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Endogenous occurrence of some anabolic steroids in swine matrices

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Abstract
Following findings of 17β-19-nortestosterone ($150–200\,\mu g\,kg^{-1}$) in pigs of unspecified gender imported into the European Union, a study to determine steroid and hormone levels in swine from six age/gender categories (uncastrated 'old' boars, cryptorchids, one intersex, barrows, gilts and sows) was initiated. Indeed, for some hormones there has been a discussion about their being endo- or exogenous. Tissue and urine samples from swine from each of the six categories were obtained in Belgium, France, the Netherlands and the USA. Samples were analysed in three laboratories. Quantitation was obtained for norandrostenedione, 19-nortestosterone and boldenone. The results give a well-documented overview of the status of the presence of these hormones in swine. The data illustrate that uncastrated 'old' boars produce the highest percentage of 'positive' matrices, followed by the cryptorchids. Concentrations in the matrices of the barrows and the gilts are lower. Also, sow matrices contain low amounts of nor-steroids. Furthermore, urine samples from an intersex pig contains a higher concentration of nortestosterone than sows and can therefore be suspected for illegal use of these hormones. Veterinarians taking samples in pig farms for the analysis of hormones need to be aware of the presence and concentrations of these substances in the different categories.

Keywords: *Swine, steroids, tissue levels, 17β-nortestosterone, norandrostenedione, 17β-boldenone*

Introduction

Since 1989, the European Union (EU) has banned the importation of meat produced with growth-promoting hormones. This ban applied without discrimination within the EU and to imports from countries outside the EU. As a result, the prohibition also applied to the use of hormonal substances for growth promotion allowed in the USA. The EU's ban on meat produced using growth-promoting hormones is a food safety issue that has been particularly contentious in US–EU agricultural trade relations. US–EU agricultural trade has been affected by differences in measures adopted to protect human, animal and plant health. Such food safety measures are ostensibly adopted to protect the health or safety of domestic consumers or of domestic livestock, fish and crops, but they can also be used as trade barriers.

For some hormones, there has been a discussion about their 'dual' nature. Are they endogenously present or is their presence due to an illegal administration? Nortestosterone (NT) and boldenone (Bol) have been topics of discussion. NT is a steroid that belongs to the group A substances according to Council Directive 96/23/EC (European Commission 1996). The 17β form of NT is also one of the most powerful androgenic anabolic steroids. In the past, 17β-NT was often used for fattening purposes due to the improved weight gain and the
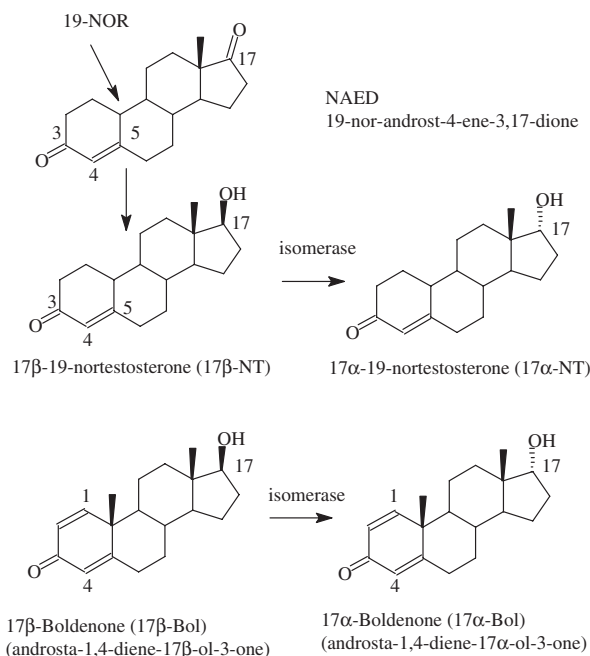
Figure 1. Chemical structures of 17β-nortestosterone (17β-NT), its 17α-isomer (17α-NT) and its precursor 19-norandrost-4-ene-3,17-dione (NAED) and of 17β-boldenone (17β-Bol) and its 17α-isomer (17α-Bol).

feed efficiency in animals. It is also used in sports doping.

