



## Detection of illegal treatment of barrows with nandrolone ester; effects on growth, histology and residue levels in urine and hair

Maria Groot, Johan Lasaroms, Eric van Bennekom, Thijs Meijer, Ester Vinyeta, Jan-Dirk van Der Klis, Michel Nielen

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**Detection of illegal treatment of barrows with nandrolone ester; effects on growth, histology and residue levels in urine and hair**

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| Abstract:                     | <p><b>Abstract</b></p> <p>To evaluate the effects of 17<math>\beta</math>-19-nortestosterone (17<math>\beta</math>NT ) treatment of barrows on residue levels and on growth results, an animal experiment was conducted. Five barrows were treated 3 times during the fattening period with 17<math>\beta</math>NT phenylpropionate (Nandrosol, nandrolone phenylpropionate 50 mg/ml, 1 mg/kg BW). Another 5 barrows were not treated, and 5 boars (not treated) were kept as a positive control. Boars and treated barrows showed 13 % and 9 % better growth than not-treated barrows, leading to mean final body weights of 121.6, 117.8 and 109.0 kg respectively. Moreover the bulbourethral glands of the treated barrows were 3 times heavier than the not-treated barrows. The histology of the prostate and bulbourethral gland of the treated barrows was comparable to the boars, whereas the control barrows showed atrophic glands. Levels of 17<math>\beta</math>NT ester in hair from treated barrows were high, whereas</p> |

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|  | boars and non-treated barrows did not show levels above LLQ. It is concluded that analysis of hair can provide a method for detection of illegal treatment with 17 $\beta$ NT ester in barrows. The size of the bulbourethral gland can be used for screening in the slaughterhouse. |
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**Detection of illegal treatment of barrows with nandrolone ester;  
effects on growth, histology and residue levels in urine and hair**

Groot<sup>1\*</sup>, M.J., J.J.P. Lasaroms<sup>1</sup>, E.O. van Bennekom<sup>1</sup>, T. Meijer<sup>1</sup>, E. Vinyeta<sup>2</sup>, J.D.  
van der Klis<sup>2</sup> and M.W.F. Nielen<sup>1,3</sup>

<sup>1</sup>Maria Groot, RIKILT Institute for Food Safety, Wageningen University and  
Research centre, PO Box 230, 6700 AE Wageningen Netherlands. Email:  
maria.groot@wur.nl

<sup>2</sup>Schothorst Feed Research, Lelystad, Netherlands

<sup>3</sup>Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB  
Wageningen, The Netherlands

**Abstract**

To evaluate the effects of 17 $\beta$ -19-nortestosterone (17 $\beta$ NT) treatment of barrows on  
residue levels and on growth results, an animal experiment was conducted. Five  
barrows were treated 3 times during the fattening period with 17 $\beta$ NT  
phenylpropionate (Nandrosol, nandrolone phenylpropionate 50 mg/ml, 1 mg/kg BW).  
Another 5 barrows were not treated, and 5 boars (not treated) were kept as a positive  
control. Boars and treated barrows showed 13 % and 9 % better growth than not-  
treated barrows, leading to mean final body weights of 121.6, 117.8 and 109.0 kg  
respectively. Moreover the bulbourethral glands of the treated barrows were 3 times  
heavier than the not-treated barrows. The histology of the prostate and bulbourethral  
gland of the treated barrows was comparable to the boars, whereas the control  
barrows showed atrophic glands. Levels of 17 $\beta$ NT ester in hair from treated barrows

1 were high, whereas boars and non-treated barrows did not show levels above LLQ. It  
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3 is concluded that analysis of hair can provide a method for detection of illegal  
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5 treatment with 17 $\beta$ NT ester in barrows. The size of the bulbourethral gland can be  
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8 used for screening in the slaughterhouse.  
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14 *Corresponding author: e-mail: maria.groot@wur.nl; tel. +31(0)3174280277, fax*  
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16 *+31(0) 317 417717*  
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21 *Key words: 17 $\beta$ -19-nortestosterone, barrows, hair analysis, histology, urine analysis*  
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25 **Introduction**  
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27 In male pigs 17 $\beta$ -19-nortestosterone (17 $\beta$ NT) is a naturally occurring compound in  
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29 urine (Poelmans *et al.*, 2005). In boars high levels can be measured but in barrows  
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31 and gilts only low levels occur. In practice however sometimes high levels of 17 $\beta$ NT  
32  
33 are found in urine from barrows. This can be due to cryptorchid animals, having one  
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35 testicle in the abdomen that produces hormones. But illegal treatment with 17 $\beta$ NT for  
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37 growth promotion is also a possibility, since 19-nortestosterone (nandrolone) has a  
38  
39 long history for abuse as growth promoter in livestock (Rico, A.G., 1983).  
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41  
42 The use of hormonal substances for fattening purposes in livestock is banned in the  
43  
44 EU since 1988 according to Council Directive 96/22 EC (Offic. J. Eu. Comm.,1996).  
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46 Steroids having estrogenic, androgenic, progestagenic action are forbidden as well as  
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48 thyreostatics and  $\beta$ -agonists. Residue analysis on target compounds is carried out by  
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50 control laboratories to enforce the ban.  
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1 Some hormones that were considered as exogenic appeared to occur naturally in some  
2 species (Scarth *et al.*, 2009). This applies to  $17\beta$ NT which occurs in urine of stallions  
3 (Houghton *et al.*, 1994) and in urine from boars (Debruyckere *et al.*, 1990).  
4 Boars grow faster and show a better feed conversion with 20 to 30% less fat than  
5 barrows (Field, 1971; Mulvaney *et al.*, 1988; Kudson *et al.*, 1985). Gonadally intact  
6 males also have 8 to 15% greater muscle mass than castrated males (Knudson *et al.*,  
7 1885; Prescott & Lamming, 1967). In boars, testis and accessory genital glands are  
8 normally developed, whereas barrows are castrated at a young age and have atrophied  
9 accessory genital glands. Treatment with androgens leads to increase in growth of the  
10 seminal vesicles and bulbourethral glands in barrows (Lauwers *et al.*, 1983; Lauwers,  
11 1984).

