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# Impact of increasing levels of condensed tannins from sainfoin in the grower–finisher diets of entire male pigs on growth performance, carcass characteristics, and meat quality

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ABSTRACT

Sainfoin is a protein-rich legume with an ideal amino acid profile and therefore could partly replace soybeans in the diets of growing pigs. However, sainfoin also contains a non-negligible amount of condensed tannins (**CTs**), which can act as antinutritional factors. Bioactive plant compounds, like hydrolysable tannins, have been suggested to be suitable in entire male (**EM**) production, as they impair the development of accessory sex glands and, by that, reduce boar taint compound levels without negatively impacting growth. It is unknown whether, similar to hydrolysable tannins, CTs from sainfoin reduce the incidence of boar taint without impacting growth performance, carcass traits, and meat quality. For the experiment, 48 Swiss Large White EM were assigned within litter to one of four grower (25–60 kg BW) and finisher (60–105 kg BW) diets supplemented with 0 (T0), 5 (T5), 10 (T10), and 15% (T15) sainfoin meal, respectively. The four diets were designed to be isocaloric and isoproteic. Increasing the dietary sainfoin level had no negative effect on growth performance or the carcass characteristics. Despite leading to a similar feed intake between the treatment groups, increasing the dietary sainfoin levels tended ($P \leq 0.08$) to reduce the number of feeder visits but increased the time spent at the feeder as well as the feed intake per visit during the finisher period. By increasing sainfoin intake, the levels of C18:3n-3 and long-chain homologs linearly increased ($P < 0.01$) in the backfat and intramuscular fat (**IMF**), whereas in the backfat, but not the IMF, the 18:2n-6 levels decreased ($P < 0.01$). The latter triggered a greater ($P < 0.01$) desaturation rate (C18:1n-9/C18:0) of the saturated fatty acids, resulting in a greater ($P < 0.01$) proportion of monounsaturated fatty acid. Apart from a linear decrease ($P = 0.02$) in the androstenone levels in the longissimus thoracis (**LT**), increasing the sainfoin intake had no effect on the level of boar taint in the LT and backfat. As determined by the elevated correlation coefficient, skatole and indole levels, but not androstenone levels, in the adipose tissue seem to be reliable proxies for their respective levels in LT and, therefore, in pork. In conclusion, sainfoin is a suitable homegrown protein source for grower finisher pigs and can be included at up to 15% in the diet to replace 7% of soybean in a diet without producing any noteworthy effects on growth, whereas the impact of CTs on boar taint was limited.



## Implications

Sainfoin as a home grown protein source is an interesting plant for grower finisher pigs. Not only is the protein content of this forage legume relatively elevated, but its essential amino acid profile is rather ideal for the growing pig. However, the content of antinutritional factors of sainfoin, like condensed tannins, poses some limitations to its use as a feed ingredient in diets. The results of this experiment revealed that up to 15% sainfoin in the ration had no negative effect on growth performance or the carcass characteristics but impaired water-holding capacity and tenderness of the loin muscle.

## Introduction

There is clear evidence that skatole production and, ultimately, its deposition in the backfat of pigs can be affected by dietary means (Wesoly and Weiler, 2012). Results from recent studies suggest that secondary plant compounds, such as hydrolysable tannins, impaired the accessory sex glands development and have the potential to reduce the production of skatole and indole in the colon, which, in turn, results in a lesser accumulation in the backfat of entire males (**EM**) (Čandek-Potokar et al., 2015; Bee et al., 2016). Likewise,

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Bilić-Šobot et al. (2016) observed that the inclusion of 3% hydrolysable tannins in the diet of EM lowered the apoptosis of intestinal epithelial cells, limiting the availability of L-tryptophan from cell debris and, consequently, microbial-mediated skatole production. In addition, Tretola et al. (2019) reported that hydrolysable tannins affected both the quantity and quality of bacteria in a way that could explain effects observed in boar taint levels. The often reported antinutritional effects of tannins, like reduced feed intake and reduced performance, were not observed when the hydrolysable tannin inclusion levels reached 30 g/kg of feed (Bee et al., 2016). On the contrary, as reviewed by Mueller-Harvey (2006), others have reported the effect of condensed tannins (**CTs**) as capable of affecting the feed intake and growth performance of pigs through a reduction of the apparent digestibility of nutrients, especially proteins and amino acids. Jin et al. (2012) observed that proanthocyanidin composition can vary among cultivars, within a species and between different grain legumes. The size of the polymers and the procyanidins-to-prodelphinidin ratio are the main parameters modifying protein–CT interaction. For example, in the case of faba beans, it has been shown that the major CTs are procyanidins, specifically catechins (Merghem et al., 2004), and that these may have different modes of action when compared with other CTs sources (Girard and Bee, 2020).

Two reasons underlined using this legume in the diet of pigs: 1) sainfoin has, compared to other CTs-containing legumes, an elevated CTs content [up to 100 g/kg DM based on Girard et al., 2016] and 2) sainfoin, as a home-grown protein-rich legume, can be considered a complement to soy protein alone and, by that, reduce reliance on imported soybean. Moreover, as opposed to faba beans, sainfoin contains lower procyanidin and greater prodelphinidin levels (Azuhnwi et al., 2013). A. greater proportion of prodelphinidin (i.e. a reduced procyanidin-to-prodelphinidin ratio) increases the biological activity of CTs and predisposes them to bind more strongly with macromolecules (Brunet and Hoste, 2006). Furthermore, to the best of our knowledge, there is no study available on the impact of CTs from sainfoin (*Onobrychis viciifolia*) offered to pigs and their possible effects on meat quality. In view of this, the purpose of the present study was to investigate the impact of increasing the amount of CTs from sainfoin, included in a grower–finisher diet of EM pigs, on growth performance, carcass characteristics, and meat quality, with special emphasis on the accumulation of boar taint compounds in backfat and intramuscular fat (**IMF**).

## Material and methods

### *Animals, diets, and slaughtering procedures*

Forty-eight Swiss Large White EM, originating from eight litters (six per litter) and weighing 24.8 ± 5.1 kg (average ± SD), were assigned within litter to four experimental treatments. The four experimental diets consisted of a control group (T0) with no added sainfoin and three diets supplemented with 5 (T5), 10 (T10), and 15% (T15) dehydrated sainfoin (*Perly* cultivar) (Table 1). The grower (25–60 kg BW) and finisher (60–105 kg BW) diets were formulated to be isocaloric and isonitrogenous according to the Swiss feeding recommendations for swine (Agroscope, 2017). The pigs were reared in a large group pen (pen size: 16.7 $m^2$; 1.4 $m^2$/pig) equipped with four automatic feeders (one for each diet) and an individual pig recognition system (Schauer Maschinenfabrik GmbH & Co. KG, Prambachkirchen, Austria). The feeding system recorded all daily visits to the feeder, feed intake (**FI**) per visit, and time spent at the feeder. For data evaluation, only feeder visits that coincided with the intake of feed (but not sham visits) were considered. In addition, intervals between meals shorter than five minutes were considered as one meal and one visit (De Haer and Merks, 1992). As proposed by Carcò et al. (2018), the day, not the single meal, was considered the temporal basis for describing the feeding behavior of the pigs during the experimental period. Thus, the total feed intake, total feeder visits, and total feeding time per day per pig were calculated. From these data, the average total time feeding per day (**TTF** expressed in min), average frequency of feeder visits (**FFV**), average time per visit (**TV** = TTF/FFV expressed in min), mean feed intake per visit (**FIV** = average daily feed intake/FFV expressed in g), mean rate of feed intake (**RFI** = average daily feed intake/TTF expressed in g/min), and interval between two meals (**FI** expressed in min) were calculated.

After 48 d, the pigs were switched from the grower to the finisher diet, on which they stayed for 52 d. In the grower and finisher period, they had *ad libitum* access to the diets and water. Within two consecutive weeks, all pigs were slaughtered at 172 ± 3.9 d of age at the research station abattoir after being fasted for approximately 12 h. Prior to slaughter, live BW was determined. A detailed description of the slaughter and sampling methods was previously given by Bee et al. (2016). Briefly, 30 min after exsanguination, the weights of the hot carcasses, liver, kidneys, testis, and bulbo-urethral gland, salivary glands (mandibular and parotids) were assessed. Subsequently, the carcasses were chilled at 2 °C for 24 h. One day post-mortem, the left cold carcass weight was determined, and the carcass was subsequently dissected into the major primal cuts (loin, ham, shoulder, and belly). Carcass yield, expressed as the proportion of hot carcass weight over BW at slaughter, was calculated. Lean and backfat percentage were calculated as previously described (Bee et al., 2004).

