EFFECT OF HCG STIMULATION ON ANDROSTENONE, SKATOLE AND INDOLE LEVELS IN ENTIRE MALE PIGS

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Background

Skatole, indole and androstenone are the compounds responsible for boar taint, an offensive odour detectable when cooking pork. Due to their lipophilic properties, they accumulate in adipose tissue of some entire male pigs. Skatole and indole are formed from tryptophan in the colon of pigs (Claus et al., 1994). Skatole passes through the liver where most of it is metabolised by cytochrome P450. The activity of this enzyme system, therefore, affects skatole levels (Babol et al., 1998). Environmental factors and nutrition are also important in the regulation of skatole levels (Walstra et al., 1999).

Androstenone is a testicular steroid and its production is linked to the synthesis of anabolic testicular hormones. Puberty development results in increased androstenone levels (Claus et al., 1994). Environmental factors such as season, photoperiod (Andersson et al., 1998) and nutrition levels can influence the time of puberty and thus androstenone levels in adipose tissue at slaughter.

HCG injection causes an abrupt increase in androstenone both in plasma and fat (Carlström et al., 1975; Andresen, 1975; Bonneau et al., 1982), but to our knowledge an eventual effect on skatole has not been studied. High level of androstenone may inhibit the expression of CYP 2E1 in liver (Doran et al., 2002), one of the enzymes involved in skatole metabolism. Subsequently, much skatole remains unmetabolised and can then accumulate into fat. Some researchers showed that androstenone levels correlated positively with skatole levels in adipose tissue at slaughter age as reviewed by Walstra et al. (1999). A positive correlation between androstenone and skatole levels in plasma at 20 weeks of age was also found (Zamaratskaia et al., 2004a). Generally, however, results vary on the relationship between androstenone and skatole levels.

Objectives

The aim of the present study was to investigate the effect of hCG stimulation and the relationships of androstenone, skatole and indole levels in plasma and fat after hCG stimulation in entire male pigs.

Materials and methods

Animals

A total of 34 entire male pigs of a crossbred (Swedish Yorkshire dams x Landrace sires) were used in this study. Animals were raised in single-sex pens with 7 pigs in each. All pigs were fed the same commercial diet according to the standard feeding regimen for finishing pigs in Sweden (restricted, 12 MJ ME per kg, digestible CP 13%).

HCG injection was performed (Pregnyl, 30 IU/kg body weight) 4 days prior to slaughter. Control pigs were injected with sterile saline the same day. Blood samples were taken from all pigs twice: before injection and the day before slaughter. Plasma samples were kept in -80°C until analysis. Back fat was taken at slaughter and kept in -20°C until analysis.

Analysis of skatole, indole and androstenone

Skatole and indole levels in plasma were measured with HPLC as described by Zamaratskaia et al. (2004a). Skatole levels in fat were measured with a colorimetric procedure (Mortensen and Sørensen, 1984). Androstenone levels in plasma and fat were measured with an ELISA method described by Squires and Lundström (1997). Androstenone was extracted from plasma with ethyl acetate and from fat with methanol.

Statistical analysis

All data were analysed with the Statistical Analysis System, version 8.2 (SAS Institute, Cary, NC, USA). Procedure Mixed was used for evaluating the results. The model included treatment and time of sampling as fixed factors, and individual pig within sire, dam and treatment as random factors. A logarithmic transformation was applied to the levels of skatole, indole and androstenone to normalise the distributions of observed values. Results are presented after back transformation.

Results and discussion

Androstenone analysis in plasma with ELISA method has been used by many researchers. The direct plasma analysis (without extraction), however, led to overestimation and extreme values could be observed (above 200 ng/ml). To avoid this overestimating effect, extraction procedure was used prior to analysis in this study.

Three pigs were used in a pilot study to evaluate the experimental design. The blood samples were taken at five occasions: before hCG injection, each day during three days after injection and the day prior to slaughter. The results showed a rapid increase in androstenone level, a plateau on the second day and a subsequent decline. This is in line with the former Swedish results (Carlström et al., 1975), where maximum levels of androstenone in plasma were found the second day after hCG-injection. Overall, no effect on skatole or indole levels was observed; however, a pronounced increase in both compounds was observed within some individuals causing a high variation (Figure 1).

HCG stimulation significantly increased androstenone levels in plasma ($P < 0.001$; Table 1), whereas skatole levels in plasma did not differ between treatments. Surprisingly, plasma indole levels increased significantly ($P = 0.03$; Table 1). This increase is difficult to explain. Probably, high androstenone levels inhibit the enzymes involved in indole metabolism more efficiently than skatole levels. Both skatole and indole are metabolised in the liver with the same enzymatic system (Gillam et al., 2000). However, the precise metabolism of indole has not been the subject of intensive research, and factors affecting indole metabolism are not yet known. The effect of hCG administration on both skatole and indole levels needs to be further studied.

