A. Ü. Vet. Fak. Derg.
39 (1-2): 215-231, 1992

THE DETERMINATION OF DIETHYLSTILBESTROL (DES) IN THE FAECES AND TISSUES OF CHICKENS TREATED WITH DES AND IN THE FAECES AND TISSUE SAMPLES OF CALVES, LAMBS AND CHICKENS COLLECTED FROM VARIOUS AREAS OF TURKEY*

Ethem Ersoy[1] **Olaf Agthe**[2] **Hilal Ergun**[1]
Tevhide Sel[3] **Tayfun Güldür**[3]

Diethylstilbestrol (DES) verilen tavukların doku ve dışkılarında ve Türkiye'nin çeşitli bölgelerinden toplanan tavuk, kuzu ve dana doku numunelerinde ve dışkılarında DES tayini.

Özet: *Yedigün süreyle oral olarak 5 mg DES/gün verilen 20 deney ve 20 kontrol broilerlerin dışkı, kas, karaciğer ve böbrek dokularında DES analizleri yapıldı. Numuneler radioimmunoassay metoduyla analiz edildi. Dokulardan DES'in atılma süresi tesbit edildi. DES'in son uygulanmasından 5 gün sonra dışkıdaki konsantrasyonu 151 ppb olarak bulundu. Bununla beraber, son verilmeden 7 gün sonra DES konsantrasyonu tekrar yükseldi. Son DES uygulamasından sonra ilk gün karaciğer, kas ve böbrekteki DES konsantrasyonu sırasıyla 0.78, 0.74 ve 1.33 ppb iken denemenin sonunda 0.35, 0.24 ve 0.22 ppb olarak bulundu. Son gün değerleri kontrol değerleri sınırları içersindedir. Denemenin sonunda plazmada ki DES konsantrasyonunda bir artış gözlenmiştir.*

Türkiye'nin çeşitli bölgelerinden toplanmış dana, kuzu ve piliçlere ait kas, karaciğer, böbrek, dışkı ve yem numunelerinde toplam 1811 numunenin DES yönünden analizleri yapılmıştır. Piliç yem numunelerinde % 36.9 DES pozitif bulunmuştur. Piliç dışkı numunelerinde de % 1.9 pozitif numune tesbit edilmiştir. Diğer numunelerde DES bulunamamıştır.

* This study was carried out as part of the scientific link between Ankara University Faculty of Veterinary Medicine and Honnover Veterinary highschool and was supperted by the BMZ - GTZ and the Ankara University Research Fund.

1 Prof. Dr. Ankara University, Veterinary Faculty, Biochemistry Department, Ankara.

2 Prof. Dr. Fachhochschule Wilhelmshaven, West Germany.

3 Dr. Ankara University, Veterinary Faculty. Biochemistry Department Ankara.

Summary: *Diethylstilbestrol (DES) analyses were carried out on muscle, liver, kidney and faeces samples of* 20 *control and* 20 *experimental broilers to which* 5 *mg DES/day had been given orally for a period of* 7 *days. The treated samples were analysed using the Radioimmunoassay method. The removal time of DES from the tissues was determined. Five days following the final administration of DES, its faecal concentration was* 151 *ppb. However* 7 *days after the final administration faecal DES concentrations increased again. On the first day after the final DES administration, DES concentrations in the liver, muscle and kidney were* 0.78, 0.74 *and* 1.33 *ppb respectively. While these values measured on the final day were within the range of the control values. There was an increase of DES in plasma at the end of the experimental period.*

A total of 1811 *muscle, liver, kidney and faeces samples of calves, lambs and chickens and feed samples collected from various areas in Turkey were analysed for the presence of DES positive samples for chicken feed was* 36.9 %. *Also* 1.9 % *of the chicken faeces samples were DES positive. All other samples were negative for DES.*

Introduction

Diethylstilbestrol (DES) is a non-steroid synthetic, exogenous anabolic substance (5,10,21,23,27,29). It has been used for many years as a growth promoter in poultry, cattle and lambs by addition to the feed, injection or implantation (5,10,11,16,21,24,26). It affects feeding efficiency and growth rate ,when used at 5–29 % (17,24). This effect is produced by DES stimulating the production of Growth hormone, Insulin and Insuline like Growth Factor I (IGF–1) (22,23, 24,31).

Endogenous oestrogens, when given orally are largely metabolized during their first passage through the liver. DES, on the other hand is resistant to hepatic metabolism and when administered orally shows high oestrogenic activity (26,29). It is effective for a long period due to the enterohepatic circulation during its metabolism (15,20, 27,29,30).

The elimination of DES from the body occurs primarily by way of the faeces where residues are detected at the highest level and for the longest period. Therefore it has became important to screen for DES residues in the faeces (1,2,22,29).

Because of its easy administration and being the cheapest oestrogenic substance (21,29), DES is used according to the indication limits for treatment in human medicine (prostate cancer, breast cancer, osteoporosis etc.) (8,18,26,28) and veterinary medicine (pyometra, vaginitis, anoestrus, prostate hypertrophy etc.) in small animals (26,27).

