# Analysis of the presence of mycotoxins in swine feed and its possible effects on semen quality in a rural property of Santa Catarina

*Análise da presença de micotoxinas em rações para suínos e seus possíveis efeitos na qualidade do sêmen em uma propriedade rural de Santa Catarina*

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## SUMMARY

The presence of mycotoxins in swine feed can trigger off serious problems. Besides influencing the quality and quantity of semen produced, it can also cause weight loss, as well as alterations of the immune and reproductive systems. This study reports on an assessment of male swine reproductive parameters over the years 2010 to 2011, at a small rural property in Santa Catarina State, Brazil, seeking to correlate them with possible contamination of the swine's diet by different mycotoxins. Out of a total of 12 samples of feed collected between August 2010 and February 2011, 58.3% were found to be contaminated with fumonisins (FBs). Only one sample of feed was contaminated by zearalenone (ZON) (64.10 μg $kg^{-1}$). No contamination by aflatoxins (AFLs), ochratoxin A (OTA) and sterigmatocystin (EST) was detected. 75 swine semen samples were collected and analysed in relation to reproductive parameters (sperm motility, volume and density). The findings were correlated to the levels of mycotoxins in feed given to the sample donors. In spite of the levels of mycotoxin contamination found in the feed (FBs and ZON), no significant variations in sperm motility, volume or density were observed in this study.

**KEY WORDS:** reproduction, feed, mycotoxins, semen, swine.

## RESUMO

A presença de micotoxinas em alimentos para suínos pode desencadear sérios problemas. Além de influenciar na qualidade e quantidade do sêmen produzido, pode também causar a perda de peso, assim como alterações no sistema imunológico e reprodutivo. Este estudo relata uma avaliação dos parâmetros reprodutivos de machos suínos ao longo dos anos de 2010 a 2011, em uma pequena propriedade rural localizada em Santa Catarina, Brasil, buscando correlacionar estes dados com a possível contaminação das dietas de suínos por diferentes micotoxinas. Do total de 12 amostras coletadas entre agosto de 2010 a fevereiro de 2011, 58,3% estavam contaminadas por fumonisinas (FBs). Apenas uma amostra de ração estava contaminada por zearalenona (ZON) (64,10 μg $kg^{-1}$). Nenhuma contaminação foi detectada para aflatoxina (AFLs), ocratoxina (OTA) e esterigmatocistina (EST). Foram coletadas 75 amostras de sêmen suíno e analisadas em relação aos parâmetros reprodutivos (motilidade, volume e densidade espermática). Os resultados foram correlacionados com os níveis de micotoxinas da ração fornecida aos reprodutores. Apesar dos níveis de contaminação encontrados nas rações (FBs e ZON), não foi observado neste estudo variações significativa na motilidade, volume ou densidade espermática.

**PALAVRAS-CHAVE:** reprodução, ração, micotoxinas, sêmen, suíno.

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## INTRODUCTION

The improvement in the efficiency of swine production of recent years, is the result of the implementation of several new biotechnologies and production practices (GERRITS et al., 2005). Artificial insemination (AI), for example, can increase the quantity and quality of swine bred. AI has replaced natural mating because it is a faster, easier and cheaper means of introducing superior genes in sow herds, while minimizing the risk of disease (VAZQUEZ et al., 2008; MAES et al., 2008). To control and improve the quality of swine reproduction, it is necessary to evaluate genetic and environmental factors such as nutrition (including food safety) and health (EWUOLA and EGBUNIKE, 2010). Mycotoxins found in animal feed are among the known causes of some reproductive swine disorders (BIRÓ et al., 2003).

Mycotoxins are toxins produced as secondary metabolites by filamentous fungi which, when present in food, can cause disease and even death in human beings and other animals (ZAIN, 2011). Aflatoxins (AFLs), fumonisins (FBs), zearalenone (ZON), ochratoxin A (OTA) and sterigmatocystin (EST) are the major mycotoxins whose presence has been associated with reproductive problems. They contribute to reproductive disorders in swine breeding units by causing the animal to reduce food intake and delay growth or by affecting vital organs, reducing weight gain, lowering immunity, or causing reproductive alterations that influence semen quantity and quality (BIRÓ et al., 2003).

AFLs cause a direct lyses of the sperm cell membrane, which results in the loss of lysozyme, an enzyme which facilitates the penetration of the ova by spermatozoa (SHUAIB et al., 2010). EST is similar to AFLs in various ways. However, it is less toxic. Diets formulated with ingredients contaminated with FBs can negatively influence swine spermatogenesis (EWUOLA and EGBUNIKE, 2010). In breeding, ZON decreases production of testosterone, testicle weight, libido (D'MELLO et al., 1999; ZINEDINE et al., 2007; BENZONI et al., 2008) and causes germinal epithelial degeneration, altering sperm formation in animals (ZAIN, 2011). OTA can also potentially affect sperm production and semen quality of boars (SOLTI et al., 1999; DUARTE et al., 2011).

