*Agronomy Research* **20**(X), xxx–ccc, 2022
https://doi.org/10.15159/AR.22.038

# The effect of feed supplementation with inulin on boar taint levels and meat quality of entire male pigs

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Received: March 19th, 2022; Accepted: May 3rd, 2022; Published: July 5th, 2022

**Abstract.** Skatole and androstenone are the two main compounds responsible for the foul odour in entire male pigs' meat, known as boar taint. This study evaluated the effect of feed supplementation with inulin on the boar taint levels of 30 entire male pigs. Two months before slaughter, the animals were allocated into three groups ($n$ = 10). The control group received a standard commercial diet. The other groups were fed the same commercial diet with 3% and 6% added inulin, respectively. Results showed that inulin addition to the feed significantly reduced skatole levels in the pigs' adipose tissue compared with the control group. The levels of androstenone were not affected by the dietary approach. Although there were differences in some parameters, the supplementation with inulin did not promote extensive changes in the meat quality parameters between the tested groups. When raising entire males, supplementation with inulin in finishing diets could be considered to reduce the boar taint perception by the consumer.

**Key words:** androstenone, entire male pigs, inulin, meat quality, skatole.

## INTRODUCTION

The surgical castration of male piglets, a common procedure in most European countries, has become an animal welfare concern due to the pain and stress associated with the procedure (EFSA, 2004). Using behavioural and physiological parameters, it has been scientifically proven that castration is a painful surgical intervention even when performed on very young animals, and it is a costly operation for producers (Fredriksen et al., 2011).

The main reason to castrate male piglets is to prevent the development of boar taint, an unpleasant odour found in the carcasses of entire males (EFSA, 2004; Fredriksen et al., 2011; Wauters et al., 2017). This odour becomes especially intense when cooked (Mathur et al., 2012) and is mainly associated with the presence of skatole

(3-methylindole) and androstenone (5α-androst-16-en-3-one), which, due to their lipophilic characteristics, tends to accumulate in adipose tissue. Skatole is a metabolite derived from the amino acid tryptophan produced in the lower part of the intestine by the intestinal microbiota and is associated with a faecal and naphthalene smell (Furnols & Oliver, 1999). Androstenone is a steroid produced in the testes with a smell often associated with urine and sweat (Furnols & Oliver, 1999). Skatole levels are usually low in castrated and female animals; also, androstenone is known to interfere with the hepatic elimination of skatole (Doran et al., 2002; Whittington et al., 2004). In contrast to androstenone, skatole is perceived by 99% of consumers (Weiler et al., 2000). However, the average consumer does not know why sometimes pork has a foul smell. A study in four European countries (France, Germany, the Netherlands, and Belgium) reported that 88.7% of consumers were unaware of boar taint or did not know what it was (Vanhonacker & Verbeke, 2011). Also, in a recent survey of Portuguese consumers, nearly half of the respondents did not know what boar taint was or had even never heard about it (Pereira Pinto & Vaz-Velho, 2021).

There are different realities in Europe. In major pig producing countries such as Germany, France, and Denmark, around 75% to 93% of commercial male piglets are surgically castrated (Van Ferneij, 2022), while in countries such as Spain, Portugal, Ireland, and the United Kingdom, male pigs are slaughtered before they reach puberty (approximately 5 months of age), weighing less than 100 kg, being raised without castration (Weiler & Bonneau, 2019). However, producers lose efficiency due to reduced carcass size.

Stakeholders in the production of pigs have been defending the ban on surgical castration in the European Union, which represents a challenge for the pork production chain (Morlein et al., 2015). All over the world, several alternatives to surgical castration are applied, such as raising uncastrated males (entire males) or immunocastration. The application of immunocastration inhibits testosterone production, reducing the boar odour without surgical intervention and the risk of infection. It requires at least two injections into the animal at least 4 weeks apart, with the second dose at least 4 weeks before slaughter (Heyrman et al., 2019). A major disadvantage of this method is that the EU catalogues this chemical in the therapeutic area of sex hormones and modulators of the genital system (EMA, 2020), which is currently unfeasible in organic production modes.

Breeding entire males have some advantages, such as lower production costs, a more natural approach, and improved animal welfare. When compared with uncastrated male pigs, it was found that in castrated males, feed efficiency decreased by 10%, muscle content decreased by 5%, while carcass fat increased by 26% (Bonneau et al., 1994). The disadvantages of breeding entire males are mainly related to the possible formation of boar and meat odour with less advantageous characteristics for the production of meat products. Aluwe et al. (2013) compared meat from entire and castrated males and found differences in pH, colour, drip loss, and cooking loss; these differences did not significantly influence the quality or palatability of the meat.

