Financially supported by:

Den Europæiske Landbrugsfond for Udvikling af Landdistrikterne: Danmark og Europa investerer i landdistrikterne

LDP 2020

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Den Europæiske Landbrugsfond for Udvikling af Landdistrikterne

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ANDROSTENONE IN INTACT MALES INCREASES AS LIVE WEIGHT INCREASES

TRIAL REPORT NO. 1102

Androstenone levels in back fat in intact males increase as live weight increases. Research reveals correlations between androstenone levels recorded in the individual pig at 60, 70, 80, 90, 100, 110 and 120 kg live weight and in the carcass at slaughter.

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PUBLISHED: 18 MAY 2017

Abstract

Androstenone levels at increasing live weight

This trial consisted of two parts. In the first part, androstenone levels were measured in biopsies taken from intact males at 10 kg intervals in the 60-120 kg growth period. All pigs were slaughtered at 120 kg live weight. On average, androstenone levels in back fat increased from 0.4 to 1.2 ppm (mg/kg) from 60 to 120 kg live weight. Intact males with a high androstenone level at 60 kg maintained a high level at 120 kg. Analyses demonstrated a significant correlation between the levels in biopsies taken the day before slaughter and the levels recorded in the carcass. Differences in the levels recorded the day before slaughter and in the carcass are attributed to analysis and sampling methods.

Boat taint in pigs slaughtered at increasing live weight

In the second part of the trial, intact males were slaughtered at increasing weight: 80, 90, 100, 110, 120 kg live weight. Biopsies were taken the day before slaughter and boar taint was subsequently assessed after slaughter. Analyses reveal that androstenone levels increased averagely from 1.01 to 1.49 ppm as slaughter weight increased, and skatole levels were low independent of the increased carcass weight (58-89 kg). In all weight groups, a significant correlation was observed between androstenone in the live pig and in the carcass. However, results show no correlation between skatole and androstenone in the carcass. We found no correlation between human nose score and androstenone levels in the 60-100 kg growth period, whereas in the 100-120 kg growth period a correlation was observed between androstenone levels in fat and human nose score.

Background

In 2010, the Danish Agriculture & Food Council joined a voluntary European declaration on alternatives to surgical castration of male pigs, which included a declaration of intent to cease castration of male pigs by January 1, 2018. Subsequently, at the Animal Welfare Summit in 2014, a voluntary agreement was made with The Danish Minister for Food to end castration of male pigs without the use of anaesthesia by the end of 2018. Consequently, as of January 1, 2019, Danish pig producers must either produce intact male pigs or introduce local anaesthesia/full anaesthesia during castration. Today (2017), intact males account for roughly 1.5-2% of all slaughterings in Denmark. A screening made in 2014 of 900 intact male pigs revealed that roughly 2% are rejected on the basis of skatole levels above 0.25 ppm. This is the method used today and approved by the Danish slaughter inspection. If pigs are assessed on the basis of the human nose method (score > 2), 12% would be rejected at slaughter. If the rejection limit for androstenone is lowered to 1.0 ppm, 38% of all intact male pigs would be rejected [9]. There is today not international agreement on the rejection limit for androstenone nor is it internationally agreed to use the human nose method. Consequently, several different methods (tests at the slaughter line or lab tests) and different scales and rejection limits are currently in play.

Several important export markets are still reluctant to buy pork from intact males due to the risk of boar taint. There are multiple pros of production of intact males provided we can reach an agreement internationally on measurement methods and detection thresholds for boar taint that generate market acceptance and do not lead to unrealistically high rejection rates at slaughter. Intact males have a far greater potential for lean meat gain and have a feed conversion ratio level with or better than female pigs and far better than castrates [7][8].

Several feeding concepts have been tested in recent years in the attempt to affect boar taint. Experience shows that it takes only a few days to reduce skatole in fat tissue, which is an effect of the bacterial production of tryptophan in the gut [10]. Androstenone in fat tissue, however, is correlated to

maturity: the older and the heavier the pig, the greater the risk of high androstenone levels [5], but it is unclear at which age or weight androstenone levels start increasing. Furthermore, there is a genetic variation and variations in general between the pigs. It is unclear how skatole and androstenone affect each other, but we do know that both compounds are metabolized in the liver by the same enzyme system. That may explain why a high androstenone level in the blood probably can inhibit the degradation of skatole and thereby indirectly increase skatole levels in blood and fat.