12  
13 In recent years, apart from urine analysis, hair has been analyzed for the presence of  
14 esters of anabolic steroids (Sauer *et al.*, 1998; Duffy *et al.*, 2009; Nielen *et al.*, 2006,  
15 Stolker *et al.*, 2009). For growth promoting purposes steroid hormones are usually  
16 administered with an ester side-chain attached in order to facilitate the steroid  
17 reaching the site of activity within the animal's body. Because steroid hormones do  
18 not occur naturally in an ester form, detection of intact steroid esters demonstrates  
19 illegal administration of these drugs.

20  
21 To investigate if treatment with  $17\beta$ NT is anabolic in barrows can lead to high levels  
22 of  $17\beta$ NT in urine and hair we treated barrows with  $17\beta$ NT and compared them with  
23 untreated barrows and untreated boars.

## 24 25 **Material and methods**

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3 1 *Experimental*  
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5 2 The animal experiment was performed by Schothorst Feed Research BV in Lelystad,  
6  
7 3 Netherlands. The trial comprised three treatments according to sex and Nandrosol  
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9 4 injection: 1) non-injected boars, 2) non-injected barrows and 3) barrows injected with  
10  
11 5 17 $\beta$ NT phenylpropionate (nandrolone phenylpropionate 50 mg/ml, 1 mg/kg BW,  
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13 6 Nandrosol, REG NL 10067, Alfasan, The Netherlands). A total of 15 pigs were  
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15 7 distributed in 5 replicates per treatment group.  
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20 8  
21 9 Experimental animals were grower pigs (Tempo x (Great Yorkshire x Finnish  
22  
23 10 Landrace)) of 60-70 days of age at the start of the experiment and approximately 25  
24  
25 11 kg live body weight. Pigs were housed in three separate pens (one pen/treatment) of  
26  
27 12 1.80 x 1.00 m. One spare pig per treatment was also allocated within the experimental  
28  
29 13 pigs and the control barrows and was fed and weighed in case a pen mate dropped out.  
30  
31 14 The animals were fed ad libitum and had free access to water. The animals were fed  
32  
33 15 commercial growing/finishing diets (Arkevaart-Twente Coöperatie, Nijkerk, The  
34  
35 16 Netherlands) commonly used in Schothorst Feed Research in practical conditions.  
36  
37 17 The animals were injected at 19, 47 and 75 days after the start of the experiment and  
38  
39 18 were slaughtered on day 93 of the experiment. Once a month urine and hair was  
40  
41 19 sampled and every two weeks the animals were weighed. Sampling times for hair and  
42  
43 20 urine were day 12, 33, 61 and 81, respectively time points 1, 2, 3 and 4. All pigs were  
44  
45 21 delivered to a commercial slaughterhouse at a body weight around 100-115 kg.  
46  
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48 22 Treated animals were condemned for destruction. This study was approved by the  
49  
50 23 Ethical Committee of Animal Science Group Lelystad, part of Wageningen  
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52 24 University Research Centre, Netherlands.  
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## 1 *Histology*

2 At slaughter the animals were inspected for gross pathology. Testes (only in boars),  
3 prostate and bulbourethral gland were sampled for histological investigation. After  
4 weighing the bulbourethral glands, samples of the prostate and bulbourethral gland  
5 were immediately fixed in 10 % phosphate buffered formalin, whereas samples of the  
6 testis were fixed in Bouin Hollandais fixative, which gives a better morphology. After  
7 the usual dehydration series with alcohols and xylol, the tissues were embedded in  
8 paraffin. Sections, 5 µm thick, were made using a rotating microtome. Sections were  
9 stained with heamatoxylin-eosin (HE) according to Mayer (Bancroft & Stevens, 1990)  
10 for routine staining and the prostate and bulbourethral gland were also stained for  
11 mucins with the combined Alcian Blue/periodic Acid Schiff (ABPAS) technique  
12 staining both acidic (blue) and neutral mucins (red) (Bancroft & Stevens, 1990).

## 14 *Chemicals*

15 The chemicals and solutions used were analytical reagent grade. Water was purified  
16 using a Milli-Q system from Millipore (Bedford, MA, USA). 17βNT  
17 phenylpropionate ester and 17βNT were obtained from Steraloids (Newport, Rhode  
18 Island, U.S.A.). The isotope-labeled internal standard d<sub>3</sub>-testosterone  
19 phenylpropionate ester and d<sub>3</sub>-17βNT were custom-synthesized at Wageningen  
20 University and Research Centre (Wageningen, The Netherlands). Stock solutions  
21 were prepared in methanol at 1 mg/ml. Bondelut LRC-C18 solid-phase extraction  
22 (SPE) columns (100 mg and 500 mg) were from Varian (Harbor City, CA, USA) and  
23 ) and IST Isolute NH<sub>2</sub> solid-phase extraction (SPE) columns (100 mg) were from  
24 Sopachem (Eke, Belgium). β-Glucuronidase/arylsulfatase (from Helix Pomatia) was  
25 obtained from Merck (Darmstadt, Germany).





