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INFLUENCE OF MYCOTOXIN ZEARELENONE ON THE SWINE REPRODUCTIVE FAILURE

ABSTRACT: Reproductive failure in swine is often a difficult diagnostic problem. If diagnoses of infectious disease or management related problems are not obtained, feed quality and safety may be questioned. Mycotoxins are often present in swine feed in the amount that can have detrimental impact on production and reproduction. Problems are expressed only as alterations of the reproductive cycle, reduced feed intake, slow growth or impaired feed efficiency. In Serbia, generally speaking, high concentrations of mycotoxins were noticed, especially mycotoxin zearalenone. High presence of zearalenone in swine feed is probably due to climatic influence and should be monitored constantly. This paper includes field observations regarding the influence of moldy feed containing mycotoxin zearalenone on the occurrence of the reproductive failure in swine breeding categories (sows, gilts and boars).

The material for this research was obtained from four swine farms where certain reproductive disorders and health problems in breeding animals were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: anamnestic and clinical evaluation, pathomorphological examination, standard laboratory testing for detection of aerobic and anaerobic bacteria, and microbiological feed testing, in order to examine the presence of fungi and mycotoxins by applying the method of thin layer chromatography.

On the basis of the obtained results, it could be concluded that mycotoxin zearalenone was detected in all examined feed samples. The presence of mycotoxin in feed was directly related to the reproductive failures in the examined swine categories (vulvovaginitis, endometritis, rebreeding, infertility). Swine reproduction represents the base for intensive swine production. The presence of mycotoxins in swine feed have influence on the reproduction and health status of pigs and under certain conditions may significantly disturb the production process.

KEY WORDS: mycotoxins, swine reproductive disorders, zearalenone

INTRODUCTION

Mycotoxins are structurally a diverse group of mostly small molecular weight components, produced mainly by the secondary metabolism of some filamentous fungi or molds which, under suitable temperature and humidity

conditions, may develop on various food and feeds, causing serious risks for human and animal health. Today, more than 300 mycotoxins have been detected and scientific attention is focused mainly on those that have proven to be carcinogenic and/or toxic. Examples of mycotoxins that are considered to be of significance for public health and agro-economy include aflatoxins (AF), ochratoxins (OCT), trichothecenes, zearalenone (ZEA), fumonisins (F), tremorgenic toxins and ergot alkaloids (Z a i n, 2011). Zearalenone is a mycotoxin produced by *Fusarium graminearum* and other *Fusarium* molds using corn, wheat, barley, oats and sorghum as substrates. Swine are among the most sensitive species to this mycotoxin (D i e k m a n and G r e e n, 1992).

Reproductive failure in swine is often a difficult diagnostic problem. If diagnoses of infectious disease or management problems are not obtained, then the feed quality and safety may be questioned. Experimental studies conducted with the aim of supporting the role of mycotoxin in swine reproduction diseases are limited (O s w e i l e r et al., 1990). Sometimes, conclusions could be drawn from herd observations rather than planned experiments, and mycotoxins are often not measured in the feeds involved (E t i e n n e and J e m m a l i, 1982). This paper included field observations regarding the influence of moldy feed containing ZEA and some other mycotoxins on the occurrence of reproductive failure in swine breeding categories (sows, gilts and boars).

MATERIAL AND METHODS

The material for this research was obtained from four swine farms where certain reproductive disorders and health problems in breeding animals (sows, gilts, boars) were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: anamnestic and clinical evaluation, pathomorphological examination, standard laboratory testing for detection of the presence of aerobic and anaerobic bacteria in the samples of reproductive organs derived from slaughtered sows and gilts, and microbiological feed testing, performed in order to examine the presence of fungi and mycotoxins by applying the method of thin layer chromatography.

RESULTS AND DISCUSSION

On the first examined swine farm, based on the anamnestic data on sows that were excluded and reasons for their exclusion, the following reproductive disorders were discovered: rebreeding (27%), infertility (20%), anestrus (10.6%) and frequently observed endometritis. Due to the reproductive problems, the parity structure of the herd was altered. The highest percent of exclusion was related to the first litter sows, i. e. with the first and the second parity (43%) and in the herd the maiden gilts were introduced. Most of the rebreedings were in relation to the first and second expected oestrus. The oc-

