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Skatole metabolism in the intact pre-pubescent male pig: The relationship between hepatic enzyme activity and skatole concentrations in plasma and fat

F. Lanthier, Y. Lou, E.J. Squires *

Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

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Abstract

The objective of this study was to evaluate, in the pre-pubescent intact male pig, the relationship between skatole levels and the activity of hepatic cytochrome *P*4502E1 (CYP2E1), cytochrome *P*4502A (CYP2A), aldehyde oxidase (AO), and phenol sulfotransferase 1A1 (SULT1A1). The activity of these enzymes has been positively associated with skatole clearance in mature boars. Twenty-four intact male pigs were weaned at 28 days of age and slaughtered 2 weeks postweaning, at which time caecal contents, blood, fat, and liver samples were collected. Caecal contents and fat were analyzed for skatole concentrations, and plasma was analyzed for skatole and steroid hormone (testosterone (T), dehydroepiandrosterone (DHEAS), estrone sulphate (E_1S)) concentrations. CYP2A, CYP2E1, and AO, as well as SULT1A1 activities were evaluated in liver samples. Stepwise regression was utilized considering plasma or fat skatole concentration as the dependent variables and hormone concentrations and enzyme activities as independent variables. The activities of the enzymes CYP2A, CYP2E1, and AO and concentrations of the hormones T, DHEAS, or E_1S were not correlated with concentrations of skatole in plasma or fat. However, SULT1A1 activity was negatively correlated with plasma ($r=-0.70$, $P<0.05$) and backfat ($r=-0.41$, $P<0.05$) skatole concentrations. Furthermore, this correlation was improved in plasma ($r=-0.88$, $P<0.05$) and fat ($r=-0.63$, $P<0.05$) when the concentrations of skatole in caecal contents was included as an independent variable in the multiple regression analysis, demonstrating the importance of measuring skatole production in these studies. T, DHEAS, and E_1S concentrations in plasma were not correlated with the activity of any of the enzymes evaluated. This study suggests that SULT1A1 is important in the metabolism of skatole in pre-pubescent pigs and the overall metabolism of skatole in the pre-pubescent pig differs from that in the mature boar.

Keywords: Boar taint; Prepubescent; Pig; Steroid hormone; CYP2E1; CYP2A; Aldehyde oxidase; SULT1A1

1. Introduction

3-Methylindole (skatole) is produced by the microbial degradation of tryptophan in the hindgut of pigs (Yokoyama and Carlson, 1979) and is a major component of the fecal-like odor and bitter taste known as boar taint (Bonneau et al., 2000; Spoelstra, 1977). Elevated concentrations of skatole have been observed in estrous sows (Claus et al., 1993) and in barrows fed diets favouring skatole production (Claus et al., 2003); however, under normal physiological and rearing conditions, the accumulation of skatole in fat resulting

* Corresponding author. Tel.: +1 519 824 4120x53298; fax: +1 519 836 9873.
E-mail address: jsquires@uoguelph.ca (E.J. Squires).

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in taint is a problem associated with entire (uncastrated) male pigs. Skatole can be absorbed through the skin or the lungs if the pigs are left to lie in feces or if ventilation is inadequate (Hansen et al., 1994). Under proper management, the major route of absorption for skatole is from the gastrointestinal tract into the portal circulation (Jensen et al., 1998). There is some discrepancy in the literature in terms of the relationship between skatole production in the hindgut and the resulting accumulation in fat. While some groups report no significant correlations between skatole concentrations measured in the hindgut and concentrations in fat (Hawe et al., 1992; Hansen et al., 1994), others have found significant positive correlations between concentrations of skatole in the hindgut and fat (Agergaard and Jensen, 1993; Jensen and Jensen, 1993). At best it seems that the concentrations of skatole measured in the hindgut account for approximately 51% (Jensen and Jensen, 1993) to 58% (Agergaard and Jensen, 1993) of the variation of skatole in fat, suggesting that other factors influence the degree of skatole accumulation. Utilizing a multicatheter model, Agergaard and Jensen (1993) reported that skatole concentrations in the hepatic vein are less than 50% of the concentration in the portal vein, suggesting significant hepatic clearance of skatole in pigs. Indeed, a number of hepatic Phase I and Phase II enzymes have been associated with the effective clearance of skatole. In post-pubescent pigs, the activities of cytochrome *P*4502A (CYP2A; (Diaz and Squires, 2000a), cytochrome *P*4502E1 (CYP2E1; Squires and Lundström, 1997), aldehyde oxidase (AO; Diaz and Squires, 2000b), and phenol sulfotransferase (SULT1A1; Babol et al., 1998) are negatively correlated with skatole concentrations in fat. While high hepatic enzyme activity is consistently associated with low skatole concentrations in fat, low enzyme activity can result in both high and low skatole accumulation (Squires and Lundström, 1997). Thus, the accumulation of skatole in fat is likely the result of two factors: the degree of skatole production and absorption from the hindgut, and the degree of skatole metabolism and clearance in the liver.

Babol et al. (2004) reported that increased plasma skatole concentrations coincide with puberty, a time during which plasma steroid hormones also increase (Ford, 1983; Tan and Raeside, 1980). The onset of puberty as well as the ensuing rise in steroidogenesis is highly variable between individual boars. This is likely because the onset of puberty is influenced by factors such as stress, social rank (DeJonge et al., 1996), nutritional status (Brown, 1994) and breed (Babol et al., 2004). Unfortunately, the onset of puberty and increase in skatole concentrations often occurs at a time where male pigs reach market weight. For this reason, male pigs destined for meat are usually castrated in order to eliminate the chance of taint; however, this practice increases production costs and reduces carcass advantages as a result of decreased lean meat yield and feed efficiency (Babol and Squires, 1995). Furthermore, due to the wide time span over which puberty occurs, studying boar taint during pubertal development is impractical.

Lanthier et al. (2006) have shown that the pre-pubescent, or weanling pig produces a predictable elevation in plasma skatole, and thus, the present study was conducted using just weaned piglets. The objective of this study was to assess the relationship between skatole concentrations in tissues of pre-pubescent intact male pigs and the activities of CYP2A, CYP2E1, AO, and SULT1A1 in the liver in order to further evaluate the potential of this animal as an *in vivo* model for studying skatole metabolism.

2. Materials and methods

2.1. Animals and sampling

Thirty-four Yorkshire just weaned piglets obtained from the Arkell Swine Research Station at the University of Guelph were used in the following studies. After weaning at 28 days of age, the piglets were fed a grower diet containing 22.2% protein and 14.6MJ DE *ad libitum*. Five piglets were slaughtered the day of weaning, 24 were slaughtered at 14 days postweaning, while the remaining 5 pigs were slaughtered at 28 days postweaning. Blood, liver, backfat and caecal content samples were collected at slaughter. Samples were centrifuged (1100×*g*) at 4 °C to collect plasma and stored at −20 °C until analyses were performed. All experiments were done in accordance with the University of Guelph Animal Care and Utilization Committee regulations.

2.2. Biochemical analyses

Concentrations of skatole in plasma, fat, and caecal contents were determined by an HPLC method modified after Claus et al. (1993) and Denhard et al. (1993). Briefly, skatole was first extracted from 0.5 mL of plasma with 2 mL of diethylether and the ether fraction was mixed with 1 mL of 40% acetonitrile. The ether was evaporated in a water bath at 47 °C and 200 μL of the extract were analyzed for skatole concentration by HPLC. For the determination of skatole concentrations in fat, 1 mL hexane was added to 100 μL of liquid fat