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DIETHYLSTILBOESTEROL (DES) HORMONE IN THE EGYPTIAN BOVINE MUSCLES

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SUMMARY

One hundred bovine thigh muscle samples were examined for detection of diethylstilboesterol residues using spectrophotometric technique. Extraction of the samples was carried out according to the method recommended by Umberger et al (1963). Diethylstilboesterol standard solution was prepared at concentration 20µ/ml. For preparation of the working solution for spectrophotometric method 25ml from the prepared solution were mixed with 18% potasium monohydrogen phosphate solution (V/V).

Finally the assay solution required for spectrophotometric method was prepared as well as the iradiation steps.

Each sample extraction was measured and calculated.

The obtaited results revealed that all samples were free from diellylstilboesterole residues. The results were discussed.

INTRODUCTION

Diethylstilboesterol (DES) hormone is one of the important anabolic steroids which are forbidden for using as growth promotor.

Growth promotors are substances added to the animal feeds to improve the daily body gain. Such illegal additives may include some chemical or hormonal substances.

The detection of the hormonal residues is one of the roles applied for protection of the consumer because of the dangerous effect of the synthetic hormones which are toxic and carcinogenic.

The first records of the carcinogenicity of diethylstilboesterol (DES) as a result of its use in the veterinary practices was at 1938 by Lacassagne. Shinkin and Grady (1941) recorded mamary adinocarcinoma of male mice as effect of administration of diethylstilboesterol.

Hartwell (1951) mentioned many records about the toxicity and carcinogenicity of human due to consumption of meat of treated animals with diethylstilboesterol.

Coulston and Wills (1975) recorded that administration of growth promotor hormones may result in higher incidence of cervical and vaginal cancers to pregnant women. On the other hand, Kurman (1979) mentioned that over 300 young women in USA were suffering from neoplasia of their lower genitalia due to exposure to diethylstilboesterol . In addition to that; Fara et al (1979) recorded an outbreak of breast enlargement in girls, and boys in a school in Melano-Italy due to uncontrolled consumption of poultry and beef.

Jukes-Thomas (1979) mentioned that food additive hormones specially diethylstilboesterol should receive good attention because of its carcinogogenic effect on the consumer. Also, it was recorded that hormones were the cause of growth defects in young American childs (Moishezon-Blank 1992) Therefore ,WHO (1988), FAO (1998a) and (1998b) put an important rule to control the use of hormones as growth promoters.

Swilam (1995) examined a total number of 200 samples of fresh, frozen, minced meats and some meat products as balady sausage, luncheon and basterma for detection of diethylstilboesterol residues; he ensured that all examined samples were free from diethylstilboesterol residues.

Fahmy (1998) stated that it is necessary to apply strict control measures on the feeds, drugs and methods of meat production as well as the routien hormonal control on the imported meats.

For detection of hormonal residues in animal tissues Hernricks et al (2001) used Radio Immuno Assay (RIA).

Marie et al (2002) examined meat for detection of illegal growth promotor hormones using receptor binding assay which was based on a direct binding assay of steroid hormones to their respective receptors. The assay revealed that the obtained receptor proteins retained a high affinity for their corresponding native ligand.

Pas et al (2003) studied the relationship between the growth hormones and the blood plasma according to the concentrations of the applied hormones. They ensured the relationship betweer the growth rate and the growth hormones in the blood plasma according to the concentrations o

the applied hormones.

Bing Shao et al (2005) applied a method for determination of residues of illegal natural and synthetic steriods in food of animal origin. The examined samples were subjected to quantitative analysis by liquid chromatography using phenyle column coupled to an electroscop ionization tendum mass spectrometer and the consumption of mobile phase and additive were also optimized to enhance detection sensitivity; they got a wide range of readings and fined their target.

Aly (2006) examined sheep and sheep variety meats for detection of hormonal residues using Radio Immuno Assay (RIA), she found that all residues detected in the examined samples were within the permissible limits.

MATERIALS AND METHODS

One hundred bovine thigh muscle samples were collected from Kafr El-Sheikh abattoirs and transferred separately in polyethelyne sacs to the laboratory for detection of diethylstilboesterol residues using septrophometric technique.

Extraction of samples:

According to the method recommended by Umberger et al (1963) by homogenization of 500gm ground meat with equal weight of chromatographic siliceous earth followed by homogenization with 1.5 litre of absolute ethyl alcohol .The supernatant fluid was filterated and combined with 50ml of 2N Hcl, then the mixture was boiled for reduction of the volume to be 100-150ml. The acid was omitted. The concentrated mixture was washed with 70ml of chloroform, shaked; 300ml of water were added carefully and the separated chloroform layer was removed to another separator containing 100ml of water. The extraction and the washings were carried out twice with 50ml portions of chloroform. The chloroform layers were treated by sodium carbonate-sodium hydroxide.

Then the treated extraction was washed again by 30ml of 10% sodium carbonate with shaking by distilled water .The same steps were repeated by 1% sodium hydroxide for 2 times .The combined sodium hydroxide layers were acidified with 2N Hcl and extarcted for 3 times by chloroform 30ml portions each. The treatment with chloroform was repeated till the alkaline phenolate solution became free from the yellow colour . The final chloroform mixture was then washed and filtered through saturated cotton with chlorform into a glass beaker. The extract was then centrifuged to break the refractory emulation. The residue which remained after evaporation of the solvent from the final chloform extract was dissolved in 2.5ml ethyl alcohol.

