ACTIVITY OF SOY PHYTOESTROGENS AND ZEARALENONE ON MAMMALIAN REPRODUCTION: A REVIEW*

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The present review summarizes the findings about the mechanism and the effects of soy phytoestrogens and zearalenone acting. These substances commonly occur in plant feeds for animals. Owing to their estrogenic effects, they can disturb physiological functions in organism, mainly in the area of reproduction. Consequently, the negative impact of these substances is reflected in the overall economy of the livestock breeding.

endocrine disruptors; estrogenic effects; reproduction; gametes

INTRODUCTION

Endocrine disruptors are substances occurring in the environment which can interfere with hormonal regulation in the organism. These are chemical compounds of either natural or synthetic origin which can variously affect the organism and cause serious health problems. By origin they can be divided into two main groups – synthetically produced substances, which are released into the environment (phenols, pesticides, plastics, etc.), and natural substances. The most important natural endocrine disruptors are natural xenoestrogens (environmental estrogens), structurally similar to molecules of estrogen, which get into the organism through food, and especially phytoestrogens, substances naturally occurring in plants, and zearalenone with its derivates, a product of Fusarium mould (Rosselli et al., 2000; Burton, Wells, 2002).

The analogous mechanisms of action of xenoestrogens and endogenous estrogens on the human and animal organism suggest that these disruptors affect mainly the area of reproduction (Gallo et al., 1998; Rosselli et al., 2000). The activity of these substances, especially phenolic compounds, influences the development and function of the genital tract not only in many wild animals, but also in humans (Gallo et al., 1998; Hoyer, 2001; Burton, Wells, 2002).

Phytoestrogens

In their chemical structure and function, phytoestrogens are similar to the endogenous steroid hormone 17β-estradiol (Knight, Eden, 1995; Tham et al., 1998; Steensma, 2006), which determines their similar effects on the organism. Phytoestrogens have been identified in more than 300 plant species (Knight, Eden, 1995; Vrzáňová, Heresová, 2003), where their presence and amount serve the plant as a defence mechanism. In the case of stress conditions the concentration increases (Barrett, 1996) up to 15-fold (Kalač, Míka, 1997).

Based on their characteristics and structure, phytoestrogens can be divided into several groups, with a very important subgroup being isoflavonoids, which include soy phytoestrogens (Cornwell et al., 2004). The estrogenic activity of isoflavonoids is relatively low; however, after structural changes in the digestive tract it increases significantly (Kalač, Míka, 1997). The greatest amount of isoflavonoids is found in legumes, clovers, beans, and other plants rich in fibre, and their major producers are soybeans (Glycine max) and red clover (Trifolium pratense) (Moravcová, Kleinová, 2002; Burton, Wells, 2002).

The non-conjugated soy isoflavonoids – genistein and its structurally similar daidzein, also known as a glycosides genistin and daidzin, belong among the most studied and the most frequent phytoestrogens (Kalač, Míka, 1997; Kurzer, Xu, 1997). Other important isoflavonoids are the metabolic precursors of both the above-mentioned substances – formononetin and biochanin A, respectively.

Genistein belongs to the most effective and the most widely occurring isoflavone. It is unique in its ability to inhibit the group of enzymes of tyrosin protein kinasis and MAPK, which control the cell cycle and are important for meiotic maturation of oocytes, fertilization, and early stages of embryo development (Kinsley, 1997; Fritsche, Steinhart, 1999; Rosselli et al., 2000; McGinnis et al., 2011). It was recorded that genistein inhibits meiotic matura-

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Mechanism of action of phytoestrogens and zearalenone

Phytoestrogens and zearalenone can have both estrogenic and antiestrogenic effects, due to interaction with transport proteins, membrane and estrogen receptors or with other mechanisms of action. Biological functions at the cellular and molecular level – proliferation, differentiation and synthesis of proteins in the various target tissues are influenced in this way (Benassyag et al., 2002; Moravcová, Kleinová, 2002; Malekinejad et al., 2005).