DES was first used for anabolic purposes in chickens in 1947 but its use in these animals was prohibited in 1959 (7,19,26,32). It was first used in cattle in 1954 and was used in the U.S.A. as an anabolic substance by addition to feed until 1979 (23,26,32).

In Germany the percentage of DES positive samples rose from 4 % in 1977 to 16 % in 1978 and 40 % in 1979. After the discovery of DES in babyfoods strict controls with sensitive methods were introduced the result being a drop in DES-positive samples to 1 % in 1980 (3,22).

Several methods have been developed for the quantitative and qualitative determination of DES in materials of animal origin. In routine use, Radioimmunoassay (RIA) has proven to be the most sensitive and cheapest method for the fast detection of residue level in the nanogram and even picogram range in large series of samples (21).

With the introduction of sensitive techniques, DES residues were detected in tissue samples. The discovery of the teratogenic and carcinogenic effects of DES and other stilbene derivatives led to the prohibition of their use in animals used for food production in the USA and the EC countries (5,26,32). In EC countries production and marketing of DES are also prohibited (26, 32). Although the use of DES in the USA and EC countries has been prohibited for food producing animals, it is suspected that DES is being used secretly and illegally as an anabolic agent (3, 5, 21, 22, 23). Recently trace amounts of DES in meat exported to the USA from Germany have been discovered and reported upon (3).

In the EC countries the presence of illegal anabolic substances in live animals used for food production is monitored for in their urine or faeces and in tissue samples after slaughter using sensitive methods regularly (22). However there is no information regarding DES analyses in Turkey.

The use of hormone and antihormone preparations as food additives in Turkey is prohibited by law (4). But in veterinary medicine DES is being used as oestrogen preparation for treatment in food producing animals.

Therefore, the first aim of the study was to determine the removal time of DES from the various tissues of treated chickens and its detection limit by RIA.

Secondly the presence of DES in meat exported from or imported into Turkey and also the use of DES as anabolic substance in Turkey were monitored.

Material and Method

Materials

a) *Equipment*

Rack Beta Liquid scintillation counter 1211/1212 Instrument Manuel (LKB), Vacuum Drying oven (Heraeus) Centrifuge at 6000 rpm with cooling system (Heraeus), Magnetic stirrer (Janke and Kunkel, IKA labortechnik).

b) *Chemicals*

All chemicals used were analytical grade. Gelatine, Sodium azide (Natriumazid), Titriplex III, NaCl, NaOH, di-natriumhydrogenphosphate dihydrat, Kaliumdihydrogenphosphate, Chloroform, Ethanol, Diethylether and Methanol were obtained from Merck chemical company. Dextran T 70 was obtained from GmbH Co. Chem. Fabrik, Norit-A was obtained from Serva chemical company, Diethylstibestrol was obtained from Sigma chemical company.

DES Antibody was kindly provided by Prof. Dr. H. Karg, Institut für Physiologie der südd. Versuch. und Forschungsanstalt für Milch Wirtschaft Weinhenstephen-München. (Monoethyl-^{3}H) DES (250 u Ci/ml) was obtained from Amershan International plc. England. Scintillation coctails were obtained from J.T. Baker Company.

Experimental Procedure

The study was performed in two parts; as an experiment and as a monitoring across Turkey.

A) In the experiment; 40 broiler chickens eight weeks old were used, 20 of these chickens were kept as controls, the other 20 broilers were treated with 5 mg/ml in ethanol DES per day given orally. These chickens were treated with DES for 7 days. 1 ml of ethanol was administered to controls during DES administration period. The experiment was continued for 16 days and chickens were slaughtered at intervals from day 7 to day 16. Faeces samples were collected before DES administration and on days 1 to 16 following DES administration. Following slaughter plasma, liver, kidney and muscle tissues were collected and analysed by RIA for DES. Chickens were allowed free access to food and water throughout the experiment and placed in seperate cages.

B) For the monitoring; Muscle, liver, kidney and faeces samples of lambs, chickens and calves were collected from slaughter-houses; faeces and feed samples were collected from 50 farms. The samples were kept at −20°C. A total of 926 faeces samples (260 chicken, 238 lamb and 428 calf) were analysed for DES. DES analyses were also carried out on 245 chicken muscle, 220 chicken kidney, 181 chicken liver, 44 calf muscle and 195 feed samples.

Method

DES concentrations in muscle, liver and kidney tissues in feed and faeces samples were determined using the technique of Radioimmunoassay (RIA) (1,2). In brief, free DES obtained by diethylether extraction and various solvent-solvent partition steps was measured by RIA using radioactive DES and DES antibody. DES standards ranging from 12.5–800 pg/0.1 ml were prepared from a stock solution of DES in ethanol at a concentration of 800 mg/ml, the stock solution being prepared from 1 mg DES/ml ethanol. Results were obtained with the use of the standart curve.

Statistical Analysis

The significant differences between values obtained from the DES analyses was determined using the student "t" test (13).