HCG injection also caused a significant increase in androstenone levels in fat ($P < 0.001$; Table 1). The mean androstenone level for the control group was 1.03 µg/g and for hCG treatment group 4.22 µg/g, approximately four fold higher than the control. Skatole levels in fat were significantly higher in the hCG-treated group compared to the control. It should be noted that skatole levels in fat in this study were measured by the colorimetric method. This method is not specific for skatole, but measures the total amount of indolic compounds. Therefore, the observed differences between treatments in fat might rather be due to indole than skatole concentrations. Indole may also partially contribute to boar taint (Garca-Regueiro and Diaz, 1989). However, the level above which consumers can perceive indole odour is not specified. It is assumed that indole is not of the same importance for boar taint as skatole and androstenone because of weaker odour.

The mean skatole levels in fat in control and hCG injection groups were 0.09 µg/g and 0.13 µg/g, with 1 and 4 pigs exceeding the rejection level of 0.20 µg/g respectively. The androstenone levels were relatively high even in pigs without hCG-stimulation. The mean value for androstenone in the control group was 1.03 µg/g. In this group, 13 and 8 out of 17 pigs exceeded the threshold levels for androstenone in fat of 0.5 and 1.0 µg/g respectively.

The correlation coefficients between skatole and androstenone levels in plasma at the two sampling occasions were 0.22 ($P = 0.44$) before hCG stimulation, and 0.03 ($P = 0.88$) after hCG stimulation. The relationship between skatole and androstenone levels in plasma after hCG stimulation has not been studied previously. Androstenone might be involved in skatole metabolism by the inhibition of CYP2E1 in vitro (Doran et al., 2002). High androstenone levels would then lead to high skatole levels. However, in an in vivo study, high androstenone levels did not result in immediately increased skatole levels (Zamaratskaia et al., 2004a, b). In these studies, the pubertal increases in testicular steroids preceded the rise in skatole levels. In the present study, skatole and androstenone in plasma were measured three days after hCG stimulation.

Probably, this time was not enough for androstenone to perform its action on liver enzymes. In this case, the increase in skatole levels can be delayed. The other explanation could be that androstenone levels after hCG stimulation still did not reach a sufficient level to inhibit CYP2E1 in vivo. The androstenone levels used in the in vitro study by Doran et al. (2002) were probably much higher than can be achieved in the liver in vivo. If so, no increase in skatole as a result of inhibiting action of testicular steroids could be anticipated.

The positive correlation between skatole and indole levels in plasma (r = 0.49) is probably due to the same intestinal origin and the linked metabolic pathway. Skatole levels in plasma and fat strongly correlated (r = 0.83). This is in agreement with previous studies (Zamaratskaia et al., 2004a). The correlation between androstenone and skatole levels in fat at slaughter was 0.47 in control animals and 0.39 after hCG stimulation. The lower correlation coefficient in the hCG-treated group may be related to the fact that androstenone levels in this group increased rapidly, whereas no such high increase in skatole levels was observed.

Conclusions

HCG stimulation significantly increased androstenone levels in both plasma and fat. Skatole levels in fat were also higher in hCG-treated pigs. This increase might be related to the variations in indole levels, since indole but not skatole levels in plasma were higher after hCG stimulation. High androstenone levels did not cause increased skatole levels in plasma with the sampling pattern used in this study. This would have been expected if androstenone inhibited the expression of the main enzymes of skatole metabolism.

References

Andersson, H., Wallgren, M., Rydhmer, L., Lundström, K., Andersson, K. and Forsberg, M. 1998. Photoperiodic effects on pubertal maturation of spermatogenesis, pituitary responsiveness to exogenous GnRH, and expression of boar taint in crossbred boars. Anim. Reprod. Sci. 54:121-137.

Andresen, Ø. 1975. 5α-androstenone in peripheral plasma of pigs, diurnal variation in boars, effects of intravenous hCG administration and castration. Acta. Endocr. 78: 385-391.

Babol, J., Squires, E.J. and Lundström, K. 1998b. Relationship between oxidation and conjugation metabolism of skatole in pig liver and concentrations of skatole in fat. J. Anim. Sci. 76:829–838.

Bonneau, M., Meusy-Dessolle, N., Léglise, P.C. and Claus, R. 1982. Relationships between fat and plasma androstenone and plasma testosterone in fatty and lean young boars during growth and after hCG stimulation. Acta. Endocr. 101: 119-128.

Carlström, K., Malmfors, B., Lundström, K., Edqvist, L.E. and Gahne, B. 1975. The effect of HCG on blood plasma levels of 5α-androstenone and testosterone in the boar. Swedish J. Agric. Res. 5:15-21.

