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PRELIMINARY SCREENING OF OESTROGENIC ACTIVITY IN THE IMMATURE AND MATURE GREEN MAIZE FODDER

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Oestrogenic activity of fresh green maize fodder harvested during the months of July through October was estimated by feeding the test diets to immature female mice and determining the increase in weight of their freshly excised uteri. The mature and immature maize extracts were found respectively to possess a potency equivalent to 1.43 and 2.38 micrograms of diethylstiboestrol per 100 gram of dried fodder. There seems a possible relationship between the higher maize oestrogenic contents and the high incidence of prolapse uteri in buffaloes of Lyallpur observed during the immature maize feeding season.

INTRODUCTION

A great many species of plants including clovers, oats, wheat barley and grasses etc., are known to contain substances which when consumed by the animals, produce effects on the reproductive system similar to those induced by animal oestrogens (Bennets *et. al.*, 1946; and Bradbury and White, 1954). Recently symptoms of excessive oestrogenic intake were observed in cows and heifers fed mainly on maize and clover silage. When this feed was withheld and these animals were treated with a group of hormones, conception occured within 7-8 weeks (Lotthammer, *et. al.*, 1970).

The most common reproductive disorders in order of their prevalence encountered in buffaloes of Lyallpur during the years 1971-73 were anoestrous, metritis, prolapse uteri, retained placenta, dystocia and abortion (Ahmad, 1973). In this report, the author has further pointed out that an alarmingly high incidence of prolapse uteri was recorded in the months of June through October. According to him it was a matter of great concern and required a large scale survey on provincial basis to know the magnitude and etiology of this problem. While recording his observations on the etiology of this syndrome, the author has suggested that either (i) a calcium-phosphorus imbalance prodisposed these animals to such an attack, or (ii) certain feed stuffs including fodders utilized by these animals contained significant amounts of oestrogens which

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after their accumulation in the body resulted in the initiation of this disorder, or (iii) certain invading micro-orgrnisms caused irritation of the genitalia and ultimately led to this serious episode.

Green maize is the major fodder consumed by the buffaloes during the period when the incidence of prolapse uteri has been found to be the highest. It was for this reason that the present experiment was designed to estimate the oestrogenic contents of the local maize so that the role of the forage oestrogens in the causation of prolapse uteri in buffaloes of this region could be defined.

MATERIALS AND METHODS

Collection of Fodder Material :

Several random samples of maize were obtained from field plots of the University of Agriculture, Lyallpur. The immature lot was harvested during the month of July, 1973 while the mature maize sample were obtained during the month of November, 1973. Fresh samples were brought to the laboratory and refrigerated until extraction of oestrogens was carried out.

Preparation of E tracts for Oral Administration :

Freshly harvested plant material was cut into ½ inch lengths and thoroughly mixed. Several 10 Gram portions were then removed for moisture determinations. A 400 Gram portion was placed in a paper bag and refrigerated until extraction for assay. Oestrogen concentrates was prepared from the forage sample by thoroughly disintegrating the fresh tissue with 1400 ml. of acetone in a blender for about five minutes. The entire content of the blender bowl was filtered through a coarse, sintered glass funnel 9 cm. in diameter, coated with a thin layer of diatomaceous earth. The filtered cakes were washed with an additional 400 ml. of acetone and the combined filterate concentrated under vacuum at a temperature of 60°C to a volume of about 30 ml. The aqueous concentrate was transferred to a separatory funnel and extracted 4 times with 60 ml. portions of ethyl ether. The ether extracts were added to the flask employed for the evaporation and taken to dryness under vacuum. The solid residue now uniformly coating the inside of the flask employed for evaporation was dissolved in a minimum volume of acetone and alchol and added to the mouse ration. The solvent was removed in a current of air with continuous stirring to assure uniform distribution of oestrogens in the ration. Last traces of solvent were removed by keeping the sample under high vacuum for several hours. The test sample were adjusted to given 15, 20, 25 and 30 grams equivalents of forage per 100 Gram of test ration so that the consumption of 50 Grams of each ration by five mice of a group have 1.5, 2. 2.5 and 3 grams of forage/mouse respectively.