currence of the increased number of dead born and mummified piglets was not detected. However, in certain number of boars high percent of litters with small piglets was evident. Besides this, the occurrence of neonatal diarrhea in suckling piglets was detected already in the first 3 days of life after farrowing. The most prominent clinical sign in large number of farrowed piglets was vulvovaginitis (swelling and reddening of the vulva). By patomorphological examination of genital organs (ovaria, uterus) from excluded females in the slaughter-house, the following lesions were discovered: 24% ovaries in the luteal phase, 16% ovaries in the follicular phase and 2% with pathological changes (cysts and fibrosis). Only 20% of the examined ovaries was recorded to have the ovulation rate 20 and above. Application of pathological control of reproductive organs of excluded sows, a significant percent of endometritis (24%) was discovered (presence of liquid muddy content in the uterus with small pieces of destroyed tissue or content that looked like a sour cream). Bacteriological tests of tissue samples from the dams genital organs showed the presence of *Streptococcus dysgalactiae subsp. equisimilis*, *Staphylococcus haemolyticus*, *Escherichia coli*, *Pasteurella aerogenes*, *Streptococcus uberis*. Having in mind the clinical and pathological symptoms observed, especially the signs of vulvovaginitis in just farrowed piglets, a justified suspicion on the presence of mycotoxins in feed was made. After laboratory tests a total number of fungi in the large number of microbiologically examined feeds was discovered: corn (887×10^3 *Aspergillus*, *Rhizopus*), feed for pregnant sows (123×10^3 *Penicillium*, *Fusarium*), feed for lactating sows (526×10^3 *Aspergillus*, *Penicillium*, *Mucor*) and feed for boars (177×10^3 *Aspergillus*, *Penicillium*, *Mucor*). The presence of mycotoxins was detected: ZEA in the feed for pregnant sows (0.72 mg/kg) and OCT (0.08 mg/kg) in the feed for boars.

Although the level of detected mycotoxins in the feed for pregnant sows and boars, obtained from the first examined farm, was lower when compared to the level set by the regulation, a cumulative effect of mycotoxins in organisms should be considered as well. Many toxigenic strains of molds can occur in grains without any production of mycotoxins, and there is little correlation between spore counts or degree of fungal growth and presence of mycotoxins. Conversely, absence of molds does not mean that feed is safe from mycotoxins (Oswieiler, 2006).

Combinations of some mycotoxins may potentiate the action of one another, or at least exert an additional effect. With respect to the known mycotoxins that are of clinical importance, the response is usually subacute or chronic and the presenting signs are often subtle and vague (Prodano et al., 2009). Problems are frequently expressed only as alterations of the reproductive cycle, reduced feed intake, slow growth or impaired feed efficiency (Oswieiler, 2006). Zearalenone affects the reproduction of livestock most seriously because it possesses estrogenic activity. Other mycotoxins affect the reproduction in livestock via indirect means such as reduced feed intake or reduced growth, or by damaging other vital organs of the body (Dekman and Green, 1992). We need to consider that feed samples available at the

time of anestrus or return to service may not represent the contaminated feed that initiated the problem (O s w e i l e r, 2006).

On the second examined swine farm, clinical examination of swines in the farrowing house showed that the most prominent clinical sign in the piglets of different age was vulvovaginitis. The litter size varied significantly, whilst the small litter dominated in the recently farrowed sows and gilts. In about 30% of farrowed sows the mastitis-metritis-agalactiae syndrome was discovered. In weaned piglets and fatteners the vulvovaginitis in the almost all female pigs was noticed as well as low weight gain. By clinical examination in the mating and waiting place, the impression was that all animals were in the heat, while the boars were uninterested for the jump (weakened libido). Consequently, the lower number of sows was moved to the farrowing house. There were no rebreedings and dead born piglets because there were no pregnant females. The mating and waiting house was overcrowded and the number of fatteners progressively decreased. Organoleptic control of the farm storage space showed, approximately, 70–80% of moldy ear – corn. After laboratory tests of corn samples the presence of ZEA from 14 to 36 mg/kg, depending on the sampling place, was detected. Three weeks after the introduction of artificially dried corn in swine feed, the signs of vulvovaginitis became less evident, the mating was normalized but the farrowing house was empty. The initial number of females on the farm significantly decreased because there were no signs of heat and no pregnancy, and gilts and sows were slaughtered. Pathological control performed in the slaughter house on all examined females detected cystic degeneration of ovaries, enlargement of the uterus and hyperemic mucosa. Frequently, mucopurulent content in uterus was discovered.

Mycotoxin ZEA has a unique nonsteroidal resorcyclic acid lactone structure. This structure resembles steroid hormones and allows ZEA to bind to estrogen receptors, where it acts as an agonist and partial antagonist to estradiol. ZEA induces estrogenic effects, often reported as hyperestrogenism, in all species tested, particularly in pigs (M a l e k i n e j a d et al., 2007). ZEA inhibits the release and secretion of follicle stimulating hormone (FSH) to depress the maturation of ovarian follicles during the preovulation stage. This results in atresia of follicles becoming the prominent histological trait and resulting in atrophy of ovaries (D i a z – L l a n o and S m i t h, 2007). Clinical signs of ZEA mycotoxicosis vary with dosage and age of swine exposed. In prepubertal gilts, ZEA causes vulvovaginitis, which is characterized by tumescence and edema of the vulva and vagina and precocious mammary development. As with other estrogens, ZEA is luteotropic in swine and can induce anestrus in sows if consumed during the middle portion of the estrous cycle. Piglets born from sows receiving ZEA may have enlarged external genitalia and uteri (O s w e i l e r, 2006). ZEA and its metabolites, alpha and beta ZEA, can cross the placenta and be present in milk of exposed sows, risking the exposure of the embryo and neonate (M a l e k i n e j a d et al., 2007) and contributing to estrogenic effects in piglets (perinatal hyperestrogenic syndrome). Lower conception rate, increased number of repeated breeders, decreased litter size, increased number of stillbirths are frequently observed. Clinical signs

