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ESTRADIOL HORMONE RESIDUES IN MUTTON AND ITS VARIETY MEATS

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SUMMARY

One hundred fifty samples were collected from slaughtered sheep and examined for detection of Estradiol 17-Beta residues; 30 samples from each muscles, liver, kidneys, spleen and heart from El-Basatin, Giza and Beni-Suef abattoirs. Collected samples were subjected to the diagnostic kits of Radio Immuno Assay (RIA). It was found that samples obtained from El-Basatin abattoir were lower in hormonal residues than those of Giza and Beni-Suef, which they were nearly similar to each other. The concentration of hormonal residues was going in descending manner in liver, kidney, spleen, heart and muscles respectively. All results were found to be within the permissible limit. Muscle samples proved to be positive for Estradiol 17-Beta residues were subjected to grilling and boiling for 30 minutes. Boiling was significantly reduced the Estradiol 17-Beta residues in muscle at $p < 0.05$. Grilling has no effect on the hormone. The public health significance of Estradiol residues was discussed.

INTRODUCTION

Hormonal residues in meats appeared as a problem for the consumer. The extended use of estrogen derivatives to increase the body gain, raised public health questions on the quality of produced meat. Estradiol hormone is one of the important anabolic hormones used as growth promoter among farm animals. The bad effect of the use of the hormones in animal production caused public health hazard. The administration of the growth hormones has effect on protein metabolism as well as improvement of the growth rate and nitrogen retention in the body (Galibraith 1980 and Velle 1982). The hazardous of anabolic hormone and their effect on atherogenic lipid and their deposition in the different edible parts of the receiving animal was discussed (Gielen et al.1988 and El- Guindy 1991). The withdrawal period for the used growth promoting hormone in sheep was estimated by Daxenberger et al. (2001). Henricks et al. (2001) used Radio Immuno Assay (RIA) for detection of hormonal residues in the tissues of

different species which had the hormonal growth promotor as ear implantation. They found that the amount of residues in the liver and kidney was greater than in other tissues of heifer and greater in liver and fat than in muscles in steers.They stated that the minimum hormonal residues was present in muscular tissues, in comparison with other organs. Marie et al. (2002) examined meat for detection of illegal growth promoters using receptor binding assays which 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. In addition, competition studies confirmed that each of the four receptors display a specificity profile for a series of analogs.

Pas et al.(2003) studied the relationships between the growth hormones and the changes in the blood plasma according the concentrations of the applied hormones in pigs. They insured the relationship between the growth rate and the concentration of the growth hormones in blood plasma. Bing Shao et al (2005) developed a method for simultaneous determination of residues of illegal natural and synthetic steroids in foods of animal origin. The samples were subjected to quantitative analysis by liquid chromatography using a phenyl column coupled to an electrospray ionization tendom mass spectrometer and the composition of mobile phase and additives were also optimized to enhance detection sensitivity. They get wide range of readings and find their target.

The present work was planned to estimate the Estradiol 17-Beta hormone residues in slaughtered sheep and its variety meats. The effect of different cooking methods on such residues was studied.

MATERIAL AND METHODS

Part (I): Estradiol 17 - Beta hormonal residues in mutton and its variety meats.

I-Collection of samples.

One hundred fifty samples were collected from slaughtered sheep; 30 samples of muscles; in addition to 120 samples of variety meats (30 each of liver, kidneys, spleen and heart). The samples were collected from Cairo, Giza and Beni-Suef abattoirs (10 from each). Each sample was identified and transferred in ice- box to the laboratory without delay.

II- The Extraction.

The samples were extracted according to the technique recommended by Rapp and Mayer (1985). The samples from mutton thigh, liver, whole spleen, heart and two kidneys were separately ground with equal amount of siliceous earth and absolute ethyl alcohol, then homogenized, filtrated and added 50ml of 2- N HCL. The volume of mixture was reduced by boiling to 100 - 150 ml. Free Estradiol- 17 Beta hormone only recovered through washing the concentrated mixture with 70 ml chloroform into 500 ml separating funnel after shaking, then 300 ml of water was carefully added and the separated choloform layer was removed to another separator funnel containing 100 ml water. Extraction and washing steps were repeated twice with 50 ml of choloform. The choloform layer was treated by washing with 30 ml sodium carbonate 10% ,then a careful succes-

sive shaking with distilled water. Washing was repeated twice by using 30 ml of sodium hydroxide 1%. The combined sodium hydroxide layers which contain impure phenols were acidified with 2 N- Hcl and extracted three times with chloroform 30 ml each. The treatment with choloform was repeated till the alkaline phenolates solution become particularly free from the yellow colour. The final mixture was washed and filtered through saturated cotton with choloform into a suitable beaker. The extracts occasionally were centrifuged to break the refractory emulsion. The residue after evaporation of solvent from the final choloform extract was dissolved in 2 -5 ml ethyl alcohol.