The estrogenic effectiveness of phytoestrogens and ZEA is significantly lower (10⁻²–10⁻³) in comparison with that of estrogens. However, they can reach very high concentrations in the organism (100–1000 times higher than the concentrations of endoestrogens) and evoke a response as strong as endogenous estrogens at their physiological level in the organism (Benassyag et al., 2002). At a concentration of 1µM, the estrogenic strength of genistein is actually higher than in 17β-estradiol. At the same time, it is important to take into consideration the influence of the concentration of both phytoestrogens and endogenous estrogens, receptor position and the kind of target organ or cell (Tapiero et al., 2002). Considering the key role of estradiol in regulation of the reproductive function, mainly female genital organs are affected at the level of ovarian follicles. It was recorded that genistein and daidzein inhibit viability of follicular cells under in vitro conditions (Titmann et al., 2007; Galleati et al., 2010).

Among the most frequent and the most studied mechanisms of action of phytoestrogens and zearalenone belongs their ability to bind on α- and β-estrogen receptors (ER) (Whitten, Patisaul, 2001; Tapiero et al., 2002; Dusza, Ciereszko, 2006). When binding to estrogen receptors α- and β-, estrogenic compounds exert two basic mechanisms of action – genomic and non-genomic (Anderson et al., 1999). Natural endogenous estrogens act most often genomically through the estrogen receptors localized in the nucleus, after ligand binding (Anderson et al., 1999; Singleton, Sohaib, 2003; Li et al., 2004), resulting in the origin of homo- or heterodimer, which interacts with specific palindromic sequences of DNA (estrogen response elements – ERE) and creates a complex ligand – ERE, which is translocated to the nucleus. Therefore, the ER function as an active transcription factor and upregulates or downregulates gene expression of the target genes (Kuiper et al., 1998; Anderson, 1999; Benassyag et al., 2002; Singleton, Sohaib, 2003). Non-genomical action proceeds more quickly and consists in the ligand binding on an appropriate membrane receptor, by means of which a specific cell response is achieved – activation of transduction signal pathways and production of secondary messengers (e.g. cAMP) (Benassyag et al., 2002).

The binding ability of phytoestrogens to ER is closely associated with differences in the affinity of specific estrogen substances to the individual ER. Generally, phytoestrogens have, in comparison with 17β-estradiol, lower binding affinity to both types of ER (Kurzer, Xu, 1997; Benassyag et al., 2002), and significantly higher affinity to ERβ than to ERα.
Effects on the female reproductive cycle and juveniles

Phytoestrogens and ZEA can cause various developmental and morphological abnormalities in the female genital tract and changes in the estrous cycle and in sexual behaviour (Chang et al., 1979; D'Mello et al., 1997; Cassidy, Faughnan, 2000; Gromadzka et al., 2008).

Whereas ZEA affects the organism only negatively, phytoestrogens can play also a positive role (Opletal, Šimerda, 2006). Especially in human medicine their positive effects e.g. on bone metabolism and the prevalence of occurrence of osteoporosis (Kurzer, Xu, 1997; Matthai et al., 2003; Cornwell et al., 2004) as well as their anticancerogenic effects (Knight, Eden, 1995; Moravcová, Kleinoval, 2002; Duncan et al., 2003; Cornwell et al., 2004) are emphasized, and they are widely used as an alternative treatment for suppression of menopause symptoms (Kurzer, Xu, 1997; Romero et al., 2008). Unlike in the human organism, the effects of phytoestrogens in animals are more commonly considered as negative, significantly interfering mainly with reproduction (Adams, 1995b). Phytoestrogens can cause male and female infertility. Of all livestock animals, pigs are the most sensitive to the effects of these substances (Fink-Gremmels, Malekinejad, 2007).