Until the early 1980s, whenever residues of 17β-NT or its metabolites were identified in the urine of bovines, race horses, body-builders or athletes, the exogenous administration was considered to be proven. The literature did not show indications of the endogenous character of this molecule in bovine, horse and man. Tuinstra et al. (1986) described 17α-NT and the oestranediols as the most important metabolites of NT in bovine. The chemical structures of 17β-NT and its precursor NAED and its metabolites are shown in Figure 1. These products could only be detected in animals treated with 17β-NT or one of its derivates (e.g. esters of 17β-NT, Laurabolin® or Durabolin®).

However, Houghton et al. (1984) demonstrated that 17β-NT was naturally present in the urine of stallions. A short time afterwards, Belgian and Dutch investigators found that 17β-NT occurs in the urine and in edible parts of boar. These results were first discussed in work groups and were afterwards published by Maghuin-Rogister et al. (1988), Van Ginkel et al. (1989) and Debruyckere et al. (1990). Rizzo et al. (1993) reported the endogenous origin of 17β-NT by the analysis of boar testicles. Hereby, the absence of an isomerase in the pig should be mentioned, i.e. 17β-NT cannot be converted to 17α-NT in pigs. In bovine, on the other hand, this isomerase is present. Since then the presence of

17β-NT in boar matrices is no longer considered as being of exogenous origin. Therefore, pork and meat products based on pork can no longer be controlled for the exogenous administration of 17β-NT until the acceptance of non-ambiguous criteria based on another diagnostic metabolite concentration and isotopic composition.

The problematic nature of NT in bovines has gained much attention. The first observations on the endogenous presence of 17β-NT in calves were based on an analytical artefact and confusion between 17β-testosterone and 17β-NT (De Ridder 1989). Rapp & Meyer (1989) described the possibility of the presence of 17β-NT in non-treated calves by feeding them with milk substitutes contaminated with 17β-NT. Vandenbroeck et al. (1991) first suggested the endogenous presence of 17β-NT (but not 17α-NT) in the urine of pregnant bovines. Meyer et al. (1992) reported the presence of 17α-NT in relatively high concentrations in the urine of a cow in the period of calving and in the newborn calf itself. These findings could be confirmed and supplemented with more data during a large-scale experiment involving recognized European laboratories (De Brabander et al. 1994).

After a short period of silence in 1999, a new NT problem emerged. Two long-distance swimmers were accused of abusing nortestosterone during a competition in Brazil. Both athletes claimed not to have abused drugs and ascribed the positive results of their urine sample to the consumption of 'Sarapatel'. This is a typical Brazilian dish based on pork, which could also contain intact boar tissue. The swimmers' attorneys called in advice from experts, who carried out some experiments. In one test three volunteers consumed meat and organs of a mature boar and their urines were tested for the metabolites of 17β-NT, 19-norandrosterone and 19-noretiocholanolone (Le Bizec et al. 2000). During approximately 1 day, 17β-NT and metabolites could be identified in the urine samples of the three test subjects at a concentration > 2 μg kg^{-1} (the official IOC cut-off level for norandrosterone). The meat and the organs of the consumed boar and of other boars were examined and 17β-NT and its precursor 19-norandrostenedione (NAED) were identified (De Wasch et al. 2001). NAED is a reduced testosterone precursor without the methyl group in the 19th position. When the NAED molecule is processed by the liver; a reduction of the 17-keto group occurs and this leads to 17β-NT. This conversion uses the 17β-hydroxysteroid dehydrogenase enzyme. The same enzyme used to convert androstenedione (AED) to 17β-testosterone.

In Belgium during a routine examination on a pig farm, 17β-NT was identified in faeces samples of an

apparent female animal. Since the presence of 17β-NT in 'non-boars' has not been documented, the samples were suspected of being the result of a possible illegal use of 17β-NT as a growth promoter. Further research, however, showed the presence of an intersexual pig in the farm (Van Cruchten et al. 2002).

In addition, the endogenous nature of Bol needs to be investigated and documented for swine matrices. Bol, also called 1-dehydrotestosterone or androsta-1,4-diene-17β-ol-3-one, differs from testosterone (T) by only one double bond at the 1-position. For a number of years, Bol has been increasingly detected in a number of biological samples (mostly veal calves). Again, the question arose about whether this increased number of Bol findings was due to the illegal treatment of animals or whether, in some circumstances, Bol could be of endogenous origin. The current knowledge of the presence of Bol in different animal species is presented by a European Working Group in a recent review (De Brabander et al. 2004). Recent research was also performed to the endogenous nature of 17β-Bol in horses (Ho et al. 2004).

