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Determination of zeranol residues and the serum testosterone oestrogene and progesterone levels in lambs around Ankara region

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Summary: Zeranol is a synthetic derivative of Zearalenone which has been used as an anabolic substance in sheep and cattle to increase growth of zeranol in food producing animals. The usage of zeranol is prohibited in most countries of the European Union and in Turkey. In the illegal use of zeranol it is difficult to determine its presence because the amount of zeranol given and period is not known. As well as this, the age and breed of the animal plus individual variations affect the period of removal of zeranol from the body. Because of this rapid, sensitive and accurate techniques are needed and Radioimmunoassay (RIA) has been proposed as an reference method for anabolic agent residues.Faeces, serum and tissue samples of lambs from Ankara and the surrounding countryside were collected and the analyses of zeranol, oestrogen, progesteron and testosteron were carried out using Radioimmunoassay. A total of 153 faeces samples of lambs collected from Ankara and the surrounding countryside were analysed for the presence of zeranol positive samples for lamb faeces were 22%.

Key words :, Eostrogen, lambs, progesterone, RIA, testosterone, zeranol

Ankara ve çevresinde kuzularda zeranol artık düzeyleri ile serum testesteron, östrojen ve progesterone düzeyleri

Özet: Zearalenone'un sentetik bir derivesi olan Zeranol, koyun ve sığırlarda besi performansını artırmak amacıyla anabolik madde olarak kullanılmıştır. Avrupa Birliği ülkelerinde ve Türkiye'de tüketiciye ulaşabilecek kalıntıları nedeniyle hayvanlarda kullanımı yasaklanmıştır. Yasal olmayan kullanımlarında, verilen miktar ve süre bilinmediğinden tesbiti güçtür. Bunun yanında hayvanın yası, cinsi ve bireysel farklılıklar da zeranol'ün vücuttan uzaklaşmasında etkilidir. Bu nedenle hızlı, duyarlı teknikler kullanılmalıdır ve Radioimmunoassay, referans metot olarak önerilmektedir. Ankara çevresinde çiftlik ve mezbahalardan toplanan dışkı ve doku örnelerinde zeranol analizleri ile serum örneklerinde testosteron, progesteron ve östrojen hormon analizleri Radioimmunoassay ile yapılmıştır. Toplam 153 kuzu dışkı örneği zeranol yönünden analiz edildi. Kuzularda % 22 zeranol pozitif bulundu.

Anahtar sözcükler: Kuzu, östrojen, progesteron, RIA, testosteron, zeranol.

Introduction

Zeranol is a non-steroidal oestrogenic growth promoter that increases the live weight gain in food animals. It is a semi-synthetic product derived from the naturally occurring mycotoxin zearalenone (4). Its administration has been banned within the European Union (EU) (Council Directive 96/22/EEC)(6) and Member States are required to monitor food-producing animals for possible abuse (Council Directive 96/23-EC) (7).

The meat production industry plays an important part in animal husbandry. Anabolic agents are given to food-producing animals to improve meat production and feeding efficiency (19,26,27,28). Although the use of anabolic agents increases production, it poses serious health problems to those who may consume the meat containing traces of these agents (2,4,11,24,29).

The use of zeranol in food producing animals is prohibited in most countries of the EU (6,7) and in Turkey. Also the use of hormone and antihormone preparations as food additives in Turkey is prohibited by law (1).

However, periodic controls are not made for the presence of the anabolic agents and other drugs residues in meat sold for human consumption.

The aim of the study was to monitor the use of zeranol as anabolic substance in Turkey; to determine the, serum testosterone, progesterone and estrogens levels of zeranol positive lambs.

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Materials and Methods

Zeranol residue analyses were carried out on a total of 153 faeces samples collected from lambs farms and slaughter-houses from Ankara and its countrysides (Sincan,Golbasi, Cubuk and Polatlı). A total of 19 tissue samples of Zeranol positive lambs (muscle, liver and kidney) were also analysed for zeranol. Testosteron, progesteron and oestrogen analyses were carried out on 31 zeranol positive lambs serum samples. The samples were kept at -20° C until analyses were performed

Zeranol concentrations in muscle, liver, kidney and faeces samples were determined using commercial RIA kit (Laboratoire D'hormonologie, Belgium). The extraction of the zeranol was performed as previously described (10).