1 ng/ml. Blanks and controls were spiked with the internal standard  $d_3$ -17 $\beta$ -19-nortestosterone at 2 ng/ml. The control urine samples were prepared by spiking blank urine with 17 $\beta$ -19-nortestosterone at 0, 0.5, 1.0, 2.0, 3.0 and 5.0 ng/ml. Prior to hydrolysis, the urine samples were adjusted to pH 4.8. Following the addition of 10  $\mu$ L  $\beta$ -glucuronidase/arylsulfatase (ready to use, Merck), enzymatic deconjugation was carried out in a water bath at 37 °C for 16 hr. The hydrolyzed sample was applied to a C18 solid-phase extraction (SPE) cartridge, pre-activated with methanol and sodium acetate buffer. The vacuum dried SPE column was eluted with 4 ml of methanol. The eluate was applied to a NH<sub>2</sub> SPE cartridge and the eluate was evaporated to dryness under a gentle stream of nitrogen. The residue obtained was re-dissolved in 225  $\mu$ L (methanol/water 20/80 v/v) and 200  $\mu$ L was injected into a HPLC system consisting of a vacuum degasser, an autosampler, a binary pump, an UV detector and a fraction collector from Agilent (Santa Clara, California). The fraction where 17 $\beta$ -19-nortestosterone eluted was collected and evaporated to dryness under a gentle stream of nitrogen and re-dissolved in 300  $\mu$ L methanol/formic acid (0.5%) (50/250 v/v). 40  $\mu$ L was injected into LC-MS/MS system 2.

#### 18 *LC-MS/MS systems*

19 System 1: The analysis of the hair samples was carried out using a UFLC liquid chromatography system, consisting of a vacuum degasser, an auto sampler and a binary pump from Shimadzu Scientific Instruments (Colombia, MD, USA) equipped with a reversed phase Waters Acquity UPLC BEH C18 analytical column of 100\*2.1 mm and 1.7  $\mu$ m particle size kept in a column oven at 50 °C. The LC gradient (solvent A, water-acetonitrile-methanol-formic acid (300/350/350/20; v/v/v/v); solvent B, acetonitrile-methanol-formic acid (500/500/20; v/v/v)) with a flow of 0.4

1 ml/min was: 0 min 10% B; 0.1-2 min linear increase to 70% B; 2-8 min linear  
2 increase to 100%B; 8-9.5 min 100% B. Injection volume was 40 µL. The UFLC  
3 system was connected to a AB Sciex (Concord, Ontario, Canada) mass spectrometer  
4 model QTRAP 5500 equipped with an electrospray interface operating in the positive  
5 ionization mode. IonSpray voltage was set at 5500V and the source temperature was  
6 set at 250°C. Curtain gas, gas 1 and gas 2 were 20, 55 and 60 psi respectively. The  
7 desolvation gas and the collision-induced dissociation (CID) gas was nitrogen.  
8 Entrance potential (EP) for all analytes was 10V. For 17β-19-nortestosterone  
9 phenylpropionate ester the most abundant MRM transition (used for the  
10 quantification) was acquired using the following conditions: precursor ion mass (m/z)  
11 407.1, Product ion mass (m/z) 105.2 with a Collision Energy of 33 (eV). A second  
12 MRM transition, used for the confirmation of the identity of 17β-19-nortestosterone  
13 phenylpropionate ester, was acquired using the following conditions: precursor ion  
14 mass (m/z) 407.1, Product ion mass (m/z) 133.1 with a Collision energy of 25 (eV).  
15 For d<sub>3</sub>-17β-testosterone phenylpropionate the most abundant MRM transition was  
16 acquired using the following conditions: precursor ion mass (m/z) 424.2, product ion  
17 mass (m/z) 105.1 with a collision energy of 30 (eV).  
18  
19 The concentrations of 17β-19-nortestosteronephenylpropionate in hair samples were  
20 calculated using the isotope dilution method and a standard addition of 5 ng/g 17β-19-  
21 nortestosterone phenylpropionate to each individual sample.  
22  
23 System 2: The LC-MS/MS system consisted of a Waters (Manchester, UK) Acquity  
24 UPLC model LC system equipped with a Waters model Quattro Premier XE triple-  
25 quadrupole mass spectrometer. The mass spectrometer was operated in the positive

electrospray ionization (ESI) mode at a capillary voltage of 2.7 kV, a desolvation temperature of 450 °C, a source temperature of 120 °C and a cone voltage of 30V. The desolvation gas was nitrogen and the collision-induced dissociation (CID) gas was argon. A Waters Acquity UPLC BEH C18 analytical column of 50\*2.1 mm and 1.7 µm particle size was used and kept in a column oven at 40 °C. The gradient (solvent A, water/formic acid 980/20 v/v); solvent B, water-acetonitrile-formic acid (80/900/20; v/v/v)) with a flow of 0.4 ml/min was: 0 – 1 min 30% B; 1-5 min linear increase to 50% B; 5-5.1 min linear increase to 100%B; 5.1-7 min 100% B. Injection volume was 40 µL. For 17β-19-nortestosterone the most abundant MRM transition (used for the quantification) was acquired using the following conditions: precursor ion mass (m/z) 275.1, Product ion mass (m/z) 109.2 with a collision energy of 25 (eV). A second MRM transition, used for the confirmation of the identity of 17β-19-nortestosterone, was acquired using the following conditions: precursor ion mass (m/z) 275.1, product ion mass (m/z) 145.1 with a collision energy of 20 (eV). For d<sub>3</sub>-17βNT the most abundant MRM transition was acquired using the following conditions: precursor ion mass (m/z) 278.2, product ion mass (m/z) 109.0 with a collision energy of 30 (eV).

The concentrations of 17βNT in urine samples were estimated using the isotope dilution method and a linear calibration curve were constructed for 17βNT by fortifying blank urine aliquots at six different concentration levels (matrix-matched standards, MMS).

## Results and discussion

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1 The controlled treatment experiment was performed without problems, the animals  
2 were in good health and all animals were suitable for slaughter at the end of the  
3 experimental period. Including the spare animals we ended up with 6 treated barrows,  
4 6 control barrows and 5 boars for investigation.