The present investigation gives the results of an international study to determine endogenous hormone levels in swine from six age/gender categories. Matrices from the different categories of swine from four different countries were distributed amongst three European laboratories: the Laboratory of Chemical Analysis (LCA) of Ghent University, Belgium; LABERCA in Nantes, France; and the National Institute for Public Health and Food Safety (RIVM) in Bilthoven, the Netherlands. The results give a well-documented overview of the status of the presence of 17β-NT, NAED and 17β-Bol in swine. This information is of utmost importance for the European market where there is a total ban on hormones, but also for the USA and its trade relations with the EU. It can be used as a guideline for inspection services to interpret results of analyses of swine matrices for 17β-NT, NAED and 17β-Bol.

Definitions

The six age/gender categories are defined below:

- An 'old' boar is an uncastrated male swine. Uncastrated boars develop a boar taint that can hamper the taste of the produced meat.
- A cryptorchid is a male pig with both testicles that have not descended into the scrotum upon maturity. This abnormality causes infertility. An older cryptorchid pig may still develop boar taint like a normal boar.
- An intersex is defined as a pig with both male and female sexual characteristics and organs, at

brith an unambiguous assignment of male or female cannot be made. The pig shows frequently intersex, from 0.1 to 2% in some pig populations (Hunter et al. 1982). Most intersexual pigs are infertile, show often aggressive behaviour, are more hairy, have a more rigid skin and can spread an odour of boars beginning at their puberty (Hunter and Greve 1996). The behaviour of these intersexes often vary strongly. These qualities can lead to great economic loss in the pig industry. Furthermore, these animals can show increased levels of steroidal hormones that can be mistaken for exogenous administered hormones, resulting in severe sanctions for the farmer.
- A barrow is a castrated male swine. Virtually all male pigs destined for meat production are castrated, usually within the first 4 or 5 weeks of life, to eliminate the boar taint. These barrows produce fattier meat than boars and require more feed to make weight.
- A gilt is a young female pig after puberty before farrowing.
- A sow is an adult female swine. The animal is named in allusion to its fecundity.

Samples

Samples of swine from six age/gender categories ('old' boars, cryptorchids, one intersex, barrows, gilts and sows) were taken for the determination of the levels of endogenous hormone concentrations. Matrices (meat, liver, kidney and urine) from all the different categories of swine and testicles of the old boars, cryptorchids and the intersex were sampled from four different countries (Belgium, France, the Netherlands and the USA) and distributed amongst three European laboratories (Laboratory of Chemical Analysis of Ghent University, LABERCA, Nantes, and the National Institute for Public Health and Food Safety, Bilthoven) for analysis.

Materials and methods

Extraction–purification steps

- LCA developed different methods depending on the matrix. A total of 5 g of meat and testicle matrices were digested with subtilisine, extracted with n-hexane and purified onto an aminopropyl solid-phase extraction (SPE) cartridge. A total of 25 g of liver and kidney were directly extracted by solvent and purified onto aminopropyl SPE cartridge. Urine (25 ml) was hydrolysed with glucuronidase-sulfatase before

performing a liquid-liquid extraction (LLE). Purification was performed onto an aminopropyl SPE column and finally fractionated onto a HPLC system.

- LABERCA clean-up procedure was performed on a sample size of 10 g for tissue, liver and kidney, 10 µg for urine and 2 g for testicle. Tissue, liver, kidney and testicle samples were lyophilized and ground into a powder. After a LLE, all samples were hydrolysed using β-glucuronidase. SPE (Envi-chromP and silica columns) was used for purification.
- National Institute for Public Health and the Environment based its strategy onto two different extraction procedures. Urine (5 ml) was hydrolysed with a *Helix pomatia* preparation, then purified onto a C_{18} and NH_2 SPE cartridge. A total of 5 g of meat, liver, kidney and testicle was digested with subtilisine and hydrolysed enzymatically with *H. pomatia*. Final purification was achieved with *t*-butylmethylether (TBME) LLE and on semipreparative HPLC.

Derivatization before GC-MS and GC-MS/MS

For LCA and LABERCA, derivatization was achieved with 20 µl N-trimethyl-silyltrifluoroacetamide (MSTFA)/trimethyliodosilane (TMIS)/ dithioerythriol (DTE) (1000:5:5, v/v/w). For the National Institute for Public Health and the Environment, heptafluorobutyric anhydride (HFBA) was used to acylate the steroids.