The analyses of serum testosteron, were also carried out by commercial RIA kit (DSL-400 Diagnostic Systems Laboratories, USA).

The serum oestrogen and progesterone levels were measured by RIA using radioactive oestrogen and progesterone and their antibodies.

Equipment

Liquid scintillation counter (LKB-Beta), Vacuum drying oven (Heraeus), cooling centrifuge (Heraeaus), magnetic stirrer (Labtec), gamma counter (Sesa), Analytical balance (Sartorious), Homogenisator (Ultra turax). Usual laboratory equipment and glass wear.

Chemicals used

Gelatine, Natrium azide, Titriplex III, NaCl, NaOH, di-sodium hydrogen phosphate, Potassium dihydrogen phosphate, chloroform, ethanol, diethyl ether, methanol, dextran T70, PPO, POPOP, Triton X100, n-Hexane and ethyl-acetate were obtained from Merck chemical company, Charcoal norit-A was obtained from Serva chemical company, Toluene was obtained from Atabay chemical company, C-18 octadecyl colon was obtained from J.T. Baker, oestrogene and progesterone standarts were obtained from Sigma, ^{3}H oestrogen and ^{3}H Progesterone tracer, (1.0 mCi/ml) were obtained from Amersham-England), Oestrogen and Progesterone antibody were kindly provided by Prof.Dr. Carlos Romero,Universidad Autonoma Metropolitana, Mexico. All chemicals were of analytical grade.

The significant differences between serum hormone levels of zeranol positive and zeranol negative lambs were determined using the T test (12).

Results

The results of analyses carried out on faeces, muscle, liver and kidney samples of lambs are shown in Tables 1 and 2. A total of 153 faeces samples were analysed for zeranol and 22% of the samples were found to be positive. In the 19 tissues samples of the zeranol positive lambs collected from slaughter-houses were analysed for zeranol. In the muscle, kidney and liver samples of the zeranol positive lambs, the percentage of zeranol-positive samples was 5%, 5% and 42% respectively (Table 2). The percentage of zeranol-positive liver samples were found to be high. The detection limits of zeranol positive samples using RIA are given in Table 3.

The serum oestrogen, progesteron and testosteron levels of zeranol positive lambs are given in Table 4. Testosteron levels were found to be 1.35 ng/ml in the zeranol positive lambs and 0.25 ng/ml in the zeranol negative lambs. The decreased levels of testosteron were found in zeranol negative animals than positive ones ($p<0.001$). Oestrogen levels were not changed in the lambs. Progesterone levels were found to be decreased in the zeranol positive animals ($p<0.05$).

Table 1. Zeranol concentrations in faeces samples of lambs collected from Ankara and its countryside.
Tablo 1. Ankara ve çevresinden toplanan kuzulara ait dışkı örneklerinde Zeranol % oranları

	Number of samples	Positive samples	% Positive	ng/g
Polatlı	14	2	14	3.7-6.3
Sincan	29	3	10	4.3-5.8
Gölbaşı 1	41	6	14	3.5-5.2
Gölbaşı 2	18	0	0	-
Gölbaşı 3	6	0	0	-
Gölbaşı 4	14	10	71	4.2-8.3
Çubuk	31	13	42	3.8-6.3
Total	153	34	22	3.5-8.3

Table 2. Zeranol concentration in liver, kidney and muscle samples of zeranol positive lambs collected from Ankara and its countryside.
Tablo 2. Ankara ve çevresinden toplanan zeranol pozitif danalara ait kas, karaciğer ve böbrek doku örneklerinde zeranol konsantrasyonları.

Sample	Number of samples	Positive samples	% Positive	ng/g
Muscle	19	1	5	0.016
Liver	19	8	42	0.03-0.08
Kidney	19	1	5	0.04

Table 3. Limit of detection of positive samples by RIA
Tablo 3. Pozitif örneklerin RIA ile deteksiyon limitleri.

Sample	Concentrations
Faeces	0-3.3
Muscle	0.00016-0.007
Liver	0.005-0.007
Kidney	0.007-0.016

Table 4. The serum oestrogen, progesteron and testosteron levels of zeranol positive lambs (ng/ml)
Tablo 4. Kuzulara ait serum örneklerinde östrojen, progesterone ve testestosteron hormon düzeyleri.

