Phyto-estrogens: lessons from their effects on animals

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Summary

Phyto-estrogens are widespread in nature, but do not usually have obvious effects on animals unless the plants that contain them form a relatively large component of the diet. Over the past 50 years, the major phyto-estrogens have been isolated and their mode of action defined. Some major epidemiological questions remain unanswered; for example, we do not know why cattle sometimes can appear unaffected when grazed on pastures that have severe effects on sheep. More recently, epidemiological studies have focussed attention on possible beneficial effects of phyto-estrogens for humans with a relatively large intake of soyabean products. Scientific analysis of the question has commenced, but a true understanding of the significance of phyto-estrogens in the human diet remains unclear.

Introduction

Phyto-estrogens can be defined as plant products that bring about effects on animals by binding to the estrogen receptor. Phyto-estrogens are a major cause of reproductive failure in sheep, particularly in the winter rainfall regions of southern Australia where subterranean clover is the base pasture legume, and can also impair reproduction in cattle in many parts of the world. However, not all plant compounds that increase or decrease mammary development, impair reproduction or promote abortion are phyto-estrogens. Recently, the importance of phyto-estrogens in human food has gained prominence because of increasing use of soyabean products that contain phyto-estrogens, and epidemiological evidence indicating that populations which consume large amounts of soy products have a reduced incidence of death from breast or prostate cancer.

Phyto-estrogens

The phyto-estrogens are generally either isoflavones (eg, genistein, biochanin A, daidzein, formononetin) or coumestans such as coumestrol, trifoliol or repensol (1-3). The estrogens produced by mammals are steroids, the primary ones being oestradiol-17β, oestrone and oestriol. The chemical structure of phyto-estrogens mimics that of oestradiol-17β sufficiently for them to bind weakly to the estrogen receptor (1, 2).

Plants may contain other estrogenic compounds such as dihydrochalcones (phloretin) or steroidal estrogens such as estrone (4), but these have not been reported to cause problems in animals. Fusarium fungi growing on corn or dry pasture may produce estrogenic resorcylic lactones (zearalenone, ß-zearalenol) that cause reproductive problems in pigs and grazing sheep (5), but their significance to other populations remains to be established. Other plant flavonoid compounds include lignans such as enterodiol and enterolactone, which have the potential to reduce the amount of estrogen in the body by inhibiting the aromatase enzyme (6). As yet, there is limited information on their effects on physiological function (7). Other compounds that cause estrogenic effects may occur in the environment (eg, DDT), but these lie outside the scope of this review.

The relative activity of the phyto-estrogens is only 1/1000 (zearalenone, some coumestans) to 1/100000 (isoflavones) that of oestradiol-17ß. The actual figure depends on both the extent to which the phyto-estrogen is metabolised, and its ability to activate the estrogen receptor. It is likely that concentrations below the effective Km of the receptor will have negligible biological

activity. However, phyto-estrogens may occur in sufficient concentrations in plants to cause detectable effects in animals. Although phyto-estrogens bind with the estrogen receptor, they are unable to bind to receptors for progesterone, androgens or glucocorticoids (4). Their function in plants is unknown, but they probably confer resistance to attack by insects or fungi.

Sources

Although phyto-estrogens are widespread throughout the plant kingdom, they are most common in legumes (3). Leguminous species such as clovers have been widely used in pasture improvement programs, because leguminous plants that fix nitrogen are a major source of plant protein. Pasture dominance by particular cultivars has sometimes led to problems in grazing livestock. Herbivorous mammals can be at risk if their dietary source of protein is not diversified.

Table 1 lists the most commonly reported sources of plant estrogens affecting domestic animals. The table over-simplifies the true position; for example, related estrogenic compounds often occur in similar concentrations, so the total concentration of phyto-estrogen in the plant material may be much greater than shown in this table. Further details can be found in a number of reviews (1-3). The concentration of isoflavone phyto-estrogens is mostly affected by the genotype of the plant, but environmental conditions may also cause a two-fold difference in concentration. In contrast, the concentration of coumestans depends primarily on environmental conditions (particularly plants affected with foliar pathogens), with genotype playing a lesser role.

Table 1. Common plant sources of phyto-estrogens that affect animals

	Plant	Major phyto-estrogen	Concentration	Determinant of estrogenicity
Isoflavones	Subterranean clover	Formononetin	0.1 - 1.5%	Cultivar
	Red clover	Formononetin	0 - 1%	Cultivar
	Soyabean	Genistein, daidzein	0.1 - 0.3%	Cultivar
		Coumestrol	0 - 80 ppm	
Coumestans	Lucerne	Coumestrol	0 - 140 ppm	Leaf infection
	White clover	Coumestrol	0 - 50 ppm	Leaf infection
	Medics	Coumestrol	0 - 500 ppm	Senescence

Most outbreaks of estrogenic infertility in sheep have been reported in animals grazing cultivars of subterranean clover or red clover that contain concentrations of formononetin greater than 0.5%, although other estrogenic isoflavones such as genistein or biochanin A are also present. In contrast, most problems in cattle have occurred in animals exposed to coumestans. Estrogenism in pigs usually results from animals fed mouldy corn that contains zearalenone. Commercial rat and mouse feeds in the USA routinely contain sufficient phyto-estrogen from soybean to cause estrogenic stimulation of the uterus (8). Indeed, it is likely that most work on laboratory animals has been carried out against a background of exposure to plant estrogens. Sharma suggested that male rats would suffer much more prostatitis if their feeds did not contain phyto-estrogen (9). Soybeans contain the glycosides of the isoflavones genistein and daidzein and their hulls and sprouts may also contain coumestans. Coumestans can also occur in alfalfa sprouts.

