

EFFECT OF MYCOTOXIN ON REPRODUCTIVE PERFORMANCE IN DAIRY CATTLE

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ABSTRACT

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The present study was determined the effect of combined mycotoxins caused by Aflatoxin and zearalenone exposure on reproductive performance and their relation to the incidence of ovarian cysts in dairy cattle. The present study used 28 lactating cows kept at dairy farm in Dakahlia governorate. Cows were classified into two groups First group (10 cows) were received ration where the percentage of aflatoxin and zearalenone not exceed permissible limit which is (5 ppb and 200 ppb respectively) and show normal estrous behavior and have mature large follicles >22mm<25mm in diameter and have corpus luteum. The second group (18 cows) received ration containing aflatoxin which found exceed permissible limit (19.7 ppb) and zearalenone which was present at high levels (400 ppb), these cows exhibit "constant" estrus (nymphomania), excessive vaginal discharge, until day 45 postpartum and have ovarian cysts based on the presence of follicular structure (≥ 25 mm in diameter) with anovulation and the absence of corpus luteum. Blood samples was collected from each cow around 45 days postpartum for evaluation of serum progesterone (P_4), estrogen (E_2), Luteinizing hormone (LH), triiodothyronine (T_3), thyroxin (T_4), prolactin (PRL) total protein, calcium, inorganic phosphorus, Liver enzymatic activities alanine aminotransferase (ALT) and aspartate aminotransferase (AST) immunoglobulins (IgG, IgM, IgA). The results of hormonal assay showed significant increase ($p < 0.05$) in levels of prolactin and estrogen, while levels of LH, progesterone, T_3 and T_4 showed significant decrease ($p < 0.05$) in cows received contaminated ration with zearalenone and aflatoxins in compared with control one. Regarding to results of biochemical and immunological parameters revealed a significant increase ($p < 0.05$) in enzymatic activities (AST), (ALT), while total protein, calcium, inorganic phosphorus and immunological parameters (IgG, IgM, IgA) showed significant decrease ($p < 0.05$) in cows received contaminated ration with zearalenone and aflatoxins compared with control one. Our results using transrectal ultrasonography revealed that mean diameter of follicular cyst was 42.3 ± 3 mm of cows received contaminated ration with zearalenone and aflatoxins while mean diameter of mature follicles was 22.1 ± 1.7 mm of control cows. We concluded that zearalenone have been shown to competitive bind to oestrogen receptors and affect reproductive performance generating hormonal imbalance inducing cell toxicity, immunosuppression due to the combined effect of aflatoxins which may predispose cows to the incidence of ovarian cysts.

Key words: Mycotoxin, Reproductive performance, Dairy Cattle.

INTRODUCTION

Mycotoxin contaminations pose growing problem in animal production from the economic and toxicological point of view (Marczuk *et al.*, 2012). The adverse effects of mycotoxins manifest both on the health status, production and reproduction in ruminants, (dairy cows specifically) (Violeta-Elana *et al.*, 2010).

Feed stuff can be infected by more than one fungus, each of them can produce several mycotoxins

consequently, and it is common that many mycotoxins occur simultaneously in feed (Sardjono *et al.*, 1998). This combination can cause more adverse effects than a single mycotoxin due to additive or synergistic interaction.

It appears that zearalenone and aflatoxins are able to directly affect reproductive performance generating hormonal dysfunction and inducing cell toxicity, while other mycotoxins show only indirect effect on health and fertility of dairy cows, through the

reduction of dry matter intake and decrease of gut nutrient absorption (Rossi *et al.*, 2009).

Many phytoestrogens are now recognized as endocrine disruptor compounds capable of interfering with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body which are responsible for reproduction (Retana-Márquez *et al.*, 2012). These compounds are structurally similar to natural estrogens such as 17 β -estradiol allowing them to bind with estrogen receptors and thereby to induce biologically detectable effects (Navarro, 2005).

Zearalenone is a mycoestrogen produced by various fungi of genus *Fusarium* species, and it is one of the key lactones of fungal origin the biotransformation of Zearalenone in cattle involved the formation of two metabolites α zearalenol and β zearalenol which are subsequently conjugated with glucuronic acid in liver. It was suggested that the glucuronic of ZEA was substantially excreted in bile to re-absorbed and metabolized further entering liver and systematic circulation via the portal supply, the reduced form of Zearalenone, zearalenol has increased estrogenic activity (Haggler *et al.*, 2001).

