HORMONES IN ANIMAL PRODUCTION

THE USE OF HORMONES IN ANIMAL PRODUCTION

by

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1. INTRODUCTION

Hormone-dependent sex differences in growth rate have been known for a long time. It has also been known that growth rate and FCE (feed conversion efficiency) are higher in intact males than in castrates. It was natural, then, that the availability of hormones and other natural or synthetic substances displaying hormonal activity led to experiments aiming at their use to increase production. Beginning in the mid-1950s, DES (diethylstilboestrol) and hexoestrol were administered to cattle increasingly in the US and the UK respectively, either as feed additives or as implants, and other types of substances also gradually became available. In general, such treatment has resulted in 10–15% increases in daily gains, similar improvements in FCE and improvement of carcass quality (increased lean/fat ratio). Thus there has been a substantial reduction in the amount of energy required per unit weight of protein produced (1,2), and the economic implications of this have been great.

While the use of hormonally active substances in animal production rose, opposition to their use also increased, because of the theoretical possibility that residues in edible tissues might endanger consumers. The factors leading to the ban on DES in the US, first imposed in 1973, have been described (3). Several reports confirm that DES endangers the health of animals and man, when repeatedly used in large doses (4,5). However, as regards risks due to the presence of residues in meat produced according to regulations,

no documented deleterious effects have ever been reported in man, either from DES or any other substance with hormonal activity.

A distinction should be made between the hormones as such, for which the metabolism in the body is relatively well known, and synthetic or other substances for whose metabolic inactivation the body may not possess the enzymes necessary. When natural hormones are used in animal production, claims of zero-tolerance residue levels are not meaningful, since these compounds occur in detectable and highly variable concentrations in body fluids as well as in the tissues of all animals, treated or not (6,7). For other substances with hormonal activity the situation is different. However, when residue levels are extremely low, it seems reasonable to weigh the potential risks against the undisputed positive effects some of these compounds have in animal protein production.

This paper will discuss types of substances with hormonal activity currently in use or under investigation, their effects, mechanism of action, metabolism/elimination, tissue levels, risks to the consumer and their economic importance. Finally, other avenues to increased animal production as alternatives to use of hormones will be briefly envisaged. For the sake of simplicity the term *hormone* will be used, even if incorrectly, to cover all substances with hormonal activity, whether natural or synthetic. Since much information on the question collected before 1975 has been reviewed previously (8), the main emphasis will be placed here on research since that time.

2. HORMONE PREPARATIONS USED IN ANIMAL PRODUCTION

2.1 Hormones of endogenous origin

These comprise the "classical" steroid sex hormones, oestradiol-17β, testosterone and progesterone. The two former are used either in the free form or as esters, mainly those of propionic or benzoic acid. Esterification generally causes prolongation of the half-life of the compounds in the body by 40 to 50%. The natural hormones having low bioavailability when administered orally, owing to rapid conjugation and metabolic transformation in the liver, they are therefore administered by subcutaneous implantation.

2.2 Hormones of exogenous origin

Of the *oestrogens*, the stilbene derivatives diethylstilboestrol (DES) and hexoestrol possess high biological activity and have been used most widely. They are active orally as well as by implantation. Other orally active oestrogens include ethynyl-oestradiol, a more slowly metabolized derivative of the true hormone, with higher activity. An oestrogen with an entirely different structure is zeranol, a derivative of a resorcylic acid lactone occurring in the fungus *Giberella zeae*.

The *synthetic androgens* comprise a large number of substances, most of which are steroids. Of these, trenbolone acetate (TBA) possesses strong anabolic properties and has

received much attention during recent years, used alone or in combination with an oestrogen. Another anabolic steroid is methyl-testosterone.

Of *synthetic gestagens*, only one will be mentioned here: melengestrol acetate, which stimulates growth in heifers but not in steers, and which can also be used for the suppression of oestrus. Numerous other gestagens also exist, but at present few other than progesterone and melengestrol acetate are used to stimulate growth.

In addition to these substances, numerous others exist, and some of them are used more or less frequently in clinical veterinary medicine. However, clinical applications of hormones are not considered to be of consequence to the consumer, since such treatment is much less frequent than the use of hormones to promote growth.

Hormone preparationsin current use as growth stimulants are listed in Table 1, which also shows modes of application, dosages, etc. It will be noted that almost all preparations currently in use are based on implantation, the site usually being the base of the ear, or less frequently, the dewlap.

3. RANGE OF APPLICATION

In *cattle* the use of hormones is limited to veal calves and beef cattle. *Veal calves* are produced mainly in continental Europe, to an extent of about 8 million per year. Research has demonstrated that hormone treatment improves growth rate, nitrogen retention and FCE during the five- to six-week period before slaughter (9,10). *Beef cattle*, including steers as well as heifers, were treated in large numbers, especially in the USA and the UK, with DES or hexoestrol, administered orally, until the use of these compounds was restricted. During the last several years, practice has changed dramatically in the direction of increased use of implants of natural steroids, synthetic anabolic steroids and the phyto-oestrogen zeranol.

Table 1. Hormonally-active substances used in animal production

Substances	Dose levels	Form	Main use - Animals	Trade name
Oestrogens alone:				
DES	10–20 mg/day	feed additive	steers, heifers	
DES	30–60 mg/day	implant	steers	
DES		oil solution	veal calves	
Hexoestrol	12–60 mg	implant	steers, sheep, calves, poultry	
Zeranol	12–36 mg	implant	steers, sheep	Ralgro

Gestagens alone:				
Melengestrol acetate	0.25–0.50 mg/day	heifers		
Androgens alone:				
TBA	300 mg	implant	heifers, culled cows	Finaplix
Combined preparations:				
DES + Testosterone	25 mg 120 mg	implant	calves	Rapigain
DES + Methyl-testosterone		feed additive	swine	Maxymin
Hexoestrol + TBA	30–45 mg 300 mg	implant	steers	
Zeranol + TBA	36 mg 300 mg	implant	steers	
Oestradiol-17β + TBA	20 mg 140 mg	implant	bulls, steers calves, sheep	Revalor
Oestradiol-17β benzoate + Testosterone propionate	20 mg 200 mg	implant	heifers, calves	(Synovex H (Implix BF
Oestradiol-17β benzoate + Progesterone	20 mg 200 mg	implant	steers	(Synovex S (Implix BM

In *sheep*, especially in wether lambs, some increase in gain has been reported (11), but results are somewhat ambiguous.

In *swine*, hormone treatment may increase growth rate, FCE and lean/fat ratio of the carcass in male castrates.

Poultry generally do not appear to respond to oestrogens by increased gain but by changes in lipid deposition. In male and female turkeys, androgens have recently been reported to increase growth rate as well as FCE (13).

4. MODES OF APPLICATION

When DES was used as a feed additive, a usual procedure was to start treatment of steers at a body weight of 360 kg and continue administration for 120 to 170 days. Since restrictions on its use were imposed, most preparations have been administered as implants, whose effect is usually limited to 80 to 100 days. Practice varies with management systems. Animals may be implanted at live weights from 270 to 450 kg.

Depending upon the age and weight at the time of implantation, the animals are either slaughtered at the end of this first period, or fed for an additional period, either without further treatment or after a second implant to act for another 80 to 100 days. Most types of implants in use are not removable, but removable types have recently been tested and their effects described (114). When tested in steers, no reduction in performance was recorded when the implants were withdrawn 32 and 39 days before slaughter.

Implantation is subcutaneous, usually at the base of the ear, thus eliminating the risk that residues of the implantation site will be present in edible tissue.

5. EFFECTS OF HORMONES

5.1 Veal calves

In veal calves, hormone treatment may begin at a body weight of about 65 kg, the animals being slaughtered at about 170 kg. Implants of 20 mg oestradiol-17β + 200 mg progesterone in males and 20 mg oestradiol-17β + 200 mg testosterone in females resulted in a 20% increase in daily gain and 21% higher nitrogen retention in the period studied (14). In other studies, improvements of 10 to 12% in gain and 10% in FCE have been reported (15, 16, 17, 18). Nitrogen retention is about 70% in the very young veal calf, but decreases gradually to below 40% at the age of about 15 weeks. For ages of 10 to 15 weeks, the average conversion of feed protein to body protein is about 40%; this rate can be increased to about 60% by hormone treatment. The effective preparations were DES, oestradiol-17β, and the combination of TBA + oestradiol-17β (9). More recently, positive effects have been reported (19, 99) for zeranol alone (36 mg) and for zeranol (36 mg) + TBA (140 mg), with increases in nitrogen retention of the same order as for DES and E_2 + TBA. When zeranol + TBA was implanted at the age of 56 days, the growth rate up to day 106 increased by 18% (19).

5.2 Steers

The most extensive studies of the effects of hormones on growth and FCE have been carried out on steers, under strictly controlled conditions as well as in the field. Since 1975, most studies have involved implants of oestrogens alone, androgens alone, or combined oestrogen/androgen preparations, although many trials have also been based on oestrogen/progesterone combinations during recent years.

Oestrogen implants have included DES, hexoestrol, oestradiol-17β and zeranol. *DES implants* have, as in previous studies, resulted in an increase of about 12% in gain and in improvement in FCE of the order of 10% (20, 21, 22). *Hexoestrol implants*, usually in doses of 30 to 60 mg, have been shown in numerous experiments to lead to considerable improvement in growth rate and FCE (23, 24, 25, 26, 27, 28, 29). In 19 trials carried out over the years on experimental husbandry farms in the UK, the overall average increase in gain produced by 45 or 60 mg hexoestrol implants was 0.16 kg a day, and in only 2 of the trials was it less than half that figure (2). *Oestradiol - 17β implants* alone (30 mg) have resulted in a 24% increase in gain and a 13% improvement in FCE (30). *Zeranol*

implants, usually at 36 mg, have consistently improved gain as well as FCE (20, 23, 24, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41). In a series of 21 UK trials over several years, the average response to zeranol implants alone was an increase in daily gain of 0.15 kg. Only in one trial was there no response (23). Similar results have been obtained in Ireland (*cit. 2*). Positive effects on gain in steers have been observed under a variety of experimental conditions, under controlled feeding, on *ad lib* feeding of standardized rations, and on pasture.

