BIOCHEMICAL STUDIES ON MYCOTIC ABORTION IN COWS AS A FIELD PROBLEM

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ABSTRACT

Approximatly 15% of a herd of cows aborted at early stages of pregnancy after consuming moldy ration. Further investigation revealed presenee of vaginal prolapse and hyperemic external genetalia of the rest of herd. Results of serum biochemical analysis indicated an elevation of female hormones estradoil and progesterone, significant increase in liver enzymes (aspartate aminotransferase AST and alanine aminotransferase ALT), no changes in calcium and phosphorus levels when compared with control. Analysis of ration ingredients revealed presence of zearalenone at level 450 ug / kg. Detoxification of the ration by ammoniation using anhydrous ammonia 3% and stored for 4 weeks resulted in decrease in the level of zearalenone to 72.8 ug/Kg.

INTRODUCTION

In recent years, the occurrence of estrogenic substances in livestock ration, either as natural constituents or as contaminants, has received increasing attention because of speculation concerning their possible etiological role in outbreaks of certain diseases. Zearalenone is a natural metabolite produced by *Fusarium. roseum, F. tricinctum, F. oxysporum and F. moniliforme.* Naturally occurring zearalenone is produced primarily by *Fusarium roseum* (**Christensen, 1979**). Natural contamination with zearalenone is most frequently and at highest levels in corn. Barely, sorghum, wheat and hay have also been found to contain the toxin. Zearalenone toxicity causes a condition commonly referred as hyperestrogenism. It has been associated with infertility problem in dairy cattle (**Mirocha *et al.*, 1968**), hyperestrogensim in swine (**Nelson *et al.*, 1971**) and uterine proliferation in rats (**Christensen *et al.*, 1965**). Zearalenone is a uterotropic fusarium mycotoxin which may produce hyperestrogenism phenomena by interacting with estrogen receptors as has been shown in rat mammary cystol (**Boyd and Wittliff, 1978**) and mouse uterus cystol (**Greenman *et al.*, 1979**). Typical

signs of hyperestrogenism are prolonged estrus, changes in libido, infertility, increased incidence of pseudopregnancy, increased udder or mammary gland development and abnormal lactation (**Mirocha and Christensen, 1974**). Stillbirths, abortions, mastitis, vulvovaginitis and rectal or vaginal prolapses are secondary complications associated with zearalenone ingestion (**Sundlof and Strickland, 1986**).

Zearalenone appears to be fairly rapidly absorbed following oral administration (**Dailey *et al.*, 1980 and Olsen *et al.*, 1985**), the major route of excretion for most species is via the feces (**Hidy *et al.*, 1977**). Fusarium species require moist conditions and warm temperatures (20 - 25°C) for growth while cool temperature (8 - 10°C) are required for optimal zearalenone production (**Christensen and Kauffmann, 1969**). Zearalenone was not destroyed at 80 - 120°C in a neutral medium, but was destroyed at high temperature at pH 11 and above (**Lasztity *et al.*, 1977**). It was also destroyed by aqueous ammonia or hydrogen peroxide (**Tamas and Woller, 1977**). **Chelkowski *et al.*, (1981)** reported that ammoniation of grain not only detoxifies several mycotoxins including zearalenone but also inhibit mold growth.

In this investigation, the occurrence of a series of early abortions, recatal and vaginal prolapse in a herd of cattle kept at a private farm in Giza Governorate has been described. The herd did not respond to different therapeatic drugs. The aim of the present investigation was to elucidate the effect of zearalenone contaminated feed on some blood biochemical parameters in cattle and studying the effect of ammoniation as a mean of detoxification of zearalenone.

MATERIAL AND METHODS

8 Friesian cows out of 35 cows' herd at early stage of pregnancy were reported to be aborted, the non pregnant cows of the same herd were recorded to have swollen and hyperemic external genitalia, no response to therapeutic drugs. Blood samples were collected from 8 aborted cows as well as from 8 apparently healthy pregnant cows of the same herd to be served as control (as they have no clinical symptoms of abortion or vaginal prolapse). Serum samples were separated for biochemical determination of estrogen and progesteron (**Cohon and Bates, 1947**), calcium (**Gingler and King, 1972**), inorganic phosphorus (**Kilchling and Freiburg, 1951**), AST and ALT (**Reitman and Frankel, 1957**). The post mortum lesions of aborted foeti were recorded.

