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5α-ANDROSTENONE IN FAT FROM BOARS SELECTED FOR RATE OF GAIN AND THICKNESS OF BACK FAT, AND FROM BOARS USED IN ARTIFICIAL INSEMINATION SERVICE

By

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ANDRESEN, ØYSTEIN and HÅVARD BAKKE: *5α-Androstenone in fat from boars selected for rate of gain and thickness of back fat, and from boars used in artificial insemination service.* Acta vet. scand. 1975, *16*, 492—502. — Large variations in the level of 5α-androstenone in fat from different boars have been found. No significant difference in the level of 5α-androstenone was detected in fat from boars selected for high rate of gain and low back fat (HP-line), low rate of gain and high degree of fatness (LP-line) and a control group maintained without deliberate selection (CL-line). In boars used in artificial insemination service relatively high levels of 5α-androstenone in fat were observed, and in these animals a significant ($P < 0.05$) positive regression of 5α-androstenone level on age was found. Positive but non-significant regression coefficients were found between number of services which the boars had performed and level of 5α-androstenone in fat from the same animals.

5α-androstenone; fat; boar taint; selection.

In various tissues from mature boars strong smelling C_{19}-16-unsaturated steroids have been detected. *Prelog & Ruzicka* (1944) identified the musk smelling an-α* and an-β in pig testes.

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* Abbreviations and trivial names used:
5α-androstenone: 5α-androst-16-en-3-one;
an-α: 5α-androst-16-en-3α-ol;
an-β: 5α-androst-16-en-3β-ol;
androstenedione: 4-androstene-3,17-dione;
dehydroepiandrosterone: 3β-hydroxy-5-androsten-17-one;
testosterone: 17β-hydroxy-4-androsten-3-one;
17α-hydroxyprogesterone: 17α-hydroxy-4-pregnene-3,20-dione;
17α-hydroxypregnenolone: 3β,17α-dihydroxy-5-pregnene-20-one;

5α-Androstenone, which has an intense urine-perspiration like smell, was first identified in boar fat by *Patterson* (1968a). The general occurrence of this steroid in adipose tissue from entire male pigs has later been confirmed (*Claus* 1970, *Fuchs* 1971), and other C_{19}-16-unsaturated steroids have also been detected in this tissue (*Beery & Sink* 1971, *Thompson et al.* 1972). 5α-Androstenone is believed to be a major contributor to the sex odour, or boar taint, which can be detected from heated fat from some boars. The presence of this taint in boar meat is the reason why meat from uncastrated males in general is considered unsuitable for human consumption. *Fuchs* found a correlation coefficient of 0.75 between the intensity of boar taint as evaluated subjectively and the concentration of 5α-androstenone in the same samples of boar fat. *Newell et al.* (1973) and *Malmfors & Andresen* (1975) found a somewhat lower correlation, namely $r = 0.53$ and $r = 0.51$, respectively.

The physiological function of these odorous steroids in the pig might be as pheromones (*Sink* 1967, *Melrose et al.* 1971, *Reed et al.* 1974). Of special significance in this respect is the presence of C_{19}-16-unsaturated steroids in boar saliva and salivary glands (*Patterson* 1968b, *Claus, Katkov et al.* 1972).

C_{19}-16-unsaturated steroids are produced and secreted by the boar testes (*Saat et al.* 1972). The biosynthesis of these compounds seems to follow biosynthetic pathways different from those used in the formation of other C_{19} steroids as androstenedione, dehydroepiandrosterone and testosterone (for review see *Gower* 1972). In in vitro studies with minced boar testes thus neither 17α-hydroxyprogesterone (*Ahmad & Gower* 1968) nor 17α-hydroxypregnenolone (*Gower & Ahmad* 1967) seem to be intermediates in the biosynthesis of C_{19}-16-unsaturated steroids.

5α-androstenone has been identified in free form in boar spermatic vein plasma (*Gower et al.* 1970) and its concentration in pig peripheral plasma has been determined (*Claus, Andresen* 1975a, *Carlstrøm et al.* 1975). Due to its low polarity this steroid seems to accumulate in adipose tissue.