Apparatus

LCA instrument. For urine analysis, an ion-trap mass spectrometer (Polaris, ThermoFinnigan, Austin, TX, USA) coupled to a trace GC 2000 (ThermoFinnigan) authorizing GC–MS^n was used. An apolar 5% phenyl-siloxane SGE BPX-5 (SGE Incorporated, 25 m × 0.22 mm i.d., film thickness 0.25 µm) was used. Helium was set as carrier gas at 1.1 ml min^{-1}; the splitless injector port was set to 250°C. The temperature gradient started from 100°C, increased to 250°C at 17°C min^{-1}, then increased to 300°C at 2°C min^{-1}. The transfer line was maintained at 275°C and the ion source at 200°C. Electron ionization was used.

For tissue, liver, kidney and testicle analysis, chromatographic separation was achieved using a Symmetry C_{18} column (Waters, 5 µm, 150 mm × 2.1 mm). A 1100 series quaternary gradient pump (Agilent Technologies, Palo Alto, CA, USA) was used. The mobile phase (methanol: A/0.02 M formic acid in water: B; 55:45) was maintained for 20 min

and increased to A/B; 100 : 0 (v/v) in 4 min. The flow rate was 0.3 ml min^{-1}. The MS detector was a Finnigan LCQ deca ion-trap (ThermoFinnigan, San Jose, CA, USA) equipped with an APCI interface in the positive-ion mode MS/MS full scan.

LABERCA instrument. An HP-6890 (Agilent Technologies) gas chromatograph equipped with an optima MN-δ3 capillary column (Macherey Nagel, 30 m × 0.25 mm i.d., film thickness 0.25 µm). Helium was used as carrier gas at 1.1 ml min^{-1}; the injector was set to 250°C; purge splitless was set at 1.5 min. Temperature programming started from 120°C (2 min), increased to 250°C at 15°C min^{-1} (0 min) and to 300°C at 5°C min^{-1} (10 min).

The quadrupole mass spectrometer was a HP-5973 (Agilent) used in the electron ionization (EI) mode and selected ion monitoring (SIM) acquisition mode. The transfer line was set at 305°C and ion source temperature at 250°C.

A JEOL (Tokyo, Japan) SX-102A double-focusing mass spectrometer coupled to a Hewlett-Packard Model 5890 gas chromatograph was used (Hewlett-Packard, Palo Alto, CA, USA). The mass spectrometer was operated at middle-high resolution in SIM acquisition mode after EI ionization.

The instrument used for MS/MS analysis was a triple quadrupole from Waters-Micromass (VG-Quattro II, Manchester, UK), the source temperature was set at 250°C and argon was used as the collision gas; selected reaction monitoring (SRM) acquisition was used.

National Institute for Public Health and the Environment instrument. Gas chromatography coupled to a quadrupole mass spectrometer was used in all cases. An HP-6890 gas chromatograph was equipped with a CPSIL-24CB capillary column (Varian, 30 m × 0.25 mm i.d., film thickness 15 µm). Helium was used as carrier gas at 1.1 ml min^{-1}; the splitless injector was set to 250°C. The temperature programme started from 80°C (1 min), increased to 255°C at 15°C min^{-1} and to 340°C at 35°C min^{-1}. The transfer line was set at 280°C. The quadrupole mass spectrometer was a HP-5973N (Agilent) operated in the EI and SIM acquisition modes. Ion source temperature was set at 250°C.

Results and discussion

Because of their impact on routine control, the results of the endogenous presence of 17β-NT, NAED and 17β-Bol in the different swine matrices are discussed here. Other analytes are still under study.

Table I. 17β-NT concentration range (number of analysed samples) in meat, liver, kidney, urine and testicles of 'old' boar, cryptorchid, intersex, barrow, gilt and sow.

17β-NT (μg kg^{-1})	'Old' boar	Cryptorchid	Intersex	Barrow	Gilt	Sow
Meat	0.7–13.4 (11)	0.1–2.4 (11)	<(1)	0.7–11.8 (11)	<(11)	0.4–0.5 (11)
Liver	1–63 (11)	0.2–12.3 (14)	<(1)	<(11)	0.1–0.9 (11)	<(11)
Kidney	2.5–232 (11)	1.3–78 (14)	1.6 (1)	0.1 (10)	0.2–0.5 (11)	0.2–1.5 (11)
Urine (μg l^{-1})	51–344 (11)	8.6–343 (14)	27 (1)	0.5–16.3 (11)	1.3–2.8 (11)	1.3–1.9 (9)
Testicle	24–144 (5)	2.2–101 (11)	5.3 (1)	–	–	–

–, no material available. <, < CC α: decision limit (Decision 2002/657/EC) (European Commission 2002).