Results

In the experimental study, DES concentrations in faeces samples collected after DES administration were found to be significantly high ($P < 0.001$) during the 16 days of the experimental period (Table 1). One day after the initial administration of DES, the con-

Table 1. Statistical evaluation of DES levels (ppb) of faeces in chickens treated with DES (5 mg DES/day).

	Days	n	$\bar{x}$	$S\bar{x}$	t
Control		55	2.15	0.31	—
DURING DES TREATMENT	1. Day	4	1375	875.00	-6.60***
	2. Day	5	5840	3211.11	-6.63***
	3. Day	5	15400	8602.17	-6.53***
	4. Day	5	9300	2119.03	-16.00***
	5. Day	5	17400	2912.86	-21.71***
	6. Day	3	12333	7584.93	-8.38***
AFTER DES TREATMENT	7. Day	4	12937	1466.20	-37.13***
	8. Day	2	1500	250.04	-43.59***
	9. Day	5	500	193.66	-9.37***
	10. Day	4	179	64.56	-11.48***
	11. Day	5	151	67.89	-7.98***
	12. Day	5	264	60.14	-15.84***
	13. Day	3	5550	450.01	-63.52***
	14. Day	5	5200	600.85	-31.54***
	15. Day	2	4475	1525.23	-21.36***
	16. Day	2	3025	275.04	-79.99 ***

*** $p < 0.001$.

centration of this substance in the faeces was 1375 ppb ($P < 0.001$). On the fifth day of DES administration the faecal concentration of this anabolic agent reached its highest level during the experimental period, 17.400 ppb ($p < 0.001$). From the 8 th day of the experiment, 2 days after the final administration of DES, faecal DES concentrations dropped dramatically to a minimum of 151 ppb on the 11th day, rising to a second maximum of 5550 ppb on day 13. On the final day of the experiment (day 16), faecal DES concentrations began to decrease once more with a value of 3025 ppb (Figure 1). The mean value for the control group not given DES was 2.152 ppb (Table 1). One day following the final DES administration DES concentrations in the liver. (Table 2 and Figure 2) and kidney (Table 2 and Figure 3) were found to be significantly high ($p < 0.001$). Although during the following days no significant amounts of DES were detected in the liver, in the kidney DES residues rose on day 4 ($P < 0.05$) and day 6 following the final DES administration ($p < 0.01$). One day following the final DES administration, DES concentrations in the muscle samples were 0,738 ppb though this value was not significantly higher than the control value of 0.410 ppb when analysed statistically (Table 2, Figure 4). Plasma DES levels remained near the control value between days 7 and 13 of the experiment (i.e. 1 to 7 days after the fi-

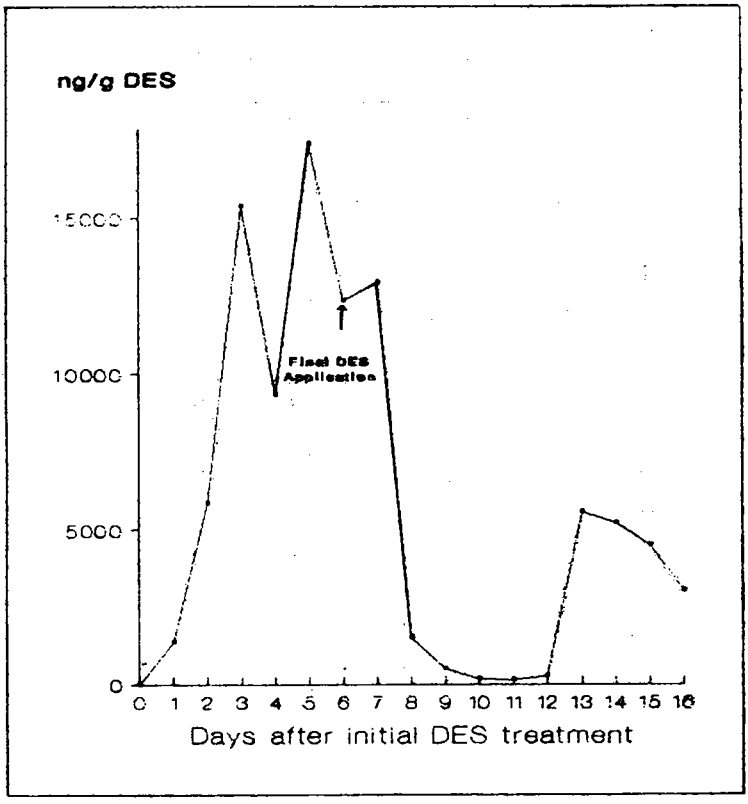

Figure 1. Excretion of DES in faeces of chickens after oral application of 5 mg DES/day for a 7 day period.

nal administration of DES) increasing to 1.46 ppb ($P < 0.01$) on day 16 (Figure 5). In the control group not administered DES, DES concentrations in the muscle, liver, kidney and plasma samples were 0.410, 0.278, 0.150 and 0.324 ppb respectively (Table 2). The detection limith of DES positive samples using RIA are given in Table 3.