Despite non-steroidal structure, Zearalenone which it activates estrogen receptors resulting in functional and morphological alteration in reproductive organs include prolonged estrus intervals decreased fertility represented by cystic ovarian disease (Kuiper-Goodman *et al.*, 1987).

Aflatoxins are highly toxic metabolites produced by *Aspergillus flavus*, Aflatoxin B₁ is converted to Aflatoxicol, the negative effect of dairy cows is not attributable to a direct activity on oocyte or embryo but it is to altered maternal homeostasis, reduction of DMI, decrease rumen motility, liver damage and reduction on immune response (Casteel, 2006) therefore there is a lack of nutrients and liver disorders that directly interfere with ovarian and uterine activity.

Reproductive toxicity in cows, infertility and hyperestrogenism have been associated with *Fusarium* producing this mycotoxin, Zearalenone causes alteration in the reproductive tract (Mello *et al.*, 1999) and various oestrogenic effects like decrease fertility changed weight of adrenal, thyroid and pituitary glands change in serum levels of progesterone and estradiol, have been observed (JECFA, 2000).

So, the aim of the present study was to determine the effect of combined mycotoxicosis caused by ZEA & Aflatoxin exposure on reproductive performance, and their relation to incidence of ovarian cyst in dairy cattle.

MATERIALS and METHODS

Animals:

The present study used 28 lactating cows kept at dairy farm in Dakahlia governorate. All animals in the present study were fed total mixed rations (TMR), nutrient concentration, met nutritional requirement for lactation according to NRC (2001). Cows were classified into two groups. First group (10 cows) were received TMR mixture where the percentage of aflatoxin and Zearalenone not exceed permissible limit which is (5 ppb and 200 ppb respectively), (FAO, 2004) and show normal estrous behavior and have mature large follicles >22mm<25mm in diameter and have corpus luteum. The second group (18 cows) received ration containing aflatoxin which found (19.7 ppb) and Zearalenone which was present at high levels (400 ppb), these cows exhibit "constant" estrus (nymphomania), excessive vaginal discharge, until day 45 postpartum and have ovarian cysts based on the presence of follicular structure (≥ 25 mm in diameter) with anovulation and the absence of corpus luteum

Blood Samples:

Blood samples collected via jugular vein puncture from each cow around 45 days postpartum twice weekly for two weeks after rectal palpation and ultrasonography observation from Cows with at least 1 large (≥ 25 mm) follicular structure, no detectable signs of luteinization or corpus luteum (cyst) and Cows with at least 1 large mature follicle (>22mm<25mm), corpus luteum in ovary. Blood samples were taken in plain centrifuge tubes for serum separation and evaluation of serum progesterone (P₄), estrogen (E₂), Luteinizing hormone (LH), triiodothyronine (T₃), thyroxin (T₄), prolactin (PRL) total protein, calcium, Inorganic phosphorus, Liver enzymatic activities alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and immunoglobulins (IgG, IgM, IgA).

Hormonal and biochemical determination:

Hormonal assay:

Serum samples were analyzed by commercial kits solid phase radioimmunoassay for the determination of sex steroids (progesterone and estradiol-17 β) levels and Triiodothyronine, thyroxin and Prolactin levels the kits were purchased from DSL, (USA). Progesterone was determined according to Abraham (1981) and estradiol-17 β estimation was carried out according to Hammond (1990), Triiodothyronine and thyroxin according to Larsen *et al.* (1981) and Prolactin according to Sinha (1996). Serum quantitative measurement of LH in serum by (ELISA) according to the method of Wide (1976) by using Mab IMA immune enzymatic kits-manufactured by Echobar France.

Biochemical determination:

The obtained sera were also used for assay of total proteins according to (Sonnen wirth and Jarett 1980), calcium according to Glinder and King (1972) Inorganic phosphorus, according to Kilichling and Freiburg (1951) Liver enzymatic activities alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities according to Reitman and Frankel (1957), spectrophotometrically by using standardized test-kits supplied from Bio-Merieux (Bains/France).

Mycotoxins analysis in feed:

Mycotoxins (aflatoxin and zearleone) were analyzed in feed by high performance liquid chromatography (HPLC-CBC-7210H Austerila) with fluorescent detection (FLD). We offer a variety of clean up columns according to Scott (1997). Mycotoxins (aflatoxin and zearleone) were estimated on feed by HPLC technique according to Howell and Taylor (1981)

Determination of serum immunoglobulin levels:

The development of sandwich ELISA was carried out according to Erahard *et al.* (1992).

