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Feeding and housing boars after puberty without castration allows for good performance and low boar taint

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ABSTRACT. Finishing entire male pigs after puberty is not common in Europe due to aggression and boar taint. Alternatives to surgical castration or immunocastration should be explored as the production of entire males can also increase the productivity and sustainability of swine production. This study evaluated the performance, welfare, health, and meat quality of heavier pigs (from 95 to 135 kg) raised without castration. A factorial experiment consisting of 2 housing conditions (H1 control, H2 improved) × 3 diets (D1 – control diet, D2 – 10% beet pulp, D3 – 5% beet pulp + 4% Fibrofos) with 60 entire males randomly allocated to 6 pens, 23 to 30 weeks of age, was conducted using a Pietrain × (Large White × Landrace) cross. Treatment effects were evaluated with respect to growth, carcass yield, skin lesions, cortisol, hemogram, skatole, androstenone and meat physicochemical characteristics. Improved housing with more headspace, larger feeders, extra drinkers, environmental enrichment (organic toys), and group stability until slaughter led to a significant decrease in androstenone levels and benefited animal health and welfare without compromising performance. In addition, diets with inulin from Fibrofos or sugar beet pulp allowed to significantly reduce skatole content. Due to the price, beet pulp is more sustainable and its 10% inclusion did not negatively affect either intake or performance. Raising entire male pigs for carcasses with low boar taint is be possible if adequate space, environmental enrichment, and specific feeds are provided.

Introduction

Despite available evidence suggesting that surgical castration is painful at any age, procedures in male piglets are still common in most European countries, (EFSA, 2004). However, this practice is being questioned in an increasing number of countries due to animal welfare concerns (Fredriksen et al., 2011). Pork production stakeholders have advocated for a ban on surgical castration by 2018 in the European Union (EU), which posed a challenge for the pork production chain (Morlein et al., 2015), but the implementation of this declaration is still ongoing and efforts are being made to achieve this goal by 2024 (Eurogroup for Animals, 2018). However, the petition from the organisation Eurogroup for Animals was non-binding. Its results have yet to be demonstrated as the EU institutions have not made new commitments to renew the European Declaration on Alternatives to Pig Castration (European Commission, 2015). In the EU, castration remains dominant, representing

approx. 31.5% of pigs slaughtered in 2020 compared to 17% of entire male pigs. Individual European countries face different realities. The top five pig producers in Europe are Spain, Germany, France, Denmark, and the Netherlands, and have 14, 80, 75, 93 and 35% of their commercial male piglets surgically castrated, respectively (Van Ferneij, 2022). In countries such as Spain, Portugal, Ireland or the United Kingdom, male pigs are frequently slaughtered before puberty, weighing less than 100 kg, thus they are raised without castration (Weiler and Bonneau, 2019), but producers lose the hypothetical efficiency of finishing male pigs at a more mature age. The potential ban on surgical castration in Europe makes the major advantage of this practice (the elimination of boar taint) a big challenge for the pig industry (Meinert et al., 2017). Male piglets are castrated primarily to prevent the development of an undesired sensory odour or flavour of boar taint in their carcasses (EFSA, 2004; Fredriksen et al., 2011; Wauters et al., 2017). Boar taint is described as a penetrating animal-, urine-, faecal- or sweat-like unpleasant odour that becomes particularly intense when entire male pork is cooked (Mathur et al., 2012) that is mainly associated with the presence of skatole (SKA) and androstenone (AND) compounds. Skatole (3-methylindole) is a metabolite derived from the amino acid tryptophan produced in the lower gut by intestinal microbiota, and androstenone (5α androst-16-en-3-one) is a steroid produced in the testis (Aldal et al., 2005; Chen et al., 2007; Lunde et al., 2010). AND interferes with SKA clearance by the liver (Doran et al., 2002; Whittington et al., 2004), which explains lower SKA levels in castrates and gilts compared to entire male pigs. Due to the lipophilic properties of SKA and AND, redistribution from blood to adipose tissue occurs readily with prolonged accumulation in fat tissues (Aldal et al., 2005; Wauters et al., 2016). Moreover, animal tissues contain varying levels of other compounds, such as indole (IND) and other steroids, which may affect the perception of the main contributors to boar taint (Annor-Frempong et al., 1997; Morlein et al., 2016). Immunocastration against gonadotropin-releasing hormone (GnRH) is sometimes used as an alternative to eliminate boar taint without the need for surgical castration. However, consumers may be sceptical about food safety and prices (Kress et al., 2019). In addition, the available authorised pharmaceutical in the European Union (Improvac®, Zoetis, Louvain-la-Neuve, Belgium) is catalogued by the EU in the therapeutic area of sex hormones and modulators of the genital system (EMA, 2020), which limits its use in organic production systems, where traditional swine breeds are slaughtered at an older age. Pork production from entire male pigs under enriched housing conditions also seems to be a promising alternative to castration, as improved husbandry aims to increase animal welfare standards and eliminate mutilations (Holinger et al., 2015). Raising entire males can increase productivity and sustainability of swine production. In comparison to barrows, boars show faster growth (+ 13%), leaner meat (+ 20%), more efficient feed conversion (+ 14%) and lower feed requirements (− 9%) (EFSA, 2004). The environmental impact of entire male pork production is not only lower compared to barrows, but also lower than immunocastrates (Kress et al., 2019). However, meat from entire males, when compared to castrates, is tougher, with reduced intramuscular fat content, inferior water holding capacity and deviations in pH and colour (Bonneau and Weiler, 2019; Skrlep et al., 2020). There is evidence that management practices such as batch rearing, use of specific feed ingredients, and prevention of pigs wallowing in excrements may reduce boar taint (EFSA, 2004). Several studies (Zamaratskaia and Squires, 2009; Bilić-Šobot et al., 2014; Wang et al., 2019) showed that fermentable carbohydrates, such as inulin obtained from chicory, effectively reduced the concentration of SKA in the hindgut. There is accumulated evidence that chicory inulin successfully reduces boar taint, but its cost is a major drawback. Beet pulp supplementation can be a more cost-effective alternative. Considering the higher risk of aggressive behaviour in heavier pigs, leading to stress and body lesions, improved animal welfare practices and housing conditions can be applied to overcome these problems (Cornale et al., 2015). Therefore, the present work aimed to evaluate dietary supplementation with inulin or sugar beet pulp, in parallel with improved housing, in raising heavier entire male pigs, their performance, welfare, and meat quality.

