



Journal Homepage: -www.journalijar.com
**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/7499
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/7499>



RESEARCH ARTICLE

DETERMINATION OF SOME ANABOLIC HORMONE RESIDUES IN CATTLE MEAT CONSUMPTION IN VAN, TURKEY.

***Ufuk Mercan Yücel¹, Nurhayat Atasoy², Özgür İşleyici³ and İdris Türel⁴.**

1. Van Yüzüncü Yıl University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Van / TURKEY.
2. Van Yüzüncü Yıl University, Faculty of Science, Department of Biochemistry, Van / TURKEY.
3. Van Yüzüncü Yıl University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Van/ TURKEY.
4. Adıyaman University, Faculty of Pharmacy, Department of Pharmacology, Adıyaman / TURKEY.

Manuscript Info

Manuscript History

Received: 03 June 2018
 Final Accepted: 05 July 2018
 Published: August 2018

Keywords:-

Meat, anabolic hormones, residue, ELISA, Van.

Abstract

The aim of this study was to determine some anabolic hormone residues (zeranol, trenbolone, DES, and testosterone) in cattle meat offered in the markets of the Van province. Cattle meat samples used in the study were collected from supermarkets, butchers, and slaughterhouses in Van Province between September 2013 and September 2014. A total of 80 samples consisting of approximately 50-150 g fat-free portions of cattle meat were collected and transported to the laboratory in the shortest time possible, after they were stored at -20° C until the analysis. Residual amounts of zeranol, trenbolone, DES and testosterone in the samples were determined by competitive enzyme immunoassay (ELISA) technique. Preparative kits (RIDESCREEN® Test Kits, R-Biopharm AG, Darmstadt, Germany) were used for the extraction and residue detection procedures, as recommended by the manufacturer company. Of all the samples in the study, the number of positive samples corresponding to each of the inspected residues were as follows: 37 (46.25%) trenbolone, 34 (42.5%) zeranol, 22 (27.5%) testosterone, and 11 (13.75%) DES. The highest residual values were determined as 230.7 ng/kg for the trenbolone in August, 1810.0 ng/kg for zeranol in September, 2004.49 ng/kg for testosterone in October, and 171.83 ng/kg for DES in November. In this study, trenbolone, zeranol, testosterone and DES residues were detected in the cattle meat offered in Van province markets. Residue quantities determined in cattle meat samples do not exceed acceptable residue limits. However, prohibited except for cases of anabolic veterinary treatment applications. The results obtained in this study show that different anabolic materials are being used for increased meat production, even though they are forbidden. For this reason, effective monitoring and inspections must be carried out at all stages from production to consumption with regard to the use of anabolic agents in livestock. It is important to remember that ELISA provides good results and rapid screening, but incorrect results may be obtained due to cross reactions.

Corresponding Author: Ufuk Mercan Yücel

Address: Van Yüzüncü Yıl University, Faculty of Veterinary Medicine, Department of Pharmacology

Introduction:

With the rapid growth of the human population in the world, the food demand constantly rising depending on the health, wealth and quality of life expectations of this population. The food industry has the expertise and technology to meet this demand. However, consumers' attitudes towards high technology production and know how get less favorable with each passing day (Webb and Erasmus, 2013). Many factors like the price of the product, the country of production, the certifications it has, the type of production, and the characteristics of the product are influential in the shaping of consumer preferences for meat and meat products. However, the increase in public knowledge about the risks such as BSE, hormones, Salmonella, and bird flu has further increased consumer concerns, and the health and nutritional characteristics of the meat have become even more important for consumers (Font- Furnols and Guerrero, 2014). In a study conducted in Belgium, it was reported that the most important factor influencing consumers' meat preferences was the food safety and that there was a decrease in recent years in market share due to the decrease of consumer confidence, especially for cattle meat products. Food safety and welfare of butchery animals are also increasingly important issues for the consumers (Verbeke and Viaene, 1999).

At least one of the three of the protein consumed daily by a sufficiently balanced diet must be derived from sources of animal origin (Uran, 2013). Meat and meat products are important food types as high-quality protein sources with high biological values. For this reason, demand for quality meat and meat products is steadily increasing. In developing countries, the consumption of food originating from animal sources has recently approached the levels of developed countries. The production of animal origin foods, especially meat and meat products, has substantially increased throughout the world to meet the increased demand (Bender 1992; Boland et al., 2013; Wolk, 2017).

Meat and meat products, which are increasingly consumed all over the world, are types of food that are open to various manipulations with the purpose of by passing, legal regulations. The reasons for the cheating attempts on meat products are the high prices, the difficulties of their production, and the inability of the current production capabilities to meet the demand adequately (Barai et al., 1992; Cawthorn et al., 2013). In order to increase the production of meat and meat products, and to close the animal protein barrier, some fertilizers were used all over the world. Antibiotics, chemotherapeutics, ionophores, hormones and hormone-like anabolic substances were used for this purpose. While these hormones and hormone-like anabolic substances increase the weight-gain performance and meat yield of the animals, when used unconsciously in overdoses, they accumulate in various organs and tissues and leave residues, which can lead to various health problems (Akıllı, 1996; Saraç et al., 1999; Şanlı, 1989). In recent years there is growing suspicions and evidence about a connection between the consumption of high amounts of red meat, especially processed meat products, with some important and common diseases such as diabetes, coronary heart disease, heart failure, stroke and cancer (Wolk, 2017). With these worries, the European Union Food Safety Agency (EFSA) has reported that all six types of hormones can cause endocrine, developmental, immunological, neurobiological, immunotoxic, genotoxic, and carcinogenic effects for the susceptible risk groups, and has forbidden the import of meat obtained from countries which do not regulate their meat productions accordingly (Donovan, 2015).

