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Research article

The use of sugar beet pulp in pig diet to control skatole analysed by HPLC quantification method

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ABSTRACT: This study aimed to determine the efficacy of a finishing diet added with sugar beet pulp to reduce backfat skatole of entire male pigs, using the optimised high-performance liquid chromatographic (HPLC) method. The study comprised 72 males Pietrain (Large White × Landrace), divided into two groups of 36 animals each. Pigs in group A (treatment) were fed a supplemented formula (addition of 10 % beet pulp). while animals in group B (control) received a commercial feed, both for a period of 14 days before slaughter. The isocratic HPLC method achieved the chromatographic separation of indolic compounds in approximately 3 min. Skatole was significantly lower (p = 0.002) in group A, showing that beet root supplementation reduced skatole levels in pig fat. In addition, the optimised HPLC method was reliable, less time-consuming, and showed a resolution suitable for small amounts of skatole.
Keywords: boar taint, entire male pigs, feed, high-performance liquid chromatography

Introduction

Boar taint can be described by the presence of off-odour and off-flavour compounds creating an unpleasant perception of pork quality by the consumer, mainly due to the presence of androsterone and skatole. These compounds have different odours (urine or sweat like for androstenone and faecal for skatole), and consumer non-acceptance of meat flavour is due to both. However, the foul odour is mainly associated to high skatole levels (Mörlein et al., 2016; Parunović et al., 2010) since it is perceived by 99 % of consumers, in contrast to androstenone (Weiler et al., 2000). Anaerobic bacteria produce skatole (3-methylindole) from tryptophan in the animal intestinal tract (Ma et al., 2021). Although entire males present higher skatole values, it can be found in smaller amounts in barrows and gilts (Aldal et al., 2005) and is the main taint contributor at low weights at slaughter (Tuomola et al., 1996). In a comprehensive review, Wesoly and Weiler (2012) summarise skatole formation via higher amounts of the amino acid L-tryptophan: skatole forms instead of microbial protein, leading to deposits in the adipose tissues due to high absorption, especially in slow digestive transit rates with reduced degradation in the liver and kidneys. Reduced digestive transit rates are responsible for increased skatole contents (Deslandes et al., 2001).

Strategies to reduce boar taint encompass grouping by gender, siblings or allowing lower stocking densities (Squires et al., 2020), genetic selection (Larzul, 2021), immunocastration (Škrlep et al., 2020), husbandry (Dalmau et al., 2019), and feeding (Heyrman et al., 2018). In terms of feeding strategies, the addition of feed components, such as the chicory root or the sugar beet pulp, rich in fermentable carbohydrates, reduces skatole concentration effectively in backfat (Aluwé et al., 2017; Hansen et al., 2008; Kjos et al., 2010; Øverland et al., 2011; Zammerini et al., 2012).

Traditionally, boar taint is detected by sensory analysis (Trautmann et al., 2014), either using a trained panel or the hot iron method (Jarmoluk et al., 1970) in the slaughter lines. However, these methods are subjective and hinder the quantitative measurement of skatole and androstenone contents in pig fat. High-Performance Liquid Chromatography (HPLC) with a fluorescence detector has been the main instrument to quantify skatole in pig backfat (Brunius and Zamaratskaia, 2012; Dehnhard et al., 1993; Regueiro and Rius, 1998; Tuomola et al., 1996). Liquid Chromatography-Mass Spectrometry (LC-MS) was also used by some authors (Verheyden et al., 2007; Woźniak et al., 2020; Zamaratskaia and Jastrebova, 2006) and can be faster, more sensitive, and specific, but also more expensive when compared to HPLC. This study aims to determine the efficacy of a finishing diet supplemented with beet pulp to reduce backfat skatole of entire male pigs using the optimised SI-HPLC method.

Materials and Methods

Animals and diets

Seventy-two males Pietrain (Large White × Landrace) were randomly allocated to two groups of 36 animals. The animals were kept in group housing with concrete floor and straw was used in beddings. The stable contained nipple drinkers and feed was available *ad libitum*. Fourteen days before slaughter, pigs in group A were fed a supplemented formula with 10 % beet pulp added. The control group B received a traditional commercial concentrate. Feed ingredients are presented in Table 1. Pigs had similar husbandry, were grown, and finished on the same farm and were sent for slaughter at 23 weeks of age. In Portugal, producers opt to sell sexually immature males (usually between 21 and 24 weeks of age), avoiding the development of boar taint while reducing production costs. Therefore, animals were slaughtered at approximately 23

Table 1 – Feed ingredients and chemical composition of the diets (% in the feed).