Material and methods

Experimental design, management and feeding

This experiment was approved under Directive 2010/63/UE and Decreto-Lei 113/2013 by the Animal Welfare Committee (ORBEA) of Escola Superior Agrária of Coimbra Polytechnic, Portugal. An experimental design with 2 factors, housing, and diet was implemented in 60 healthy males at 30 weeks of age, from a Pietrain terminal sire, crossed with a Large White, Landrace crossed sow.

All pigs were acquired from a local farm after being reared on the same diet and similar housing. At the beginning of the experiment and after allocation to six experimental pens, each group of ten boars had a one-week transition period to the new diet. Two housing treatments, standard or improved, and three diets were tested. The labels used for the treatments are shown in Table 1; the standard and improved housing systems were named H1 and H2, respectively. The improved housing differed from the standard by increased area, feeder length and objects for pig manipulation (Table 2). The feeders had a double front space in H2 and two drinkers (water nipple) instead of one. All pens had a plastic toy hanging in the centre, but the improved housing had three extra toys. One of these was changed each week, to attract the pigs' attention to different toys. Additional toys were made of organic matter or chewable material, such as wood sticks, rubber balls, cotton ropes, and sackcloth. Housing temperature ranged from 12 to 18 °C; airflow was low and relative humidity was high (80–90%) throughout the experiment.

Table 1. Housing conditions, feed type and number of pigs in each pen

Pen	Housing	Diet	Number of pigs
A	H1	D1	10
B	H1	D2	10
C	H1	D3	10
D	H2	D1	10
E	H2	D2	10
F	H2	D3	10

H1 – control (standard housing), H2 – improved housing, D1 – control diet, D2 – 10% beet pulp, D3 – 5% beet pulp + 4% Fibrofos

Table 2. Experimental housing conditions

Housing	Pen area, m^2	Slatte, %	Toys	Feeder space, m	Drinkers
H1	10	100	1	1.2	1
H2	12.5	70	4	2.4	2

H1 – control (standard housing), H2 – improved housing

Two diets, one with 10% sugar beet pulp (by-product after sugar extraction from *Beta vulgaris* L. (Beet & Feed, Bratislava, Slovakia), and the other containing 5% sugar beet pulp and 4% Fibrofos (by-product from *Cichorium intybus* L. roots with 60% inulin Fibrofos 60 purchased from Speerstra, Eesterga, Nederland) were compared to a control diet (percentages as fed). Table 3 lists dietary ingredients and chemical composition on a dry matter basis. The control diet and the diets supplemented with beet pulp and beet pulp mixed with Fibrofos were designated D1, D2, and D3. In this factorial experiment, 60 entire crossbred male pigs (Large White × Landrace gilts sired by Pietrain) were randomly allocated to 6 pens (Table 1) at 23 weeks of age and an average weight of 94.5 ± 5.7 kg. The trial lasted seven weeks (21 November 2018 to 8 January 2019). The average weight of the animals on the day before slaughter (30 weeks of age) was 134.8 ± 9.1 kg.

Diets were formulated (Table 3) to provide the same net energy (NEg 11 MJ/kg dry matter), protein (17.7% dry matter) and essential amino acids.

Table 3. Ingredient and chemical composition of experimental diets, % dry matter (DM)

Diets	D1	D2	D3
% DM	87.6	87.6	87.8
Ingredients, g/kg DM			
wheat	377	325	421
corn	300	369	233
soybean meal 47	120	170	173
barley	187	97	112
Fibrofos 60	-	-	46
beet pulp	-	114	57
rapeseed meal	57	34	6
wheat bran	16	-	59
molasses	11	8	6
sunflower meal	46	-	
animal fat	-	-	4
Chemical composition, g/kg DM			
crude protein	178	178	177
neutral detergent fibre	157	164	157
acid detergent fibre	66	69	61
non-fibre carbohydrates	559	525	517
calcium	8	8	8
phosphorus	5	5	5
lysine	11	12	11
methionine + cysteine	7	7	7
threonine	8	8	8
inulin	36	36	69
NEg MJ/kg DM	11.0	11.0	11.0
Cost of production, euro/ton (October 2018)	21	17	57