### *Meat quality measurements*

Temperature and pH were monitored at 30 min, three hours, and 24 h post-mortem in the longissimus thoracis (LT; at the 10th rib level), using a pH meter (WTW PH196-S, WTW, Weilheim, Germany) equipped with a WTW Eb4 electrode. One day after slaughter, the LT was excised from the left carcass side at the 8th to 10th rib level, and 4 × 1.5 cm thick chops were cut and labeled as A, B, C, and D. On chops A and C, drip loss was assessed as the quantity of purge generated during storage at 4 °C for 48 h, expressed as a percentage of the initial sample weight (Honikel, 1998). After a 20 min bloom period, L* (lightness), a* (redness), and b* yellowness values for the LT were measured for the B and D chops using a spectrophotometer (model CM-2600d, Minolta, Dietikon, Switzerland). Three replicated measurements were performed on each sample. Afterward, chops B and D were vacuum-packaged, kept for 24 h at 2 °C, and subsequently frozen and stored at −20 °C. Within one month after slaughter, these chops were thawed for 24 h at 2 to 4 °C in their vacuum plastic bags, dabbled with a paper towel, and weighed to assess the thaw loss. The chops were then cooked for five minutes on a preheated (170 °C) grill plate (Hungentobler Indu-Griddle HG 3000) to an internal temperature of 70 °C, reweighed, and cooking loss was determined. After being kept at room temperature for 2 h, Warner-Bratzler shear force was measured in these chops using a Stable Micro System TA.XT2 Texture Analyzer (Godalming, Surry, UK) equipped with a 2.5-mm-thick Warner-Bratzler shear blade. The LT from the right carcass side was also removed, vacuum-packaged, and stored a −20 °C for chemical analysis.

### *Chemical analysis of feed and meat*

Feed samples were collected weekly and pooled to three pool samples (two weekly feed samples as one pool sample) for each treatment and for each growth period. Prior to laboratory analysis, feed samples were ground to pass a 1-mm screen (Brabender mill, no. 880804, Brabender, Duisburg, Germany) and were freeze-dried thereafter. Dry matter (3 h at 105 °C) and ash content (constant mass at 550 °C) were determined according to the ISO 6496 and ISO 5984 methods, respectively. The nitrogen (N) content was determined by the Dumas method (ISO 16634-1), and CP was calculated as N × 6.25. Cell wall constituents were analyzed with the ANKOM 200/220 Fiber Analyzer (ANKOM Technology, Fairport, NY, USA). Dietary crude fat contents were determined as petrol ether extract after an

**Table 1**
Composition and analyzed nutrient content of the grower and finisher diets of entire male pigs.

| | Grower diet[1] | | | | Finisher diet[1] | | | |
|---|---|---|---|---|---|---|---|---|
| | T0 | T5 | T10 | T15 | T0 | T5 | T10 | T15 |
| Barley, % | 42.20 | 29.30 | 16.30 | 3.40 | 10.00 | 10.00 | 10.00 | 10.00 |
| Oats, % | – | – | – | – | 10.20 | 6.80 | 3.40 | – |
| Wheat ground, % | 13.40 | 16.70 | 20.10 | 23.40 | 8.90 | 22.40 | 35.80 | 49.30 |
| Corn, % | 17.40 | 23.20 | 29.00 | 34.80 | 53.20 | 38.80 | 24.40 | 10.10 |
| Wheat flour, % | 0.39 | 0.39 | 0.39 | 0.39 | 0.40 | 0.41 | 0.42 | 0.43 |
| Wheat starch, % | 5.00 | 5.00 | 5.00 | 5.00 | – | – | – | – |
| Fat blend, % | 0.96 | 1.61 | 2.25 | 2.91 | 0.13 | 1.30 | 2.46 | 3.61 |
| Potato protein, % | – | 0.54 | 1.09 | 1.64 | – | | 0.04 | 0.12 |
| Soy extraction meal, % | 16.20 | 13.90 | 11.50 | 9.20 | 13.20 | 11.50 | 9.70 | 7.80 |
| Wheat bran, % | 0.02 | 0.05 | 0.06 | 0.08 | – | – | – | – |
| Sainfoin meal, % | – | 5.00 | 10.00 | 15.00 | – | 5.00 | 10.00 | 15.00 |
| L-lysine-HCl, % | 0.356 | 0.384 | 0.414 | 0.444 | 0.252 | 0.286 | 0.320 | 0.352 |
| DL-methionine, % | 0.050 | 0.056 | 0.064 | 0.072 | – | – | – | – |
| L-threonine, % | 0.086 | 0.096 | 0.104 | 0.112 | 0.032 | 0.048 | 0.060 | 0.074 |
| Tryptophan, % | – | 0.008 | 0.014 | 0.022 | – | – | – | – |
| Dicalcium phosphate, % | 1.470 | 1.508 | 1.546 | 1.584 | 1.098 | 1.076 | 1.052 | 1.028 |
| Calcium carbonate, % | 0.874 | 0.692 | 0.510 | 0.328 | 0.856 | 0.712 | 0.570 | 0.426 |
| NaCl, % | 0.284 | 0.282 | 0.284 | 0.286 | 0.390 | 0.396 | 0.398 | 0.404 |
| Pellan, %[2] | 0.300 | 0.300 | 0.300 | 0.300 | 0.300 | 0.300 | 0.300 | 0.300 |
| Mineral-vitamin premix, %[3] | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 | 0.400 |
| Natuphos 5000 G, % | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 |
| Mikrogrit, % | 0.600 | 0.600 | 0.600 | 0.600 | 0.600 | 0.600 | 0.600 | 0.600 |
| Analyzed nutrient content, g/kg DM | | | | | | | | |
| DM | 90.4 | 90.5 | 90.5 | 90.8 | 90.3 | 90.3 | 90.1 | 90.0 |
| Total ash | 56.7 | 56.2 | 54.7 | 55.1 | 51.1 | 51.3 | 51.9 | 52.5 |
| Crude fiber | 33.9 | 36.6 | 42.6 | 43.0 | 36.2 | 39.0 | 44.7 | 50.0 |
| CP | 182.5 | 181.6 | 177.5 | 178.4 | 174.0 | 171.8 | 170.6 | 168.5 |
| Crude fat | 41.5 | 49.4 | 59.0 | 64.8 | 41.8 | 50.9 | 57.0 | 67.6 |
| Condensed tannins[4] | – | 4.0 | 8.0 | 12.0 | – | 4.0 | 8.0 | 12.0 |
| Calculated DE, MJ/kg[5] | 14.9 | 14.9 | 14.9 | 14.9 | 14.9 | 15.0 | 15.0 | 15.0 |
| Fatty acid profile[6] | | | | | | | | |
| C14:0 | 0.84 | 1.07 | 1.25 | 1.44 | 0.27 | 0.79 | 1.25 | 1.67 |
| C16:0 | 20.19 | 20.31 | 20.30 | 20.44 | 16.73 | 18.34 | 20.43 | 22.76 |
| C18:0 | 5.86 | 7.31 | 8.27 | 9.20 | 2.91 | 6.23 | 8.90 | 11.20 |
| SFA | 27.46 | 29.55 | 30.76 | 32.09 | 20.31 | 25.98 | 31.49 | 36.69 |
| C16:1n-7 | 0.86 | 1.12 | 1.31 | 1.45 | 0.30 | 0.82 | 1.26 | 1.68 |
| C18:1n-9 | 24.96 | 26.77 | 28.53 | 29.57 | 29.75 | 29.22 | 28.98 | 29.81 |
| C18:1 t11 | 1.57 | 1.81 | 2.00 | 2.09 | 1.08 | 1.54 | 2.06 | 2.45 |
| MUFA | 27.96 | 30.60 | 32.86 | 34.24 | 31.54 | 32.29 | 33.36 | 35.18 |
| C18:2n-6 | 41.98 | 36.85 | 32.72 | 29.19 | 46.55 | 39.29 | 31.84 | 24.22 |
| C18:3n-3 | 2.59 | 2.99 | 3.66 | 4.48 | 1.60 | 2.44 | 3.31 | 3.91 |
| PUFA | 44.57 | 39.85 | 36.39 | 33.67 | 48.15 | 41.73 | 35.15 | 28.13 |
| C16:1n-7/C16:0 | 0.04 | 0.06 | 0.06 | 0.07 | 0.02 | 0.04 | 0.06 | 0.07 |
| C18:1n-9/C18:0 | 4.26 | 3.67 | 3.45 | 3.22 | 10.48 | 4.70 | 3.26 | 2.66 |
| C18:2n-6/C18:3n-3 | 16.21 | 12.35 | 8.93 | 6.51 | 29.11 | 16.11 | 9.63 | 6.19 |