Ingredient (% dry matter)	Supplemented	Commercial
Wheat	28.5	33.0
Corn	32.3	26.3
Soybean meal 47	14.9	10.5
Barley	8.47	16.4
Beet pulp	10.0	-
Rapeseed meal	3.0	5.0
Sunflower meal	-	4.0
Wheat bran	-	1.44
Molasses	0.7	1.0
Dicalcium Phosphate	0.5	0.4
Vitamins	0.4	0.4
L-Lysine	0.35	0.42
Salt	0.35	0.35
L-Threonine	0.15	0.15
DL-Methionine	0.08	0.05
Limestone	0.3	0.59
Chemical composition (% dry matter)		
Moisture	12.37	12.38
Crude protein	15.57	15.56
Raw fibre	4.72	4.54
NDF	14.39	13.72
ADF	6.02	5.77
Raw ash	4.36	4.32
Fat	1.94	2.00
Starch	41.93	45.1
Lysine	1.02	1.00
SID Lysine	0.91	0.90
Methionine + Cysteine	0.62	0.61
SID Methionine + Cysteine	0.55	0.55
Threonine	0.71	0.69
SID Threonine	0.63	0.62
Tryptophan	0.17	0.18
SID Tryptophan	0.15	0.16
Energy (kcal kg^{-1})	2309	2309

NDF = Neutral Detergent Fibber; ADF = Acid Detergent Fibber; SID = Standardized Ileal Digestibility.

weeks to bring this trial closer to the production system used in Portugal. The animals fasted for approximately 20 h (including transport time) before slaughter.

Sample collection and preparation

Adipose tissue from the dorsal subcutaneous fat of the neck was collected from 71 carcasses after slaughter. One animal in Group B was rejected due to injury. Fat samples were stored at –18 °C until analysis. Liquid fat was extracted using microwave heating (800 W, 2 min). A sample of water-free liquid fat (1 g) was placed in Falcon tubes and 1 mL of methanol was added. After vortexing for 30 sec, tubes were incubated for 10 min at 40 °C in an ultrasonic bath (Sonica® Ultrasonic Cleaner). Samples were centrifuged (JP Selecta Mixtasel) for 15 min at 1100 g and placed in an ice-water bath for 20 min.

Simple Isocratic High-Performance Liquid Chromatography

A Thermo Scientific™ UltiMate 3000 HPLC equipped with a Hypersil ODS C18 5 µm column with particle diameter 250 × 4.6 mm was used, operating at 40 °C. Elution was achieved with an isocratic gradient of 0.1 % acetic acid (45 %) and acetonitrile (55 %) at 2 mL min^{-1}, with a total run time of 4 min. Skatole native fluorescence was monitored using a fluorescence detector tuned to an excitation wavelength of 285 nm and emission at 350 nm.

Method performance

The study design considered the following validation parameters: linearity, detection limits, quantification, and recovery. Calibration using the external standard method was applied. The method linearity was studied using nine standard solutions of skatole in methanol ranging from 0.31 ng mL^{-1} to 244.00 ng mL^{-1}, obtaining a correlation of ($r > 0.999$). Recovery was evaluated by the standard addition of skatole to a fat sample matrix with a known concentration of skatole at two levels: 10 ng mL^{-1} and 100 ng mL^{-1}.

Statistical analysis

Data were tested for normality using the Kolmogorov-Smirnov test. In case a lack of normality occurred, a non-parametric approach was used. The Mann-Whitney U-test was used to test the statistical difference between the skatole levels in groups A and B. The level of significance was set to $p < 0.05$.

The statistical analysis was performed using TIBCO® Statistica®, v.14.0.0, TIBCO Software Inc.

Results and Discussion

The HPLC method

The Simple Isocratic HPLC (SI-HPLC) method achieved the chromatographic separation of indolic compounds (Figure 1), where skatole eluted at minute 3.0 of the chromatogram. The limits of detection (LoD) and quantification (LoQ) were 6.29 ng g^{-1} and 19.06 ng g^{-1}, respectively, and the analyte recovery after extraction was 82.7 ± 6.3 % (Table 2). The chromatogram also showed a pronounced peak eluting at minute 2.5; although not quantified, this compound is indole. This compound contributes to boar taint; nevertheless, to a lesser extent (Heyrman et al., 2021). Therefore, most studies quantifying boar taint determine the two major contributors, skatole and androstenone. The elution time achieved in this method was relatively faster than in similar studies, where the HPLC with a fluorescence detector was used: 4.2 min (Dehnhard et al., 1993), 4.4 min (Regueiro and Rius, 1998), 8.9 min (Tuomola et al., 1996), and 9.8 min (Brunius and Zamaratskaia, 2012).