TBA implants administered alone at a dose of 300 mg have also had positive effects on growth (23, 24, 25, 26, 27, 29, 37, 40, 41), even if combination with an oestrogen has yielded better responses (*vide infra*). In a series of 8 trials in the UK, the average additional daily gain amounted to 0.09 kg, with considerable variation among trials (23). Similar results have been reported from Ireland (*cit.* 2).

Combined preparations. A number of trials have been carried out with implants containing two hormones. The combination of an oestrogen with an anabolic steroid, or with progesterone, has met with the greatest responses. *Synovex-S* has consistently increased gain as well as FCE, with responses averaging about 20% and 17% respectively (20, 21, 22, 34, 35, 36, 37, 42, 43, 44, 45, 46, 47, 48). *Hexoestrol + TBA* (usually 30 or 45 mg hexoestrol + 300 mg TBA) has resulted in marked increases in gain (24, 25, 26, 29, 49, 50, 51, 52, 53), of the order of 30% and in FCE (25, 49, 50, 51, 53) of the order of 20%. *Oestradiol-17β + TBA* (20/140 mg) has given similar results (27, 28, 37, 54, 55), as has *Zeranol + TBA* (36/300 mg), also recently tested (27, 37, 38, 39, 40, 41, 56).

Hormone preparations have also been tested in combination with substances such as monensin, which increase FCE by promoting propionic acid formation in the rumen. Results have varied from no effect (38, 52) to marked additional gain (43).

Reimplantation, tested under various forms of management with varying results (25, 26, 32, 36, 52, 56, 57, 58), has not gained general acceptance. Lamming (45) has stated that "repeat implantation of hormone is not likely to produce the benefits obtained from its initial use, since a second dramatic change in the endocrine balance of the animal is not likely to occur. In addition, double implantation increases the possibility of exceeding the optimum dose rate and the chance of deleterious side effects occurring."

The evidence for highly significant positive effects on the growth rate and FCE of steers is thus beyond dispute, the most marked effects being provoked by implants combining an oestrogen with an androgen of high anabolic activity.

5.3 Bulls

Since the entire male animal produces its own anabolic androgen, testosterone, an effect of additional hormones similar to that for steers is not to be expected. The number of trials with bulls is also limited. Positive effects on gain have been reported using DES alone (2, 58, 59) and combined oestrogen/TBA implants (60); in other studies, no effect

on gain has been recorded (49, 61), while a certain increase in the deposition of fat in the carcass has been observed (61).

5.4 Heifers

Recent trials with beef-producing heifers have mostly been based on the use of an androgen, although oestrogens have been tested, alone or in combination. Thus, zeranol has been reported to increase gain (62, 63), while in other trials no response has been observed (37, 62). TBA administered alone (300 mg) has led to increases in weight gain and FCE of the order of 36% and 25% respectively (24, 27, 37, 64, 65, 66, 67, 68, 69, 70). In other trials, combinations of an oestrogen with TBA (68) or testosterone (62) have yielded significant growth responses. In general, it appears that the effect of TBA alone in heifers corresponds closely to the effect of combined oestrogen/TBA implants in steers.

5.5 Sheep

Trials have mainly concerned wether lambs, and positive effects of hormonal treatment have been reported using DES (69), hexoestrol (71) and zeranol (72, 73), although other reports have indicated that zeranol yields no significant effects (74, 75). Wether lambs implanted with TBA + oestradiol-17β have shown increases in gain, carcass weight and FCE (11, 69). In general, however, the results obtained in sheep thus far do not warrant the same clear-cut conclusions as for steers and heifers.

5.6 Swine and poultry

There is little evidence that existing hormonal preparations influence the growth rate and FCE to an extent that would be interesting from a practical point of view. The lean/fat ratio in male castrate and female pig carcasses may be increased by the use of oestrogen/androgen combinations (76). In poultry, redistribution of fat in the body is a known effect of oestrogens. Recent research indicates improved growth rate and FCE using androgens in young male and female turkeys (13, *cit.* 27).

5.7 Undesirable side effects in treated animals

Reported side effects of hormone treatment for growth stimulation are few and generally concern the use of oestrogens in steers. Changes in body conformation such as feminization and raised tail-heads were described as early as 1958 (118). Similarly, bulling has occurred with increased frequency (57, 118, 119), although in most animals it is limited to the first few days after implantation (46). However, it has been reported from Kansas that 2.2% of all steers fed in pens have to be removed, at an estimated loss of $23 per head (119). In a study of the effect of reimplantation of oestrogens in steers, all animals were given a 30 mg DES implant at a live weight of 260 kg, and then reimplanted 91 days later, with either 30 mg DES or Synovex S. Following the second implant, the frequency of the steer-buller syndrome was 1.65% for the DES-DES group, and 3.36% for the DES-Synovex S group. The economic advantage of using DES + DES

was estimated at $1.15 per head (57). The steer-buller syndrome is a special problem in feedlots.

6. MECHANISM OF ACTION OF HORMONES

No reliable explanation of how the growth-promoting hormones act has yet been furnished. Some observations indicate an indirect influence through changes in the balance of endogenous hormones. Thus there have been reports of DES and TBA increasing the levels of growth hormone and/or of insulin in plasma (51, 63); these hormones are known to stimulate amino acid transport across the cell membrane. However, others have found no such effect (49, 60, 67, 77, 82). Bulls fed DES (10 mg/day) over two years had significantly higher plasma testosterone levels than controls (78); those levels are positively correlated with growth (78, 79, 80). Recent experiments indicate that DES reduces the rate of muscle catabolism in steers (81).

As regards the anabolic androgens, evidence exists indicating competition with glucocorticoids for receptor sites on the muscle cell membrane. Since glucocorticoids have a catabolic effect on tissues, their displacement from muscle cells would reduce catabolism. TBA alone, and even more when combined with oestradiol-17β, causes a marked decrease in the concentration of total thyroxin in plasma of steers (82). In another study, combined oestradiol-17β-progesterone implants (20 + 200 mg) in steers caused a uniform but slight increase in thyroxine binding capacity (44). The significance of these findings is not yet clear.

For a fuller discussion of possible mechanisms of action of the hormones, see references 2, 27 and 83.

7. LEVELS OF ENDOGENOUS HORMONES IN BODY FLUIDS AND TISSUES

Any discussion of possible health hazards connected with the use of hormones in animal production must take into account the normal occurrence of hormones and their metabolites in body fluids and tissues, and the fact that the levels of these hormones vary greatly, according to the physiological state of the animal. Thus, oestrogen levels in the blood of female farm animals may vary from a few pg up to 5–6 000 pg per ml plasma (6). As to males, the plasma of stallions and entire male pigs contains high levels of oestrogens, although mainly in the conjugated form. Milk also contains oestrogens in very high concentrations in the first drawings after parturition; in non-pregnant animals, levels in the range of 80–100 pg/ml have been reported (6, 84). More recently, reliable data have also become available concerning concentrations in edible tissues; some of these are presented in Table 2. For the sake of comparison, levels of oestrogen activity normally present in products of plant origin widely used in human nutrition are included.

Table 2. Concentrations of endogenous hormones in edible tissues of farm animals

Animal/tissues	Oestrone pg/ml	Oestradiol-17β pg/ml	Testosterone pg/g	Progesterone pg/g
Veal calf				
muscle		< 100	70	
liver		< 100	47	
kidney		< 100	685	
fat		< 100	340	6
Bull				
muscle			335	
liver			749	
kidney			2 783	
fat			10 950	
Heifer				
muscle		12–13	92	
liver	20–40	38–71	193	16
kidney		40–71	595	
fat		6	250	
Cow, pregnant				
muscle		370–860 2 500–5		336
fat	3 870	500		
Steers				
muscle	6	14		
liver	20	14		
fat	23	10		
Wheat germ oil	4 000 pg/g DES equivalent			
Soy-bean oil	2 000 000 pg/g DES equivalent			

Sources: 85, 86, 87, 88, 89.

8. METABOLISM, ROUTES AND RATES OF ELIMINATION

The general patterns of metabolism and elimination of endogenous hormones in farm animals have been outlined (90). In ruminants, testosterone and oestradiol-17β are rapidly converted to their epimers, biologically much less active, epitestosterone and oestradiol-17α. Progesterone is partially converted to androgens before excretion. In the pig, epimerization of testosterone and oestradiol-17β does not appear to take place to a significant degree. The faecal route of elimination dominates in ruminants, while in the pig urinary excretion is more important.

8.1 Progesterone

After repeated injections of progesterone to cows and steers over 2 to 3 weeks followed by ^{14}C-progesterone for 2 to 5 days, the animals were slaughtered 2 to 3 hours after the last injections. Activity levels were 2 to 7 times higher in the fat, 3 times higher in the kidneys, and 13 times higher in the liver than in the muscle. Excretion of radioactivity amounted to 50% and 12% in faeces and 2.0% and 1.2% in urine in cows and steers respectively. About 50% of the activity in muscle and milk was associated with unchanged progesterone, most of the remaining activity being associated with a mono-hydroxy compound. Cooking or frozen storage did not affect the nature or quantity of metabolites (91).