Bioassay :

The method of Bickoff *et. al.*, (1959) was followed for the bioassay of extracted oestrogens. Immature Swiss albino female mice reared in the Department of Physiology and Pharmacology, University of Agriculture, Lyallpur were used in the present study. Animals of 20-22 days of age were selected so that their weight ranged from 8-10 grams. The mice in groups of 5 were caged and fed the test diet *ad libitum* until a total of 50 Grams of ration had been consumed by five mice. This usually took about 6-8 days. The mice were sacrifled and the fresh excised uteri were carefully trimmed and weighed without blotting or slitting. The oestrogenic activity of the forage was expressed in terms of diethylstilboestrol equivalence by comparing with previously constructed dose response curve prepared by feeding graded increments of diethylstilboestrol incorporated in the control diet to mice in groups of 5.

RESULTS

The comparison of results of the mouse bioassays of immature maize extracts, together with controls with and without added diethylstilboestrol (D.E.S.) are shown in Table 1.

TABLE 1. *Comparison of oestrogenic activity in the extract of Immature maize fodder with the control and diethylstilboestrol diets*

Diet	Quantity fed per mouse	No. of Bioassays	No. of mice/Assay	Average Body wt. Start	Average Body wt. End.	Average uterine weights (Mean ± S.E.)
				Gram	Gram	Milligrams
Control	—	5	5	9.10	10.12	9.73 ± 0.33
	Microgramms					
Diethyl–	0.025	3	5	8.80	9.12	10.75 ± 0.14
stilboestrol	0.050	3	5	9.30	9.90	15.61 ± 0.41
	0.075	3	5	9.10	9.82	23.00 ± 0.65
	0.100	3	5	8.90	9.63	31.83 ± 0.65
	Grams*					
Maize	1.5	3	5	10.12	10.31	9.76 ± 0.71
extract	2.0	3	5	10.11	10.32	13.56 ± 0.98
	2.5	3	5	9.75	10.15	18.98 ± 1.50
	3.0	3	5	9.90	10.23	23.50 ± 2.6

S.E = Standard Error.
* = Equivalent weight of dried maize fodder.

The dose response curves of the immature maize extract and D.E.S. are linear and their slopes are very similar. From these data it can be seen that this typical maize has a potency equivalent to 2.38 micrograms of diethylstilboestrol per 100 Gram of fodder (Fig.1). The results of bioassay of mature maize fodder together with control are shown in Table 2.

If we assume that the diethylstilboestrol is the predominent oestrogenic substance in this forage, then we can calculate that it was present in the mature maize fodder at a concentration of 1.43 micrograms/100 Grams. The average body weight of all the mice used in this study varied between approximately 9-11 Grams in the start and at the end of the assay.

TABLE 2. *Comparison of Oestrogenic Activity in the extract of mature maize fodder with control and diethylstilboestrol diets*

Diet	Quantity fed/mouse	No of bioassay	No. of mice/assay	Average body weights Start	End	Average uterine weight (Mean ± S.E)
				Grams	*Grams*	*Milligrams*
Control	—	5	5	9.10	10.12	9.73 0.33
	Micrograms					
Diethyl-	0.025	3	5	8.80	9.12	10.75 ± 0.14
stilboes-	0.050	3	5	9.30	9.90	15.61 ± 0.41
trol.	0.075	3	5	9.10	9.82	31.00 ± 0.85
	0.100	3	5	8.90	9.63	31.83 ± 0.65
	*Grams**					
Maize	1.5	3	5	9.38	9.43	2.78 ± 0.18
extract	2.0	3	5	9.18	9.38	10.12 ± 0.11
	2.5	3	5	9.22	9.82	12.32 ± 0.23
	3.0	3	5	9.48	9.48	14.11 ± 0.31
	3.5	3	5	9.11	9.78	15.15 ± 0.42

