# PERFORMANCE TESTING FOR ANDROSTENONE, SKATOLE AND INDOLE: A PIVOTAL STEP TOWARDS GENETICALLY REDUCING BOAR TAINT

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Abstract - This study describes the establishment of a performance test for boar taint in the Swiss terminal sire line PREMO®. A biopsy device for tissue sampling selection candidates is introduced, data obtained from biopsy cores is presented and repeatability is validated. Statistical models for estimation of variance components based on biopsy data are tested, and models suitable for routine breeding value estimation are identified. Quantification of androstenone, skatole and indole from small samples was accurate and repeatable; estimates and heritability other genetic were plausible. parameters With the establishment and validation of a performance test suitable for use in large scale breeding programmes, a pivotal step has been made towards genetically resolving the problem of tainted meat and ending surgical castration.

Key Words – meat quality, pig breeding, variance component estimation

## I. INTRODUCTION

Boar taint, predominantly caused by the accumulation of androstenone (A), skatole (S) and indole (I) in fat tissue of boars, is a strong, unpleasant smell which occasionally emanates from heated pork. Although raising intact finishing males has several advantages over finishing castrates, the problem of boar taint has prevailed in the pork industry for decades and has hindered production of pork from intact males in many countries. The incidence of boar taint is commonly circumvented by surgically castrating male pigs shortly after birth. Due to increasing animal welfare concerns, however, surgical castration of pigs is likely to be banned in Europe in the near future; a viable alternative to castration is therefore required.

A great deal of research has been done to better understand the multi-factorial phenomenon of boar taint (see [1, 2, and 3] for reviews). Genetics, management practices, season, age and body weight have been identified as clear causal factors for tainted meat. Because of the distinct genetic variation between and within breeds, attention is focussing on breeding as a long term, non-invasive solution to the problem. To incorporate boar taint into breeding programmes, however, a number of issues must be addressed. Firstly, the definition of boar taint is still somewhat generic (reference methods and levels are not standardized). The identification of live male selection candidates with a high and low propensity for tainted meat must be warranted (performance testing). Furthermore, evidence suggests that A, S, and I may have unfavourable correlations to other economically important traits. Nevertheless, the incorporation of boar taint into current breeding programmes via statistical modelling has the potential to genetically improve meat quality and to reduce prevalence of tainted meat while avoiding surgical castration and hormone or vaccine use. In 2010, the Swiss service center for pig production (SUISAG) began working on a breeding programme against boar taint. Together with several scientific partners and a retailer, they set the goal of establishing a performance test for boar taint in sire lines and to develop a

feasible selection strategy against boar taint. This study describes the establishment of a performance test for boar taint in the Swiss terminal sire line PREMO®. A biopsy device for tissue sampling selection candidates is introduced and explained in detail. Data obtained using tissue from biopsy cores is presented and repeatability is validated by repeated sampling. Various statistical models for the estimation of variance components based on biopsy data are tested. Finally, models suitable for incorporation into routine breeding value estimation are identified.

# II. MATERIALS AND METHODS

#### Development of the biopsy device

For performance testing, identification of male selection candidates with a high and low propensity for tainted meat must be warranted. A biopsy device for collecting adipose tissue samples from breeding candidates was developed based on previous work by Topigs (Merks and Westerhof, personal communication, 2010) (Figure 1). The device, a modified rabbit stun device, was approved for use by the Swiss Federal Veterinary Office (BVET) and the Swiss Animal Protection (SAP).

Initial tests were done on carcasses to analyse the mechanical function of the device and to determine the amount of adipose tissue extractable from the biopsy core. Because of the small sample size, special chemical extraction methods for the determination of A, S, and I contents were developed [4].

Biopsy tissue samples were taken from the neck area at a  $45^{\circ}$  angle to the skin. Behavioural observations (vocalisation and movement) were made on live boars biopsied directly before slaughter. After ensuring that the biopsy technique evoked no or only minor reactions from test animals, further boars were biopsied 10 days before slaughter and wound healing was observed. The effect of disinfectant (D) prior to sampling and the application of antibiotic spray (A) after sampling was investigated (Group 1: 8 individually-housed boars with D + A treatment, Group 2: 6 boars in group housing with D treatment). The biopsy device was then implemented in a field setting.

## Model testing

Once the device function and lab methods were established, phenotypic data obtained from adipose tissue sampled by biopsy from the neck of 516 boars (9 herd book farms) was collected; some boars were biopsied more than once. In

order to replicate a performance testing scenario, only animals between 100 and 130 kg live weight were included in the analysis. After data editing. 528 observations remained. Transformations (log, ln) were tested to achieve a normal distribution of A, S and I. A total of 36 different mixed linear models were tested for each boar taint compound using the nlme package of R-2.13.0 [5]. No permanent environment effect was considered because only a very limited number of boars had multiple observations. Example model variations included consideration of age and / or live weight as covariables or as categorical fixed effects and various combinations thereof. The AIC and BIC were used to rank the models in terms of their information content. Significance of fix effects (categorical live weight at biopsy, categorical age at biopsy and farm/season) was tested using the ANOVA procedure of R-2.13.0 [5]. Based on these results, one model per boar taint compound was selected for further analysis.



