Reproductive Function of Breeding Gilts Exposed to Zearalenone and a Mycotoxin Deactivator

PEER REVIEWED

Mycotoxins are toxic substances produced by naturally occurring metabolic processes in fungi. Mycotoxins can invade the seeds before the actual harvest whilst the crop is still on the field, or alternatively, mould growth can occur during storage at the feed mill or on the farm. As a result, high numbers of mycotoxins could already be present in the ingredients before they are received in feed mills or farms. Mould can also grow during feed processing, especially when the temperature and humidity in the feed is increased during mixing. Finally, mould growth and mycotoxin production can also occur at the farm level from improperly cleaned silos, transport systems and feeders. The production of mycotoxins is enhanced by factors such as the moisture of the substrate (10 to 20 %), the relative humidity (≥ 70 %), the temperature (0 to 50˚C, depending on the fungus species) and the availability of oxygen (Kanora and Maes, 2009). The most important role of feed mills is to keep the levels of mycotoxins as low as possible, while multi-mycotoxin contamination should be also avoided. Most of the mycotoxins occur concurrently and a commodity usually contains more than one mycotoxin at the same time.

The most frequently concurrently occurring non-specific symptom of mycotoxin contamination in fattening pigs and piglets is decreased feed intake or feed refusal - very typical for deoxynivalenol (DON) contamination and diarrhoea. It is known that DON is capable of compromising several intestinal barrier functions, including a decreased surface area available for nutrient absorption and potentiation of intestinal inflammation. Both feed refusal and diarrhoea might contribute to decreased daily weight gains and low FCR in growing pigs. The splay leg syndrome is the major congenital cause of lameness in suckling piglets. It is characterised by a temporarily impaired functionality of the hind leg muscles immediately after birth, resulting in inability to stand and walk (Papatsiros, 2012). Aetiology and pathogenesis of splay leg syndrome are complex and still remain poorly understood. Infectious factors might also be involved in the aetiology. Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes late-term reproductive failure in sows, which is characterised by increased number of stillbirths, and weak, lightweight and splay-legged piglets (Papatsiros et al., 2006). Exposure of piglets to another Fusarium mycotoxin - fumonisin B1 (FB1) - increased the risk for PRRSV disease (Bane et al., 1992). Various management and genetic factors have been connected with the aetiology of splay leg syndrome, such as farrowing induction, low birth weight, short gestation lengths, slippery floors and breeds (e.g., Large White and Landrace pigs) (Ward, 1978). In addition, nutrition can play a role in pathogenesis, as choline or methionine deficiency in sow diets are correlated with the presence of splay leg syndrome. Some researchers suggest that one cause of splay leg is a deficiency of choline and methionine in the diet of the sow, which are essential for normal myelin production (Kornegay and Meacham, 1973). In contrast, the addition of 3g choline and 5g methionine to the sows' daily ration feed had no effect on the occurrence of splay leg (Dobson, 1971). Finally, nutrition is also involved in the aetiological factors, especially the zearalenone toxicity. The contamination of feed in sows with more than 4 ppm zearalenone can result in an increase in the number of piglets born with splay leg (Kanora and Maes, 2009).

Zearalenone (ZEN) is a mycotoxin produced by cereal species of Fusarium fungi (including *F. graminearum*). These fungi are common, occurring in cereal plants around the world and causing zearalenone contamination in harvested grains. This toxin is frequently found in maize and its products. Reports suggest that grass, hay and straw can also contain zearalenone. Zearalenone is oestrogenic, and has various effects in different animals. The most striking clinical feature is the swollen red vulva of immature gilts. The other signs are dependent upon the levels present in the feed and the state of pregnancy. The following may be used as guidelines to the symptoms that may be observed:

Boar semen can be affected with feed levels above 30ppm, but not fertility. At higher levels, poor libido, oedema of the prepuce and loss of hair can occur. In pre-puberty gilts (1 - 6 months old), 1 to 5ppm in feed causes swelling and reddening of the vulva and enlargement of the teats and mammary glands. Rectal and vaginal prolapses also occur in the young growing stock. In mature gilts, 1 to 3ppm will cause variable lengths of the oestrus cycle due to retained corpora lutea and infertility. In sows, levels of 5 to 10ppm can cause anestorus, which may also be associated with pseudo-pregnancy due to the retention of corpus luteum. However, zearalenone will not normally cause abortion. If sows are exposed during the period of implantation, litter size may be reduced. In lactation, piglets may develop enlarged vulva.