NEg – net energy, D1 – control diet, D2 – 10% beet pulp, D3 – 5% beet pulp + 4% Fibrofos, beet pulp (by-product after sugar extraction from *Beta vulgaris* L.) Fibrofos (by-product from *Cichorium intybus* L. roots with 60% inulin)

All three mixes were pelleted, but Fibrofos was particularly expensive and difficult to process. A local commercial mill that supplied the mixes reported the cost of ingredients and processing conditions. Feed and water were provided *ad libitum*. New feed was given daily (30 kg per pen), and individual intake was assessed by subtracting the weight of leftovers from each feeder. Average daily body weight gain (BWG) was determined by weighing each animal at the beginning, middle and end of the trial.

Group stability was maintained during transportation and lairage (ten animals per group). European welfare regulations were applied from farm to slaughterhouse and upon arrival, the animals were subjected to stress-reducing procedures in six different pens. The waiting time for slaughter was less than two hours, and carcass yield values were determined on the basis of live weight of each pig on the day before slaughter, and carcass weight was assessed at the abattoir.

Skin lesion score

Each pig was examined for skin lesions 5 times, on days 2, 14, 24, 30 and 43 of the experiment. The method was adapted from the Welfare Quality® protocol, and the incidence of skin lesions was evaluated on the neck, shoulder, flank, back and rear leg of both sides (left and right). The results were scored on a 0–4 scale: less than five small skin lesions were scored as 0, five to 10 small skin lesions were scored as 1, 10 to 16 small skin lesions were scored as 2, more than 16 small skin lesions or more than 1 severe skin lesion (blood but superficial injury) were scored as 3, and serious injuries such as deep and bloody wounds were scored as 4. The experiment was always assessed by the same person.

Sampling

Individual blood samples were collected the day before the trial commenced and subsequently on days 25 and 44 of the experiment. At the same time, a pooled saliva collection was carried out. After containment with a nose restrainer, blood was collected individually from the jugular vein. The procedure was rapid, usually taking less than 2 minutes. Blood was centrifuged upon arrival at the laboratory (2 000 *g*, 10 min, 4 °C; Hettich Rotanta 460R refrigerated centrifuge, Beverly, MA, USA). The supernatant (plasma) was collected, aliquoted into several microtubes and stored at −80 °C (Thermo Scientific freezer, Ashville, NC, USA) until analysis. Before blood collection, pooled saliva was collected by pen from ropes made available to the animals to chew on (Pack CIVTEST® Suis oral fluids, Hidra, Girona, Spain). The strings containing saliva samples from each park were squeezed into a tube (50 ml falcon type), refrigerated until sequential centrifugations were performed (2 000 *g*, 10 min, 4 °C; MPW-350R refrigerated centrifuge MPW Med. Instruments, Warsaw, Poland) to remove as much impurities as possible (mainly food debris). After 2 to 4 centrifugations, the samples were stored at −80 °C until analysis. After slaughter, pork carcasses were transported to a meat processing factory. Subsequently, adipose tissue (from backfat and subcutaneous belly fat) and meat samples (from *biceps femoris*) of each animal were collected and frozen. Backfat and belly fat samples were collected to extract adipose tissue to determine boar taint concentration (skatole and androstenone), and meat from ham (*biceps femoris*) to analyse the physicochemical parameters of the meat. Samples were kept at −18 °C for 5 weeks until further analysis.

Health care and hemogram

Before the study, the prophylactic plan included immunisation against *Mycoplasma* sp. and circovirus at 28 days of age, against porcine reproductive and respiratory syndrome (PRRS) and Aujesky on day 60, with a booster dose at 14 weeks. Flubendazole (Flimabo, Virbac, Novo Mesto, Slovenia) was administered for five days at a dose of 2.5 mg/kg live weight at 11 weeks of age for deworming. Individual haematological parameters were determined at the beginning of the trial and at the end before slaughter. Blood samples were preserved at 4 °C and processed within 24 h using an Advia 120® Haematology System automated analyser (Siemens, Erlangen, Germany) and Advia 120 software version 6.3.2-MS. Reference intervals were considered for discussion (Koomkrong et al., 2017). Repeated measures ANOVA was applied for all variables to determine the differences between the two sampling time points, at the beginning and at the end of the assay (95% confidence interval). The effect of housing conditions and diet was assessed at the last sampling time point before slaughter.

Plasma androstenone (AND), skatole (SKA) and indole (IND) analysis

Plasma AND, SKA, and IND concentrations were determined using U-HPLC-HR-Orbitrap-MS (Thermo Fisher Scientific System, San José, CA, USA), and a validated method developed by Wauters et al. (2015) based on previous work of Bekaert et al. (2012). The limit of detection (LOD) for AND, SKA and IND was 1, 0.5, and 0.5 µg/l, respectively. The maximum method repeatability was 4.7, 6.6, and 7.5%, and the minimum within-laboratory reproducibility was 6.4, 6.1, and 10.4% for AND, SKA, and IND, respectively. Plasma samples collected in Portugal were sent at −20 °C to the Laboratory of Chemical Analysis at Ghent University (Merelbeke, Belgium), where AND, SKA, and IND were analysed using a validated method (Wauters et al., 2015).