Hormones play an extremely important role in maintaining the normal life functions of the body. This hormone balance is necessary to obtain maturation, sexual development, regeneration, and the replacement of many physiological functions. Disruption of hormone balance can cause malfunctioning of certain organs or disorders that affect the whole body. The hormone levels in children and adults can be affected by hormone consumption. The consumption of meat and meat products derived from hormone-applied animals can cause consumers to be exposed to various levels of hormone residues. There is a concern that hormones, which are very important for the human body, can cause health risks if they are consumed, even in residual quantities found in foods of animal origin. The amount of exposure to hormone residues varies individually depending on the consumer's eating habits, the amount of hormone residues in the animal's flesh, and the product's concentration (Anonymous, 2000). The presence of androgenic hormone residues in the meat (such as testosterone, trenbolone acetate), may affect virilization in women (menstruation) and cause menstrual cycle disorders; while estrogenic hormone residues have been reported to cause feminizing impotence, and infertility in men. In addition to these general hormonal activities of hormone residues, they may also act as carcinogens, and cause nervous system complications and arteriosclerosis (Hero, 2017).

Veterinary drugs with anabolic effects, even some used for therapeutic and prophylactic purposes, also may be used to enhance the ability of the animal to benefit from the feed. That being said, many of them are prohibited in the European Union and are only allowed to be used in specific situations under strict control measures (Reig and Toldra, 2008). Growth regulating substances acting like hormones are called “anabolics”.

Sex hormones such as estrogens (estradiol), androgens (testosterone) and progestogens (progesterone) are sometimes to provide more live weight gain in animals, to increase utilization from the feed, to develop better carcass and meat quality. Synthetic non-steroidal estrogens such as diethylstilbestrol (DES), hexoestrol, dienestrol and zeranol, and synthetic steroids such as trenbolone acetate (TBA), testosterone propionate, melengestrol acetate, methyltestosterone are used as hormonal effect anabolic agents (Hero, 2017). Estrogenic and androgenic agents were used in the meat industry for about 50 years to increase meat production and profitability. These agents can be used alone, or in combination with some other agents. Such estrogenic preparations are effective for the growth of the dendritic systems, while androgenic preparations are especially potent in the growth of the ovary (Dunshea et al., 2005). Weight gain in butchered cattle using anabolic hormone implants is increased by 10-20% compared to untreated animals. In addition, 8% less fat was detected in slaughtered animals using implants compared to animals not using the same implant (Dikeman, 2007). Because of these positive effects, the uncontrolled and widespread use of anabolic agents has increased. These drugs are now prohibited and the productions of some of them were stopped (Toldra and Reig, 2006). In particular, the use of 6 of the growth-promoting hormones from anabolics is risky in terms of human health. These are; 17β estradiol, testosterone, zeranol, progesterone, trenbolone acetate, and melengestrol acetate (Anonymous, 1999).

This study was designed to contribute to the identification of trace levels of these banned hormones and to statutory auditing and monitoring activities, thereby contributing to the minimization of the negative consequences of the uncontrolled hormone use. The situation is particularly of importance these days: as the date of the greater aid approaches, more breeders are tempted to use these illegal hormone medicines in order to increase the weight gain of the animals in a very short period of time.

Materials And Methods:

Meat samples were collected from large markets, butchers, and slaughterhouses. 80 samples of 50-150 g fat-free cattle meat were collected between September 2013 and September 2014 and were stored at -20 °C until analysis. Analyzes were performed using the ELISA test (Bio-tek Instruments ELX 800 UV Reader, Bio-tek Instruments ELX 50 Washer, USA) in line with the manufacturer's specifications (R-Biopharm, 2006). RIDASCREEN® Zeranol (Art. 3301), DES (Art No. R 2701), Testosterone (Art No.R 2401), and Trenbolone (Art.Solid phase extraction cartridges of RIDASCREEN® C-18 (Art. No: R2002) brand were used in the extraction step of other anabolic except for the zeranol.

In the evaluation of the data obtained from the samples in the study were used the numbers of the samples, the corresponding average amounts of these numbers, the standard errors, the minimum and maximum values.

Results:

The amounts of hormones detected in analyzed samples and the distributions according to the months are given in table 1, table 2, table 3 and table 4.

Table 1: Trenbolon levels of meat samples and distribution according to the months.