The results of analyses caried out on faeces, muscle, liver and kidney samples of calves, lambs and chickens and on chicken feed samples collected from various areas of Turkey are shown in Tables 4, 5, 6, 7. A total of 195 feed samples were analysed for DES and 36.9 % of the samples were found to be positive (Table 7). These feed samples were sent to Germany (Staatliches Chemisches Untersuc-

Table 2. Statistical evaluation of DES levels (ppb) of liver, muscle, kidney tissues and plasma in chickens treated with DES (5 mg DES/day).

	LIVER				MUSCLE				KIDNEY				PLASMA			
	n	$\bar{x}$	$S\bar{x}$	t	n	$\bar{x}$	$S\bar{x}$	t	n	$\bar{x}$	$S\bar{x}$	t	n	$\bar{x}$	$S\bar{x}$	t
Control	40	0.28	0.07	—	37	0.41	0.05	—	38	0.15	0.02		20	0.32	0.09	—
1st day	4	0.78	0.05	—5.756**	4	0.34	0.34	—1.77	4	1.33	0.19	—15.47***	2	0.32	0.16	0
4thday	6	0.36	0.09	—1.101	6	0.39	0.09	+0.14	6	0.26	0.03	—2.49*	3	0.34	0.15	—0.074
5th day	6	0.38	0.08	—1.313	6	0.31	0.04	+0.82	6	0.18	0.02	—0.76	3	0.28	0.16	+0.165
6th day	6	0.29	0.04	—0.126	6	0.45	0.10	—0.28	6	0.16	0.02	—0.30	3	0.33	0.14	—0.037
7th day	6	0.34	0.11	—0.747	6	0.41	0.06	0	5	0.25	0.06	— 2.0	2	0.49	0.05	—0.553
8th day	6	0.31	0.04	—0.474	5	0.44	0.13	—0.18	6	0.33	0.09	— 3.23*	3	0.71	0.37	—1.435
10th day	4	0.35	0.03	—0.869	4	0.24	0.02	+1.11	4	0.23	0.02	— 1.44	2	1.46	0.94	—3.112***

*** $p < 0.001$
** $p < 0.01$
* $p < 0.05$

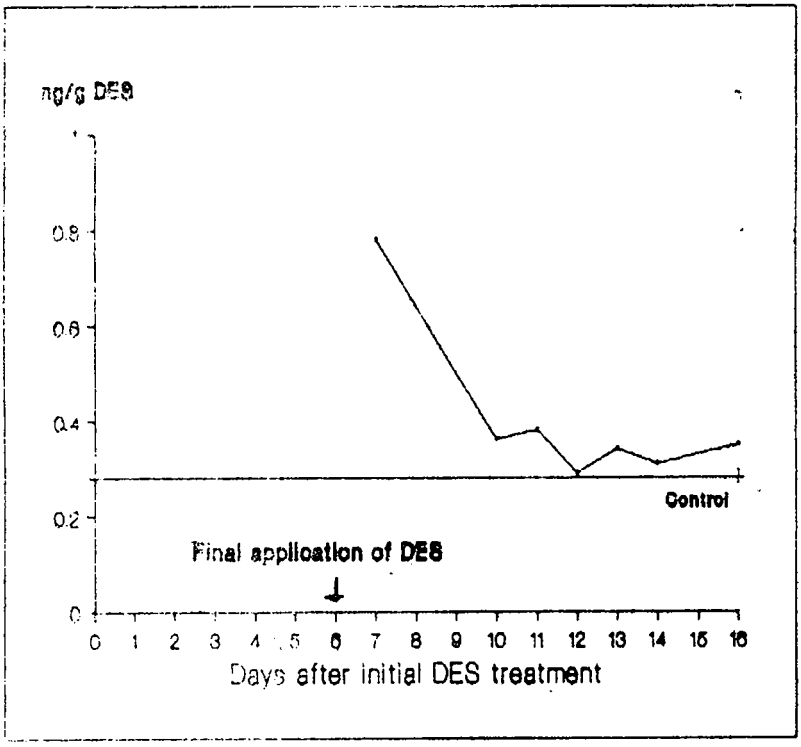

Figure 2. Removal of DES from the liver of slaughtered chickens after oral application of 5 mg DES for a 7 day period.

hungsamt and Staatliches Veterinäruntersuchungsamt, Oldenburg) where this result was confirmed by RIA and GC-MS (14). Of the positive chicken feed samples 59.7 % had a range between 10–50 ppb 23.6 % between 50–100 ppb and 16.7 % above 100 ppb DES (Table 7).

In the chicken faeces samples obtained from the counties of Bolu, Bursa and Erzurum, the percentage of DES - positive samples was 1.1 %, 4.4 % and 6.5 % respectively (Table 4). No DES - positive calf and lamb faeces samples were found (Table 5 and 6).

In the 245 muscle, 220 kidney and 181 liver samples of chickens collected by ministry of Health from 29 countries analysed no - DES positive samples were found. Forty - four calf muscle samples were also DES - negative.