Claus, R., Weiler, U. and Herzog, A. 1994. Physiological aspects of androstenone and skatole formation in the boar—A review with experimental data. Meat Sci. 38:289–305.

Doran, E., Whittington, F.W., Wood, J.D. and McGivan, J.D. 2002. Cytochrome P450IIE1 (CYP2E1) is induced by skatole and this induction is blocked by androstenone in isolated pig hepatocytes. Chem. Biol. Interact. 140:81-92.

Garca-Regueiro, J.A. and Diaz, I. 1989. Evaluation of the contribution of skatole, indole, androstenone and androstenols to boar-taint in back fat of pigs by HPLC and capillary gas chromatography (CGC). Meat Sci. 25:307-316.

Gillam, E.M., Notley, L.M., Cai, H., De Voss, J.J. and Guengerich, F.P. 2000. Oxidation of indole by cytochrome P450 enzymes. Biochem. 39:13817-13824.

Mortensen, A.B. and Srensen, S.E. 1984. Relationship between boar taint and skatole determination with a new analysis method. In Proceedings of 30th European Meeting Meat Research Workers, Bristol, UK, 394-396.

Squires, E.J. and Lundstrom, K. 1997. Relationship between cytochrome P450IIE1 in liver and levels of skatole and its metabolites in intact male pigs. J. Anim Sci. 75:2506-2511.

Walstra, P., Claudi-Magnussen, C., Chevillon, P., von Seth, G., Diestre, A., Matthews, K.R., Homer, D.B. and Bonneau, M. 1999. An international study on the importance of androstenone and skatole for boar taint: levels of androstenone and skatole by country and season, Livest. Prod. Sci. 62:15-28.

Zamaratskaia, G., Babol, J., Andersson, H. and Lundström K. 2004a. Plasma skatole and androstenone levels in entire male pigs and relationship between boar taint compounds, sex steroids and thyroxine at various ages. Livest. Prod. Sci.(in press).

Zamaratskaia, G., Babol, J., Madej, A., Squire, E.J. and Lundström, K. 2004b. Age-related variation of plasma concentrations of skatole, androstenone, testosterone, oestradiol-17β, oestrone sulphate, dehydroepiandrosterone sulphate, triiodothyronine and IGF-1 in six entire male pigs. Reprod. Dom. Anim. 39:1-5.

Table 1. Concentrations of androstenone, skatole and indole in plasma and fat from entire male pigs before and after hCG injection or at slaughter (least-square means)

	Control group			hCG injection group		
	Before injection	Day before slaughter / Slaughter day	P-value	Before injection	Day before slaughter / Slaughter day	P-value
Plasma (ng/ml)						
Androstenone	1.01	1.32	0.22	1.19	6.42	0.001
Skatole	2.97	2.75	0.59	3.54	4.10	0.29
Indole	2.25	2.66	0.33	2.74	3.98	0.03
Fat (µg/g)						
Androstenone		1.03			4.22	0.001
Skatole		0.09			0.13	0.03

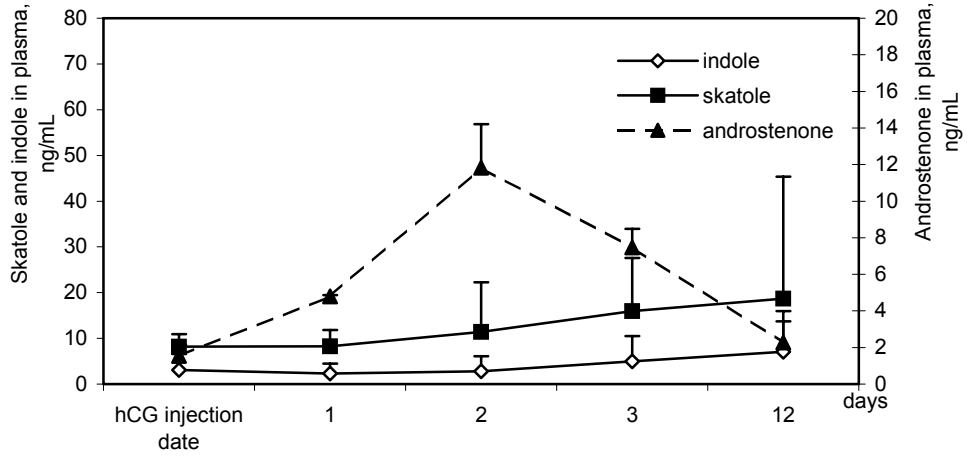

Figure 1. Androstenone, skatole and indole levels in plasma after hCG stimulation in three entire male pigs (mean value + standard deviation).