MONTHS	Amount (ng / kg)	TRENBOLOON		
		Sample Number	Mean	Min. and Max. Values
January	ND	4	-	-
	100-200	2	126.1	109.91-142.28
February	ND	4	-	-
	100-200	2	162.79	130.14-195.44
March	ND	2	-	-
	100-200	3	146.5	118.58-184.46
	200-300	2	205.5	204.69-206.42
April	ND	2	-	-
	100-200	5	137.77	123.2-147.48

May	ND	4	-	-
	100-200	2	154.13	117.43-190.82
	200-300	1	217.4	217.4
June	ND	2	-	-
	100-200	4	156.29	113.96-178.11
July	ND	3	-	-
	100-200	4	125.8	104.13-137.65
August	ND	3	-	-
	100-200	2	127.83	125.52-130.14
	200-300	1	230.7	230.7
September	ND	3	-	-
	100-200	3	121.08	103.56-131.87
	200-300	1	209.31	209.31
October	ND	7	-	-
	100-200	1	119.16	119.16
November	ND	3	-	-
	100-200	3	118.39	104.71-108.76
December	ND	6	-	-
	100-200	1	101.24	101.24

ND: Not detected

Table 2: Zeranone levels of meat samples and distribution according to the months.

MONTHS	Amount (ng / kg)	ZERANOL		
		Sample Number	Mean	Min. and Max. Values
January	ND	5	-	-
	200-300	1	263.22	263.22
	300-400	1	313.01	313.01
	500->	1	849.76	849.76
February	ND	4	-	-
	400-500	1	461.02	461.02
	500->	-	-	-
March	ND	6	-	-
	500->	1	591.38	591.38
April	ND	1	-	-
	200-300	3	265.11	251.38-272.40
	300-400	2	352.75	309.54-395.96
	400-500	1	499.11	499.11
	500->	1	517.44	517.44
May	ND	7	-	-
June	ND	2	-	-
	300-400	1	384.60	384.60
	400-500	1	477.96	477.96
July	ND	3	-	-
	300-400	1	323.65	323.65
	400-500	-	-	-
	500->	-	965.85	965.85
August	ND	3	-	-
	200-300	2	230.95	206.49-254.30
	300-400	1	330.91	330.91
	400-500	1	466.01	466.01
September	ND	3	-	-
	300-400	1	308.38	308.38
	400-500	1	497.31	497.31

October	ND	4	-	-
	300-400	1	345.87	345.87
	400-500	-	-	-
	500->	3	877.23	763.19-1105.33
November	ND	5	-	-
	200-300	2	266.61	251.38-281.84
December	ND	7	-	-
	200-300	1	215.79	215.79
	300-400	1	339.57	339.57
	400-500	1	416.61	416.61
	500->	1	572.45	572.45

ND: Not detected

Table 3: Testosterone levels of meat samples and distribution according to the months.

MONTHS	Amount (ng / kg)	TESTOSTERON		
		Sample Number	Mean	Min. and Max. Values
January	ND	7	-	-
February	ND	3	-	-
	200-300	1	248.37	248.37
	300-400	1	365.29	365.29
	500->	1	563.66	563.66
March	ND	5	-	-
	100-200	1	111.99	111.99
	500->	1	690.33	690.33
April	ND	7	-	-
May	ND	7	-	-
June	ND	5	-	-
	100-200	1	127.10	127.10
July	ND	2	-	-
	500->	5	788.03	528.66-1047.33
August	ND	6	-	-
September	ND	7	-	-
October	ND	1	-	-
	400-500	1	427.22	427.22
	500->	5	1065.60	567.24-2004.49
November	ND	3	-	-
	200-300	1	256.30	256.30
	500->	3	688.48	256-951.30
December	ND	5	-	-
	400-500	1	491.58	491.58

ND: Not detected

Table 4: DES levels of meat samples and distribution according to the months.

MONTHS	Amount (ng / kg)	DES		
		Sample Number	Mean	Min. and Max. Values
January	ND	8	-	-
February	ND	6	-	-
March	ND	5	-	-
April	ND	6	-	-
	100-200	2	106.14	105.66-106.62
May	ND	7	-	-
June	ND	4	-	-
July	ND	4	-	-

	100-200	2	122.2	116.13-128.27
August	ND	6	-	-
	100-200	1	104.40	104.40
September	ND	7	-	-
	100-200	1	105.66	105.66
October	ND	8	-	-
November	ND	3	-	-
	100-200	4	144.01	113.78-171.83
December	ND	5	-	-
	100-200	1	112.13	112.13

ND: Not detected

Discussion:

Anabolic agents are substances that increase growth in cattle, sheep and even in pet animals, and they cause substantial weight gain. These effects are induced by increasing protein synthesis and reducing fat tissue (Lone 1997; Turner et al., 1995). Although the use of hormones for weight increase purposes contributes to productivity, the opinion among European countries and the United States towards their use is vastly different. The European Union forbids the use of anabolic hormones as a growth promoter in slaughterhouse animal breeding. The FDA, on the other hand, has allowed the use of some naturally occurring hormones (estradiol and testosterone) in animal breeding (Sawaya et al., 1998b).

