A review of the Effect of Legumes on Ewe and Cow Fertility

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Executive Summary

This review focuses on the main legume forages used on UK beef and sheep farms at present, but also covers additional knowledge on other legumes to improve our overall understanding of the effects of phyto-oestrogens in legumes on livestock production and fertility.

The literature review showed that there are many different types and also different concentrations of these oestrogen-mimicking compounds present in a range of forage legumes. The concentration of phyto-oestrogens is affected by genetic and many different environmental factors, which may have led to contrary anecdotal evidence as to the effects of legumes *per se* on ruminant fertility. This, in turn, has resulted in many contradictory farmer guidelines, based on reports from many different countries, on the effects of forage legumes on ruminant fertility.

Analysis of the enquiries received at IBERS by the Grassland Development Centre (GDC) extension team showed that red clover represented 61% of all the contacts regarding the use of forages legumes. Within these calls, by far the largest proportion of enquiries were about animal health with 52% of the calls asking about infertility in ewes pre and post mating, and feeding red clover silage to pregnant ewes and cows.

From the current literature and current guidelines based on work from the UK and abroad, it is currently not possible to extend and provide further clear guidance to UK farmers on the effective use of forage legumes whilst guaranteeing to protect the fertility status of their livestock. Hence, the key industry message - to avoid grazing red clover and other forage legumes prior to and during mating - should remain unchanged until further research is completed. This review has highlighted that there is a clear need for reliable, and UK relevant, R&D on which to base up-to-date guidelines for farmers on the management and use of forage legumes whilst minimising or even eliminating the deleterious effects of phyto-oestrogens on cattle and sheep fertility.

Overall the review found that there are three key areas that require further research to successfully select for future low-oestrogen forage legume varieties and to effectively develop the current guidelines on the use of forage legumes without any detrimental effects on livestock fertility. These are: grazing strategies around mating; guidance on diet inclusion rates; and a better understanding of factors that can alter the concentrations of oestrogens present in the plant and on the plant (as myco-oestrogens) when offered fresh and conserved as hay or silage.

Introduction

In pursuit of sustainable and economically-viable livestock systems that meet consumer demands, beef and sheep farmers in the UK are under increasing pressure to maximise their use of home-grown forage-based diets for their livestock. In particular, there is renewed interest in the use of legumes, due to their ability to accumulate substantial soil nitrogen by biological fixation and thereby reduce reliance on costly inorganic fertilisers.

Research has clearly shown that forage legumes, such as red clover (*Trifolium pratense*), offered fresh or ensiled can increase growth rates in ruminants due to higher nitrogen utilisation efficiency and dry matter intakes (Speijers *et al*., 2004; Marley *et al*., 2007). However, one constraint to the use of red clover for forage-based sheep systems is that guidelines, dating back to the 1940s, suggest farmers do not graze red clover prior to and post tupping or for periods of longer than a few months, as phyto-oestrogen compounds present in the forage will reduce ewe fertility. Phyto-oestrogens are a group of naturally-occurring plant-derived non-steroidal compounds which have the ability to cause oestrogenic and / or anti-oestrogenic effects in livestock (Benassayag *et al.*, 2002). Since that time, plant breeders have developed many newer varieties of red clover, some with lower levels of the oestrogenic compounds (e.g., the variety AberRuby), and there is a need to determine the effects of these varieties to develop new management guidelines on the use of red clover for commercial farm use. Furthermore, other forage legumes used with ruminant systems contain phyto-oestrogens, including white clover (*Trifolium repens*) and lucerne (*Medicago sativa* L.). However, the different forage species contain different types and also different concentrations of these oestrogen-mimicking compounds, which may have led to contrary anecdotal evidence as to the effects of legumes *per se* on ruminant fertility. In addition, many of these species are grown within mixed species swards so interactions between the species within a mixture and their management can also alter the varying proportions of legumes and, hence, the concentrations of phyto-oestrogen during the season.

There is a need to develop clear messages on the effects of these forage legumes on ruminant fertility for the farming industry, messages which consider the availability of new varieties, current understanding and future research requirements to overcome the practical constraints for their use on UK beef and sheep farms. The aim of this review is to provide an analysis of the current knowledge on the effects of forage legumes on ewe and cow fertility, existing guidelines to farmers based on the current knowledge and to identify the key requirements for further research to facilitate the uptake of legumes as important forage crops for UK beef and sheep producers.