Skatole levels

The results showed that the skatole content was discernable (p = 0.002) between the groups analysed. Feed supplementation effectively reduced skatole. The group fed with supplemented feed (A) had an average of 104.63 ng g^{-1}, while the control group (B) received an average of 200.65 ng g^{-1} (Table 3).

Comparing these results with those other studies (Table 4), the values fall in similar intervals, between 100 and 200 ng g^{-1}, under different experimental conditions for each study.

Sensorial cut-off values for skatole can vary between 150 and 250 ng g^{-1} due to difficulties in harmonising methods (Aluwé et al., 2018). The results of this experiment show that the skatole levels of the supplemented group are below these values, which can translate into a significant increase in the acceptance of pork meat at a practical level. According to Aluwé et al. (2018), androstenone levels in pork can rise considerably without consumer rejection if skatole levels are low. Therefore, the risk of rejection when using meat from entire males can be minimized if skatole levels are controlled through the diet.

Carcass weight is often associated with boar taint (Zamaratskaia and Squires, 2009; Backus et al., 2016); however, it appears to not correlate with weight and skatole levels, as stated in some studies (Aluwé et al., 2011; Borrisser-Pairó et al., 2016; Heyrman et al., 2021; Moore et al., 2017). In this study, there were no statistical differences (p > 0.05) between groups in hot carcass weights at slaughter (Table 5).

Sugar beet pulp (SBT) is very rich in highly digestible fibre, and because this fibre is soluble, it has a unique form. However, it is not only high fibre solubility that explains the high digestibility. Fibre composition in highly digestible uronic acid (pectin substances) and arabinose contributes to its high digestibility (Anguita et al., 2007). This soluble uronic acid and arabinose deliver a readily fermentable substrate to produce volatile fatty acids (mainly acetic and propionic acids) (von Heimendahl et al., 2010). The SBT fibre is not digested in the small intestine; however, it is used as a carbon source in fermentations promoted by hindgut microflora (Diao, 2020).

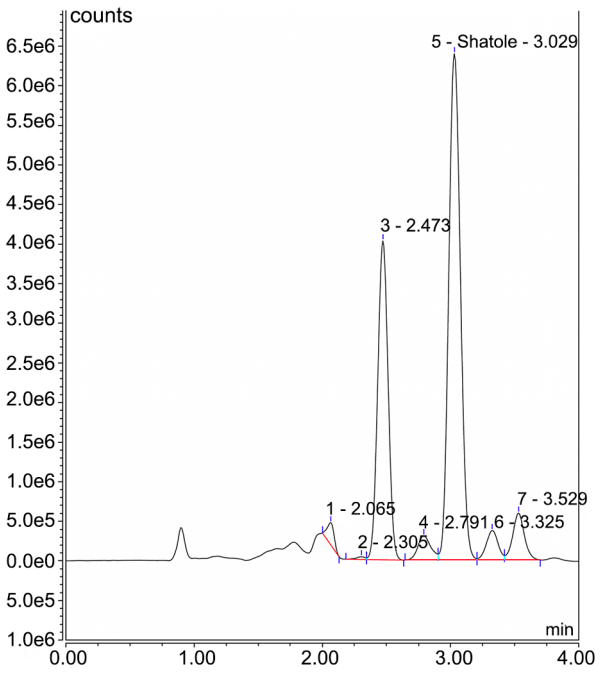

Figure 1 – Typical chromatogram of the samples, where skatole elutes at min 3.029, showing a good peak separation.

Table 2 – HPLC validation parameters.

Linearity Range (ng mL^{-1})	0.31-244.00
Slope	11483969.2
Intercept	–3746.9
Correlation coefficient (r)	0.999
LoD (ng mL^{-1})	6.29
LoQ (ng mL^{-1})	19.06
Recovery %	82.7 ± 6.3

LoD = Limit of Detection; LoQ = Limit of Quantification.

Table 3 – Skatole concentration in backfat of the groups under study.

Skatole (ng g^{-1})	Mean	SD	Median	IQR
Group A (n = 36)	104.63	80.59	72.63	[55.76; 108.79]
Group B (n = 35)	200.65	156.77	151.11	[117.20; 197.25]
p-value	0.002			

SD = Standard deviation; IQR = Interquartile range.