Several studies (Byrne et al., 2008; Hansen et al., 2008; Kjos et al., 2010; Aluwe et al., 2013; Bilić-Šobot et al., 2014; Backus et al., 2016) have shown that feed supplemented with fermentable carbohydrates, such as inulin obtained from chicory, were effective in reducing the concentration of skatole in the swine intestine. Inulin is a non-digestible fructose polymer found mainly in chicory roots (*Cichorium intybus*) or Jerusalem artichoke tubers (*Helianthus tuberosus*). It contains oligosaccharides and

polysaccharides, regarded as a source of prebiotics (Grela et al., 2021). Prebiotics are commonly used to enhance pigs fattening, changing pork physicochemical properties such as increasing the total fat content (Degola & Jonkus, 2018). Dietary inulin is not hydrolysed by mammalian enzymes but is easily fermented by the bacterial community and favours the growth of intestinal bifidobacteria (Grela et al., 2021). Fermentation of inulin by intestinal bacteria produces a large number of short-chain fatty acids, which can stimulate the production and secretion of the mucous layer covering the mucosal surface of the gastrointestinal tract (Wang et al., 2019). Dietary inulin may also alter intestinal bacterial populations due to its iron bioavailability-promoting effect (Patterson et al., 2010), and inulin-type fructans may reduce the production of potentially toxic metabolites (Meyer & Stasse-Wolthuis, 2009).

Thus, this research aimed to evaluate the effect of feed supplementation with inulin in the finishing diets of entire males, assessing meat quality parameters and, mainly, the influence of inulin on the reduction of the boar taint in carcasses.

## MATERIALS AND METHODS

### Study design and feeding conditions

Thirty entire male pigs males, offspring from a Pietrain terminal sire, crossed with a Large White, Landrace crossed sow were used for the study. The animals were fed the same commercial diet, with *ad libitum* access, up to the age of 5.5 months. At this stage, the pigs were allocated into three distinct groups ($n$ = 10), where one group received a standard commercial diet (control group). In the other groups, the same commercial diet included 3% and 6% of inulin (Fibrofos, a by-product of *Cichorium intybus* L.), respectively. Feed was constituted of wheat, corn, soybean bagasse, barley, fibrofos 60, rapeseed bagasse, wheat bran, cane molasses, dicalcium phosphate, L- lysine, salt, L-threonine, DL-methionine, and calcium carbonate. Some slight corrections were made in the relative composition of the diets containing Fibrofos in order to balance the nutritional level. Diets have similar nutritional profiles, as shown in Table 1. All groups received the same feed, 2.8 kg per day per pig, for 2 months until slaughter.

**Table 1.** Nutrient levels (%) of the standard diet (control) and the diets with added inulin

| Nutrients | Control | 3% of added inulin | 6% of added inulin |
|---|---|---|---|
| Moisture | 12.37 | 12.26 | 12.24 |
| Crude Protein | 15.55 | 15.56 | 15.55 |
| Raw fibre | 4.53 | 3.84 | 3.73 |
| NDF | 13.71 | 12.63 | 11.64 |
| ADF | 5.76 | 5.01 | 4.76 |
| Raw ash | 4.32 | 4.30 | 4.10 |
| Fat | 1.99 | 1.89 | 1.82 |
| Starch | 45.10 | 42.65 | 40.45 |
| Calcium | 0.72 | 0.74 | 0.71 |
| Phosphor | 0.48 | 0.48 | 0.49 |
| Lysine | 0.99 | 0.99 | 0.99 |
| Lysine SID | 0.90 | 0.91 | 0.91 |
| Met + Cis | 0.61 | 0.62 | 0.62 |
| Met + Cis SID | 0.55 | 0.56 | 0.57 |
| Threonine | 0.69 | 0.71 | 0.70 |
| Threonine SID | 0.62 | 0.64 | 0.64 |
| Tryptophan | 0.18 | 0.18 | 0.18 |
| Tryptophan SID | 0.16 | 0.16 | 0.16 |
| Sodium | 0.17 | 0.20 | 0.20 |
| Linoleic acid | 0.97 | 0.88 | 0.85 |
| Energy Kcal $kg^{-1}$ | 2309 | 2309 | 2309 |
| Inulin | 0.00 | 3.00 | 6.00 |