[1] Grower diet formulated for pigs in the BW range of 25 to 60 kg; finisher diet formulated for pigs in the BW range of 60 to 110 kg; T0 = standard diet without addition of sainfoin meal; T5 = standard diet with addition of 5% of sainfoin meal; T10 = standard diet with addition of 10% of sainfoin meal; T15 = standard diet with addition of 15% of sainfoin meal.
[2] Binder that aids in pellet formation.
[3] Supplied the following nutrients per kg of diet: 20000 IU vitamin A, 200 IU vitamin D3, 39 IU vitamin E, 2.9 mg riboflavin, 2.4 mg vitamin B6, 0.010 mg vitamin B12, 0.2 mg vitamin K3, 10 mg pantothenic acid, 1.4 mg niacin, 0.48 mg folic acid, 199 g choline, 0.052 mg biotin, 52 mg Fe as FeSO4, 0.16 mg I as Ca(IO)3, 0.15 mg Se as Na2Se, 5.5 mg Cu as CuSO4, 81 mg Zn as ZnO2, 15 mg Mn as MnO2.
[4] Estimated condensed tannin content based on the inclusion level of sainfoin.
[5] The digestible energy coefficients from each feed ingredient were obtained from the Swiss Feed Database (https://www.feedbase.ch), and, taking into account the relative amount of each feed ingredient in the diet, the digestible energy content was calculated.
[6] Values are expressed as % of total fatty acids; SFA = saturated fatty acids, MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

acidic hydrolysis (ISO 6492, VDLUFA 5.1.1). Concentrations of total CTs were determined in feed samples using the HCl butanol method described by Terrill et al. (1992) with the following modifications: for each sample, three aliquots of 500 mg were used, and the absorbance of the resulting anthocyanidins was measured at 550 nm on a UV/VIS Spectrometer (PerkinElmer, Schwerzenbach, Switzerland). A blank was conducted for each fraction of CTs of each sample by avoiding color development induced by heat (reaction vessel kept at 4 °C); thus, every absorbance was deduced by its corresponding blank. Purified extracts of sainfoin were used for calibration. The LT samples obtained from the right carcass sides were freeze-dried and grinded for further analysis. The IMF content was measured in triplicate via the Soxtec extraction method (Soxtec® Avanti 2050 Auto System, Foss Tecator AB, Höganäs, Sweden) using petroleum ether as a solvent. Fatty acid esters and free fatty acids were determined in feed and in freeze-dried, grinded, BF and LT samples by transmethylation/esterification under acid catalysis (5% HCl in MeOH) for three hours at 70 °C, as described by Kragten Ampuero et al. (2014). Briefly, the samples (250 mg) were placed in a polytetrafluoroethylene tube with 1 ml of internal standard solution (1 mg/ml C19,0 in toluene) and 3 ml of 5% HCl in methanol. Then, the reaction mix was neutralized with 5 ml of 6% $K_2CO_3$ and purified by solid-phase extraction. The determination of fatty acid methyl-esters was performed with a gas chromatography instrument equipped with a flame ionization detector (Agilent 6850, Agilent Technologies, Germany).

*Analysis of boar taint compounds in backfat and the intramuscular fat*

Androstenone, skatole, and indole concentrations in the backfat were analyzed according to the method previously described by Ampuero Kragten et al. (2011). Briefly, the backfat was liquefied in a microwave oven three to four times for two minutes at 300 W. The liquefied samples were then centrifuged at 11′300 × g for two minutes at room temperature, and the aqueous phase was removed. Subsequently, 0.5 ml of pure liquid fat was placed in 2.5-ml Eppendorf tubes, and 1 ml of methanol containing the internal standards (0.496 mg/l androstanone [internal standard for androstenone] and 0.050 mg/l 2-methylindole [internal standard for skatole and indole]) was added. The samples were incubated for five minutes at 30 °C in an ultrasonic water bath, cooled in an ice-water bath for 20 min, and then centrifuged at 11′300 × g for 20 min. The supernatants were filtrated (0.2 μm filter) and transferred to vials for androstenone, skatole, and indole analysis with HPLC (Agilent 1200, Agilent Technologies, Germany). The androstenone, skatole, and indole concentrations were expressed per gram of backfat. The quantification limits were 0.2 μg/g backfat for androstenone and 0.03 μg/g backfat for skatole and indole. For the analysis of androstenone, skatole, and indole in freeze-dried LT samples, the quantitative extraction of IMF from the LT samples was performed first in triplicate by a Soxtec system with petrol ether. In a following step, androstenone, skatole, and indole were extracted from 100 mg of liquid IMF, from each of the three Soxtec extractions, with 200 μl of methanol containing the internal standard. Thereafter, the procedure was analogue to the procedure for the analysis of backfat. The analytes were expressed in μg/mg of muscle by taking into account the per-sample average IMF content (tree replicates) and the loss of mass during the initial freeze drying of the LT. Androstenone, skatole, and indole were calculated using specific calibration curves, i.e. determined either in backfat or IMF matrixes, respectively. The recovery values for the determinations in the IMF were 98, 92, and 96% for androstenone, skatole, and indole, respectively. The coefficients of variation were 30, 16, and 17% for androstenone, skatole, and indole, respectively.

*Statistical analysis*

The data of one T10 and one T15 pig were excluded from the data analysis because they exhibited a very low performance and reached a slaughter weight of only 71.2 and 79.2 kg, respectively. The data were analyzed with the PROC MIXED procedure of SAS considering the experimental groups as fixed and litter as random effects. Orthogonal contrasts were used to determine the linear and quadratic effects of the increasing sainfoin inclusion levels. The effects were considered significant at $P < 0.05$ and as tendencies at $P < 0.10$. Due to lack of normality, statistical analysis was performed with log transformed data for androstenone, skatole and indole determined in the backfat, log transformed data for androstenone in the LT and inverse skatole and indole values in the LT. The reported *P*-values of the linear and quadratic contrasts are derived from the MIXED model, whereas the values presented are mean values of the raw data. The BW at slaughter was used as covariate for carcass traits and organ weight data. Pearson's correlations between the boar taint compounds in the LT and backfat, as well as between the boar taint compounds and the weight of the testes and anatomical parameters, were determined using the CORR procedure.

## Results

*Diet composition*

As planned, the four grower and four finisher diets had similar CP and DE contents (Table 1) but differed in their crude fiber and fat content, which linearly increased from T0 to T15 in both, the grower and in the finisher diets. The relative amount of saturated fatty acids and monounsaturated fatty acids linearly increased from the T0 to the T15 diets. These increases were mainly a consequence of the increasing relative proportion of C18:0 and C18:1n-9. Despite the linear increase in the level of C18:3n-3, the decrease in polyunsaturated fatty acid levels coincided with a decrease in the level of C18:2n-6, ultimately resulting in a decreasing C18:2n-6-to-C18:3n-3 ratio. The estimated CTs content of the grower and finisher diets was based on the inclusion level of sainfoin: 4.0, 8.0, and 12.0 g/kg DM in the T5, T10, and T15 diets, respectively.

*Growth performance and feeding behavior*

The inclusion of increasing amounts of CTs in the diets had no effect on average daily gain, average daily feed intake, and gain-to-feed ratio during the grower, finisher, and overall growth periods (Table 2). Despite average daily feed intake not differing among the experimental groups, during the finisher period, increasing the dietary sainfoin supply tended to linearly decrease ($P = 0.06$) the FFV and tended to increase ($P = 0.08$) the TV and FIV (Table 3). By contrast, the TTF, RFI, and FI were not affected by the dietary treatments.

*Carcass characteristics and organ weights*

The BW at slaughter was similar in all four experimental groups, whereas the hot and cold carcass weight as well as the percentage carcass yield and the loin percentage linearly decreased ($P \leq 0.05$) with increasing sainfoin inclusion (Table 4). Neither the lean meat percentage nor the backfat cover of the carcass, assessed as the percentage of total subcutaneous fat, the percentage of backfat, and the 10th-rib backfat thickness, did differ between the dietary treatments. From the T0 to the T15 treatments, the liver weight increased linearly ($P = 0.01$), whereas the weight of the other organs did not differ.