The present work was undertaken to further examine the occurrence of 5α-androstenone in boar adipose tissue. Since various routes exist for the biosynthesis of C_{19} steroids in boar testes, and the secretion capacity for 5α-androstenone seems to vary between boars (*Carlstrøm et al.*), changes in the qualitative and quantitative testicular secretion of steroids could contribute

to variations in leanness and rate of gain between lines of animals. A further aim was therefore to study if selection according to thickness of back fat and rate of gain might have influenced these pathways and caused any changes in the quantities of 5α-androstenone secreted by the testes, as mirrored in the concentration of 5α-androstenone in the subcutaneous fat depots.

MATERIAL AND METHODS

Variation within animals

The extent to which the level of 5α-androstenone in single samples is representative for the level in subcutaneous fat throughout the animal was studied in six sexually mature boars. In each of these animals a total of eight samples of subcutaneous fat were collected bilaterally at four different locations after slaughtering. Two samples were collected about 15 cm laterally to the midline in the neck, one halfway between the ear base and the shoulder blade, and the other in front of the shoulder blade. The two others were taken in the lumbar region about 10 and 20 cm from the midline.

Variation between lines of animals

Samples from 49 sexually mature boars have been analysed. Thirty-seven of the boars were young animals, 184—309 days of age, from the seventh and eighth generation of a selection experiment, and 12 were older boars, 431—890 days of age, used in artificial insemination service (AI) (Table 3). In the selection experiment the criterion was an index including rate of gain and thickness of back fat (*Standal* 1967). One line (highpoint, HP) was selected for high rate of gain and low back fat thickness, one line (lowpoint, LP) was selected in the opposite direction, and one line (control line, CL) was maintained without deliberate selection. All the boars had been used for service at the time when the samples were taken. The animals were penned individually in the same room as a number of gilts and sows. The boars from the three selection lines received the same diet, providing 2—2.5 Scandinavian feed units a day containing approx. 400 g crude protein.

After three to four generations of selection, *Standal et al.* (1973) found a difference of 12.5 mm in average back fat thickness between the LP- and HP-line, and a corresponding difference

of 8.5 % in dissected fat tissue in carcasses from pigs slaughtered at 90 kg live weight. In the seventh generation the difference in back fat thickness had increased to 18 mm. No total carcass dissections have been performed in these animals, but estimates of the difference in amount of fat tissue between the three lines was obtained on the basis of the difference in back fat thickness as described by *Aulstad* (1969). Using his regression equations the difference in fat tissue corresponding to 18 mm in back fat should be 12 % (HP-line 20 %, CL-line 25 % and LP-line 32 % fat). Similar results were also obtained by extending the relationship between back fat thickness and fat tissue found by *Standal et al.*

The boars used for AI service are selected on the basis of performance and sib testing and are expected to possess the highest genetic potential in the population for important breeding characters. The AI boars included in this study were individually penned at Stensby boar station. They were fed 2 kg concentrate a day containing 18 % crude protein. In addition 1 kg pelleted dry grass was given. Nine of these boars were of Norwegian Landrace and three were of the Yorkshire breed.

Biopsies of subcutaneous fat were collected dorsally in the neck about 10—15 cm from the midline and 5—10 cm cranial to the shoulder blade, while the boars stayed in the pens. The technique used was as described by *Lundstrøm et al.* (1973).

The concentration of 5α-androstenone was determined by a radioimmunological procedure (*Andresen* 1975b). Unlabelled and (5α-^{3}H)-5α-androstenone were generously supplied by Syntex Research, Palo Alto, California, USA. All samples were analysed in duplicate, and the mean concentration of 5α-androstenone was calculated per g ethylacetate-extractable fat.

RESULTS

Variation within animals

The average concentrations ± s of 5α-androstenone in fat from eight different anatomical locations in the six boars were 0.78 ± 0.18, 1.10 ± 0.11, 2.91 ± 0.34, 2.92 ± 0.31, 3.23 ± 0.34 and 7.31 ± 0.89 μg per g, respectively.

The analysis of variance in Table 1 showed significant differences between boars ($P < 0.001$) and between sampling positions ($P < 0.05$). The fat biopsies were collected from two se-

Table 1. Analysis of variance of 5α-androstenone concentration in fat from several anatomical positions.

