

## Determination of Diethylstilbestrol Residue in Raw Meat Sold in Burdur

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### Abstract

The aim of this study was to detect diethylstilbestrol residue in raw meat sold in Burdur. In the study, a total of 120 raw meat samples were obtained from different markets and slaughterhouse used as test materials. The results indicated that the diethylstilbestrol residue was not detected in any of the samples. In conclusion, since presence of hormones in raw meat and meat products is harmful for consumers, utilization of anabolic agents must be strictly controlled in Turkey

**Keyword: Diethylstilbestrol, Residue, Raw Meat, ELISA**

## INTRODUCTION

Diethylstilbestrol (DES) is a synthetic non-steroidal estrogen, which was first synthesized in 1938 in London. DES was widely used, both clinically and in the agricultural industry, before the health risks associated with it became known. DES can produce the same pharmacological and therapeutic effects as estradiol, a naturally occurring hormone used to treat estrogen hypothyroidism and hormonal imbalance [1]. In animals, DES was widely used as a growth stimulant to improve feed conversion efficiency and promote growth rates, resulting in increased protein metabolism and animal daily gain, and reduced fat [1]. However, there is evidence that DES has the potential for mutagenic, teratogenic, and carcinogenic effects, which has raised widespread concern [1, 2, 3, 4].

Meat and meat products, which play an important role in human nutrition should be safe and should not contain any factors or substances harmful for human health. However, the anabolic agents used for various purposes in animal husbandry for slaughter tend to leave residues and thus cause some problems in consumer health. But depending on the use of anabolics in animal feed, anabolic residues that may occur in meat and meat products present risks to human health [5]. Its veterinary use as a growth promoter for food producing animals was banned in most of countries (since 1979 in USA and since 1981 in the EU) to protect consumers from exposure to the carcinogenic residues in animal products [6]. The EU has established the minimum required performance limit (MRPL) of 0.5–2.0 µg/kg to control its abuse in food of animal origin [6]. Therefore, the development of a simple, rapid, sensitive, and specific method to detect DES residues in animal food products is desirable.

Immunoassay method based on the highly specific antigen and antibody interaction could be a sensitive tool in a field of microanalysis. Radioimmunoassay (RIA) fluorescence immunoassay (FIA), enzyme-linked immunosorbent assay (ELISA) were developed for the analysis of DES. Compared to RIA and FIA, ELISA has the advantages of safety, speediness, reliability, sensitivity and low-cost [7]. ELISA is one of the sophisticated techniques to detect residuals based on enzyme-labeled reagents. Therefore, investigations using ELISA technique to monitor residues of various drugs in meat products should be applicable to insure the safety of animal food products for human consumption.

Increasing awareness of the scientific as well as public community about the risks of the residues of such compounds on human health has been raised in the recent years. The recent life style of partially or fully dependence on fast foods and the increased consumption of such a food by the adolescents and prepubertal young generation promoted us to monitor the residues of the three natural sex steroid hormones in meats and their available processed product [8]. Diethylstilbestrol had been extensively used as potent growth promoters in livestock, but the uses were banned due to their carcinogenic effects in human and animals [9]. However, these compounds are still available illegally in the markets of certain countries and mostly used for promoting growth of livestock. In order to control the hormone residues in meat and to ensure the safety of Turkish consumers, it is imperative that a monitoring system be put in place to address the concerns. Therefore, the main objective of the present study was to determine the prevalence of diethylstilbestrol residue on raw meat sold in Burdur city, Turkey

## MATERIALS AND METHODS

### Meat samples

In this study raw meat samples were collected from the markets and slaughterhouse in Burdur. The total of 120 samples of raw meat were gathered and kept frozen until use.

### Enzyme Immuno Assay

The ELISA instrument (ELX800, Universal Microplate Reader, BIOTEK) and the ELISA test kits were obtained from Shenzhen Lvshiyuan Biotechnology Co., Ltd, (Shenzhen, China) and used for detecting the residues in sample extracts. These tests were always performed twice.

