

## Characterisation of 3-beta-hydroxysteroid dehydrogenase from pig liver and testis in relation to boar taint

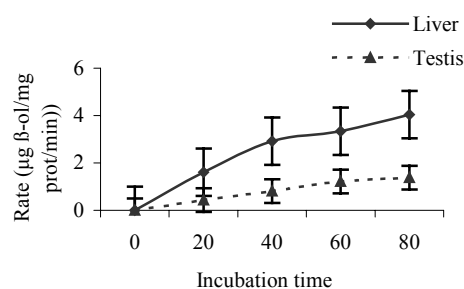
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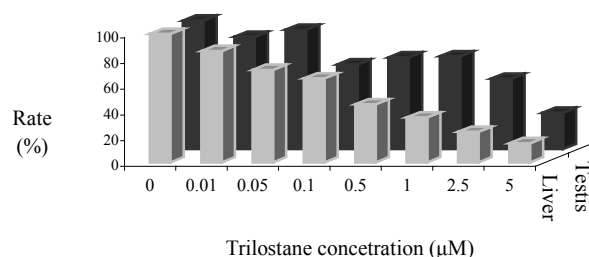
**Introduction** Boar taint is a major meat quality defect, which affects about 10% of entire male pigs. It is due to an excessive accumulation of skatole and androstenone in adipose tissue. One of the reasons for accumulation of these compounds is a low rate of their metabolism. Androstenone is metabolised in liver via the enzyme 3-beta-hydroxysteroid dehydrogenase (HSD). This enzyme is well characterised in the testis, where it participates in the synthesis of steroids, while its properties in liver are unknown. The aim of the present study was to characterise and compare properties of HSD from pig liver versus pig testis when metabolising androstenone.

**Materials and methods** Liver and testis samples were collected from 5 Large-White pigs immediately after slaughter. Microsomes were isolated by differential centrifugation and frozen at  $-80^{\circ}\text{C}$  until use. Microsomes were incubated under different conditions in order to determine HSD kinetic parameters and metabolic rates with androstenone as substrate and NADH as cofactor. Rates were estimated by recording the appearance of the main metabolite,  $\beta$ -androstenol ( $\beta$ -ol), by high-resolution gas chromatography. For kinetic experiments the incubation conditions were:  $37^{\circ}\text{C}$ , pH 7.4 and incubation time 20 min, except when different pH of the incubation media were used for determining the optimum pH. Different concentrations of androstenone as substrate and trilostane as a HSD specific inhibitor were used. The amount of HSD protein in isolated microsomes was determined by Western Blotting using specific antibodies. ANOVA was used for the statistical analysis.

**Results** The rate of androstenone metabolism was higher in liver than in testis (Figure 1). The kinetic study revealed that hepatic HSD has lower affinity for androstenone as a substrate in comparison with testicular HSD. However, hepatic HSD is able to metabolise androstenone faster than the testicular enzyme. Differences were observed in the optimum pH range as well (Table 1). Inhibition studies showed that hepatic HSD was more sensitive to trilostane than testicular HSD (Figure 2). In both cases competitive inhibition occurred. Western Blotting results showed no differences in the amount of HSD protein in pig liver and testis (Table 1).



**Fig 1** HSD time-dependent curve for androstenone metabolism



**Fig 2** Trilostane effect on hepatic and testicular HSD

**Table 1** Metabolic rates, kinetic parameters and expression of HSD in pig liver and testis

Parameter	Liver	Testis
Metabolic rate ( $\mu\text{g } \beta\text{-ol/mg prot/min}$ )	1.28*	0.24*
$K_m$ ( $\mu\text{M}$ )	253* ( $\pm 38.9$ )	59* ( $\pm 24.3$ )
$V_{max}$ ( $\mu\text{g } \beta\text{-ol/mg prot/min}$ )	2.3* ( $\pm 0.5$ )	0.5* ( $\pm 0.1$ )
Optimum pH	7	6
Kind of inhibition of trilostane	competitive	competitive
Amount of HSD (arbitrary units)	90 ( $\pm 4$ )	92 ( $\pm 9$ )

(\* denotes statistical significance,  $p < 0.05$ )

**Conclusions** The results of this study demonstrate that HSD activity and kinetic characteristics differ in pig liver and testis. However, the amount of HSD protein expression was similar in both tissues. Therefore we conclude that there might be different HSD isoforms in pigs and their distribution is tissues specific. This study will be extended to different breeds in order to identify an apparent polymorphism in HSD. Such a polymorphism could be used to develop a genetic test for boar taint.

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