5  
6 An overview of the live weights of the animals is listed in Table 1.  
7 Mean live weight at slaughter was 121.6 kg for boars, 108.9 kg for barrows and 117.8  
8 kg for the 17 $\beta$ NT treated barrows. The treated barrows showed a significant ( $p < 0.05$ )  
9 better growth performance than the control barrows, and were not significantly  
10 different from the boars. At the facility where the animals were housed it appeared  
11 that the last weeks before slaughter there was an increased growth of the treated  
12 barrows as compared to the control animals (fig. 1).

13 **Insert Table 1 and Figure 1**

14  
15 These data confirmed our suggestion that 17 $\beta$ NT has a growth promoting effect in  
16 barrows. In literature the effects of anabolic steroids in pigs are not conclusive: In a  
17 series of experiments with implants in pigs, the combination of trenbolone and 17 $\beta$ -  
18 estradiol or 17 $\beta$ -estradiol and 17 $\beta$ -testosterone in barrows adversely affected growth  
19 rate and feed intake, but the implanted barrows had less fat than the controls (De  
20 Wilde & Lauwers, 1984). Other experiments with castrated males (Van Weerden &  
21 Grandadam, 1976) using implants with trenbolone and 17 $\beta$ -estradiol showed  
22 increased protein deposition and decreased fat deposition in the treated animals.  
23 Moreover the carcass weight was 4.5 kg higher than the controls, and carcass quality  
24 was improved and backfat thickness was reduced. In our experiment carcass

characteristics were not investigated because the aim of our experiment was to examine the effects of  $17\beta$ NT on histology and residue levels in hair and urine. At slaughter there appeared a striking difference in the weight and size of the bulbourethral glands (Table 2), not only between barrows and boars, but also between control and treated barrows. Treated barrows had larger and significant ( $p < 1 \times 10^{-7}$ ) almost 3 times heavier bulbourethral glands than the controls (fig. 2). Increase of the weight of male accessory glands is a well-known effect of androgens in castrated males (Brandes, D, 1994; Booth, W.D., 1890). This increased size could be used as a screening method for illegal treatment of barrows with androgenic hormones in the slaughterhouse.

Insert Table 2 and figure 2

### *Histology*

Histological investigation of the testes of the boars revealed normal spermatogenesis as was expected in these animals. The prostate showed abundant glandular tissue and the bulbourethral glands showed mature glandular tissue (fig. 3) consisting of mucinous acini with secretion of Alcian Blue positive (acidic) secretions. The barrows showed atrophied glandular tissue in the prostate and bulbourethral gland showing immature glandular tissue presenting with rosette shaped alveoli in abundant stroma (fig. 4) and scarce PAS positive secretion. The treated barrows showed an increased amount of glandular tissue in the prostate. The bulbourethral gland presented with mature glandular tissue (fig. 5), which stained blue with Alcian Blue PAS staining. This means that treatment with  $17\beta$ NT induced maturation of the glandular tissue of the prostate and bulbourethral glands in barrows, causing histology resembling that of boars. The results of the histological findings are in accordance with what Lauwers (1984) described as androgen like changes, which he found in barrows treated with

1 allyltrenbolone. In treated barrows he observed development of the accessory glands  
2 in the physiological direction with more glandular tissue in the prostate and increased  
3 secretion in the bulbourethral gland and seminal vesicals. In barrows implanted with  
4 trenbolone acetate (López-Bote *et al.*, 1994), no changes in carcass weight were  
5 observed, but the implanted animals had less fat. They found enlargement of the  
6 prostate and bulbourethral gland with increased secretion and cyst formation. Cyst  
7 formation was not observed in our experiment, maybe because the animals were not  
8 continually exposed to hormones and the difference in androgenic and anabolic action  
9 between trenbolone and 17 $\beta$ NT.

10 **Insert figures 3-5**

11 *Residue analysis in urine*

12 All urines were analyzed for the presence of 17 $\beta$ NT. The urine was sampled at 4 time  
13 points, corresponding with day 12, 33, 61 and 81 of the trial. Levels of 17 $\beta$ NT were  
14 generally low (< 10  $\mu$ g/L) in barrows and treated barrows, except for one control  
15 barrow that had a level of 300  $\mu$ g/L. It was not clear why this animal had such a high  
16 level only at the last time point. No difference was observed between the 17 $\beta$ NT  
17 levels in the urine of the treated and control barrows. This may be due to the sampling  
18 schedule which was once a month and approximately two weeks after the day of  
19 treatment. In practice occasionally barrows and gilts are observed with high levels of  
20 urinary 17 $\beta$ NT, but possibly after treatment levels are only high in urine for a few  
21 days. In future experiments we have planned to investigate the kinetics of 17 $\beta$ NT in  
22 urine from hour to hour and from day to day after treatment. Another possibility is to  
23 examine the possible differences in metabolite profiles in treated and non-treated  
24 animals.

1 In boars high levels up to 400 µg/L of 17βNT were observed in urine, especially in  
2 older animals (table 3). Analysis of urine was in this trial not able to differentiate  
3 between treated and non-treated barrows, but most boars showed much higher levels  
4 than barrows.

5 **Insert table 3**

#### 6 *Residue analysis in hair*

7 In hair we measured the intact ester of 17βNT phenylpropionate. Hair was also  
8 sampled at 4 time points. For this method a calibration series was used from  
9 0 to 25 µg/kg. All values above this series are estimated (table 4).

11 In all non-treated barrows the levels were low <LLQ. All boars showed levels <LLQ.  
12 In non-treated animals no esters were expected in the hair, these results confirmed this  
13 expectation. The treated animals showed from the start of the trial high levels 17βNT  
14 phenyl propionate in the hair, ranging from 15 to 220 µg/kg, which increased in time  
15 reaching a maximum level of 600 µg/kg (fig. 6).