Mycotoxins have been shown to affect such functional parameters in boars as sperm stability, motility and spermatogenesis. For this reason, the aim of this study has been twofold: to analyse possible contamination of boar feed by mycotoxins (FBs, ZON, OTA, AFLs and EST) and to correlate these findings with those gathered for reproductive parameters of male swine over the years 2010 to 2011 in a small rural property in the State of Santa Catarina/Brazil.

## MATERIALS AND METHODS

### Swine farm characteristics

This study was conducted on a farm situated in the Itajaí Valley region, in Santa Catarina State, Southern Brazil, between August 2010 and February 2011.

### Assessment of parameters used for sperm quality determination

Semen collection: 75 semen samples were collected from two boars, breeder A and breeder B (Table 1).

Semen analyses: Volume of ejaculate: was measured directly as per the markings on the collection bottle (LIMA et al., 2007). Sperm density was measured on a semen sample (1 mL) collected and placed in a sperm densimeter with saline. Motility was evaluated on a scale from 0 to 100%, according to Scheid (1993).

### Assessment of feed mycotoxin contamination

Sample collection: breeder's feed samples, were collected for analysis of FBs, ZON, OTA, AFLs and EST for the period specified. The main breed feed composition can be seen in table 2. A 1 kg sample was collected from each of the 25 kg bags stored in the property's shed.

AFLs, OTA, ZON and EST contamination: analysed by the method described by Soares and

Table 1 - Breeders used in the study.

| Male swine | Collection | |
|---|---|---|
| Breeder | Number of Samples | Period (2010/2011) |
| A | 37 | August/February |
| B | 38 | August/February |
| Total | 75 | - |

Table 2 - Composition of the feed intended for swine breeding.

| Ingredients | Amount | |
|---|---|---|
| | (kg) | (%) |
| Corn | 325 | 65 |
| Soyabean meal | 80 | 16 |
| Rice meal | 75 | 15 |
| Minerals | 20 | 4 |
| Total | 500 | 100 |

Rodrigues-Amaya (1989). Briefly, each sample was extracted with methanol and potassium chloride (4%) and filtered. After that, ammonium sulphate (30%) was added, followed by moderate stirring and filtration. The resulting filtrate was transferred to a separation funnel, and toxins were extracted with chloroform. Extracts were collected in a beaker and submitted to solvent evaporation. Extracts were re-suspended in 200 µL of toluene and immediately subjected to thin layer chromatography. The analyses were performed in a saturated vat with the following solvent system: toluene, ethyl acetate, formic acid (60:40:0.5). The toxins were detected under UV light and quantified by comparison against toxin standards (λ: 256 and 365 nm). The limits of determination (LOD) and quantification (LOQ) were 1 and 2 µg $L^{-1}$, respectively.

FBs contamination: analysed by liquid chromatography and fluorescence detector (LC/FD) at 335 and 440 nm wavelength (excitation and emission, respectively), as described by AOAC (2005). LOD was 0.5 and LOQ was 1 µg $kg^{-1}$ for both, $FB_1$ and $FB_2$.

*Statistical Analysis:* performed by variance analysis (ANOVA) and Turkey´s test, to evaluate significant differences among the means ($p<0.05$) using GraphPad Prism 4.0 software. The results were expressed as the mean values and standard errors.

## RESULTS AND DISCUSSION

The first objective of this study was achieved by analyzing breeder feed to detect mycotoxins. The presence of mycotoxins in feed for swine breeding affects the reproductive system in various ways. For example, AFLs can lead to disruption of the testicular histo-architecture, spermatogenesis and even the secretion of androgens (FARIDHA et al., 2007). $FB_1$ not only affects functional parameters such as sperm chromatin stability and motility, but also reduces testicular sperm reserves (GBORE and EGBUNIKE, 2008; MINERVINI et al., 2010). Moreover, OTA increases the incidence of chromosome abnormalities and affects sperm morphology (KUMARI and SINHA, 1994). Furthermore, ZON has been reported to depress serum testosterone, inducing feminization and suppressing libido (EFSA, 2004).

The following results were obtained: 58.3% of a total of 12 samples, collected between August 2010 and February 2011, were found to be contaminated by FBs. $FB_1$, ranging from 58.5 to 531.2 µg $kg^{-1}$, with an average of 173.2 µg $kg^{-1}$, was detected in seven samples. $FB_2$ was found in three samples and contamination ranged from 81.2 to 997.6 µg $kg^{-1}$, with an average of 114.4 µg $kg^{-1}$. Only one sample of feed (8.3%) was contaminated by ZON (64.10 µg $kg^{-1}$). No

contamination by AFLs, OTA or EST was found (Table 3).