III- Measurement of Estradiol -17 Beta residues By Radio .Immuno .Assay in sheep variety meats and muscle.

Quantitative measurement of Estradiol 17- Beta residues was applied in the Animal Reproduction Research Institute by radio immunoassay Kits (RIA) and Multi-Crystal Gamma counter, LB 2103 according to method of Boursier (1985).

Part (II): Effect of different cooking methods on Estradiol -17 Beta residues in mutton.

The mutton samples proved to be positive for the presence of Estradiol 17- Beta residues were cooked by

a- Boiling in water for 30 minutes.

b- Grilling on coal by traditional method.

The cooked samples were subjected to the same procedures previously applied for detection of Estradiol -17 Beta residues by R.I.A.

RESULTS

Table (1): Estradiol 17- Beta residues (pg/g) in mutton and its variety meats samples collected from El- Basatin abattoir (n = 10)

Parameter	Positive samples Number	%	Concentration of Estradiol 17-Beta residues		
			Minimum	Maximum	Mean ± SE
Liver	10	100	12.99	42.89	32.03 ± 1.22
Kidneys	10	100	12.35	40.25	29.97 ± 1.01
Spleen	10	100	3.42	35.00	27.99 ± 1.04
Heart	10	100	0.83	12.12	9.88 ± 1.54
Muscles	3	30	0.04	9.69	4.45 ± 0.31

Table (2): Estradiol 17- Beta residues (pg/g) in mutton and its variety meats samples collected from Giza abattoir (n = 10)

Parameter	Positive samples Number	%	Concentration of Estradiol 17-Beta residues		
			Minimum	Maximum	Mean ± SE
Liver	10	100	22.37	55.00	42.03 ± 1.72
Kidneys	10	100	21.22	45.87	34.97 ± 1.21
Spleen	10	100	15.98	43.60	33.03 ± 1.00
Heart	10	100	2.00	31.00	18.20 ± 1.68
Muscles	5	50	0.95	12.12	9.72 ± 0.71

Table (3): Estradiol 17- Beta residues (pg/g) in mutton and its variety meats samples collected from Beni-Suef abattoir (n = 10).

Parameter	Positive samples		Concentration of Estradiol 17-Beta residues		
	Number	%	Minimum	Maximum	Mean ± SE
Liver	10	100	26.18	61.42	47.53 ± 9.70
Kidneys	10	100	25.00	49.00	34.00 ± 7.00
Spleen	10	100	19.00	30.62	32.99 ± 3.93
Heart	10	100	8.76	16.68	21.82 ± 2.76
Muscles	5	50	1.23	12.99	10.60 ± 0.50

Table (4): Effect of cooking methods on Estradiol - 17 Beta concentration residues (pg/g) in mutton proved to be positive.

No. samples	Fresh samples	Treated samples			
		After Boiling	Reduction %	After grilling	Reduction %
1	12.68	9.98	21.23	12.57	0.85
2	12,12	9.77	19.34	12.00	1.00
3	9.69	5.34	44.87	9.67	0.12
4	6.00	4.68	22.00	6.00	0.00
5	10.90	6.21	43.00	10.88	0.13
6	9.90	5.53	44.12	9.76	1.41
7	9.10	6.98	23.25	9.00	1.67
8	7.34	5.33	27.38	7.23	1.43
9	6.78	3.98	41.20	6.60	2.71
10	5.60	3.11	44.42	5.40	3.41
Mean ± SE	9.01 ± 0.21	6.09* ± 0.00	--------	8.93 ± 0.02	------
Frequency of reduction	---------	----------	33.08	---------	1.27