**Analysis of moisture, pH, loss on thawing, colour, hardness, and intramuscular fat**

Meat samples were collected from the ham's muscle tissue (*Biceps femoris*), frozen and kept at -18 °C until analysis. Before further analysis, meat samples were thawed to determine the thawing loss: samples were weighed, thawed at 4 °C for 24 hours, dried with a paper towel and reweighed. To determine the moisture content, samples were minced and dried in an oven at 103 ± 2 °C until constant weight, as described in A.O.A.C (2016), method 950.46. The pH was measured by potentiometry using the equipment Crison pH25+ (Crison Instruments, Barcelona, Spain), inserting the probe electrode into the meat samples. Colour measurements were performed according to the CIELAB colour system described by Honikel (1998), using a Minolta CR-300 colourimeter (Konica Minolta Tokyo, Japan). To determine the hardness, samples of meat (4 cm) were cut to analyse the texture profile (using the TA.XT2 Plus equipment from Stable Micro Systems TPA). Intramuscular fat (IMF) percentage was determined by a Soxhlet extraction procedure using petroleum ether as an extraction agent after sample hydrolysis, as described in the A.O.A.C. (2016) method 960.39. To analyse moisture, pH, thawing loss, and intramuscular fat, measurements were performed in triplicate. For colour and hardness, samples were measured 10 times each.

**Boar taint quantification**

The quantification of skatole and androstenone in pork fat was done using a high-performance liquid chromatography (HPLC) method adapted from Hansen-Moller (1994), as described in the following sections.

**Fat extraction.** Sample preparation consisted of cutting the adipose tissue from the subcutaneous layer of the belly and extracting the liquid fat after microwave heating (800 W, 2 min). A sample of water-free liquid fat (1 g) was placed in Falcon tubes, and 1 mLof 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 1,100 g and placed in an ice-water bath for 20 min. The liquid fraction was filtered through a 0.2 µm filter before sample derivatisation.

**Sample derivatisation.** Manual derivatisation of 500 µL of the sample was performed at room temperature by sequentially adding 75 µL of 0.1% dansylhydrazine, 50 µL of deionised water in methanol, and 40 µL of 14% $BF_3$ solution in methanol. After stirring for 5 min, 20 µl of the mixture was injected into the HPLC.

**HPLC operating conditions.** A ThermoFisher UltiMate 3000 HPLC system with a Hypersil ODS C18 column (5 µm particle diameter and 250×4.6 mm, Thermo Scientific Portugal), operated at 40 °C, was used. The eluent was: (A) 0.1% v/v acetic acid; (B) acetonitrile; (C) tetrahydrofuran; (D) pH 6.0 buffer solution (25 mM potassium phosphate). The following gradient profile was used: 0–5 min, 50%–60% A, 35%–45% B, 5% C; 5–6 min, 50% A, 45% B, 5% C; 6–6.1 min, 20%–50% A, 30%–45% B, 5%–30% C, 0–20% D; 6.1–12 min, 0–20% A, 30%–40% B, 30%–40% C, 20% D; 12–12.5 min, 0–60% A, 40%–35% B, 5%–40% C, 0–20% D; 12.5–13 min, 60% A, 35% B and 5% C, with a flow rate of 2.0 mL $min^{-1}$. Fluorescence detection was performed with excitation at 285 nm and emission at 340 nm (0–6.0 min) for detection of skatole; excitation at 346 nm and emission at 521 nm (6.1–13 min) for the detection of androstenone.

**Quantification of skatole and androstenone.** For quantification, a calibration method using the external method was performed. The range of standards was between 1.60 and 63.0 ng $g^{-1}$ for skatole and 37.6 and 1503.8 ng $g^{-1}$ for androstenone, using 10 standards for each. Calibration curves' linearity coefficient ($R^2$) was 0.9928 for skatole and 0.9969 for androstenone. Detection and quantification limits (LoD and LoQ) were calculated: LoD of 12.6 ng $g^{-1}$ and 63.2 ng $g^{-1}$ for skatole and androstenone, respectively, and LoQ of 38.2 ng $g^{-1}$ and 191.5 ng $g^{-1}$ for skatole and androstenone, respectively.

**Data analysis**

Statistical analysis was performed using Statistica for Windows software package, version 14.0.0.15 (TIBCO Software, Palo Alto, California, USA). Data were subjected to Kolmogorov-Smirnov test to assess normality and, depending on the result, followed by an analysis of variance (ANOVA) or the Kruskal-Wallis test. Differences between groups were determined using the Fischer's Least Significant Difference (*LSD*) *post-hoc* test. Significant differences were established in $P < 0.05$.