17 We conclude that analysis of 17βNT phenyl propionate in hair is a good method to  
18 detect treated animals.

19 **Insert table 4 and figure 6**

#### 21 **Conclusion**

22 In this experiment we showed that 17βNT phenyl propionate is anabolic in barrows in  
23 the dosage used and that this treatment yields increased size and weight of the  
24 bulbourethral gland and profound histological alterations in the prostate en  
25 bulbourethral gland making them resemble the glands of boars. Inspection of the



weight and histology of bulbourethral glands could be used as a screening method for illegal treatment of barrows with androgenic hormones in the slaughterhouse. Treatment of barrows with 17 $\beta$ NT phenyl propionate can be detected by hair analysis for the intact 17 $\beta$ NT esters. More investigation is needed to evaluate the excretion of 17 $\beta$ NT phenyl propionate in urine after treatment and the incorporation of 17 $\beta$ NT phenyl propionate in hair after treatment in barrows as well as in gilts.

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**Table 1.** Body weights at slaughter

| Body weight (kg) | Boar   | Barrow | Barrow17β19NT |
|------------------|--------|--------|---------------|
|                  | 116.4  | 103.2  | 113.4         |
|                  | 123.8  | 116.7  | 119.5         |
|                  | 119.2  | 114    | 120.1         |
|                  | 114.3  | 108.3  | 119.2         |
|                  | 134.1  | 114.1  | 122.5         |
|                  |        | 97.5   | 112.3         |
| mean             | 121.6* | 109.0  | 117.8**       |
| s.d.             | 7.8    | 7.4    | 4.0           |

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\* Boars significant heavier than barrows (p <0.05)

\*\* Barrow17βNT significant heavier (p <0.05) than control barrows

Weights barrow17βNT not significant different than boars

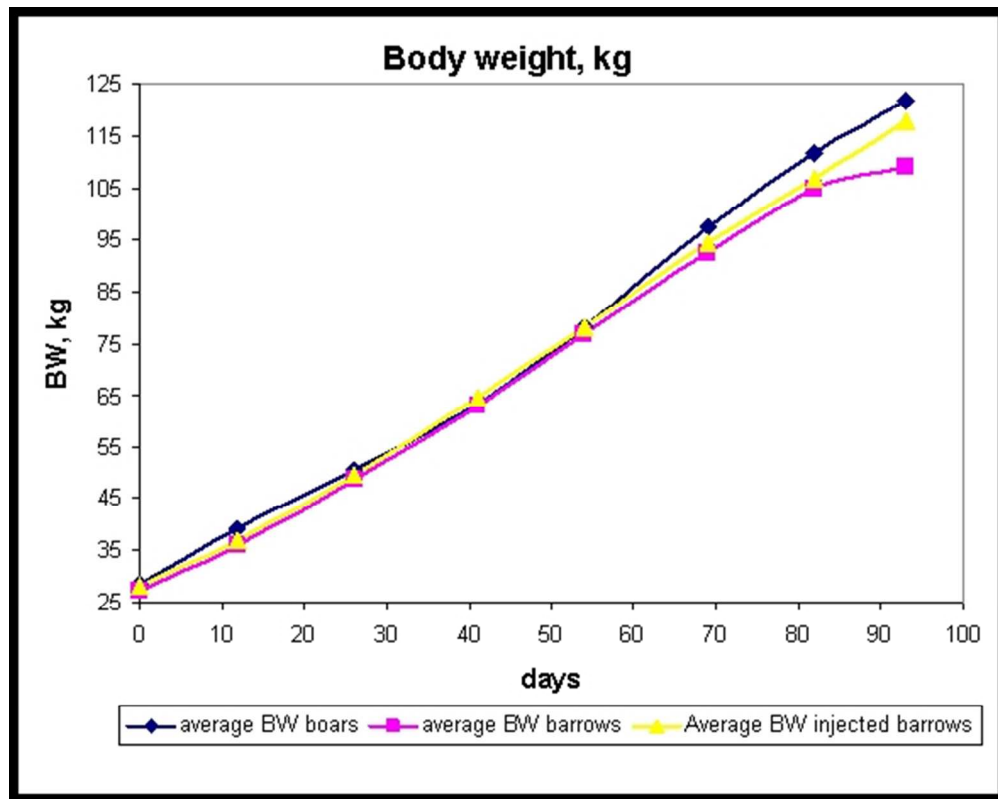


Figure 1. Growth of the animals during the trial  
156x124mm (96 x 96 DPI)

**Table 2.** Weights of the bulbourethral glands

|                | Bulbourethral gland 1<br>(grams) | Bulbourethral gland 2 |
|----------------|----------------------------------|-----------------------|
| Control boar   | 69.9                             | 65.5                  |
| Control boar   | 71.7                             | 69.2                  |
| Control boar   | 84.4                             | 90.7                  |
| Control boar   | 69.0                             | 66.3                  |
| Control boar   | 75.7                             | 70.6                  |
| <b>Mean</b>    | <b>73.3** ± 8.1</b>              |                       |
| Control barrow | *                                | *                     |
| Control barrow | 4.1                              | 3.9                   |
| Control barrow | 2.3                              | 3.3                   |
| Control barrow | 3.6                              | 3.4                   |
| Control barrow | 3.3                              | 2.8                   |
| Control barrow | 3.9                              | 3.2                   |
| <b>mean</b>    | <b>3.4** ± 0.5</b>               |                       |
| barrow17β19NT  | 9.5                              | 10.5                  |
| barrow17β19NT  | 6.4                              | 6.2                   |
| barrow17β19NT  | 9.6                              | 10.3                  |
| barrow17β19NT  | 6.5                              | 7.9                   |
| barrow17β19NT  | 7.0                              | 8.2                   |
| barrow17β19NT  | 11.6                             | 11.6                  |
| <b>mean</b>    | <b>8.8** ± 2.0</b>               |                       |

