

## THE THERAPEUTIC POTENTIAL OF GLUCOMANNAN AND CALIBRIN-Z TREATMENTS AGAINST ZEARALENONE AND THEIR IMPACTS ON IMMUNITY, SOME BIOCHEMICAL PARAMETERS AND REPRODUCTIVE PERFORMANCE IN DAIRY CATTLE

EL-GARHI M.S.<sup>1</sup>; LAILA E. KORTAM<sup>2</sup> AND MAHA A. IBRAHIM<sup>3</sup>

<sup>1</sup> Field Investigation Department (ARRI) Animal Reproduction Research Institute, Giza, Egypt.

<sup>2</sup> Immunity Unit (ARRI) Animal Reproduction Research Institute, Giza, Egypt.

<sup>3</sup> Biology of Reproduction Department (ARRI, ARC), (ARRI) Animal Reproduction Research Institute, (ARC) Agriculture Research Center, Giza, Egypt.

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### ABSTRACT

This study used 320 Holstein cows in the breeding season at the postpartum period, which began four weeks before breeding and ended with a pregnancy confirmation examination. Cows were divided into four groups that were provided with a total mixed ration (TMR) but differed in the source of corn silage formulated according to (NRC2001). Group 1 (G1) fed good corn silage with 10 gm of Calibrin-Z/head (Montmorillonite clay). Group 2 (G2) and group 3 (G3) were fed corn silage contaminated with Zearalenone and supplied with (10, 60) gm Calibrin-Z/head, respectively, while group 4 (G4) was fed the same contaminated silage and supplied with 20 gm Glucomannan/head (Yeast stimulate, *Saccharomyces cerevisiae* Cell Wall). TMR, feed stuff and corn silage were tested for mycotoxins (Aflatoxins, Zearalenone). Milk production, feed consumption, and reproductive performance were monitored. Samples of blood were drawn from 10 cows of each group for the examination of oxidative indices, some blood biochemical and immunological parameters, and to monitor DNA integrity. The results showed that (G4) showed a significantly higher reproductive response than (G3). Aspartate Aminotransferase significantly decrease in (G3) and (G4) compared with (G2). A significant increase in catalase activity in (G3) compared with others groups. Also, nitric oxide (NO) and lymphocyte transformation increased significantly in (G4) compared with (G3). The DNA integrity of whole blood was significantly lower in (G 3) and (G 4) compared with (G 2).

**Keywords:** Calibrin- Z, dairy cow, Glucomannan, reproductive performance, Zearalenone.

### INTRODUCTION

Mycotoxins, microbes, heavy metals, and other harmful substances in feed can harm an animal's health. Mycotoxins are released and seeped into agricultural produce, mostly legumes, rice, and other crops, in specific environments, such as high

humidity and temperature, as well as poor storage conditions (Gareeballah Osman Adam and Hong-Geun Oh, 2022). To reduce the number of dangerous compounds present, feed must be decontaminated before feeding.

Mycotoxins are a worldwide problem due to their significant prevalence in food for human and animal nutrition. Zearalenone (ZEN) is one of the most prevalent mycotoxins discovered in cereals and animal feed (Dorninger *et al.*, 2019). It is non-

*Corresponding author:* Laila E. Kortam

*E-mail address:* Lailakortam71@gmail.com;

[m\\_elgarhi2005@yahoo.com](mailto:m_elgarhi2005@yahoo.com)

*Present address:* Immunity Unit (ARRI) Animal Reproduction Research Institute, Giza, Egypt.

steroidal estrogenic mycotoxin produced by numerous *Fusarium* fungi, which is frequently found in grains and animal feed and causes a variety of reproductive problems, as well as genotoxic, hepatotoxic, and immunosuppressive effects, which impact human and animal health (HuezaI *et al.*, 2014), (Martha *et al.*, 2015), (Eskola *et al.*, 2019), and (Jalila *et al.*, 2008). So, any delay in ZEN detoxification and bio-inactivation in dairy cows poses a significant financial risk (De Vries, 2006; Hovingh, 2009).

In livestock, mycotoxin adsorption and bio-inactivation by feed additives that are consumed have been studied; mycotoxin binders include Lucerne, Zeolites, Bentonite, and Bleaching Clays, which prevent the toxin from being absorbed by the animal through its diet. Additionally, these additives create stable complexes with mycotoxins, lowering their bioavailability (Alexandros *et al.*, 2021). Among the problems of this approach is the risk of decreasing dietary amino acids, vitamin, and mineral availability. So to get around it, Biomass containing yeast, bacteria, lactic acid, and *Aspergillus Conidia* is used as a second-generation binder, providing numerous potential sites for mycotoxin attachment (Huwig *et al.*, 2001).

Potential adsorbents should have a high capacity for adsorption and be able to bind to a variety of mycotoxins, as well as, have low binding to nutrients (Zhu *et al.*, 2016). Agents that act as antidotes or oppose the effects of hazardous substances like ZEA are important for both medicinal and economic reasons. These absorbents have several advantages, including low cost, safety, and ease of use by incorporating them into foods (Oliveira *et al.*, 2013, Abeer *et al.*, 2014, and Pinotti *et al.*, 2016).

In recent years, *Saccharomyces cerevisiae* cell wall (Glucmannan) has been continuously improved as a dietary supplement and administered to feed to

serve as a detoxifying agent and avoid ZEA toxicity (Van Wang *et al.*, 2019). They can absorb ZEN (nearly 30%) and reduce the bioavailability of toxins in the digestive tract (Dawson *et al.*, 2001) and (Yiannikouris *et al.*, 2004). In addition, clay minerals are also a promising feed additive due to their inexpensive cost and lack of negative effects. Montmorillonite (MMT) is the most widely used clay mineral; it has good biocompatibility and biodegradability, as well as good mechanical qualities. It has a high adsorption capacity for mycotoxins, heavy metals, and bacteria in animal feed and the human body (Jia *et al.*, 2021). Wang *et al.* (2012) describe a commercial version of MMT that can help animals recover faster after being intoxicated with ZEA. In fact, the deleterious effects of mycotoxins on the production, health, and reproductive performance of ruminant cattle warrant additional examination (Pulina *et al.*, 2014).