Table II. Percentage of samples (number of analysed samples) where 17β-NT was detected in meat, liver, kidney, urine and testicles of 'old' boar, cryptorchid, intersex, barrow, gilt and sow.

17β-NT (%)	'Old' boar	Cryptorchid	Barrow	Gilt	Sow
Meat	100 (11)	91 (11)	18 (11)	0 (11)	18 (11)
Liver	100 (11)	100 (14)	0 (11)	18 (11)	0 (11)
Kidney	100 (11)	93 (14)	10 (10)	18 (11)	27 (11)
Urine	100 (11)	86 (14)	18 (11)	18 (11)	33 (9)
Testicle	100 (5)	100 (11)	–	–	–

Table III. NAED concentration range (number of analysed samples) in meat, liver, kidney, urine and testicles of old boar, cryptorchid, intersex, barrow, gilt and sow.

NAED (μg kg^{-1})	'Old' boar	Cryptorchid	Intersex	Barrow	Gilt	Sow
Meat	0.1–5.5 (11)	0.04–0.4 (11)	<(1)	0.05–0.8 (11)	<(11)	0.04–0.07 (11)
Liver	0.1–24 (11)	0.4–3.5 (14)	1 (1)	1.9–16 (11)	0.3–13 (11)	3.1–8.3 (11)
Kidney	2.3–535 (11)	0.2–159 (14)	1.1 (1)	0.1–15 (10)	8.3–25 (11)	2.7–18 (11)
Urine (μg l^{-1})	5–109 (11)	9.9–103 (14)	0.5 (1)	1.1–16 (11)	1.8–17 (11)	0.9–18 (8)
Testicle	6.2–110 (5)	1.3–25 (11)	0.9 (1)	–	–	–

–, no material available. <, < CC α: decision limit (Decision 2002/657/EC) (European Commission, 2002).

Table IV. Percentage of samples (number of analysed samples) where NAED was detected in meat, liver, kidney, urine and testicles of 'old' boar, cryptorchid, barrow, gilt and sow.

NAED (%)	'Old' boar	Cryptorchid	Barrow	Gilt	Sow
Meat	91 (11)	73 (11)	36 (11)	0 (11)	18 (11)
Liver	91 (11)	93 (14)	36 (11)	55 (11)	45 (11)
Kidney	100 (11)	93 (14)	40 (10)	36 (11)	36 (11)
Urine	91 (11)	86 (14)	18 (11)	64 (11)	75 (8)
Testicle	100 (5)	82 (11)	–	–	–

The substances were analysed in different laboratories, with different extraction and detection methods (GC-MS^n and LC-MS^n) for all the matrices. A more detailed description and comparison of the methods will be described elsewhere (Deceuninck et al. 2005). An overview of the results for 17β-NT, NAED and 17β-Bol is given in Tables I–VI. The results shown are obtained from the three laboratories.

In all matrices of the 'old' boars (meat, liver, kidney, urine and testicles), 17β-NT was detected in variable concentrations (Tables I and II). All testicles of the 'old' boar and the intersex, and nearly all testicles of the cryptorchids contained the precursor of 17β-NT, NAED (Tables III and IV). Nearly all meat (91%), liver (91%), kidney (100%) and urine (91%) samples were positive for NAED. The highest concentrations of both analytes were detected in 'old' boar matrices, but the presence of 17β-NT seemed of the same order of magnitude of concentration in the urine of the cryptorchids. Urine of the 'old' boars contained the highest concentration compared with the other swine matrices of 17β-NT (344 μg l^{-1}), followed by kidney (232 μg kg^{-1}). The highest concentration of NAED was found in kidney (535 μg kg^{-1}), followed by testicles (110 μg kg^{-1}) and urine (109 μg kg^{-1}) of the 'old' boars. The variation of the 17β-NT concentration in 'old' boar samples, analysed by all three laboratories, was comparable in urine and kidney (Figure 2). A high

Table V. 17β-Bol concentration range (number of analysed samples) in meat, liver, kidney, urine and testicles of 'old' boar, cryptorchid, intersex, barrow, gilt and sow.