According to the Communiqué of the Turkish Food Codex in our country, the following hormones and substances are prohibited for use on animals with food value, or may only be used under some specific conditions; stilbenes, stilbene derivatives, anti-thyroidal substances, steroids suitable for anabolic purposes, resorcylic acid lactones including zeranol and β -agonist substances (Anonymous, 2003).

There are two basic problems encountered during an inspection of hormone residues in animal products: to distinguish the animals own naturally occurring hormone levels from that of the residues, and the fact that each animal species has different hormone levels (Şener, 1994). Generally, the use of growth promoting substances should be inspected from three perspectives: the producer, the animal, and the consumer. It would be ideal to use a growth accelerator substance that is economic from the producer's perspective, does not adversely affect the biological functions from the animal perspective, and does not adversely affect human health from consumer's perspective. The use of a material that does not meet all of these three conditions is strictly prohibited (Alpan, 1989). Despite the fact that the use of anabolic substances is prohibited in our country, these residual hormones were detected in meat and meat products through various researches, some of which reported very amounts of residual anabolics. In terms of consumer health and safe food consumption, it is necessary to carry out hormone analyzes on animal products more frequently, to increase the number of controls, and to initiate legal proceedings on those who were determined to have used them.

There are various studies that show the presence of residual hormones and similar substances with anabolic effects in animal-derived foods. In a study conducted by Kadim et al. (2010) in order to determine the residual levels of various antibiotics and hormones in chicken meat, it was reported that the residual levels of 17β -estradiol and testosterone were above the acceptable limits.

Sadek et al. (1998) investigated the presence of anabolic agent residues in meat and poultry samples collected from supermarkets and small-scale butcher shops and found that supermarket pickles contained higher levels of TBA residues than the samples from the butcher's shops. Similarly, they detected DES and estradiol residues in cattle meat and liver samples, albeit at low levels. They also detected that none of the samples contained zeranol.

In the study conducted by Nazlı et al. (2005a), none of the samples were found to contain DES and clenbuterol residues, whereas zeranol and DES residue levels were above the limit values in all 30 of the samples. From these determinations, it is suggested that hormone residues in meat and meat products may pose a risk for human health and that the control mechanisms need to work better to prevent mass-scale health problems. In another study (2005b), the same researchers reported that they found zeranol in all 60 meat and meat products samples, TBA in 48 samples, and DES residues in 21 samples, whereas no clenbuterol was identified in any of the samples.

Yılmaz et al. (2007) reported that they have determined zeranol in 18 samples, while they detected 6 samples of testosterone, 7 samples of TBA, 1 sample of DES and 22 of 17 β -estradiol amongst 155 cattle meat in Tekirdağ. In another study (Akkaya et al., 2004), 300 chicken meat samples were analyzed for the presence of DES, zeranol, estradiol, testosterone, progesterone, and clenbuterol, and no anabolic hormone residues were found in any of the samples.

In the study conducted by Oruç et al. (2007) in Bursa, 2 of the 81 cattle meat samples were found to contain zeranol, 11 of the 80 samples were found to contain DES, while 3 of the 29 samples were found to contain testosterone. In addition, 72 of the samples were analyzed for the presence of clenbuterol and 29 for 17 β -estradiol residues and it was determined that the meat samples did not contain the residues of the corresponding hormones.

Şevik and Ayaz (2017) inspected 200 cattle meat samples that they collected from the butchers and supermarket in Kocaeli and found DES levels varying between 84,06 and 178,56 ppt, while trenbolone levels varied between 49,87 and 162,43 ppt, estradiol 17 β levels 49,87 and 334,75 ppt, zeranol levels 100, 94 and 614.06 ppt.

80 meat samples were evaluated in our study to determine the presence of various anabolics, where 37 of them were found to contain TBA, 33 contained zeranol, 21 contained testosterone, and 11 contained DES.

Trenbolone acetate is a synthetic, anabolic compound of androgenic structure, and is a 19-nortestosterone derivative (Appleve et al., 1991; Durmaz, 1997, European Commission, 2006). TBA reduces both the protein synthesis and the protein degradation. When degradation is lower than the synthesis rate, net muscle protein deposition increases (Appleve et al., 1991). Since TBA is metabolized 85% in the liver when taken orally, it is applied to ear in cattle, alone or in the range of 40-300 mg with estradiol (Appleve et al., 1991; Durmaz, 1997; European Commission, 2006). After 70 days of application of 140 mg of TBA to cattle, residue levels in various tissues were found as follows: 0.09 ppb in the muscle, 0.38 ppb in the liver, 0.28 ppb in the kidney and 0.48 ppb in the fat tissue (Durmaz, 1997).