This experiment aimed to compare two ways of treatment of ZEA's naturally contaminated diet by the addition of Glucmannan (*Saccharomyces Cerevisiae* Cell Wall) or Calibrin-Z (Montmorillonite clay) by investigating their effects on some of the serum biochemical parameters, the antioxidant status, the immune function, some reproductive, in addition to the productive performance of the dairy cows, in order to recommend the best choice for treatment.

## MATERIALS AND METHODS

### Animals, experimental design, and diets:

The experiment was carried out on a private farm called Elbaramoth dairy farm in Wadi Elnatroon, Egypt. The study included 320 multiparous Holstein dairy cows from the dairy herd. The experimental animals' average body weight (BW) was 620 kg, and their average daily milk output was 41 kg. At 184 days, the average days in milk. The cows were randomly blocked into 4 groups and fed the same TMR that covered their requirements and differed in the source of

corn silage. There were two corn silage bunkers. The first one's analysis for total aflatoxins and ZEN was below the permissible limit, and the second bunk was infected with ZEN mycotoxin. Cows were divided into four groups, Group 1 (G1) received good corn silage with 10 gm Calibrin-Z/head (MMT), Groups 2 (G2) and 3 (G3) received ZEN-contaminated corn silage with 10,60 gm Calibrin-Z/head, respectively and Group 4 (G4) received the contaminated silage with 20 gm Glucomannan/head (Saccharomyces Cerevisiae Cell Wall).

The experimental diet was designed to provide all the necessary nutrients for a 620-kg multiparous Holstein cow to produce 40

kg of milk per day (NRC 2001). The table lists the components of the experimental diet (1). A total mixed ration was given to the cows (where concentrates and roughages were mixed mechanically using a mixer wagon on the farm). The same TMR was given to cows together with various sources of corn silage in amounts that were beyond the recommended daily intake for ad libitum consumption with a feed refusal rate of 2–3%. Every day, the DMI for each group is calculated. Water was made available to cows at all times. Each day, cows were milked three times. Representative feed samples were taken at the beginning of the experiment for analysis, and then taken twice monthly for nutrient and mycotoxin content analysis.

**Table 1:** Composition and proximate analysis of the basal diet (As- fed basis).

| Rations \ Feed Stuff             | (Kg/h/d) |
|----------------------------------|----------|
| Corn Grain, Ground, Dry          | 6.5      |
| Crashed flaxseed                 | 1.0      |
| Soybean, Meal, Solv, 46% protein | 4.0      |
| Rice polish                      | 1.0      |
| Corn gluten meal                 | 0.5      |
| Calcium soaps of fatty acids     | 0.45     |
| Corn silage, mature, 30% DM      | 28.0     |
| Alfa Alfa hay                    | 2.5      |
| Wheat straw                      | 1.0      |
| Magnesium oxide                  | 0.04     |
| Sodium Bicarbonate               | 0.28     |
| Mono- basic calcium phosphate    | 0.03     |
| Premix <sup>1</sup>              | 0.07     |
| CP2                              | 17.0     |
| NEL (M Cal/kg DM)3               | 1.62     |
| NDF (%DM)4                       | 30.7     |
| ADF (%DM)5                       | 19.6     |
| Forage NDF (%DM)                 | 24.4     |
| NFC                              | 41.0     |
| TDN (% DM)6                      | 71.0     |
| E.E (% DM)7                      | 5.0      |

10000000 IU vitamin A, 2500000 IU vitamin D3, Zinc 100000 mg, 35000 mg biotin, , Mn 80000 mg, Cu 30000 mg, I 800 mg, Se 300 mg, Co 400 mg, , Caco3 to 3 kg).

2 = crude protein, 3 = net energy for lactation, 4 = neutral detergent fiber, 5 = acid detergent fiber, 6 = total digestible nutrients, 7=ether extract.

**Milk analysis:**

Individual milk yield was recorded at every milking and summed for each day using the parlor or milking system and farm management (GYA). Milk from each group was collected in individual milk tanks during milk sampling and about 50 ml milk samples from each group were taken weekly for determination of milk fat and solid not fat. Weekly milk samples of 50 mL from each group were collected for the purpose of calculating milk fat and solid fat. The Milko-Scan (FT 6000) was used to analyze the milk samples for fat and solids-not-fat.

**Analysis of mycotoxins and nutrient content:**

The percentage of DM was calculated after feed samples were dried at 60°C in a forced-draught oven for 48 hours. A Wiley mill was used to grind the dry materials (2-mm screen). The AOAC (2007) procedure was used to assess feed samples for DM, ether extract (EE), ash, and crude protein (CP). Non-acid detergent fiber (NDF) and acid detergent fiber (ADF) were the fibers identified. Using the methods described by Berthiller *et al.* (2007), feed samples from a total concentrate mixture and corn silage were taken for the determination of total aflatoxins and zearalenone using ELISA (Van Soest *et al.*, 1991).