1. A review of the literature on the effects of different legumes and their impact on fertility of sheep and cattle

1.1 Red clover

Red clover is grown across the world to produce a high quality, high protein, forage crop. It is a well-established legume in the UK where it is grown primarily as a silage crop and for grazing for finishing lambs. It is usually grown in a mixed grass clover sward but can be grown in pure stand.

To identify and critique the original source material about red clover and ewe fertility

The first study that conclusively demonstrated that red clover could affect the fertility of ewes was in 1965 from work in Australia (Barrett *et al.*, 1965), which followed a letter to Nature reporting 'reproductive disturbances' in ewes grazing red clover-dominant pastures (Ch'ang, 1961). The first paper that reported an adverse effect of legumes on ewe fertility was in 1946 with regard to ewes grazing subterranean clover (*Trifolium subterraneum*) (Bennetts *et al*., 1946). The earliest report of red clover grown in the USA having a negative impact on fertility in ewes in the USA was by Fox *et al.* (1959). Further studies in Australia showed that temporary (Ch'ang 1958, 1961; Fox and Oldfield, 1959; Jennings and Dow, 1959) and, in some cases, permanent (Barrett *et al*., 1965) infertility was induced by prolonged feeding on a red clover pasture. This led to a number of chemical and biological studies, mostly conducted on red clover grown in Australia, indicating that red clover contained oestrogen-like compounds (Bartlett *et al*., 1948; Legg *et al*., 1950; Cunningham and Hogan, 1954; Pope and Wright, 1954; Bassett and White, 1955; Pieterse and Andrews, 1956). Later, researchers identified that red clover with high levels of the phyto-oestrogens formononetin (7-hydroxy-4'-methoxyisoflavone) and biochanin A (5,7-dihydroxy-4'-methoxyisoflavone) indirectly caused the infertility. These phyto-oestrogens are metabolised by the micro-organisms in the rumen with, for example, formononetin being metabolized mainly to daidzein (7-hydroxyisoflavone) and further to the isoflavan equol (7,4'-dihydroxyisoflavandiol) (Nilsson e*t al*., 1967). It is the equol which directly causes the effect on fertility (Shutt and Braden, 1968; Cox and Braden, 1974), with equol concentrations in blood being the main method used to determine the direct levels of phyto-oestrogens in red clover phyto-oestrogen *in vivo* (Braden *et al*., 1971). Studies conducted in the 1960s and 1970s in the UK supported these earlier findings in other countries (Newton and Betts, 1968; Newton *et al*., 1973). Work in the UK concluded that feeding ensiled red clover could still lead to reduced ewe fertility when offered as 25% or more of the diet, and that feeding pelletted red clover as the sole feed also had an adverse effect on lambing percentage (Thomson, 1975). Sheep grazing pastures with red clover at less than 25 % of total dry matter could not be guaranteed not to cause fertility problems as it has been shown that sheep will selectively graze clovers over grasses in mixed swards.

Bennets *et al.* (1946) and many of the early papers on legumes and ewe fertility described the condition as “clover disease”. This is not to be confused with ‘clover sickness’ which relates to the plant itself (as first documented by Amos, 1918) and occurs when clover is grown in the same soil at intervals shorter than eight years and the crop is affected by pest, disease or nutrient balances. Red clover contains phyto-oestrogens from the isoflavone group (e.g. formononetin, daidzein, equol, biochanin A, genistein and prunetin) (Steinshamn *et al*., 2008). Formononetin and biochanin A are the main ones present, with smaller concentrations of daidzein and genistein. Although, a total of 31 different types of different isoflavones have been identified within red clover (Wu *et al*., 2003), it is unlikely that all of these are active in livestock as reports have shown that biochanin-A and genistein are inactive in ruminants (Petterson *et al*., 1984).