in neonatal gilts include swelling of the vulva and teats, edematous infiltration of the perineal region, ventral abdomen and umbilicus, usually accompanied by exudative, crusted inflammation and necrosis of the teats. An increase of splayleg and trembling piglets has been reported. Pathological lesions of hyperestrogenism included enlargement of the ovary and uterus, ovarian follicle maturation, glandular proliferation of the endometrium and epithelial proliferation of the vagina. Young boars may have reduced libido and decreased testicular size (O s w e i l e r, 2006).

In Serbia, generally speaking, alarmingly high concentrations of mycotoxins were noticed, especially increased concentration of ZEA. High presence of ZEA in swine feed is probably due to climatic influence and should be monitored constantly (P e t r u j k i ć et al., 2008).

The results from the third examined farm included prolonged gravidity period and farrowing time of sows, cases of agalactia (sudden loss of milk and lying on the udder), a small number of stillbirths and mummified piglets. The newborn piglets were described as weak, nonviable, with diarrhea. The diseased piglets lived only for 4 days after the birth. They probably died due to hypoglycemia because sows did not have enough milk or the piglets were too weak and did not have enough strength for milk suckling. Sporadically, the occurrence of splayleg was observed. The sows had good body condition in all production stages but an increase in body temperature was detected after parturition in large number of sows (48%). The abortions and rebreeding were not registered, and no connection of the above mentioned problems with the parity was observed. Conducted laboratory tests on feed showed that there was simultaneous presence of several mycotoxins (ZEA, AFB1, AFG1, OCT). Mycotoxins were detected in feed for pregnant and lactating sows (ZEA 0.8 mg/kg; AFB1 0.008 mg/kg; AFG1 0.02 mg/kg; OCT 0.2 mg/kg), in corn (ZEA 4 mg/kg; AFB1 0.008 mg/kg; AFG1 0.002 mg/kg; OCT 0.2 mg/kg), sunflower pellets (ZEA 4 mg/kg; AFB1 0.016 mg/kg; AFG1 0.008 mg/kg; OCT 1.0 mg/kg) and soyabean pellets (ZEA 2.0 mg/kg; AFB1 0.016 mg/kg; AFG1 0.008 mg/kg; OCT 1.0 mg/kg).

Mycotoxin mixtures, i.e. the combinations of several mycotoxins, can normally occur and they may affect the immunity in an additional or synergistic manner. The presence of mycotoxins in feed for pregnant sows causes the occurrence of embrional and fetal death and decreased immunological defense in piglets (P r o d a n o v – R a d u l o v i ć et al., 2011). Feeding lactating sows with grains naturally contaminated with *Fusarium* mycotoxins results in reduced feed intake and increased body weight loss in piglets, but no changes in milk composition or milk production are detected. The reduced feed intake and losses of body tissues tend to increase the weaning to estrus interval (D i a z – L l a n o and S m i t h, 2007).

Even though it is not clear whether ZEA consumption affects the onset of puberty, experimental results indicate that the estrogenic properties of ZEA are not permanently damaging and that gilts may successfully enter the breeding herd without subsequent reduction in fertility if they are given a 2 week withdrawal period. ZEA causes multiple reproductive dysfunctions in mature,

cycling gilts (constant estrus, pseudopregnancy, and ultimately, infertility) and if it is added to the diet of pregnant sows it can cause them to farrow smaller litters with smaller offspring (D i e k m a n and G r e e n, 1992).

Complex health problems, including significant reproductive disturbances, were observed on the fourth swine farm. By applying the control of anamnestic data, frequent cases of sows delivering mummified piglets, stillbirths and decreased litter size were observed. Also, the increased number of rebreeding sows at irregular intervals was discovered. The conception rate dramatically decreased and the problem with frequent abortions, occurring 2 months before farrowing, was intensified. Besides this, just farrowed piglets were non-viable and despite the medical treatment they lived only for 3-4 days after birth. Sporadically, the occurrence of severe yellowish diarrhea in piglets and apparent clinical signs of vulvovaginitis in just born piglets were evident. After conducted laboratory testing of swine feed samples the simultaneous presence of several mycotoxins was recorded: ZEA (6.4 mg/kg), AF (0.0064 mg/kg) and OCT (0.032 mg/kg).

