51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

NOVEL DATA ON DIETARY SUPPLEMENT OF RAW POTATO STARCH AND BOAR TAINT IN PUBERTAL MALE PIGS

G. Zamaratskaia[1], G. Chen[1], H. K. Andersson[2], K. Lundström[1]*

[1]Dept of Food Science and [2]Dept of Animal Nutrition and Management, SLU, Uppsala, Sweden

Key Words: Androstenone, Indole, Skatole, Raw Potato Starch

Introduction

Castration of male piglets is widely practiced in most European countries to prevent boar taint, an unpleasant odour in meat from some entire male pigs that occurs when they reach sexual maturity. Nowadays, there is a growing interest in use of entire male pigs instead of castrated because of improved animal welfare and superior carcass characteristics. However, if entire male pigs are to be used for pork production, the taint has to be eliminated to avoid consumer dissatisfaction.

Skatole (3-methylindole) and androstenone (5α-androst-16-en-3-one) are the major contributors to boar taint. Indole may also contribute to the taint, but to a lesser degree because of relatively weak odour and weak lipophilic properties. Both skatole and indole are produced from the amino acid tryptophan in the large intestine by bacteria, and feeding system affects their levels in entire male pigs (Van Oeckel et al., 1998) and castrates (Van Heugten and Van Kempen, 2002; Willig et al., 2005).

Androstenone is synthesized in the testes and mainly affected by puberty stage and individual ability of the pig to produce this steroid (Bonneau, 1998). In the regulation of androstenone levels, dietary factors are less important unless they affect puberty (Øverland et al., 1995). Studies investigating nutritional effects on boar taint, have demonstrated that specific ingredients in the diet are not involved in the regulation of androstenone levels (Whittington et al., 2004; Zamaratskaia et al., 2004).

Objectives

The aim of the present study was to evaluate the effect of live weight at slaughter and dietary supplement of raw potato starch on the levels of skatole, indole and androstenone in fat and plasma from entire male pigs. This study was the second replicate of a large project on effects of diet, raising system and live weight at slaughter on boar taint and aggression level. The results of the first replicate are presented elsewhere (Zamaratskaia et al. 2004).

Methodology

A total of 100 entire male pigs of a crossbred (Swedish Landrace sires x Swedish Yorkshire dams) were included in the study. The pigs were slaughtered at the average live weight (LW) of either 90 or 115 kg. All pigs slaughtered at 90 kg LW (n=28), and half of the pigs slaughtered at 115 kg LW (n=36), were fed commercial diet. The remaining pigs (n=36) were additionally fed 0.6 kg of raw potato starch (RPS, Lyckeby Culminar AB) per pig and day for two weeks prior to slaughter. Skatole, indole (HPLC) and androstenone (ELISA) were measured in plasma, collected the day before slaughter, and adipose tissue (HPLC for all) collected at slaughter. Androstenone levels in plasma were measured by ELISA with and without extraction with ethyl acetate.

The fixed effects of live weight (90 or 115 kg LW) and diet (with or without RPS) were evaluated using Procedure Mixed (Statistical Analysis System, version 8.2, SAS Institute, Cary, NC, USA). The effects were evaluated separately for plasma and fat levels of investigated substance. Effects of individual pig nested within dam and sire (for plasma model), or dam and sire (for fat model) were included as random factors.

Results & Discussion

The diet with RPS induced a significant reduction in skatole levels in fat and plasma ($P<0.001$), but indole levels remained unchanged ($P>0.05$) (Table 1). This reduction in skatole levels was expected since RPS is not fully digestible in the upper gastrointestinal and undergoes bacterial fermentation in the large intestine affecting the microflora and intestinal functions (Jensen et al., 1995, Claus et al., 2003). However, the precise mechanism of dietary impact on skatole levels is still unclear. Claus et al. (2003) explained RPS impact on skatole levels by the inhibition of cell apoptosis and thus reduction in tryptophan availability for skatole production. However, if skatole reduction in the pigs fed RPS is due to decreased tryptophan availability, indole reduction could also be expected. Thus, the involvement of other factors in the mechanism of RPS action in the large intestine should be considered. Firstly, diet may dramatically affect intestinal transit time and, therefore, the absorption of skatole from intestinal walls (Jensen et al., 1995). Secondly, tryptophan biotransformations in the gut are pH dependent (Jensen et al., 1995), and altered skatole/indole ratio in the present work might be due to a shift in the synthesis because of lower pH. Interestingly, Willig et al. (2005) reported lower indole concentrations in faeces when pigs were fed RPS, while Rideout et al. (2004) demonstrated no significant changes in indole levels in faeces after feeding chicory inulin. The differences in the response between skatole and indole to dietary changes require further investigations, in which qualitative and quantitative evaluations of intestinal microflora should be included. It is likely that diet affects indolic compounds through the alterations in the gut flora and pH, and potential changes in tryptophan availability are less important.

