IMPACTS OF PHYTOESTROGENS ON LIVESTOCK PRODUCTION: A REVIEW

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SUMMARY

Phytoestrogens, polyphenolic compounds, are one of the major plant secondary metabolites found abundantly in animal diets. However, these phytochemicals play initial important roles in plant growth, development and maintenance. They also have many biological roles in animal body, affecting their productivity. The richest sources of phytoestrogens in animal diets are legumes, particularly, soybeans and clover. These plants often comprise substantial part of animal feed, thus presenting a potential source of phytoestrogens. The interest in these compounds was started earlier, over 50 years ago, when they have been known to affect fertility of an Australian sheep herd, resulting in an economic loss. The putative effects of phytoestrogens are based on their structural similarity to the mammalian estrogen 17β-estradiol (E2) and thus their potency to bind with mammalian estrogen receptors. More attention has been paid to these metabolites since they can act as estrogen agonists or antagonists which make their effects are not expected. The estrogenic activity of phytoestrogens depends on many factors such as chemical structure of the compound, bioavailability, animal specie, responsive tissue and its estrogen receptor (ER) sub-type (ERα or ERβ) and metabolites resulting from their fermentation and digestion. Reports showed that phytoestrogens could affect animal fertility and growth, quality of animal products and human health. This review illustrates the impacts of phytoestrogens on the livestock production system, focusing on physiology of reproduction, as well as the knowledge obtained from research in laboratory and field scales.

Keywords: Phytoestrogens, estrogenic activity, reproduction, farm animals

INTRODUCTION

Phytoestrogens are one of many secondary metabolites producing by plants during photosynthesis, they primarily involve in host-microbe interactions, defense mechanism (Graham, 1999). Chemically, phytoestrogens include more than 100 molecules those are belonging to different chemical classes such as isoflavones, flavones, coumestans, stilbenes, and lignans (Wochlawek-Potocka et al., 2013). Effects of phytoestrogens on livestock production have been detected earlier. In the 1940s, it was noticed for the first time that red clover (a plant which is rich in the phytoestrogen coumestrol) pastures had negative effects on the fertility and fecundity of grazing sheep due to its hyperestrogenic activity, which is so-called clover disease (Bradem et al. 1967; Yildiz, 2005). About 20 years later, similar observation has been noted in cows that had fertility disturbances resulting from periods of feeding with red clover (Kallela et al., 1984). Since this time interests with phytoestrogens have been increased especially when their effects on human and animal health and reproduction were found to be inconsistent. In farm animals, consumption of phytoestrogen rich-diets disturb hormonal balance in animal body leading to silent heat (Zduńczyk et al., 2005), progesterone deficiency (Piotrowska et al., 2006), embryonic loss (Wochlawek-Potocka et al., 2005) and low semen quality (Glover and Assinder, 2006). On the other hand, inclusion of phytoestrogenic plant in diets of growing animals showed beneficial effects by stimulating weight gain and increasing the growth rate (Trenkle and Borroughs, 1978). Additionally, in human, a high intake of dietary phytoestrogens is suggested to be associated with a reduced incidence of breast and prostate cancer, cardiovascular diseases and osteoporosis (Cornwell et al., 2004) in populations whose diet contains an abundance of soy products, when compared with typical Western diets. These actions may be due to the antiestrogenic effects of phytoestrogens in human (Adlercreutz et al., 1995) and/or their antioxidant activity (Siow and Mann, 2010). Previous information about the biological role of phytoestrogen in humans and animals makes them an interesting material for more research discovering their negative and positive effects in the mammals.
Chemical Structure of Phytoestrogens

Phytoestrogens acquired their name as they have a chemical structure similar to that of mammalian estrogens which enables them to affect different mammalian estrogen responsive tissues. Most of phytoestrogens are polyphenolic compounds belonging to two main groups: flavonoids and non-flavonoids known as lignans (such as secoisolariciresinol enterolactone and enterodiol). The majority of phytoestrogens belong to the flavonoid group which is divided to three sub-groups: 1) isoflavones (genistein, daidzein, glicetin, formononetin, biochanin A); 2) flavones (quercetin and kaempferol); 3) coumestans as cumestrol (Strauss et al., 1998). Additionally, there is also estilbenes (resveratrol) which is present in plants or in their seeds; also, some fungi can synthesize mycoestrogens zearalenone and its metabolites α and β-zearalenol (Moutsatsou, 2007). Naturally, most of phytoestrogens are conjugated with carbohydrate derivatives as glucose, this form is known as glycoside phytoestrogen. This conjugated form of phytoestrogens is biologically inactive when consumed. Therefore, the bioavailability of these...
compounds is controlled by the activity and by the type of both internal gastrointestinal and gut microflora enzymes in each animal specie (Patisaul and Jefferson, 2010).