The aim of this trial was to investigate the development of and variations in androstenone levels at different slaughter weights. In addition, boar taint was assessed using different methods related to measurement of androstenone in live pigs.

Hypotheses:

- There is a curvilinear correlation between skatole levels in fat and live weight
- There is no correlation between skatole levels in fat at slaughter and live weight
- Androstenone levels are significantly lower (0.2 ppm) in fat tissue in carcasses from light intact males compared with heavy intact male pigs when there is a minimum difference in slaughter weight of 20 kg.

Material and method

All pigs for the trial were individually marked at birth and moved to trial station Grønhøj at weaning, where the trial started when the pigs reached approx. 30 kg.

In the first part of the trial, fat biopsies and blood samples were collected from 44 intact male pigs at 60, 70, 80, 90, 100, 110, 120 kg live weight (table 1a). The intention originally was to analyse both skatole and androstenone levels in blood samples as well as in biopsies to monitor the development in androstenone and skatole in each pig as live weight increased. However, it was possible only to analyse androstenone in fat biopsies. The method intended for analyses of blood remained undeveloped, and due to fixation of the biopsies with methanol at the lab at Aarhus University (AU), it was not possible to analyse skatole in the biopsies. It was subsequently – unsuccessfully – attempted to find another lab to perform these analyses. The data in this trial report therefore only include androstenone in biopsies taken at increasing live weight and skatole, androstenone and human nose score in back fat samples from carcasses.

In the second part of the trial, approx. 50-70 intact males in each group were slaughtered at different live weights: 80, 90, 100, 110 and 120 kg. Biopsies were taken from these pigs the day before slaughter (table 1b).

All pigs were weighed several times a week, and trial pigs were delivered for slaughter twice a week to meet the planned live weight.

Approval was obtained from the Danish Animal Experiments Inspectorate to take biopsies and blood samples (Approval no. 2012-15-2934-00160).

Table 1a. Biopsies taken on intact males at 10 kg intervals in the 60-120 kg period.

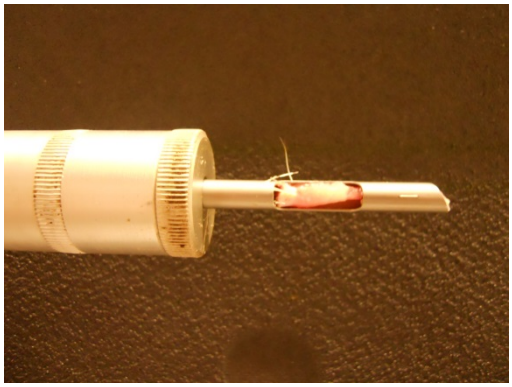
Location	Samples	Weight
Grønhøj, live pigs	Biopsy	60 kg
	Biopsy	70 kg
	Biopsy	80 kg
	Biopsy	90 kg
	Biopsy	100 kg
	Biopsy	110 kg
	Biopsy	120 kg
Slaughterhouse	Slaughter – back fat	120 kg

Table 1b. Slaughter at different live weight intervals.

Location	Samples	Weight
Slaughterhouse	Biopsy before slaughter and fat sample from carcass	80 kg
	Biopsy before slaughter and fat sample from carcass	90 kg
	Biopsy before slaughter and fat sample from carcass	100 kg
	Biopsy before slaughter and fat sample from carcass	110 kg
	Biopsy before slaughter and fat sample from carcass	120 kg

Biopsy procedure

- Pigs were moved into the inspection alley and weighed individually
- The ID and weight of each pig were recorded
- Biopsies were taken in the neck region where there is thick layer of fat using a biopsy needle developed by SUISAG (Swiss breeding company).



Fat sample taken with a biopsy needle.

- Rind and meat were cut off the sample, leaving minimum 100 mg fat per pig.
- The needle was disinfected in industrial alcohol between each pig.
  Fat samples were stored individually at -80° C.
- Pain relief (Metacam) was administered in the back. Pigs that are given pain relief are normally subject to a retention time of six days. However, the Animal Experiments Inspectorate approved the omission of pain relief when biopsies were taken the day before slaughter.
- The administration of pain relief and collection of biopsies alternated between the right and left sides of the back.

Analyses

The method used for analysis of biopsies was developed at AU's lab where androstenone levels were analysed using the HPLC method [4]. As described above, it was not possible to analyse skatole levels in biopsies, as the biopsies was fixated with methanol at Aarhus University (AU), which made the analysis of skatole impossible.