Table 4 – Observed skatole concentrations in studies using backfat of entire males.

Authors	Skatole concentration in backfat (ng g^{-1})	Observations
(Aldal et al., 2005)	120-190	Entire males slaughtered at 16 weeks of age
(Aluwé et al., 2017)	119 (inulin)-180 (control)	Entire males with supplemented feed, slaughtered at 25-27 weeks of age
(Bonneau et al., 2000)	150	Entire males (slaughter age not reported)
(Grela et al., 2020)	110.4-115.8	Entire males slaughtered at 22-24 weeks of age
(Hansen et al., 2008)	100 (chicory)-140 (control)	Entire males with supplemented feed (slaughter age not reported)
(Liu et al., 2017)	200	Entire males slaughtered at 25 ± 2 weeks of age
(Prusa et al., 2011)	197.3	Entire males (slaughter age not reported)
(Whittington et al., 2011)	171	Entire males (slaughter age not reported)

Table 5 – Hot carcass weight means, standard deviation, minimum and maximum per group.

Weight (kg)	n	Mean	Standard deviation	Min	Max
Group A	36	81.87	8.28	61.00	98.40
Group B	35	78.30	8.28	63.00	97.60
p-value		> 0.05			

Supplementation with sugar beet pulp regulates the digestive transit, decreasing its time and eventually decreasing the apparent digestibility of nutrients while stimulating a compensatory secretion of endogenous digestive enzymes, such as lactase and sucrase (Diao et al., 2020). The high SBP content in highly digestible carbohydrates also effects the regulation of the hindgut flora, promoting beneficial flora growth and inhibiting harmful bacteria growth (Diao et al., 2020). This affects the proliferation of *Clostridium* and *Bacteroides*, the two main metabolizers of L-tryptophan in indole acetic acid and the main precursor of skatole (Whitehead et al., 2008).

The hindgut flora uses the highly digestible fibre provided by SBP to produce energy through its fermentation into volatile fatty acids. In the absence of this source of energy, L-tryptophan is deviated from the production of microbial protein and is metabolized, increasing, therefore, the synthesis of skatole (Wesoly and Weiler, 2012).

The lack of fibre in the pig diet is also responsible for an increased number of mucus-degrading bacteria, increasing susceptibility of the mucus layer degradation and exposing the intestinal lumen to pathogenic bacteria (Diao et al., 2020). L-tryptophan is absorbed in the small intestine, which prevents its availability for microbial degradation in the colon. Studies have shown that an increased rate of mitosis and apoptosis in the small intestine is responsible for the increased content of gut cell debris, a source of L-tryptophan for microbial skatole synthesis in the colon (Claus and Raab, 1999). Supplements reducing the pathogenic bacterial activity in the intestines of pigs are effective in protecting the intestine lumen, decreasing the formation of gut cell debris (Huang et al., 2012).

Finally, the use of sugar beet pulp in pig feed regulates the digestive transit, diminishing its rate and, therefore, the time given to the hindgut microflora to synthesise skatole.

Conclusions

Feed supplementation with sugar beet pulp significantly lowers skatole levels in pig fat. The use of this supplement in the last two weeks before slaughter reduced the skatole concentration to half in the group of supplemented pigs in comparison with the control group. A new HPLC method was also tested to determine skatole in pig fat. This method was less time-consuming, with an LoD suitable for tpigs' skatole concentration range. Supplementation with sugar beet pulp should be considered an alternative to castration to reduce the skatole levels at slaughter. Thus, a win-win solution is emphasized in this study with the promotion of organoleptic refinement of pork quality, while ensuring animal welfare at higher standards.

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Authors' Contributions

Conceptualization: Pereira Pinto, R. **Data curation:** Pereira Pinto, R.; Mata, F. **Formal analysis:** Pereira Pinto, R.; Mata, F.; Pires, P.; Barros, M. **Investigation:** Pereira Pinto, R.; Mata, F. **Methodology:** Pereira Pinto, R.; Pires, P. **Funding acquisition:** Vaz-Velho, M. **Project administration:** Pires, P.; Vaz-Velho, M. **Resources:** Vaz-Velho, M. **Supervision:** Pires, P.; Barros, M.; Vaz-Velho, M. **Validation:** Pires, P.; Barros, M.; Araújo, J.P. **Writing-original draft:** Pereira Pinto, R.; Mata, F. **Writing-review & editing:** Pereira Pinto, R.; Mata, F.; Pires, P.; Barros, M.; Araújo, J.P.; Vaz-Velho, M.

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