Microbiological and parasitological examination: in both groups.

Ultrasonarography

Ovaries were determined by rectal palpation and ultrasography observation, (Pierson Ginther, 1988) and scanned by ultrasonography using a real-time, B mode instrument equipped with a 7.5-MHz linear array intrarectal transducer (Exy Gyne, Version 2.01, and Noveko). Transducer of penetration power 10 cm Ultrasound examinations were performed by a single operator. Cows with at least 1 large (≥ 25 mm) follicular structure, no detectable signs of luteinization or corpus lutium, and low P4 concentrations (< 1 ng/mL) were categorized, retrospectively, as having a follicular cyst. Cows with at least 1 large mature follicle (> 22 mm < 25 mm), corpus lutium in ovary and P4 concentrations (> 1 ng/mL) in control group.

Statistical analysis:

The biochemical, hormonal parameters immunoglobulin levels were subjected to T test analysis according to Senedecor & Cochran (1982).

RESULTS

Table 1: Hormonal assay in control cows compared with those received contaminated ration with zearalenone and aflatoxins.

	Control group	Treated group
OESTROGEN ng/mL	5.42± 5.25	6.32±1*
PROGESTRONE ng/mL	1.02± 0.20	0.49±0.17*
T3 ng/mL	62±3.4	34±3.2*
T4 (µg/dl)	6.5±0.33	4.9±0.15*
LH ng/mL	6.1±0.15	5.2±0.51*
PROLACTIN ng/mL	9.5±0.9	18.5±0.95*

Superscripts star within raw are significant at (p<0.05) Data were present as means ±SE

Table 2: Biochemical parameters in control cows compared with those received contaminated ration with zearalenone and aflatoxins.

	Control group	Treated group
AST (IU/ml)	13±0.2	49±1.5*
ALT (IU/ml)	18±0.94	65±0.3*
PROTIEN (g/ dl)	7.39±0.45	6.24±0.39*
CALCIUM mg/ml	10.2±1.5	7.8±0.9*
PHSPHORUS mg/ml	5.5±0.15	4.3±0.12*

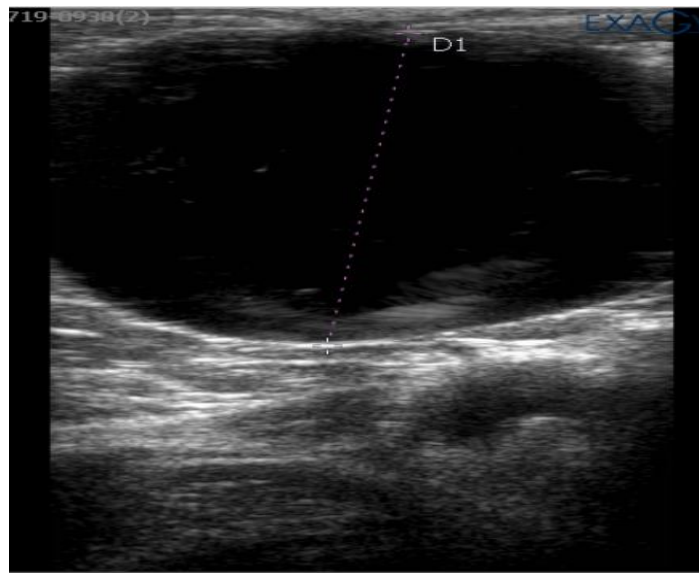
Superscripts star within raw are significant at (p<0.05) Data were present as means ±SE

Table 3: Serum immunoglobulin levels in control cows compared with those received contaminated ration with zearalenone and aflatoxins.

Control group			Treated group		
IgG	IgM	IgA	IgG	IgM	IgA
1639±30.15	205±9.28	84.6±6.11	1471±35.92*	132.5±3.52*	50.5±4.94*

Superscripts star within raw are significant at (p<0.05) Data were present as means ±SE

Fig (1) follicular cystic ovary in dairy cow received contaminated zearalenone and aflatoxins



D: 47.mm

Follicular Observations based on ultrasonography, in cows with cysts in cows received contaminated ration with zearalenone and aflatoxins. Mean diameter of cystic structure (42.3±1.50 mm), cows exhibit “constant”estrus (nymphomania).