Cortisol

Cortisol concentration was determined using an ELISA kit (ENZO, Lausen, Switzerland), which allows to determine this analyte in various biological fluids. It is based on a competitive ELISA assay, with readings performed on a Tecan's ELISA Sunrise reader (Mannedorf, Switzerland) at 405 nm. According to the manufacturer, the sensitivity limit was 56.7 pg/ml or 0.00567 µg/ml. The results were calculated by adjusting for the 4-parameter logistic curve (4PL), as recommended by the manufacturer. The intra-assay CV was 11.4%, and the inter-assay CV was 11.7%.

Meat quality

Moisture content (%), intramuscular fat, thawing loss, pH and colour determination were carried out in meat from *biceps femoris* as follows: moisture (%) was determined according to the Portuguese Standard NP 1614/2009; the meat was ground, mixed with analytical sea sand and oven-dried until constant weight (103 ± 2 °C, approx. 3 h). Intramuscular fat (IMF) percentage was determined using the Soxhlet extraction procedure and petroleum ether as an extraction agent after sample hydrolysis, according the Portuguese Standard NP 1613/1979 (Meat and meat products – Determination of total fat content, reference method). All subcutaneous fat was removed during sample preparation using only lean muscle tissue for analysis. For thawing loss determination, the samples were weighed, thawed at 4 °C for 24 h, dried with a paper towel and reweighed. The samples thawed for determinations had been frozen for two to five weeks. The pH values were determined after thawing by potentiometry using a pH meter (PH 25+, Crison Instruments, Barcelona, Spain) according to the Portuguese Standard NP 3441/2008. The colour of thawed meat was measured on a freshly cut surface using a Minolta CR-300 set (Konica Minolta, Tokyo, Japan) with D65 illuminant, recording L* a* b* values, where L* – lightness, a* – redness, and b* – yellowness (Pathare et al., 2013). The colour values were the average of ten measurements of the meat surface at different positions.

Androstenone and skatole analysis

SKA and AND contents in pig fat was determined using a rapid high-performance liquid chromatographic (HPLC) method adapted from Hansen-Moller (1994). Sample preparation for HPLC injection consisted of cutting the adipose tissue removed from the neck (backfat) and belly, and extracting the liquid fat by solid-liquid separation after microwave heating (800 W, 2 min). After sonication and centrifugation, analytical extraction from the liquid fat was carried out using methanol. Manual derivatisation of the sample (500 µl) was performed at room temperature for 5 min before injection by adding 40 µl of BF3, 50 µl of deionised water, and 75 µl of dansylhidrazine 0.1%. An HPLC system (Thermo Scientific UltiMate 3000, Waltham, MA, USA) with a Hypersil ODS C18 250 × 4.6 mm 5 µm (Thermo Scientific, Waltham, MA, USA) column operating at 40 °C was used. The composition of the mobile phase buffers was as follows: (A) acetic acid 0.1%, (B) acetonitrile, (C) tetrahydrofuran, and (D) buffer solution pH 6.0 (potassium phosphate 25 mMol). The following gradient profile was used: 0–5 min, 60–50% A, 35–45% B, 5% C; 5–6 min, 50% A, 45% B, 5% C; 6–6.1 min, 50–20% A, 45–30% B, 5–30 C, 0–20% D; 6.1–12 min., 20–0% A, 30–40% B, 30–40% C, 20% D; 12–12.5 min, 0–60% A, 40–35% B, 40–5% C, 20–0% D; 12.5–13 min, 60% A, 35% B, 5% C, with a flow rate of 2.0 ml/min. Fluorescence detection was performed with excitation at 285 nm, emission at 340 nm (0–6.0 min), followed by excitation at 346 nm and emission at 521 nm (6.1–13 min). In all assays, 20 µl of sample was injected. The limit of detection (LOD) for AND and SKA was 0.053 and 0.005 ppm, respectively.

Statistical analysis

Statistical analysis was performed using the Statistica for Windows software package, version 7 (Stat Soft Inc., Tulsa, OK, USA) and the implemented Kolmogorov-Smirnov test to assess normality. Treatment effects were tested by analysis of variance (ANOVA) performed for the two experimental factors (housing and diet), followed by Tukey's post hoc test. For some variables, a previous analysis using the non-parametric Kruskal-Wallis test was performed due to the lack of normality of the data. Housing and diet were set as independent variables, and the obtained parameters (pH, L* a* b*, IMF, moisture %, thawing loss, AND and SKA) were defined as dependent variables. Differences were considered significant at $P < 0.05$.

Results

Animal husbandry performance

Performance in relation to individual diets and housing showed no significant differences between the experimental treatments (Table 4). This indicated that the treatments had no significant effect on growth or carcass yield.