Source of variation	d.f.	Mean square
Boars	5	45.748***
Sampling position	7	0.491*
Error	35	0.174

*** Significant at 0.1 % level.
* Significant at 5 % level.

parate regions of the carcasses (neck and lumbar region), and an analysis of variance was performed to find whether the significant effect of sampling position was explained by a difference between these regions. The analysis showed a highly significant ($P < 0.001$) difference between 5α-androstenone concentration in the neck and lumbar region, while the difference among positions within these regions were not significant (Table 2). The regression of 5α-androstenone levels in samples from the lumbar region on levels in samples from the neck was found to be $y = 1.14x + 0.10$ with $R^2 = 0.92$.

Table 2. Analysis of variance of 5α-androstenone concentrations in fat from the neck and lumbar region.

Source of variation	d.f.	Mean square
Neck vs. lumbar region	1	2.506***
Among positions within regions	6	0.155
Error	35	0.174

*** Significant at 0.1 % level.

Variation between lines of animals

The concentrations of 5α-androstenone in fat from the selection experiment boars and the AI boars are presented in Table 3. A mean level ± s.e.m. 5α-androstenone of 5.4 ± 0.5, 4.7 ± 0.7 and 4.8 ± 1.0 μg per g fat was found in the HP, LP and CL boars, respectively. Analysis of variance showed no significant differences in 5α-androstenone concentration between the selection lines. Linear regression of 5α-androstenone concentration on age was calculated for the three lines separately and for all of them together. None of these calculations showed significant age effect

Table 3. Concentration of 5α-androstenone in fat from boars. HP: boars selected for high rate of gain and low backfat, LP: boars selected for low rate of gain and high degree of fatness, CL: boars maintained without deliberate selection, AI: boars used in artificial insemination service.

Group of animals	No. of animals	Age of animals in days, mean ± s.e.m.	No. of services, mean ± s.e.m.	μg 5α-androstenone per g fat, mean ± s.e.m.
HP	12	223 ± 8	5.3 ± 1.0	5.4 ± 0.5
LP	12	271 ± 7	2.7 ± 0.4	4.7 ± 0.7
CL	13	229 ± 6	4.2 ± 0.8	4.8 ± 1.0
AI	12	705 ± 36	78.6 ± 11.4	10.3 ± 3.0

on 5α-androstenone, and comparisons in this age interval (184—309 days) were therefore made without age corrections.

The mean concentration ± s.e.m. of 5α-androstenone in fat from the 12 AI boars was 10.3 ± 3.0 μg per g. In one of these boars, 864 days of age, extremely high values were detected (42.1 μg per g fat). In contrast to what was found in the younger boars in the selection experiment, a significant ($P < 0.05$) positive regression of 5α-androstenone on age was found for this group of animals, whether the extreme value found in the 864 days old boar was excluded or not (Fig. 1). Age explained approx.

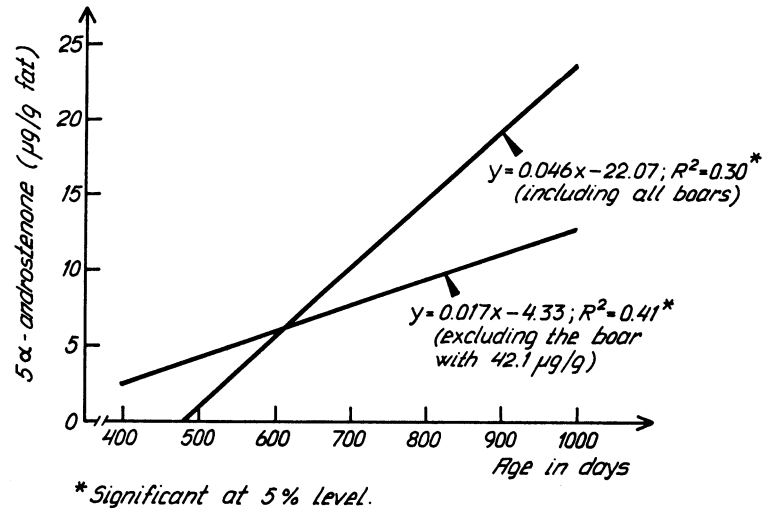

Figure 1. Age effect on 5α-androstenone concentration in fat from AI boars.

30 % of the variations in 5α-androstenone concentration. The effect of the use of the boars in breeding on 5α-androstenone concentration in fat was also calculated. Positive, but non-significant regression coefficients were found for both the young boars and the AI boars between number of services which the boars had performed and 5α-androstenone concentration.