**Reproductive parameters:**

The four groups were synchronized for estrus using a GnRH combined with PGF<sub>2α</sub> (Ovsynch complete method GPG). On day 0, cows were initially treated with GnRH (20 g, IM, Buserelin (Receptal®), MSD Animal Health, a division of Merck Animal Health and formerly Intervet, Germany), then on day 7, PGF<sub>2</sub> (25 mg, IM, Dinoprost (Dinolitic®), Pfizer, Belgium) was injected. GnRH was then given on day 9. Also, this method was used for ovarian cyst treatment. Conceptions were confirmed utilizing a 7.5 MHz linear array probe and a veterinary ultrasound scanner (Dynamic Imaging, Livingstone, UK) through a 7.5 MHz linear array probe attached to a veterinary ultrasound scanner (Dynamic Imaging,

Livingstone, UK) after 25–27 days post insemination and confirmed at (45–60) days of AI. Cows in the experimental groups after uterine and ovarian examination are classified into conception rate %, ovulation %, ovarian cyst %, and normal open cows %.

Fertility was monitored in terms of conception rate, corpus luteum %, cystic ovary %, response to cystic ovary treatment %, and normal open cows as follows:

Conception rate = number of conceived cows/numbers of mated cows multiplied by 100 (Ozyurtlu *et al.*, 2011)

Ovulation % = number of cows with ovarian luteal solid structures or luteal structures with a cavity less than 20 millimeters and a wall more than 3 millimeters thick/number of mated cows multiplied by 100 (Chiho Kawashima *et al.*, 2006)

Cystic ovary% = number of cows with cystic ovarian follicles, COF (follicle more than 20 mm persistent for 7 days, corpus luteum absent) multiplied by 100 (Vanholder *et al.*, 2006).

Response to cystic ovary treatment: % = number of cows recovered/number of cows treated for cystic ovaries multiplied by 100 (Vanholder *et al.*, 2006).

Normal Open Cows% = the number of cows with 70 to 80 open days (the calving-to-conception interval) multiplied by the number of mated cows (Harman *et al.*, 1996).

**Sampling**

Ten cows in each group were randomly selected to study the effect of each treatment on various blood biochemical markers, antioxidant levels, and some immunological responses. Blood samples were collected after two months of mycotoxin treatment from each cow before the morning feed as follows:

1-Fresh heparinized blood for Single-Cell Gel Electrophoresis (comet) and

immunological studies (Lymphocyte Transformation).

2-Serum samples for biochemical and some immunological analysis.

The obtained sera were spectrophotometrically analyzed using Bio Diagnostic (Bains, France) standardized commercial test-kits.

Total Proteins (TP) (Doumas *et al.*, 1981), Albumin (Doumas *et al.*, 1971), and Globulin values were calculated by subtracting albumin from total protein data (Eckersall, 2008).

-Liver enzymatic activities: Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were analyzed (Reitman and Frankel, 1957).

Total antioxidants: Total Antioxidant Capacity (TAC) (Koracevic *et al.*, 2001), Glutathione Peroxidase (GPX) (Pagila and Valentine, 1967), Ascorbic Acid (Vit C) (Harris and Rays, 1935) and Malondialdehyde (MDA) (Ohkawa *et al.*, 1979).

-Determination of Urea (Fawcett and Soctt, 1960).

-Comet assay (Single Cell Gel Electrophoresis). Optica Axioscope fluorescence microscope (OPTIKA-ZEISS) at 400 x (Kadam *et al.*, 2013).

### Immunological studies:

- Estimation of Nitric Oxide (NO) levels (Rajarman *et al.*, 1998) using an ELISA plate reader (Epoch, Bio Tek, Germany).

-Estimation of Lymphocyte Transformation using the MTT reduction test, the proliferation activity was determined by measuring mitochondria activity using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) using ELISA reader (BIOTEK ELX 808) reduction method, by reduction of MTT to blue formazan product (Rai *et al.*, 1985).

-Detection of Lysozyme concentration (Schultz, 1987).

### Statistical Analysis:

Analysis of variance (ANOVA) was used to examine statistical differences across groups, and then, Duncan's test using SPSS for Windows version 16.0 (SPSS 16.0., SPSS Inc., 2007), at ( $P < 0.05$ ) significant levels were considered (SPSS 16.0., SPSS Inc., 2007)

## RESULTS

Table (2) showed non-significant differences between experimental groups in dry matter intake (DMI), average milk production, milk fat, solid non-fat (SNF), and feed efficiency.

Table (3) recorded that the concentration of total Aflatoxins was below the normal level (20 ppm), while the level of zearalenone concentration was higher than normal.

### Reproductive parameters:

In table (4), the control positive group (G2) recorded a significant decrease ( $p < 0.05$ ) in conception rate, corpus luteum % (CL%), response to cystic ovary treatment %, and normal open cows compared with the negative control group and other treated groups ( $31.1 \pm 5.6$ ,  $3.8 \pm 0.77$ ,  $38.4 \pm 2.7$ ,  $23.3 \pm 2.33$ ) respectively, and a significant increase in cystic ovary % ( $33.7 \pm 4.4$ ) compared with other groups. Although, (G4) recorded a significant increase ( $p < 0.05$ ) in conception rate and normal open cows compared with (G2) and (G3) ( $43.1 \pm 6.43$ ,  $40.9 \pm 4.45$ ), respectively, while G4 recorded a significant increase ( $p < 0.05$ ) in CL% in comparison with G (2) only ( $7.9 \pm 1.09$ ). Although G (3) had a significant increase in cystic ovary% ( $p < 0.05$ ), the response to cystic ovary treatment% was 28.91.98, 81.86.22, respectively, when compared to G (4).

### Serum biochemical parameters:

Most of the serum biochemical parameters showed non-significant changes ( $p < 0.05$ ) (Table 5). However, there was a significant

decrease ( $p < 0.05$ ) in AST value in treated groups (G3) and (G4) ( $55.5 \pm 3.4$ ,  $52.5 \pm 3.8$ ) respectively when compared with (G2); Additionally, there was a significant increase ( $P < 0.05$ ) in urea concentration ( $22.1 \pm 0.57$ ,  $21.7 \pm 1.3$ ,  $22 \pm 1.3$ ) in (G2), (G3) and (G4) compared with (G1).