The effects of ZEA in mature gilts and sows were related to the period of contaminated feed intake, i.e. mating period, the concentration in the diet and the duration of administration. The most apparent effect of feeding the mature gilts with low concentrations of ZEA was increased pseudopregnancy if the contaminated diet was fed prior to mating (Y o u n g and K i n g, 1986). ZEA ingestion by mature gilts may produce two different effects which are related to estrogenic properties of this mycotoxin. When females are not pregnant, ZEA induces a pseudopregnancy state characterized by uterine hypertrophy and corpora lutea maintenance on ovaries. Sows do not cycle and cannot be bred. These consequences may be of great economical importance because they can disrupt a breeding program. Secondly, when fed during gestation, ZEA reduces development of the uterus, placental membranes and fetuses. These effects may induce lower embryonic survival or higher rate of immature piglets at birth, which, are less able to suckle and may die within a few days after farrowing (E t i e n n e and J e m m a l i, 1982).

Main difficulty in assessing the risk of mycotoxins to animal health is the multiplicity of factors affecting the production or presence of mycotoxins in feeds. Presence of molds is not necessarily accompanied with the production of toxins. Thus, the demonstration of mold contamination does not easily prove that there are etiological agents in a given veterinary health problem. The incidence of mycotoxicoses may be more common than suspected (Z a i n, 2011). It is considered that pigs with the signs of ZEA mycotoxicosis should not be selected for reproduction, i.e. as breeding animals. Residues of ZEA in pork meat represent a potential danger for humans who are the consumers of contaminated meat. Animals that consumed feed contaminated with ZEA should be slaughtered 3-4 weeks after the withdrawal of feed (P o p o v i ć, 2007). The basic preventive protection measures for animals are the use of healthy feed and proper storage and management conditions for animals feed. Certainly, when mycotoxicosis occurs or is suspected, the first action should be to change the source of feed (P r o d a n o v et al., 2009).

CONCLUSION

On the basis of the obtained results, it may be concluded that mycotoxin ZEA was detected in all examined feed samples. The presence of mycotoxin in feed was directly related to the reproductive failures in the examined swine categories (vulvovaginitis, endometritis, rebreeding, infertility). Swine reproduction represents the base for intensive swine production. The presence of mycotoxins in swine feed affects the reproduction and health status of pigs and, under certain conditions, it may significantly disturb the production process.

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УТИЦАЈ МИКОТОКСИНА ЗЕАРАЛЕНОНА НА ПОРЕМЕЋАЈЕ У РЕПРОДУКЦИЈИ СВИЊА

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Резиме

Поремећаји у репродукцији свиња представљају озбиљан дијагностички проблем. Уколико се не дијагностикују инфективно обољење или проблеми у менаџменту на фарми свиња, оправдано се поставља сумња на исправност квалитета и сигурност хране за исхрану свиња. У храни за свиње често су присутни микотоксини у количини која има штетан утицај на производњу и репродукцију. У великом броју случајева проблеми се бележе у виду репродуктивних поремећаја, слабије конзумације хране или прираста. У Србији је утврђено присуство високих концентрација микотоксина, нарочито зearаленона. Сматра се да је присуство високих концентрација зearаленона у храни за свиње вероватно последица утицаја климатских фактора, што захтева константну контролу. У раду су приказани резултати теренских испитивања утицаја плесниве хране која садржи микотоксин зearаленон на појаву репродуктивних поремећаја код приоплодних категорија свиња (крмаче, назимице, нерастови).

Материјал за испитивање је обухватао четири фарме свиња, на којима су регистровани одређени репродуктивни поремећаји и здравствени проблеми приплодних јединки. У зависности од специфичности испитиваног случаја и доступног материјала, примењене су следеће методе испитивања: анамнестичка и клиничка испитивања, патоморфолошки преглед, стандардне лабораторијске методе за утврђивање присуства аеробних и анаеробних бактерија у узорцима репродуктивних органа угинулих јединки и микробиолошко испитивање узорака хране у циљу установљавања присуства плесни и микотоксина, методом танкослојне хроматографије.

Постигнути резултати испитивања указују да је у свим испитаним узорцима хране за свиње установљено присуство микотоксина зearаленона. Присуство овог микотоксина у храни се директно доводи у везу са утврђеним репродуктивним поремећајима код испитиваних категорија свиња (вулвовагинитиси, ендометритиси, повађање, инфертилоост). Репродукција представља основу интензивне свињарске производње. Присуство микотоксина у храни утиче на репродукцију и здравствено стање свиња и у одређеним условима може знатно нарушити процес производње.

КЉУЧНЕ РЕЧИ: микотоксини, поремећаји у репродукцији свиња, зearаленон

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