*= significantly reduced at P <0.5

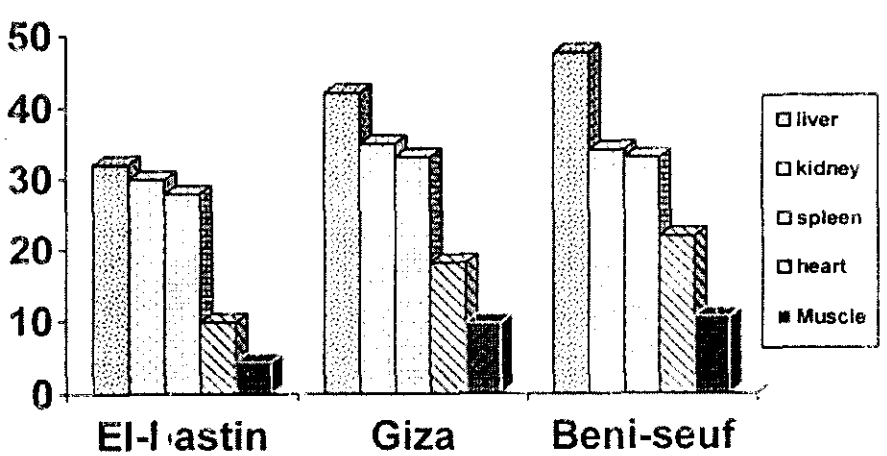

Fig.(1): Estradiol -17 Beta residues in mutton and its variety meats.

DISCUSSION

The data recorded in table (1) revealed that the mean values of Estradiol - 17 Beta levels in slaughtered sheep were 32.03 ± 1.22, 29.97 ± 1.01, 27.99 ± 1.04, 9.88 ± 1.54 and 4.45 ± 0.31 pg/g in liver, kidneys, spleen, heart and muscles respectively at El - Basatin abattoir. Such levels were 42.03 ± 1.72, 34.97 ± 1.21, 33.03 ± 1.00, 18.20 ± 1.68 and 9.72 ± 0.71 pg/g in Giza abattoir, while these levels were 47.53 ± 9.70, 34.00 ± 7.00, 32.99 ± 3.93, 21.82 ± 2.76 and 10.60 + 0.50 pg/g at Beni- Suef abattoir. Similar results were obtained by Sulttan (2002). Low figures were obtained by Fahamy (1998). All the examined muscle and variety meats samples were within the permissible limit (Table1, 2 ,3 and Figure 1), which is probably less than 1 ppb (Gracey, 1981). Moreover, Gracey (1986) reported that when anabolic steroids properly used, the residue levels in meat and other edible tissues do not exceed 1ng/g of tissue. There are several hormones and hormone like agents improve the growth rate and the efficiency of feed intake in farm animals. These hormones may be natural or synthetic. Among these natural hormones is Estradiol 17-Beta. Some countries strictly prohibit the application of hormones especially estrogens (Hoffmann and Evers, 1986). Anabolic agents may be given as subcutaneous implants to sheep or intramuscular injection which is active only for few weeks, implants are active for about 100 days. The mode of action of Estradiol - 17 Beta is increasing nitrogen retention and thereby protein formation. The most serious potential hazard arising from the use of anabolic steroids is that of tissue residues or its metabolites in muscles and other organs (Gracey,1986 and Codex Alimentarius,1995). The body gains in sheep due to the implantation of Estradiol 17-Beta may be attributed to the increasing nitrogen retention and protein formation, so produce animals with a more favorable muscle/ fat ratio (Hui et al., 2001). The low levels of Estradiol 17-Beta residues in tissue may be attributed to that its metabolic and biologically less active derivatives appear in urine and include oestrone, oestriol, 16- epioestriol and 16- hydroxy - oestrone and their conjugates with sulphate and glucuronic acid, This was explained by De Groot (1989). In this respect, Harper (1971) stated that sulphate conjugate of some steroids generally associated with inactivation mechanism in the liver for hormones. On the other hand, Gracey (1986) stated that the withdrawal time of Estradiol 17- Beta is 60 days. The hormonal residues of Estradiol 17 - Beta in the tissue depend on the dosage , withdrawal time and site of subcutaneous implants . From the present data it could be concluded that the metabolism of Estradiol 17-Beta in the live sheep produced many derivatives of metabolites which conjugated with sulphate and glucuronic acid in the liver, In addition to the short period of withdrawal may be causative agents for low residual levels (De Groot,1989; Harper,1971 and Gracey,1986). Therefore the slaughtering of sheep after sixty - ninety days from implant with Estradiol 17- Beta was recommended to confirm the withdrawal of most of the residues, as well as the application of Good Animal Practices was necessary (Codex Alimentarius, 1995).

