Use of a multi-residue method in the regulatory control on the use of anabolics

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Anabolic drugs are widely used in cattle breeding, despite prohibition. The low residue levels in the tissues of the treated animals demand methods allowing routine detection of 0.5 - 5 ppb of anabolic residues in various tissues. Thus, the efficiency of the regulatory control would be increased in sampling that tissue containing the highest residue level. Samples of urine, liver, kidney, different fat and muscle tissues, taken from cows or heifers treated with methyltestosterone and diaethylstilboestrol, have been quantitatively analysed. Among the different muscles studied, highest residue concentrations were consistently found in M. Diaphragma. However, compared to the corresponding M. Diaphragma, kidney fat contained 4 to 10 times higher residue levels of diaethylstilboestrol and methyltestosterone.
In another experiment, urine, kidney fat and M. Diaphragma were sampled in slaughterhouses from different heifers, cows or bulls. Results obtained on anabolic positive animals indicate that kidney fat may be classified as a marker tissue for the regulatory control on the abuse of anabolics.

Eine Rückstandsmethode zur Kontrolle der Verwendung von Anabolika

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Die Verwendung von Anabolika in der Rinderzucht ist trotz der bestehenden Verbote weit verbreitet. Der geringe Restgehalt an Anabolika in den Geweben der behandelten Tiere erfordert Methoden, mit denen routinemäßig Konzentrationen von 0,5 bis 5 ppb in den verschiedenen Geweben nachgewiesen werden können. Die Wirksamkeit der Kontrollen würde dabei erhöht, wenn das Gewebe mit der höchsten Rückstandskonzentration entnommen würde.
Bei Kühen und Färsen, die mit Methyltestosteron und Diäthylstilböstrol behandelt wurden, wurden Proben von Urin, Leber, Niere und verschiedenen Fett- und Muskelgeweben entnommen und quantitativ analysiert. Bei den verschiedenen untersuchten Muskeln wurden die höchsten Rückstandskonzentrationen immer in den Diaphragmen gefunden. Verglichen mit den Diaphragmen enthielt Nierenfett jedoch einen 4- bis 10-mal so hohen Gehalt an Rückständen von Diäthylstilböstrol und Methyltestosteron.
Bei einer anderen Untersuchung wurde in Schlachthäusern Urin, Nierenfett und Muskeldiaphragma bei verschiedenen Kühen, Färsen und Stieren entnommen. Die bei mit Anabolika behandelten Tieren erhaltenen Ergebnisse weisen darauf hin, daß Nierenfett als Indikatorgewebe für die Kontrolle bezüglich der mißbräuchlichen Verwendung von Anabolika klassifiziert werden kann.

5.8

Utilisation d'une méthode de dépistage dans le contrôle de l'utilisation des anabolisants

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L'utilisation des anabolisants s'amplifie dans l'élevage bovin en dépit des interdits. La faiblesse des teneurs résiduelles des anabolisants dans les viandes animales traitées demande des méthodes capables de les déceler dès 0.5 - 5 ppb. En utilisant la méthodologie existante, l'efficience du contrôle serait augmentée en échantillonnant le tissu de carcasse possédant les plus hautes teneurs en anabolisant.

Des échantillons de différents muscles, tissus adipeux et organes furent prélevés sur des carcasses de vaches et de génisses traitées au méthyltestostérone et au diéthylstilbestrol. Après analyse quantitative, au niveau musculaire les teneurs les plus élevées étaient observées dans les diaphragmes. Néanmoins, les taux de ces composés étaient 4 à 10 fois plus forts dans les graisses périrénales.

Ultérieurement, de la graisse périrénale, le diaphragme et de l'urine furent prélevés à l'abattoir de plusieures vaches, génisses et taureaux. Les résultats obtenus sur différents animaux traités aux anabolisants démontrent la sensibilité du tissu adipeux périrénal dans son utilisation comme indicateur de l'emploi abusif d'hormones.

Применение метода выявления при контроле использования анаболических веществ

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Вопреки запрещениям, применение анаболических средств в скотоводстве развивается. Ничтожность остаточных содержаний анаболических веществ в тканях таких животных требует методов, позволяющих их обнаружение уже на уровне 0,5-5 ч.н.б. При применении существующих методов, эффективность контроля может быть повышена путём взятия проб тех тканей туши, в которых остаточное содержание анаболических веществ самое высокое.