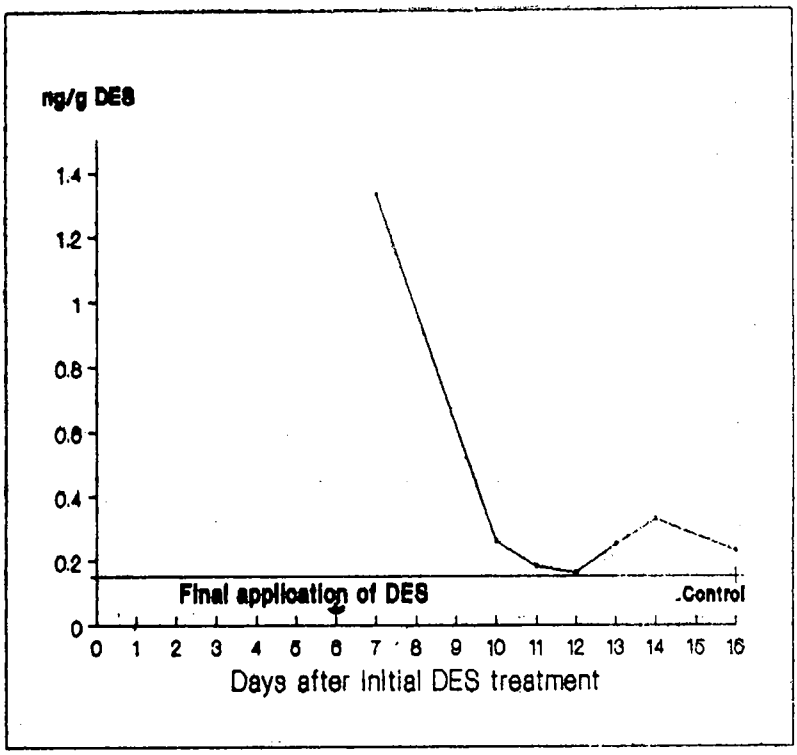

Figure 3. Removal of DES from the kidney of slaughtered chickens after oral application of 5 mg DES for a 7 day period.

Discussion and Conclusion

DES causes increases in body wieght and feeding efficiency (6, 7, 11, 16, 17, 24). Therefore meat production increases, thus the use of DES is economically viable. However due to its carcinogenic and teratogenic effects (5, 10, 19, 21, 23, 26, 32) it poses a risk for the consumers. DES is prohibited therefore it must not be found in any biological materials of animal origin.

In the experiment, on day 8 to 12 it appeared that DES had been removed from the chicken, however on day 13 there was a further dramatic increase in the faecal DES concentration (Figure 1). This may be due to the presence of metabolic intermediates of DES such as paraquinone (this being the precursor for the biosynthesis of dienestrol), or other DES metabolites such as dienestrol and w-hydroxydi-

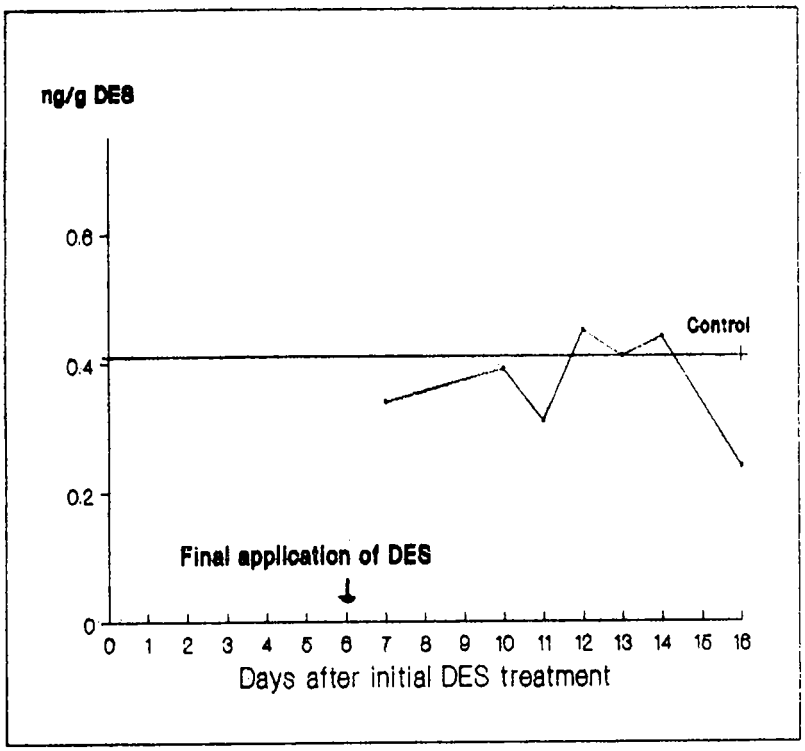

Figure 4. Removal of DES from the muscle of slaughtered chickens after oral application of 5 mg DES for a 7 day period.

enestrol which give cross reactions with DES (30) or of free DES, produced as a result of bacterial action on DES-glucuronide in the intestine (15, 27, 29). Therefore this apparent increase in faecal DES after day 13 (Figure 1) can be attributed to the enterohepatic circulation of DES and its metabolites, thereby prolonging the effects of these substances in the organism, as seen by the presence of DES in the kidney on days 7 ($p < 0{,}001$), 10 ($p < 0.05$) and 14 ($p < 0{,}05$) (Figure 3). DES concentrations in the liver on day 7 was 0.78 ppb, significantly higher ($p < 0{,}01$) than the control value of 0,278 ppb. Thereafter liver DES concentrations decreased to near the control value.