### Isolation of Diethylstilbestrol

Two g of the homogenized sample was added 6 mL Acetonitrile-Acetone, after shaken for 10 min. The content was centrifuged at above 3000 r/min at 15 °C for 10 min. Three mL supernatant was transferred into a new centrifuge tube, and dried air at 60 °C. After this, 0.5 mL of CHCl<sub>3</sub> was added and mixed by vortex for 20 sec, then 2 mL of 1 M NaOH was added mixed by vortex for 30 sec, and was centrifuged at above 3000 r/min for 5 min. One mL supernatant was taken, and was added 200 µL 6 M H<sub>3</sub>PO<sub>4</sub>, then the content was mixed by vortex for 5 sec. Three mL of Acetonitrile (CH<sub>3</sub>CN) was added for extraction, after shaken properly for 10 min, was centrifuged at above 3000 r/min at room temperature (20-25°C) for 10 min, and the upper layer was taken and dried air at 60 °C. Dry residue was dissolved in 1 mL of the diluted redissolving solution. Fifty µL water phase was taken, and added 450 µL of the diluted redissolving solution, then shaken properly. Finally, 50 µL of stop solution were added into each well, followed by gentle manual shaking. The optical densities were measured at 450 nm with a plate reader (ELX800, Universal Microplate Reader, BIOTEK).

## RESULTS AND DISCUSSION

In the study, A total of 120 raw meat samples were analyzed for level of diethylstilbestrol by Enzyme-Linked Immunosorbant Assay method. The results indicated that the diethylstilbestrol residue was not detected in any of the samples. As a result of the validation studies of the cut-off value was determined to be 1.508, and the lowest absorbance value was found to be 1.640.

DES residue in meat and meat products has been demonstrated in several studies carried out by ELISA technique in Turkey [10, 11, 12, 13]. Nazlı et al. [10] reported that diethylstilbestrol residues were detected in 21 of 60 samples (35% of the total) whereas 18 of them were fresh meat preparations and 3 of them were meat product samples in Istanbul. However, as diethylstilbestrol residues were detected only in (35% of the total test samples) and amounted to low values such as 0.01-1.0 ppb in 12 samples (85% of the total test samples) and exceeded 1 ppb in only 9 samples (15% of the total), it may be said that diethylstilbestrol is not widely used in Turkish animal husbandry. Oruç et al. [11] detected DES residues in 11 (14%) of 80 cattle meat samples. The average level of DES was 102.13 ng/kg in samples. Günşen et al [12] reported DES residues were determined from 19 (15.97%) of 119 meat and meat products in Bursa. The average level of DES was 148.961 ng/kg in samples. Sever et al [13] detected DES residues in 18 of 135 bovine meat (neck,

thoracic, leg meat) in Erzurum. As a result of studies commented in Turkey, although the use of anabolic agents are banned in Turkey, some anabolic agents have been used illegally in beef cattles to improve the growth rate and feed efficiency, and the residues impose risks for public health. In this research, the results indicated that the diethylstilbestrol residues were not detected in any of the samples. The absence of DES residues in the samples in this study is considered positive for consumer health.

ELISA is a rapid and practical method for residue detection in food products and is recommended by EU. It is mentioned that conducting recovery tests before the study will be useful for a correct test result. For this reason test results are of importance as they give some information about the use of hormone preparations in national animal husbandry and in the food industry [10].

In conclusion, so it seems that the absence of DES in raw meat in markets and slaughterhouse in Burdur is not at risk. However, the number of samples included in this survey was relatively small compared to the total sold in the markets and slaughterhouse. Considering, authorities in the Turkey need to set up strict rules and procedures to monitor the residues in the imported and local meat products. As hormones are frequently administered by unqualified farmers or paraveterinary field staff, awareness campaigns and educational programmes targeted at them on the accurate and proper use of veterinary drugs would help avoid many of the unwanted consequences.

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