Пробы различных мускулов, жировых тканей и органов брали из туш коров и тёлок, которым подскармливали метилтестостерон и диэтилстильбестрол. При количественном анализе мускулов, наивысшее содержание обнаружили в диафрагме. Однако, по сравнению с диафрагмой, почечный жир содержал 4-10 раз больше остатков этих веществ.

Взяли затем на скотобойне образцы почечного жира, диафрагмы и мочи ряда коров, тёлок и быков. Результаты, полученные для различных животных, которым подскармливали анаболические вещества, показали, что почечный жир является сигнальной тканью что касается чрезмерного использования гормонов.

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Introduction

In the last years the use of anabolics in cattle breeding has been amplified despite regulations existing in different EEC-countries. In the illicit practice often relatively high doses of various synthetic anabolics are applied at different loci of the bovine carcass without respecting adequate time intervals before slaughter. These practices result in appreciable residue levels of the hormones in muscular tissue and entail the risk that highly contaminated application sites reach the consumer. Therefore, efforts are directed towards a control discouraging the use of synthetic anabolics in cattle breeding.

In our country, samples are taken from bovine carcasses in the slaughterhouses and examined on the presence of residues of anabolics using a qualitative multi-residue method (5,6,7). If hormonal treatment would give rise to elevated and persistent residue levels in some particular tissues of the carcass, the efficiency of the control would be increased considerably through selection of these tissues. This paper describes the distribution of some anabolic residues among different tissues of the carcass after hormonal treatment. Fat around kidney is found to be an efficient marker tissue in detecting application of anabolics to fattening animals.

Materials and methods

Slaughterhouse animals were used in this work. The distribution of DES and methyltestosterone in different tissues was studied in two heifers with injection lesions in the fat of tail head. Three days after slaughter samples were taken from kidney, liver, fat around kidney, pelvic fat, fat of shoulder and flank area, M. adductor, M. diaphragma, M. gracilis, M. gastrocnemius, M. longissimus dorsi, M. sternomandibularis, M. soleus and M. trapezius. In a second experiment urine, M. longissimus dorsi and fat around kidney was sampled from heifers, cows or bulls at slaughter. Samples from DES or methyltestosterone positive animals were selected for quantitative analysis of anabolics. All samples were stored at -20 °C until analysed.

Extraction and qualitative detection of hormones from tissues or urine follow the procedure described by this author (6,7). Quantitative estimation of hormones was performed through an adaptation of the antidiagonal technique of Beljaars et al. (1). The sample and three standards, with increasing concentrations of the hormones to be determined, are applied in the right corner of the HPTLC-plate along an antidiagonal. After bidimensional development the plates are dipped in 5 % sulphuric acid in ethanol during 30 sec and then heated at 95 °C during 10 min. Fluorescent spots of the hormones in the sample and standards are situated along a line. The fluorescence intensity was scanned using a Zeiss TLC scanner with excitation at 366 nm and a cut-off filter at 546 nm. The red fluorescent derivatives of DES were scanned using a cut-off filter of 620 nm.

Results and discussion

1. Reproducibility of quantitative analysis of anabolics

Fig. 1 shows the result in scanning a fat extract contaminated with 2 µg/kg methyltestosterone and 1.3 µg/kg testosterone using the antidiagonal technique. The linear relation between

Fig. 1. Quantitative determination of testosterone and methyltestosterone in meat extracts with the aid of antidiagonal technique.