Fig (2) Normal mature follicle of control group.



D: 23.2 mm

Follicular Observations based on ultrasonography in normal cows with mature follicle mean diameter mature follicles (22.3±1.71mm), cows show normal estrous behavior.

All cows in the present study showed negative microbiological and parasitological examination. Regarding to our results of hormonal assay in table 1 showed significant increase ($p < 0.05$) in levels of prolactin and estrogen while levels of LH, progesterone, T3 and T4 showed significant decrease ($p < 0.05$) cows received contaminated ration with zearalenone and aflatoxins in compared with control one. The results of biochemical parameters present in table 2 revealed that cows received contaminated ration with Zearalenone and aflatoxins showed a significant increase ($p < 0.05$) in ALT & AST, while total protein, calcium and phosphorus showed significant decrease ($p < 0.05$) in cows received contaminated ration with Zearalenone and aflatoxins in compared with control one. The results of immunological parameters (IgG, IgM, IgA) present in table 3 showed significant decrease ($p < 0.05$) in cows received contaminated ration with zearalenone and aflatoxins than control one. Our results using transrectal ultrasonography in Fig 1 Mean diameter of follicular cyst was 42.3 ± 3 mm of cows received contaminated ration with zearalenone and aflatoxins while Fig 2 mean diameter of mature follicles was 22.1 ± 1.7 mm of control cows.

DISCUSSION

The impact of ZEA in feed on ovarian function of cattle is suggested in this study, these data confirm previous reports concerning the influence of toxic substances on cattle. The alteration of endocrine activity might induced by the cellular stress (Kolesarova *et al.*, 2011).

In the present study our results showed a significant increase at ($p < 0.05$) in levels oestrogen and prolactin while showed a significant decrease at ($p < 0.05$) in levels of progesterone LH, T3 and T4 which could be due to Zearalenone which is a mycoestrogen produced by the fungi fusarium that may be present in moldy feed (Diekman and Green, 1992). This compounds which estrogenic activity and may play a role in cystic ovarian disease. Jakimiuk *et al.* (2010) revealed that Zearalenone exhibits hormonal activity which is determined by its spatial chemical structure, concentration and time of exposure. The reproductive system is a target tissue for Zearalenone hormonal effect. Zearalenone is metabolized to hydroxyl metabolites, referred to as h-zearalenol, by ruminal protozoa, erythrocytes and liver at successive stages of mycotoxin metabolism. A-zearalenol demonstrates higher affinity to estrogen receptor and it is more hormonally active than the original substance, although α -zearalenol is absorbed in small quantities, this active substance is characterized by much higher polarity (Gajęcki *et al.*, 2010). Zearalenone and metabolites, including α -zearalenol bind to estrogen receptors to initiate an agonistic/antagonistic interaction that lead to hyperestrogenism (Marczuk

et al., 2012) the most characteristic symptoms include ovarian cysts and impaired oocyte development. Zearalenone is capable of modulating the level of enzymatic protein expression not only by interacting with estrogen receptors, but also as a substrate by way of competition in the absence of or at low concentrations of endogenous substrate. Its hormonal activity in the gonads is determined by concentrations at which the cellular ERs are reached, as well as by interactions with given ER type. Postpartum ovarian cysts developed when the hypothalamus and pituitary appeared to be less responsive in releasing LH under the influence of estradiol (Kesler & Grverick., 1982) In addition, Erb *et al.* (1973) indicates that may be there is a deficiency in release of GnRH however, the hypothalamic –pituitary axis may not be as responsive in releasing LH due to elevated concentration of estradiol (Jakimiuk *et al.*, 2009). The regulatory effect of estrogen on folliculogenesis and steroidogenesis in ovarian follicles, in particular medium-size follicles, is attributed mostly to ER β in respect of which Zearalenone demonstrates weak estrogenic activity (Schams and Berish, 2002). Yet, most importantly, tissue response, including the ovarian response, is determined by the Zearalenone dose. By affecting the transcription activity of nuclear receptors, Zearalenone and its metabolites modify the metabolism of endogenous and exogenous compounds at ovarian level. The above receptors control the expression and the activity of phase I and II enzymes (Gajęcka *et al.*, 2009). Zearalenone hormonal activity is manifested subject to the mycotoxin concentration and affinity to selected receptors. At low concentrations, Zearalenone may display only hormonal effects, while at high concentrations it may show both estrogenic and xenobiotic activity, thus affecting the enzymes involved in its bioconversion. (Minf *et al.*, 2007). A factor which enhanced the Zearalenone effect on the ovaries of experimental gilts was gonad receptors for which Zearalenone showed a high affinity (Uzumeu and Zacow, 2007). Due to its similarity to estrogen receptors, Zearalenone receptors, causing estrogenic effects in pigs (Chi *et al.*, 2010)

