) and the intensity of the corresponding bands was quantified using the ImageQuant program (Molecular Dynamics, UK). CYP2E1 protein expression was analysed using rabbit polyclonal antibody (Santa Cruz, USA) and donkey anti-rabbit IgG-HRP (Amersham) as the secondary antibody. COUP-TF1 protein expression was carried out using primary antibodies from Santa Cruz, USA (Cat. Sc-6577), and donkey anti-goat IgG-HRP as the secondary antibody (Santa Cruz, USA). In the case of COUP-TF1, the high molecular weight protein was detected in non-treated samples and the low molecular weight COUP-TF1 was detected in samples heated to 100°C for 4 min in a boiling water bath (treated samples).

Results and Discussion

Western blot analysis shows the presence of an immuno-reactive band of approximately 100 kDa for COUP-TF1 in non-treated nuclear extracts, and a lower molecular weight (approximately 50 kDa) band in the case of treated samples (Figure 1). As the molecular weight of the protein in the non-treated samples is double the size of the protein in the treated samples, this might suggest that COUP-TF1 may be present as a monomer (50 kDa band) or as a dimer (100 kDa band). These results are consistent with data of Wang et al. (1991) who also showed that low molecular weight (43-47 kDa) and high molecular weight (66-74 kDa) COUP-TFs are present in nuclear extracts from HeLa cells. It is also possible that the high molecular weight band in our experiments represents a complex of COUP-TF1 monomer with other protein of similar molecular weight. Existence of such a complex has been reported for COUP-TF1 and RXR (Kliewer et al., 1992).

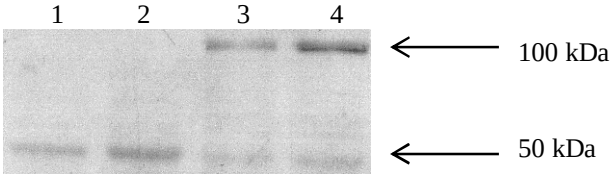

Figure 1. An example of a Western blot for COUP-TF1 in treated and non-treated samples. Lanes 1 and 2 represent treated samples, lanes 3 and 4 represent non-treated samples.

Table 1 shows that expression of the low and high molecular weight immuno-reactive proteins differed between the two breeds of pigs. LW pigs had more than a two-fold higher expression of both, 50 kDa and 100 kDa immuno-reactive proteins in liver when compared to D. The higher level of protein expression was accompanied by an increased level of CYP2E1 protein in the LW crosses (Table 1). These results suggest that a low level of CYP2E1 protein in pig liver might be related to a defective transcription of the CYP2E1 gene due to a low expression COUP-TF1.

Table 1. Expression of CYP2E1 protein in isolated microsomes, and high and low molecular weight COUP-TF immuno-reactive proteins in nuclear extracts from liver of pigs of two different breeds.

Protein expression (arbitrary units)	**Pig breed**		
	Duroc	**Large White**	**p-value**
COUP-TF1 50 kDa	32.1 ± 3.12	92.1 ± 13.7	0.011
COUP-TF1 100 kDa	21.0 ± 2.81	96.6 ± 10.9	0.001
CYP2E1	68.4 ± 4.79	102.9 ± 6.41	0.001

The results are presented as mean ± SEM (n=7).

Conclusion

1. This study has established the presence of low- and high-molecular weight COUP-TF1 immuno-reactive proteins in the nuclear extracts from pig liver.
2. Expression of both COUP-TF1 immuno-reactive proteins was higher in LW crossbred pigs when compared to D crossbred pigs.
3. A low expression of COUP-TF1 immuno-reactive protein was accompanied by a reduced level of CYP2E1 protein expression in hepatic isolated microsomes.

These data suggest that a low level of CYP2E1 protein in some pigs might be due to a defective expression of COUP-TF1 transcription factor.

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