## RESULTS AND DISCUSSION

The values of skatole and androstenone concentrations in the belly fat, determined by HPLC, are shown in Table 2. In the control group, where no inulin was added, the average content of skatole was about 2.7 times higher than in the groups supplemented with inulin (44.3 ng $g^{-1}$ *vs*. 16.4 µg $g^{-1}$), meaning that inulin addition was effective in skatole reduction independently of the tested amount.

**Table 2.** Skatole and androstenone concentrations (mean ± standard deviation) in pig belly fat (ng $g^{-1}$ fat)

| Group (added inulin) | Skatole (ng $g^{-1}$) | Androstenone (ng $g^{-1}$) |
|---|---|---|
| 0% | 44.3 ± 41.1[a] | 459.2 ± 235.4 |
| 3% | 16.4 ± 10.0[b] | 358.3 ± 244.5 |
| 6% | 15.9 ± 15.9[b] | 275.9 ± 191.8 |
| *P-value* | 0.03 | 0.36 |

[a,b] Different superscripts within columns indicate significant differences ($p < 0.05$).

Regarding androstenone, there were no significant differences ($P = 0.36$). Although a slight reduction in mean values can be noticed when inulin is increased, it is not possible to establish a link between the diet and the androstenone production. Androstenone values in fat mainly depend on genetic factors linked to sexual maturity (Bonneau, 2006).

Similar results in feeding trials with inulin were obtained (Kjos et al., 2010; Overland et al., 2011; Zammerini et al., 2012; Aluwe et al., 2017), where skatole was reduced, and there were no differences in androstenone values. Nonetheless, in a study conducted by Heyrman et al. (2018), chicory root supplementation for two to three weeks led to a reduction in both skatole and androstenone. However, the author of this study states that the significant differences in androstenone values may be due to the differences in carcass weight since androstenone concentrations were positively linked with carcass weight.

Fig. 1 represents the skatole *vs.* androstenone scatterplot, where are the values obtained for skatole and androstenone for each animal ($n = 30$) and identified according to the provided diet. Some patterns can be identified: lower skatole values in the 3% and 6% groups and higher values in the control group. A wide dispersion of results is

reflected in the standard deviation values shown in Table 2. However, this dispersion is typical when skatole and androstenone are measured in the pigs' population, as reported in similar studies (Hansen-Moller, 1994; Aluwe et al., 2013; Aaslyng et al., 2015; Morlein et al., 2016; Liu et al., 2017; Verplanken et al., 2017).

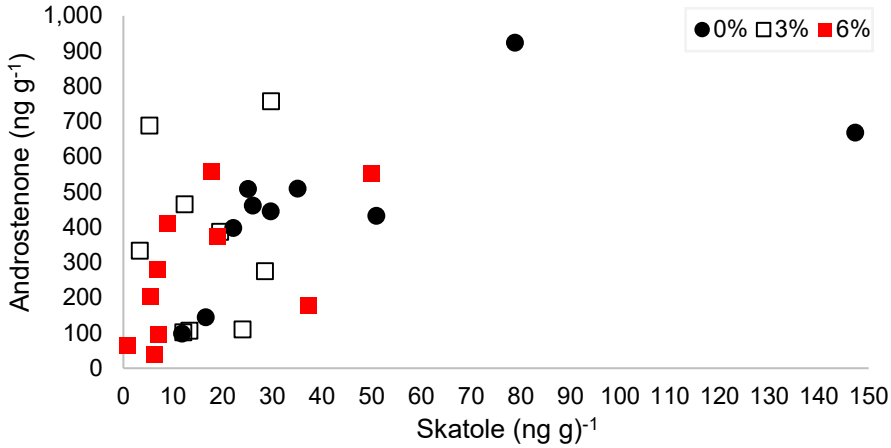


**Figure 1.** Skatole *vs*. androstenone scatterplot.

Regarding the influence of inulin supplementation on meat quality, no significant differences ($P > 0.05$) between groups were found in pH values, thawing loss, moisture, and intramuscular fat (Table 3). On the other hand, hardness values were found to be significantly different (group *P*-value was 0.002); the group with 6% of added inulin showed the lowest hardness value, which indicated softer meat. Wang et al. (2019) stated that the dietary inulin supplementation had no significant influence on meat quality, except for the expression of the *MyHC IIb* gene, which was increased by inulin supplementation. Tenderness is affected by fibre type, especially type IIb fibres, and muscles with larger type IIb fibres may be tougher or may have greater hardness (Guo et al., 2019). However, this was not verified in our study, as the increase in inulin led to lower meat hardness.