8.2 Oestradiol-17β

Following daily injections of 1 mg oestradiol-17β or its benzoate to heifers and steers for 11 days, followed by the ^{14}C-compounds on days 12, 13 and 14, the animals were slaughtered 3 hours after the last injections, when residual levels were maximal. In muscle extracts, oestradiol-17β represented the major fraction of extracted activity (38 to 71%), followed by oestrone (17 to 45%). Levels in muscle were 161 to 225 pg/g and 40 to 86 pg/g for oestradiol-17β and oestrone respectively. In fat the levels were 3 to 5 times higher. The authors conclude that residual levels are extremely low when these hormones are administered as growth stimulants to growing/finishing cattle (92). Glucosides of the 17β- and the 17α- epimers, and the glucoronide of the 17α- epimer are the major metabolites in cattle (125). When oestradiol-17β was administered orally to swine, plasma concentrations were very high 7 min after administration. Oestradiol was completely conjugated during absorption and its first passage through the liver. Some conversion to oestrone took place (93).

8.3 DES

The metabolism of DES in food-producing animals has been reviewed recently (94). The substance seems to be eliminated to a large extent in unaltered form. After oral administration of ^{14}C-DES to beef cattle, 99.5% of the radioactivity was excreted within 5 days after withdrawal. In liver extracts, radioactivity associated with DES-conjugate and free DES was found to be 75% and 25% respectively. Higher than background levels of activity were observed after withdrawal in kidney, liver, bile and urine/faeces for up to 5, 7, 9 and 11 to 12 days respectively (95). The fate of 24-mg DES implants containing ^{14}C-DES and implanted in the dewlap of calves was studied over 98 days. Free radioactivity was almost completely associated with unchanged DES. At the time of slaughter, levels were less than 0.1 ppb in muscle and fat, and 1 to 1.5 ppb in liver and kidney (96). In a study in steers implanted with ^{14}C-DES, on day 120 after implantation radioactivity in muscle was not distinguishable from background. It was above background in spleen, lung, adrenal glands and kidney, but less than levels corresponding to 0.5 ppb. In a similar study on steers, 120 days after implantation, levels in liver, kidney, lungs and salivary glands were in the range of 0.07 to 0.13 ppb of DES equivalent (98). In a recent study of DES metabolism in rhesus monkeys and chimpanzees, most of the substance was excreted with the urine. Extracts in the organic and aqueous phase mostly contained unchanged DES in the free and conjugated form respectively (121). Current

evidence indicates that the oxidative metabolism of DES leads to at least three compounds that may have cytotoxic or mutagenic activity (121), but these have not been identified as DES metabolites in ruminants, but in the mouse.

8.4 Zeranol

Using a gas chromatographic method with a sensitivity limit of 20 ppb, no residues of zeranol could be detected in edible tissue from cattle slaughtered 65 days following implantation of 36 mg, or from lambs 40 days following implantation of 12 mg (101). In another study, tritiated zeranol was implanted in cattle as part of 36-mg doses. Skeletal muscle obtained 10, 30 and 50 days following implantation contained no detectable residual activity (99). This confirms previous results based on the use of ^{14}C-labelled zeranol (100).

8.5 Trenbolone acetate (TBA)

Trenbolone is a 17β-OH steroid esterified in the 17 position with acetic acid. Upon release in the organism the ester is rapidly hydrolyzed to the free compound TB-17β-OH and acetate. In cattle the 17β-OH compound is rapidly transformed to its 17α-OH epimer, in the same manner as oestradiol-17β in this species. The 17α epimer possesses only about 5% of the biological activity of the 17β epimer. Another metabolite of TBA in cattle is the 17-keto compound, analogous to oestrone; quantitatively it appears to be of very little importance. Following intravenous injection of TBA, levels of TB-17β-OH and TB-17α-OH of 0.05 and 0.005, 0.10 and 1.0, 0 and 191 ppb have been recorded for muscle, liver and bile respectively. Other metabolites occurred in extremely small quantities in cattle (102, 103). Similar findings have been reported in studies based on the use of implants (*cit.* 102). The major route of excretion is by faeces. Metabolism studies of TBA thus clearly show that the substance is rapidly subjected to biological inactivation in cattle, mainly by epimerization of the free steroid to the 17α-compound, and that the major route of excretion is via the bile.

9. RESIDUES IN EDIBLE TISSUES OF HORMONE-TREATED ANIMALS

Much work has been devoted to the development of sensitive methods of detecting hormone residues in meat from hormone-treated animals. As regards compounds given orally, it should in principle be possible to realize claims of zero-tolerance residue levels, by selecting the proper withdrawal time. During recent years, the use of implants has, however, gained in importance. While removable implants have been tested in steers, with no decrease in performance when withdrawn 32 and 39 days before slaughter (104), the wide use of non-removable implants makes residue studies important. Determination of normal levels of endogenously produced natural hormones is also important, to enable risk evaluation to be carried out in realistic terms.

Several residue studies have been made of synthetic as well as natural compounds, mainly in cattle. When regulations governing dose, sites of implantation and timing in relation to slaughter are adhered to, residue levels of DES (88, 95, 96, 97, 98), hexoestrol (105) and oestradiol-17β (106, 107) in edible tissues have generally been in the lower ppb to the ppt range, i.e. from a few ng/g down to some hundred pg/g of tissue. In the latter case there was almost complete overlap between values for untreated and treated steers after 105 days (107). Zeranol implants have so far not left detectable residues in edible tissue (99, 100, 101).

Most studies of androgens have concentrated on TBA. The ester being rapidly hydrolyzed, measurements of residues have been limited to the free compound and/or its major metabolite. Results based on radio-immunoassay of extracts or on radioactivity measurements (88, 102, 103, 106, 108, 109, 122) have indicated levels in edible tissue of the order of 1 ppb or below. In a recent study using implants containing tritiated TBA in heifers, it was found that when slaughter took place 60 days after implantation, the major proportion of tritium-containing residues was not extractable with organic solvents. In muscle 95.5%, in liver 94.4%, in kidney 98.8% and in fat 59.1% of the radioactivity remained in the aqueous phase, not quantifiable by radio-immunoassay. This suggests that the major part of the residues after TBA implantation occurs in a non-extractable, possible covalently bound form in tissues (123).

Residue levels of gestagens have been also measured, in connection with their use as growth stimulants. Residues of melengestrol acetate used as a feed additive in daily doses of 0.25 to 0.50 mg per head have consistently been below the sensitivity levels of the methods used (i.e., below 10 ppb in fat, liver, muscle and kidney), whether or not the compound was withdrawn 48 hrs before slaughter (124).

10. HORMONES IN FOOD: MEAT FROM HORMONE-TREATED ANIMALS VERSUS OTHER SOURCES

According to the Agricultural Research Service, United States Department of Agriculture (ARS), the average per caput consumption of beef is 157 g per day in the US (110). Calculations show that 157 g of beef from an animal implanted 61 days before slaughter with a combined implant containing 20 mg oestradiol-17β + 200 mg progesterone or testosterone will contain 3.43 ng oestrogen and 19.5 ng progesterone or 16 ng testosterone. Table 3, which provides data on normal levels of these hormones in certain dairy foods, shows that some foods represent hormone sources vastly richer than meat from hormonetreated animals. Based on these values, and averages for consumption of various foods, the relative contribution of meat from hormone-treated animals to the total consumption of hormones has been calculated on the assumption of proper use of the hormones (see Table 4). It is clear that in most cases the contribution from meat of treated animals is insignificant when hormones have been properly used, and must be considered to be biologically without impact. This becomes even more evident when seen in relation to normal endogenous hormone production in man, as illustrated in Table 5. It

will be seen that even for oestrogens, the hormones considered the greatest risk, the maximal contribution from meat (assuming proper use of the hormones) is less than 0.01% in the prepubertal boy who represents the lowest endogenous oestrogen production.

Table 3. Hormones in certain dairy foods

	Oestrogens (pg/ml)	Progesterone (ng/ml)
Milk, from non-pregnant cows	80	9.5
Milk, from pregnant cows	126	
Cream		73
Butter		133

Source: 103.

Thus far the discussion has been limited to the natural hormones. For synthetic substances the situation may be different. But again, considering the very low residue levels found *when* hormones have been properly used, the question may be raised whether the risk to the consumers is being grossly overestimated.

Table 4. Relative contribution of meat from hormone-treated steers to total hormone intake via food
(per cent)

	Oestrogens	Progesterone
Child under 1 year	0.22	0.014
Child 6 to 8 years	1.56	0.1
Adult male	7.69	0.5

Source: Condensed from 103.

Table 5. Contribution of hormones from hormone-treated steers relative to total daily hormone production in man[1]
(per cent)

	Oestrogens	Progesterone	Testosterone
Prepuberal girls	0.00636	0.00078	0.005
Prepuberal boys	0.00826	0.00130	0.00244
Women			
Follicular phase	0.00018	0.00047	
Luteal phase	0.00007	0.01	0.0004
Men	0.00025	0.00048	0.00003

[1] The figures represent effective fractions (i.e. 10% of real fractions), to take into account the low bio-availability of the hormones absorbed orally.

11. ECONOMIC IMPLICATIONS OF THE USE OF HORMONES IN ANIMAL PRODUCTION

In the production of meat for human consumption, a hormonally-induced increase in growth rate of the order of 10% evidently has major economic implications. The improvement in FCE which usually accompanies the increase in gain adds to the economic benefits, and at the same time makes possible greater production of edible protein per unit energy used, and this in itself is of importance in a world lacking in protein supplies. Some of the hormones that have become available recently appear on average to increase gain as well as FCE considerably beyond the 10% level, and in examining whether they should be approved for use in animal production, the risk/benefit analysis must take this fact into account.

Few analyses of the economic advantages of using hormones as growth stimulants appear to have been made. For the UK, a recent calculation (see Table 6) is based on the estimated increased return to producers for 1 350 000 cattle treated over a 12-month period (111). Assuming that 1 155 000 of these were steers and 195 000 were heifers, and that the estimated daily gain was only 0.06 to 0.11 kg for steers and 0.05 to 0.06 kg for heifers, depending on the preparation used, the overall gross increased return was calculated at £21 306 000, without taking into consideration improvements in FCE.