1.1.1 Factors affecting phyto-oestrogen levels in red clover

The phyto-oestrogen content of red clover is primarily under genetic control but environmental factors can also have an effect. Different cultivars have been shown to contain different concentrations of phyto-oestrogens (Jones, 1986) and plant breeders have developed many new varieties of red clover, recognised for their lower levels of the oestrogenic compounds (e.g., the variety AberRuby). In Australia and New Zealand, pasture contains higher levels of phyto-oestrogen in spring, with oestrogen content declining after flowering. Nutrient deficiencies that impair plant growth have also been shown to increase the concentrations of formononetin, and hay and silage have been shown to retain oestrogenicity in some cases (Adams, 1995). In particular, one study highlighted that the oestrogen content of red clover silage was higher than that of the raw material and that the type of silage inoculant used during ensiling could affect phyto-oestrogen levels (Sarelli *et al*., 2003). Kallela (1984) showed that silage containing 0.56% formononetin in the dry matter of spring red clover caused fertility disturbances in dairy cows. More recently, a field experiment conducted in China investigating the effects of the rate of sowing, the rate of an application of molybdenum and the row spacing on phyto-oestrogen content of red clover showed that plant phyto-oestrogen concentrations increased with an increase in sowing rates (Yu *et al*., 2009).

1.1.2 Effect of high phyto-oestrogen levels in red clover on ewes

Severe clinical abnormalities - ‘clover disease’

Bennetts *et al.* (1946) described severe clinical abnormalities in sheep grazed on highly oestrogenic “subterranean” clover pastures in Australia. This syndrome, described as clover disease, resulted in very low lambing rates, prolapsed uteri and dystocia in ewes, together with observations of severe metritis and pyometron. Red clovers containing varieties with high levels of formononetin have also been implicated in ’clover disease‘, particularly if ewes graze pure stands. Reports dating from 2005 stated that ‘clover disease’ and severe

infertility are now rare in Australia (Croker *et al*., 2005 - Government of Western Australia Farmnote No. 41/2005). The literature search did not find any reports of severe clinical abnormalities resulting from feeding red clover in the UK.

Permanent infertility

When ewes are exposed to high oestrogen red clover for prolonged periods permanent infertility may occur. Clover infertility results in permanent damage to the reproductive tract which becomes worse with each year's exposure to oestrogenic pastures (Barrett *et al.*, 1965). The oestrogen affects the structure of the cervix and sperm transport and conception rates are reduced (Lightfoot *et al.*, 1973). There are often no visual signs that ewes are suffering from permanent infertility as ovarian function remains normal and ewes will show normal oestrous cycles (Adams, 1995). The lack of visual signs means that the condition often goes unnoticed and it is estimated that in Australia 1 million ewes may be affected and that, on average, affected flocks have around a 10% increase in non-pregnant ewes (Adams, 1990). Diagnosis of the problem is difficult and depends primarily on detection of pathological changes in the cervix, which is normally identified on material collected from ewes at abattoirs, rather than history or clinical signs.

Temporary infertility

Ewes fed high oestrogen red clover may suffer temporary infertility that will normally resolve within one month after cessation of grazing (Adams 1995). As in permanent infertility, ewes will exhibit normal oestrus cycles, although some breeds of ewe develop swelling of the vulva and development of the mammary glands. Some breeds have a tendency to exhibit visual signs more frequently than others but absence of visual signs is common in certain breeds (e.g., Merino ewes) (Adams, 1995). An accurate diagnosis of temporary infertility depends on the oestrogen concentration in the pasture at the time the sheep are being mated (Adams, 1995). Research from the UK examined the effects of grazing red clover (var. Hungaropoly) four weeks before and during the eight-week tupping period. The ewes were on a predominantly perennial ryegrass/white clover sward at other times. There was no apparent adverse effect of red clover on ovulation rate, conception rate or embryo mortality (Dickson *et al.,* 1977).

1.1.3 Effect of high phyto-oestrogen levels in red clover on rams

Bennetts *et al.* (1946) showed that "clover disease" did not affect ram lambs. Further work (George and Turnbull, 1966) showed that at 6 months old, ram lambs on red clover pasture showed no negative effect on puberty, liveweight or reproductive traits, and that by 12 months of age, liveweight and reproductive characteristics, including sperm numbers, were significantly better than on grass-dominant pastures. The fertility of rams on red clover for 60 days over tupping was not found to be affected, as determined from microscopic examination of the number of live sperm, sperm motility and morphology (Fox *et al*., 1959).

1.1.4 Effect of high phyto-oestrogen levels in red clover on cows

There are contradictory reports on the effect of red clover silage on cows. In one report, plant oestrogens in a pure red clover silage were found to be the most likely cause of fertility problems in cows (Kallela *et al*., 1984). Other research (Austin *et al*., 1982) found no evidence to indicate that herd fertility was suppressed by red clover silage.

