Effects of phyto- and mycoestrogens in domestic animals

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Abstract

Phyto- and mycoestrogens are compounds of plant and fungal origin with estrogenic effects in animals. A wide range of phytoestrogens have been characterized, while so far only one mycoestrogen of importance have been isolated. Phytoestrogens are particularly widespread in legumes like soy and clover, but other plants like grain and alfalfa also contain phytoestrogens. The presence of estrogenic substances in pasture plants have been associated with decreased fertility and oestrogen-like effects, particularly in the female reproductive organs, in grazing sheep and cattle. The condition has been named clover disease, since it was first observed in sheep grazing on clover pastures. Both a permanent strong decrease in fertility and temporary infertility has been observed in ewes. The phytoestrogens affect the hypothalamus-pituitary axis and the feed-back regulation of the hormonal regulation in sheep. Little information is available on the effects of phytoestrogens on other production animals. The mycoestrogen zearalenone is produced mainly by Fusarium fungi. Zearalenone is commonly found in cereals, particularly on maize. Fusarium species is also commonly found in grass species. Pigs are very sensitive to zearalenone. Reproductive disturbances and alterations in female reproductive organs in pigs have been reported both from farms and experiments where the feed has contained zearalenone. Also sheep seem to be sensitive to zearalenone, but few studies are available for this species, while other species seems to be more resistant towards zearalenone.

Background

Phyto- and mycoestrogens are compounds of plant, respectively fungal origin with estrogenic activity in humans and animals. There is a wide range of phytoestrogens described, including isoflavones such as genistein, formononetin, daidzein and biochanin A, coumestans and lignans. Only a few of them have a documented estrogenic effect in production animals. Phytoestrogens are widespread in legume plants and the most important sources of phytoestrogens in...
animal feed are probably soy, some clover species and alfalfa. Species like white clover and alfalfa normally contain low concentrations of phytoestrogens, but the concentrations may increase to significant amounts if the plants are damaged by insects or fungi (1,2). Cereals, important in food and feed, contain lignans. Lignans are also found in particularly high concentrations in flaxseed (2). Zearalenone, produced by fungi of the Fusarium genus, is the only mycoestrogen of importance. The fungi grow on cereals like wheat and maize and on grass species and legumes.

**Phytoestrogens**

*Effects on grazing animals*

The reproduction was dramatically reduced in grazing sheep in the southwestern region of Australia in the 40s and fell below 30% of the normal reproduction rate. The herds also had a high incidence of red and swollen vulvas. The observed condition was named clover disease since the symptoms were observed in animals grazing on clover pastures (3). No estrogenic effects were observed in cattle grazing on the same fields, and it was assumed that cattle were less sensitive for the red clover disease than sheep. Later, similar effects have also been reported from cattle given alfalfa (4) or red clover silage (5).

*Metabolism*

The metabolism of phytoestrogens in production animals has been reviewed by Lundh (6). After an initial adaptation period of 3-7 days, biochanin A and genistein are generally degraded to the non-estrogenic p-ethyl phenol in the rumen of sheep and cattle (fig. 1). Formononetin and its metabolite daidzein are both transformed to equol in the rumen (fig. 1). This compound, which is extensively absorbed from the gastrointestinal tract (GI), has more potent oestrogen-like effects than its maternal compounds. The absorption of equol from the GI is similar in cattle and sheep and equol is to a large extent conjugated in the epithelial layer of the GI during the process of being absorbed. The conjugation capacity is higher in sheep than cattle. This does not fit with the proposed hypothesis that cattle are less sensitive to phytoestrogens than sheep due to differences in detoxification capacity. A lower fraction of formononetin and daidzein is converted to equol in pigs than in ruminants (7) but the levels of unconjugated equol is similar levels to the levels found in ruminants fed comparable amounts of phytoestrogens (6,8).