Boar taint was analysed in back fat samples taken the day after slaughter:

- At Danish Crown's slaughterhouse in Ringsted:
  - Detection using the Human nose method [2] according to the below scale:
    - 0 = No boar taint
    - 1 = Faint boar taint
    - 2 = Pronounced boar taint
- At Aarhus University where skatole, indole and androstenone were subject to HPLC analysis [4]

Analysis methods and detection thresholds.

Method		Unit	Detection thresholds
Slaughterhouse	Human nose	Score 0, 1, 2	≥ 2 [1]
Laboratory, HPLC	Skatole	ppm = mg/kg	> 0.25 [1]
	Indole	ppm = mg/kg	-
	Androstenone	ppm = mg/kg	> 1.00 / 2.00 [6]

Scientists across the world are still discussing the correct detection thresholds for rejection based on androstenone from different viewpoints such as consumer responses. Several levels are currently in play: >1.00 ppm and >0.50 ppm androstenone [6], but also 2.00 ppm is being considered. One common rejection limit for boar taint (measured on skatole, androstenone and/or the human nose method) has yet to be agreed.

Feeding

All pigs were fed a standard finisher diet from 30 kg until slaughter that complied with the Danish nutrient recommendations.

Health

Recordings in this trial did not include diarrhoea and mortality.

Statistical analyses

- Skatole and androstenone were log transformed before they were analysed to obtain normal distribution, stabilize the variance, and subject to analysis in a linear model.
- Analysis of androstenone from live pigs take into consideration repeated measurements on pigs
- Results: values are back-transformed.

Results and discussion

Androstenone levels at increasing live weight

Androstenone increases with increasing weight (and age) (figure 1). Results show large variations between individual pigs. There is a significant correlation between the recordings on each pig. It is in particular interesting that pigs with low androstenone levels at 60 kg also have low androstenone levels at 120 kg (figure 2, table 2). This also indicates the high heritability for androstenone [11].

Table 2. Androstenone median (ppm) in male pigs at increasing live weight.

Weight interval	Actual weight, av kg (min-max)	Pigs	Age, av days (min-max)	Analyses	Androstenone median, ppm	Confidence interval median	
						Min	Max
60	60.7 (56-71)	44	111.7 (83-125)	43	0.40	0.32	0.51
70	69.5 (64-77)	44	118.5 (90-130)	44	0.53	0.42	0.66
80	79.9 (76-85)	40	126.9 (95-140)	37	0.67	0.54	0.83
90	89.8 (86-96)	44	134.1 (104-156)	44	0.82	0.65	1.02
100	99.6 (96-106)	43	141.6 (111-168)	43	0.97	0.77	1.21
110	109.6 (106-115)	43	148.3 (116-170)	43	1.11	0.89	1.38
120	119.2 (116-126)	44	155.4 (123-175)	44	1.22	0.97	1.55