Confirming our results, Fink-Gremmels and Malekinejad (2007): reported that Zearalenone has a resorcylic acid lactone structure and can cross cell membranes binding to the cytosolic 17 β -estradiol (E₂) receptors and forming a Zearalenone E₂R complex. This complex (Zearalenone-E₂R) is transferred into the cell nucleus and binds to specific nuclear E₂ receptors activating the gene responsible for mRNA synthesis (normally generated by E₂). These estrogen-like effects cause anabolic and reproductive activity. Zearalenone interacts not only with both types of estrogen receptors but is also a substrate for hydroxysteroid dehydrogenases, which convert it into two stereo-isomeric metabolites, alpha-zearalenol and beta-zearalenol. Alpha-hydroxylation results in an

increase of estrogenic potency and explains the species specific sensitivity towards Zearalenone intoxications, whereas glucuronidation capacity inactivates Zearalenone (Haggler *et al.*, 2001)

Although doses of zearalenol required to alter pituitary secretion were greater than those required with estradiol, the resulting profiles for the hormones (LH and PRL) were similar, presumably, zearalenol and estradiol exert their effects through common mechanism that involve interaction between zearalenol, estradiol and estrogen receptors within hypothalamus and pituitary resulting in reduced sensitivity and responsiveness to the estrogen – induced PRL secretion and decreased acute pituitary responsiveness to estrogenic stimulation of gonadotropins and PRL (Elasser *et al.*, 1983).

Regarding to thyroid function our results highlight that the decrease levels of thyroid hormones, T3 and T4 inconsistent with Susic-Jurjevic *et al.* (2006) who referred to adverse effect of zearalenone which disrupt the pituitary thyroid axis leading to decrease levels of thyroid hormones, on the other hand TSH cells are estrogen responsive-ER α has been detected, previous studies reported that pharmacological doses of estradiol decreased the TSH immunopositive cells in female rats, other researchers have reported that higher doses of E2 inhibited TSH B mRNA levels. Moreover, Franklyn *et al.* (1987) reported that the biological significance of this mechanism is still unclear, thought it might affect the free thyroid hormone concentrations, resulting in altered tissue availability and disturbed feedback to the pituitary (Schutzler *et al.*, 2007). While, Hopkin (1997) stated that *Aspergillus flavus*, a common indoor mold, has been linked to thyroiditis and possible hypothyroidism.

Regarding to immunological parameters our results revealed that significant decrease at ($p < 0.05$) in IgA, IgG and IgM which could be due to mycoestrogen (zearalenone) become the cause of the decreased efficacy of the action of environmental endogenous estrogens e.g. 17 β -estradiol. They bind to (block) estrogen receptors and they probably cause the transfer of a receptor-ligand complex from cytoplasm to the nucleus and provoke synthesis of particular proteins. The phenomenon of binding (blocking) of these environmental chemical compounds to new estrogen receptors is possible (Arcaro *et al.*, 1999). Mycoestrogen that simulate natural hormones (mimicry) block and change the effect of binding of the hormone to receptor. They can also change the metabolism of natural estrogens (Soto *et al.*, 1995 and Withanage *et al.*, 2001). The problem of a double action of mycoestrogen on the immunological system, which is often noticed, (Yurino *et al.*, 2004). Ample studies have shown that hormones of the reproductive system influence the morphology of thymus and other