**Binding Affinity of Phytoestrogens to Mammalian Estrogen Receptors**

In mammals, there are two sub-types of estrogen receptors (ERs) known as ERα and ERβ. ERα is expressed predominantly in endometrium, breast cancer cells, ovarian stroma cells, efferent duct epithelium, and the hypothalamus, whereas ERβ is expressed in kidney, brain, bone, heart, lungs, intestinal mucosa, prostate, and endothelial cells. Phytoestrogens exert their effects primarily through binding to the estrogen receptor (ER) sub-types α or β (ERα or ERβ). Phytoestrogens have lower affinity for ERs than estradiol (E₂), and most of them exhibit a higher affinity for ERβ than for ERα by approximately 30 fold (Whitten and Naftolin, 1998; Pettersson and Gustafsson, 2001; Turner et al., 2007). Generally, the affinity of different phytoestrogens to the E₂ sub-types was studied using receptor binding affinity test. The ranking of the estrogenic potency of phytoestrogens for both ER sub-types in the transactivation assay is different; that is, E₂ > zearalenone = coumestrol > genistein > daidzein > apigenin = phloretin > biochanin A = kaempferol = naringenin > formononetin = ipriflavone = quercetin = chrysirin for ERα and E₂ > genistein = coumestrol > zearalenone > daidzein > biochanin A = apigenin = kaempferol = naringenin > phloretin = quercetin = ipriflavone = formononetin = chrysirin for ERβ (Kuiper et al., 1998).

The affinity of different phytoestrogens to ERs depends on many chemical structure elements including the presence of a phenolic ring that is indispensable for binding to estrogen receptor, low molecular weight near to that of estrogens (MW=272), distance between two hydroxyl groups at the isoflavonones nucleus similar to that occurring in estradiol and optimal hydroxylation pattern (Yildiz, 2005). For example, genistein (4',5,7-trihydroxyisoflavone) has a higher binding affinity for the ERs than daidzein (4',7-dihydroxyisoflavone), the differential potency between genistein and daidzein could be ascribed to the presence of the 5-hydroxyl group of genistein which provides more identical chemical similarity to mammalian E₂ (Bickoff, 1961; Shutt and Cox, 1972). Also the binding affinity of phytoestrogens could be attenuated by methylation of hydroxyl groups of isoflavones, instantly, the methoxy derivative of daidzein or formononetin has less estrogenic activity compared to daidzein (Bickoff, 1961). According to the previous facts, phytoestrogens are known to have a weak estrogenicity compared with endogenous estradiol (E₂). This is due to the lower affinity of phytoestrogens for ERs and the higher concentrations of isoflavones required to induce transcriptional activity of E₂ (10³ fold than E₂). However, there are other aspects controlling their effects in vivo. Free circulating phytoestrogens is more than 50% versus 4% for E₂, also the magnitude of concentration of phytoestrogen is one order higher than E₂ (ng/ml versus pg/ml) (Retana-Márquez et al., 2012). This greater bioavailability to ERs may explain why phytoestrogens have in vivo potential effects. Interestingly, the transcriptional activity of phytoestrogens is not the only mechanism by which they disturb hormonal balance, but also they have non-genomic effects by modulating the concentration of endogenous estrogens by binding or inactivating some enzymes related to estrogen biosynthesis, such as P450 aromatase, 5α-reductase, 17β-hydroxysteroid dehydrogenase (17β-OHDD), topoisomerases, and tyrosine kinases (Watson et al., 2007; de Souza et al., 2010).