Effects on pregnancy: Embryo survival to implantation does not appear to be affected at levels less than 30ppm but above this, complete loss between implantation and thirty days occurs, followed by pseudo-pregnancies. Low levels of 3 to 5ppm do not appear to affect the mid-part of pregnancy, but in the latter stages, piglet growth in utero is depressed, with weak splay-legged piglets born. Some of these may have enlarged vulvas.

Effects on lactation: 3 to 5ppm has no effect on lactation but the weaning to service interval may be extended. The clinical signs are distinctive. Rations that are suspected of contamination should be examined for both the presence of zearalenone and also other oestrogen-like substances. Removal of the suspect feed will be followed by the regression of symptoms within three to four weeks.

Experimental Design

The aim of the experiment was to evaluate if an application of mycotoxin deactivation product could have diminishing effect on the toxicity of zearalenone in piglets.

Thirty Large White gilts, six months of age, were allotted to three groups:

- Control: good quality compound feed (10 animals)
- Zearalenone: feed contaminated with 660 ppb zearalenone (10 animals)
- Treatment: zearalenone + Mycotoxin deactivation product MDP (TOXY-NIL® Plus) 1 kg/ton (10 animals)

The experimental period lasted two months, with gilts being given feed based on 86% barley and 14% protein, vitamin and mineral supplements. Gilts were weighed once a month.

Oestrus signs were visually observed twice a day (behavioural changes and genital redness) and standing reflex was measured three times a day. After the trial, at eight months of age, five gilts from each group were slaughtered for evaluation of the reproduction system. The parameters measured were: length of uterus, size of vagina vestibule and length of vagina, length of cervix, ovarian size and volume.

Results

Gilts fed with zearalenone-contaminated diet had significantly prolonged oestrus time (hours) compared to the control group and the contaminated group with MDP included (Fig.1). Mycotoxin also resulted in extended standing heat, however this effect was fully moderated by application of MDP (Fig.2).

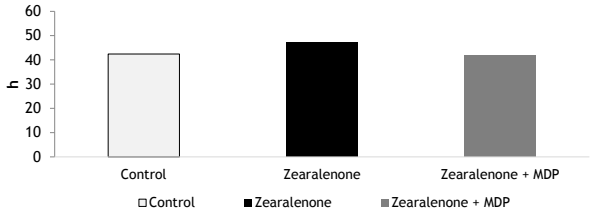

Figure 1 – Oestrus time (hours) in gilts fed ZEN-contaminated feed and protective effect of MDP product. [a-b] Different superscripts above the columns mean statistically significant differences (P≤0.05).

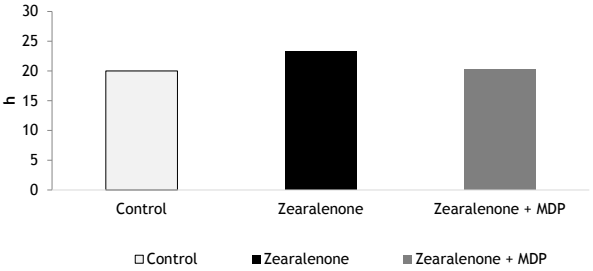

Figure 2 – Standing heat (hours) time in gilts fed ZEN-contaminated feed and protective effect of MDP product. [a-b] Different superscripts above the columns mean statistically significant differences (P≤0.05).

	Control	Zearalenone	Zearalenone + MDP
Length of uterus (m)	1.2[a]	1.02[b]	1.2[a]
left side	1.2[a]	0.9[b]	1.1[b]
right side			
Weight of genital organs with urinary bladder (g)	506.0[a]	496.6[a]	505.6[a]
Size of vagina vestibule (cm)	7.3[a]	6.9[b]	6.1[c]
Length of vagina (cm)	12.2[a]	11.5[a]	12.5[a]
Length of cervix (cm)	9.8[a]	10.3[ab]	11.4[b]
Volume of ovaries (cm^3)	16.2[a]	9.71[b]	14.2[a]
Weight of ovaries (g)	18.3[a]	11.98[b]	15.3[ab]

Table 1 - Status and functionality of the reproductive system.
[a-b] Different superscripts above the row mean statistically significant differences (P≤0.05).

Inspection of the reproductive tract of gilts after slaughtering indicated significant influence of 660 ppb zearalenone on the uterus length, size of vagina vestibule and volume and weight of the ovaries (Table 1). These observations confirmed suboptimal development of the reproductive system in gilts which were showing clear oestrus signs and prolonged standing heat. When gilts consumed contaminated feed with simultaneous inclusion of MDP, the length of uterus and volume of ovaries were similar to the control gilts. The similarity showed high statistical significance.