#### Some antioxidant enzymes activities and oxidative Indices:

In table (6), the (G1) group recorded significantly higher at ( $p < 0.05$ ) levels of vitamin C, GSH-Px, and SOD ( $51 \pm 4.2$ ,  $167.9 \pm 13.7$ ) respectively, when compared with other groups. The animals receiving Glucomannan treatment (G4) showed a significant increase ( $p < 0.05$ ) in (T-AOC) ( $0.86 \pm 0.021$ ) compared with G2 and G3, in addition to a significant increase ( $p < 0.05$ ) in MDA level ( $11.1 \pm 0.8$ ) compared with (G2). On the other hand, (G2) showed a significant decrease in vitamin C (GSH-Px) and (T-AOC) ( $28.4 \pm 3.2$ ,  $100.1 \pm 10.5$ ,  $0.72 \pm 0.04$ ) respectively compared with (G1).

#### Some blood immunological parameters:

In our study, table (7) showed a significant decrease ( $p < 0.05$ ) in NO concentration in

(G2) ( $26.2 \pm 1.69$ ), compared with (G1), while lysozyme recorded a non-significant difference ( $p < 0.05$ ) between experimental groups. On the other hand, the lymphocyte transformation assay recorded a significant decrease ( $p < 0.05$ ) in (G2) ( $0.21 \pm 0.012$ ) compared with (G1) and (G4). Moreover, (G4) recorded a significant increase in NO level and lymphocyte transformation level ( $38.3 \pm 8.7$ ,  $1.1 \pm 0.07$ ), respectively compared with (G2) and (G3).

In table (8), DNA integrity damage in whole blood cells in (G2) was demonstrated by a significant increase ( $p < 0.05$ ) in comet%, tail length, DNA in tail%, tail moment%, and olive tail moment% ( $26.9 \pm 1.04$ ,  $8.7 \pm 0.34$ ,  $9.07 \pm 0.42$ ,  $1.0 \pm 0.074$ ,  $1.01 \pm 0.01$ ) respectively in comparison with other groups. Although, (G3) and (G4) recorded a significant decrease ( $p < 0.05$ ) in comet %, tail length, DNA in tail%, tail moment%, and olive tail moment% ( $18.2 \pm 1.2$ ,  $7.3 \pm 0.49$ ,  $8 \pm 1.14$ ,  $0.89 \pm 0.14$ ,  $1.1 \pm 0.11$ ), ( $17.5 \pm 1.3$ ,  $7.2 \pm 0.46$ ,  $7.9 \pm 0.59$ ,  $0.74 \pm 0.08$ ,  $0.74 \pm 0.06$ ) respectively compared with (G2)

**Table 2:** dry matter intake (DMI), average milk production, milk fat, solid not fat (SNF) and feed efficiency of the experimented dairy cow groups,  $m \pm SE$ .

| Groups<br>parameters       | Control negative<br>group (G1) | Control positive<br>group (G2) | Calibrin-z<br>group (G3) | Glucomannan<br>group (G4) |
|----------------------------|--------------------------------|--------------------------------|--------------------------|---------------------------|
| DMI                        | $29.2 \pm 1.18$                | $27 \pm 0.8$                   | $27.7 \pm 0.3$           | $28.6 \pm 0.94$           |
| Average milk<br>production | $47.9 \pm 1.13$                | $43.2 \pm 1.2$                 | $44.4 \pm 0.93$          | $46.4 \pm 0.9$            |
| Feed efficiency            | 1.6                            | 1.6                            | 1.6                      | 1.6                       |
| Milk fat                   | $3.4 \pm 0.15$                 | $3.3 \pm 0.2$                  | $3.45 \pm 0.1$           | $3.5 \pm 0.11$            |
| SNF                        | $9.0 \pm 0.24$                 | $8.9 \pm 0.1$                  | $8.9 \pm 0.07$           | $9.0 \pm 0.24$            |

Group 1 (control-ve) Calibrin-Z 10 gm, Group 2 (Control +ve) Calibrin-Z 10 gm, Group 3 (Treatment 1), Calibrin-Z 60 gm, Group 4 (Treatment 2), 15 gm of yeast stimulant (cell wall extract)

**Table 3:** Feed stuff Aflatoxins and Zearalenone concentration:

| Feed stuff parameters  | Silage bunk1 | Silage bunk 2 | Concentrate mixture |
|------------------------|--------------|---------------|---------------------|
| Total Aflatoxins (ppb) | 3.0          | 2.25          | 5.11                |
| Zearalenone (ppb)      | 395.0        | 70.0          | 95.0                |

**Table 4:** Some reproductive performance parameters of treated groups (Calibrin-z (G3) and Glucomannan (G4) of dairy cows were compared to control negative (G1) and control positive (G2) groups, m  $\pm$  SE.

| Parameters                           | Control negative group (G1)   | Control positive group (G2)  | Calibrin-z group (G3)        | Glucomannan group (G4)       |
|--------------------------------------|-------------------------------|------------------------------|------------------------------|------------------------------|
| Conception rate                      | 49.45 <sup>a</sup> $\pm$ 2.44 | 31.1 <sup>d</sup> $\pm$ 5.6  | 39.4 <sup>c</sup> $\pm$ 3.55 | 43.1 <sup>b</sup> $\pm$ 6.43 |
| corpus luteum %, (Cl %)              | 9.8 <sup>a</sup> $\pm$ 0.55   | 3.8 <sup>c</sup> $\pm$ 0.77  | 6.5 <sup>b</sup> $\pm$ 1.1   | 7.9 <sup>b</sup> $\pm$ 1.09  |
| Cystic ovary %                       | 4.3 <sup>d</sup> $\pm$ 1.0    | 33.7 <sup>a</sup> $\pm$ 4.4  | 28.9 <sup>b</sup> $\pm$ 1.98 | 6.8 <sup>c</sup> $\pm$ 1     |
| Response to cystic ovary treatment % | 87.4 <sup>a</sup> $\pm$ 5.5   | 38.4 <sup>d</sup> $\pm$ 2.7  | 81.8 <sup>b</sup> $\pm$ 6.22 | 66.6 <sup>c</sup> $\pm$ 6.3  |
| Normal Open cows                     | 47.2 <sup>a</sup> $\pm$ 4.8   | 23.3 <sup>c</sup> $\pm$ 2.33 | 21.0 <sup>d</sup> $\pm$ 3.76 | 40.9 <sup>b</sup> $\pm$ 4.45 |