Table 6. Estimated increased return to producers from the use of hormones in animal production (12 months)

	Ralgro	Finaplix
1. Number of animals treated[1]		
Steers	675 000	480 000
Heifers	75 000	120 000
2. Average increase in daily gain (kg)[2]		
Steers	0.11	0.06
Heifers	0.05	0.06
3. Average increase in slaughter weight (kg)[3]		
Steers	10	5
Heifers	6	3
4. Estimated total increase in slaughter weight (carcase weight) (tonnes)	5 478	
5. Estimated overall increased gross margin per head to the producer (£)[3]		
Steers	21.60	10.25
Heifers	10.40	8.55

6. Estimated gross return (£)	14 580 000	4 920 000
Steers	780 000	1 026 000
Heifers	15 360 000	5 946 000
Total	21 306 000	
7. Estimated price per dose (£)	1.20	1.80
Total (£)	900 000	1 080 000
8. Net return (£)[4]	14 460 000	4 866 000
Total (£)[4]	19 326 000	

[1] Estimated from sales of the preparations during a year.
[2] Based on results from Meat and Livestock Commission trials using yard finishing cattle receiving only one implant.
[3] Based on 1978 data showing that 0.1 kg increase in daily gain gave an increase in gross margin of £13 per head, and that increase in slaughter weight averaged 85 p per head. From these figures are subtracted the cost of treatment.
[4] Does not include costs of veterinary services, etc.
Source: 111

These calculations must be taken as an example only. Availability of the various feeds, variations in feed and product prices as well as in types of management from time to time and from place to place may play an important role. However, shortening the time required for producing a certain weight at slaughter will represent an economic advantage, especially under feedlot conditions, since non-feed costs also contribute significantly to the total cost of production (10 to 18 cents per head per day in the USA).

12. ALTERNATIVES TO THE USE OF HORMONES

Growth rates are influenced by many factors, especially genetic constitution and feeding. Over time, selection as well as improvements in management systems, feed composition and feeding programmes have contributed much to increasing productivity in meat as well as milk. Although it is difficult to evaluate the exact relative contributions of these factors, the overall improvements have been dramatic. An example is the increase in milk yield per head in US dairy cattle. In the period 1944–1975, the number of dairy cows decreased by 33%, while the average yield per cow increased by 60%. These gains represented a saving of about 23 billion kg of total digestible nitrogen per year, the volume of milk produced remaining relatively constant. The saving is equivalent to about 1.1 billion bushels of maize (112). Data illustrating progress in beef production over the

years are scarce, but increases in productivity similar to those for milk production are unlikely.

In addition to the use of hormones, many avenues are still open for increasing productivity in meat and milk production (see 115), including breeding programmes, regulation of rumen fermentation, optimalization of the balance between the indirect and direct feeding of the ruminant organism proper, and disease control.

12.1 Breeding programmes

Systematic selection of high-quality sires, combined with an increase in the number of offspring from high-yielding females through embryo transfer, may bring about further improvements in beef and milk production. In many countries, development along these lines has hardly begun. However, the establishment of effective breeding associations and the strict organization of programme planning and execution are prerequisites for realizing the potentials in this sector.

12.2 Regulation of rumen fermentation

The microbial systems in the rumen are extremely complex, and the balance between the various strains of bacteria is susceptible to changes brought about by many factors. The recent introduction of substances such as monensin offers great promise in altering the fermentation pattern to the benefit of productivity by increasing FCE. Since the very extensive breakdown of carbohydrates and protein represents loss of much energy, research is currently being conducted in many laboratories in order to find new methods of increasing FCE.

12.3 Optimalization of the balance between the indirect and the direct feeding of the ruminant organism proper

To a large extent, feeding a ruminant means feeding the rumen microbes which then themselves serve as feed for the organism proper. This is indirect feeding, expensive in energy. On the other hand, the ruminant possesses, in the postruminal part of its digestive tract, all the enzymes necessary for utilizing all types of nutrients except cellulose. The rumen microbes are necessary for the utilization of cellulose, which globally represents an enormous source of energy. However, it is possible to sustain an adequate microbial population in the rumen even when ruminal breakdown of part of the easily digestible nutrients is prevented. Enabling nutrients to bypass the rumen will increase the utilization of feed for production, and also create a more adequate supply of amino acids. Increased rumen bypass of nutrients can currently be brought about by several means, including formaldehyde and heat treatment of protein-rich feeds. A third method, aiming more at specific substances that may be rate-limiting for production (e.g. certain amino acids), or of significance in treatment of diseases, is protection against rumen degradation by such means as incorporation into the ration of long-chain fatty acid mixtures in the form of small pellets (113, 114).

In the future, new methods of increasing rumen bypass will undoubtedly contribute significantly to increased productivity of ruminants.

12.4 Disease control

Whatever management system is adopted, effective disease control is essential for productivity. In many areas of the world, infectious and parasitic diseases inflict heavy losses on animal production. A recent study has disclosed nearly a one-to-one relationship between investment in agricultural research and annual productivity of edible protein in ruminants. An increase of about 45% in scientist/man years and a corresponding increase in funding for research and development is considered sufficient to raise productivity in this sector by 50% (115). Investment in disease control is an important aspect of this work. Annual world mortality losses from disease exceed 50 million cattle and buffalo, and 100 million sheep and goats. Non-lethal diseases are believed to lead to an equivalent reduction in production (115). Thus, investment in disease control holds great promise for future augmentation of animal protein production.

In these perspectives, the significance of hormones in animal production may seem marginal, leading to the question of what priority to give to the various efforts to increase productivity and production. In the global context it is, however, at least at present, impossible to adopt one approach to the exclusion of others. As long as preparations exist that combine positive effects on yield and feed utilization with low or non-existing risk to the consumer, there will be a market for them. What is more, the use of hormonally-active substances in the future may not be limited to those currently available. Common to the present compounds, natural or synthetic, is that they are degraded in the body only to a limited extent. An entirely different situation exists for proteid hormones, which are broken down completely to amino acids, leaving no residues whatever. An example is the growth hormone which not only stimulates growth (116) but also milk secretion, even in high-yielding cows (117, 126). This anabolic hormone is currently available only in small quantities for research. However, a recent breakthrough in the use of recombinant DNA technique (see 127) has made large-scale microbial production of species-specific peptide hormones a realistic possibility. Combined with the development of miniaturized automatic delivery systems for subcutaneous use, a new era may be visualized as regards the use of hormones in animal production.

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METHODS OF MEASURING HORMONE LEVELS IN ANIMAL PRODUCTS

by

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1. INTRODUCTION

The question of the use in animal production of anabolic agents, including the natural steroid hormones and their derivatives, has been -- and continues to be -- badly stated, and therefore misunderstood. Those who brought the problem before the public had neither the information nor the technical competence, especially in biology, to speak with perfect objectivity.

Hormones are found naturally in many animal products (6, 7) and, as phytohormones, in many plants (3, 5). Only some of them raise public health problems, and these should be distinguished from the others. Indeed, a study of the practical and health aspects of the use of hormones in animal production should underlie all regulation of the matter, and therefore any decision on the measurement of hormone levels.

A considerable volume of documentation exists on the problem (6), which is as important for the developed as for the developing countries. Items 1 to 11 in the Bibliography of this paper indicate a number of general studies.

2. THE USE OF HORMONES IN ANIMAL PRODUCTION, AND ITS CONSEQUENCES

A reading of many popular, and even official, papers reveals that different types of anabolic agents, whatever their structure, are grouped together under the general name of hormones, among them those whose activity as oestrogens is emphasized and those which reinforce masculine traits.

Published material and research results make it possible to distinguish three types of anabolic agents.

The first type includes those not found in mammals and birds but obtained exclusively through organic synthesis: diethylstilboestrol (DES) or stilboestrol, dienoestrol, hexoestrol and their derivatives. These compounds, active when administered orally or as implants, are harmful for laboratory animals, whether given directly or as residues in the flesh and offal of treated animals. The use of these substances, a potential danger to human health, is prohibited in many countries, particularly since the methods of measuring DES levels in meat are unreliable, so that measurements must be made on the excreta.

The second type of anabolic agents consists of the natural steroids, normally present in all animals: testosterone, oestradiol, oestrone, progesterone. Secreted by the endocrine glands, these substances and their derivatives, although absorbed by the intestines, are deactivated in the liver. They produce measurable effects only when implanted.

The third type, found in the higher plants and moulds, consists of substances with a more or less clear oestrogenic and, at times, anabolic activity. Among these phytoestrogens, only zearalenone has been isolated for use in animal production: as zeranol, it gives good results, but has an effect on genital formation and the level of thyroxine in plasma (23). It should be added that Sharaf and Gomaa (21) attribute oestrogenic effects to Vitamins E, B_6, C and perhaps A, while Nelson*et al*. (20) have studied the same effects in O-p'-DDT.

This paper discusses only the consequences for the consumer of the use in animal production of the first two types: the synthetic agents such as DES and the natural steroids oestradiol, progesterone and testosterone.

First mention should be made of cases of cancer of the vagina and hypogonadism observed in the offspring of women treated with DES. Such observations (17, 18, 22) are verified by research based on the so-called relay methodology (13), based on the fact that methods of analysis for a residue and its metabolites fail to reflect practical realities, since they may lead to findings of residues without bioavailability or vice versa. Further, the individual biological evaluation of each metabolite fails to reflect the overall consumption of all of them. The relay, a farm animal, changes this situation.