Figure 1. Biopsy device for collecting adipose tissue samples from breeding candidates.

## Estimation of Genetic Parameters

The same data set described above was expanded by including a pedigree containing 2245 animals. Pedigree information was obtained from the herdbook computing center (SUISAG) in Sempach, Switzerland and included non-biopsied ancestors of biopsied animals (10 generations). Biopsied boars descended from 70 sires; the number of biopsied sons per sire ranged from 1 to 41, with an average family size of 5.9 sons per sire. On the maternal side, biopsied boars descended from 180 sows; the number of biopsied sons per sow ranged from 1 to 8, with an average family size of 2.4 sons per sow. The following mixed linear model was applied in VCE [6][7] to estimate variance components for A, S and I:

$$\mathbf{y}_i = \mathbf{X}\mathbf{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{W}\mathbf{v} + \mathbf{e},$$

where  $\mathbf{y}_{(mx1)}$  is the vector of phenotypic observations (A, S or I) for boar i,  $\beta_{(kx1)}$  is the vector of fixed effects (categorical weight at biopsy (for A only), categorical age at biopsy and herd/season),  $\mathbf{u}_{(nx1)}$  is a vector of random additive genetic effects,  $\mathbf{v}_{(rx1)}$  is a vector of random litter effects and e contains residual effects. random Subscripts in parenthesis of the vectors and matrices denote their dimensions; m is the number of observations. k is the number of levels of fixed effects, *n* is the number of animals in the full pedigree and r is the number of unique litter effects. The model also contains the known incidence matrices  $\mathbf{X}_{(m \times k)}$ ,  $\mathbf{Z}_{(m \times n)}$  and  $\mathbf{W}_{(m \times gam)}$ .

# III. RESULTS AND DISCUSSION

#### **Biopsy device**

With respect to wound healing, no discharge, redness or swelling was observed after 3 (5) days in Group 1 (2) animals. Furthermore, over 90% of animals tested showed no behavioural response to the biopsy procedure



Figure 2. Correlation between androstenone, skatole and indole values of two separate adipose samples from same individuals (Biopsy 1 and Biopsy 2, n = 18)

Although core size was only 200-300 mg of tissue (hair, skin, adipose tissue and muscle), quantification of A, S and I from these small

samples proved accurate and repeatable. For example, correlation of A values obtained using standard extraction methods and those obtained using biopsy cores were high (r = 0.938), and repeated sampling showed correlations close to unity for doubled biopsies (Figure 2).

Mean levels of A (mean = 0.578,  $\sigma$  = 0.527), S (mean = 0.033,  $\sigma$  = 0.002) and I (mean=0.032,  $\sigma$  = 0.002) were found to be within plausible ranges.

#### Estimation of Genetic Parameters

Univariate analysis of the data set resulted in heritability estimates for A comparable to those in the literature (Table 1). Surprisingly, heritabilities for S and I were higher than that of A. Standard errors for heritability ( $h^2$ ), however, were considerable, which is explained by the relatively small amount of data available.

Table 1 Phenotypic variance  $(\sigma_p^2)$ , heritability $(h^2)$ , litter  $(c^2)$  variance as a proportion of phenotypic variance and standard error (SE) from univariate analyses of boar taint compounds

Model	$\sigma_p^2$	$h^2$	SE(±)	<i>c</i> <sup>2</sup>	SE(±)
lnA	0.905	0.453	0.108	0.163	0.067
lnS	0.548	0.524	0.041	0.115	0.067
lnI	0.344	0.571	0.099	0.012	0.071

Multivariate analysis provided very similar results. Genetic and phenotypic correlations were comparable to those found in the literature (Table 2). Standard errors (SE) from multivariate analyses ranged from 0.063 to 0.122.

Table 2. Phenotypic (lower diag.) and genotypic correlations (upper diag.), and heritabilities (diag.) from multivariate analysis

	Androstenone	Skatole	Indole
A	0.452	0.110	0.354
S	0.278	0.495	0.902
Ι	0.256	0.739	0.550

These results show that data on boar taint compounds obtained from small adipose samples provide similar genetic parameters as described in the literature for larger samples.

#### IV. CONCLUSION

With the establishment and validation of a performance test suitable for use in large scale breeding programmes, a pivotal step has been made towards genetically resolving the problem of tainted meat and ending surgical castration. The performance test is imperative for incorporating boar taint components into current breeding programs and provides the basis for selection of animals with a low risk of boar taint. Routine sampling for regular breeding value estimation has begun. Once more data has been collected, parameters can be re-estimated and standard errors are expected to decrease. Reliable genetic correlations to production and reproduction traits will be calculated with this expanded data set.

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