Effect of dietary zearalenone on the performance, reproduction tract and serum biochemistry in young rats

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ABSTRACT
The present study was conducted to determine the toxic dose response of a chronic dietary Zearalenone (ZEA) in weaned young rats. Sixty, 21-day-old, Sprague Dawley female rats were randomly allocated to five groups of four replicate cages containing three rats. Rats were fed diets with increasing amounts of ZEA (0, 0.5, 0.9, 1.8 and 3.6 mg/kg) for 4 weeks. Daily feed intake was reduced (P < .05) by feeding the ZEA diets with 0.9 and 3.6 mg ZEA/kg feed. Rats fed the diet containing 1.8 mg ZEA/kg increased (P < .05) the body weight gain (BWG) and reduced (P < .05) feed conversion rate (FCR) as compared to the control group. The two highest levels of dietary ZEA also increased (P < .05) the weight of the uterus. However, ovaries' weight, timing of vaginal opening and the inter-ovestrous interval were not affected by increasing the doses of dietary ZEA (P > .05). Similarly, serum concentrations of total protein, follicle-stimulating hormone and alanine aminotransferase, aspartate aminotransferase and alkaline phosphate activities were not altered by the ZEA treatments. In conclusion, our results indicated that a chronic dietary consumption of ZEA at concentrations of 1.8 mg ZEA/kg increases the BWG and the uterus weight of weaning female rats.

1. Introduction
Zearalenone (ZEA) is a non-steroidal estrogenic fusariotoxin which is produced by Fusarium graminearum and Fusarium culmorum in grains, mainly corn and wheat (Kuiper-Goodman et al. 1987). It is considered a public concern associated with morum in grains, mainly corn and wheat (Kuiper-Goodman et al. 1987). It is hypothesized that the clinical signs will depend on factors such as type and concentration of ZEA and the duration of exposure. The aim of this study was to investigate the deleterious dose response of dietary ZEA on growth, reproductive tract and behaviour, internal organs' weight and serum biochemistry in weaning rats as an animal model.

2. Material and methods
2.1. Chemicals and feed contamination
Pure crystalline ZEA (Sigma-Aldrich Chimie S.A.R.L France) was incorporated into the diets by dissolving ZEA in absolute ethanol (w/vol) followed by mixing the solution with appropriate quantities of ground commercial feed. The contaminated feed was left overnight at room temperature for the solvent to evaporate and was then mixed into basal diet to provide the desired levels of mg ZEA/kg feed. The diet containing ZEA was analysed by high-performance liquid chromatography to ensure the ZEA concentrations in the experimentally contaminated diets.

2.2. Animals and treatments
The experiment was performed at the Animal Facility Research Centre of the Universitat Autònoma de Barcelona (UAB) and it
was conducted according to the guidelines for animal experimentation of UAB and approved by the Ethical Committee. Sixty weaning (21 day old) Sprague Dawley female rats (60 ± 1.3 g, initial body weight) were randomly allocated to five groups of four replicate cages containing three rats. Rats were fed diets with different doses of ZEA (0, 0.5, 0.9, 1.8 and 3.6 mg ZEA/kg feed). Rats were housed in wire cages with filter tops at 24°C, 55–60% of humidity and a 12 h light/12 h dark cycle. Standard Certified Rodent Chow diets (SAFE-Scientific Animal Food Engineering – France) and water were offered ad libitum.

### 2.3. Experimental procedures

Body weight gain (BWG), feed intake (FI) and feed conversion rate (FCR) per cage were recorded weekly. FCR was calculated as FI (g) per weight gained. Timing of vaginal opening was monitored daily from the day of vaginal opening to the end of the experiment by examination of vaginal smears to determine the time of the oestrus cycle. Electric impedance of the vaginal mucous membrane was measured following the method described by Bartas (1977). Animals were killed for taking blood samples by an overdose of sodium pentobarbital after 2 days of oestrus in the interval of 43–50 days of age to exclude the effects of oestrus on the weight of reproductive organs including uterus and ovaries and the concentration of the follicle-stimulating hormone (FSH). After ketamina-xilacina (80 mg/kg) anaesthesia, the blood samples were collected from eight animals from each treatment via heart for haematological and serum biochemical determination. Blood samples were placed on ice during collection. Within 1 h, the serum was obtained by centrifugation (2500×g for 15 min) and stored at −80°C until further analysis. Liver, kidneys, spleen, gastrointestinal tract, small intestine and urinary bladder and the reproductive tract, including the uterus (cervix and corpus uteri) and the ovaries, were dissected and weighed.

### 2.4. Biochemical analysis

Serum biochemical parameters were measured by using Olympus System Reagents (Olympus, Clare, Ireland) and an automatic clinical chemistry analyser (Olympus AU 400, Hamburg, Germany). The concentration of total protein (TP) was measured by following the Biuret method; uric acid (UA) by following the uricase method; the enzymatic activities of alkaline phosphatase (ALP), γ-glutamyltransferase by using the recommended International Federation of Clinical Chemistry and Laboratory Medicine reference methods. The FSH concentration was studied by RIA.