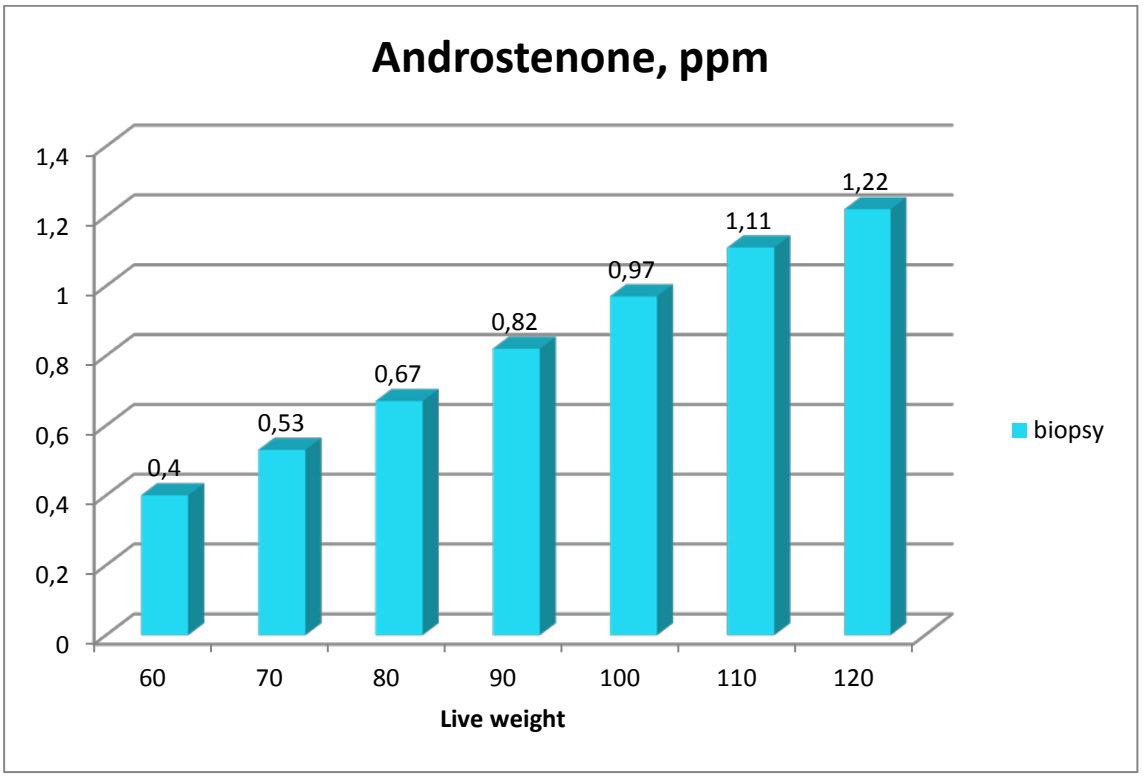


Figure 1. Medians, androstenone (ppm) at increasing weight (live pigs).

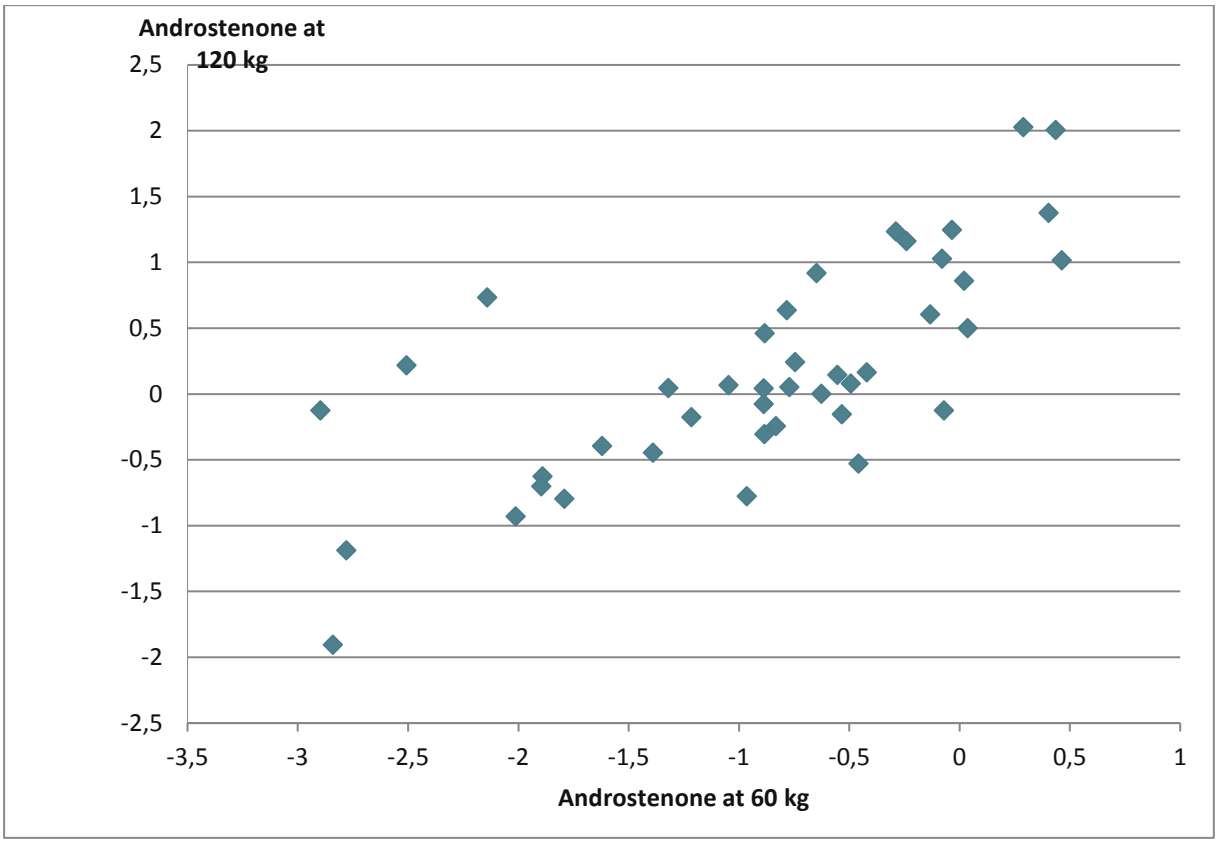


Figure 2. Correlation between androstenone at 60 and 120 kg live weight in the same male pig.