In order to determine whether this was true of the first two types of anabolic agents, rats and mice of both sexes received (13) balanced diets containing 20% of the flesh or 6% of the liver of male and female calves implanted with DES (24 mg in each of two implants), oestradiol and progesterone (20 + 200 mg in two doses) or oestradiol + testosterone (20 + 200 mg in two doses). Animals were implanted at the beginning of the growth period and 60 days later, 38 days prior to slaughter.

Chemical measurement of residues showed DES levels of 30 g/kg in the flesh, but none in the liver. Levels of natural steroid hormones did not exceed levels in untreated animals.

Feeding rats and mice on the flesh of DES-implanted calves led to growth in both sexes, sterilized the females and sharply atrophied the testicles, seminal ducts and penis of the males (12, 15). Liver behaviour was less clear. Pokrovski*et al*. and Nesterin (2) obtained similar results with flesh. It was also observed that DES persisted for nearly 40 days in the environment and was found in alfalfa (5, 14).

Animals fed on the flesh and liver of calves implanted with oestradiol + progesterone or oestradiol + testosterone behaved in the same manner as controls fed on the flesh and liver of control calves. After 24 months, the percentage of tumors (primarily mammary adenomata, frequent in old Wistar rats) was the same in both groups.

Trenbolone acetate (TBA), a testosterone derivative, leaves little or no residue. Measured by radio-immunoassay, Gropp*et al*. (16) also studied their biological effects by the relay toxicity method, on Sprague-Dawley rats fed on the flesh of calves implanted with a mixture of 20 mg oestradiol-17β + 140 mg TBA, as well as with doses 10 and 25 times higher. The results of this study, which continued for 110 weeks and covered two generations, showed the absence of any unfavourable influence on growth, organ weight, reproduction, teratogenicity and cancerogenicity. Only females fed on the flesh of control calves to which TBA had been added in doses 25 times normal, had significantly lower growth($P < 0.05$). The biochemical constants in the blood were the same in both groups.

These experiments show that compounds of natural steroid male and female hormones, and their derivatives, appear to be without danger for the consumer. They are useful in animal production, obviating a number of often costly and polluting therapeutic operations. On the contrary, the use of DES-type non-steroid anabolic agents has serious disadvantages. It is difficult to detect their residues in meat. Relay toxicity results show that analyses and biological behaviour fail to agree.

The indiscriminate prohibition of all types of anabolic agents may be leading to the use of DES and similar substances. Analyses of urine in which they can be identified most easily, have revealed them in 20% of cases. In the United States, McClung (19) estimates that 500 000 bovines are being treated illegally. In Europe, illegal use appears to be fairly general, but is tending to decrease. The use of DES and similar substances should thus be prohibited, while that of the steroids and their derivatives should be authorized under veterinary control. This was the view of many writers as early as 1967, and their conclusions were confirmed by the FAO/WHO Joint Committee of 1975 (2) and a recent international symposium (Warsaw, 27–30 April 1980) (9). Any new anabolic agent should be severely tested before it is distributed widely.

There is a need to develop practical, economic and rapid -- but very exact -- methods of detecting residues, particularly those originating in products chemically different from the natural steroid hormones and their derivatives.

3. METHODS OF MEASURING HORMONE LEVELS IN ANIMAL PRODUCTS

It should be clearly understood that this discussion concerns only measurement methods intended to detect residues remaining in flesh and viscera. These methods are, however, not very reliable, and other biological material must be examined to determine whether the animals have been treated. Specialists agree that there are still difficulties in testing for residues in the tissues of animals intended for use as food.

Many different methods have been proposed and discussed at the EEC level. While the trend is increasingly toward the use of radio-immunoassay, it is undeniable that the techniques are highly complex and still far from being perfected.

DES and similar substances should preferably be looked for in urine and faeces. Instructions to this effect were issued in the United States in 1979 and confirmed in 1980 (48).

Account should also be taken of the fact that no difference may be found between animals properly implanted with anabolic agents and untreated animals. This problem has received insufficient attention. The proceedings of the FAO/WHO Joint Meeting of March 1975 (2) contain reports emphasizing this lack of difference and the relatively high, but physiological, levels of testosterone and progesterone in the flesh of bulls and gestating cows, respectively. This being the case, how can the natural presence of these substances be distinguished from their presence due to the use of natural products?

Further, it should not be forgotten that very many animal feeds contain phytohormones which can cause modifications in the morphology and histology of certain organs.

After summarizing and stating the principles of the four main groups of useful techniques enumerated by Kroes *et al.* (39) and Richou-Bac and Pantaléon (42) -- histological, biological, physico-chemical and radioimmunoassay methods -- this paper will study in detail the techniques that can be applied in practice for routine control.

a. Histological methods (Kroes *et al.* (39)), used specially in young bovines, consist in the observation of modifications in the male and female genitalia. The prostate and the bulbo-urethral glands are particularly examined in male calves and lambs, while in females attention is focused on the glands of Bartholin, the teats, the vagina and the cervix. Modifications are obvious when DES and zeranol, as well as hexoestrol, have been used. In pigs the effects are less discernable. It is generally agreed that the histological method makes it possible to conclude that an oestrogen has been used at some earlier time. The use of natural steroid oestrogens, in combination with testosterone or progesterone, leads only to slight histological modifications. The phyto-oestrogens may also have some effect and lead to modifications; this is the case of Fusariuminfected maize.

b. The biological methods used are those of Astwood (26), which measures the increase in uterus weight of pre-puberal mice and rats, and of Allen and Doisy (25), based on modifications of the cytology of the vagina of adult mice after ovarectomy. The latter test, applied by Stob *et al.* (47) in 1954, is the most sensitive, but it is responsive only to compounds with oestrogenic activity. Martin (40) reports that the use of concentrated extracts placed in the vagina of mice also gives good results.

c. Physico-chemical methods which make it possible to identify with precision the anabolic agent sought for, require a fairly long extraction, followed by thin-layer or gaseous-phase chromatography. Hans and Abraham (31) report that the latter techniques, combined with mass spectometry, can now be contemplated. Sensitivity is high. Further off is the possible use of liquid/liquid chromatography. Thin-layer chromatography is currently used, and Verbeke gives details in an EEC document (50), while Waldschmidt (52) and Schuller and Stephany (46) have described the method.

d. Radio-immunoassay, first described for the oestrogenic steroids by Jiang and Ryan (38), is based on competition between tritium-labelled and unlabelled antigens for the corresponding specific antibodies. This technique, increasingly used, is highly sensitive, making it possible to detect 0.05 to 0.005 ppb of DES and 0.1 ppb of TBA. It is, however, complex and long, although it is being improved. It has the same advantages as all uses of radioactive compounds. The technique has been well studied by Hoffmann (34) and Hoffmann and Karg (35).

Two new methods may also be mentioned: high-pressure liquid chromatography which, according to Richou-Bac and Pantaléon (42) does not appear to be sufficiently sensitive, and the so-called Elisa method, applied by Ruitenberg *et al.* (44) in the Netherlands to detect pig infection by *Trichinella spiralis*. This method, which uses an enzyme for labelling, followed by measurement with 450 nm spectrophotometry, needs further development, but it may be possible to automatize its use. Kroes, working at Bilthoven (Netherlands) is studying how it can be used to measure hormone levels.

Table I, completed from an original kindly furnished by Richou-Bac, indicates the sensitivity of all these new methods.

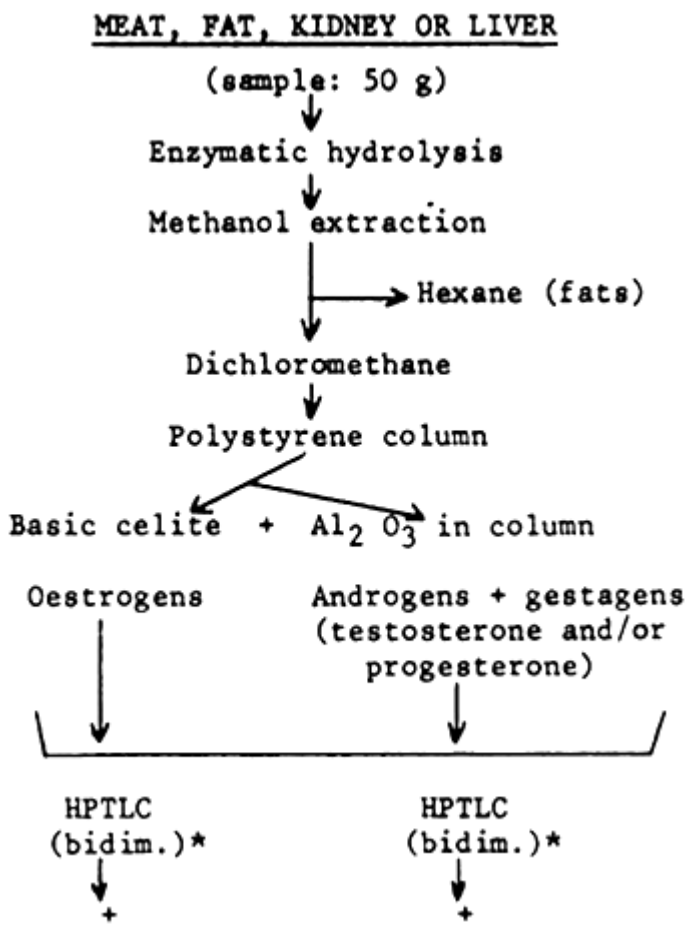
TABLE I. Sensitivity of techniques for testing for "hormones" and anabolic agents (ppb)

Substance	Radio-immunoassay				Thin-layer chromatography			Biological		Other
	Muscle	Liver	Urine	Plasma	Meat	Urine	Faeces	Astwood	Histology (prostate)	
DES	0.05–0.09	0.05–0.09		0.2	0.5 to 20	6	10	5 (oral)	+ + + +	High-pressure
Hexoestrol	0.04			0.03	0.5 to 80			-	+ + +	liquid chromatography