*Meat quality and fatty acid composition*

The pH at three hours post-mortem linearly increased ($P = 0.05$) and initial muscle temperature linearly decreased ($P = 0.03$) with increasing sainfoin inclusion, whereas the pH at 45 min and ultimate pH, as well as the temperature at three hours and one day post-

**Table 2**
Effects of increasing levels of sainfoin inclusion in the grower and finisher diets on the growth performance of entire male pigs.[1,2]

| | Treatments[1] | | | | | Contrasts[2] | |
|---|---|---|---|---|---|---|---|
| | T0 | T5 | T10 | T15 | SEM | L | Q |
| BW, kg | | | | | | | |
| At the start of the grower period | 24.7 | 25.1 | 25.6 | 25.3 | 1.74 | 0.66 | 0.77 |
| At the start of the finisher period | 62.9 | 60.3 | 64.0 | 61.8 | 2.98 | 0.97 | 0.96 |
| At slaughter | 112.0 | 106.6 | 114.5 | 111.9 | 3.21 | 0.57 | 0.63 |
| Average daily gain, kg/d | | | | | | | |
| Grower period | 0.91 | 0.84 | 0.92 | 0.87 | 0.039 | 0.85 | 0.77 |
| Finisher period | 0.96 | 0.91 | 0.99 | 0.99 | 0.041 | 0.29 | 0.45 |
| Grower–finisher period | 0.94 | 0.87 | 0.96 | 0.94 | 0.034 | 0.56 | 0.53 |
| Average daily feed intake, kg/d | | | | | | | |
| Grower period | 1.70 | 1.61 | 1.75 | 1.65 | 0.103 | 0.99 | 0.94 |
| Finisher period | 2.38 | 2.24 | 2.52 | 2.36 | 0.137 | 0.66 | 0.92 |
| Grower–finisher period | 2.07 | 1.95 | 2.18 | 2.04 | 0.113 | 0.78 | 0.93 |
| Gain to feed ratio kg/kg | | | | | | | |
| Grower period | 0.54 | 0.43 | 0.53 | 0.54 | 0.017 | 0.86 | 0.45 |
| Finisher period | 0.41 | 0.41 | 0.40 | 0.43 | 0.018 | 0.51 | 0.31 |
| Grower–finisher period | 0.46 | 0.45 | 0.45 | 0.47 | 0.015 | 0.71 | 0.29 |

[1] T0 = standard diet without addition of sainfoin meal ($n = 12$); T5 = standard diet with addition of 5% of sainfoin meal ($n = 12$); T10 = standard diet with addition of 10% of sainfoin meal ($n = 11$); T15 = standard diet with addition of 15% of sainfoin meal ($n = 11$).
[2] Contrasts: L = Linear; Q = Quadratic.

**Table 3**
Effects of increasing levels of sainfoin in the grower and finisher diets on the feeding behavior of entire male pigs.

| | Treatments[1] | | | | | Contrasts[2] | |
|---|---|---|---|---|---|---|---|
| Item[3] | T0 | T5 | T10 | T15 | SEM | L | Q |
| Grower period | | | | | | | |
| TTF, min | 69.8 | 61.8 | 67.5 | 63.3 | 3.71 | 0.27 | 0.50 |
| FFV, n | 9.5 | 7.8 | 9.6 | 7.7 | 0.77 | 0.18 | 0.88 |
| TV, min | 8.3 | 9.0 | 7.8 | 9.3 | 0.83 | 0.50 | 0.47 |
| FIV, g | 226 | 256 | 212 | 262 | 28.2 | 0.55 | 0.70 |
| RFI, g/min | 27 | 28 | 28 | 28 | 2.20 | 0.90 | 0.95 |
| FI, min | 79.0 | 91.3 | 76.8 | 90.3 | 6.56 | 0.37 | 0.90 |
| Finisher period | | | | | | | |
| TTF, min | 56.5 | 51.3 | 58.9 | 53.5 | 3.25 | 0.89 | 0.95 |
| FFV, n | 5.8 | 5.4 | 5.5 | 4.6 | 0.46 | 0.06 | 0.59 |
| TV, min | 11.4 | 10.9 | 11.8 | 13.8 | 1.19 | 0.08 | 0.22 |
| FIV, g | 525 | 519 | 560 | 664 | 60.1 | 0.08 | 0.33 |
| RFI, g/min | 47 | 49 | 47 | 49 | 3.1 | 0.51 | 0.97 |
| FI, min | 115.1 | 122.3 | 121.6 | 132.8 | 9.09 | 0.12 | 0.79 |

[1] T0 = standard diet without addition of sainfoin meal ($n = 12$); T5 = standard diet with addition of 5% of sainfoin meal ($n = 12$); T10 = standard diet with addition of 10% of sainfoin meal ($n = 11$); T15 = standard diet with addition of 15% of sainfoin meal ($n = 11$).
[2] Contrasts: L = Linear; Q = Quadratic.
[3] TTF = average total time feeding per day; FFV = average frequency of feeder visits; TV = average time per visit (TTF/FFV); FIV = average feed intake per visit (average daily feed intake/FFV); RFI = average rate of feed intake (average daily feed intake/TTF); FI = interval between two meals.

mortem, were not affected by the diets (Table 5). Water holding capacity, determined as drip loss or as the sum of thaw and cooking losses and tenderness determined as shear force, tended to linearly increase ($P \leq 0.06$) from T0 to T15. Meat color traits were not affected by the diets.

The diet had no effect on the IMF content of the LT (Table 6). However, the saturated fatty acid levels tended to linearly decrease ($P = 0.09$), and the monounsaturated fatty acid levels increased quadratically

**Table 4**
Effects of increasing levels of sainfoin in the grower and finisher diets on the carcass characteristics and organ weights of entire male pigs.

| | Treatments[1] | | | | | Contrasts[2] | |
|---|---|---|---|---|---|---|---|
| | T0 | T5 | T10 | T15 | SEM | L | Q |
| Hot carcass weight, kg | 88.4 | 87.3 | 86.9 | 86.0 | 0.37 | <0.01 | 0.89 |
| Cold carcass weight, kg | 85.6 | 84.6 | 84.3 | 83.3 | 0.36 | <0.01 | 0.91 |
| Carcass yield, % | 79.56 | 78.62 | 78.29 | 77.42 | 0.334 | <0.01 | 0.89 |
| Cold Loss, %[3] | 3.12 | 3.12 | 3.08 | 3.08 | 0.043 | 0.45 | 0.92 |
| Lean meat, %[4] | 57.61 | 58.30 | 56.22 | 57.44 | 0.785 | 0.12 | 0.46 |
| Loin | 26.87 | 26.96 | 26.24 | 26.48 | 0.348 | 0.05 | 0.71 |
| Ham | 18.22 | 18.62 | 17.60 | 18.24 | 0.353 | 0.26 | 0.54 |
| Shoulder | 12.51 | 12.72 | 12.37 | 12.69 | 0.208 | 0.66 | 0.54 |
| Belly, % | 16.23 | 15.89 | 16.22 | 15.91 | 0.322 | 0.55 | 0.96 |
| Subcutaneous fat, %[5] | 13.15 | 13.26 | 14.38 | 13.43 | 0.533 | 0.20 | 0.13 |
| Backfat, % | 7.70 | 7.83 | 8.51 | 7.78 | 0.404 | 0.42 | 0.09 |
| 10th rib backfat thickness, mm | 19.1 | 17.4 | 20.2 | 18.0 | 1.60 | 0.95 | 0.87 |
| Organ weight, g | | | | | | | |
| Liver | 1696 | 1781 | 1819 | 1855 | 45.2 | 0.01 | 0.56 |
| Kidney | 386 | 405 | 394 | 405 | 14.4 | 0.40 | 0.71 |
| Testis | 516 | 539 | 489 | 529 | 29.2 | 0.92 | 0.72 |
| Bulbourethral gland | 164 | 200 | 167 | 165 | 18.0 | 0.70 | 0.28 |
| Salivary gland | 89 | 99 | 81 | 97 | 7.1 | 0.78 | 0.62 |
| Parotid gland | 214 | 213 | 205 | 229 | 13.8 | 0.41 | 0.20 |

[1] T0 = standard diet without addition of sainfoin meal ($n = 12$); T5 = standard diet with addition of 5% of sainfoin meal ($n = 12$); T10 = standard diet with addition of 10% of sainfoin meal ($n = 11$); T15 = standard diet with addition of 15% of sainfoin meal ($n = 11$).
[2] Contrasts: L = Linear; Q = Quadratic.
[3] Weight loss of the hot carcass during chilling at 2 °C for 24 h.
[4] Sum of denuded shoulder, loin, and ham weight as a percentage of cold carcass weight.
[5] Sum of external fat from the shoulder, loin, and ham expressed as a percentage of cold carcass weight.