Results show a very good correlation between repeated recordings (table 3) and between androstenone levels recorded a different live weight intervals and the levels in the carcass on the individual pig. In particular the correlation of 0.78 and 0.72, respectively, between the first recording at 60 kg and subsequently at 110 kg and 120 kg (table 3) is interesting as this indicates that it is possible to predict the potential androstenone level in the carcass at an early point in the pig's life. There was a positive correlation between pigs' androstenone level at 60 kg and 120 kg live weight and androstenone levels in the carcass (table 4). Androstenone levels and correlations increase as weight increases as slaughter approaches.

Results revealed no correlation between androstenone levels in biopsies taken at 60-120 kg and skatole at slaughter. The correlation between androstenone in the live pig at 100-120 kg and Human nose score at slaughter is 0.41-0.49 (significant – see table 4). This indicates that the score remains largely unaffected until androstenone reaches a high level at a high live weight.

Table 3. Correlation matrix for androstenone in live pigs at increasing weight (***all significant***).

	Weight	Pigs	Live						
			60	70	80	90	100	110	120
Live weight	60	44	1.00	0.89	0.79	0.75	0.79	0.78	0.72
	70	44	0.89	1.00	0.77	0.72	0.80	0.81	0.74
	80	40	0.79	0.77	1.00	0.70	0.83	0.78	0.70
	90	44	0.75	0.72	0.70	1.00	0.78	0.82	0.80
	100	43	0.79	0.80	0.83	0.78	1.00	0.95	0.87
	110	43	0.78	0.81	0.78	0.82	0.95	1.00	0.93
	120	44	0.72	0.74	0.70	0.80	0.87	0.93	1.00

Table 4. Correlation between androstenone in the live pig at live weight and androstenone, skatole, Human nose in the carcass *= significant.

		Androstenone ppm in biopsy (day before slaughter at 120 kg)		
Live weight	Pigs	Androstenone, ppm, carcass	Skatole ppm, carcass	Human nose, cracass
60	42	0.75*	0.22	0.17
70	41	0.71*	0.16	0.17
80	37	0.71*	0.29	0.29
90	44	0.74*	0.29	0.20
100	43	0.90*	0.38*	0.49*
110	42	0.91*	0.42*	0.44*
120	44	0.93*	0.37	0.41*

Boar taint in pigs slaughtered at increasing live weight

Androstenone levels increase as live weight at slaughter increases: levels are 0.3 ppm higher in carcass samples compared with biopsies taken on live pigs. This difference may be attributed either to differences in water content in the fat samples or to differences between labs using the same method (table 5 and figure 3). Skatole levels remain stable at increasing slaughter weight when allowing for analysis inaccuracies. A previous study [12] found no differences in skatole when pigs were slaughtered at 75 and 95 kg live weight, respectively, and also revealed that increasing slaughter weight led to an increase in androstenone levels [5].

Table 5. Skatole (ppm), androstenone (ppm), boar taint (human nose score) in intact males at increasing slaughter weight.

Live weight at slaughter	Pigs	Age, days	Weight, delivery kg	Carcass weight, kg	Lean meat %	Skatole fat, ppm	Androste-none fat, ppm	Androste-none biopsy, ppm
80	71	132	80.7	58.1	62.6	0.08 (0.07-0.09)	1.03 (0.91-1.16)	0.74 (0.66-0.84)
90	65	139	90.1	65.5	62.5	0.07 (0.07-0.08)	1.14 (1.04-1.24)	0.85 (0.78-0.93)
100	51	146	99.7	73.2	61.3	0.07 0.06-0.07)	1.26 (1.17-1.35)	0.97 (0.91-1-04)
110	64	153	109.8	81.0	60.5	0.06 (0.06-0.07)	1.39 (1.28-1.52)	1.11 (1.02-1.21)
120	76	160	119.7	88.9	60.2	0.06 (0.05-0.06)	1.54 (1.36-1.74)	1.27 (1.13-1.43)
P-value						0.0005	<0.0001	<0.0001