1.1.5 Effect of high phyto-oestrogen levels in red clover on bulls

No reports of studies on the effect of red clover on bull performance have been found in this search.

1.2 White clover

White clover (*Trifolium repens*) is a species of clover native to Europe, North Africa and West Asia. It has been widely introduced worldwide as a pasture crop. White clover is usually grown in a mixed grass clover sward and is valued as a palatable source of home - grown protein that can fix nitrogen, replacing purchased nitrogen fertiliser. It can also produce excellent livestock growth rates. Plant breeders have developed new high performance varieties of white clover that are flexible and well suited to modern management systems, exhibiting grazing and stress tolerance, improved pest and disease resistance and compatibility with modern ryegrass varieties. White clover can produce isoflavones (daidzein, formononetin, genistein, biochanin-A) and coumarine (coumestrol) (Nykänen-Kurki *et al*., 1993), with 10 of the isoflavones found being the same as those present in red clover (i.e. daidzein, formononetin, genistein, pseudobaptigenin, glycitein, calycosin, prunetin, biochanin A, irilone and pratensein) (Wu *et al*., 2003). Coumestrol was more oestrogenic than isoflavones when given to rats and its effects were cumulative (Whitten *et al*., 1992).

1.2.1 Factors affecting phyto-oestrogen levels in white clover

White clovers mostly produce phyto-oestrogens when grown under stress. The most common factor stimulating coumestrol production is leaf infection by fungal diseases (Wong *et al.,* 1971). However, it is also worth noting that soil-borne fungi that live on pasture plants can also produce oestrogenic compounds (called myco-oestrogens) (Markiewicz, 2008). Insect damage, virus infection and poor plant nutrition can also lead to increased coumestrol levels. Coumestrol levels in white clover do not vary with the stage of growth. Legume density will influence the oestrogen concentration of the pasture so the presence of grasses and weeds will dilute the coumestrol concentration (Croker *et al*., 2005).

1.2.2 Effect of high phyto-oestrogen levels in white clover on ewes

The impacts of coumestrol on ewe fertility are less severe than those caused by formononetin. The main effects are reduced ovulation and delayed oestrus. Recovery of fertility is usually rapid following removal of sheep from oestrogenic pasture (Croker *et al*., 2005). Ch'ang 1961, who investigated the effects of red and white clover on the reproductive performance of ewe lambs, found no effects of white clover on ewe lamb fertility. However, in the UK, oestrogenic activity in white clover had been recorded (Newton and Betts, 1968; Saba *et al*., 1974). A further study in 1974 showed considerable variations in oestrogenic activity of white clover, which did not seem to be associated with a specific time of year or quantitatively related to the coumestrol content of the pasture, and suggested that other factors may be influencing oestrogenic activity (Saba *et al.,* 1974).

1.2.3 Effect of high phyto-oestrogen levels in white clover in cows

One research paper attributed reduced conception rates in and milk-like secretion in Jersey heifers in part to the oestrogenic content of white clover (Wada *et al.,* 1988).

1.2.4 Effect of high phyto-oestrogen levels in white clover on rams and bulls

There have been no references found in this search recording any effect of coumestrol on infertility in rams and bulls.

1.3 Lucerne (alfalfa)

Lucerne is a flowering plant of the pea family Fabaceae, cultivated as an important forage/ensiling crop in the US, Canada, Argentina, France, Australia, the Middle East, South Africa, and increasingly in the UK and New Zealand. It superficially resembles clover, with clusters of small purple flowers. A perennial forage legume which normally lives 4-8 years under UK growing conditions, reaching a height of up to 1 metre (3 ft), with a deep root system, thriving in free draining, higher pH soils, lucerne is a small seeded crop, and has a slow-growing seedling, but after several months of establishment forms a tough 'crown' at the top of the root system. This crown contains many shoot buds that enable lucerne to re-grow many times after being grazed or harvested. The high protein and fibre content of the crop makes ensiled lucerne an excellent complement to maize or ryegrass silage.

Lucerne produces coumestrol, a phyto-oestrogenic compound.