Effects of phytoestrogens and zearalenone

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Effects on the female reproductive cycle and juveniles

Phytoestrogens in feed lead to changes in the estrous cycle and in female sexual behaviour and decrease the chance of becoming pregnant (Adams, 1995b; Opletal, Šimerda, 2006). In general, they have hyperestrogenic effects on the organism (Gajecka et al., 2011), leading to e.g. swelling and reddening of the vulva (Young et al., 1986; Obremski et al., 2003), and to the interference with the level of endogenous estrogens (Adams, 1995a; Opletal, Šimerda, 2006). Markers of estrogenization were also observed in the uterus and vagina (Chang et al., 1979; Whitten et al., 1995; Adams, 1996; Gallo et al., 1998). In ruminants irregular intervals between estrous, occurrence of false estrous, nymphomania, anestrous and decreased number of ovulations were observed (Adams, 1995b; Opletal, Šimerda, 2006). The length of the estrous cycle in rats and mice was prolonged depending on the dosage of phytoestrogens given (Gallo et al., 1998).

Phytoestrogens and ZEA also affect the organism of young animals, both in the prenatal and postnatal period. Transplacental transmission of phytoestrogens makes it possible to influence the metabolism of estrogens in the uterus (Cassidy, Faughnan, 2000), and in this way they can significantly interfere with the development of estrogen-sensitive tissues and therefore the sexual differentiation of foetuses (Adams, 1996) and neurohumoral direction of ovulation (Barret, 1996; Kurzer, Xu, 1997; Benassayag et al., 2002). Transplacental transmission of ZEA can also negatively affect embryos, which are especially sensitive 7–10 days after fertilization. Prenatal exposure to a mixture of isoflavones with a predominant share of daidzein and daidzin caused premature puberty in female rats and irregularities in the estrous cycle (e.g. prolongation) in adults (Romero et al., 2008). The toxicity of phytoestrogens and ZEA can be demonstrated by embryonic or later on foetal mortality or possibly by the birth of less viable or dead piglets (Nelson et al., 1994; Romero et al., 2008). Phytoestrogens and ZEA can postnatally affect the progeny via breast milk. The manifestations of toxicosis are similar to those in adult animals – edema and reddening of the vulva and nipples, enlargement of the mammary gland in immature females and males, hypertrophy of the vulva and uterus, possible prolapase of vagina or rectum (Biehl et al., 1993; Adams, 1996; Gallo et al., 1998; Burton, Wells, 2002). ZEA in young gilts negatively affects sexual behaviour, because the reflex of immobility during ovulation is not evoked (Kummer et al., 2001). In prepubertal gilts similar symptoms to those in the cycle of sows occur, but often at lower concentrations of ZEA. In gilts ZEA causes prolongation of the estrous cycle and persistence of corpus luteum (Edwards et al., 1987).
clover, which contains phytoestrogens, the development of ovarian cysts was observed (Adam s, 1995b; Op le ta l, Šim er da, 2006). The syndrome of polycystic ovaries is associated with anovulation, increased secretion of androgens and luteinizing hormone insulin. Abnormal metabolism of IGF was also observed (Knight, Eden, 1995). Phytoestrogens can cause the development of an enormous number of small and medium-sized follicles, and follicle atresia (Adam s, 1995a; Burton, Wells, 2002), in addition, accompanied by hypertrophy of granulose cells in mice (Mak arevi c h et al., 1997). In mice ovaries a lower number of Graafian follicles and the absence of corpus luteum were observed. In sheep grazing on clover with isoflavone content, small numbers of follicles mostly without antra and subsequent early atresia were developed (Burton, Wells, 2002).

Long-term exposure to low concentrations of ZEA leads to impairment of proliferative activity of the follicle granulose cells and connective tissues of the ovarian stroma in prepubertal gilts (Gajecka et al., 2011). In gilts, ZEA caused follicle atresia and apoptotic changes in granulose cells (Obremski et al., 2003). In pregnant and lactating sows exposed to the action of ZEA, the number of follicles with normal morphology was decreased. This reduction of reserves of the early stages of follicles can cause the premature depletion of healthy follicles and shorten the reproductive period in sows (Schoevers et al., 2012). The derivates of ZEA, a- and β-zearenalol, inhibit progesterone synthesis in porcine granulose cells (Tiemann et al., 2003).