**Metabolism of Phytoestrogens in Ruminants**

Dietary consumed phytoestrogens are exposed to many chemical reactions in the elementary tract. Hydrolysis of the conjugation between phytoestrogen and its carbohydrate moiety producing aglycone phytoestrogen (biologically active form) is the most important chemical reaction (Fig. 2). This process takes place in rumen and/or intestine by the action of the β-glycosidase enzymes producing by gut microorganisms or intestinal epithelial glands, additionally it could be induced by the gastric hydrochloric acid (Lindsay and Kelly, 1970). However, re-conjugation of free phytoestrogens could be occurred in the epithelial cells of the gastrointestinal tract during the absorption. Following absorption, most of the free circulating phytoestrogens are re-conjugated mainly to glucuronic acid and to a lesser degree to sulphuric acid (Lundh, 1995; Branca and Lorenzetti, 2005). Also, they undergo further chemical reactions including demethylation, methylation, hydroxylation, chlorination, iodination, and nitration. In ruminants, phytoestrogens, mainly isoflavones, are bio-converted by the action of ruminal microflora to other metabolites. Both biochanin A and genistein are transformed to the p-ethyl phenol, whereas formononint and daidzein are transformed to more potent estrogenic compound equol (Fig. 3; Benassyay et al., 2002; D'Alessandro et al., 2005).

**Sources of Phytoestrogens in Animal Feed Stuffs**

There are reports indicating that most of the legumes commonly used for feeding livestock contain from 5 to 25 % phytoestrogens. The concentrations of phytoestrogens in plants vary depending on many environmental factors, such as: temperature, humidity, light, age of the plant, amount of fertilizer, and pathogens (Adams, 1995). The plant family most abundant in phytoestrogens is Leguminosae. Isoflavones as daidzein, genistein and glyceitin are the predominant in soybeans. Biochanin A and
formonontin are isolated from clover. Lignans are the main phytoestrogens in the flaxseeds, while coumestans are found in germs; for example beans sprouts and also in fodder crops (Price and Fenwick, 1985).

Figure (2): Conjugated and free forms of different phytoestrogens (A) and transformation of genistin (B) from glycoside form (a, biologically inactive) into aglycone form (b, biologically active) by the hydrolysis action of β-glycosidase enzyme.
**Impacts on Animal Productivity:**

**Reproduction in Females**

Because endogenous estrogen receptors are widely distributed in the entire reproductive axis (hypothalamus, pituitary, ovary and reproductive tract), it is expected that phytoestrogens can alter reproduction of females on different levels. Many studies have reported that phytoestrogens in legumes may act as antagonists or agonists of endogenous estrogen (Usui et al., 2002). Phytoestrogens generally inhibit endogenous estrogens production, leading to disturbances in the follicular development and lack of the occurrence of estrus (Rosselli et al., 2000). Further, it has been suggested that phytoestrogen compounds may disturb estrus and ovulation through their effects on the central nervous system (Wocławek-Potocka et al., 2005). So for example genistein, an isoflavone phytoestrogen, can stimulate the expression of immunoreactive ERα in the pituitary LH-cells, but not in the FSH-cells, and changes the endocrine activity of LH-producing cells of anestrous ewes (Polkowska et al., 2004).

In this context, a correlation was detected between blood plasma concentration of isoflavones and the incidence of silent heat in cows, where, Zdueczyk et al. (2005) compared between three herds of cows those had different levels of blood plasma of total isoflavones. It was found that the highest concentration of blood plasma total isoflavones (4.9 ± 0.5 μmol/l vs. 2.6 ± 0.9 μmol/l and 1.0 ± 0.3 μmol/l) was detected in the herd with the highest incidence of silent heat (40.9% vs. 35.0% and 7.0%). In ewes, Hashem and Sallam (2012) suggested that feeding Egyptian clover to seasonal anestrous ewes around period of mating and early pregnancy might implicate with other environmental factors leading to an increase in silent heat and decrease in conception rate following estrous synchronization.

In heifers, higher concentrations of active metabolites of phytoestrogens in the corpus luteum (CL) tissues collected from heifers receiving soy diet have been detected compared to animals fed with standard fodder. These high concentrations of phytoestrogen metabolites were associated with lower concentrations of P₄, leading to higher early pregnancy losses. Phytoestrogens and its metabolites could also affect establishment of pregnancy during early stages by increasing PGF₂α/PGE₂ ratio (Piotrowska et al., 2006). During early pregnancy, an increased concentration of PGE, and thus lower PGF₂α/PGE₂ ratio, is required which helps in blood vessels relaxation and improves blood flow to the uterus, preparing it for proper embryo implantation (Okuda et al., 2002). More recent study by Cools et al. (2014) showed that dairy cows fed soybean meal throughout three estrous cycles had reduced area occupied by steroidogenic and endothelial cells in the luteal biopsies compared to those fed rapeseed meal (low phytoestrogenic seeds). The reduction in the steroidogenic and endothelial cells in soybean fed cows was negatively correlated with blood concentrations of equol and glycitein.