<sup>a, b, c</sup> Means in the same row with different superscripts differ significantly at (P < 0.05).

Group 1 (control-ve) Calibrin-Z 10 gm, Group 2 (Control +ve) Calibrin-Z 10 gm, Group 3 (Treatment 1), Calibrin-Z 60 gm, Group 4 (Treatment 2) 15 gm of yeast stimulant (cell wall extract).

**Table 5:** Blood biochemical parameters of treated groups (Calibrin-z (G3) and Glucomannan (G4) of dairy cows were compared to control negative (G1) and control positive (G2) groups, m  $\pm$  SE, n=10.

| Groups parameters    | Control negative group (G1)  | Control positive group (G2)  | Calibrin-z group (G3)        | Glucomannan group (G4)       |
|----------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| Total Proteins(g\dl) | 6.0 $\pm$ 0.13               | 6.3 $\pm$ 0.05               | 6.1 $\pm$ 0.14               | 6.0 $\pm$ 0.04               |
| Albumin(g\dl)        | 3.3 $\pm$ 0.14               | 3.3 $\pm$ 0.08               | 3.07 $\pm$ 0.12              | 3.2 $\pm$ 0.09               |
| Globulin(g\dl)       | 2.6 $\pm$ 0.23               | 2.9 $\pm$ 0.11               | 3.06 $\pm$ 0.15              | 2.7 $\pm$ 0.12               |
| AST(U\ml)            | 57.6 $\pm$ 4.70 <sup>b</sup> | 72.8 $\pm$ 3.60 <sup>a</sup> | 55.5 $\pm$ 3.40 <sup>b</sup> | 52.5 $\pm$ 3.8 <sup>b</sup>  |
| ALT(U\ml)            | 27.4 $\pm$ 1.30              | 30.10 $\pm$ 3.10             | 35.3 $\pm$ 4.30              | 31.7 $\pm$ 1.80              |
| Urea(mg\dl)          | 17.1 $\pm$ 1.50 <sup>b</sup> | 22.1 $\pm$ 0.57 <sup>a</sup> | 21.7 $\pm$ 1.30 <sup>a</sup> | 22.0 $\pm$ 1.30 <sup>a</sup> |

<sup>a, b</sup> Means in the same row with different superscripts differ significantly at (P < 0.05).

Group 1 (control-ve) Calibrin-Z 10 gm, Group 2 (Control +ve) Calibrin-Z 10 gm, Group 3 (Treatment 1), Calibrin-Z 60 gm, Group 4 (Treatment 2) 15 gm of yeast stimulant (cell wall extract)

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

**Table 6:** Some antioxidant enzymes activities and oxidative Indices of treated groups (Calibrin-z (G3) and Glucomannan (G4) of dairy cows were compared to control negative (G1) and control positive (G2) groups,  $m \pm SE$ ,  $n=10$ .

| <b>Groups<br/>parameters</b>      | <b>Control<br/>negative group<br/>(G1)</b> | <b>Control<br/>positive group<br/>(G2)</b> | <b>Calibrin-z<br/>group (G3)</b> | <b>Glucomannan<br/>group (G4)</b> |
|-----------------------------------|--|--|----------------------------------|-----------------------------------|
| Catalase activity(U/l)            | 762.4 $\pm$ 21.30 <sup>b</sup>             | 778.6 $\pm$ 8.80 <sup>b</sup>              | 871.8 $\pm$ 1.30 <sup>a</sup>    | 787.5 $\pm$ 6.70 <sup>b</sup>     |
| Vitamin C (mg/l)                  | 51.0 $\pm$ 4.20 <sup>a</sup>               | 28.4 $\pm$ 3.20 <sup>b</sup>               | 35.5 $\pm$ 2.80 <sup>b</sup>     | 30.8 $\pm$ 4.50 <sup>b</sup>      |
| Glutathione peroxidase (mU/ml)    | 167.9 $\pm$ 13.70 <sup>a</sup>             | 100.1 $\pm$ 10.50 <sup>b</sup>             | 129.9 $\pm$ 14.80 <sup>b</sup>   | 119.2 $\pm$ 13.20 <sup>b</sup>    |
| Super oxide dismutase (U/ml)      | 130.5 $\pm$ 12.50                          | 99.0 $\pm$ 7.70                            | 127.6 $\pm$ 14.80                | 116.8 $\pm$ 13.30                 |
| Total antioxidant capacity (mM/l) | 1.01 $\pm$ 0.10 <sup>a</sup>               | 0.72 $\pm$ 0.04 <sup>b</sup>               | 0.74 $\pm$ 0.04 <sup>b</sup>     | 0.86 $\pm$ 0.021 <sup>ab</sup>    |
| Malondialdehyde (nmol/ml)         | 10.4 $\pm$ 0.70 <sup>b</sup>               | 13.6 $\pm$ 0.70 <sup>a</sup>               | 10.2 $\pm$ 0.60 <sup>b</sup>     | 11.1 $\pm$ 0.80 <sup>b</sup>      |

<sup>a, b</sup> Means in the same row with different superscripts differ significantly at ( $P < 0.05$ ).