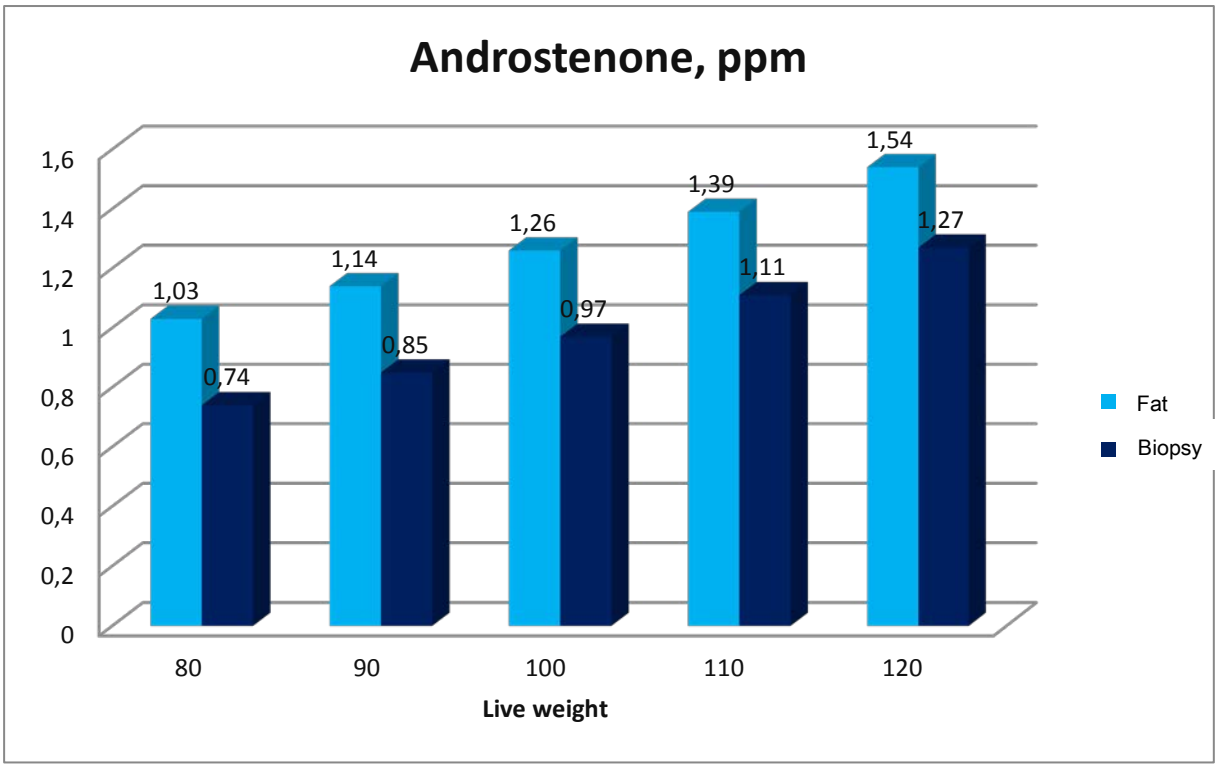


Figure 3. Median values for androstenone (ppm) at increasing weight in live pigs (biopsy dark blue) and carcasses (neck fat light blue).