**Table 3.** Values (mean ± standard deviation) of physicochemical determinations.

| Group (added inulin) | Hardness (N) | pH | Thawing loss (%) | Moisture (%) | IMF (%) |
|---|---|---|---|---|---|
| 0% | 6.60 ± 2.24[b] | 6.01 ± 0.35 | 4.75 ± 3.09 | 73.38 ± 1.45 | 2.28 ± 0,40 |
| 3% | 6.83 ± 1.99[b] | 5.87 ± 0.30 | 6.41 ± 2.95 | 71.52 ± 3.84 | 2.29 ± 0.62 |
| 6% | 5.58 ± 1.86[a] | 5.91 ± 0.28 | 5.72 ± 2.29 | 72.20 ± 4.60 | 2.60 ± 0.67 |
| *P-value* | 0.002 | 0.18 | 0.18 | 0.30 | 0.12 |

[a,b] Different superscripts within columns indicate significant differences ($p < 0.05$).

Another parameter of meat quality is its colour. Instrumental determination was achieved through the CIELAB system. Results of the parameters L* (brightness), a* (red colour) and b* (yellow colour) are shown in Table 4 and indicate statistically significant differences in red and yellow, particularly in the group supplemented with 3% of inulin.

Altmann et al. (2022) measured colour in raw pork loin and found that a change along the b* axis (yellowness) in CIELAB colour space is most discernible, followed by the * axis (redness) and then the L* axis (lightness). However, the difference between the colour coordinate values is minimal, in the order of 1.5 on average, which is too low for a the colour discrimination threshold of the human eye. According to Konica-Minolta (2019), the human eye cannot differentiate some colours even if they are numerically (L*a*b* coordinates) different, depending on the saturation and hue. The L* values showed no significant differences between groups. A similar conclusion was drawn in a study by Gispert et al. (2010). Also, results of the meat colour are inconsistent in the literature since muscle fibre characteristics may depend on genotype as well as environmental factors, such as nutrition, preslaughter and slaughter conditions (Aluwe et al., 2013).

**Table 4.** Meat colour determinations (mean ± standard deviation)

| Group (added inulin) | L* | a* | b* |
|---|---|---|---|
| 0% | 44.83 ± 4.40 | 14.18 ± 2.79$^{b}$ | 4.64 ± 2.11$^{b}$ |
| 3% | 45.53 ± 6.41 | 12.57 ± 2.26$^{a}$ | 3.19 ± 2.23$^{a}$ |
| 6% | 44.03 ± 5.53 | 13.81 ± 2.14$^{b}$ | 4.12 ± 2.07$^{b}$ |
| *P-value* | 0.16 | 0.00 | 0.00 |

$^{a,b}$ Different superscripts within columns indicate significant differences ($p < 0.05$).

Albeit there are statistically significant differences in hardness and colour values (a* and b*), little is known about the influence of inulin on meat quality (Wang et al., 2019). Available studies indicate that inulin addition did not affect meat properties: Grela et al. (2021) reported that the addition of inulin from different sources as prebiotics to pig feed did not have a negative effect on the physicochemical properties of meat; Aluwe et al. (2013) found no significant differences in the meat quality parameters of boars fed with chicory when compared to control. Also, Wang et al. (2019) stated that dietary inulin supplementation had no significant influence on meat quality.

## CONCLUSIONS

Commercial feed supplemented with inulin significantly reduced skatole levels present in the entire male pigs' fat, the proportion of 3% of added inulin being sufficient for the purpose. Androstenone values were not altered by the prebiotic. Regarding meat quality, the addition of inulin affected some meat characteristics such as hardness and colour compared to the control group. Feed supplementation with inulin could be considered an alternative to reducing skatole levels and consequent organoleptic improvement.

ACKNOWLEDGEMENTS. Project PIGS+CARE (POCI-01-0247-FEDER-017626) was co-financed by the European Regional Development Fund (FEDER) through COMPETE 2020 (Operational Program for Competitiveness and Internationalisation). To project TECH - Technology, Environment, Creativity and Health, Norte-01-0145-FEDER-000043, supported by Norte Portugal Regional Operational Program (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). The feeding trials were conducted by Escola Superior Agrária de Coimbra of the Polytechnic Institute of Coimbra (ESAC-IPC), Portugal, a partner in this project. To CISAS - Center for Research and Development in Agrifood Systems and Sustainability - UIDB/05937/2020 and UIDP/05937/2020, financed through the FCT - Fundação para a Ciência e a Tecnologia.

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