Preparation of Diethylstilboesterol standard solution:

Diethylstilboesterol was dissolved in absolute ethyl alcohol to the accurate dilution to get a concentration of 20ug/ml.

Preparation of the working solution for spectrophotometric method:

From the prepared working solution 25ml were mixed with 1.8% potassium monohydrogen phosphate solution (volume/volume)

Preparation of the assay solution for spectrophotometric method:

The applied method was adapted for oily extraction containing not more than 2mg Diethylstilboesterol. The extract of each sample was transfered to a separator containing 50ml iso-octane; the mixture was shaked with 10 ml sodium hydroxide 1/N . The defined aqueous layer was transferred to another separator containing 50ml iso-octan. The mixture was vigorously shaked then the aqueous layer was transferred to third separator. The extraction of the two iso-action layers were repeated successively with portions of 10ml 1/N sodium hydroxide and the aqueous layer of the 3rd separator was collected while the iso-action layer was discarded.

The combined aqueous extract was acidified by 3ml sulphoric acid (H_2SO_4) (1+1) then the solution was cooled.

Diethylstilboesterol was extracted with 30ml chloroform for 3 times; chloroform extract was washed successively in two seporators; the 1st one containing 20ml of 1% sodium carbonate and the 2nd 20 ml distiled water then the washed chloroform extract was filtered through cotton pledget moistened with chloroform into 100ml volumetric flask for dilution to its volume by chloroform and mixed.

Ten ml of chloroform containing Diethylstelboesterol were transferred to a small erlenmyer and were evaporated for dryness. Finally 10ml from the absolute ethyl alcohol were added. The residue was dissolved by swirling and after 15 minutes, 10ml of 1.8% potassium monohydrogen phosphate (K2HPO4) were mixed to prepare the assay solution.

IRRADIATION

Irradiation was applied by testing the transparency of several irradiation containers by putting suitable amount of the working solution to the cuvets which was fixed for 7cm distance from the germicidal lamb (a15 watt) and the solution was irradiated transversely for exactly 10 minutes.

Measured (A) of yellow solution at 4. Irradiation was after 1-3 minutes between each time. time re-

quired for developing of the colour was recorded. Irradiation process with varying distances of the tubes from the lamp was repeated for determination of the suitable status for developing fixed repeatable colours.

Measuring and calculation:

Diethylstelboesterol residues in the samples was calculated as $U = \frac{STXB}{A} = \frac{ST \times B}{A}$

Where U = Concentration of unknown sample.
St = Concentration of standard solution.
A= Reading of standard solution.
B= Reading of unknown sample.

RESULTS

The results obtained from the examination of 100 samples of bovine thigh muscles by spectrophotoometric methods revealed undetectable residues of diethylstelbpesterol (DED).

DISCUSSION

The obtained results in this study are considered as a confirmation to the similar studies which were a reported by Swilam (1995) and Aly, (2006) who found that the hormonal residues in the examined samples were within the permissible limits.

Our conclusion was based on the obtained results in the necessity of application of strict rules for prevention of using of hormonal growth promotors due to their toxic and carcinogenic growth effect on the consumer. Such conclusion is going with those of Lacassagne (1938), Shinkin and Grady (1941), Hartwell (1951) Coulston and Wills (1975), Fara et al (1979), Kurman (1979).

Fahmy, (1998), Henricks et al N(2001), Marie et al (2002) Pas et al (2003) and finally Bing Shao et al.(2005).

At the same time, our conclusion is going strongly with the rules given by WHO (1988 and FAO (1995a) and (1995).

Therefore, this study is one of the recent studies carried out for the detection of diethylstilboesterol (DES) to ensure the necessity for prevention of its use as growth promotor to meat producing animals.

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هرمون داى إيثيل ستلبيستيرل فى عضلات الأبقار المصرية

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فرع كفر الشيخ

مائه عينة من عضلات الأفخاد البقرية فحصت للكشف عن بقايا هرمون أيثيلستلبوسيترول باستخدام طريقة التحليل الطيفى وذلك بتحضير مستخلص العينات طبقا لطريقة أومبرجر وآخرين عام ١٩٦٣ وكذلك بتحضير المحلول القياسى للهرمون بتركيز ٢٠ ميكرون/مل ولتحضير المحلول الفعال للقياس الطبيعى اضيفت ٢٥ مل منه الى مثلها من محلول بوتاسيوم مونوهيدروجين فوسفات تركيز ١ر٨٪ . وأخيرا تم تحضير المحلول اللازم لأختبار التحليل الطيفى مع خطورة التشعيع.

وقد تم قياس وحساب بقايا الهرمون بكل عينة على حده بتطبيق المعادله الخاصة بالإختبار.

النتائج المستخلصة أوضحت أن جميع العينات المستخدمه كانت خاليه تماما من أى بقايا للهرمون وقد نوقشت النتائج بمقارنتها بنتائج الباحثين الآخرين.