*Mechanism*

The estrogenic effects of phytoestrogens are mediated through binding with the oestrogen receptors. Equol has a higher affinity to the receptors than other isoflavones (9). Coumestrol has higher affinity to the oestrogen receptors than the
isoflavones, but are normally present in much lower concentrations. However, the concentrations of coumestrol in white clover and alfalfa may increase significantly if the plants are damaged, for example by insects or fungi (2).

**Clinical effects in ruminants**

Occurrence of clover disease in ruminants have been reported both from several occasions from farms and from experimental work where animals have been given clover, alfalfa or soy (reviews in 2, 10). The clinical effects on ruminants have been divided into temporary and permanent effects.

**Permanent effects**

A condition that has been called permanent infertility has been reported from ewes after 5-7 months of grazing on estrogenic feed. The term is not accurate since the condition is a permanent strong decrease in fertility, but not infertility. The condition has been studied in ewes exposed to estrogenic pastures for 5-7 months per year for three or more years. After exposure, the animals were given non-estrogenic feed for 6-18 months to allow recovery from reversible effects.

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**Figure 1.** Metabolism of some phytoestrogens in ruminants.
The infertility results primarily from a failure of ewes to conceive due to impaired transport of spermatozoa through the cervix (11). Other parameters such as twinning rate, embryo mortality, duration of breeding season etc are only slightly affected. The impaired transport of spermatozoa through the cervix is accompanied by a variety of morphological changes, leading to a cervix that resembles the uterus (10,11). These changes described from adult ewes exposed to phytoestrogens are similar to effects in other species exposed to oestrogen or estrogenic substances during organogenesis (10).

**Temporary effects**

A range of reversible clinical changes associated with exposure to phytoestrogens has been described from grazing ruminants, including clinical changes like enlarged mammary glands, milk production in non-milking animals, swelling of vulva, reddening of the vaginal mucosa membrane, decreased or no ovulation, decreased twinning rate and histological changes in the cervix, mucosa and muscular layers in the mucosa and cellular hypertrophy in the pituitary, adrenal, and thyroid glands. These changes resemble the effects of oestrogen in ewes. The effects on grazing ewes in South-Western Australia have been most extensively studied, but similar clinical findings have been reported from both ewes, cows and heifers exposed to phytoestrogens in the feed as well as in heifers given clover under experimental conditions (5,12). The ovulation rate in both cattle and sheep may decrease with no visible external sign. A low reduction in the fertility is difficult to detect unless the herd is carefully examined due to high background variation in the reproduction rate. The effects of clover may therefore go undetected in herds (13). Efforts such as the development of new varieties of clover low in phytoestrogens and increased proportion of grass in the pastures have been put in place to restrict the exposure of grazing animals to phytoestrogens, but phytoestrogens were still estimated to cause a 7-10% decrease in sheep reproduction in Australia (13).

**Effects on hormone levels**

The estrogenic effects in the reproductive organs are probably caused by alterations in the hormone levels in the animals. In addition to the estrogenic effects in reproductive organs, phytoestrogens affect the hormone levels in ewes. Phytoestrogens bind to the oestrogen receptors in the hypothalamus and pituitary glands of ewes, indicating that the compounds can interfere with the normal feed-back loop regulating the hormonal axis (14). The ability of phytoestrogens to interfere with the hormonal feed-back regulation have been further studied in a series of experiment where genistein was infused into the intracerebroventricular region of the brain. This treatment increased the level of LH mRNA as well as ERα mRNA in the pituitary gland in anoestrus ewes, showing that the phytoestrogens are able to activate the genes regulated by the ER in the brain of the ewe (15). Direct infusion of genistein into the brain also decreased both plasma LH concentrations and LH pulse frequency and increased the plasma concentration of
prolactin (16). Ewes treated in this way showed a biphasic response to infusion of genistein; an initial decrease in plasma LH concentration during the first six hours, followed by an increase LH (17). Even serum level of thyroid hormone T3 and the number of ER in the thyroid gland was increased after infusion of genistein into the brain (18). The effects of phytoestrogens in these experiments mimic the effects of estradiol in the same experimental systems. In ewes fed alfalfa and coumestrol, the amplitudes of LH pulses were reduced during breeding season, but not during anoestrus season (19).