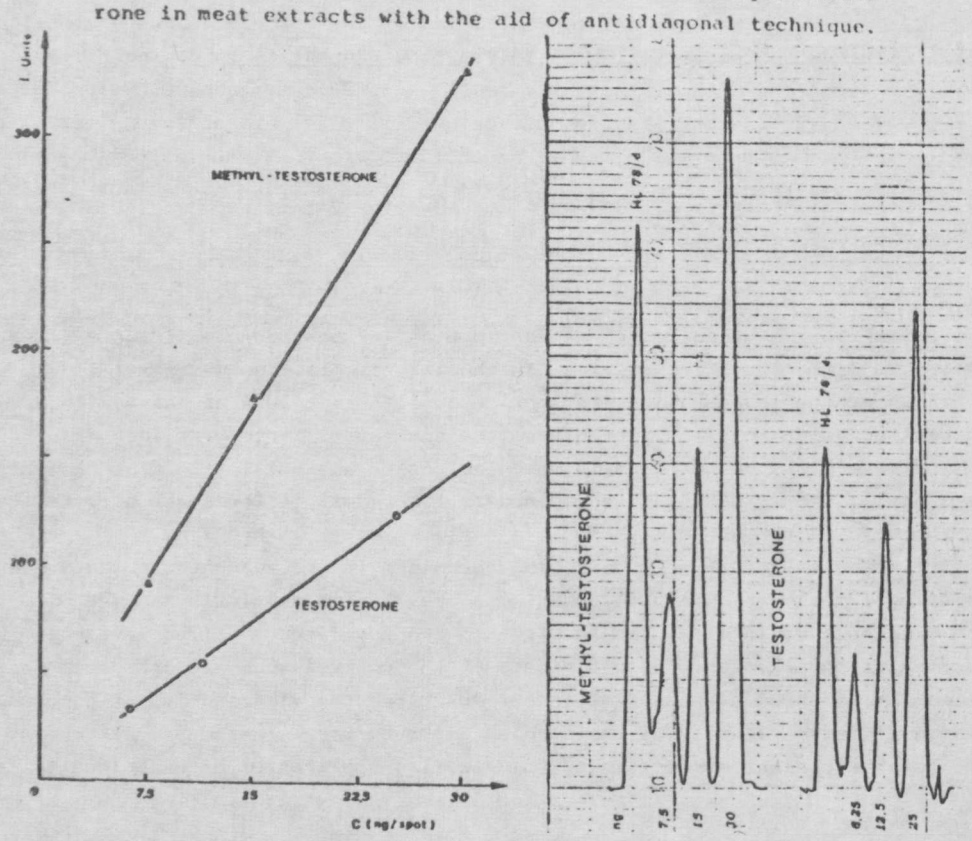

Table 1

Residue concentrations (ug/kg) in different tissues of heifers treated with DES and methyltestosterone (MeT)

	Heifer 7813		Heifer 791	
Residual anabolic (ug/kg)	MeT	DES	MeT	DES
Tissue examined				
Fat around kidney	33	20	15	8
Fat of flank area	3.0	<0.1	<0.1	<0.1
Pelvic fat	2.8	<0.1	<0.1	<0.1
Fat of shoulder	1.2	<0.1	2	<0.1
Kidney	3.5	0.55	2.7	
Liver	1.7	0.3	>1	0.3
M. Diaphragma	4.2	2.2	2.3	<0.1
M. L. Dorsi	1.2	0.1	0.33	<0.1
M. Sternomandibularis	0.48	0.33	1.7	<0.1
M. Gastrocnemius	0.67	0.15	1.0	<0.1

fluorescence intensity of the spot and concentration of the hormone allows estimation of most steroids with a standard deviation of 10 %. Since DES splits up during chromatography in two spots corresponding with cis- and trans-DES, scanning of trans-DES was more variable (standard error : 20 %). In most tissues, detection limits for DES and methyltestosterone were at 0.1 µg/kg. Due to interfering spots on the chromatograms, the detection of DES and methyltestosterone was found to be less sensitive in urine samples (detection limit 1 µg/l).

2. Hormone residues in different tissues of animals treated with DES and methyltestosterone

From literature data (2,3,4) it is expected that residue levels would approach the detection limit of our method if the animals in the illicit practice would be treated with a normal dose and if an adequate time interval before slaughtering is respected. However, residue formation in tissues of the same animal may be a reflection of several factors operative in a particular tissue : solubility of the hormone, metabolic activity, blood perfusion rate, e.a. Therefore it was expected that different muscles and fats of the same animal would contain distinct residue levels. Moreover, in selecting that tissue of the carcass containing the highest residue concentration, the efficiency of the regulatory control would be increased considerably.