\* not available

\*\* means of the paired organs

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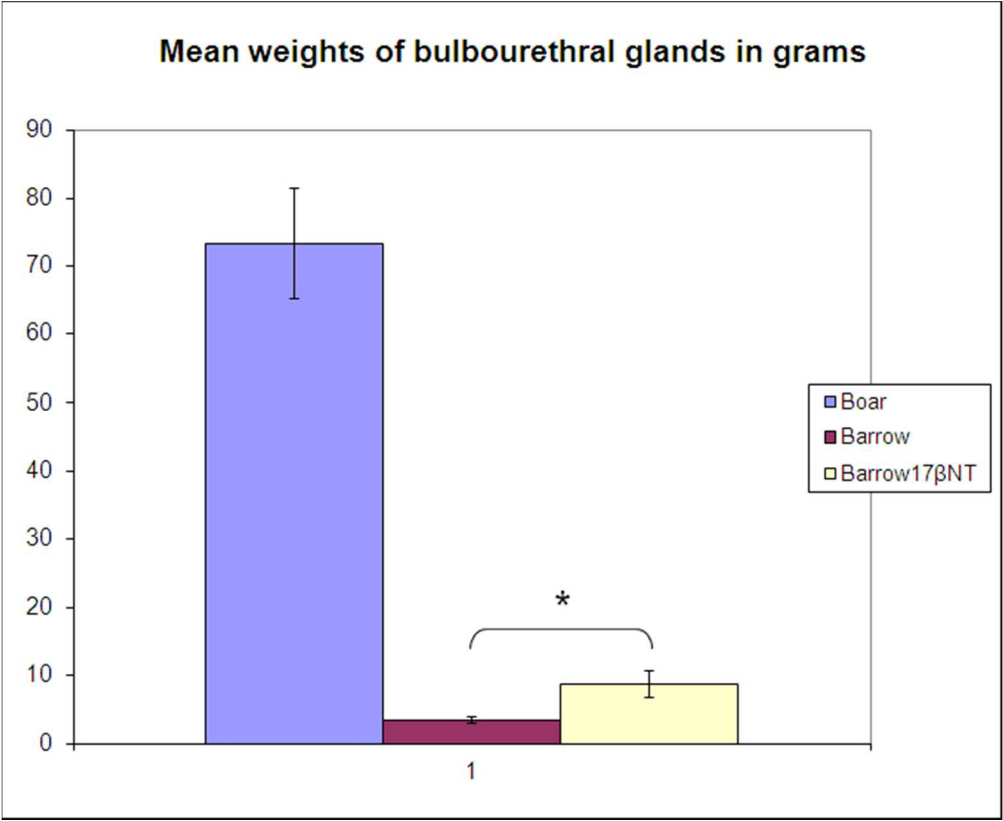


Figure 2: Mean weights of the bulbourethral glands

**Table 3.** Levels of 17 $\beta$ -19-nortestosterone in urine per animal

| Concentration 17 $\beta$ -19-nortestosterone ( $\mu$ g/L) |                   |   |              |              |              |
|---|-------------------|---|--------------|--------------|--------------|
| (LLQ = 0.5 $\mu$ g/L for pig urine)                       |                   |   |              |              |              |
| Animal number   | Sex/<br>treatment | Time point 1<br>(prior to<br>treatment) | Time point 2 | Time point 3 | Time point 4 |
| 1087  | Barrow            | 35                                      | 1            | 5            | 300          |
| 6504  | Barrow            | 2                                       | 2            | 8            | 3            |
| 7630  | Barrow            | n.d.*                                   | 1            | 20           | 10           |
| 7702  | Barrow            | n.d.                                    | 1            | 13           | 7            |
| 9975  | Barrow            | n.d.                                    | 3            | 5            | 10           |
| 9950  | Barrow            | n.d.                                    | 2            | 6            | No sample    |
| 9373  | Barrow+**         | 12                                      | 2            | 4            | 4            |
| 8111  | Barrow+           | n.d.                                    | 1            | 2            | 3            |
| 5133  | Barrow+           | n.d.                                    | 0.5          | 1            | 3            |
| 6185  | Barrow +          | n.d.                                    | 2            | 4            | 5            |
| 6559  | Barrow+           | n.d.                                    | 2            | 1            | 5            |
| 7177  | Barrow+           | 7                                       | 3            | 3            | 2            |
| 1210  | Boar              | 15                                      | 100          | 125          | 175          |
| 4473  | Boar              | 185                                     | 165          | 150          | 2            |
| 7805  | Boar              | -                                       | 130          | 120          | 40           |
| 8775  | Boar              | 55                                      | 140          | 130          | 100          |
| 9631  | Boar              | 50                                      | 80           | 190          | 400          |