**Table 5**
Effects of increasing levels of sainfoin in the grower and finisher diets on the meat quality traits of entire male pigs.

| | Treatments[1] | | | | | Contrasts[2] | |
|---|---|---|---|---|---|---|---|
| | T0 | T5 | T10 | T15 | SEM | L | Q |
| pH | | | | | | | |
| 45 min | 6.54 | 6.52 | 6.59 | 6.60 | 0.035 | 0.12 | 0.69 |
| 3 h | 6.23 | 6.15 | 6.35 | 6.40 | 0.086 | 0.05 | 0.44 |
| 24 h | 5.44 | 5.49 | 5.43 | 5.46 | 0.031 | 0.99 | 0.74 |
| Temperature, °C | | | | | | | |
| 45 min | 38.8 | 38.5 | 38.2 | 37.9 | 0.33 | 0.03 | 0.93 |
| 3 h | 20.8 | 20.1 | 21.3 | 20.7 | 0.79 | 0.85 | 0.95 |
| 24 h | 2.6 | 2.4 | 2.6 | 2.6 | 0.12 | 0.83 | 0.20 |
| Color[3] | | | | | | | |
| L* | 47.4 | 46.1 | 47.7 | 47.2 | 1.12 | 0.79 | 0.64 |
| a* | 5.4 | 5.4 | 6.1 | 5.5 | 0.32 | 0.29 | 0.19 |
| b* | 2.7 | 2.4 | 3.1 | 2.6 | 0.26 | 0.71 | 0.59 |
| Chroma value | 6.0 | 5.9 | 6.9 | 6.1 | 0.38 | 0.38 | 0.26 |
| Water holding capacity, % | | | | | | | |
| Drip loss | 1.54 | 1.82 | 1.77 | 2.07 | 0.180 | 0.02 | 0.85 |
| Thaw loss | 9.45 | 10.22 | 9.02 | 10.76 | 0.537 | 0.23 | 0.34 |
| Cook loss | 22.61 | 22.92 | 23.07 | 23.69 | 0.598 | 0.14 | 0.75 |
| Total loss | 30.00 | 30.91 | 30.07 | 32.09 | 0.785 | 0.05 | 0.34 |
| Shear force, kg | 6.6 | 7.3 | 6.8 | 7.7 | 0.56 | 0.06 | 0.81 |

[1] T0 = standard diet without addition of sainfoin meal ($n = 12$); T5 = standard diet with addition of 5% of sainfoin meal ($n = 12$); T10 = standard diet with addition of 10% of sainfoin meal ($n = 11$); T15 = standard diet with addition of 15% of sainfoin meal ($n = 11$).
[2] Contrasts: L = Linear; Q = Quadratic.
[3] L* = a measure of darkness to lightness (higher L* values indicates a lighter color), a* = a measure of redness (higher a* value indicates a redder color), and b* = a measure of yellowness (higher b* value indicates a more yellow color).

($P = 0.05$), with no dietary effect on the polyunsaturated fatty acid concentrations in the IMF. The changes in the saturated fatty acid levels were mainly due to linear decreases ($P \leq 0.05$) in the levels of C17:0 and C18:0. The increase in the monounsaturated fatty acid levels was mainly the result of a quadratic increase ($P = 0.04$) in C18:1n-9. Despite the lack of change in polyunsaturated fatty acid concentrations, the C18:3n-3 and C22:5n-3 levels linearly increased ($P < 0.01$) from T0 to T15. As the C18:2n-6 levels were unaffected by the diet, the level of two of its long-chain homologs (C20:2n-6 and C22:4n-6), the C18:2n-6/C18:3n-3, and the $\sum$ n-6-to-$\sum$n-3 fatty acid ratio linearly decreased ($P \leq 0.03$) with increasing dietary sainfoin levels. The desaturation index, defined as the C18:1n-9/C18:0, linearly increased ($P = 0.03$) from T0 to T15.

As in the IMF, the fat content of the backfat was not affected by the diet (Table 6). By contrast, the fatty acid profile of the subcutaneous fat differed when the dietary sainfoin levels increased. The proportion of MUFA, especially that of C16:1n-7 and C18:1n-9, linearly increased ($P < 0.001$) with increasing dietary sainfoin inclusion. These changes were compensated by a linear decrease ($P \leq 0.01$) in polyunsaturated fatty acid, C18:2n-6, C20:2n-6, C20:4n-6, and C22:4n-6 levels. Similar to the IMF, the levels of C18:3n-3, C20:3n-3, C20:5n-3, and C22:5n-3 in the backfat linearly increased ($P < 0.01$) with increasing dietary sainfoin inclusion. Consequently, the C18:2n-6/C18:3n-3 linearly decreased ($P < 0.001$). The C16:1n-7/C16:0 and C18:1n-9/C18:0 desaturation indexes linearly increased ($P < 0.01$) and the polyunsaturated fatty acid to saturated fatty acid ratio of the backfat linearly decreased ($P < 0.01$) from the T0 to the T15 group. Despite being significant, the linear decrease ($P \leq 0.05$) in C16:0, C18:0, and C20:0 and the linear increase ($P < 0.01$) in C 17:0 and C14:0 were small.

### *Boar taint compounds in the longissimus thoracis and backfat*

In the LT, the concentration of androstenone linearly decreased ($P < 0.01$) with increasing dietary sainfoin intake (Table 7). By contrast, the skatole and indole levels in the LT, as well as the androstenone,

**Table 6**
Effect of increasing levels of sainfoin in the grower and finisher diets on the intramuscular fat content of the longissimus thoracis muscle and the fatty acid composition of the intramuscular fat and backfat of entire male pigs.[1,2]

| | Treatments[3] | | | | | Contrasts[4] | |
|---|---|---|---|---|---|---|---|
| | T0 | T5 | T10 | T15 | SEM | L | Q |
| Intramuscular fat, g/kg | 30.3 | 25.3 | 29.5 | 28.5 | 1.47 | 0.88 | 0.32 |
| Fatty acid profile of the longissimus thoracis muscle, g/100 g total fatty acids | | | | | | | |
| C14:0 | 1.24 | 1.15 | 1.25 | 1.21 | 0.042 | 0.99 | 0.50 |
| C16:0 | 23.27 | 22.69 | 23.11 | 22.58 | 0.343 | 0.17 | 0.99 |
| C17:0 | 1.77 | 2.20 | 1.94 | 2.27 | 0.014 | <0.01 | 0.57 |
| C18:0 | 11.74 | 11.79 | 11.55 | 11.26 | 0.272 | 0.05 | 0.37 |
| C20:0 | 0.14 | 0.12 | 0.15 | 0.15 | 0.011 | 0.18 | 0.29 |
| SFA | 36.56 | 35.97 | 36.26 | 35.42 | 0.525 | 0.09 | 0.76 |
| C16:1n-7 | 3.67 | 3.11 | 3.40 | 3.32 | 0.140 | 0.71 | 0.34 |
| C18:1n-9 | 40.40 | 38.88 | 40.76 | 41.34 | 0.584 | 0.04 | 0.04 |
| C18:1 t11 | 4.12 | 3.91 | 4.11 | 4.10 | 0.107 | 0.59 | 0.14 |
| C20:1n-9 | 0.70 | 0.66 | 0.67 | 0.67 | 0.031 | 0.54 | 0.55 |
| MUFA | 49.23 | 47.22 | 49.68 | 50.21 | 0.803 | 0.06 | 0.05 |
| C18:2n-6 | 10.82 | 12.65 | 10.53 | 10.41 | 0.787 | 0.21 | 0.11 |
| C20:2n-6 | 0.38 | 0.43 | 0.36 | 0.33 | 0.023 | 0.03 | 0.07 |
| C20:4n-6 | 1.75 | 2.17 | 1.63 | 1.63 | 0.194 | 0.19 | 0.17 |
| C22:4n-6 | 0.28 | 0.31 | 0.24 | 0.23 | 0.023 | 0.03 | 0.49 |
| C18:3n-3 | 0.33 | 0.48 | 0.57 | 0.86 | 0.045 | <0.01 | 0.02 |
| C20:3n-3 | 0.27 | 0.32 | 0.27 | 0.28 | 0.024 | 0.85 | 0.32 |
| C22:5n-3 | 0.18 | 0.27 | 0.24 | 0.33 | 0.027 | <0.01 | 0.99 |
| PUFA | 14.23 | 16.84 | 14.07 | 14.43 | 1.074 | 0.53 | 0.15 |
| C16:1n-7/C16:0 | 0.15 | 0.14 | 0.15 | 0.15 | 0.005 | 0.26 | 0.24 |
| C18:1n-9/C18:0 | 3.48 | 3.30 | 3.55 | 3.68 | 0.113 | 0.03 | 0.08 |
| PUFA/SFA | 0.30 | 0.37 | 0.31 | 0.33 | 0.02 | 0.74 | 0.28 |
| C18:2n-6/C18:3n-3 | 36.53 | 27.80 | 19.25 | 12.05 | 2.003 | <0.01 | 0.70 |
| Fat content of the backfat g/kg | 757.4 | 722.3 | 779.7 | 730.2 | 23.89 | 0.75 | 0.62 |
| Fatty acid profile of the backfat, g/100 g total fatty acids | | | | | | | |
| C14:0 | 1.26 | 1.29 | 1.33 | 1.34 | 0.024 | <0.01 | 0.53 |
| C16:0 | 22.35 | 22.32 | 22.25 | 21.76 | 0.330 | 0.05 | 0.28 |
| C17:0 | 0.27 | 0.34 | 0.36 | 0.41 | 0.015 | <0.01 | 0.56 |
| C18:0 | 11.90 | 11.78 | 11.18 | 11.25 | 0.323 | <0.01 | 0.61 |
| C20:0 | 0.18 | 0.18 | 0.17 | 0.15 | 0.006 | <0.01 | 0.15 |
| SFA | 36.29 | 36.28 | 35.72 | 35.40 | 0.580 | 0.03 | 0.60 |
| C16:1n-7 | 2.00 | 2.08 | 2.30 | 2.30 | 0.072 | <0.01 | 0.46 |
| C18:1n-9 | 37.19 | 37.62 | 39.38 | 39.80 | 0.286 | <0.01 | 0.97 |
| C18:1 t11 | 2.56 | 2.67 | 2.93 | 3.06 | 0.048 | <0.01 | 0.82 |
| C20:1n-9 | 0.81 | 0.82 | 0.83 | 0.83 | 0.034 | 0.45 | 0.94 |
| MUFA | 43.63 | 44.45 | 46.85 | 47.59 | 0.339 | <0.01 | 0.86 |
| C18:2n-6 | 17.83 | 16.60 | 14.36 | 13.33 | 0.521 | <0.01 | 0.77 |
| C20:2n-6 | 0.74 | 0.65 | 0.56 | 0.52 | 0.020 | <0.01 | 0.12 |
| C20:4n-6 | 0.25 | 0.24 | 0.21 | 0.20 | 0.014 | <0.01 | 0.74 |
| C22:4n-6 | 0.09 | 0.08 | 0.07 | 0.07 | 0.005 | <0.01 | 0.38 |
| C18:3n-3 | 0.73 | 1.00 | 1.32 | 1.74 | 0.036 | <0.01 | <0.01 |
| C20:3n-3 | 0.09 | 0.09 | 0.08 | 0.08 | 0.004 | <0.01 | 0.88 |
| C20:5n-3 | 0.02 | 0.03 | 0.03 | 0.06 | 0.004 | <0.01 | 0.22 |
| C22:5n-3 | 0.05 | 0.07 | 0.09 | 0.10 | 0.006 | <0.01 | 0.96 |
| PUFA | 20.13 | 19.23 | 17.36 | 16.86 | 0.590 | <0.01 | 0.59 |
| C16:1n-7/C16:0 | 0.09 | 0.09 | 0.10 | 0.11 | 0.003 | <0.01 | 0.72 |
| C18:1n-9/C18:0 | 3.14 | 3.21 | 3.55 | 3.55 | 0.109 | <0.01 | 0.52 |
| PUFA/SFA | 0.48 | 0.46 | 0.41 | 0.40 | 0.03 | <0.01 | 0.83 |
| C18:2n-6/C18:3n-3 | 24.74 | 16.60 | 10.83 | 7.53 | 0.195 | <0.01 | <0.01 |