The maximum permissible levels of TBA in tissues by the US Food and Drug Administration (FDA, 1991) were reported as 50 $\mu\text{g}/\text{kg}$. In this study, TBA was detected in 100-200 ng/kg levels in 32 of 80 samples and in 200-300 ng/kg (6.25%) levels in 5 samples. These values, however, are well below the residue limit set by the FDA. The highest TBA value based on the months was found in the August samples. Sawaya et al. (1998a) found TBA levels in the range of 20-50 ng/kg in 30 cattle meat in Kuwait. Nazlı et al. (2005b) reported 0.10-0.5 $\mu\text{g} / \text{kg}$ TBA in 4 (40%) of the 10 readymade mince meat samples collected from markets in Istanbul, while they reported 0.01 to 0.1 $\mu\text{g}/\text{kg}$ in 5 (50%) samples. 10 pre-carcass samples were found to have 0.11-0.5 $\mu\text{g}/\text{kg}$ TBA; while another 10 were found to contain TBA between 0.01 and 0.1 $\mu\text{g}/\text{kg}$. Mahgoub et al. (2006) reported that sheep and goat meat samples contained 0-0.16n /kg of TBA in Maskata. Mor et al. (2011) reported that in the samples of cattle liver, kidney and meat presented to consumption in Burdur, 3 of the 30 meat samples (10%) contained 50-100 ng/kg TBA, while 21 (70%) had 100-150 ng/kg and 6 (20%) had 151-200 ng/kg TBA. The TBA levels in our study were found to be lower compared to the study of Nazlı et al. (2005b), close to the levels of Mor et al. (2011) and Şevik and Ayaz (2017) and higher than the levels found by Mahavoub et al. (2006) and Sawaya et al. (1998a).

Zeranol (α -zeranol) is a non-steroidal resorcylic acid lactone formed of zearalenone, a mycotoxin, and has estrogenic effects (Aksoy and Dagoglu 1998; Durmaz 1997; Daxenberger et al 2000). A joint committee between the World Health Organization and World Agriculture Organization has determined a tolerance level of 2 $\mu\text{g}/\text{kg}$ zeranol for edible tissues of animals. In this study, it was found that of the 80 samples, 9 had 500 ng/kg (11.25%) zeranol, while 6 had 400-500 ng/kg (7.5%), and 9 had 300-400 ng/kg 300 ng/kg (11.25%) of zeranol. When inspected on a per month basis, the highest values were found to be that of September and October. Even these values, however, do not exceed the limit of 2 $\mu\text{g} / \text{kg}$ (2000 ng/kg). Nazlı et al. (2005b) have identified zeranol residues in all 60 meat samples sold on the Istanbul market and determined 23 of those posed high for risk to human health. Sever et al. (2012), found zeranol levels between 200-694 ng/kg in 44 meat samples (29.3%) and Oruç et al. (2007) found levels of 456.7 and 1501.3 ng/kg in 2 of the 81 meat samples. Finally, Mor et al. (2011) found the average zeranol levels of 179 ng/kg in 11 out of 30 meat samples. The results obtained from this study are lower than that of Nazlı et al. (2005b), fairly close to the results obtained by Sever et al. (2012), Oruç et al. (2007) and Şevik and Ayaz (2017), and are higher than the results obtained by Mor et al. (2011).

In this study, analyzes of anabolic materials were performed by ELISA technique. It is known that zeranol analysis in biological materials can shift towards positive direction (Cooper *et al.*, 2003). In zeranol analyzes made on the meat samples, zeranol can be detected in low amounts even if the samples contain no zeranol. Positivity values can be much higher than normal when extraction is not performed appropriately. For these reasons, the results can be misinterpreted if they are not evaluated carefully.

Testosterone, although allowed to be used under certain circumstances in some countries such as the US, it is prohibited in Turkey and the EU countries. Testosterone-implanted animals were reported to have a residual level of 0.031-0.360 µg/kg of testosterone, and animals with no hormones were reported to have a level of 0.006-0.029 µg/kg (Jeong *et al.*, 2010). In this study, over 500 ng/kg testosterone was found in 15 of the 80 samples (19%): 400-500 ng/kg in 2 samples, 300-400 ng/kg in 1 sample, 200-300 ng/kg in 2 samples and 100-200 ng/kg in 1 sample. When evaluated in a per-month basis, testosterone was not detected in samples belonging to January, April, May, August, and September. It was also determined that the highest testosterone level in meat samples occurred in October. Oruç *et al.* (2007) found testosterone levels at a mean value of 329.96 ng/kg in three out of 29 samples (7%). The results obtained in our study are higher compared to the results found by Oruç *et al.*

Diethylstilbestrol is a synthetic estrogenic compound with a potent accelerating effect on physical development. DES is not allowed at any levels in animal tissues since it has a carcinogenic effect and can't be metabolized by the organism (Durmaz 1997; Van Peteghem and Van Haver 1986). According to the Codex Alimentarius Commission (CAC) and the Turkish Food Codex, the use of DES is prohibited and is not allowed in foods (Food and Agriculture Organization of the United States 1997; Anonymous 2003). In this study, DES was detected in 11 samples (14%) at the levels of 100-200 ng/kg while 69 samples (86.25%) were DES-free. No DES was detected in the samples belonging to January, February, March, May, June and October. It was detected in 1 sample of August, September, and December each, 2 samples of April and July each, and 4 samples belonging of November. The highest values of DES were also determined in meat samples of the November. Nazlı *et al.* (2005b) reported that 21 out of 60 meat samples sold in Istanbul (35%) contained DES at a level of ≥ 10 µg / kg, and Oruç *et al.* (2007) reported that they found DES at a level of 102.13 ng/kg in 11 of 80 meat samples they inspected, and Şevik and Ayaz (2017) reported that 200 meat samples sold in Kocaeli contained DES at level of 84,06-178,56 ppt . When compared to the data obtained by Nazlı *et al.* (2005b), the presence of DES detected in our study is much lower but is close to the values found by Oruç *et al.* (2007) and Şevik and Ayaz (2017). The determination of DES in meat samples, however, also indicates that the meats offered to consumption in the market pose a risk to the public health.