The liver plays an important role in DES metabolism. DES is metabolised in the liver and excreted via the urine and faeces (12,

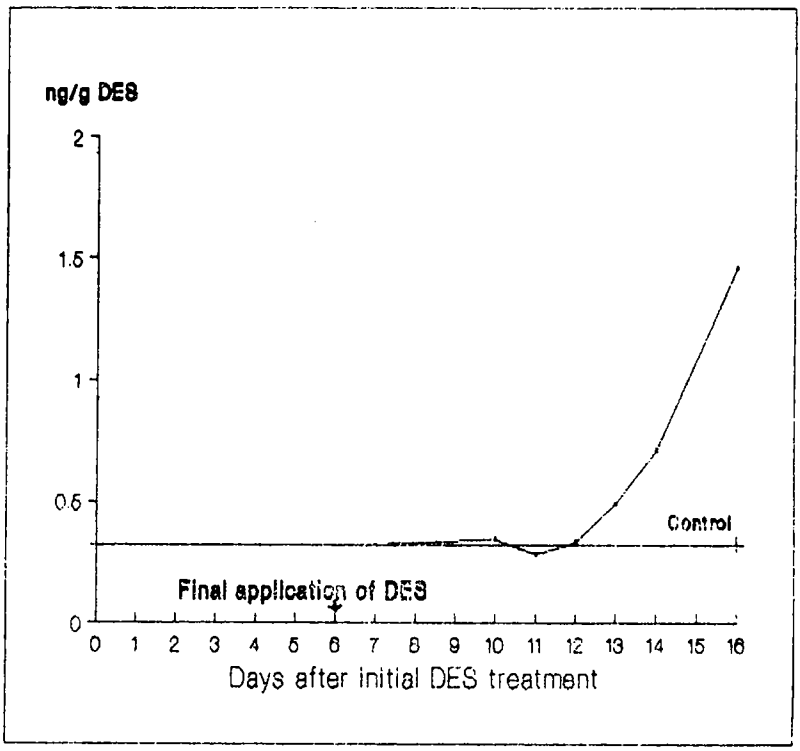

Figure 5. Removal of DES from the plasma of slaughtered chickens after oral application of 5 mg DES for a 7 day period.

Table 3. Limit of detection of positive samples by RIA.

Sample	Concentrations
Muscle	0.08 – 1.1 ppb
Liver	0.08 – 0.68 ppb
Kidney	0.06 – 0.68 ppb
Faeces	0 – 10 ppb
Feed	0 – 10 ppb

19, 20, 22, 27, 29, 30). Therefore accumulation occurs mainly in the liver and kidneys (20, 22, 30). DES has been detected in the liver 60 days after implantation at a level of 0,1 ppb (30). DES was cleared very rapidly from the blood, however a further increase in plasma DES concentration was observed thereafter (19, 20), which is consistent with our results (Figure 5). DES is found for the longest time and at the

Table 4. DES concentrations in faeces samples of chickens collected from various areas of Turkey.

Counties	Number of Samples	Positive Samples	% Positive	ppb (ng/g)
Bolu	90	1	1.1	20.
Bursa	46	2	4.4	16.5; 42.5
Erzurum	31	2	6.5	11.3; 53
İzmir	59	0	0	--
Ankara	34	0	0	--
Total	260	5	1.9	

Table 5. DES concentrations in faeces samples of calves collected from various areas of Turkey.

Counties	Number of Samples	Positive Samples	% Positive	ppb (ng/g)
Adapazarı	80	0	0	—
Ankara	91	0	0	—
Bolu	61	0	0	—
Bursa	24	0	0	—
Erzurum	36	0	0	—
İzmir	88	0	0	—
Van	48	0	0	—
Total	428	0	0	—

Table 6. DES concentrations in faeces samples of lambs collected from various areas of Turkey.

Counties	Number of Samples	Positive Samples	% Positive	ppb (ng/g)
Ankara	62	0	0	—
Bolu	27	0	0	—
Bursa	5	0	0	—
Erzurum	52	0	0	—
İzmir	56	0	0	—
Van	36	0	0	—
Total	238	0	0	—

Table 7. DES concentrations in chicken feed samples collected from various areas of Turkey.