[1] Only fatty acids that accounted for >0.1/100 g of total are presented.
[2] Fatty acids were designated by the number of carbon atoms followed by the number of double bonds. The position of the first double bond relative to the methyl (n) end of the molecule was also indicated. The sums of the main fatty acid series are represented as SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.
[3] T0 = standard diet without addition of sainfoin meal ($n = 12$); T5 = standard diet with addition of 5% of sainfoin meal ($n = 12$); T10 = standard diet with addition of 10% of sainfoin meal ($n = 11$); T15 = standard diet with addition of 15% of sainfoin meal ($n = 11$).
[4] Contrasts: L = Linear; Q = Quadratic.

**Table 7**
Effect of increasing levels of sainfoin the grower and finisher diets on the androstenone, skatole, and indole content in the intramuscular fat of the longissimus thoracis muscle and the backfat of entire male pigs.[1]

| | Treatments[2] | | | | Contrasts[3] | |
|---|---|---|---|---|---|---|
| | T0 | T5 | T10 | T15 | L | Q |
| Intramuscular fat, μg/mg longissimus thoracis muscle | | | | | | |
| Androstenone | 345.5 (39.2) | 272.0 (40.8) | 307.8 (67.7) | 187.9 (23.0) | <0.01 | 0.84 |
| Skatole | 3.6 (1.1) | 3.8 (0.9) | 2.3 (0.4) | 2.8 (0.4) | 0.71 | 0.90 |
| Indole | 1.2 (0.1) | 0.7 (0.2) | 0.5 (0.1) | 0.6 (0.1) | 0.66 | 0.07 |
| Backfat, μg/mg backfat | | | | | | |
| Androstenone | 532.6 (188.0) | 414.3 (80.6) | 551.7 (251.6) | 332.4 (57.9) | 0.99 | 0.72 |
| Skatole | 133.4 (57.8) | 127.2 (46.1) | 59.0 (17.3) | 81.5 (18.9) | 0.72 | 0.28 |
| Indole | 30.2 (19.0) | 20.2 (8.7) | 13.3 (3.1) | 14.0 (1.8) | 0.83 | 0.58 |

[1] Due to lack of normality, statistical analysis was performed with log transformed data for androstenone, skatole and indole determined in the backfat, log transformed data for androstenone in the intramuscular fat and inverse skatole and indole values in the intramuscular fat. The reported *P*-values of the linear and quadratic contrasts are derived from the MIXED model. The values presented are mean values and in brackets the respective standard errors of the raw data.
[2] T0 = standard diet without addition of sainfoin meal ($n = 12$); T5 = standard diet with addition of 5% of sainfoin meal ($n = 12$); T10 = standard diet with addition of 10% of sainfoin meal ($n = 11$); T15 = standard diet with addition of 15% of sainfoin meal ($n = 11$).
[3] Contrasts: L = Linear; Q = Quadratic.

skatole, and indole levels in the backfat, were unaffected by the diets. The androstenone levels in the backfat were positively correlated with the skatole and indole levels in the backfat (skatole-$_{backfat}$: $r = 0.52$; indole-$_{backfat}$: $r = 0.59$; $P < 0.01$) and the LT (skatole-$_{IMF}$: $r = 0.48$; indole-$_{IMF}$: $r = 0.53$; $P < 0.01$). The skatole levels in the backfat highly correlated with the indole levels in the backfat and IMF (indole-$_{backfat}$: $r = 0.82$; indole-$_{IMF}$: $r = 0.82$; $P < 0.01$) and the skatole levels in the LT (skatole-$_{IMF}$: $r = 0.93$; $P < 0.01$). The concentrations of androstenone in the backfat, but not in the LT, were positively ($P < 0.01$) correlated with the weight of the testis ($r = 0.52$), bulbourethral gland ($r = 0.72$), and mandibular gland ($r = 0.54$). The skatole and indole levels in the backfat and LT were positively ($P \leq 0.03$) correlated with the weight of the bulbourethral gland (skatole-$_{backfat}$: $r = 0.39$; indole-$_{backfat}$: $r = 0.378$; skatole-$_{IMF}$: $r = 0.40$; indole-$_{IMF}$: $r = 0.31$). No significant relationships were found between the levels of skatole and indole and the weight of the testis, salivary gland, and parotid gland.

## Discussion

### *Growth performance and feeding behavior*

Depending on the concentration (>50 g/kg DM), tannins in animal diets have been reported to have anti-nutritional effects, including reduced feed intake, nutrient digestibility, and growth rates (Acamovic and Brooker, 2005). Depression in feed intake is thought to be caused by the formation of tannin–salivary protein complexes in the mouth or signals of gut distension resulting from tannin interactions with proteins of the gut wall (Acamovic and Brooker, 2005). In the present study, EM fed up to 15% sainfoin, which amounts to a total CTs intake of 12.0 g/kg DM, exhibited growth rates comparable to those of the T0 group. Similarly, Kotrotsios et al. (2012) found no negative effects on BW development and feed efficiency when diets contained up to 9.7 g/kg of CTs from carob pots. Thus, one can conclude that pigs are relatively resistant to the consumption of tannin-rich feed up to those concentrations, especially if the diets are formulated isocaloric and isoproteic. To counteract the astringency of tannins, pigs can adapt by inducing hypertrophy of the parotid gland, thereby increasing the salivary secretion of proline-rich proteins that bind tannins (Cappai et al., 2013). This adaptation seems to not have occurred in the present study, as the weight of the parotid gland was similar among the groups.