When compared to the other months, the highest TBA level in meat samples was found in August, the highest zeranol value of the meat samples was detected for September and October samples, the highest testosterone value was in October, and the highest value of DES was found in November. Considering that the great eid of 2013 was in October, this may indicate that some breeders took the advantage of approaching great aid by using illegal hormone medicines. Even though the sales and use of hormonal agents are forbidden in Turkey, it's possible that some contraband may be arriving from Iran.

Conclusions:

As a result; this study concluded that hormones or similar substances were actually being used in animals and while the zeranol and TBA levels detected in the samples did not exceed the tolerance limits effective control mechanisms still have to be developed to prevent the use of anabolic agents. In light of the findings obtained from the study, it was concluded that anabolic agents such as zeranol, DES and testosterone were being used in certain amounts even though they are forbidden in Turkey. The results indicate that consumption of the meat offered in the market may pose a risk for public health as some samples contain the residues of these substances. As a matter of fact, developed countries have implemented many regulations to protect consumer health from the threat of anabolic substances in animal breeding, and to control their presence in foodstuffs. Information on whether or not anabolic is used in animal breeding in our country is not enough. The use of anabolic substances that are regarded as harmless in terms of consumer health should be allowed. Those allowed should be effectively inspected. Such agents should be licensed by the relevant ministry sold by veterinary prescriptions, and should be monitored under veterinary surveillance during their use, and if necessary after their use.

Considering ELISA tests may return false or higher-than-normal positive results due to cross-reactions for many of the substances, verification of the levels of hormones determined in this study by further verification tests is recommended. Many researchers have emphasized the necessity of confirming the hormone and drug residue values

obtained by ELISA method for meat products using more advanced devices and techniques simultaneously (Seo et al., 2005; Fuh et al., 2004; Sun et al., 2010; Chaoyang et al., 2008).

Acknowledgments:-

The authors would like to express their gratitude towards the Van YüzüncüYıl University Scientific Research Projects Directorate for their financial support of this research project under the registry no 2015-HIZ-VF206.

Conflict of interest

The authors have no conflict of interests to declare.

References:-

1. Akıllı A. (1996). Gıdalarda veteriner ilaç ve anabolizan maddelerin kalıntı düzeylerinin tespiti. Gıdalarda katkı, kalıntı ve bulaşanların izlenmesi. T.C. Tarım ve Köy İşleri Bakanlığı Koruma Kontrol Genel Müdürlüğü Yayınları.
2. Akkaya, R., Akıllı, A., Gürel, Y., Çmar, S., Koç, F., Turhan, E., Daş, Y.K., Yiğit, Y. and Başsatan, A. (2004). The research of pollution by anabolic hormones, beta- agonists and pesticides in meat and other organs of broilers in. Etlik Vet. Mikrob. Enst. Derg., 15(1- 2); 37-48.
3. Aksoy, A. and Dağoğlu, G. (1998). The effect of Zeranol and Nandrolone (19 nortestosteronhekzafenilpropiyonat) on the weight gain, FSH, LH, total testosterone and some biochemical parameters of Akkaraman Lambs. YYÜ Vet Fak.Derg, 9, 17-28.
4. Alpan, O. (1989). Büyüme hızlandırıcı maddelerin hayvancılıkta kullanımı ve ekonomik değeri. TUBİTAK-VHAG, İhtisas Komisyonu Toplantısı-X, Ankara, 25-30.
5. Anonymous (1999). European Commission, unit B3-management of scientific committees II: Opinion of the scientific committee on veterinary measures relating to public health: Assessment of potential risks to human health from hormone residues in bovine meat and meat products. 30 April 1999.<http://www.europa.eu.int>
6. Anonymous (2000). Food Series 43, FAO Food and Nutrition 41/12. http://www.who.int/ipcs/food/jecfa/summaries/en/summary_52.pdf.
7. Anonymous (2003). Turkish Food Codex Regulation, Communiqué on Hormones and Similar Substances Prohibited to be Applied to Food-Rated Animals and Linked to Certain Conditions. Communiqué No: 2003/18, 19.06.2003.
8. Apple, J.K., Dikeman, M.E. and Simms, D.D. (1991). Effects of synthetic hormone implants singularly or in combinations on performance carcass traits, and longissimus muscle palatability of Holstein steers. J of Animal Science, 69: 4437-4448.
9. Barai, B.K., Nayak, R.R., Singhal, R.S. and Kulkarni, P.R. (1992). Approaches to the detection of meat adulteration. Trends Food Sci Tech 3, 69-72.
10. Bender, A. (1992). Meat and meat products in human nutrition in developing countries. Food Nutr. Pap., 53:1-91.
11. Boland, M.J., Rae, A.N., Vereijken, J.M., Meuwissen, M.P.M., Fischer, A.R.H., van Boekel, M.A.J.S., Rutherford, S.M., Gruppen, H., Moughan, P.J. and Hendriks, W.H. (2013). The future supply of animal-derived protein for human consumption. Trends Food Sci. Tech., 29,62-73.
12. Cawthorn, D.M., Steinman, H.A. and Hoffman, L.C. (2013). A high incidence of species substitution and mislabelling detected in meat products sold in South Africa. Food Contro., 32, 440-449.
13. Chaoyang, L., Ximei, W., Binghui, Z. and Xiaoyong, Z. (2008). Determination of diethylstilbestrol in chicken meat by HPLC internal standard method. Chemistry, 6, 1-4.
14. Cooper, K.M., Ribeiro, L., Alves, P., Vozikis, V., Tsitsamis, S., Alfredsson, G., Lovgren, T., Tuomola, M., Takalo, H., Litia, A., Sterk, S., Blokland, M. and Kennedy, D. (2003). Interlaboratory ring test of time resolved fluoroimmunoassays for zeranol and α -zearalenol and comparison with zeranol test kits. Food Add. Contam., 20(9):804-812.
15. Daxenberger, A., Lange, I.G. and Meyer, H.H.D. (2000). Detection of anabolic residues in misplaced implantation sites in cattle. Journal of AOAC International, 83 (4) : 809-819.
16. Dikeman, M.E. (2007). Effectes of metabolic modifiers on carcass traits and meat quality. Meat Sci., 77,121-135.
17. Donovan, C. (2015). If FDA Does Not Regulate Food, Who Will? A Study of Hormones and Antibiotics in Meat Production. American Journal of Law and Medicine, 41(2-3): 459-482.