Counties	Total Number of Samples	Positive Samples	% Positive	ppb (ng/g)
Various areas of Turkey. (Erzurum, Van, Ankara, Bolu)	195	72	36.9	*

* Of the positive chicken feed samples, 59.7 % had a range between 10-50 ppb 23.6 % between 50-100 ppb and 16.7 % above 100 ppb DES.

greatest concentration in the faeces (1, 2, 22). Infact it has been reported that DES can be detected in the faeces 3 months after administration (22). Because the concentration of DES in the urine and faeces is 100–1000 times greater than in the tissues (1, 2, 22), it has become important to analyse urine and faeces samples while screening for DES (1, 2, 22). This not only allows in vivo detection of DES during feeding and before slaughter but faeces is also relatively easy to collect (22).

In the illegal use of DES, it is difficult to prove its administration since the amount of DES given, the period, site and method of application are not known (9, 22, 24, 27). The age and breed of the animal plus individual differences affect the removal of the drug from the body (9,27). Therefore samples to be collected should be selected carefully and the analytical method used to detect illegal uses of DES must be well developed and sensitive (21, 25). Restrictions alone are not effective. With the aid of sensitive and developed methods for residue screening, the use of these illegal substances has decreased (22).

Chicken faeces samples from various areas of Turkey screened for DES using RIA were found to be DES positive with the percentage of positive samples being 1,1 % in Bolu, 4.4 % in Bursa and 6.5% in Erzurum, giving a national average of 1.9 %.Of the chicken feed samples analysed, 36.9 % were found to be DES positive (Table 4).

Upon the detection of DES positive feed samples from 2 factories in Turkey (14), the Ministry of Health ordered the collection of chickens from across the country for analysis in the Department of Biochemistry, Ankara Üniversity Faculty of Veterinary Medicine. Chickens collected from 29 counties were analysed for DES and 245 muscle, 220 kidney and 181 liver samples were found to be DES negative.

From the present results (Table 4 and 7), it can be concluded that there is still a risk of DES being used in Turkey.

The results obtained can serve as a good basis for the calculation of the risk of DES use in Turkey but also underline the necessity to prevent illegal use of DES and similar compounds in the future. As a conclusion, the experimental part of the study will provide a basis for the determination of the detection limit of DES for other labora-

tories to be established in the future where analyses will be carried out regularly. Also, the present data should be considered as an indicator of the seriousness of the matter in Turkey.

Acknowledgement

We would like to thank Prof. Dr. H. Karg of the München-Weihenstephan Institute for providing the DES antibody.

References

1. **Agthe, O.** (1980). *Die Anwendung des Radioimmunoassay für Diäthylstilbestrol auf Kotproben von Mastkalbern.* Arch. für Lebensmittelhyg. 31–102.

2. **Agthe, O., Frenne de D., Sperveslage, C.M.** (1979). *Über die Verbreitung der Adwendung von Östrogenen in der Kalbermast im Raum Weser ems.* Arch. Für Lebensmittelhyg. 30–67.

3. **Anonim** (1989). *American survey Ashort history of hormones.* The Economist January 29–30.

4. **Anonim** (1973). *Yem Kanunu ve Yem Yönetmeliği, Kanun no:* 1734 *Yem yönetmeliği. Ekler,* 3 *Nolu Liste. Karma Yemlemere katılması yasak olan maddeler (A).* 13 sayfa 450.

5. **Bağnati, R., Castelli, M.G. and Airddi, L.** (1990). *Analysis of diethylstilbestrol, dienestrol and hexestrol in biological samples by immunoaffinity extraction and gas chromatography-negative-ion chemical ionization mass spectrometry.* J. Chromatogr., 527, 267–278.

6. **Baker, F.H. and Arthaud, V.H.** (1972). *Use of Hormones or hormone active agents in production of slaughter Bulls.* J. Anim. Sci. 35: 4, 752–754.

7. **Bassila, M.K., Adams, R.L., Pratt, D.E. and Staddmann, W.,** (1975). *Effects of sex, strain and oestrogens on quality of chicken roaster.* Poultry Sci. 54, 696–702.

8. **Bishop, M.C., Selby, C. ,Taylor, M.** (1985). *Plasma Hormone levels in Patients with Prostatic Carcinoma Treated with DES and Estramustine.* British J. of Urology, 57: 542–547.

9. **Brahmakshatriya, R.D., Snetsinger, D.C. and Waibel, P.E.** (1969). *Effects of exogenous estrogen and /or Androgen on Performance, Egg shell characteristics and Blood Plasma Changes in Laying Hens.* Poultry Sci., 48: 2, 444–451.

10. **Coffin, D.E. and Pilon, J.C.** (1973). *Drug Residues in Animal tissues. Gas chromatographic Determination of Diethylstilbestrol Residues in Animal Tissues.* Journal of the AOAÇ 56: 2, 352–357.

11. **Creger, C.R., Mithchell, R.H., Jones, M.L., Atkinson, R.L., Quisenberry, J.H., Couch, J.R.** (1960). *The effects of Administration of diethiylstilbestrol and Dienestrol Diacetate on Growth Rate and Efficiency of Feed Utilization of Beltsville small White Turkey Broilers.* Poultry Sci., 39 (4): 1041–1045.