Despite the lack of no difference in average daily feed intake due to increasing dietary sainfoin levels, feeding behavior in the finisher period tended to be altered, as the pigs went fewer times to the feeder (FFV), stayed longer (TV), and ingested more feed per visit (FIV). Various factors, like feed composition and appetence, the housing and feeding system, health of the pigs, and the environment affect feeding behavior (Maselyne et al., 2015). Since, in the present study, all pigs were kept in the same room, shared the same large pen, and no health-related issues were observed, solely feed composition or feed appetence could have played a role. As intended, the diets were isonitrogenous and isocaloric. Thus, only the CTs content, together with the crude fiber and fat content, differed. Quemeneur et al. (2019) observed that increasing the level of crude fiber by 2% and NDF by 4% lowered meal frequency but also reduced meal size and the interval between two meals. The authors linked the altered feeding behavior pattern to fiber-induced increased satiety. In the present study, the amount of ingested dietary fiber increased from T0 to T5, T10, and T15 on average by 2.6, 8.1, and 10.7 g/kg. Compared to the values reported in the aforementioned study, differences among treatments were smaller and increase in dietary fiber intake were not sufficiently large to reach bulking properties that impact meal size (Maljaars et al., 2007). Dietary fat content linearly increased from T0 to T5, T10, and T15 on average by 7.7, 16.0, and 23.7 g/kg, respectively. Apart from reducing overall feed intake when pigs are continuously fed dietary fat levels exceeding 10% (Azain, 2001), there is, to our knowledge, no evidence that feeding behavior is affected by the dietary fat content, especially at the moderate levels used in this trial. Therefore, the most plausible explanation for the fewer feeder visits and longer feeding time per visit is the different dietary CTs content. Astringency, a known trait of tannin-rich plants affects oral-born somatic sensing, and, when the stimulus of somatosensing is too strong, feed intake can be impaired (Roura and Tedó, 2009). Because overall feed intake was unaffected by the CTs content of the diet, one can hypothesize that either astringency was not sufficient to markedly affect the total feed intake or was sufficient to slow the eating speed.

*Carcass characteristics and meat quality*

Although the BW at slaughter was similar, hot and cold carcass weights and consequently carcass yield decreased linearly ($P < 0.01$) with increasing sainfoin supplementation. As the pigs were withdrawn from feed for at least 12 h before slaughter and final live BW was determined one hour before harvesting, the difference in carcass yield can solely be explained by the difference in the weight of the digestive tract and/or the residual digestive contents. The aforementioned increasing dietary fiber level could have resulted in an increased gut fill, as gastric emptying is slowed down when dietary crude fiber content increases (Asmus et al., 2014; Coble et al., 2018). The linear decrease in relative loin weight was reflected in a numerical linear decrease in the carcass leanness. These findings cannot be explained by differences in the diet composition but rather reflect the numerical increase in the amount of subcutaneous fat.

Huff-Lonergan et al. (2002) reported that a greater percentage of drip loss, a greater percentage of cook loss, and lower marbling were related to lower tenderness. In agreement, the current data follow a similar pattern with respect to water holding capacity traits and the instrumental measurement of tenderness but was unrelated to the IMF content. Despite the observed relationships being consistent, there is no clear evidence as to why increasing sainfoin inclusion affected these traits.

*Fatty acid composition of the intramuscular fat and backfat*

The tissue level of polyunsaturated fatty acid is highly correlated with dietary polyunsaturated fatty acid intake in pigs, whereas the tissue level of saturated fatty acid and monounsaturated fatty acid is mainly determined by de novo lipogenesis and the desaturation of the saturated homologs and only marginally by the intake of these fatty acids (Wood et al., 2008). The inclusion of sainfoin increased the dietary content of C18:3n-3, which is the main fatty acid in sainfoin (Girard et al., 2016). Thus, compared to T0 pigs, T5, T10, and T15 pigs ingested 151, 257, and 318% greater amounts of C18:3n-3, respectively. However, the concentration of C18:3n-3 increased only by 145, 173 and 261% in the IMF and by 137, 181, and 238% in the backfat of T5, T10, and T15 pigs, respectively. Assuming a linear increase in the tissue with a dietary C18:3n-3 intake increase, the concentrations were lower, as expected, suggesting a lower tissue incorporation rate with greater dietary supply (Bee et al., 2008). However, it appeared that an additional part of the dietary C18:3n-3 was elongated and desaturated, as the C22:5n-3 in the IMF and the C20:5n-3 and C22:5n-3 concentrations were consistently greater (ranging from 50 to 83% for C22:5n-3 in the IMF and ranging from 50 to 200% for C20:5n-3 and 40 to 100% for C22:5n-3 in the backfat). Bee et al. (2008) found that, when greater C18:3n-3 levels were fed (21.7 vs 18.0 g/100 total fatty acids), the activities of the Δ-6- and Δ-5-desaturase and elongase were limited, resulting in no significant differences in the levels of the long chain n-3 homologs. In the present study, it appears that the C18:3n-3 intake was not so elevated to impair enzyme activities but was sufficiently high to result in a dose-dependent increase in the long-chain n-3 homologs. The intake of C16:0 and C18:0 increased with increasing sainfoin intake, whereas their levels slightly but significantly decreased in the backfat and, to some extent, in the IMF. Concomitantly, the proportion of their elongation and desaturation products increased in the tissues. As total fat deposition was independent from the dietary treatments, one could hypothesize that the de novo lipogenesis of C16:0 and C18:0 was unaffected. We did not determine the activity of the stearoyl-CoA desaturase activity, but the increasing desaturation indexes (C16:1n-7/C16:0 and the C18:1n-9/C18:0) suggest that, with increasing sainfoin intake, an increasing portion of C16:0 and C18:0 was desaturated to monounsaturated fatty acid. Both C18:2n-6 and C18:3n-3 inhibit the stearoyl-CoA desaturase activity (Kouba and Mourot, 1998; Bee et al., 2002). Compared to the C18:3n-3, the C18:2n-6 tissue levels were greater (IMF: 12- [T15] to 37 times [T0]: backfat: 8- [T15] to 25 times [T0]) regardless of dietary treatment. It can reasonably be concluded that the influence of C18:2n-6 on the stearoyl-CoA desaturase activity was greater when compared to that of C18:3n-3. Furthermore, due to the decreasing dietary intake of C18:2n-6 with increasing sainfoin inclusion, the C18:2n-6 levels in the IMF and backfat decreased, thereby reducing the inhibiting effect on the stearoyl-CoA desaturase activity.

*Boar taint compounds in the longissimus thoracis and backfat*

It was hypothesized that increasing the amount of CTs from sainfoin could reduce the deposition of boar taint-related compounds in the backfat. This hypothesis was based on previous results of Čandek-Potokar et al. (2015), who observed that the fat accumulation of skatole decreased when EM were fed diets supplemented with hydrolysable tannins from chestnut. Similarly, Bee et al. (2016) reported that indole concentrations in the backfat linearly decreased with the increasing inclusion of chestnut extract. The results of our study, although using CTs instead of hydrolysable tannins, are partly in agreement with the aforementioned findings, as the levels of skatole and indole in the LT and backfat decreased numerically with increasing dietary CT levels. It is believed that dietary tannin supplementation may impair skatole and indole production by affecting either the gut microflora (Tretola et al., 2019) or the process of enterocyte proliferation and apoptosis (Čandek-Potokar et al., 2015). Interestingly, and in agreement with the studies of Čandek-Potokar et al. (2015) and Bee et al. (2016), the androstenone

levels decreased with increasing dietary CTs intake. As CTs form complexes with various substances, like proteins, carbohydrates, and minerals, in the small intestine (Girard and Bee, 2020), androstenone, originating from the enterohepatic circulation, that is conjugated androstenone (a more polar molecule), might be bound to CTs and hence not be available for reabsorption.

Apart from the known positive correlations between androstenone, skatole, indole, and the weight of the testes and accessory sex glands (Bee et al., 2016), the skatole and indole levels of the LT and the backfat were highly correlated. As expected, the levels of skatole and indole in the LT are around 3 to 4% of the levels in backfat. Since the amount of IMF in the LT is approximately 3% (Table 6), the expected skatole and indole levels in the LT should be around 3% provided that those compounds are essentially present in the IMF lipids as predicted by their lipophilic characteristics. These results and especially the high correlations between the levels of skatole and indole in the backfat and the LT suggest that the levels determined in the backfat are a good proxy for those in the LT. The androstenone levels in the LT were greater than the expected 3% of the androstenone levels in the backfat. The reason is probably the presence of androstenone as a conjugated more polar molecule, which is better solved in a more polar environment than the backfat. The latter is mainly composed of triglycerides with a relative lower amount of the more polar phospholipids. Nevertheless, these findings questions the validity of using the levels or thresholds of androstenone in the backfat as a proxy for boar taint sensory attributes in the LT.

In conclusion, sainfoin included in grower finisher diets can substitute up to 7% of soy meal with no or minimal impact on growth performance and carcass characteristics, and, therefore, can be regarded as a valuable home-grown protein source. Although CTs might have affected feeding behavior, the CTs content had only minimal impact on the aforementioned traits. The effect of CTs on the expression of boar taint compounds was minimal, probably also because the boar taint concentrations were already low in the T0 group. The skatole and indole but not the androstenone levels in the backfat are valid proxies for those in the IMF and, by extension, in meat and its sensory characteristics.

## Ethics approval

All procedures contributing to this work complied with the ethical standards of the Swiss Federal Committee for Animal Care and Use. The reference number of the committee approval was 27654/2016_13_FR.

## Data and model availability statement

None of the data and none of the statistical models were deposited in an official repository. Upon reasonable request the data and statistical models are available from the corresponding author.