18. Dunshea FR, D'Souza DN, Pethick DW, Harper GS, Warner RD, 2005. Effects of dietary factors and other modifiers on quality and nutritional value of meat. *Meat Sci.*, 71, 8-38.
19. Durmaz, F. (1997). Durmaz F. *VeterinerUygulamalıFarmakoloji*. Medisan yayın serisi No:28, Ankara, Cilt 2 (I. Baskı), 266–269, 1997.
20. European Commission, unit-management of scientific committees II (2016). Opinion of the scientific committee on veterinary measures relating to public health: Assessment of potential risks to human health from hormone residues in bovine meat and meat products. 30 April 2016. <http://www.europa.eu.int>.
21. Font-i Furnols, M. and Guerrero, L. (2014). Consumer preference, behaviour and perception about meat and meat products: An overview. *Meat Sci.*, 98(3): 361-371.
22. Food and Agriculture Organization of The United Nations (1997). European ban on hormone-treated cattle rejected by WTO. <http://www.fao.org/news/1997/970601-e.htm>
23. Food and Drug Administration (FDA) (1991). Center for Veterinary Medicine, Summary of NADA 140-897: Revalor®-S(trenbolone acetate and estradiol). <http://www.fda.gov/cvm/efoi/section2/140897.html>.
24. Fuh, M.R., Huang, S.Y. and Lin, T.Y. (2004). Determination of residual anabolic steroid in meat by gas chromatography-ion trap-mass spectrometer. *Talanta*, 64, 408-414.
25. https://www.stb.org.tr/Resimler/Buyuk/files/1582017_hayvansal_gidalardaki_antibiyotik.pdf
26. Jeong, S.H., Kang, D., Lim, M.W., Kang, C.S. and Sung, H.J. (2010). Risk assessment of growth hormones and antimicrobial residues in meat. *Toxicol. Res.*, 26(4):301-13.
27. Kadim, I.T., Mahgoub, O., Al-Marzooqi, W., Al-Maqbaly, R., Annamali, K. and Khalaf, S.K. (2010). Enzyme-linked immunosorbent assay for screening antibiotic and hormone residues in broiler chicken meat in the Sultanate of Oman. *J. Muscle Foods.*, 21, 243-254.
28. Kahraman, C. (2017). Hayvansal gıdalardaki antibiyotik ve hormon kalıntılarının insan sağlığı üzerine olası etkileri.
29. https://www.stb.org.tr/Resimler/.../1582017_hayvansal_gidalardaki_antibiyotik.pdf
30. Lone, K.P. (1997). Natural sex steroids and their xenobiotic analogs in animal production: growth, carcass quality, pharmacokinetics, metabolism, mode of action, residues, methods and epidemiology. *Crit. Rev. Food Sci. Nutr.*, 37(2):93-209.
31. Mahgoub, O., Kadim, I., Ann, M. and Annamalai K, (2006). Use of Enzyme Linked Immunosorbent Assay (ELISA) for detection of antibiotic and anabolic residues in goat and sheep meat. *World J. Agr. Sci.*, 2, 298-302.
32. Mor, F., Şahindokuyucu, F., Kav, K. and Köker, A. (2011). Determination of zeranol and trenbolone residues in tissue samples of cattle. *Eurasian J. Vet. Sci.*, 27(4); 235-239.
33. Nazlı, B., Çolak, H., Aydın, A. and Hampikyan, H. (2005b). The presence of some anabolic residues in meat and meat products sold in Istanbul. *Turk J. Vet. Anim. Sci.*, 29, 691-699.
34. Nazlı, B., Çolak, H. and Hampikyan, H. (2005a). A study on the presence of some anabolic residues in offals marketed in Istanbul. *J. Fac. Med Istanbul univ.*, 31(1), 83-92.
35. Oruç, H.H., Cengiz, M., Bağdaş, D. and Uzunoğlu, I. (2007). Zeranol, Diethylstilbestrol (DES), Clenbuterol, 17β-oestradiol and Testosterone Residues in Cattle Meat. *Uludag Univ. J. Fac. Vet. Med.*, 26 (1-2), 11-15.
36. Reig, M. and Toldra, F. (2008). Veterinary drug residues in meat: Concerns and rapid methods for detection. *Meat Sci.*, 78, 60-67.
37. Sadek, I.A., Ismail, H.M., Salam, H.N. and Salem, M. (1998). Survey of hormonal levels in meat and poultry sold in Alexandria Egypt. *Eastern Mediterranean Health J.*, 4, 239-243.
38. Şevik, S.E. and Ayaz, N.D. (2017). Investigation of hormone residues in beef. *Vet Hekim Der. Derg.*, 88(1): 13-20.
39. Saraç, B.M., Zerrin, M. and Altunçul, V. (1999). Deneysel zeranol enjeksiyonunun tavşanlardaki kan tablosu üzerine etkisi. *Bornova Vet. Kont. Araşt. Enst. Derg.*, 24, 11-14.
40. Sawaya, W.N., Lone, K., Saeed, T., Husain, A. and Khalafawi, S. (1998a). Application of an enzyme-linked immunosorbent assay for screening of sheep urine and animal tissue for the androgenic steroid trenbolone acetate in the state of Kuwait. *Food Addit. Contam.*, 15, 151-156.
41. Sawaya, W., Lone, K.P., Hasain, A., Dashti, B. and Al-Zenki, S. (1998b). Screening for estrogenic steroids in sheep and chicken by the application of enzyme-linked immunosorbent assay and a comparison with analysis by gas chromatography-mass spectrometry. *Food Chem.*, 63 (4): 563-569.
42. Seo, J., Kim, H.Y., Chung, B.C. and Hong, J. (2005). Simultaneous determination of anabolic steroids and synthetic hormones in meat by freezing lipidfiltration, solid phase extraction and gas chromatography-mass spectrometry. *J. Chromatog. A*, 1067, 303-309.