Concentrations required to bring about an estrogenic effect

The dietary concentration of phyto-estrogen required to affect animals is relatively similar across species, except that relatively low doses have produced detectable effects on humans (Table 2). Sheep weigh slightly less than a human, but appear to require much greater exposure to isoflavones to have detectable effects. However, the position in humans is not yet clear, as a 4-fold larger dose than that cited in Table 2 (165 mg) has been reported to have no detectable effects on post-menopausal women (10). The concentrations listed in Table 2 are those of the major phyto-estrogen, and probably underestimate the total exposure.

Table 2. Minimum estimated minimum dietary intake of phyto-estrogen (mg/kg/day) reported to produce detectable effects in mammals

Compound	Species	Daily intake	Effect observed	Reference
Formononetin	Sheep	40	Low conception	(2)
Formononetin	Cattle	80	Low conception	(11)
Isoflavones	Human	0.6	Prolonged menstrual cycle	(12)
Coumestrol	Sheep	1	Reduced twinning	(13)
Coumestrol	Rat	4	Uterine growth	(8)
Coumestrol	Cattle	0.37	Infertility	(14)
Zearalenone	Sheep	0.06	Reduced twinning	(5)

Metabolism of phyto-estrogens

Coumestans appear relatively resistant to microbial degradation in the gut, but the isoflavones are extensively metabolised by gut flora in all species, with substantial effects on the resulting estrogenicity. In the rumen of sheep (15) and cattle (16) genistein and biochanin A are mostly degraded to non-estrogenic phenols, but formononetin is demethylated to more estrogenic compounds, and is absorbed mostly as equol and excreted in the urine. These metabolic changes may vary between individuals, so that some sheep excrete the less estrogenic metabolite 5'methoxy-equol (15), which may well reduce the severity of the estrogenic effects.

Metabolic conversions are also extensive in monogastric animals. Formononetin is not common in human diets, but degradation of genistein, daidzein and biochanin A by gut flora is less complete than in ruminants, so these compounds contribute to the estrogenicity. A proportion of daidzein or genistein may be converted to equol, although this may depend on the extent to which the microbial population has adapted to metabolism of the isoflavones. As with sheep, human subjects appear to differ substantially in the extent to which their gut flora degrade or metabolise isoflavones (17), and it is likely that this will influence the degree of estrogenic challenge to which individuals are exposed.

Estrogenic and anti-estrogenic activity

Phyto-estrogens can have estrogenic or anti-estrogenic effects, depending on the type and amount of phyto-estrogen relative to the concentration of endogenous steroid estrogen. Isoflavone and coumestan estrogens bind more weakly to the estrogen receptor than oestradiol-17b (18), so they may compete with the endogenous steroid and have anti-estrogenic effects. Folman and Pope (19) showed in the immature mouse that at very low doses of steroid and plant estrogen, the resultant response was additive, while at higher doses of plant or steroidal estrogen,

competition was observed such that the response to both was decreased. Plant estrogens given alone were estrogenic, but they became anti-estrogenic in the presence of increasing amounts of steroid.

These results may explain some of the differences observed between species. The concentration of oestradiol-17ß in plasma of sheep is approximately 10-fold lower than that observed in pre-menopausal women. Only estrogenic effects have been reported in sheep, but we may expect that in humans the amount of plant estrogen will be less and the amount of endogenous steroidal estrogen will be greater, so that anti-estrogenic effects of dietary phyto-estrogens might be more obvious.

Effects on mammals

Estrogens have a number of minor species-specific effects on metabolism; for example, estrogens stimulate protein deposition and growth in ruminants (20), but in humans their effects are observed more on lipid and bone metabolism. These effects are often relatively slight, so that most studies on the effects of phyto-estrogens have not detected them. Generally, phyto-estrogens and steroidal estrogens act similarly through the estrogen receptor to bring about the classical estrogenic effects described below.

Reproductive tract

Typical estrogenic stimulation of the reproductive tract has been observed in sheep and cattle exposed to estrogenic pastures on many occasions (11, 21). The mammary glands enlarge and secrete a milk-like fluid. The uterus undergoes hypertrophy. In cattle and some breeds of sheep the external genitalia may appear swollen and increased secretion of cervical mucus may be visible from the vulva. Castrate male sheep develop enlarged bulbo-urethral glands. Intact male sheep have been reported to lactate when exposed to phyto-estrogens, but their reproductive efficiency appears unimpaired (22).