The second objective of this study involved attempting to correlate the presence of mycotoxins to the existence of alterations of the quantity and quality of breeder sperm (sperm motility, volume of the ejaculate and sperm density).

AFLs, OTA and ZON can deteriorate semen quality, not only by altering sperm morphology coiled/double tails, small/tapering heads (SALEM et al., 2001), but also by decreasing its motility (BOSE and SINHA, 1994; ALM et al., 2002; MATHURIA and VERMA, 2008; BENZONI et al., 2008; TSAKMAKIDIS et al., 2008). Sperm motility is considered one of the most important parameters in evaluating the fertilizing ability of sperm (BENZONI et al., 2008).

In our results, motility remained at 80% and was, therefore, not affected by FBs or ZON (Figure 1). This is consistent with Rajkovic et al. (2007), which shows no semen alterations by FBs and EST. Differently, Alm et al. (2002) show that ZON was able to decrease sperm motility. The fact that a very low level of ZON was present in our sample, may explain the fact that motility was not affected in our study.

The average semen volume for breeders A and B was 280.88 (± 26.13) and 286.16 (± 25.75) (mL), respectively. Statistical analysis revealed no significant variation of this parameter in the relevant months (Figure 2). Our results are in agreement with Kozink et al. (2004), i.e. volume of an ejaculate ranges from 75 to 400 mL.

Likewise, sperm density did not change significantly (Figure 2). In our study, contamination levels found in the feed, ranged from 58.5 to 531.2 µg $kg^{-1}$ for $FB_1$ to 81.2 to 997.6 µg $kg^{-1}$ for $FB_2$. Gbore and Egbunike (2008) found that levels of 5000 µg $kg^{-1}$ of feed lowered sperm production. Our results may be interpreted as too low a level of contamination to cause any alterations. Furthermore, factors such as temperature, photoperiod, humidity and nutrition (EWUOLA and EGBUNIKE, 2010; PURDY et al., 2010) being under control may have contributed to preventing or minimizing the risk of spermatic pathologies, even in the presence of feed contaminants.

Table 3 - Assessment of swine breeder feed samples for mycotoxins.

| Feed | Mycotoxins (µg $kg^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | $FB_1$ | $FB_2$ | ZON | OTA | AFLs | EST |
| Total samples (n) | 12 | 12 | 12 | 12 | 12 | 12 |
| Positive samples (n) | 7 | 3 | 1 | 0 | 0 | 0 |
| (%) | 58.3 | 25.0 | 8.3 | 0 | 0 | 0 |
| Average (µg $kg^{-1}$) | 173.2 | 114.4 | 5.3 | 0 | 0 | 0 |
| Max (µg $kg^{-1}$) | 531.2 | 997.6 | 64.1 | 0 | 0 | 0 |
| Min (µg $kg^{-1}$) | 58.5 | 81.2 | 0 | 0 | 0 | 0 |

$FB_1$ and $FB_2$: Fumonisins; ZON: zearalenone; OTA: Ochratoxin; AFLs: Aflatoxins; EST: Sterigmatocystin.

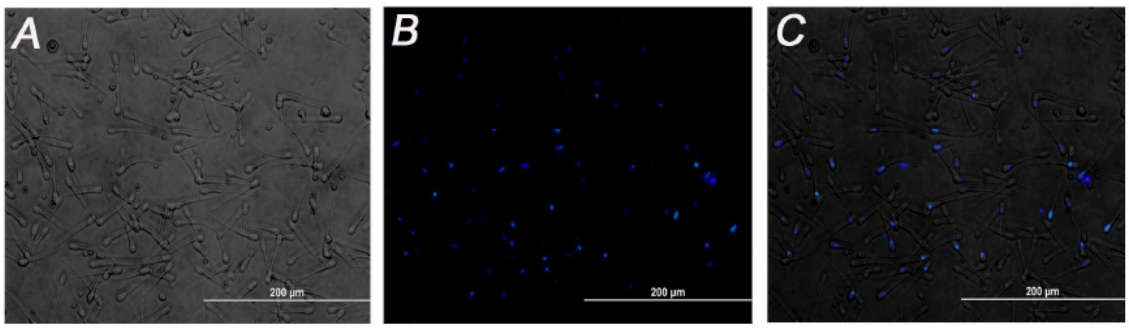


Figure 1 - (A) Photomicrograph of boar semen; (B) sperm nucleus staining by DAPI and (C) overlap of A and B.