	Control				Zeranol Positive				
Hormone	N	X	± Sx	Min-Max	n	X	± Sx	Min-Max	p
Oestrogen	10	0.05	+0.005	0.025-0.08	30	0.04	+0.002	0.01-0.06	p>0.05
Progesteron	10	0.33	+0.074	0.17-0.88	31	0.14	+0.12	0.04-0.35	P<0.05
Testosteron	8	0.23	+0.091	0.2-0.65	31	1.35	+0.344	0.2-6.8	p<0.001

Diccussion and Conclusion

Zeranol was found to be 22 % positive in the faeces of the lambs. This could be a sign of the usage of these materials in Ankara region. Zeranol residue was also seen in the tissue samples of zeranol positive animals.

Elsaser (1983) and Flowers (1987) reported that levels of endogen sex hormons in the zeranol positive animals are changeable according to the species and the race of animals. The amount of anabolic agents given also effected the endogen hormones levels (9). Testosteron levels were found to be 1.35 ng/ml in the zeranol positive lambs. Testosterone levels were 0.25 ng/ml in the zeranol negative lambs. That was indicated the decreased levels of testosteron in zeranol negative animals than positive ones. Oestrogen levels were not changed in the lambs. However, progesterone levels were found to be decreased in the zeranol positive animals.

There are several views and the applications for the usage of Zeranol (Ralgro) as anabolic agent. Anabolic agents including zeranol were inhibited in some of the European countries (Germany, Belgium, Denmark, Holland and Greece) and in Austria and Switzerland. Usage of the anabolic agents is under the control of law in total 35 countries including USA, France, England and Ireland. IN limit of the physiological clearance period and the slaughter time of 60-70 days are necessary to wait in these countries. Moreover, residues of the anabolic agents in the ralgro applied animals are investigated using the sensitive analysis techniques (3, 22, 25, 30), and then the permition for the consumption of the meat is given in these at the 35 countries. However, zeranol levels must be zero level in the tissue of Ralgro applied animals. All these applications show that some of the the countries permitted for the usage of the Ralgro and similar anabolic agents have the strict law and scientific regulations for the human safety (15, 21).

Ralgro (zeranol) is not a drug for the protection and treatment, but it is useful for the gain of weight and food consumption by the animals (13, 16, 18, 27). As a result of this, it has an economical importance and it could be use as an abaloic agents for animals under the control of law. The EU has recently revised the technical criteria that must be applied in the screening and confirmation of veterinary drug residues in food of animal origin (Commission Decision 2002/657/EC) (8). These criteria lave replaced those that lave applied in the EU for the last 10 years (Commission Decision 93/256/EEC) (5). A simple and rapid analytical methods were established for detection of anabolic agent. residues in tissues,faeces and urine samples of food producing animals (3, 22, 25, 30).

In the illegal use of anabolic agents, it is difficult to determine its presence because the amount of anabolic agents given, its formulation, period, method and area of application is not known. As well as this, the age and breed of the animal plus individual variations affect the period of removal of anabolic agents from the body (23, 24). Zeranol crossed the placental barrier and were detectable in foetal tissue. The extent of tissue concentration varied depending on the compound and tissue analysed. Gender differences were observed in some instances (24). Zeranol passes the placenta unrestrictedly. After zeranol treatment, the female foetuses showed higher residues in their tissues than males. In placenta, however, the zeranol contents were lower in the female fetuses (24).

Anabolic agents are found for the longest time and at the highest level in the faeces, therefore it has become practically important to look for anabolic agents residues in the faeces (10).

In the EU 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 method regularly (20).

As a result of analyses on lamb faeces samples collected from Ankara, zeranol was detected. The presented data should be considered as an indicator of the seriousness of the usage of the anabolic agents in Turkey.

The results of our studies have enable us to review the situation regarding the use of zeranol in Turkey and this situation may end up with the protection in the usage of illegal substances in Turkey in the future. Periodic controls are necessary to show the presence of anabolic agents and similar compounds residues in meat sold for human consumption. The obtained results will provide a basis for the determination of the detection limit of Zeranol for other laboratories to be established in the future where analyses will be carried out regularly.

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