Table 4. Effect of experimental diets and housing on pig performance related to animal husbandry

Treatment	Intake, kg/day	BWG, kg/day mean ± SD	Carcass yield, % mean ± SD
Diet			
D1 (n = 20)	2.9	0.91 ± 0.14	81.0 ± 0.021
D2 (n = 20)	3.0	1.00 ± 0.19	81.2 ± 0.026
D3 (n = 20)	2.8	0.94 ± 0.19	80.6 ± 0.024
P-value		0.256	0.672
H1 (n = 30)	2.8	0.91 ± 0.16	81.4 ± 0.018
H2 (n = 30)	3.0	0.98 ± 0.19	80.5 ± 0.027
P-value		0.130	0.156

BWG – body weight gain, D1 – control diet, D2 – 10% beet pulp, D3 – 5% beet pulp + 4% Fibrofos, H1 – control (standard housing), H2 – improved housing; SD – standard deviation; $P > 0.05$

Skin lesion results

In general, lesion scores were low and very few injuries with blood were noticed. The results (Figures 1 and 2) showed a maximum score at the midpoint of the trial. There was a trend towards fewer lesions after the third observation time point.

The results showed higher scores for standard housing and lower for improved housing. However, the incidence of skin lesions for the diets with beet pulp and Fibrofos (D2 and D3), was higher than for control (D1) at the end of the trial.

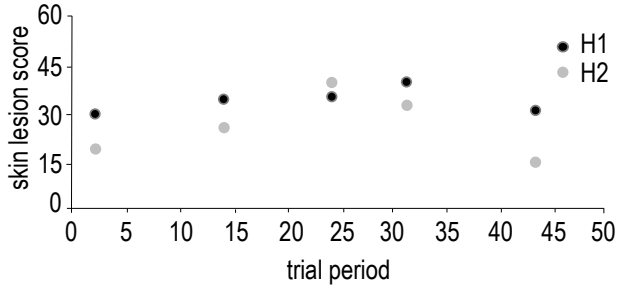

Figure 1. Skin lesion score for different housing conditions during the experiment

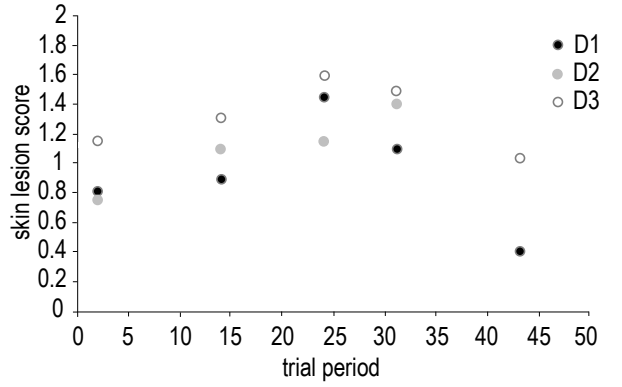

Figure 2. Skin lesion score for different diets during the experiment

Plasma androstenone, skatole and indole analysis

Initial results for plasma IND and SKA levels (samples from day 0) were 1.1 ± 1.3 µg/l and 0.9 ± 1.0 µg/l, respectively. However, the second and final analysis showed results below LOD for all samples (< 0.5 µg/l). For AND, the results demonstrated (Table 5) that the effect of diet was significant at the end of the experiment; it was found that diets with the addition of beet pulp and Fibrofos (D2 and D3) reduced AND. The effect of housing on AND was significant (Table 5) at the end of the experiment, and animals in improved housing conditions (H2) had significantly reduced AND levels.

Table 5. Effect of experimental diets and housing on pigs plasma androstenone levels (µg/l) before the study, on day 25 and 44

Diet	0 days	25 days	44 days
	mean ± SD	mean ± SD	mean ± SD
D1 (n = 20)	1.3 ± 0.4	1.6 ± 1.1	3.4 ± 3.6^{a}
D2 (n = 20)	1.4 ± 0.5	1.9 ± 1.3	1.5 ± 0.8^{b}
D3 (n = 20)	1.7 ± 1.1	1.7 ± 1.3	1.5 ± 1.1^{b}
P-value	0.317	0.759	0.0007
Housing			
H1 (n = 30)	1.5 ± 0.6	1.7 ± 1.3	3.2 ± 3.0^{a}
H2 (n = 30)	1.4 ± 0.9	1.7 ± 1.2	1.0 ± 0.04^{b}
P-value	0.317	0.759	0.0007

D1 – control diet, D2 – 10% beet pulp, D3 – 5% beet pulp + 4% Fibrofos, H1 – control (standard housing), H2 – improved housing; SD – standard deviation, ^{ab} – means within columns with different superscripts are significantly different at $P < 0.05$

Haematological parameters

Before slaughter, all blood parameters in the samples were normal according to the pre-determined standards. Moreover, compared to the control diet (D1), the eosinophil count was significantly lower ($P < 0.05$) in group D3 and the platelet count was significantly higher ($P < 0.05$) in group D2, indicating a positive effect of both the mix with beet pulp and Fibrofos and the 10% beet pulp mixture, respectively (Table 6).

Table 6. Effect of experimental diets on blood parameters of pigs at the end of the study

Diet	Eosinophils, % mean ± SD	Platelets, × 10^3/µl mean ± SD
D1 (n = 20)	5.4 ± 2.5^{a}	337.0 ± 62,0^{b}
D2 (n = 20)	4.4 ± 3.3^{ab}	402.5 ± 83.7^{a}
D3 (n = 20)	3.3 ± 3.3^{b}	350.1 ± 71.3^{ab}
P-value	0.022	0.015

D1 – control diet, D2 – 10% beet pulp, D3 – 5% beet pulp + 4% Fibrofos; SD – standard deviation, ^{ab} – means within columns with different superscripts are significantly different at $P < 0.05$

Plasma cortisol

Table 7 presents plasma cortisol levels on days 25 and 44 of the experiment. No significant differences were found between animals fed the experimental diets or kept in different housing conditions after study day 25. On day 44, animals kept in the standard housing (H1), irrespective of the diet, showed significantly higher circulating cortisol levels than animals in the improved housing (H2). This was consistent with the skin lesion score calculated during the trial, as it decreased significantly after 25 days for the improved housing (Figure 1).