DISCUSSION

A significant difference in the concentration of 5α-androstenone in fat originating from different anatomical locations in the same animal was found (Table 1). The analysis in Table 2 showed that most of the variation was explained by the difference between neck and lumbar region, and that the variations within these two areas within individuals were small. The level of 5α-androstenone in one fat biopsy sample collected at a defined location should thus provide a good estimate of the general level of 5α-androstenone in subcutaneous fatty tissue of the boar, and should be a reliable parameter to use in a comparison of individuals. Regarding intensity of boar taint from heated fat, *Malmfors & Hansson* (1974) were unable to detect significant differences in back fat from the shoulder, middle and lumbar regions of boar carcasses, while *Walstra* (1974) observed differences both in boar taint intensity and concentration of 5α-androstenone in samples of back fat from various regions.

There was a difference in average age between selection lines but due to the differences in rate of gain, the average live weights at sampling were approximately the same in the three lines. No significant difference in the absolute concentrations of 5α-androstenone was detected between the selection lines. As the boar carcasses were not dissected, no accurate estimates of the total amount of fatty tissue in the animals are available. But calculations according to *Aulstad* (1969) would indicate an increasing total amount of 5α-androstenone in fat in boars from the CL-line to the HP-line to the LP-line. Significant differences in lipid mobilization have been found between the three lines (*Standal et al.* 1973). This could indicate differences in the turnover rate for the adipose tissues making the absolute concentration of 5α-androstenone in fat an unreliable parameter for the amount of steroid secreted by the testes. The data presented do, however, not indicate that selection for rate of gain and

thickness of back fat has caused major changes in the ability of the testes to produce and secrete 5α-androstenone or in the endocrine factors regulating the production of this steroid. Other studies with pigs from the selection experiment have shown that some endocrine characters (*Lund-Larsen & Bakke,* in press), and serum levels of glucose, non-esterified fatty acids and cholesterol (*Bakke* 1975) have been influenced by the selection.

In accordance with previous observations (*Fuchs* 1971, *Malmfors & Andresen* 1975) large variations were detected between individual boars in the level of 5α-androstenone in fat. The level of 5α-androstenone found in the present material was, however, substantially higher than the values reported by *Malmfors & Andresen.*

For the boars in the three selection lines no relationships were found between age and concentration of 5α-androstenone. In fat from the AI boars larger concentrations of 5α-androstenone than in the three selection lines were observed. The AI boars were older (431—890 days) than the boars in the selection experiment, and a significant positive regression of level of 5α-androstenone in fat on age was detected in this group of animals. *Booth* (1975) has detected large fluctuations with age in the level of various C_{19}-steroids in testes and submaxillary glands of boars. Much higher level of 5α-androstenone were found in 2 years old boars than in boars 36 weeks of age.

One might expect the use of the boars in breeding to influence the 5α-androstenone concentrations, but calculations showed that number of services had less effect on the concentrations than age, and multiple regression of level of 5α-androstenone on age and number of services did not explain a larger part of the variation ($R^2 = 0.31$) than age alone ($R^2 = 0.30$).

Work is in progress to elucidate both the effect of age and sexual exitement on the level of 5α-androstenone in peripheral plasma and subcutaneous fat.

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SAMMENDRAG

5α-androstenon i fett fra råner selektert for veksthastighet og ryggspekktykkelse og fra råner brukt i kunstig sædoverføring.

Det ble funnet store variasjoner i 5α-androstenonkonsentrasjonen i fett fra forskjellige råner. Det ble ikke påvist signifikante forskjeller i 5α-androstenonnivå i fett fra råner selektert for høy veksthastighet og tynt ryggspekk, lav veksthastighet og tykt ryggspekk og en kontrollgruppe. I fett fra råner brukt i kunstig sædoverføring, fant en

relativt høye konsentrasjoner av 5α-androstenon og hos disse fant en signifikant ($P < 0,05$) positiv regressjon av 5α-androstenonnivå på alder. Positive, men ikke signifikante regressjonskoeffisienter ble funnet mellom antall parringer rånene var benyttet til og nivå av 5α-androstenon i fett fra de samme dyr.

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