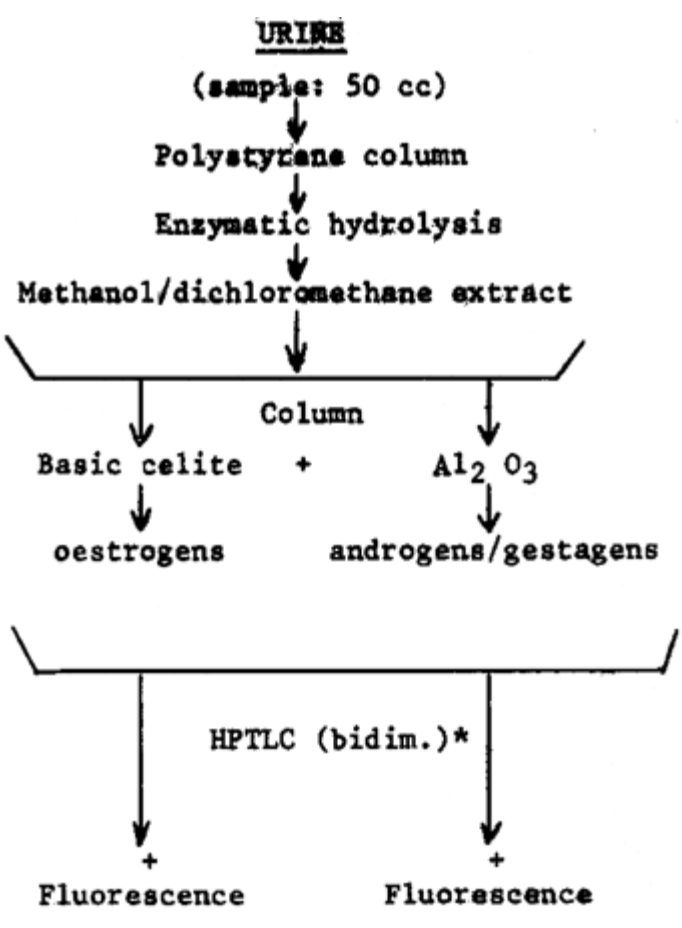
Dienoestrol	-	-		-	0.5 to 40			-	+ +	Elisa method
Ethynyl-oestradiol	-	-		0.02	4 to 40			5	+ + + +	
Zeranol					3 to 80			+ (subcutaneous)	+ + + +	
Zearalenone					3					
Oestradiol-17β	<0.05	<0.05	0.1	0.02	1	10		20	+ +	
Oestradiol-17α	-	-		0.02	0.5	10		?	?	
Oestrone	≤0.5	≤0.5		0.02	2			40	+	
Oestriol	0.05 to 1	0.05 to 1		-						
Oestradiol-17β + Progesterone									+ +	
Progesterone	?	?		0.05						
Medroxy-progesterone					(1 to 10					
α orβ-methyl progesterone					2 to 10					
Testosterone	?	?	≤0.02	≤0.02	2 to 8					
19-Nortestosterone				0.02						
Methyl testosterone				-	0.5 to 4					
Trenbolone	0.1	0.1		0.02	0.2 to 10					
Trenbolone + oestradiol-17β									+	
1–6 or 5 Dehydrotestosterone	0	0			1 to 3					

Figure A

Figure B



Source: V erbeke (50)



Source: V erbeke (49)

Figure C

URINE

(sample: 20 ml, diluted with 10 ml H_2O)

↓

Hydrolysis

↓

Ether extraction and washing of ether phase

↓

Silica gel column

↓

Eluate evaporated in N atmosphere
and collected in µl acetone

↓

HPTLC (bidim.)*

↓

Observation under UV 366 nm
(pink spot reveals presence of DES)

Source: French veterinary services.

* High-performance thin-layer chromatography (bi-dimensional).

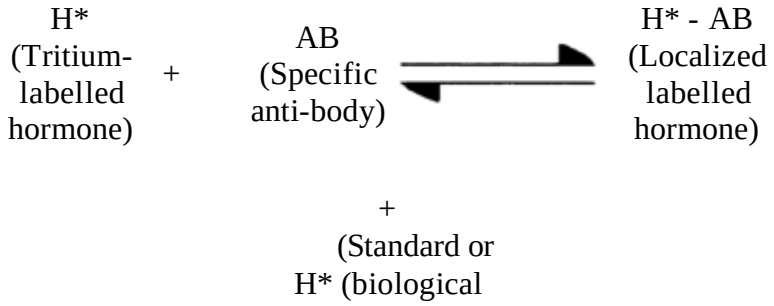
In practice, the most urgent problem appears to be the detection of DES. Bories *et al.* (27) and other authors have shown that it is eliminated in the urine and faeces. It has also been demonstrated (30) that the saliva does not contain DES but that it does contain progesterone. DES cannot be detected in the flesh of animals treated three weeks before slaughter. It can be detected in the urine after one month by thin-layer chromatography and radio-immunoassay.

The bladder need only contain from 20 to 50 ml of urine for the thin-layer chromatographic method to be applied. The method, used in 1973 (29) to detect oestradiol residues, was modified and improved by Verbeke (50) in 1979. Vogt and Oehrle (51) have employed it for detecting steroid oestrogen residues and Stilbene in calves' urine. Ryan and Hoffmann (22) use it, together with radio-immunoassay, to study linked trenbolone residues.

For the latter compound, according to Richou-Bac and Pantaléon (42), Schuller and Stephany believe it possible to use thin-layer chromatography to attain a sensitivity level of 0.005 ppb, comparable to that for radioimmunoassay; this figure has not been included in Table I.

Figures A and B show, respectively, outlines of Verbeke's analytical methods for meat, fat and viscera and for urine (49, 50). Figure C shows the outline of a technique for urine used in the laboratories of the French veterinary services for the detection of DES.

Radio-immunoassay is based on competition between labelled and unlabelled antigens, i.e. a labelled hormone (indicated below by H*) and an unlabelled hormone (H), for the same antibody molecule. The diagram below shows the process of the reaction.



(Tritium-labelled hormone)

H – AC

(Localized unlabelled hormone)

The levels of AB and H* being constant, any increase in H provokes a reduction in the labelled hormone localized in the specific antibody (H* - AB). The measurement, established by calculating the relationship

$$\frac{H^* - AB}{H^*}$$

depends on the measurement of radioactivity levels between the localized labelled hormone and the free hormone.

Kroes *et al*. (39) and Richou-Bac and Pantaléon (42) give details of extraction and measurement techniques. Hoffmann and Laschuetza (37) have used the method to examine blood plasma and flesh for DES.

The hormone is rendered antigenic by localization on seric albumen. Labelling the hormone should in no case alter the molecule. The specificity of immunoassay depends on the intrinsic characteristics of the antibody and the efficiency of the hormone purification method. Many techniques exist for separating the free H*, but immunoprecipitation appears to be the method most commonly used. The immunological behaviour of the hormone present in its matrix should be identical with that of the reference standard.

Authors such as Exley *et al*. (28), Hoffmann and Karg (35) and Heinritzi (32) have used this method for the natural oestrogens, Abraham *et al*. (24) and Rombauts *et al*. (43) for DES, and Pottier *et al*. (41), Heitzman and Harwood (33) and Hoffmann and Oettel (36) for TBA.

It is not yet clear what techniques are the most exact and economical and are therefore practical for large-scale control.

The basic material which can be inspected also needs to be determined.

Flesh, fat and viscera cannot be used for DES, which can be identified only in the urine, with a sensitivity of about 6 ppb. Many analyses can be made by thin-layer chromatography and radio-immunoassay. The French control services use only the former, estimating the cost of one analysis at 25 FFr (= approx. US $5). The possibility of techniques applicable to flesh is under study, but in this regard the techniques speak of

"chemical torture". They concede that routine control of meat for all hormones appears to be difficult.

The same services also use urine for measuring natural hormones, applying Verbeke's technique (50). No results are obtained from flesh, even for natural hormones, over 3 weeks following treatment, levels being too low. Thus, as compared with untreated calves, thin-layer chromatography yields no information.

Tables 1 through 4 of ref. 50 show sensitivity levels for the various hormones, according to the solvent system used, as determined by Verbeke, and also the RF values and spot colours under UV fluorescence at 366 nm.

It can be concluded that routine control in flesh is very difficult in all cases, whatever the anabolic agent. Above all, the use of DES should be avoided, and for this purpose, using urine as the basic material, high performance bidimensional thin-layer chromatography is the method to be preferred.

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CURRENT NATIONAL LEGISLATION RELATING TO THE USE OF CERTAIN HORMONES IN ANIMAL PRODUCTION

An annotated chronological index of legislative and regulatory provisions of various countries

(FAO Legislation Branch, Legal Office)

INTRODUCTION

In view of the concern expressed by the Intergovernmental Group on Meat in December 1980 with regard to the use of hormones, the Director-General of FAO requested the Legal Office of the Organization to prepare a short annotated chronological index of legislation on the subject, and to submit it to the Joint FAO/WHO Expert Committee on Food Additives at its 25th Session, held in Geneva in March-April 1981. The Animal, Plant and Food Legislation Section of the Legislation Branch, Legal Office, prepared the index, which contains references to legislation enacted or amended in certain countries over the last two decades, the text of which has been received by, or brought to the attention of, FAO Headquarters, Rome.

The Index does not purport to be exhaustive: the selection of countries and of legislative provisions was determined solely by the availability of documentation. In federal countries, state or provincial legislations are cited as examples only. It will be recalled that important legislation on this subject is currently published or abstracted within the general framework of FAO's *Food and Agricultural Legislation* (FAL), which appears twice yearly.

AFRICA

CONGO

Decree No. 63–393, regulating the production and marketing of compound feeds in the territory of the Republic of Congo. - 30.XI.1963 - *Journal officiel* No. 28, 15.XII.1963, p. 1021.