This study confirmed that the growth rate is not normally affected by the presence of zearalenone in feed. The average daily weight gain between the three groups was not statistically different (Fig. 3).

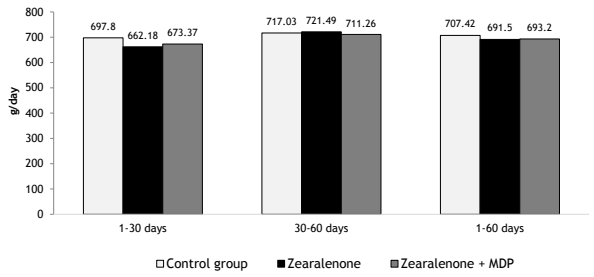

Figure 3 - Average daily gain, g/day of gilts fed ZEN-contaminated feed and protective effect of MDP product.

Conclusion

The results of this study in breeding gilts showed that there was a significant influence of oestrogen like mycotoxin zearalenone at 660 ppb on the reproductive function (uterus and vagina development, volume and weight of ovaries and oestrus signs). The negative effects of zearalenone on the reproductive function of gilts were effectively reduced by the inclusion of mycotoxin deactivation product (TOXY-NIL® Plus) at 1 kg/t in the feed.

Four Steps to Successful Mycotoxin Management

The best practical way to control mycotoxin levels is to use rapid test kit systems for the analysis of mycotoxins in raw ingredients which are not yet in silos. Different rapid test kit systems are validated for different mycotoxins and commodities and offer a very quick and effective way of raw material screening before they enter the feed mill. Once the

levels are known, every feed mill can estimate the quality of its raw ingredients in terms of mycotoxin contamination and can effectively and more precisely (through dosage adjustment) apply mycotoxin deactivator during feed production.

Another strategy of mycotoxin risk management is to test for the presence of mycotoxins in finished feeds. This method has some advantages and disadvantages. The most important advantage is that as every raw ingredient can bring its own mycotoxins into the finished feed and by only testing some raw ingredients by rapid test kits, some important raw ingredients - whose inclusion is not high (5-10 %) and which can still cause significant contamination of finished feed - can be missed.

Since the 1960s, many analytical methods have been developed for the testing of mycotoxins in human food and animal feeds due to the concern of toxicity for human health. Among them, the methods of thin-layer-chromatography (TLC), enzyme-linked immunosorbent assay (ELISA) and immunosensor-based methods have been widely used for rapid screening, while high-performance liquid chromatography (HPLC) with fluorescence detection (FD) and mass spectrometry detection (MS) have been used as confirmatory and reference methods. An accredited laboratory service is required for this step. The most important disadvantage is that analysis of finished feed takes quite a long time, such that the tested feed is likely to have been fed to the animals by the time the results from the analysis are known.

Storage mycotoxin contamination (ochratoxins, aflatoxins) can be prevented by keeping temperature and moisture content in silos low, whilst grain is regularly aerated. In case perfect storage conditions cannot be guaranteed, use of mould inhibitor is highly recommended.

The final possible step in mycotoxin management is the application of a mycotoxin deactivator. These products work strictly *in vivo* and will not counteract or mask mycotoxin in stored feed or raw ingredients. It is highly recommended to apply effective mycotoxin deactivator, which offers an opportunity to significantly improve animal health, performance, productivity and profit impaired by mycotoxins. Depending on the target performance, different mycotoxins can be more or less problematic. Therefore, using different products for different animal groups becomes a rational trend.

References

1. Bane D.P., Neumann E.J., Hall W.F., Harlin K.S. and Slife, R.L.N. 1992. Relationship between fumonisin contamination of feed and mystery swine disease-a case-control study. Mycopathologia; 117, 121–124.
2. Dobson, K.J. 1971. Failure of choline and methionine to prevent splay leg in piglets. Austral. Vet. J., 47: 587-590. PMID: 5137938.
3. Kanora, A. and Maes, D. 2009. The role of mycotoxins in pig reproduction: a review. Veterinarni Medicina, 54, 2009 (12): 565–576.
4. Kornegay, E.T. and Meacham, T.N. 1973. Evaluation of supplemental choline for reproducing sows housed in total confinement on concrete on in dirt lots. J. Anim. Sci.37:506-509.
5. Papatsiros, V. 2012. The splay leg syndrome in piglets: A Review. American Journal of Animal and Veterinary Sciences 7 (2): 80-83.
6. Ward, P. 1978. The Oxford companion to Spanish literature. 1st Edn., Clarendon Press, Oxford, pp: 629.

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