Table 7. Effect of experimental diets and housing on plasma cortisol (ng/ml) levels in pig samples on day 25 and 44 of the study

Diet	25 days mean ± SD	44 days mean ± SD
D1 (n = 20)	133.4 ± 50.7	160.7 ± 46.4
D2 (n = 20)	129.1 ± 41.5	149.2 ± 55.5
D3 (n = 20)	110.2 ± 26.6	139.4 ± 35.0
P-value	0.170	0.313
Housing		
H1 (n = 30)	129.2 ± 41.6	165.8 ± 46.7[a]
H2 (n = 30)	119.2 ± 41.3	133.8 ± 40.9[b]
P-value	0.346	0.006

D1 – control diet, D2 – 10% beet pulp, D3 – 5% beet pulp + 4% Fibrofos, H1 – control (standard housing), H2 – improved housing; SD – standard deviation, [ab] – means within columns with different superscripts are significantly different at $P < 0.05$

Meat quality

The values of the analysed meat quality traits are shown in Table 8. Feed supplementation affected some parameters, such as meat moisture content (%), intramuscular fat percentage (IMF%) and pH. Meat from groups fed beet pulp and Fibrofos showed lower moisture, lower IMF, and lower pH. Regarding the housing conditions, thawing loss was significantly lower for H1. Interactions between factors for all parameters analysed were not significant ($P > 0.05$). In terms of colour coordinates, no significant differences were found in comparison to the groups scored according to diets. Housing conditions seemed to affect the colour of the meat as the colouration was darker (L*) and redder (a*) in group H1.

Boar taint compounds in fat

The results obtained in HPLC analysis of the neck (backfat) and belly fat revealed statistically significant differences ($P < 0.05$) in SKA and AND levels, depending on the factor analysed. A factorial ANOVA was performed (diet vs housing conditions) and there was no evidence of a statistically significant interaction (0.531 for SKA and 0.852 for AND in belly; 0.621 for SKA and 0.607 for AND in backfat). Table 9 presents SKA and AND quantification results, depending on the factors analysed. SKA levels were significantly lower in groups with supplemented feed, while it could be noted that feed did not affect AND concentrations. However, the housing factor seemed significant as the animals under the improved conditions had lower AND concentrations. Housing conditions showed no clear effect on SKA levels.

AND and SKA concentrations differed slightly in the two adipose tissue samples analysed. However, the obtained results were proportional (Table 9), leading to the same conclusions about the effectiveness of the experimental factors.

Table 8. Effect of experimental diets and housing on parameters of pig meat quality

	Moisture, %	IMF, %	Thawing loss, %	pH	L*	a*	b*
Diet, mean ± SD							
D1 (n = 20)	71.8 ± 2.6[ab]	4.4 ± 1.3[a]	6.8 ± 3.2	5.66 ± 0.14[a]	44.4 ± 7.8	11.8 ± 3.9	8.7 ± 2.7
D2 (n = 20)	72.1 ± 2.8[a]	4.0 ± 1.2[a]	8.0 ± 3.0	5.64 ± 0.17[ab]	45.6 ± 8.5	11.5 ± 4.3	8.3 ± 2.9
D3 (n = 20)	70.9 ± 2.4[b]	3.3 ± 1.0[b]	7.4 ± 3.0	5.56 ± 0.21[b]	46.0 ± 10.3	11.3 ± 8.0	8.5 ± 3.6
P-value	0.039	0.001	0.141	0.009	0.242	0.684	0.476
Housing, mean ± SD							
H1 (n = 30)	71.8 ± 2.7	4.0 ± 1.1	6.3 ± 2.7[a]	5.6 ± 0.2	43.7 ± 6.3[a]	12.6 ± 3.6[a]	8.4 ± 2.9
H2 (n = 30)	71.4 ± 2.5	3.8 ± 1.4	8.4 ± 3.1[b]	5.6 ± 0.2	47.0 ± 10.8[b]	10.4 ± 7.0[b]	8.7 ± 3.2
P-value	0.372	0.210	0.000	0.211	0.000	0.000	0.717

IMF – intramuscular fat, L* – lightness, a* – redness, b* – yellowness, D1 – control diet, D2 – 10% beet pulp, D3 – 5% beet pulp + 4% Fibrofos, H1 – control (standard housing), H2 – improved housing; SD – standard deviation; [ab] – means within columns with different superscripts are significantly different at $P < 0.05$

Table 9. Effect of experimental diets and housing on SKA and AND concentrations (µg/g) in pig backfat and belly fat