Ovarian function and reproductive cycles

Sheep grazing pastures that contain phyto-estrogens have reduced frequency of twins, with total cessation of follicular development if concentrations of phyto-estrogen are high (13). A similar infertility syndrome is caused by isoflavones, coumestans or the fungal estrogen zearalenone. Infertility in cattle is due to anovulation, or the development of cystic follicles in their ovaries. Of course, careful studies have been required to distinguish the effects of phyto-estrogen on twinning frequency from effects of the supply of nutrients by the pasture. In women exposed experimentally to isoflavones the length of the follicular phase of the menstrual cycle was increased (12), but there are no records of reduced fertility.

There are no substantiated reports of phyto-estrogens causing estrus sexual behaviour, and it is likely that phyto-estrogens do not cross the blood-brain barrier readily (23). After exposure to phyto-estrogens, decreases in the plasma concentrations of gonadotrophic hormones in sheep are relatively slight (24), but in women decreased secretion of LH and FSH appears to be more marked (12). Effects on gonadotrophins may result from altered estrogenic feedback onto the pituitary gland or the hypothalamus.

Sexual development

Estrogen plays a role in normal defemininisation and masculinisation of the fetus during the development of the male phenotype. Coumestrol injected during sexual development may also cause this effect on mice (25). The sheep is unusual among mammals in that female sheep exposed to estrogen may undergo slow sexual redifferentiation throughout adult life (26). The permanent infertility which results from such re-differentiation in sheep after prolonged

exposure to estrogenic clover pastures ('clover disease') is still common in Western Australia (2). Although defeminisation can be detected in all the estrogen-sensitive target organs in affected sheep, the infertility results mainly from disruption of the structure in the cervix, which becomes more like the uterus. This causes impaired transport of spermatozoa and so reduced conception rates.

Transfer of phyto-estrogens across the placenta appears relatively unimportant, at least in sheep (27), so it is unlikely that effects on sexual development will be important for other species that complete sexual differentiation before birth. However, there is still a possibility that exposure to phyto-estrogens during early life may have untoward effects. Such exposure might occur either through excretion of phyto-estrogens in milk, or through soy-based milk replacers.

Epidemiology

The history of research on phyto-estrogens is one of attempts, not always successful, to account for phenomena observed in the field and to moderate or alter their effects.

In the 1940's there was a serious outbreak of estrogenic infertility in sheep grazed on subterranean clover pastures in Western Australia. Sheep had been grazed on such pastures for over a decade previously without obvious ill-effects, so initial studies focussed on the effects of reduced application of superphosphate, which had resulted from a shortage of fertilizer during the war (28). It was found that lack of phosphate fertilizer did increase the concentration of estrogenic isoflavones in the plant (28), but the phosphate deficiency also decreased the amount of plant material grown, so the total amount of estrogen available to sheep did not increase greatly unless stocking rates were rapidly reduced. The reason for the sudden appearance of the problem, and its association with newly cleared land, is still unknown.

Cattle appear to be as sensitive to coumestans as sheep. However, cattle frequently graze clover pastures that contain sufficient estrogenic isoflavones to cause problems in sheep without any detectable effects on their fertility. It was initially assumed that cattle have more efficient metabolism of isoflavone phyto-estrogens than sheep, but the availability of more sophisticated analytical methods in recent years has shown that, if anything, plasma concentrations of estrogenic isoflavones are even higher in cattle than in sheep on the same feed (16). The reason why sheep are more severely and frequently affected than cattle remains unclear.

The effects of exposure to phyto-estrogens on human populations are not yet clear. There is little evidence of frank estrogenism, and the amounts of phyto-estrogen consumed are low, relative to those that cause infertility in sheep (Table 2). Epidemiological studies have linked the lower incidence of death from breast or prostate cancer in oriental populations with the consumption of large amounts of soyabean products containing phyto-estrogens (29) and a number of studies in laboratory animals provide some support for such a hypothesis (cited in 12). A range of other potentially beneficial effects of phyto-estrogens have also been proposed on the basis of animal studies, eg reduced osteoporosis and reduced risk of atherosclerosis (30). There is a clear need to establish which of the potential effects on the human population are of practical significance. As described above, it is probable that the phyto-estrogens act primarily as anti-estrogens in humans, because of relatively low intakes and high endogenous steroid levels. If this is so, studies on effects in humans will need to calibrate the intake of phyto-estrogen against the concentrations of endogenous estrogen, to permit the results to be extrapolated to other populations.

There are examples where laboratory models have uncovered effects of phyto-estrogens that would otherwise have remained undetected by epidemiology. For example, the permanent infertility that occurs after prolonged exposure to phyto-estrogens in sheep is difficult to measure because the damage to the reproductive tract accumulates over several years. Permanent sexual redifferentiation may be measured by examining histological sections of the

cervix to determine the extent it has changed to become like uterus (26). We have used this test to show that subclinical infertility is still widespread in sheep in Western Australia today, although clinically apparent estrogenism is rare. There was a linear relationship between the fertility of commercial flocks across a broad area of the State and their likely cumulative exposure to estrogenic subterranean clover pastures (31). Similar detailed epidemiological studies will be needed to define potential subclinical effects in humans and relate these to exposure to phyto-estrogens. Even then, the history of research on phyto-estrogens suggests that many questions will remain unanswered.

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