17β-Bol (μg kg^{-1})	'Old' boar	Cryptorchid	Intersex	Barrow	Gilt	Sow
Meat	0.5–2.5 (11)	0.7 (11)	< (1)	< (11)	< (11)	< (11)
Liver	1.3–4.9 (11)	0.5–2.3 (11)	< (1)	< (11)	< (11)	< (11)
Kidney	0.8–9.2 (11)	0.3–8.1 (14)	< (1)	< (10)	< (11)	< (11)
Urine (μg l^{-1})	5.1–120.5 (11)	0.9–57.6 (14)	< (1)	1.1 (11)	0.5–0.6 (11)	< (11)
Testicle	2.1–16 (5)	0.6–15.1 (11)	< (1)	–	–	–

–, No material available. <, < CCα: decision limit (Decision 2002/657/EC) (European Commission 2002).

Table VI. Percentage of samples (number of analysed samples) where 17β-Bol was detected in meat, liver, kidney, urine and testicles of 'old' boar, cryptorchid, barrow, gilt and sow.

17β-Bol (%)	'Old' boar	Cryptorchid	Barrow	Gilt	Sow
Meat	36 (11)	9 (11)	0 (11)	0 (11)	0 (11)
Liver	45 (11)	36 (11)	0 (11)	0 (11)	0 (11)
Kidney	82 (11)	57 (14)	0 (10)	0 (11)	0 (11)
Urine	91 (11)	50 (14)	9 (11)	27 (11)	0 (11)
Testicle	100 (5)	73 (11)	–	–	–
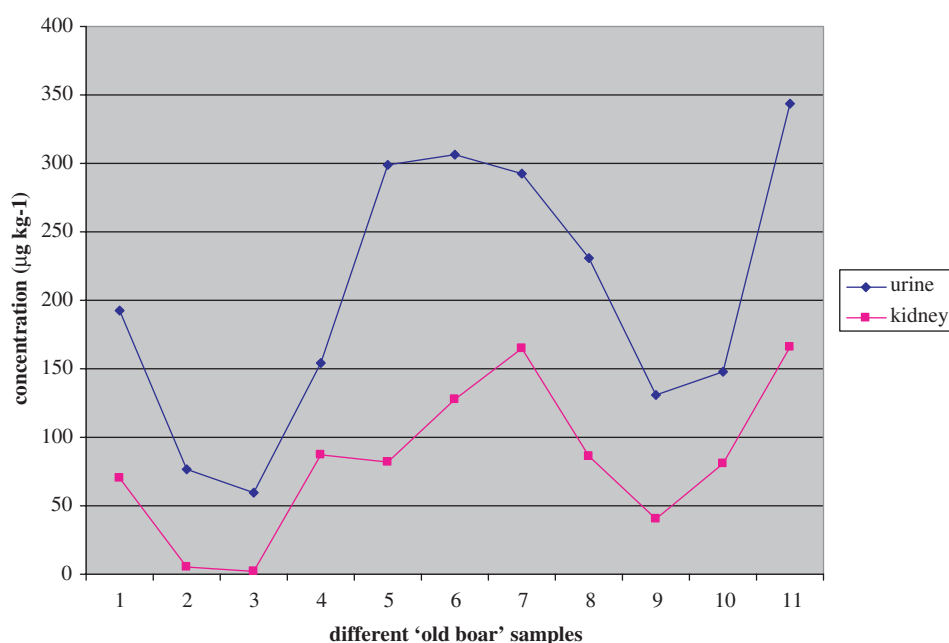
Figure 2. Comparison of the 17β-NT concentration in urine (μg l^{-1}) and kidney (μg kg^{-1}) of the 'old' boars in samples analysed by the three laboratories.

kidney concentrations corresponded to high urine concentrations. The urine concentrations were two to four times the kidney concentration except for 'old' boar sample numbers 2 and 3, where the kidney concentrations were 5 and 3 μg kg^{-1} respectively.

Similar conclusions can be drawn from the cryptorchid data. Only the percentage of positive samples were slightly lower compared with the samples from 'old' boars. In cryptorchids, the maximum measured kidney 17β-NT concentration (78 μg kg^{-1}) was also lower than the urine concentration (343 μg kg^{-1}). For NAED, the kidney concentration was the highest (159 μg kg^{-1}).