On the other hand, Cole and Sweeney (1980) reported that Estrogens have been shown to stimulate liver cell proliferation. The results of several studies have described the appearance of hepato cellular carcinoma in women using oral contraceptive steroids for prolonged periods of time (Henderson ,1983).

The results recorded in Table (4) revealed that boiling of mutton positive for Estradiol 17- Beta residues for 30 minutes was significantly reduces (at $p < 0.05$) the levels of such residues. The mean reduction rate for all meat samples was 33.08%. Nearly similar results were obtained by Sadek et al. (1998). On the contrary, Sulttan (2002) stated that there is no effect of boiling on such residues. Grilling of mutton has no effect on Estradiol 17- Beta residues. The reduction of hormonal residues in some meat samples by boiling may be attributed to the storage of hormone in fat, as well as during boiling the fat melted in boiling water with their hormonal content, therefore the level of reduction in boiling (33.08%) is greater than grilling (1.27%). From the present data, it could be concluded that the efficient home cooking of meat could get rid of Estradiol 17- Beta residues to a certain extent.

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متبقيات هرمون الاستراديول في لحوم الضان واحشائه

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الملخص العربى

تم جمع مائة وخمسون عينة من الأغنام المذبوحة ، ٣٠ عينة من كلا من العضلات والكبد والكلاوى والطحال والقلوب من مجازر البساتين والجيزة وبنى سويف بحيث فحصت لمعرفة مدى تواجد متبقيات هرمون الاستراديول ١٧٠ بيتا بها باستخدام الطريقة المناعية الاشعاعية (RIA) بجهاز عداد جاما.

بينت النتائج ان متوسط تواجد هرمون الاستراديول ١٧٠ بيتا في العضلات كان ٤٥ ،٤ ±٣ بينما في كلا من الكبد والكلاوي والطحال والقلوب كان ٠٣ ،٣٢ ± ٢٢ ،١ ، ٩٧ ،٢٩ ± ١٠١ ،١ ± ٩٩ ،٢٧ ± ٠٤ ،١ ، ٨٨ ،٩ ± ٥٤ ،١ من مجزر البساتين ، بينما في مجزر الجيزة أظهرت النتائج ارتفاع متوسط تواجد هرمون± الاستراديول – ١٧ بيتا فكانت في العضلات ٧٢ ،٩±٧١ والكبد والكلاوي والطحال والقلوب كانت ٠٣ ،٤٢± ٧٢ ،١ ،٩٧ ،٣٤± ٢١ ،١ ، ٠٣ ،٣٣ ± ١ ، ٢٠ ،١٨ ± ٦٨ ،١ أما مجزر بني سويف فكان متوسط تواجد الهرمون في العضلات والكبد والكلاوي والطحال والقلوب ٦٠ ،١٠ ± ٥، ٥٣ ،٤٧ ± ٧٠ ،٩ ، ٣٤ ± ٧ ،٩٩ ،٢٢ ± ٩٣ ،٣، ٨٢ ،٢١ ± ٧٦ ،٢ علي التوالي. وقد كانت كل النتائج التي تم الحصول عليها في اطار الحد المسموح به.

تم تعريض عينات اللحوم التي ثبت ان بها متبقيات هرمون الاسترادول – ١٧ بيتا للسلق (في الماء المغلي لمدة ٣٠ دقيقة) وللشواء. فوجد ان الغليان قد اختزل متبقيات هرمون الاستراديول – ١٧بيتا في اللحوم اختزال معنوي وكانت نسبة الاختزال ٨ ،٣٣٪ عند درجة حرية أقل من ٠٥ ،٠ وان الشواء ليس له تأثير معنوى علي متبقيات الهرمون عند درجة حرية اقل من ٠٥ ،٠. تم مناقشة المخاطر الصحية لمتبقيات هرمون الاسترايول – ١٧ بيتا علي صحة المستهلك.