Only four categories of "auxiliary substances", not including hormones, may be added to feeds with a view to enhancing yields or protecting animals against stated diseases.

KENYA

Legal Notice No. 213: The Meat Control (Export Slaughterhouse) Regulations 1973. - 6.X.1973 - *Kenya Gazette* No. 50, 2.XI.1973, Supplement No. 75 (Legislative Supplement No. 56), p. 467.

Schedule B-F (3) prohibits the export of animals which have received oestrogen hormones within times prior to slaughter determined by the importing country.

MOROCCO

Decree No. 2-63-253, prohibiting the use of arsenicals, antimonials and oestrogens in the feeding or raising of specified animals. - 22.VII.1963 - *Bulletin officiel* No. 2649, 2.VIII.1963, p. 1240.

Concerns animals the flesh or products of which are consumed by man. Feeds intended for these animals, and foodstuffs obtained from them, may not be held for sale, placed on sale or sold if they contain, *inter alia*, oestrogens other than those administered for therapeutic purposes.

TUNISIA

Order of the Ministers of Agriculture and Public Health, regulating the use of oestrogens in veterinary medicine. - 26.XII.1980 - *Journal officiel de la République tunisienne* No. 78, 30-31.XII.1980, p. 3382.

Prohibits the administration of oestrogens to animals the flesh and products of which are intended for use as food, except as prescribed and administered exclusively by authorized veterinarians and for curative purposes for adult female animals with a view to controlling their oestral cycle. See full text in FAO's *Food and agricultural legislation*, Vol. XXX, No. 2.

ZIMBABWE

The Farm Feeds (Amendment) Regulations, 1970 (No. 1), made in terms of Section 24 of the Fertilizers, Farm Feeds, Seeds and Remedies Act and notified by Rhodesia Government Notice No. 306 of 1970. - (Undated) - Supplement to *Rhodesia Government Gazette* No. 18, 24.II.1971, p. 1341.

Constituents claimed to have growth-stimulating properties may be registered at the discretion of the Registering Officer, acting on the advice of the competent animal husbandry and veterinary authorities. In the case of hormones, the content of the active constituent must be stated in any such application for registration as well as in the labelling.

AMERICA, NORTH, CENTRAL AND SOUTH

ARGENTINA

Decree No. 4224, concerning the treatment of livestock with oestrogens. - 26.V.1961 - *Boletín Oficial* No. 19.545, 2.VI.1961, p.1.

Prohibits the treatment of livestock by whatever means with oestrogenic substances as growth stimulants. The therapeutic use of oestrogens in livestock for export will be covered by special regulations.

BARBADOS

Statutory Instruments 1970, No. 212: The Health Services (Control of Drugs) Regulations 1970. - Supplement to the *Official Gazette* No. 87, 29.X.1970.

Reg. 9, (5) and (6), provide that the milk or flesh of an animal to which certain drugs, including six hormones has been administered shall not be sold or supplied for human consumption except under a labelling bearing the appropriate instruction or direction.

BRAZIL

Decree No. 57 824, approving the Regulations for the industrial, food quality and sanitary inspection of products intended as feed for domestic animals. - 18.IX.1965 -*Diário Oficial,* Section 1, Part 1, No. 222, 22.XI.1965, P. 11.871.

Article 4 (4) prohibits, subject to the current conditions under law, the addition of hormones to feedstuffs.

CANADA

The Feeds Regulations (SOR/65-280). - 1.VI.1967 - *Canada Gazette* II, Vol. 101, No. 11, 14.VI.1967, p. 922 (as amended, *inter alia*, by SOR/73-258 of 22.V.1973 and SOR/77-144 of 11.II.1979 - *C.G.* II, Vol. 107, No. 11, 13.VI.1973 and Vol. 111, No. 4, 23.II.1977).

Regulate the importation, registration, standardization, etc., of "medicated feeds", expression which includes,*inter alia*, "a hormone the function of which is to promote growth in animal body". No feed may contain DES.

- (British Columbia)

The Veterinary Drugs and Medicated Feed Regulation (No. 808/74), made under the Pharmacy Act by Order in Council 3857. - 4.XII.1974 -*The British Columbia Gazette* II, Vol. 17, No. 26, 24.XII.1974, p. 1228.

DES is removed from the table of permitted feed additives.

COSTA RICA

Executive Decree No. 2769-A-SPPS. - 12.I.1973 - *La Gaceta* No. 16, 24.I.1973, p.349.

Prohibits the importation, processing and use of DES as part or ingredient of, or as additive in, feed mixes for livestock the meat or byproducts of which can be used for human consumption.

MEXICO

Regulations for the control of biological, pharmaceutical and food products for livestock. - 15.IV.1963 - *Diario Oficial*, No. 24, 29.V.1963, p.1.

Regulation 7 subjects hormone preparations, among other biological substances, to the control prescribed by the regulations.

PERU

Ministerial Resolution prohibiting the importation and sale of hormones for fattening poultry. - 13.IX.1960 - *El Peruano*, No. 5822, 16.IX.1960, p.1.

Prohibits the importation into, and the sale in, Peru, of oestrogenic hormones for this purpose.

UNITED STATES OF AMERICA

The Animal Drug Amendments of 1968 to the Federal Food Drug and Cosmetic Act (Public Law 90-399). - 13.VII.1968 - 62 *Stat.* 351.

A new Sec. 512 (k) relates to approval of animal feeds containing "new animal drugs".

- Order revoking all New Animal Drug Applications (NADAs) for the manufacture of DES premixes. - 4.VIII.1972 - *Federal Register*, Vol. 37, p. 15747.
- Order revoking the regulations which allowed the use of DES implants alone or in combination with testosterone.
  - 27.IV.1973 - *F.R.*, Vol. 38, p. 10926.

Note: Both these Orders were revoked on 24 June 1974 by the U.S. Court of Appeals for the District of Columbia Circuit. The production, sale and use (oral use and implants) of DES could accordingly be resumed.

URUGUAY

Decree regulating the prohibition and use of oestrogens for the sexual sterilization and fattening of animals the meat and by-products of which are intended for human consumption. - 5.IV.1962 - *Diario Oficial,* Vol. 227, No. 16390, 8.V.1962, p. 186 A.

Prohibits the production, importation, sale or use of oestrogens for these purposes. The prohibition does not extend to therapeutic purposes under certain conditions. The possession, sale or distribution of fresh or preserved meat or any products whatever of animals having been treated with natural or synthetic oestrogens is also prohibited.

ASIA

JORDAN

Ordinance No. 7 of 1961 in application of the Animal Diseases Act. - (undated) - *Official Gazette,* No. 1572, 19.IX.1961, p. 1254.

Prohibits the use of any natural or synthetic hormonal substances intended to promote growth or to sterilize animals the flesh of which is intended for human consumption. Also prohibits the sale and export of such meat and of milk and milk products derived from animals so treated, including domestic animals and live poultry intended for food or for sale.

LEBANON

Ordinance No. 46/1 of the Ministry of Agriculture, prohibiting the introduction of concentrated feedstuffs containing DES and similar hormones. - 21.II.1973 -*Official Gazette,* No. 19, 5.III.1973, p. 151.

PHILIPPINES

Administrative Order No. 194 of 1973 (Office of the Secretary, Department of Health), prohibiting the use of DES. - 18.IX.1973 - *Official Gazette* Vol. 69, No. 42, 1.X.1973, p.9964.

The ban concerns the rearing of farm animals and poultry and extends to the production of DES premixes.

EUROPE

(EEC Countries)

EUROPEAN COMMUNITIES

Council Directive (70/524/EEC) concerning additives in feedstuffs. - 23.XI.1970 - *Official Journal of the European Communities* No. L 270/1, 14.XII.1970, p.840.

The Annex contains a positive list of feed additives. The use of other feed additives may, on a temporary basis (up to 1975), also be permitted by Member States, except, however, in the case of substances "having a hormonal or anti-hormonal effect"*.

BELGIUM

Crown Order declaring meat, fat and offal, and poultry meat and edible offal obtained from animals to which hormone or anti-hormone preparations have been administered, to be unwholesome. - 3.IX.1973 - *Moniteur belge* No. 202, 18.X.1973, p. 11788.

Since the administration of these preparations may cause alterations in the product, the Minister for Health may prescribe laboratory techniques designed to reveal such alterations and may approve laboratories for the relevant tests.

* According to a press report (*The Guardian, Frankfurter Allgemeine,* 8 January 1981), the EEC Commission is considering a specific ban on the use of hormones in animal feeding, with the proviso that excepted hormone use shall be subject to precise veterinary instructions.

- Crown Order relative to certain operations concerning substances having a hormonal anti-hormonal or antibiotic action. - 12.IV.1974 -*M.B.* No. 87, 7.V.1974, p. 6592.

Imports and exports of these substances are subject to general authorization and the obligation of prior notification. The general authorization requirement, and a record-

keeping obligation, apply to their manufacture and to holding for industrial manufacture, and wholesale marketing. In other cases of holding, and for their transport, sale, offer for sale, consignment or acquisition, the general authorization is sufficient.

DENMARK

Order No. 369, prohibiting the use of thyreostatic preparations intended for the fattening of domestic animals. 23.IX.1965 - *Lovtidende A* XXIII, 27.IX.1965.

- Order No. 496, restricting the use of medicaments for domestic animals. - 28.IX.1978 - *Lt. A* 51, 26.X.1978, p. 1692.

Seven hormones and similar products are included among the medicaments which may be used only by veterinarians.

FRANCE

Order of the Minister for Agriculture, giving the list of countries which have prohibited the use of the substances contemplated in Article 1 of Decree No. 62-827 of 21 July 1962. - 12.VIII.1976 - *J.O.* No. 201, 28.VIII.1976, p. 5212.