12. **Dixon, S.N. and Heitzman, R.** 3. (1981). *Residues of Exogenous Anabolic Agents in Beef Cattle and sheep.* (58–69) *İn: Anabolic agents in beef and veal production.* Proceedings of a workshop held at Brussles, March 5 th and 6th.

13. **Düzgüneş, O.** (1963). *Bilimsel Araştırmalarda İstatistik Prensipleri ve Metodları,* Ege Üniversitesi Matbaası, İzmir.

14. **Ersoy, E., Agthe, O., Ergün, Ş.H. and Üresin, T.** (1988). *Etlik piliçlerde ve yemlerinde Diethylstilbestrol (Hormon benzeri etkili madde) yönünden en çalışmalar.* A.Ü. Vet. Fak. Dergisi 35: 2–3 Supplement.

15. **Fıscher, L.J. and Millburn, P.** (1970). *Stilbestrol Transport and Glucuronide formation in everted sacs of rat intestine.* The journal of Pharmacology and Experimental Therapeutics. 175: 2, 267–275.

16. **Galloway, J.H.** (1966). *The effects of steroid Hormones on the Growth Rate of young rabbits.* Vet. Rec. 79: 5, 126–128.

17. **Hafs, H.O., Purchas, R.W. and Pearson, A.M.** (1971). *A review: Relationships of some hormones to growth and carcass quality of ruminants.* J. Anim. Sci. 33: 1, 64–71.

18. **Haryley, H.A.J. Mason, R. and Phillips, P.J.** (1983). *Profound hypocalcaemia associated with oestrogentreatment of carcinoma of the prostate.* The Medical J. of Australia 2 (1): 41–42.

19. **Hopwood, M.L. and Gassner, F.X.**(1962). *Metabolism of C^{14} - Diethylstilbestrol in the chicken: Retention and Excretion.* Endocrinology, 70, 898–885.

20. **Hopwood, M.L. and Gassner, F.X.** (1962). *Metabolism of C^{14} - Diethylstilbestrol in the chicken: Conversion in vivo,.* Endoctrinology, 70 886–889.

21. **Jansen, E.H.J.M., Van Den Berg, R.H., Van Blitterswiyk, H., Both, Miedema, R. and Stephany, R.W.** (1985). *A highly specific detection method for diethylstilbestrol in bovine urine by radioimmunoassay following high performance liquid chromatography.* Food Additives and Contaminants, 2: 4, 271–281.

22. **Karg, H. and Vogt, K.** (1981).*Residues of Diethylstilbestrol (DES) in veal calves* (70–83) *İn: Anabolic agents in beef and veal production.* Proceedings of a workshop held at Brussels, March 5th and 6th.

23. **Metzler, M.** (1989). *Metabolism of Some Anabolic Agents: Toxicological and Analytical aspects.* Journal of Chromatography, 489: 11–21.

24. **Meyer, H.H.D. and Karg, H.** (1989). *Growth Stimulators for Farm Animals: Mode of action, effects on meat quality and potential risks originating from residues.* Proc. FAO/CAAS Workshop on Biotechnology in Animal Production and Health in Asia and Latin America, Beijing, Oct. 9–13, 49–58.

25. **O'Keffe. M. and Hopkins, J.P.** (1989). *Application of Sorbent Extraction chromatography to the Purification of Diethylstilboestrol Extracted from muscle Tissue and Determined by Radioimmunoassay.* J. Chromatogr. 489, 199–204.

26. **Page, SW.** (1991). *Diethylstilboestrol historical background and current regulatory status.* Australian Veterinary Journal, 68: 7–224–226.

27. **Page, SW.** (1991). *Diethylstilboestrol clinical pharmacology and alternatives in small animal practice.* Australian Veterinary Journal, 68: 7, 226–230.

28. **Parfitt, A.M.** (1965). *Changes in serum calcium and phosphorus during stillboestrol Treatment of osteoporosis.* The J. of Bone and Joint Surgey 47 B: 1, 137–139.

29. **Preston, R.L.** (1975). *Biological responses to estrogen additive in meat producing cattle and lambs.* J. of Anim. Sci.. 41: 5.

30. **Rico, A.O., Burgat-Sacaze, V., Braun, J.P.** (1981). *Metabolism of Endogenous and Exogenous Anabolic Agents in Cattle.* (45–57). *In: Anabolic agents in beef and veal production.* Proceedings of a workshop held at Brussels, March 5th and 6th.

31. **Roche, J.F., Hartz, F.J, Joseph, R.L. and Davis, W.D.** (1981). *The use of growth promoters in beef production.* (27–44). *In: Anabolic agents in beef and veal production.* Proceedings of a workshop held at Brussels, March 5th and 6th.

32. **Ross, D.B.** (1981). *Toxicology of exogenous anabolic agents* (139–154). *In: Anabolic agents in beef and veal production.* Proceedings of a workshop held at Brussels, March 5th and 6th.