Concentrated ration used for feeding of cattle consisted of yellow corn grain, cottonseed meal, soybean meal, sunflower meal, linseed meal and rice

bran. Ration was subjected to detection of zearalenone according to **European communities (1976)**. Fresh new ration sample was served as control. Detoxification of ration ingredients by ammoniation (3% anhydrous ammonia w/w) was carried out as described by (**Tilley and Terry, 1963**) and stored for 4 weeks and were analyzed for detection of zearalenone after storage period.

Data obtained for both diseased and control animals were simultaneously analyzed and statistically operated with the tested samples according to **Petrie and Watson, (1999)**.

RESULTS

1- Clinical signs and post mortum lesions:

There is obvious edema and hyperemia of the vaginal mucosa, rectal and vaginal prolapse, inflammation of the mammary gland in all affected cows. The pregnant cows aborted at early stage of pregnancy showing uterine prolapse after abortion, weakness and loss of body weight. The dead foeti showed inflammation, oedema and congestion of the carcasses, moreover abnormal development was recorded in one foetus.

2- Biochemical parameters of serum:

Table (1) demonstrated the biochemical changes in serum of affected cow when compared with control animals, there was a significant increase in estrogen (measured as estradiol) and progesterone levels. No significant changes were recorded in calcium and inorganic phosphorus levels. On the other hand, AST and ALT activities were highly significantly increased when compared with control levels ($P< 0.001$).

Table (1): Biochemical changes in the affected and non-affected cow.

Parameter / Animal	Estrsadoil (pg/ml)	Progesteron (ng/dl)	Calcium mg/dl)	Phosphorus mg/dl)	AST (i.u/l)	ALT (i.u/l)
Affected cow	47 ± 0.12*	4.6 ± 0.03*	9.89 ± 0.9	4.67 ± 0.18	121.82 ± 0.24**	93.61 ± 1.03*
Control cow	12 ± 0.09	1.4 ± 0.01	9.17 ± 0.22	5.0 ± 0.01	20.36 ± 0.57	69.00 ± 1.96

* $P < 0.05$

** $P < 0.001$

3- Analysis and detoxification of ration:

Ration analysis revealed presence of zearalenone toxin in a level of 450 ug/kg. Detoxification of ration by means of ammoniation using anhydrous ammonia (3%) and stored for 4 weeks resulting in decrease in zearalenone level reaching to 72.8 ug/kg. (Detoxification rate is 83.8%).

DISCUSSION

Zearalenone is a nonsteroidal estrogenic mycotoxin produced by *Fusarium* species. It is associated with reproductive problems in animals. It is mainly contaminant in corn which require moist condition and cold temperature for production, therefore, zearalenone contamination of feed stuff can be a major problem in moist climate areas and bad storage condition which resulted in high economic losses (**Goodman *et al.*, 1987**).

Results in Table (1) showed significant increase in both of estradiol and progesterone level in aborted and affected cows when compared with control group. Zearalenone may induce this response by interacting with estrogen receptors as has been shown in rat (**Boyed and Wittliff, 1978**), mouse (**Greenman *et al.*, 1979**) and bovine (**Kiang *et al.*, 1978; Ingerowski and Stan 1979**). Meanwhile, **Weaver *et al.*, (1986); Kallela and Ettala, (1984); Coppoek *et al.*, (1990)** and **Skrinjar *et al.*, (1995)** reported a variety of signs of hyperestrogenism and abortion at different stages of pregnancy in cattle fed moldy feed contaminated with zearalenone. **James and Smith, (1982)** mentioned that zearalenone induced uterine enlargement by the effect of endogenous estrogen production. This may be explain the increase in both estradoil and progesterone level. **Ueno, (1984)** reported that zearalenone mimics the effects of the hormone estrogen and induce abortion, embryonic death and inhibition of fetal development

Serum calcium and phosphorus were monitored as a measure of an estrogenic response to zearalenone. No significant differences were observed between the affected and non-affected cows as shown in table (1), the same results were reported by ***Weaver et al., (1986)***. Nevertheless, **Common *et al.*, (1984)** mentioned that estrogen increase the transfer of calcium from gut to blood stream and so, increase serum calcium level in affected animals.