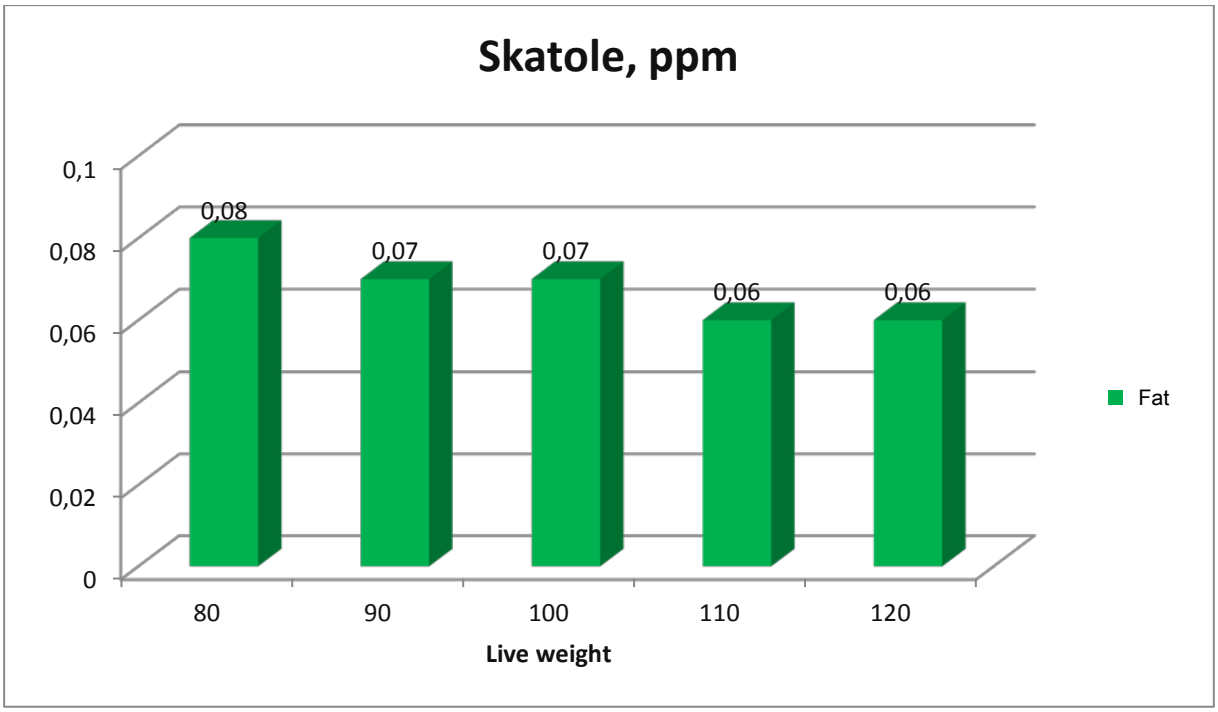


Figure 4. Median values for skatole (ppm) at increasing slaughter weight (neck fat).

Overall, for all weight groups we see a positive correlation between androstenone in the live pig and in the carcass (correlation = 0.93-0.97 ($p<0.0001$)). Results shows no correlation between skatole and androstenone at slaughter at a live weight of 80-100 kg regardless of whether androstenone was measured in the live pig or in the carcass. There was a significantly positive correlation between skatole in the carcass and androstenone, regardless of whether androstenone was recorded in the live pig or the carcass of a pig slaughtered at 110 or 120 kg live weight. Results show a significant correlation between human nose score recorded at slaughter and skatole levels recorded at different live weight in all groups, whereas a significant correlation between androstenone and human nose is only observed at 110 and 120 kg (table 6).

Table 6. Correlation between androstenone (ppm), skatole (ppm), and human nose in intact males slaughtered at different live weight (*= significant).

		Androstenone biopsy			Androstenone slaughter		Skatole slaughter
Liv weight at slaughter, kg	Pigs	Androstenone at slaughter	Skatole at slaughter	Human nose at slaughter	Skatole	Human nose	Human nose
80	69	0.93*	0.19	0.16	0.22	0.16	0.55*
90	64	0.93*	0.13	0.19	0.09	0.12	0.70*
100	51	0.93*	-0.07	0.19	-0.00	0.16	0.63*
110	63	0.97*	0.29*	0.43*	0.28*	0.47	0.75*
120	74	0.95*	0.30*	0.41*	0.29*	0.42*	0.57*

Confirmation of hypotheses:

1. Results show a significant correlation between androstenone levels in fat and live weight
2. Results show no correlation between skatole levels in fat and live weight
3. Androstenone levels were significantly lower (0.2 ppm) in fat tissue from light, young intact males compared with heavy, old intact males when slaughter weight differed by minimum 20 kg.

Conclusion

Androstenone levels increase with increasing live weight, and results show that intact males with a high androstenone level at 60 kg maintain a high androstenone level at slaughter at 120 kg.

When live weight increased, androstenone levels in fat increased distinctly, while skatole levels were low regardless of weight. Analyses show a positive correlation between androstenone levels in fat in the live pig and in the carcass, and no correlation between androstenone and skatole, neither in the live pig nor in the carcass, at 80-100 kg, whereas a significant correlation was observed at 110 and 120 kg.

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Trial no. 1203
File no.: 48-400560
Journal no.: 3663-11-00182

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