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\* n.d. = not detected

\*\* barrow+ = barrow17β19NT

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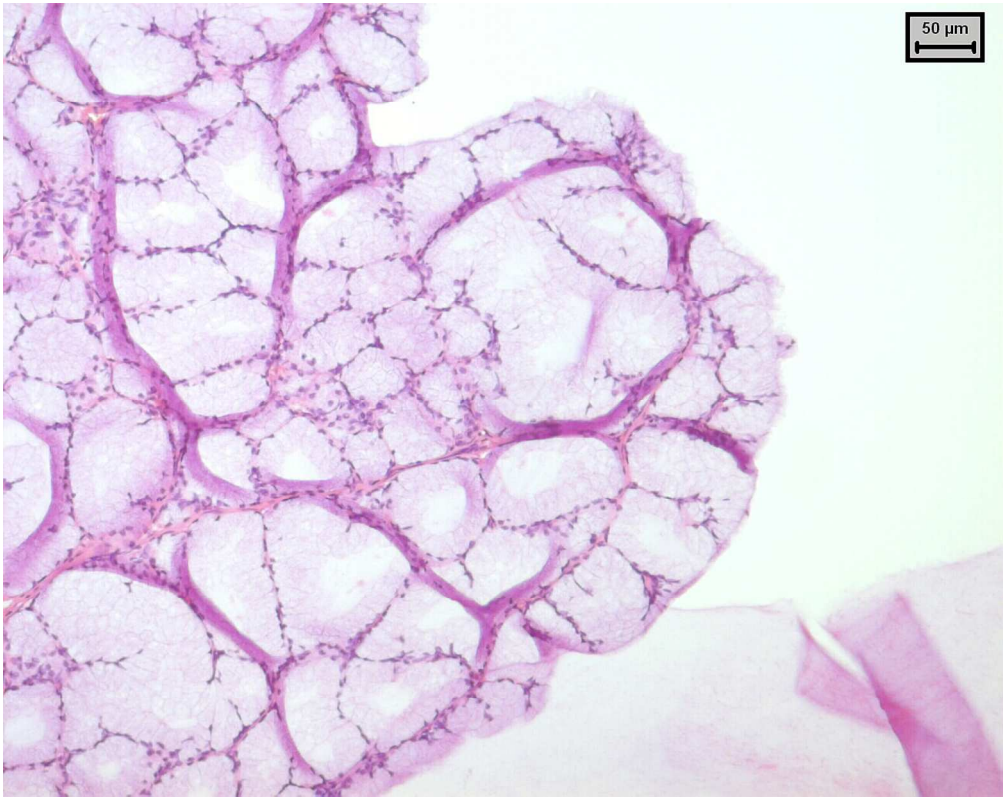
**Table 4.** Levels of 17 $\beta$ -19-nortestosterone phenylpropionate in hair per animal

Concentration 17 $\beta$ -19nortestosterone phenylpropionate  
( $\mu$ g/kg), (LLQ = 2.5  $\mu$ g/kg for pig hair)

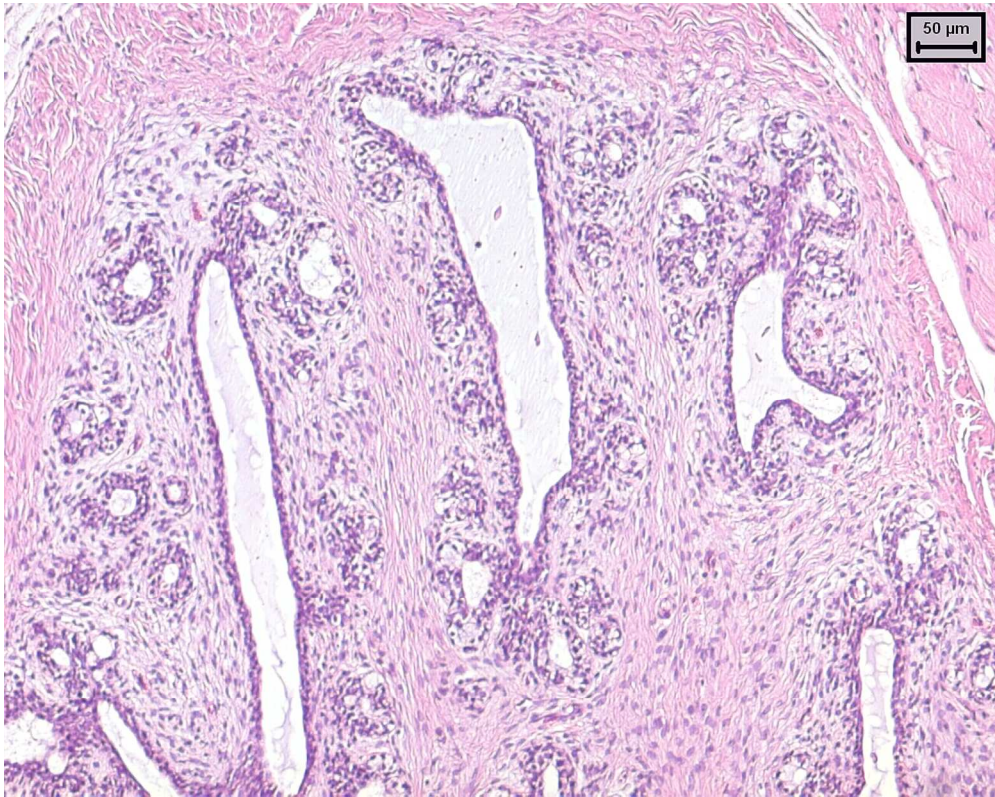
| Animal<br>number | treatment | Time point 1<br>Prior or<br>treatment | Time point 2 | Time point 3 | Time point 4 |
|------------------|-----------|---------------------------------------|--------------|--------------|--------------|
| 1087             | Barrow    | < LLQ                                 | < LLQ        | < LLQ        | < LLQ        |
| 6504             | Barrow    | < LLQ                                 | < LLQ        | < LLQ        | < LLQ        |
| 7630             | Barrow    | < LLQ                                 | < LLQ        | < LLQ        | < LLQ        |
| 7702             | Barrow    | < LLQ                                 | < LLQ        | < LLQ        | < LLQ        |
| 9950             | Barrow    | < LLQ                                 | < LLQ        | < LLQ        | < LLQ        |
| 9975             | Barrow    | < LLQ                                 | < LLQ        | < LLQ        | < LLQ        |
| 8111             | Barrow+*  | < LLQ                                 | 17           | 240          | 330          |
| 9373             | Barrow+   | < LLQ                                 | 220          | 15           | 340          |
| 5133             | Barrow+   | < LLQ                                 | 15           | 60           | 200          |
| 6185             | Barrow+   | < LLQ                                 | 365          | 125          | 600          |
| 6559             | Barrow+   | < LLQ                                 | 13           | 175          | 265          |
| 7177             | Barrow+   | < LLQ                                 | 120          | 20           | 90           |
| 7805             | Boar      | < LLQ                                 | < LLQ        | < LLQ        | < LLQ        |
| 8775             | Boar      | < LLQ                                 | < LLQ        | < LLQ        | < LLQ        |
| 9631             | Boar      | < LLQ                                 | < LLQ        | < LLQ        | < LLQ        |
| 1210             | Boar      | < LLQ                                 | < LLQ        | < LLQ        | < LLQ        |
| 4473             | Boar      | < LLQ                                 | < LLQ        | < LLQ        | < LLQ        |