Effects on oocytes

The effects of phytoestrogens and ZEA are also manifested at the level of oocytes. The degree of impact on the morphology of oocytes, markers of meiotic and developmental competence, varies in specific estrogenic substances depending on the dose, estrogenic effectiveness, animal species or the particular cultivation system. Generally, compared to phytoestrogens, the effects of ZEA are more prominent and marked at lower concentrations (Adam s, 1995a; Op le ta l, Šim er da, 2006). Genistein, due to its effects, is a very often monitored phytoestrogen which affects, a.o., meiotic maturation and early embryogenesis in cattle, rabbits, and pigs. In bovine oocytes genistein does not cause blockade of Germinal Vesicle Break-Down (GVBD), but the meiotic maturation is altered and in a statistically significant portion of oocytes, meiotic maturation stops stage or prometaphase I in the late diakinesis (Makarevich et al., 1997). In pigs and mice, in vitro experiments proved the ability of genistein to block, in a dose-dependent manner, GVBD at meiotic maturation of oocytes (Jung et al., 1993; Vodková et al., 2008). Contrary to this study, Makarevich et al. (1997) recorded that in low concentrations genistein stimulated nuclear maturation of pig oocytes.

Jung et al. (1993) explain the effects of genistein ability to inhibit tyrosine protein kinases (TPK), whereas some studies suggest that the effects of phytoestrogens are not dependent only on this inhibition. Van Cauwenberg, Alexandre (2000) tested the effects of another inhibitor of TPK – lavendustin A – and concluded that resumption of meiosis in mice oocytes was not inhibited. Simultaneously, Vodková et al. (2008) recorded, although at higher concentrations and with substantially lower efficiency, similar inhibitory effects of genistein also in genistin – a phytoestrogen, which does not inhibit the activity of TPK. The results of these experiments suggest that inhibition of meiotic maturation of oocytes is also achieved in other ways than just by inhibition of the activity of TPK. This fact is supported by the results of in vitro experiments which describe the ability to inhibit meiotic maturation in pig oocytes at the level of GVBD, also by 17β-estradiol (Bing et al., 2001; Li et al., 2004).

On the other hand, low doses of another soy phytoestrogen, daidzein, did not affect nuclear maturation, rapidness of fertilization, or even the number of fertilized oocytes like in the subsequent embryonal development in pigs (the part of the embryos at the blastocyst stage or the blastomer/blastocyst ratio). However, decrease of progesterone synthesis by cumulus cells was determined (Galeata et al., 2010).

The effects of ZEA on pig oocytes cultivated in vitro caused a decrease in the number of oocytes capable of undergoing GVBD (Schoevers et al., 2012). The cultivation of porcine cumulus oocyte complex in the presence of ZEA caused a significant decrease in the number of oocytes which achieved MII stage after 44 h. An increased incidence of nucleus abnormales in oocytes, which leads to aneuploidy, was also observed (Malekinejad et al., 2007). The defects in spindle apparatus and aneuploidies in porcine oocytes are most frequently induced by bivalent separation failure or by premature segregation of sister chromatids, and they have negative effects on the subsequent fertilization and early embryonal development (Alm et al., 2002, 2006; Malekinejad et al., 2007; Hornak et al., 2011). At the cytoplasmic level ZEA induced vacuolization of ooplasm, which is considered a manifestation of autophagia (Malekinejad et al., 2007; Schoevers et al., 2012). In pig oocytes cultivated in IVM medium supplemented with ZEA (1000 µg/l) the impairment of zona pellucida sperm penetration after in vitro fertilization was observed (Sambuu et al., 2011).