**Other species**

Limited information is available for non-ruminant species such as pigs. Pigs are sensitive to the mycoestrogen zearalenone and care should therefore be taken if plants containing phytoestrogens are mixed into pig feed. The possibility of using estrogenic feed as a “natural growth promoter” in pigs has been reported with mixed results. Daidzein supplement increased the birth weight of male offspring significantly. The increase in birth weight was accompanied by a downregulation of ER in the hypothalamus and an upregulation of insulin-like growth factor 1 receptor in skeletal muscle (20). No effect on growth performance or meat quality where however found in male pigs given phytoestrogens in the feed (7). Phytoestrogens may act agonistic at low concentrations of estradiol and antagonistic at high estradiol concentrations, promoting growth in males. Less information about the effects of phytoestrogens on pig reproduction performance is available, but pigs are affected by soy.

**Zearalenone**

Zearalenone is a mycotoxin produced by *Fusarium spp*. *Fusarium* species are commonly found on cereals, including major feed sources such as wheat and maize, as well as on grass species.

**Metabolism**

The metabolism of zearalenone has been reviewed (21,22). Zearalenone is reduced *in vivo* to form α- and β-zearalenol. The proportion of the α and β isomers formed varies between species and a comparably higher proportion of the α-isomer is formed in pigs than in most other species studied. β-zearalenol has a stronger estrogenic potential than zearalenone, while α-zearalenol is less estrogenic. In addition, both zearalenols and the maternal compounds are extensively glucuronide conjugated in most species. Furthermore, the zearalenols can be further reduced to form zearalanols. α-zearalanol is also called zeranol and is being used as a growth promoter in cattle due to its strong estrogenic effects. The formation of zearalanol has been demonstrated in sheep (23) and cattle (24), while only trace amount of zearalanol was found in pigs (25).
**Effects of zearalenone**

The effects of zearalenone on animals were reviewed by JECFA (22) and EFSA (26). Pig is the species most sensitive to zearalenone. There is also indications that sheep is sensitive to zearalenone, while cattle seem to be less vulnerable.

**Pigs**

A range of clinical symptoms typical for estrogenic substances have been observed in pigs exposed to zearalenone, including swelling of uterus, delayed puberty, increased cycle duration, increased plasma progesterone, prolonged maintenance of corpora lutea and decreased litter size (reviews in 22, 26). Growing pre-pubertal female pigs are most sensitive for zearalenone and lowest observed effect levels (LOEL) in pigs have been reported from 0.05 mg/kg feed – more than 400 mg/kg feed. Boars seem to be more resistant to zearalenone, but precocious spermatogenesis and damage to germinal epithelium in young boars and decreased libido in adult boars have been reported. Data on the reproductive performance of pigs exposed *in utero* is limited.

**Sheep**

Sheep is also sensitive to zearalenone and typical signs of estrogenism have been reported from ewes exposed to zearalenone in the diet. Reduced ovulation rate was observed in ewes given 3 mg/kg feed, while increasing levels of zearalenone lead to range of effects including increased duration of oestrus, increased uterine, liver and ovary weight as well as reduced fertilisation rate (27).

**Other species of production animals**

A few studies of the effects of zearalenone in poultry and cattle have also been reported. Estrogenic effects have only been reported when these animals have been exposed to very high levels of zearalenone (from 50 mg/kg feed) and are not likely to occur if the animals are not given heavily mould infected feed. Data on the reproductive performance is however scarce.

**Conclusions**

Both phyto- and mycoestrogens have a potential disrupt the endocrine balance and cause reproductive disturbances in domestic animals under farm conditions. Measures to control these substances in feed and pastures are necessary, at least in certain parts of the world. Measures in use include the development of new varieties of plants containing lower amounts of phytoestrogens, control of the amount of phytoestrogen-containing plant species in feed and the introduction of other plant species in pastures. The presence of phyto- and mycoestrogens in certain plant species should be taken into consideration when vegetable feed is being used to new species, such as in aquaculture.
References