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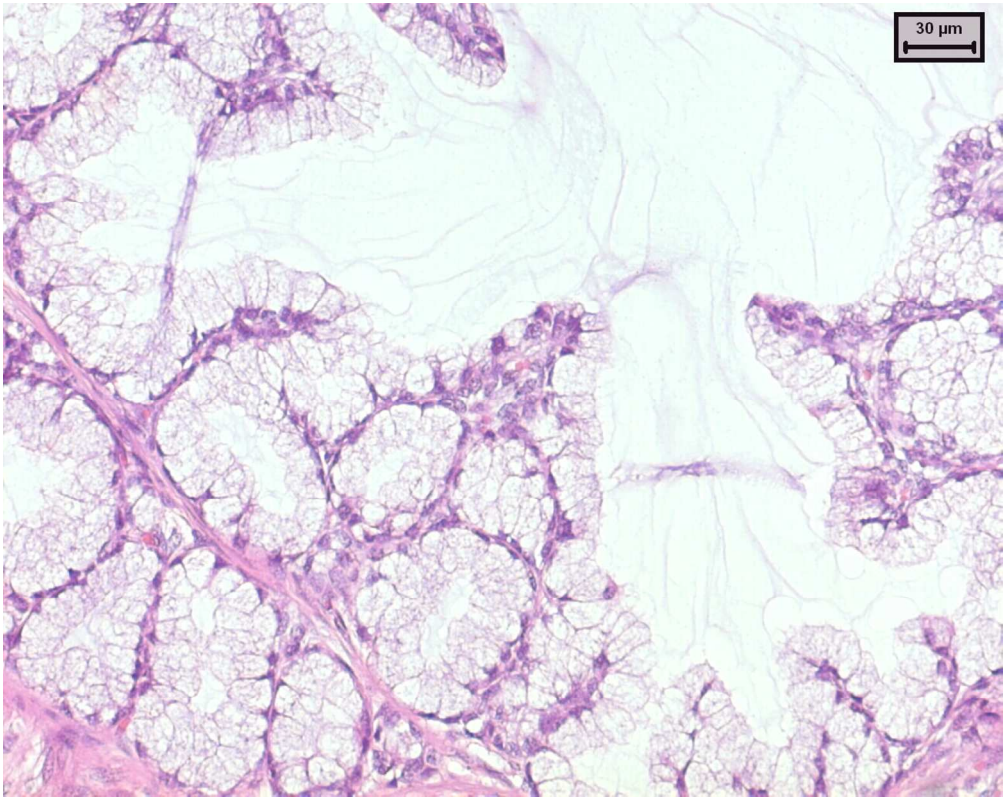


Bulbourethral gland boar (HE), showing mature glandular tissue  
343x272mm (96 x 96 DPI)



Bulbourethral gland barrow, showing immature glandular tissue (HE)  
343x272mm (96 x 96 DPI)





Bulbourethral gland of a treated barrow, showing mature glandular tissue (HE).  
343x272mm (96 x 96 DPI)

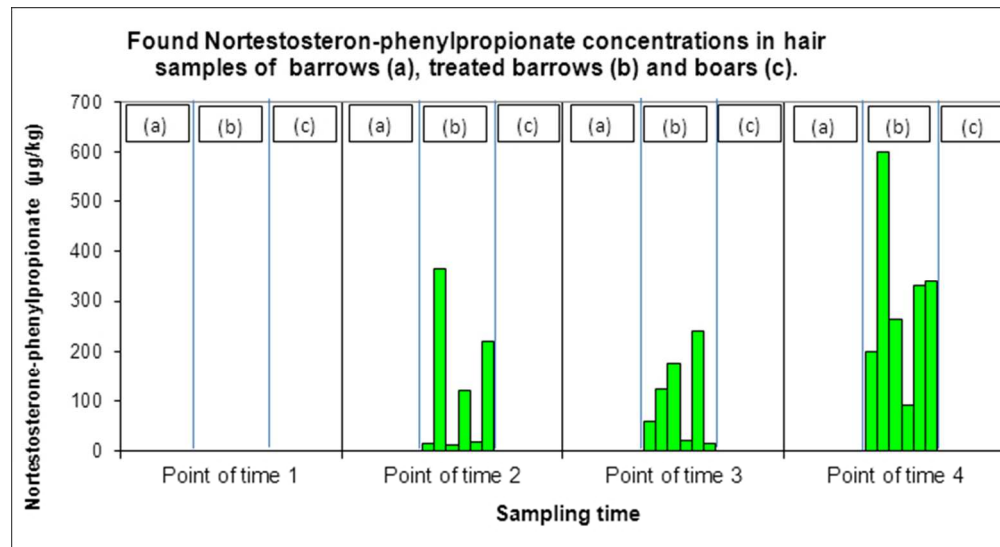


Figure 6.  $17\beta$ NT phenylpropionate in hair  
181x98mm (96 x 96 DPI)