43. Sever, E., Okumuş, B. and İnce S. (2012). Investigation of Residual 17 β -estradiol, Diethylstilbestrol, and Zeranol in Red Meat Sold in Erzurum Province, Turkey. *J. of the Fac. of Vet. Med., Kafkas Uni.*, 18 (2): 267-272.
44. Sun, M.M., Zhao, Y.W., Liang, Y., Qian, J.R., Li, L.H. and Wang, S.H. (2010). Determination of residual diethylstilbestrol in chicken by fluoroimmunoassay. *Phys. Test Chem. Anal.*, B5, 1-4.
45. Şanlı, Y. (1989). Et üretiminin tavukçulukla ilişkileri: Anabolik hormon, *Çiftlik Dergisi*, 66.
46. Toldra, F. and Reig, M. (2006). Methods for rapid detection of chemical and veterinary drug residues in animal foods. *Trends Food Sci. Tech.*, 17, 482-489.
47. Turner, N.D., Greene, L.W., Byers, F.W. and Kenison, D.C. (1995). Influence of incremental zeranol implant doses on the chemical and physical characteristics of third metacarpal bone and chemical composition of liver and soft tissues from feedlot steers. *J. Anim. Sci.*, 73:1-8.
48. Uran, H. (2013). A research on the determination of quality characteristics of chicken burgers produced by the addition of transglutaminase. Namık Kemal University Graduate School of Natural and Applied Sciences, Ph.D. Thesis, pp 71, Tekirdağ, Turkey.
49. Van Peteghem, C.H. and Van Haver, G.M. (1986). Chromatographic purification and radio-immunoassay of diethylstilbestrol residues in meat. *Analytica Chimica Acta*, 182:293-298.
50. Verbeke, W. and Viaene, J. (1999). Beliefs, attitude and behaviour towards fresh meat consumption in Belgium: empirical evidence from a consumer survey. *Food Quality and Preference*, 10:437-445.
51. Webb, E.J. and Erasmus, L.J. (2013). The effect of production system and management practices on the quality of meat products from ruminant livestock. *South African Journal of Animal Science*, 43(3): 413-423.
52. Wolk, A. (2017). Potential health hazards of eating red meat. *Journal of Internal Medicine*, 281(2): 106-122.
53. Yılmaz, D., Durmaz, G. and Oz, B. (2007). The presence of some anabolic residues in beef meats. 5 International Congress on Food Technology Greece "Consumer Protection through Food Process Improvement & Innovation in The World" Proceedings Volume 1, 502-505.