The 17β-NT concentrations of the different matrices of the barrow were lower. In the liver no 17β-NT was identified. Some of the samples of meat, kidney and urine showed concentrations in the range 0.7–11.8 and 0.1–0.5 μg kg^{-1}, respectively. The NAED concentrations were slightly higher, and also the percentage of samples in which NAED was found to be present was higher.

The gilt and sow produced the lowest concentration of 17β-NT, with urine still being the matrix with the highest concentrations. 17β-NT and NAED were not detected at or below the decision limit CCα in the meat of the gilt. The NAED

concentrations in liver, kidney and urine of the gilt and sow were higher than the 17β-NT concentrations for the respective matrices. The urine of the intersex showed a relatively high concentration of 17β-NT ($27 \, \mu g \, kg^{-1}$). 17β-NT was also identified in the testicle and kidney. Low concentrations of NAED were observed in different matrices of the intersex pig. Only the meat seemed to contain no NAED. However, only limited intersex samples were available.

Besides 17β-NT and its precursor NAED, 17β-Bol was also investigated in different swine matrices. The study of the possible precursors of 17β-Bol (androstadienedione or ADD) is ongoing. 17β-Bol was detected in all matrices of the 'old' boar and cryptorchid. The highest concentrations were found in the urine, up to $120.5 \, \mu g \, kg^{-1}$ in the 'old' boar and up to $57.6 \, \mu g \, kg^{-1}$ in the cryptorchid. The measured concentrations in different matrices diminished in both the 'old' boar and the cryptorchid from urine to testicles, kidney and liver. 17β-Bol was not detected in the sow, and only one urine sample of the barrow and two of the gilt showed the presence of a low concentration. The endogenous presence of 17β-Bol is more sex dependent than 17β-NT because only the 'old' boar and the cryptorchid contain this analyte in relatively high concentrations in all of their matrices.

It can be summarized that the 'old' boar contains all the investigated substances in all its matrices, followed by the cryptorchid. The matrices with the highest endogenous concentration of the analytes are the urine, kidney and testicles. This study also makes clear that endogenous concentrations of the investigated analytes in matrices of different kinds of swines can vary widely. This is especially the case for the matrices of the cryptorchid and the 'old' boar, where the variations of the endogenous concentrations can be very high. These observations make it very difficult or nearly impossible to discriminate between abuse of 17β-NT or 17β-Bol and its natural presence based on the concentration.

Moreover, the conversion from an administered analyte to its metabolites complicates this situation. When any of the investigated analytes is administered, it is possible that the analyte itself is not present in higher concentrations than the endogenous presence because of a fast metabolization of the analyte. Therefore, more investigation is needed before criteria for concentrations of certain analytes can be established to prove the abuse of an analyte.

Conclusions

In this investigation, samples of different matrices from pigs from four different countries (Belgium, France, the Netherlands and the USA) were analysed in three different European laboratories in order to obtain an overview of the endogenous presence of 17β-nortestosterone, of its precursor norandrostenedione and of 17β-boldenone in different swine matrices. The data illustrate that the uncastrated 'old' boars contain all three substances in all matrices. In cryptorchids lower, but analogous, results were obtained. In accordance with the absence of an isomerase in pigs, the 17α-forms were not detected. Concentrations of 17β-NT and NAED in the matrices of the barrows and the gilts were lower and also sow matrices may contain low amount of these substances. Inspection services should take into account these facts using analytical data from laboratories with very low limits of decision for these substances. Furthermore, urine samples from intersex pigs contain a higher concentration of 17β-NT than the 17β-NT concentration range of sows and can therefore be suspected of an illegal use of nandrolone: every non-compliant sample for NT should be strictly correlated to the gender (also intersex) and age of the animal in question.

17β-Boldenone is also endogenous in male entire pigs, but it seems to be more sex dependent than 17β-nortestosterone. Surprisingly, 17β-Bol was also detected in the urine of some barrows and gilts at very low concentrations. However, care should be taken with the interpretation of these results since the mechanism of formation of 17β-Bol in urine contaminated with faeces during sampling is still unknown.

The data give a well-documented overview on the status of the presence of 17β-NT, NAED and 17β-Bol in swine. The results indicate that the inspection services should be very careful with the interpretation of results for 17β-NT NAED in swine matrices. In most cases it is impossible to discriminate between treated and untreated animals only based on the presence and concentrations of the substances.

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