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## Author's contributions

Eleonora Seoni and Giuseppe Bee validated the data and carried out the main statistical analyses. Giuseppe Bee and Frigga Dohme-Meier conceived the study design and secured substantial funding. Eleonora Seoni and Giuseppe Bee performed the animal experiment, data recording during the growth trial and slaughter, and collected and processed feed, meat and backfat samples. Silvia Ampuero Kragten performed the boar taint analysis in the backfat and muscle samples. Giuseppe Bee, Eleonora Seoni, Frigga Dohme-Meier, and Gianni Battacone supervised analyses, drafted and reviewed critically the manuscript. All authors read and approved the final manuscript.

## Declaration of interest

None.

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## References

Acamovic, T., Brooker, J., 2005. Biochemistry of plant secondary metabolites and their effects in animals. Proceedings of the Nutrition Society 64, 403–412.
Agroscope, 2017. Fütterungsempfehlungen und Nährwerttabellen für Schweine. Lehrmittel Zentrale, Zollikofen, Switzerland.
Ampuero Kragten, S., Verkuylen, B., Dahlmans, H., Hortos, M., Garcia-Regueiro, J.A., Dahl, E., Andresen, O., Feitsma, H., Mathur, P.K., Harlizius, B., 2011. Inter-laboratory comparison of methods to measure androstenone in pork fat. Animal 5, 1634–1642.
Asmus, M.D., DeRouchey, J.M., Tokach, M.D., Dritz, S.S., Houser, T.A., Nelssen, J.L., Goodband, R.D., 2014. Effects of lowering dietary fiber before marketing on finishing pig growth performance, carcass characteristics, carcass fat quality, and intestinal weights. Journal of Animal Science 92, 119–128.
Azain, M., 2001. Fat in swine nutrition. Swine Nutrition 95, 106.
Azuhnwi, B.N., Hertzbergm, H., Arrigo, Y., Gutzwiller, A., Hess, H.D., Mueller-Harvey, I., Torgerson, P.R., Kreuzer, M., Dohme-Meier, F., 2013. Investigation of sainfoin (*Onobrychis viciifolia*) cultivar differences on nitrogen balance and fecal egg count in artificially infected lambs. Journal of Animal Science 91, 2343–2354.
Bee, G., Gebert, S., Messikommer, R., 2002. Effect of dietary energy supply and fat source on the fatty acid pattern of adipose and lean tissues and lipogenesis in the pig. Journal of Animal Science 80, 1564–1574.
Bee, G., Guex, G., Herzog, W., 2004. Free-range rearing of pigs during the winter: adaptations in muscle fiber characteristics and effects on adipose tissue composition and meat quality traits. Journal of Animal Science 82, 1206–1218.
Bee, G., Jacot, S., Guex, G., Biolley, C., 2008. Effects of two supplementation levels of linseed combined with CLA or tallow on meat quality traits and fatty acid profile of adipose and different muscle tissues in slaughter pigs. Animal 2, 800–811.
Bee, G., Silacci, P., Ampuero-Kragten, S., Candek-Potokar, M., Wealleans, A., Litten-Brown, J., Salminen, J., Mueller-Harvey, I., 2016. Hydrolysable tannin-based diet rich in gallotannins has a minimal impact on pig performance but significantly reduces salivary and bulbourethral gland size. Animal 11, 1617–1625.
Bilić-Šobot, D., Kubale, V., Škrlep, M., Čandek-Potokar, M., Prevolnik Povše, M., Fazarinc, G., Škorjanc, D., 2016. Effect of hydrolysable tannins on intestinal morphology, proliferation and apoptosis in entire male pigs. Archives of Animal Nutrition 70, 378–388.
Brunet, S., Hoste, H., 2006. Monomers of condensed tannins affect the larval exsheathment of parasitic nematodes of ruminants. Journal of Agricultural and Food Chemistry 54, 7481–7487.
Čandek-Potokar, M., Škrlep, M., Batorek Lukač, N., Zamaratskaia, G., Prevolnik Povše, M., Velikonja Bolta, Š., Kubale, V., Bee, G., 2015. Hydrolysable tannin fed to entire male pigs affects intestinal production, tissue deposition and hepatic clearance of skatole. The Veterinary Journal 204, 162–167.
Cappai, M.G., Wolf, P., Rust, P., Pinna, W., Kamphues, J., 2013. Raw hulled shredded acorns from Downy Oak (Quercus pubescens) in the diet of pigs: effects on digestibility and faeces characteristics. Journal of Animal Physiology and Animal Nutrition 97, 1–5.
Carcò, G., Gallo, L., Dalla Bona, M., Latorre, M.A., Fondevila, M., Schiavon, S., 2018. The influence of feeding behaviour on growth performance, carcass and meat characteristics of growing pigs. PLoS ONE 13, e0205572.
Coble, K.F., DeRouchey, J.M., Tokach, M.D., Dritz, S.S., Goodband, R.D., Woodworth, J.C., 2018. Effects of withdrawing high-fiber ingredients before marketing on finishing pig growth performance, carcass characteristics, and intestinal weights. Journal of Animal Science 96, 168–180.
De Haer, L., Merks, J., 1992. Patterns of daily food intake in growing pigs. Animal Science 54, 95–104.
Girard, M., Bee, G., 2020. Invited review: Tannins as a potential alternative to antibiotics to prevent coliform diarrhea in weaned pigs. Animal 14, 95–107.
Girard, M., Dohme-Meier, F., Silacci, P., Ampuero Kragten, S., Kreuzer, M., Bee, G., 2016. Forage legumes rich in condensed tannins may increase n-3 fatty acid levels and

sensory quality of lamb meat. Journal of the Science of Food and Agriculture 96, 1923–1933.
Honikel, K.O., 1998. Reference methods for the assessment of physical characteristics of meat. Meat Science 49, 447–457.
Huff-Lonergan, E., Baas, T.J., Malek, M., Dekkers, J.C.M., Prusa, K., Rothschild, M.F., 2002. Correlations among selected pork quality traits. Journal of Animal Science 80, 617–627.
Jin, A.L., Ozga, J.A., Lopes-Lutz, D., Schieber, A., Reinecke, D.M., 2012. Characterization of proanthocyanidins in pea (*Pisum sativum* L.), lentil (*Lens culinaris* L.), and faba bean (*Vicia faba* L.) seeds. Food Research International 46, 528–535.
Kotrotsios, N., Christaki, E., Bonos, E., Florou-Paneri, P., 2012. Dietary carob pods on growth performance and meat quality of fattening pigs. Asian-Australasian Journal of Animal Sciences 25, 880.
Kouba, M., Mourot, J., 1998. Effect of a high linoleic acid diet on D9-desaturase activity, lipogenesis and lipid composition of pig subcutaneous adipose tissue. Reproduction Nutrition Development 38, 31–37.
Kragten Ampuero, S., Collomb, M., Dubois, S., Stoll, P., 2014. Composition des acides gras dans l'alimentation animale—méthodes d'analyse. Recherche Agronomique Suisse 5, 330–337.
Maljaars, J., Peters, H., Masclee, A., 2007. The gastrointestinal tract: neuroendocrine regulation of satiety and food intake. Alimentary Pharmacology & Therapeutics 26, 241–250.
Maselyne, J., Saeys, W., Van Nuffel, A., 2015. Review: quantifying animal feeding behaviour with a focus on pigs. Physiology & Behavior 138, 37–51.
Merghem, R., Jay, M., Brun, N., Voirin, B., 2004. Qualitative analysis and HPLC isolation and identification of procyanidins from *Vicia faba*. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques 15, 95–99.
Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. Journal of the Science of Food and Agriculture 86, 2010–2037.
Quemeneur, K., Labussiere, E., Le Gall, M., Lechevestrier, Y., Montagne, L., 2019. Feeding behaviour and pre-prandial status affect post-prandial plasma energy metabolites and insulin kinetics in growing pigs fed diets differing in fibre concentration. British Journal of Nutrition 121, 625–636.
Roura, E., Tedó, G., 2009. Feed appetence in pigs: an oronasal sensing perspective. In: Torrallardona, D., Roura, E. (Eds.), Voluntary feed intake in pigs. Wageningen Academic Publishers, Wageningen, NL, pp. 105–140.
Terrill, T.H., Rowan, A.M., Douglas, G.B., Barry, T.N., 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. Journal of the Science of Food and Agriculture 58, 321–329.
Tretola, M., Maghin, F., Silacci, P., Ampuero Kragten, S., Bee, G., 2019. Effect of Supplementing hydrolysable tannins to a grower–finisher diet containing divergent PUFA levels on growth performance, boar taint levels in back fat and intestinal microbiota of entire males. Animals 9, 1063.
Wesoly, R., Weiler, U., 2012. Nutritional influences on skatole formation and skatole metabolism in the pig. Animals 2, 221–242.
Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I., Whittington, F.M., 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Science 78, 343–358.