	Backfat		Belly fat	
	SKA	AND	SKA	AND
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Diet				
D1 (n = 20)	0.069 ± 0.042[a]	0.272 ± 0.194	0.078 ± 0.062[a]	0.114 ± 0.086
D2 (n = 20)	0.028 ± 0.024[b]	0.194 ± 0.119	0.021 ± 0.016[b]	0.102 ± 0.104
D3 (n = 20)	0.022 ± 0.015[b]	0.200 ± 0.161	0.030 ± 0.032[b]	0.097 ± 0.085
P-value	0.000	0.354	0.000	0.512
Housing				
H1 (n = 30)	0.036 ± 0.036	0.263 ± 0.190[a]	0.039 ± 0.044	0.128 ± 0.109[a]
H2 (n =3 0)	0.043 ± 0.036	0.181 ± 0.118[b]	0.045 ± 0.051	0.080 ± 0.059[b]
P-value	0.228	0.050	0.863	0.046

SKA – skatole, AND – androstenone, D1 – control diet, D2 – 10% beet pulp, D3 – 5% beet pulp + 4% Fibrofos, H1 – control (standard housing), H2 – improved housing; SD – standard deviation; [ab] – means within columns with different superscripts are significantly different at $P < 0.05$

Discussion

This study evaluated the performance, welfare, health, and meat quality of non-castrated heavier pigs raised in two different housing conditions and fed three different diets. Several studies have previously been conducted to evaluate the effect of diet on the reduction of boar taint that tested ingredients such as chicory inulin (Fibrofos) and sugar beet pulp, with varying outcomes (Bee et al., 2020). Housing conditions also play an important role in the development of boar taint, thus combining this factor with feeding supplementation is of importance. Muscle tissues with higher water losses generally have lower pH values and a paler colour (Bee et al., 2007; Seiquer et al., 2019). The results of this study showed a trend towards higher BWG for diets D2 and D3 (with beet pulp and Fibrofos) when compared to control (D1), as well as higher BWG for the improved housing (H2) in relation to the standard housing (H1). For group H2, food intake was higher and water access easier, and pigs under improved conditions presented significantly lower levels of plasma cortisol, an indicator of less stress. Furthermore, throughout the trial, the lesion scores tended to be lower for group H2, indicating lower stress levels in these animals. The cost of raising pigs on diet D2 was lower as this feed was cheaper, and feed conversion was similar (3.0) to D3 but lower than D1 (3.2). The results showed a positive effect of the new diets (D2 and D3) on the haematological profile, with a decrease in the total leukocyte count (21 700/µl on day 0 to 18 000/µl on day 44 of the trial). A statistically significant result was also recorded for the introduction of Fibrofos in combination with beet pulp (D3), which exerted an anti-inflammatory effect, expressed in improved eosinophil counts (1 300/µl on day 0 to 790/µl on day 44 of the trial). Pigs fed 10% beet pulp (D2) showed a significantly better platelet count, which in some contexts have protective immune functions. No direct effect was observed, but it was similar to that described by Tretola et al. (2019). Our study showed a clear effect of housing conditions on lowering AND plasma levels, indicating that low stress or an enriched environment may have contributed to the decrease in AND. Careful transportation and slaughter could be associated with the low fat levels of AND. Aggression due to social ranking or sexual behaviour and stressful environments is normally associated with high AND levels in entire males (Squires et al., 2020). Diverting their attention to food, water or toys, and maintaining group stability may have contributed to lower AND concentrations in the plasma. The transport of AND in plasma is a poorly understood process that precedes AND accumulation in adipose tissue (Squires et al., 2020). It is suggested that the degree of AND binding to albumin in plasma affects AND concentration in fat, and unconjugated plasma AND has been shown to be positively correlated with AND fat levels (Bone and Squires, 2021). This experiment demonstrated that improved housing (H2) caused a decrease in both plasma and fat AND concentrations. The inclusion of beet pulp and Fibrofos in diets D2 and D3, both rich in carbohydrates fermentable in the large intestine, could activate healthy microbiota, leading to less cell debris and availability of L-tryptophan fermentation initiated by SKA (Pieper et al., 2014; Tretola et al., 2019). This study found that meat quality parameters were affected by housing conditions and diet supplementation, but the interaction of these factors was not statistically