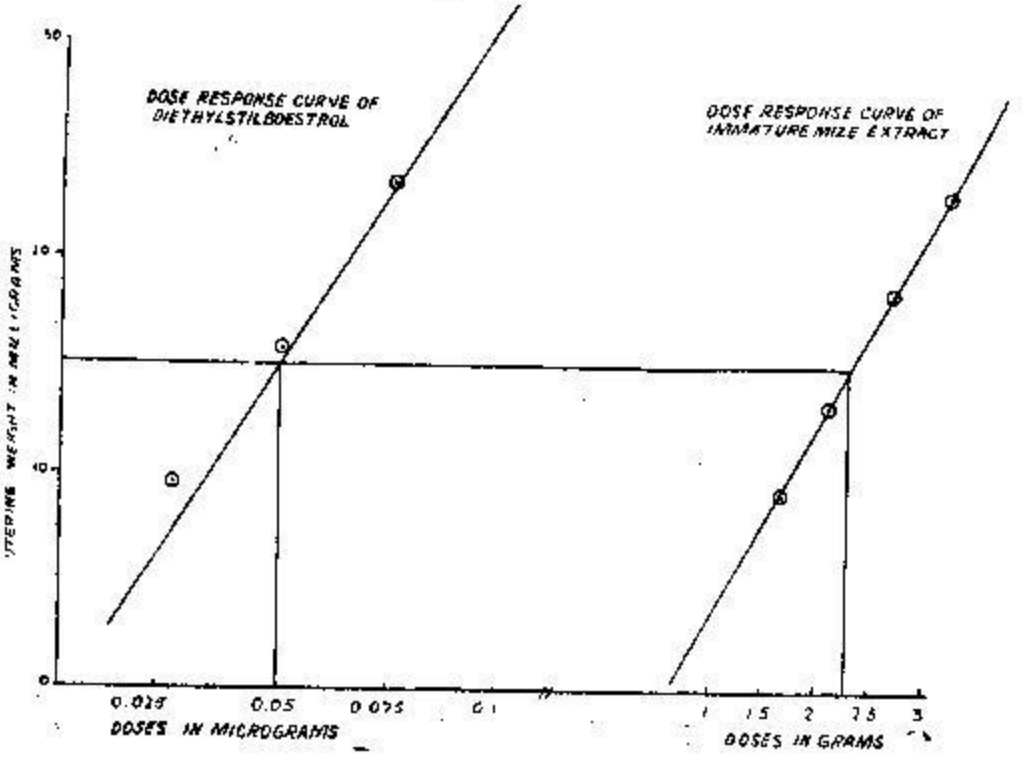
S.E = Standard Error.
* = Equivalent weight of dry maize fodder.

DISCUSSION

The occurence of oestrogenically active substances in plant materials is well established (Bradbury *et. al.*, 1954). The data presented here indicates that the oral intake of green immature maize fodder produced a well marked uterine response in immature mice administered in various doses of the plant

extract and this typical maize fodder has a potency equivalent to 2.38 Micrograms of diethylstilboestrol per 100 Grams. The mature maize fodder harvested in the month of October, however, exhibited a much reduced activity (1.43 micrograms/100 gram diethylstilboestrol equivalents). This difference in mature and immature fodder can be explained on the basis of reports of Alexander and Watson (1951) and Kitts *et. al.*, (1959), who observed seasonal variations in the oestrogenic activity of plants. They showed that in general the oestrogenic activity of the forages is relatively high during the early phases of vegetative growth and decline at full bloom stage. The present findings of existance of oestrogenic activity in maize are in conformity with those of Lotthammer *et. al.*, (1970).

Subterranean clover containing 4.4 micrograms diethylstilboestrol equivalents/100 grams of dried fodder caused infertility in Australia sheep (Bennetts, 1949 and Barrett *et. al.*, 1961). Lotthammer, *et. al.*, (1970) reported that the feeding of corn silarge containing 4.9 micrograms of oestradiol equivalents produced severe symptoms of hyper oestrogenicity for several weeks in cause. In comparison, the present detected level of oestrogenic activity



in immature maize (2.38 micrograms/100 gram of dried fodder) also appears to be quite high to cause reproductive disturbances. For example, an adult buffalo consumes daily about 50 Kg. of this fodder. This means, she will daily consume about 1.19 mg. of diethylstilboestrol equivalents. It has also been reported that repeated doses of diethylstilboestrol may cause post-parturient straining with prolapse of the vagina or even of the uterus (Jones, 1965). As maize is one of the major fodder offered to the cattle during the months of July to November, it can, therefore, be suspected that the maize oestrogens are probably responsible for the high incidence of prolapse uteri and other syndromes of oestrogenic stimulation observed during this season. The absence of similar reproductive disturbances in cows can be explained on the basis of the observation that the water buffaloes of this region are much more sensitive to oestrogens than the cows (Ahmad, M., 1973-b).

This is only a preliminary screening and any final conclusion drawn from the present study is subjected to definite limitation as several factors not only alter the oestrogenic contents of various plants but also the response of the animals to oestrogenic intake. The experimental animals used in this study were mice, while the implications are in livestock, therefore, a trial of a wider scale is suggested. The maize samples from different plots/fields and regions and the plants at different stages of maturity should be examined. Controlled studies in buffaloes will also be required to confirm the etiology of the reported episode.

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