Phytoestrogens also inhibit in vitro expansion of cumulus cells surrounding the oocyte. Tirone et al. (1997) determined that a concentration of 60µM genistein in a cultivation medium completely blocked the FSH and EGF stimulation effect on expansion in
mice oocytes. The same effect in pigs, at a concentration of 148µM, was confirmed by Vodkova et al. (2008). The ability to suppress the expansion of cumulus cells of porcine oocytes by 17β-estradiol was also demonstrated (Li et al., 2004).

**Effect on the male**

Although most of the effects described concerned females, the effects of environmental estrogens on males were demonstrated as well. ZEA in prepubertal boars caused a reduction of libido and a decrease of testosterone level in the plasma; however, the development of sexual behaviour was not affected. In young castrates it can result in the hypertrophy of the mammary gland or edema of the prepuce. In boars ZEA can cause reduced mass of the testicles. Long-term exposure to a low concentration of ZEA can cause the reduction of epididymis and seminal vesicles development in young males (Bergen et al., 1981; Tsakmakidis et al., 2006; Benzoni et al., 2008; Minervini, Dell’Aquila, 2008; Kanora, Maes, 2009; Bhat et al., 2010).

It has been demonstrated that phytoestrogens and ZEA also affect male gametes – spermatozoa. The action of phytoestrogens during incubation results in a decrease in the induction of acrosomal reaction in sperm and inhibition of the binding of sperm to the zona pellucida, whereas in bovine spermatozoa motility, viability and capacitation are not affected (Hägeler et al., 1998; Hinsch et al., 2000; Menezel et al., 2007). Studies by Mohamed et al. (2011) showed that the effects of phytoestrogen genistein on the capacitation of porcine sperm are comparable to those of endogenous estrogens. On the contrary, in human sperm the phytoestrogen genistein significantly accelerates capacitation and acrosome loss, which can lead to the inability to fertilize the oocyte (Fraser et al., 2006; Cederroth et al., 2010). Genistein, in comparison to 17β-estradiol, has stronger effects on stimulation of acrosomal reaction in pig sperm (Mohamed et al., 2011). On the other hand, Furuya et al. (1992) and Park et al. (2011) mention that the addition of genistein strongly inhibits capacitation in mice sperm.

The above-mentioned authors agree that genistein has no effect on motility; however, Kumi-Diaka, Towns ed (2001) recorded that higher concentrations of genistein result in significant interference with the number of motile spermatozoa, whereas morphology is not affected. Park et al. (2011) recorded that genistein (100µM in medium) decreased the reactivity of murine sperm acrosome after short-term exposure (15 min) contrary to prolonged exposure (30 min), which had a stimulative effect. In the case of bovine sperm, after 25 min of genistein exposure (7.4µM in medium), acrosome reaction was inhibited (Hinsch et al., 2000).

ZEA can negatively affect the stability of chromatin and the motility of boar spermatozoa (Benzoni et al., 2008) as well as damage the plasmatic membrane of sperm, and reduce the percentage of sperm with reactive acrosome (Tsakmakidis et al., 2006).

The effect of ZEA on qualitative parameters for IVF systems was examined by a long-term (one week) incubation of boar spermatozoa with this contaminant, and no significant changes in penetration as a whole, or in monospermy and polyspermy oocyte fertilization, were recorded. In the same way, there were no negative effects on the cleavage and development of blastocystes after in vitro fertilization. After three weeks of incubation the motility, viability, and plasma membrane integrity were significantly reduced compared with non-stored sperm (Sammuel et al., 2013).

**CONCLUSION**

Phytoestrogens and zearalenone affect the reproduction of animals at the level of changes in the function of the reproductive organs, even at the level of gametes – oocytes and spermatozoa. Spermatozoa can be affected by these estrogen substances not only in male gonads during their own formation and maturation, but also in the female genital tract after ejaculation. The effects of these substances in the form of impact on reproductive efficiency are especially prominent in pigs, which are often exposed to phytoestrogens and zearalenone in feedstuffs.

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