As expected, the diet with RPS did not affect androstenone levels in fat ($P>0.05$) (Table 1). As mentioned above, androstenone levels primarily depend on maturity stage (Bonneau, 1998), and diet does not affect the levels (Whittington et al., 2004; Zamaratskaia et al., 2004a). However, the effect of RPS on plasma androstenone levels differed depending on the method used to measure androstenone. Even if the correlation

coefficient between these two methods was high (r=0.70), androstenone values obtained by direct ELISA were much greater compared to those obtained after including the extraction step. This difference might be because most androstenone in plasma exists in sulphated form (Sinclair et al, 2005). It is likely, that ELISA without extraction procedure measured total androstenone (free and conjugates), whereas extraction separates free and sulphated forms, and obtained final values reflect free androstenone concentrations in plasma. Androstenone levels measured by direct ELISA (without extraction procedure) were not affected by diet. Conversely, the levels measured by ELISA including extraction step were significantly lower in the pigs fed RPS compared to those in the pigs fed only commercial diet. This was unexpected and difficult to explain. Generally, in the discussion about androstenone metabolism, only hepatic metabolism is considered. However, androstenone metabolism might occur in other tissues, e.g. the intestine. The presence of phase II enzymes, including sulphotransferase, in the intestine has been demonstrated (Falany, 1997), and these enzymes can be either up-regulated or inhibited by components of the diet. We speculate that dietary changes accelerate intestinal sulphation of androstenone through the activation of the enzyme sulphotrasferase. Thus, the ratio free/conjugated androstenone was changed, whereas the levels of total androstenone remained constant. This is an important finding. Upon sulphation, sulphostransferase substrates become polar and thus suitable for rapid excretion. We suggest that manipulation of intestinal enzymes by dietary changes can increase androstenone sulphation and thus reduce fat levels of androstenone. Interestingly, androstenone levels in fat in the present study slightly decreased after feeding RPS (0.56 vs. 0.81 µg/g), although this decrease did not reach statistical significance. These results about a possible impact of RPS on androstenone levels in plasma have never been shown before, but probably more than 14 days of RPS feeding is needed to reach a significant reduction in fat. More research is certainly needed to investigate the dietary impact on androstenone metabolism and excretion.

Slaughter at lower weight (90 kg) did not affect the levels of skatole, indole and androstenone in plasma, and skatole and indole in fat (Table 1). An age-related increase in the levels of those compounds was demonstrated in our previous studies (Babol et al., 2004 and Zamaratskaia et al., 2004). It was suggested that the increase in skatole levels might be due to decreased activities of enzymes CYP2A6 and CYP2E1 in older pigs (Zamaratskaia et al., 2005). In the present study, however, the activities of CYP2A6 and CYP2E1, measured in the liver from the same animals, did not differ between pigs slaughtered at 90 and 115 kg (unpublished). This might explain the absence of age-related increase in the levels of indolic compounds. Additionally, an increase in skatole levels often coincides with onset of puberty (Zamaratskaia et al., 2004), and the levels of circulating endogenous steroids, particularly androstenone, are implicated in the increase in skatole levels (Doran et al., 2002). Given that plasma androstenone levels in the present study did not vary with live weight, no variations in skatole levels could be expected. Increased androstenone levels in fat in the pigs at 115 kg suggest that androstenone accumulation in fat does not ultimately depend on plasma levels. This suggestion is supported by conflicting results from the correlation analysis between androstenone levels in fat and plasma obtained in different studies. Sinclair et al. (2001) explained this observation by the ability of androstenone to accumulate differently in the

fat with different composition. However, the nature of this effect is not clear and should be further studied.

Conclusions

Dietary supplement of raw potato starch significantly reduced skatole levels in fat and plasma, but indole levels remained unchanged. The levels of free androstenone in plasma were significantly lower in pigs fed RPS although the levels of total androstenone were not affected by diet. Androstenone levels in fat were slightly lower in pigs fed RPS; however, this decrease did not reach statistical significance. Therefore, dietary supplement of raw potato starch can be used to manipulate skatole and perhaps also androstenone levels in entire male pigs. The effects of potato starch on the levels of boar taint compounds need to be further investigated.

Slaughter at lower weight (90 kg vs. 115 kg) did not reduce the levels of skatole, indole and androstenone in plasma, and skatole and indole levels in fat, but fat androstenone levels were significantly lower in pigs at 90 kg.

References

Bonneau, M., 1998. Use of entire males for pig meat in the European Union. Meat Sci., 49, S257–S272.

Babol, J., Zamaratskaia, G., Juneja, K., Lundström, K., 2004. The effect of age and distribution of skatole and indole levels in entire male pigs in four breeds: Yorkshire, Landrace, Hampshire and Duroc, Meat Sci. 67, 351–358.