significant. Differences were mainly observable between different dietary groups. The results showed that moisture %, IMF% and pH led to significant variations. The group supplemented with beet pulp and Fibrofos (D3) presented the lowest moisture percentage, while the group fed beet pulp (D2) the highest. Although previous research has shown that diet may affect water holding capacity, and thus meat moisture content (Watanabe et al., 2018), these parameters are mainly influenced by housing and handling factors, especially in the immediate pre-slaughter period (Cheng and Sun, 2008). However, no significant differences were found between the housing groups in this experiment. On the other hand, thawing loss was higher in meat from animals kept in better maintenance conditions (8.44%). In some studies (Seiquer et al., 2019; Skrlep et al., 2019a, b), an average thawing loss above 10% was considered a normal value for entire males. Nevertheless, other studies that used similar methods to determine thawing losses reported values lower than 5% (Aaslyng et al., 2003; Ku et al., 2014). In comparison with D3, the group without supplementation had higher pH values, and the groups with different housing conditions presented very similar pH results. The pH values determined in the present study were in line with those reported in other studies (Aaslyng et al., 2003; Hansen et al., 2008; Grela et al., 2020). There is no conclusive evidence in the available literature that diet is able to significantly alter meat pH. This type of changes may be due to *post-mortem* changes induced by glycolytic potential (Li et al., 2015). The lower IMF percentage in diet D3 could be due to inulin supplementation promoting muscle hypertrophy, thereby increasing the lean percentage of growing-finishing pigs (Wang et al., 2019). However, the percentage of lean meat in similar studies was not significantly affected by the applied treatments (Aluwe et al., 2009; 2017). Housing conditions were the only factor that led to colour changes. Meat from the group kept under standard conditions was darker and redder in colour (a* values were higher). The long pre-slaughter period and physical activity associated with aggressive behaviour of entire males can lead to darker meat (Xue et al., 1997). Yet these characteristics are generally considered more attractive for consumers (Norman et al., 2003; Straadt et al., 2013). SKA and AND levels were determined by extracting liquid fat from adipose tissue of the neck (backfat) and belly, and the results showed that D2 and D3 effectively reduced SKA levels. Feeding 10% beet pulp and mixes of beet pulp (5%) with Fibrofos (4%) reduced the level of SKA in backfat by 60 and 68%, respectively (from 0.069 ± 0.042 μg/g to 0.028 ± 0.024 μg/g and 0.022 ± 0.015 μg/g). The same trend was observed in belly fat, with D2 reducing SKA concentration by 74% and D3 by 62% (from 0.0779 ± 0.0623 μg/g to 0.021 ± 0.016 μg/g and 0.030 ± 0.032 μg/g, respectively). Similar findings were also reported by other authors (Hansen et al., 2006; Byrne et al., 2008; Kjos et al., 2010; Overland et al., 2011; Zammerini et al., 2012; Aluwe et al., 2017; Heyrman et al., 2018). In our study, fat AND levels were not affected by the feed, but other studies reported that diet reduced AND levels (Rasmussen et al., 2012; Heyrman et al., 2018). However, in most experiments, the type of feeding was not associated with a reduction of this pheromone levels in pig fat. Therefore, these measures are not efficient in controlling AND levels (Bonneau and Weiler, 2019). On the other hand, the housing factor led in the present study to significant differences in AND levels as the improved housing conditions caused a reduction of AND content in fat, detected in both backfat ($P = 0.050$) and belly fat ($P = 0.046$). However, the AND values were higher in backfat, thus it could be inferred that the area where the tissue was removed might have affected the final result, as described by Meinert et al. (2017). Fredriksen and collaborators (2006) also reported that using a farrow-to-finish system led to a reduction in AND levels. Still other authors argued that housing conditions did not affect AND levels in boars (Fabrega et al., 2011; Kress et al., 2020). However, insufficient studies support these conclusions because recommendations for housing entire male pigs are scarce, especially for unconventional systems (Holinger et al., 2015). The perception of boar taint by meat consumers is a complex issue related to AND and SKA concentrations in meat fat. Most of the fat samples in this experiment never exceeded the level accepted as normal (Prusa et al., 2011) sensory detection threshold for AND (1 μg/g) and SKA (0.2 μg/g). Morlein et al. (2015) indicated a high risk of reduced consumer acceptance associated with AND and SKA fat levels of 2 and 0.3 μg/g, respectively. The same authors, however, reported that AND and SKA concentrations lower than 0.5 and 0.1 μg/g, respectively, in entire male fat caused no negative perception associated with boar taint. On average, for diets with beet pulp and beet pulp mixed with

Fibrofos, the results for AND and SKA levels were below 0.5 and 0.1 μg/g, respectively (Table 9). For backfat AND, 2 of 20 pigs fed D3 exceeded 0.5 μg/g. For SKA, 1 out of 20 animals fed D2 slightly exceeded 0.1 μg/g, while 6 of 20 were outside the 0.5 and 0.1 μg/g threshold for AND and SKA, respectively, in the control diet. For SKA in belly fat, 1 out of 20 pigs fed D3 exceeded 0.1 μg/g, while 4 of 20 were outside the 0.1 μg/g threshold for SKA level in the control diet.

Conclusions

The obtained results showed that beet pulp or a mix of beet pulp and chicory inulin reduced skatole levels. On the other hand, the improvement in living conditions led to a reduction in androstenone concentrations. Regarding other meat quality parameters, standard housing conditions resulted in a darker red colour but lower thawing losses. Although the overall meat quality parameters were mainly affected by housing and handling conditions, the results showed that diets with beet pulp or inulin from chicory positively affected pH values, moisture, and intramuscular fat content parameters. Only 5% of the pigs fed 10% beet pulp showed backfat skatole or androstenone levels slightly exceeding human perception threshold, which was an improvement in comparison to 30% fed the control diet. Administering the mix with Fibrofos (chicory inulin) gave similar results to 10% beet pulp feed, but it was much more expensive, so the latter would be preferred. Improved housing conditions allowed for a significant decrease in fat androstenone content, as it was approximately 30% lower than in pigs kept in standard conditions. Animal health and welfare signs were assessed to be higher under improved housing conditions. Animal performance was not impaired either by altered maintenance or the use of beet pulp, therefore we conclude that it is possible to grow entire male pigs for heavier carcasses without castration and an occasional low perception of boar taint.

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Conflicts of interest

The Authors declare that there is no conflict of interest.

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