parts of the lymphatic system. Administration of estrogens causes thymus involution, (Ansar Ahmed *et al.*, 1999 and Walker *et al.*, 1999). Estrogens regulate the synthesis of serum and uterus immunoglobulins IgM, IgA and IgG, (Wira and Sandoe 1987; Makkonen *et al.*, 2001 and Gajęcka *et al.*, 2004). They also evoke an increase in the production of specific and non-specific antibodies (Kurup *et al.*, 2000). From the biochemical point of view and taking interactions into account it can be concluded that both those forms are not clear. It is likely that hormones and cytokines serve a very important function in the transmission of information between the two systems: the reproductive and the immune one (Krakowski *et al.*, 2004). This probably strict co-operation assumes that the influence of the natural estrogens on the tissues in the reproductive system can also affect the immunological system. It is still controversial, however, if this result is produced by the direct or indirect action of the natural estrogens. It should also be considered if the immunological system is a real aim for all EDs or environmental estrogens in particular. In addition Fusarium mycotoxins appear to act at different cellular and molecular levels that affect viability, proliferation and differentiation of immune system cells (George *et al.*, 2008), these effects may be a result of inhibited protein synthesis (Desjardins *et al.*, 2006) or impairment of the activity or secretory functions of immune system cells (Oswald *et al.*, 2005) as well as synthesis of cytokines that regulate the communication network of immune system (Swamy *et al.*, 2004). In addition aflatoxins can cause organ damage or immunosuppression. Recent investigations have reported that several alterations of immunological parameters were found in vitro associated with Zearalenone concentrations (Marin *et al.*, 1996), the depressed immunological status such as IgG, IgM and serum globulins had higher values.

Concerning to our biochemical results, some biochemical parameters exceeded the normal physiological level which may be the results of a defense reaction of the organism to the dairy cows the biochemical parameters change their values which are characteristic for certain pathological affections that a biochemical panel is recommended each time the suspicion of mycosis and mycotoxicosis (Simion *et al.*, 2010). Moreover Zearalenone and aflatoxin have been shown to be hepatotoxic haematotoxic and genotoxic our results of protein level showed significant decrease at ($p < 0.05$) in group received contaminated ration with Zearalenone and aflatoxin compared with control one (Doll *et al.*, 2003 and Cheng *et al.*, 2006) which could be due to inhibition of protein synthesis at cellular level and will therefore predominantly damage quickly proliferating cells as found in the immune system (Goyards *et al.*, 2006). Moreover aflatoxin inhibits protein synthesis and cell proliferation (Sharma, 1993). This inhibition may not

be the primary mechanism involved in their immunotoxic effects; but may have selective effects on various subpopulations of lymphocytes. Since immunoglobulins are proteins, a decrease in total proteins and globulins might result in reduced antibody production which ultimately results in decreased immunity. On the other hand, Fusarium mycotoxins directly affect globulin synthesis in the liver and compromise the immune response of cows (Rotter *et al.*, 1994).

Blood parameters might be used for an additional estimation of toxic effects on live animals. It is assumed that elevated activities of serum enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) might reflect the organ damage (Cheng *et al.*, 2001; Cheng *et al.*, 2006 and Chytor *et al.*, 2010) mycotoxins induced hepatotoxicity (Bergsjö *et al.*, 1993). The alterations of liver enzyme activities due to abnormal excretion of liver metabolites due to mild liver damage (Chytor *et al.*, 2010) and hepatocellular cytoplasmic vacuolation with early portal fibrosis and bile duct hyperplasia (Harvey *et al.*, 1989) In the studies of Mikami *et al.* (2004) the results indicated that Fusarium induces apoptosis through the caspase-3 activation pathway and causes functional disorder in hepatocytes.

Regarding the estimated levels of Ca, Ph, in of cows received contaminated ration with zearalenone and aflatoxins animals. They were significantly decreased compared to that clinically normal cows. The reported lower levels could be attributed to decrease food intake and absorption due to affection similar results were reported by Ramos *et al.* (1996), Amal and Abo El-Maged, (2003), Dede *et al.* (2003) and Aatish *et al.* (2007). These results indicate Liver and tissues destruction due to aflatoxin similar results were recorded by Harvey *et al.* (1995), Fernandez *et al.* (1997), Magda (2001), Manal *et al.* (2004), and Pinar *et al.* (2009). However, there are link between mineral status and infertility. Gonadotropin-releasing hormone stimulation to LH release from pituitary gland involve calcium –dependent mechanism. No cAMP is involved and LH is not released in the absence of calcium or blocking agent (Hurley and Doane, 1989). Phosphorus deficiency induced irregular estrus, anestrus, decreased ovarian activity, increased incidence of cystic follicles and generally depressed fertility (Morrow, 1980).

We concluded that zearalenone have been shown to competitive bind to oestrogen receptors and affect reproductive performance generating hormonal imbalance inducing cell toxicity, immunosuppression due to the combined effect of aflatoxins which may predispose cows to the incidence of ovarian cysts.