### 2.5. Statistical analysis

Statistical analyses were performed with SPSS for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). Results of the parameters were analyzed by ANOVA with the GLM procedure of SPSS by using the following model: $Y_{ij} = \mu + \alpha_{i} + \delta_{j} + \epsilon_{ij}$, where $\mu$ is the overall mean, $\alpha$ ($j = 1, 5$) is the effect of ZEA in the diet, and $\epsilon$ is the unexplained random error. Means are separated by Tukey’s multiple range tests when ANOVA was significant ($P < .05$), data are resented as means and SEM. Polynomial contrasts (linear, quadratic and cubic) were used to test the effect of ZEA levels on the various parameters measured.

### Table 1. Effects of dietary ZEA on growth parameters in young rats.

| Measurements            | ZEA Treatment (mg/kg) | SEM | $P$ | Effects |
|-------------------------|-----------------------|-----|-----|---------|
|                         | (Control) 0          | 0.9 | 1.8 | 3.6     |        |
| Age (d)                 | 48.4 3.70            | 48.2| 48.4| 48.7    | 0.364  |
| Feed intake (g/d)       | 15.23a               | 14.47b| 14.99b| 14.82b  | 0.051  |
| Body weight gain (g/d)  | 3.70b                | 3.79b| 4.00b| 3.75b   | 0.012  |
| Feed gain ratio         | 4.11a                | 3.98b| 3.89b| 3.74b   | 0.033  |

Note: *Means within the line with different superscripts differ significantly ($P < .05$). *Linear, Cubatic effect ($P < .05$).

NS, not significant; L, linear; Q, quadratic; C, cubic effects; SEM, standard error of the mean.
reduction. The control rats exhibited a regular inter-oestrus interval of 3.6 days, while ZEA-treated groups showed inter-oestrus intervals of 4.2, 5.9, 3.7 and 4.7 days for 0.5, 0.9, 1.8 and 3.6 mg ZEA/kg, respectively. Timing of vaginal opening is an external signal of sexual development in female rats and has been used as a biomarker of pubertal onset (Marty et al. 1999). Nikaido et al. (2004) referred to an earlier vaginal opening and persistent oestrus simultaneous to structural changes in the ovary in neonatal prepuce rodents fed on dietary ZEA. In our study, supplementation with increasing amounts of ZEA up to 3.6 mg ZEA/kg feed for 4 weeks did not promote changes in the vaginal opening but enlarged the time of the oestrus cycle (P > .05). In mice experiments, the effects of ZEA on the vaginal opening were dependent on the age and way of administration (Ito & Ohtsubo 1994). When ZEA (30 µg/animal) was administered to neonatal (1–5 days) animals in daily pulse doses a tendency to a delayed vaginal opening was observed, but a pulse dose at 10 days of age promoted an earlier opening. The reason could be related with the fate and kinetics of the toxin in the animals, and the competitive binding to the reproductive receptors (Powell-Jones et al. 1981). The way, time and the administrated dose of ZEA may be also reasons for such a discrepancy (El-Makawy et al. 2001). The weights of liver, kidney, spleen, uterus, ovaries and intestinal tract are shown in Table 2. Weights of liver, kidney, spleen and ovaries were not affected by treatments (P > .05). On the other hand, uterus weight was higher (P < .001) in rats fed the highest doses of ZEA in diet (1.8 and 3.6 mg ZEA/kg) than rats fed the Control diet. Our results of the present paper confirm the effects of ZEA on the reproductive organs of weaning rats, especially by promoting an estrogenic response (Kissing 1982; Ito & Ohtsubo 1994; Perez-Martinez et al. 1996; Yuri et al. 2004). In rats, the reproductive consequences of ZEA exposure include decreased fertility, resorption or deformities of foetuses, and abortion at high dietary concentrations (Kuiper-Goodman et al. 1987). ZEA has been mainly characterized by its estrogenic properties in a number of species (Etienne & Dourmand 1994; Doll et al. 2005). Most of the effects have been observed on peripheral reproductive organs especially uterus and ovaries (Fitzpatrick et al. 1989). It is known that ZEA is mostly metabolized in liver and intestinal mucosa to α- and β-zearenalol and zearalanol (Olsen 1989). ZEA metabolites have been referred limited or no-binding to carrier proteins, allowing their easier access to oestrogen target sites and a higher oestrogen activity (Leffers et al., 2001). The effect of ZEA most worth mentioning in the present study was the increase on the uterus weight, as previously described (Ito & Ohtsubo 1994, Yamini et al. 1997). The fact that the low level of ZEA increased also uterus weight suggests that this organ may be more sensitive to the toxicity of ZEA than other organs including brain, liver and kidney (Turcotte et al. 2005). The intestinal tract weight was also higher (P < .05) in the group fed with 3.6 mg ZEA/kg feed than rats fed the Control diet. Serum biochemical parameters are given in Table 3. All ZEA levels in diet increased (P < .05) the serum bilirubin concentration. In addition, serum UA concentration was lower (P < .05) in rats fed the highest level of ZEA (3.6 mg ZEA/kg feed) as compared to rats fed the Control diet. Decreased serum UA concentrations may be associated with the amino acid utilization, changes in enzyme systems (Kubena et al. 1988) and amino acids for energy utilization, leading to excess UA synthesis (Swamy et al. 2002). However, serum ALP, ALT and AST activities, TP and FSH concentrations were not affected by dietary treatments (P > .05). Results from our experiment indicate that the concentration of bilirubin was increased by all levels of dietary ZEA, but no changes were observed on the ALP, ALT or AST activities. Increases of the bilirubin concentration in the blood may indicate a certain degree of liver toxicity caused by dietary ZEA. Maaroufi et al. (1996) reported a significant increase in the activities of blood markers such as bilirubin, ALT, AST and ALP which are rather in favour of direct toxic effects on the liver leading to