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**Figure (3):**

Metabolism of some isoflavones in ruminants.
Interestingly, however, the estrogen-like effects of phytoestrogens and their metabolites during early pregnancy are not desirable. In non-pregnant cows, these effects may help in resumption of cyclicity and ovulation in post-partum cows (Woelawek-Potocka et al., 2005). During luteolysis, stimulation of PGF$_{2a}$ secretion by estrogen-like substances accelerates the positive feedback loop between PGF$_{2a}$ and other regulators of luteolysis, such as, for example, oxytocin (Goff, 2004) or TNFα (Skarzyński et al., 2009), leading to the pulsatile PGF$_{2a}$ output from the endometrium during luteolysis in ruminants (McCracken et al., 1999).

**Reproduction in Males**

The effects of phytoestrogens on male fertility has increased in recent years as it has been demonstrated that estrogens play an important role in the male reproductive system (Rochira et al., 2005). Although there are only few reports, it has been shown that phytoestrogens also cause reproductive disruption in males. Some phytoestrogen exert an inhibitory effect on steroidogenic enzymes (Strauss et al., 1998). For example, isoflavonoids and lignans inhibit 5α-reductase activity, thereby reducing the conversion of testosterone to the active form DHT (Evans et al., 1995). A high phytoestrogen diet in male rats can also block spermatogenesis, induce germ cell apoptosis (Assinder et al., 2007), and decrease the expression of ERβ in the cauda epididymis as well as increase lipo-peroxidation in epididymal sperm. These effects are possible mediated by disruption of the steroid regulation of the epididymis, resulting in decreased quality of sperm, and thereby reducing fecundity (Glover and Assinder, 2006). On the other hand, chronic dietary exposure to genistein in combination with vinclozoline (a fungicide considered an endocrine disruptor) reduces sperm count and motility, litter size and increased post-implantation loss (Eustache et al., 2009). In cryopreserved bull spermatozoa, genistein can affect a protein tyrosine phosphorylation-independent signal transduction pathway that is involved in sperm capacitation, the acrosome reaction and sperm-pellucid zone binding, thereby decreasing 40-50% the acrosome reaction (Menzel et al., 2007). Besides, mouse and human spermatozoa exposed in vitro to different levels of genistein and daidzein, alone or in combination, accelerate capacitation (Adeoya-Osiguwa et al., 2003) and acrosome loss, which may possibly impair fertility (Fraser et al., 2006). In bovines, ingestion of fodder with high quantities of coumestrol causes glandular metaplasia in both prostate and bulbourethral glands (Lenis et al., 2010), gynecomastia and even galactorrhea (Romero et al., 1997).

**Animal Growth**

It is interesting to know that there are evidences on beneficial anabolic actions of the phytoestrogenic plants observed in the growing animals, but not unequivocally established. Phytoestrogens showed beneficial effects when fed to monogastric or ruminant animals, stimulating weight gains and increasing the growth rate (Trenkle and Borroughs, 1978). Previous works indicated that growing rabbits fed on commercial diets supplemented with 0.1 g/kg of a subterranean clover extract compared to a group supplemented with 1 mg/kg of clenbuterol (β-agonist) showed very close average daily gain values and meat percentages (Pace et al., 1994). Also, Moorby et al. (2004) found that red clover-fed lambs had a significantly higher live weight gain than grass-fed lambs with no substantial differences on carcass quality. This enhancement was associated with higher concentrations of growth hormone and insulin like growth factor-1 in red clover-fed lambs compared to grass-fed lambs. Same results were obtained by Pace et al. (2006) who reported that male and female lambs fed subterranean clover selected for low formononetin content had a significant higher body weight gain compared with those fed Italian ryegrass (non-estrogenic roughage).

**CONCLUSION**

Despite the abundant researches that carried out discussing the biological roles of phytoestrogens in humans and farm animal, the paradox effects of phytoestrogens still present a good material for further studies. Specially, the effects of these compounds depend on multifactor such the chemical form of the phytoestrogen, the route of administration, the metabolism, the physiological status, the age, and the time and the level of exposure. Additionally, the researches on the effects of other phytoestrogens rather than soybean isoflavones desire more considerable evaluation in humans and animals.

**REFERENCES**


Tátárfatok az étvészületek növényi hatása a reproduktív teljesítménnyel: Áttekintés