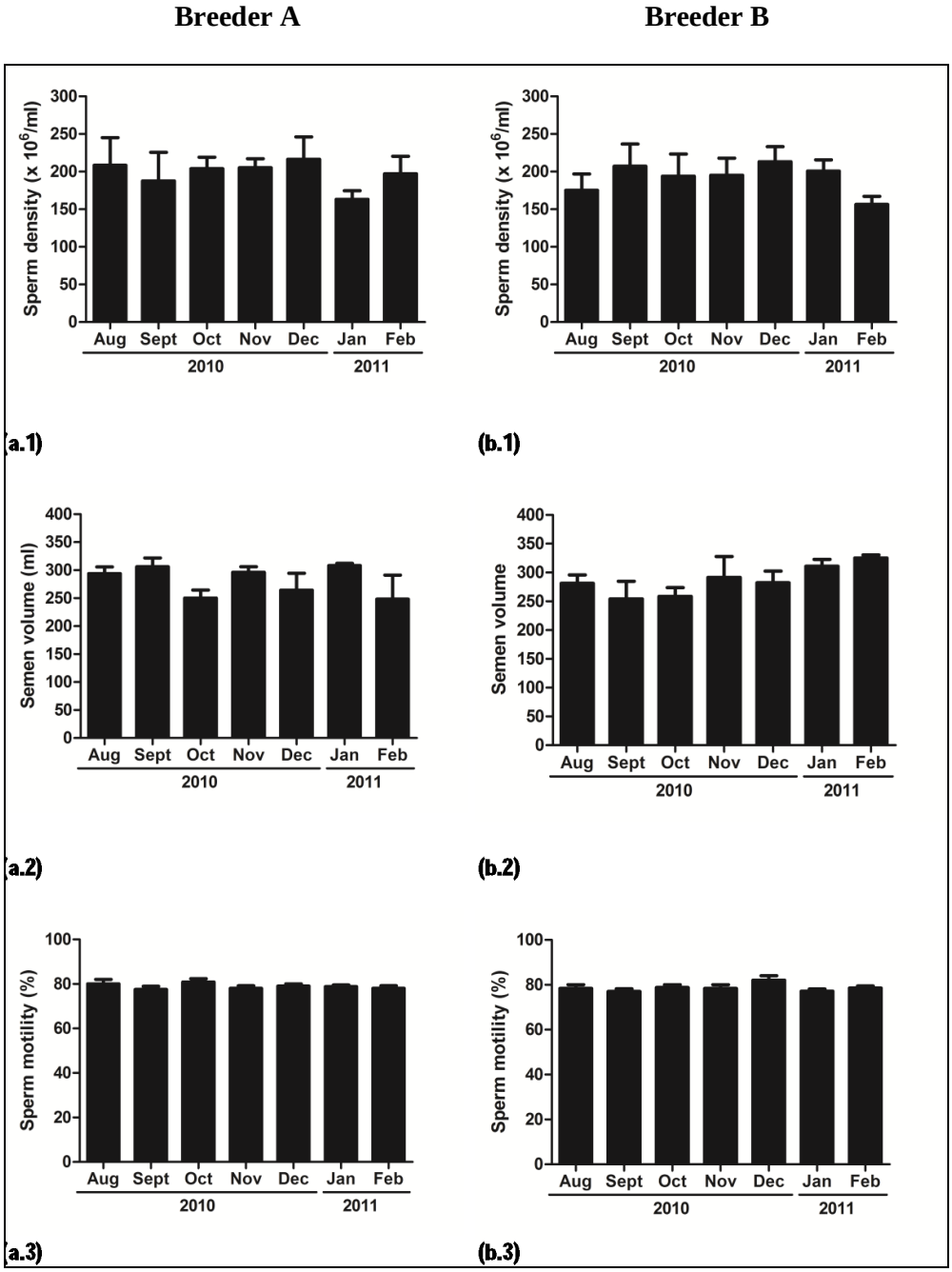


Figure 2 - Breeders A and B semen analysis (a.1 and b.1 - sperm density; a.2 and b.2 - semen volume; a.3 and b.3 - sperm motility).

## CONCLUSION

Our results show the presence of mycotoxins (FBs and ZON) in breeder feed given to boars in a small property in the South of Brazil between 2010 and 2011. In spite of this, no changes were found in semen quality (sperm motility, volume and density). Therefore, no correlation between these factors can be asserted. Other factors that may be considered in future studies include higher concentrations of contaminants than those found in this article, follow up for longer periods of exposure to contaminated feed, or focus on parameters other than those already covered in this study. Any or all of these may eventually be shown to cause observable reproductive alterations.

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