Group 1 (control-ve) Calibrin-Z 10 gm, Group 2 (Control +ve) Calibrin-Z 10 gm, Group 3 (Treatment 1), Calibrin-Z 60 gm, Group 4 (Treatment 2)15 gm of yeast stimulant (cell wall extract).

**Table 7:** Some blood immunological parameters of treated groups (Calibrin-z (G3) and Glucomannan (G4) of dairy cows were compared to control negative (G1) and control positive (G2) groups,  $m \pm SE$ ,  $n=10$ .

| <b>Groups<br/>parameters</b> | <b>Control<br/>negative group<br/>(G1)</b> | <b>Control<br/>positive group<br/>(G2)</b> | <b>Calibrin-z<br/>group (G3)</b> | <b>Glucomannan<br/>group (G4)</b> |
|------------------------------|--|--|----------------------------------|-----------------------------------|
| NO (nitric oxide) (uM/ml)    | 49.4 $\pm$ 5.10 <sup>a</sup>               | 26.2 $\pm$ 1.69 <sup>c</sup>               | 29.1 $\pm$ 3.30 <sup>c</sup>     | 38.3 $\pm$ 8.70 <sup>b</sup>      |
| Lysozymes ug/ml              | 181.2 $\pm$ 5.50                           | 184.3 $\pm$ 5.60                           | 180.4 $\pm$ 3.40                 | 183.3 $\pm$ 3.80                  |
| Lymphocytes transformation   | 2.1 $\pm$ 0.18 <sup>a</sup>                | 0.21 $\pm$ 0.012 <sup>c</sup>              | 0.30 $\pm$ 0.003 <sup>c</sup>    | 1.1 $\pm$ 0.07 <sup>b</sup>       |

<sup>a, b, c</sup> Means in the same row with different superscripts differ significantly at ( $P < 0.05$ ).

Group 1 (control-ve) Calibrin-Z 10 gm, Group 2 (Control +ve) Calibrin-Z 10 gm, Group 3 (Treatment 1), Calibrin-Z 60 gm, Group 4 (Treatment 2),15 gm of yeast stimulant (cell wall extract).

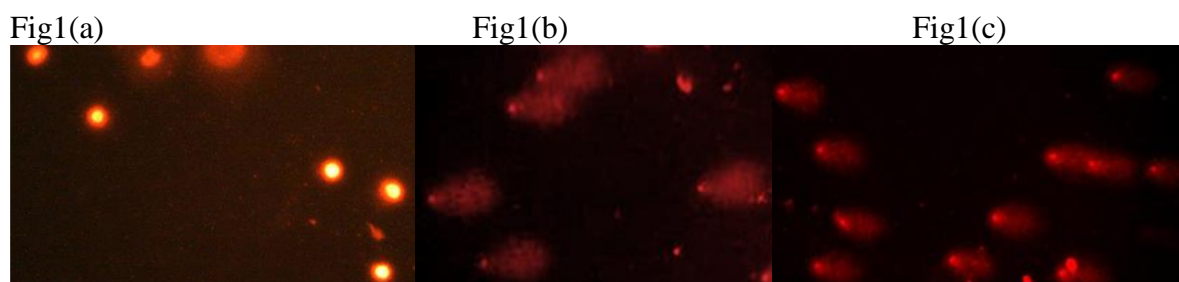


**Table 8:** DNA integrity of whole blood cells of treated groups (Calibrin-z (G3) and Glucomannan (G4) of dairy cows were compared to control negative (G1) and control positive (G2) groups,  $m \pm SE$ ,  $n=10$ .

| Groups<br>parameters | Control<br>negative group<br>(G1) | Control<br>positive group<br>(G2) | Calibrin-z<br>group(G3)      | Glucomannan<br>group (G4)    |
|----------------------|-----------------------------------|-----------------------------------|------------------------------|------------------------------|
| Comet %              | 12.2 $\pm$ 0.50 <sup>c</sup>      | 26.9 $\pm$ 1.04 <sup>a</sup>      | 18.2 $\pm$ 1.20 <sup>b</sup> | 17.5 $\pm$ 1.30 <sup>b</sup> |
| Tail length (px)     | 6.2 $\pm$ 0.49 <sup>b</sup>       | 8.7 $\pm$ 0.34 <sup>a</sup>       | 7.3 $\pm$ 0.49 <sup>b</sup>  | 7.2 $\pm$ 0.46 <sup>b</sup>  |
| DNA in tail%         | 6.9 $\pm$ 0.30 <sup>c</sup>       | 9.07 $\pm$ 0.42 <sup>a</sup>      | 8.0 $\pm$ 1.14 <sup>b</sup>  | 7.9 $\pm$ 0.59 <sup>b</sup>  |
| Tail moment%         | 0.63 $\pm$ 0.47 <sup>c</sup>      | 1.0 $\pm$ 0.074 <sup>a</sup>      | 0.89 $\pm$ 0.14 <sup>b</sup> | 0.74 $\pm$ 0.08 <sup>b</sup> |
| Olive tail moment %  | 0.67 $\pm$ 0.05 <sup>b</sup>      | 1.01 $\pm$ 0.01 <sup>a</sup>      | 1.1 $\pm$ 0.11 <sup>a</sup>  | 0.74 $\pm$ 0.06 <sup>b</sup> |

<sup>a, b, c</sup> Means in the same row with different superscripts differ significantly at ( $P < 0.05$ ).

Group 1 (control-ve) Calibrin-Z 10 gm, Group 2 (Control +ve) Calibrin-Z 10 gm, Group 3 (Treatment 1), Calibrin-Z 60 gm, Group 4 (Treatment 2) 15 gm of yeast stimulant (cell wall extract) .