Claus, R., Lösel, D., Lacorn, M., Mentschel, J., Schenkel, H., 2003. Effects of butyrate on apoptosis in the pig colon and its consequences for skatole formation and tissue accumulation, J. Anim. Sci. 81, 239–248.

Doran, E., Whittington, F.W., Wood, J.D., 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.

Falany, C.N., 1997. Enzymology of human cytosolic sulfotransferases. FASEB J., 11, 206–216.

Jensen, M.T., Cox, R.P., Jensen, B.B., 1995. Microbial production of skatole in the hind gut of pigs given different diets and its relation to skatole deposition in backfat, Anim. Sci., 61, 293–304.

Rideout, T.C., Fan, M.Z., Cant, J.P., Wagner-Riddle, C., Stonehouse, P., 2004. Excretion of major odor-causing and acidifying compounds in response to dietary supplementation of chicory inulin in growing pigs. J. Anim. Sci., 82, 1678–1684.

Sinclair, P.A., Squires, E.J. and Raeside, J.I., 2001. Early postnatal plasma concentrations of testicular steroid hormones, pubertal development, and carcass leanness as potential indicators of boar taint in market weight intact male pigs. J. Anim. Sci. 79, 1868–1876.

Sinclair, P.A., Squires, E.J., 2005. Testicular sulfoconjugation of the 16-androstene steroids by hydroxysteroid sulfotransferase: its effect on the concentrations of 5alpha-androstenone in plasma and fat of the mature domestic boar. J. Anim. Sci., 83, 358–365.

Van Heugten, E., Van Kempen, T.A., 2002. Growth performance, carcass characteristics, nutrient digestibility and fecal odorous compounds in growing-finishing pigs fed diets containing hydrolyzed feather meal. J Anim Sci., 80, 171–178.

Van Oeckel, M.J., Warnants, N., De Paepe, M., Casteels, M. and Boucque, Ch.V., 1998. Effect of fibre-rich diets on the backfat skatole content on entire male pigs. Livest. Prod. Sci., 56, 173–180.

Whittington, F., M., Nute, G. R., Hughes, S. I., McGivan, J. D., Lean, I. J., Wood, J. D., Doran, E., 2004. Relationships between skatole and androstenone accumulation, and cytochrome P4502E1 expression in Meishan × Large White pigs. Meat Sci, 67, 569–576.

Willig, S., Lösel, D., Claus, R., 2005.Effects of resistant potato starch on odor emission from feces in swine production units. J. Agric. Food Chem., 53, 1173–1178.

Zamaratskaia, G., Babol, J., Andersson, H.K., Andersson, K., Lundström, K., 2004. Effect of live weight and dietary supplement of raw potato starch on the levels of skatole, androstenone, testosterone and oestrone sulphate in entire male pigs. Livest. Prod. Sci. (in press).

Zamaratskaia, G., Squires, E.J., Babol, J., Andersson, H.K. Andersson K., Lundström, K., 2005. Relationship between the activities of cytochromes P4502E1 and P4502A6 and skatole content in fat in entire male pigs fed with and without raw potato starch. Livest. Prod. Sci. (in press).

Øverland, M., Berg, J., Matre, T., 1995. The effect of feed and feeding regime on skatole and androstenone levels and on sensory attributes of entire male and female pigs. In: Proceeding of Meeting EAAP Working Group, Production and Utilisation of Meat from Entire Male Pigs. Milton Keynes, UK, 6 pp.

Table 1. Least-squares means and 95% confidence intervals (within brackets) of skatole, androstenone and indole levels from entire male pigs at two slaughter weights and diets

		Slaughter weight /diet			
Site of measurements	Compound	90kg	115kg no RPS	115kg + RPS	P-value
Plasma, ng/mL	Skatole	4.80[a] (3.43-6.70)	3.88[a] (2.68-5.62)	0.74[b] (0.51-1.07)	0.001
	Indole	1.44 (1.03-2.01)	1.67 (1.17-2.39)	1.69 (1.18-2.43)	0.223
	Androstenone (direct ELISA)	15.01 (11.78-19.12)	16.10 (11.97-21.66)	17.98 (13.41-24.12)	0.253
	Androstenone (ELISA with extraction step)	4.02[a] (3.02-5.36)	4.52[a] (3.12-6.56)	2.09[b] (1.45-3.02)	0.001
Fat, µg/g	Skatole	0.06[a] (0.04-0.11)	0.05[a] (0.03-0.09)	0.01[b] (0.01-0.01)	0.001
	Indole	0.02 (0. 01-0.03)	0.02 (0.01-0.03)	0.02 (0.01-0.03)	0.682
	Androstenone	0.40 [a] (0.21-0.78)	0.81 [b] (0.43-1.51)	0.56 [ab] (0.30-1.04)	0.072

Data are presented after back-transformation. Least-squares means with different superscripts differ ($p<0.05$).