### Table 2. Effects of dietary ZEA on the weight of the reproductive tract and other internal organs in young rats.

| Measurements                        | ZEA treatment (mg/kg) | Effects |
|-------------------------------------|-----------------------|---------|
|                                    | (Control) 0 | 0.5 | 0.9 | 1.8 | 3.6 | SEM1 | P | L | Q | C |
| Uterus weight                       | 0.147<sup>a</sup> | 0.182<sup>b</sup> | 0.183<sup>b</sup> | 0.204<sup>a,b</sup> | 0.208<sup>a</sup> | 0.006 | .001 | * | NS | NS |
| Ovaries weight                      | 0.063 | 0.068 | 0.066 | 0.070 | 0.072 | 0.001 | .767 | * | NS | NS |
| Intestinal weight                  | 7.63<sup>b</sup> | 7.87<sup>b</sup> | 8.22<sup>b</sup> | 8.64<sup>a,b</sup> | 8.81<sup>a</sup> | 0.137 | .028 | * | NS | NS |
| Liver weight                        | 4.36 | 4.47 | 4.69 | 4.75 | 4.73 | 0.089 | .645 | NS | NS | NS |
| Kidney weight                       | 0.89 | 0.98 | 0.87 | 0.87 | 0.87 | 0.025 | .583 | NS | NS | NS |
| Spleen weight                       | 0.272 | 0.300 | 0.291 | 0.270 | 0.284 | 0.004 | .0004 | NS | NS | NS |

Note: <sup>a,b</sup>Means within the line with different superscripts differ significantly (P < .05). *Linear, Quadratic, Cubic effect (P < .05). NS, not significant; L, linear; Q, quadratic; C, cubic effects; SEM, standard error of the mean.

### Table 3. Effects of dietary ZEA on serum biochemistry in young rats.

| Measurements     | ZEA treatment (mg/kg) | Effects |
|------------------|-----------------------|---------|
|                  | (Control) 0 | 0.5 | 0.9 | 1.8 | 3.6 | SEM1 | P | L | Q | C |
| AST (IU/L)       | 86.0 | 78.4 | 78.7 | 81.5 | 75.0 | 2.970 | .142 | NS | NS | NS |
| ALT (IU/L)       | 37.3 | 34.7 | 34.1 | 37.1 | 38.5 | 2.463 | .644 | NS | NS | NS |
| ALP (IU/L)       | 343.4 | 357.2 | 320.3 | 328.1 | 353.2 | 22.2 | .721 | NS | NS | NS |
| Total protein (g/dL) | 6.08 | 5.92 | 5.99 | 6.21 | 5.91 | 0.084 | .082 | NS | NS | NS |
| Uric acid (mg/dL) | 26.4<sup>a</sup> | 20.8<sup>a,b</sup> | 21.7<sup>a,b</sup> | 24.9<sup>a,b</sup> | 20.5<sup>b</sup> | 0.084 | .082 | NS | NS | NS |
| Bilirubin (mg/dL) | 0.10<sup>a</sup> | 0.13<sup>a</sup> | 0.13<sup>a</sup> | 0.14<sup>a</sup> | 0.13<sup>a</sup> | 0.006 | .001 | * | NS | NS |
| FSH (ng/mL)      | 5.63 | 3.85 | 5.19 | 5.58 | 6.53 | 0.662 | .108 | * | * | NS |
limited hepatolysis after a single i.p. administration by ZEA. Similarly Minervini et al. (2001) reported that a pulse dose of 1.5 and 6 mg ZEA/kg b.w. causes toxic effects on the reproductive tract and liver. In this respect, it is important to mention that ZEA is mainly metabolized in the liver, which appears to be one of the main targets. However, differences in the liver toxicity could be associated with the level of and the way ZEA is administered as well as the age of the animals.

4. Conclusion

In conclusion, our results indicated that dietary ZEA at concentrations above 1.8 mg ZEA/kg can induce toxic effects in weaning rats, which was mainly explained by significant increases of the uterus weight.

Disclosure statement

No potential conflict of interest was reported by the authors.

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