Lists 23 countries which have prohibited the use, in poultry raising and feeding, of arsenicals, antimonials and oestrogens. The Decree cited prohibits, under all customs regimes other than transit, the importation of poultry products originating in or shipped from countries where the use of these substances is not prohibited.

- Act No. 76-1067, prohibiting the use of oestrogens in veterinary medicine. - 27.XI.1976 - *J.O.* No. 278, 28.XI.1976, p. 6335.

Prohibits the administration of oestrogens to animals whose meat or products are intended as food, save for oestrus control in adult females. Food of animal origin containing oestrogens (whether or not steroids), at all levels exceeding those to be prescribed by decree taking into account normal physiological levels, are to be banned as food.

- Order prohibiting the use of oestrogens in veterinary medicine. - 2.II. 1978 -*J.O.* 25.II.1978, No. 48, p. 825.

Made under Act No. 76-1067 cited above. Animal products and products of animal origin, intended for food, may not contain synthetic oestrogens (DES, dienoestrol, hexoestrol and their derivatives, and ethynyloestradiol). The maximum rate of incorporation of natural oestrogens (oestradiol, oestrone and their derivatives) in these products is prescribed at 0.01 mg/kg in animals of reproductive age and at 0.0002 mg/kg in calves and other young animals.

- Order relating to withdrawal from consumption of meat and offal from slaughter animals to which prohibited anabolic preparations have been administered. - 20.X.1980 - *J.O.* No. 249, 24.X.1980, p. 2476.

Proof of such administration may be constituted by evidence of illicit implants or suspect residues in any tissues, secretions or excreta.

GERMANY (F.R.)

Ordinance concerning substances having a pharmacological effect. - 3.VIII.1977. - *Bundesgesetzblatt,* Part I, No. 53, 10.VIII.1977, p. 1479.

Under Article 1 and the Schedule, it is prohibited to administer oestrogens (in particular the stilbenes) and their derivatives, salts and esters, to livestock. Article 2, however, authorizes, under certain conditions, the administration of substances having an oestrogenic, androgenic or gestogenic effect and having received type approval to animals from which foodstuffs are obtained.

GREECE

Crown Decree No. 176, prohibiting the release for human consumption of meat from oestrogen-treated livestock, including poultry. - 24.II.1968 - *Ephemeris tes Kuberneseos* I, No. 48, 11.III.1968, p. 416.

Applies to both home and imported meats. Oestrogen treatment is understood to mean both the introduction of these products into the animal by whatever means (injection, implantation) and their administration, alone or mixed in feeds, with a view to influencing meat yield and/or the lean/fat composition thereof.

IRELAND

The Animal Remedies (Control of Oestrogenic Substances) Regulations, 1962, made under Section 7 of the Animal Remedies Act, 1956 (No. 41 of 1956). - I.VI.1962 - *S.I.* No. 96 of 1962.

Prohibit the manufacture, preparation, packing or sale of 12 substances specified in the Schedule and of any oestrogenic substance or preparation, natural or synthetic, which, on being administered to animals or incorporated in feeds, produces oestrogenic effects.

ITALY

Law No. 3, prohibiting the use of oestrogens as growth stimulants or sex inhibitors in animals whose meat or products are intended for human consumption. - 3.II.1961 - *Gazzetta Ufficiale* No. 43, 18.II.1961, p. 714.

Prohibits the use of synthetic and natural oestrogens as growth stimulants or sex inhibitors; the prohibition also covers poultry and other farm animals marketed live, as well as imported products.

- Ministerial Decree prohibiting the holding by stockbreeders, or the administration by them to animals, of substances having a hormonal or anti-hormonal action. - 15.I.1969 - *C.U.* No. 16, 20.I.1969, p. 366.

Prohibits the holding or administration, under any form whatsoever, of substances having a hormonal action (e.g., oestrogens, androgens, progestins) or anti-hormonal action (e.g. thyreostatics).

LUXEMBOURG

Grand-Ducal Regulation issuing revised rules governing certain substances intended for use in animal feeding. - 28.I.1971*Mémorial A*, No. 9, 12.II.1971, p. 78.

Prohibits the import, transport for sale, and sale of feedstuffs containing hormones and anti-hormones, and of foodstuffs obtained from animals to which they have been administered. The presence of residues of such substances in food renders the latter unfit for consumption.

NETHERLANDS

Act No. 363, regulating trade in antibiotics, hormones, thyreostatics and chemotherapeutical products intended for or susceptible of use with livestock. - 1.VIII.1964 - *Staatsblad* 1964, p. 914.

Such products and preparations may be sold only to persons prescribed (e.g., veterinarians), and to approved veterinary establishments.

- Order No. J. 3328 (as amended by Order of 28.XII.1964), implementing Act Stb1. 363 of 1 August 1964 (see above). *Staatscourant* Nos. 239/1964 and 1/1965.

UNITED KINGDOM

Four sections of the Medicines Act 1968 respecting medicated animal feedstuffs, viz. Sec. 40 (general provisions), 42 (supplementary provisions), 62 (prohibition of sale, supply of importation) and 90 (labelling, marketing, leaflets, containers, etc.). - 25.X.1968 -*Eliz.* 2, ch. 6.

These provisions also apply, under the Medicines (Feeding Stuffs Additives) Order 1975 (S.I. 1975, No. 1349), to non-medicinal substances or articles incorporated in feeds for medicinal purposes.

- The Medicines (Labelling of Medicated Animal Feeding Stuffs) Regulations, 1973. - 29.VIII.1973 - *S.I.* 1973, No. 1530.

Detailed labelling provisions. Also regulate bulk sale or supply under specified circumstances, etc.

EUROPE

(Other Countries)

FINLAND

Resolution of the Board of Agriculture, No. 44/221-68, made under the Feeds and Fertilizers Act No. 335/68, as amended, concerning the buyer's notification of the quality of trace elements, vitamins, hormones and similar preparations, pharmaceutical substances and substances to be considered as poisons in feeds, etc. - 4.III.1969.

- Ordinance No. 281 on feed additives. - 2.V.1969 - *Finlands Författningssamling* No. 275-281, 8.V.1969, p. 507.

Prohibits the use of hormones in feeds.

POLAND

Order (Text No. 352) of the Minister for Agriculture amending Order (Text No. 114) of 5.XI.1952 on the control of certain animal feedstuffs. - 11.X.1962. - *Monitor Polski* No. 75, 20.X.1962, p. 632.

Prohibits the addition of hormonal substances to animal feeds.

SPAIN

Order prohibiting the use of arsenicals, antimonials and oestrogens in the preparation of compound feeds for use in poultry breeding, and the trade in and sale of eggs, birds and poultry for consumption when imported from countries that do not prohibit the use of such substances. - 4.III.1964 - *Boletín Oficial* No. 69, 20.III.1964, p. 3677.

Also prohibits the marketing of home-produced poultry (and eggs thereof) fed with these substances. For all eggs and poultry, in particular those imported, the absence of any hormonal treatment must be certified previous to shipment.

- Resolution of the Agricultural Production Department regulating the use of hormones in animal production. - 7.VII.1980 - *Boletín Oficial* No. 174, 21.VII.1980, p. 16 550.

Marketing and use are subject to veterinary prescription or control. Hormone preparations must be type-approved before they may be marketed.

SWITZERLAND

Order of the Federal Council amending the Federal Meat Inspection Ordinance. - 21.I.1970 - *Recueil des lois fédérales* No. 7, 20.II.1970, p. 160.

No substance or product liable to have an inadmissible effect on the condition or keeping quality of meat (in particular oestrogenic or thyreostatic substances) may be administered to slaughter animals.

YUGOSLAVIA

Feed Quality Regulations. - 3.IV.1978 -*Sluzbeni List* SFRJ No. 31, 9.VI.1978, Text No. 479, p. 1319.

Prohibits the addition to feeds of hormones, sedatives, thyreostatics or similar substances.

- Act on the wholesomeness of foodstuffs and articles of everyday use. - 3.X.1978 -*S.L.* SFRJ No. 55, 13.X.1978, Text No. 845, p. 220.

Hormones as well as other substances likely to have a prejudicial effect on the health of the consumer, if present in any food, render that food unwholesome. The authorities will establish permitted levels of hormones and other specified substances, as well as other conditions respecting wholesomeness, required for foods marketed in Yugoslavia.

OCEANIA

AUSTRALIA (SOUTH AUSTRALIA)

Amendments to the Stock Foods Regulations 1967. - 28.XI.1968 - *South Australian Government Gazette* No. 54, 28.XI.1968, p. 2325.

Schedule One prohibits the presence of anabolic agents and natural and synthetic hormones in any animal feed.

NEW ZEALAND

The Stock (Insecticides and Oestrogens) Regulations, 1961, as amended by Amendment No. 1, 1963 (*SR* 1961/101, 30.VIII.1961 and 1963/128, 10.VII.1963).

Except as specified, stock (cattle, sheep, or swine, of any age or sex) may not be treated with or exposed to any insecticide or oestrogen, nor may these substances be used in any slaughtering place, meat-packing house or cannery. No stock exposed or treated in accordance with regulations may be slaughtered or sold for slaughter for human consumption during the following 30 days.

- The Stock Remedies (Biochemical Substances) Regulation (Reprint of the 1951 Regulations). - 11.IV.1967 - *Statutory Rules* 1967/81.

The Second Schedule restricts the sale, dispensing and prescribing of hormones, as well as any preparation containing any hormone as a biochemical substance for the treatment of stock.

- The Animal Remedies Act 1967. - 16.XI.1967 - *Act* No. 51/1967.

Consolidates and amends the Stock Remedies Act 1934. Hormones are included in the definition of "animal remedies" and "biochemical substances", the manufacture, importation, sale and use of which are controlled.

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