**Fig (1a):** undamaged DNA of whole blood cells in (G1), (G3), (G4) measured by comet assay where DNA remains within the core .DNA is tightly compressed and maintained the circular disposition of the normal nucleus.

**Fig (1b, 1c):** marked degree of DNA damage of whole blood cells of (G2) measured by comet assay. The increase in DNA damage was mostly evidenced by an increase of comet tail.

## DISCUSSION

Data presented in our work studied the effects of dietary treatments of ZEN toxicity by either Calibrin-Z or Glucomannan on some reproductive performance parameters. When compared to the other groups, (G2) had a significant decrease in conception rate, CI%, response to cystic ovary treatment %, and normal open cows. When dealing with mycotoxins on dairy farms, it's typical to concentrate on the direct effects on productivity and health. Concerns about ZEN are different: ZEN, which has a structure that is extremely similar to estrogen, can bind to receptors in several parts of the body, including the mammary gland, the uterus, the pituitary gland, and

hypothalamus, causing delays in reproduction, spontaneous abortions, and other reproductive challenges.

Our results agreed with Fushimi *et al.* (2014, 2015), who claimed that when ZEN circulates in the bloodstream and binds to estrogen receptors, it can cause an endocrine disturbance. More follicles are recruited with ZEN exposure than usual, but only one follicle can mature for ovulation. Even low levels of contamination, such as 200 ppb of diet, might result in the formation of ovarian cysts due to the buildup of immature follicles (Mahmoud *et al.*, 2013).

Due to ZEN exposition, alterations in the concentrations of progesterone can occur.

Progesterone is also known as "the pregnancy hormone" because once an ovum has been successfully fertilized, it ensures that the physiological conditions in the cow are created to preserve the embryo. A possible alteration to this set of conditions can cause spontaneous abortions, mainly in the first third of the pregnancy (Fiorenza Minervini and Maria Elena Dell'Aquila, 2008). In our work (G4), we recorded a significant increase in conception rate and normal open cow compared with other ways of treatment (G3). These findings are consistent with those of Nasiri *et al.* (2018), who demonstrated that cows fed diets enriched with Glucomannan had higher plasma IGF-I, E-17 $\beta$  and P4 concentrations, shorter estrous cycles, larger ovulatory follicles, and enhanced reproductive efficiency. As a result, yeast dietary supplementation improves the fertility of lactating dairy cows by promoting the development of larger ovulatory follicles.

In the present work, there was a significant increase in AST levels in the positive control group compared to the other groups, which may indicate that the ration contaminated with ZEN had a negative impact on liver function. Battacone *et al.* (2009) agreed with our results and mentioned that mycotoxins can affect liver function while glucomannan can mitigate fescue toxicosis.

Our results observed that serum urea concentrations were significantly different between mycotoxin-affected groups. These findings are consistent with Smith *et al.* (2007), who discovered that the serum urea concentrations are continuously raised when animals are fed feedstuffs contaminated with mycotoxins. Also, our results agreed with those of Chaiyotwittayakun (2010) and Huang *et al.* (2018), who mentioned that cows fed a mycotoxin-contaminated diet had significantly increased serum levels of metabolites such as urea and AST.

On the other hand, Korosteleva *et al.* (2007, 2009) and Xiong (2015) found that mycotoxicosis had no effect on the

concentrations of ALT, AST, GGT, or ALP in dairy cattle, and they did not record any changes in the hematological profile of the animals affected by ZEN toxicity, which is in disagreement with our results.

In addition to elevating MDA levels, serum antioxidant data indicated that ZEN decreased levels of vitamin C, GSH-Px, and TAC. These findings are in line with the hypothesis that mycotoxin-induced defects in antioxidant defense by attacking the unsaturated bonds of membrane phospholipids and damaging the liver cells. 2018; Shuai Huang *et al.*).

Also, the Glucomannan-treated group (G4) revealed a moderately significant level between the control negative group and other groups in T-AOC levels, which may be due to the ameliorating effect of Glucomannan on myotoxicity that was also in agreement with Laura *et al.* (2017).

Generally, we noticed that most of the blood biochemical parameters and the antioxidant activities in the two treated cattle groups were higher than the control positive group but not significantly different in most of them, which may be due to the health improvement caused by these treatments, which act as a cascade of damaging effects of ZEN toxicity.

In our study, nitric oxide showed a significant decrease in cows that received naturally contaminated rations with ZEN in comparison with the control negative group. This finding agreed with Jalila *et al.* (2008) who reported that ZEN had hepatotoxic, genotoxic, and immunotoxic effects in addition to several alterations of immunological parameters. As opposed to the control positive group and the Calibrin-Z treated group, cattle treated with Glucomannan displayed a significantly higher level of NO. These findings are consistent with those of Abeer *et al.* (2014) and Muhammad *et al.* (2020), who attributed this finding to the presence of mannan oligosaccharide in Yeast Cell Wall,

Furthermore, Glucomannan activated cells, T cells, and macrophages, which had indirect impacts on cellular immunity. While Lysozyme recorded a non-significant difference between experimental groups, Lysozyme is a hydrolytic enzyme which cleaves the glycosidic linkages of bacterial peptidoglycan. Animals' exocrine secretions contain Lysozyme, which serves as their main line of defense against bacterial infection.