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تأثير السموم الفطرية على الكفاءة التناسلية في الأبقار الحلابة

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اجريت هذه الدراسة لبيان تأثير التعرض لسموم الأفلاتوكسين والزيرونيون على الاداء التناسلى وعلاقتها بحدوث تكيس المبايض فى الأبقار الحلاب وقد استخدم لهذا الغرض ٢٨ بقرة حلاب من مزرعة فى محافظة الدقهلية وفحصت هذه الأبقار لتأكد من خلوها من الامراض الطفيلية والامراض الميكروبيولوجية وقسمت هذه الحيوانات الى مجموعتين: المجموعة الاولى (١٠ ابقار حلاب): غذت على عليقة متزنة حيث نسبة الأفلاتوكسين زيرونيون لا تتجاوز الحد المسموح به وهو (٥ جزء فى البليون , ٢٠٠ جزء فى البليون على التوالي) وتظهر سلوك الشبق الطبيعى والمبيض عليه جربات حويصلية مبيضية، >٢٥م فى قطر وبها الجسم الأصفر حتى يوم ٤٥ والمجموعة الثانية (١٨ بقرة حلاب) غذت على عليقة متزنة تحتوي على زيرونيون الذى عند مستويات مرتفعة (٤٠٠ جزء فى البليون) الأفلاتوكسين (١٩.٧ جزء فى البليون). وهذه الأبقار تظهر سلوك الشبق غير طبيعى ومتكرر (الشهوة)، والإفرازات المهبلية المفرطة حتى يوم ٤٥ بعد الولادة والمبيض عليه تكيسات مبيضية ≤ ٢٥ ملم فى القطر ولا يحمل الجسم الأصفر ولا يحدث تبويض تم جمع عينات الدم من كل بقرة فى اليوم ٤٥ بعد الولادة لتقييم هرمون البروجسترون فى الدم، وهرمون الاستروجين، ثلاثي يودوثيرونين، التيروتوكسين، البرولاكتين البروتين الكلى والكالسيوم والفوسفور غير العضوية، والأنشطة الأنزيمية الكبد ((AST، ALT)) والاميونوجلوبولين وفى هذه الدراسة أظهرت الأبقار فحص سلبي للميكروبيولوجي والطفيليات وأظهرت نتائج الفحص الهرموني زيادة معنوية (P > ٠.٠٥) فى مستويات البرولاكتين وهرمون الاستروجين فى حين أظهرت مستويات LH البروجسترون، T3 و T4 انخفاض معنوي (P > ٠.٠٥) فى الأبقار المغذاه على عليقة ملوثة بالزيرونيون والأفلاتوكسين فى مقارنة بالمجموعة الطابطة كشفت نتائج القياسات البيوكيميائية أن الأبقار المغذاه على عليقة ملوثة بالزيرونيون والأفلاتوكسين اظهرت زيادة كبيرة (P > ٠.٠٥) فى والأنشطة الأنزيمية الكبد ((AST، ALT)) بينما أظهر البروتين الكلى والكالسيوم والفوسفور انخفاض معنوي (P > ٠.٠٥) فى الأبقار المغذاه على عليقة ملوثة بالزيرونيون والأفلاتوكسين فى مقارنة بالمجموعة الطابطة كشفت نتائج الفحص المناعية) انخفاض معنوي (P > ٠.٠٥) فى الأبقار المغذاه على عليقة ملوثة بالزيرونيون والأفلاتوكسين فى مقارنة بالمجموعة الطابطة كشفت نتائجنا باستخدام الموجات فوق الصوتية عبر المستقيم ان متوسط قطر الكيس الجريبي المنكيس كان ٤٢.٣ ± ٣ ملم من الأبقار المغذاه على عليقة ملوثة بالزيرونيون والأفلاتوكسين فى حين كان متوسط قطر متوسط قطر الكيس الجريبي الناضج ٢٢.١ ± ١.٧ ملم فى المجموعة الطابطة نستخلص من هذه الدراسة الى ان الزيرونيون يتنافس للارتباط بمستقبلات هرمون الاستروجي مما يؤثر بشكل مباشر على الكفاءة التناسلية مخلفا اضطرابات هرمونية وتأثيرا سلبي على الخلايا مع تثبيط المناعة بسبب التأثير المشترك مع الأفلاتوكسين الذى قد يؤهب الأبقار إلى حدوث التكيسات المبيضية.