The activity of liver enzymes (AST, ALT) showed significant increase when compared with control values as in table (1). **Ray *et al.*, (1986)** reported liver damage in the affected cows with zearalenone. Meanwhile, **Olsen *et al.*, (1981)** have identified the liver enzyme responsible for reduction and metabolism of zearalenone in rats resulting in changes in the activity of liver enzymes.

The economic losses due to mycotoxins are multifaceted, involving direct crop and livestock losses through reduced health and reproduction efficiency. Mycotoxins specially, zearalenone cause considerable health hazards to man and animals associated with reproductive problems. The high incidence of zearalenone detected in our examined feed samples can be attributed to the bad storage condition and high moisture content in climate. The tested feed samples were found highly contaminated with zearalenone

(450 ug/kg). Our results supported the findings obtained by **Shotwell *et al.*, (1980)** and **Sundlof and Stricklaud, (1986)**.

In the present study, detoxification of the contaminated ration by ammoniation and storage for 4 weeks resulted in decrease in the level of zearalenone to reach to 72.8ug/Kg. **Weaver *et al.*, (1986)** found that cows are resistant to dietary concentration of zearalenone up to50ug/Kg ration. **Chelkowski *et al.*, (1981)** and **Charmley *et al.*, (1996)** confirmed that ammonia solution was not completely effective in destroying zearalenone in grain unless post 28 days treatment was used. Also, **Park *et al.*, (1988)** approved ammoniation as an effective method for detoxification of many mycotoxin including zearalenone. **Park *et al.*, (1988)** explained the mechanism for the action of ammoniation appears to involve a chemical conversion of parent zearalenone to products that exhibit greatly decreased toxicity.

Recommended control measures for mycotoxicosis of feed should be include application of mould inhibitors as quickly as possible when the ingredients are still fresh and control of moisture during storage. Ammoniation can be used as a detoxifying agent of moldy feed to compensate economic losses.

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الملخص العربي

ظهرت حالات اجهاض مبكر في حوالي ١٥ % في قطيع من الأبقار في مزرعة خاصة في محافظة الجيزة بينما ظهرت حالات انقلاب في المهبل و التهاب في الأعضاء التناسلية الخارجية في معظم بقية القطيع. وبدراسة هذه الأعراض اظهرت نتائج التحليل الكيميائي لدم الحيوانات المصابة مقارنا بدم الحيوانات الضابطة (التي لم تظهر عليها أي أعراض مرضية) ارتفاع في نسبة الهرمونات الأنثوية (الاستروجين و البروجستيرون). كانت هناك زيادة معنوية في انزيمات الكبد (الأسبرتات و الألانين أمينوترانسفيراز) ولم يحدث أى تغيير في نسبة الكالسيوم و الفوسفور.

أظهرت نتائج تحليل العليقة وجود سموم الزيرالينون بنسبة ٤٥٠ ميكروجرام/كجم. وهذا هو السبب الرئيسى لهذه المشكلة. كانت هناك محاولة لتقليل نسبة سموم الزيرالينون عن طريق معالجة العليقة باستخدام أمونيا لا مائية و حفظ العليقة لمدة ٤ أسابيع و بعد هذه المعالجة انخفضت نسبة سموم الزيرالينون الى ٧٢,٨ ميكروجرام/ كجم.

يمكن استخلاص أن معالجة العليقة المصابة بسموم الزيرالينون بواسطة الأمونيا اللامائية ٣% تؤثر بشكل ملحوظ على نسبة سموم الزيرالينون.