Since in our country the combination DES and methyltestosterone is widely used in the illicit practice of cattle breeding, the distribution of DES and methyltestosterone among different tissues of two heifers was studied (Table 1). Liver, kidney and M. diaphragma contained a two to five fold higher concentration of DES and methyltestosterone as compared to most muscles studied. Essentially similar findings have been reported earlier for trenbolone, testosterone and estrone (2,3,4). However, the residue levels of DES and methyltestosterone in fat around the kidney were consistently higher than observed in the other tissues and amounted to 8 - 10 times the concentration observed in M. diaphragma. The accumulation of DES and methyltestosterone was typical for kidney fat. Analysis of fat of the flank area, fat of shoulder or pelvic fat showed that methyltestosterone concentrations in these fat tissues were always less than observed in diaphragma or kidney. In contrast with most muscles studied, no DES could be detected in pelvic fat or fat from shoulder or flank area. These results suggest that fat around the kidney may be used as a sensitive marker tissue in the control on the abuse of anabolics in cattle breeding.

3. Use of multi-residue method in the control of illicit administration of anabolics to cattle

In our laboratory, more than 600 samples of urine, muscular tissue, fat or organs of cattle have been analysed over the last 3 years on the presence of anabolics. In urine samples, residue levels of 2 - 40 µg/l of various anabolics were often found. The residue concentrations detected in meat and fat were lower and rarely exceeded 5 µg/kg. Since excretion of the injected or implanted anabolic is fairly rapid, the relatively high residue concentrations suggest that in the illicit practice some animals may be injected shortly before slaughter. From our analysis, it became evident that a high percentage of the anabolic positive animals were treated with DES or with combination of DES and methyltestosterone. Since in the illicit practice a variety of dosage forms of the hormones are used at different periods before slaughter the efficiency of a control, based on analysis of fat around kidney or M. diaphragma, was studied in slaughterhouse cattle.

Kidney fat, M. diaphragma and urine of heifers, cows or bulls were quantitatively analysed and classified according to the residue contents of urine (Table 2). The results obtained for methyltestosterone indicate consistent higher residue concentrations in fat around the kidney as compared with the corresponding contents in M. diaphragma. Essentially similar

results were found with DES if the urine of the animals contained less than 10 µg/l DES. Recent administration of DES, as indicated by excessive levels in urine, resulted in low residue levels in kidney fat. These results may be largely explained if accumulation of residues occurs faster in M. long. dorsi than in fat around the kidney. Conversily, the persistent and relatively high residue levels in kidney fat as compared with other tissues are indicative for a slower elimination rate of residues from kidney fat. Kidney fat may thus be especially suited in detecting the illicit use of anabolics at a distant time from slaughter.

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References

1. P.R. BELJAARS, C.A.H. VERHULSTDONCK, W.E. PAUSCH and D.H. LIEM (1973) J. Ass. Offic. Anal. Chem., 56, 1444.
2. B. HOFFMANN and H. KARG (1976) In : Anabolic Agents in Animal Production FAO/WHO, Rome ; Environmental Quality and Safety, Suppl. Vol V, F. Coulston and F. Korte (Editors), G. Thieme, Stuttgart, pp 181 - 191.
3. B. HOFFMANN and G. OETTEL (1976), Steroids, 27, 509.
4. B. HOFFMANN and E. RATTENBERGER (1977), J. Anim. Sci., 45, 635.
5. R. VERBEKE (1975) BENELUX Workgroup Hormones, SP/LAB/h(1975)1.
6. R. VERBEKE (1978) XXIVth European Meeting of Meat Research Workers, Kulmbach, Section L11.
7. R. VERBEKE (1979) J. Chromatogr. : In Press.

Table 2
Concentrations of residues of anabolics in urine, kidney fat and M. diaphragma in heifers or bulls treated with methyltestosterone or DES

Methyltestosterone (µg/kg)			Diethylstilboestrol (µg/kg)		
Urine	Kidney fat	M. diaphragma	Urine	Kidney fat	M. diaphragma
10 to 2	90	3	50 to 10	0.8	2.0
	22	<0.1		<0.1	0.6
	14	1		<0.1	<0.1
	13	10			
	2.5	<0.1	9 to 2	15	0.1
				8	<0.1
1.9 to 1	13	25		4.8	1.7
	12	12		0.2	<0.1
	7	0.4			
	2.5	15	1.9 to 1	8	<0.1
	3	<0.1		6	<0.1
	3	<0.1		5	5
	2	<0.1		0.7	0.5
	0.6	<0.1		<0.1	0.4
	<0.1	3		<0.1	0.4