Our results recorded a significant decrease in the Lymphocyte Transformation assay in the (G2) compared with the (G1) because ZEA affects the spleen's immune system, changes the phenotypes of the lymphocytes, and even results in lymphocyte atrophy (Carlos *et al.*, 2018). Additionally, ZEA can cause immunosuppression by lowering serum immunoglobulins and lymphoid organ cytokines (Van Wang *et al.*, 2019). These results are consistent with those of Carlos *et al.* (2018). Furthermore, the Glucomannan-treated group recorded a significant increase in the Lymphocyte Transformation assay compared with (G2) and (G3). This result agreed with Abeer *et al.* (2014), as Glucomannan can overcome the effect of ZEN as an immunosuppressive agent and stimulate the immune system by activating pattern recognition receptors such as toll-like receptor 2 (TLR2). Carpenter *et al.* (2013) studied the effect of Yeast Cell Wall supplementation on post-exercise immunosuppression and discovered that it increased the ability of blood leucocytes to produce IL-2, IL-4, IL-5, and IFN- (Liu *et al.*, 2013) and (Takada *et al.*, 2014).

Carlos *et al.* (2018) observed an elevation in the concentration of lymphocytes and heterophils accompanied by the addition of Yeast Cell Wall, which indicates its ability to increase the cell populations of lymphocytes, and play a crucial part in the immunological reaction. Based on the YCWs' chemical composition, which is primarily composed of sugars, and the sugars' role as lectin-like receptor ligands, which has been characterized in cell

populations of lymphoid origin, this may be the case (Devegowda and Murthy 2005), (Gómez-Verduzco *et al.*, 2009). It was discovered that the mannans found in the cell walls of *S. cerevisiae* stimulate TLR4, TLR2, and TLR6, as well as boost some immunological responses (Carlos *et al.*, 2018).

The group exposed to ZEN toxicity demonstrated DNA integrity damage in whole blood cells, as evidenced by a significant increase in comet%, tail length, DNA in tail%, tail moment%, and olive tail moment% compared with the (G1). These results support those of Zhu *et al.* (2012) and Martha *et al.* (2015), who discovered that micronucleate cells increased and underwent apoptosis at high concentrations of ZEA of between (30 mM) and (120 mM). Qin *et al.* (2015) stated that ZEA increased the amount of reactive oxygen in whole blood cells and caused mitochondrial transmembrane dysfunction. Furthermore, ZEA caused considerable oxidative DNA damage in whole blood cells, inhibited the growth of T and B lymphocytes, and subsequently increased the rate of cell death (Vlata *et al.*, 2006).

On the other hand, (G3) recorded a significant decrease in comet percentage and tail length compared with (G2), Supplementing with Calibrin-Z counteracted ZEA's effects on serum MDA (Wang *et al.*, 2012). The dietary supplement by probiotic preparation (G4), on the other hand, showed a significant decrease in comet percentage and tail length, DNA in tail%, tail moment %, and olive tail moment % compared with (G2). As *Saccharomyces cerevisiae* can cause protection against the damaging effect of ZEN, this result was consistent with that mentioned by Oliveira *et al.* (2013) and Abeer *et al.* (2014) who found that the dietary supplement by probiotic preparation decreased the extent of DNA damage of blood lymphocytes caused by mycotoxin in addition to their ant-mutagenic and antigen-toxic effects.

## CONCLUSION

The results of this study may offer suggestions for the treatment of naturally contaminated diets with ZEN above the feed limits. This study revealed that the addition of Glucomannan (20 grams/head/day) or Calibrin-Z (60 grams/head/day) to the ration for two months could improve to varying degrees the adverse effect of ZEN on the reproductive performance, general health, and immunity of affected dairy cows. We found that Glucomannan provides more effective and better results concerning cystic ovary (%) and some biochemical and immunological aspects in the affected cattle, consequently having more therapeutic and economic importance.

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## التأثير العلاجي للجلوكومنان والكالبرزين على التسمم الغذائي بفطر الزيرالينون علي بعض المعايير البيوكيميائية والمناعية بالإضافة للكفاءة التناسلية للأبقار الحلابة

محمد سامي محمد عبد المعز ، ليلى اسماعيل عبدالمجيد ، مها عبد الحميد ابراهيم

E-mail: [Lailakortam71@gmail.com](mailto:Lailakortam71@gmail.com)

Assiut University web-site: [www.aun.edu.eg](http://www.aun.edu.eg)

أجريت هذه الدراسة على ٣٢٠ بقرة هولشتاين في موسم التكاثر خلال فترة ما بعد الولادة وتم تقسيم الأبقار الي أربع مجموعات تتغذى علي نفس الحصة من العليقة المختلطة المتكاملة (تى إم أر). المجموعة الأولى تم تغذيتها بسيلاج الذرة مع إضافة ١٠ جرام لكل حيوان من الكالبرزين والمجموعة الثانية تم تغذيتها علي السيلاج الملوث طبيعياً بفطر الزيرالينون بالإضافة الي علف الذرة والمجموعة الثالثة تتغذى مثل المجموعة الثانية بالإضافة الي ٦٠ جرام من الكالبرزين لكل حيوان في حين تم إضافة ٢٠ جرام من الجلوكومنان علي السيلاج الملوث طبيعياً لكل حيوان من المجموعة الرابعة .

إنتاج اللبن وإستهلاك الأعلاف بالإضافة الي الأداء التناسلي للأبقار تم متابعتهم وتسجيل مستوياتهم خلال فترة التجربة. أيضاً تم تجميع وتحليل عينات الدم لمتابعة التغيرات التي طرأت علي بعض القياسات البيوكيميائية ومضادات الأكسدة والقياسات المناعية ومراقبة سلامة الحمض النووي .

أظهرت النتائج أن المجموعه الرابعه أظهرت إستجابة فى الكفاءة التناسلية أعلي من المجموعه الثالثه كما زاد مستوى حمض النيتريك وتحول الخلايا الليمفاوية في المجموعه الرابعه أيضاً بالمقارنه بالمجموعه الثالثه. كما أوضحت النتائج سلامة الحمض النووي لخلايا الدم في كلاً من مجموعتي العلاج الثالثه والرابعه.