1.3.1 Factors affecting phyto-oestrogen levels in lucerne

The physiology of phyto-oestrogen (coumestrol) production in lucerne is poorly understood, but it is thought that the formation and/or accumulation of isoflavones in red clover and white

clover are strongly influenced by environmental and nutritional factors. The greatest content of phyto-oestrogen in lucerne plants is often found at the budding stage of growth, at which time environmental and nutritional factors are likely to have their greatest influence over the crop. As with other stress factors in other forage legumes, pests and diseases have been shown to increase the coumestrol production in lucerne (Hawk *et al*., 1967; Kain and Biggs, 1980).

1.3.2 Effect of high phyto-oestrogen levels in lucerne on ewes

Many examples of research, across a wide range of countries, into the effects of phyto-oestrogen levels in lucerne on ewes have been identified as part of the review (albeit none identified in the UK), with several stating that the effect with regards to fertility has been found to be minimal. Research using Finnish Landrace ewes (Sormunen-Cristian *et al*., 1998) compared ewe fertility and performance between a lucerne-based silage and a timothy-based silage. Results from the research indicated that moderate coumestrol content of lucerne had no detrimental influence on ewe conception or "prolificacy" and that plant oestrogens in yellow-flowered lucerne might hasten oestrus activities in ewes and thus shorten the mating period. Secondly, work by Ruttle and Goret (1968) found that ewes either grazing lucerne or fed baled lucerne did not have reduced conception rates at first oestrus or lambing percentage, and rams used in the same study showed no measurable changes in semen quality.

However, over a prolonged period of time, research work conducted in Spain on Manchego ewes contradicts this (Cantero *et al*., 1996). Anatomical studies showed that "43% of ewes from a group of 28 fed on lucerne-based diets displayed macroscopic changes within the genital tract" when compared to a control group fed on a non-leguminous diet. The research also showed that a "greater than normal development of the cervical folds was observed" indicating that a prolonged exposure to coumestrol was seen to have permanent effects on a ewe's reproductive organs. Limited evidence was also found on the effect of feeding lucerne to immature females - in a group of ten ewe lambs fed on lucerne silage the ovaries were seen to be smaller than those of a group of ten fed on Italian ryegrass (Valderrábanoa *et al.,* 1988). Microscopic examination showed specific features affecting the uterus, cervix and vagina; however, no difference in ovarian activity was detected.

1.3.3 Effect of high phyto-oestrogen levels in lucerne in cows

The number of research reports detailing the effects of the levels of phyto-oestrogen on fertility in cows has proven to be limited. The main research article found focuses on the effect of lucerne silage high in coumestrol on conception rates in dairy cows (Romero *et al*., 1997). This study was performed for one year on 608 cows in which 1264 inseminations achieved only 376 gestations. Measurement of coumestrol concentration in the lucerne ingested by the animals demonstrated a considerable level of this compound (66.8 mg/kg

dry alfalfa, without considering the losses during the extraction and purification procedures), which will undoubtedly explain the occurrence of the described syndrome. Although this report clearly indicates that there has been a connection between the levels of coumestrol found in lucerne and infertility in dairy cows, there appears to be little to be found in the way of supporting or contradicting reports.

1.3.4 Effect of high phyto-oestrogen levels in lucerne on rams and bulls

Ruttle and Goret (1968) found that rams grazing lucerne or fed baled lucerne did not have measurable changes in semen quality. No evidence was found in the search of the effects of phyto-oestrogen levels in lucerne on males of either cattle or sheep.

1.4 Lupins

Lupins belong to a diverse genus of the legume family that is characterised by long flowering spikes with a range of different colours. During the 20th century they were domesticated for modern agriculture and have become an important protein source in many parts of the world. Being a legume, lupins fix atmospheric nitrogen via a rhizobium-root nodule symbiosis, which, together with a deep root system, explains their tolerance of infertile soils. By far the majority of global lupin production is utilised by stock feed manufacturers for animal feed. Ruminants (cows and sheep) have been the biggest users followed by pigs and poultry. Spring lupins can provide a high protein grain of known provenance and source of oils and balanced energy for optimised ruminant and non-ruminant livestock production. There are three distinct species of lupins grown in the UK at present - white, blue and yellow - all suitable for grain or livestock feed. White lupins tend to have higher protein content than blue, but require a longer growing season. Yellow lupins fall between the two on both accounts.

1.4.1 Factors affecting phyto-oestrogen level in lupins

Unlike other legumes reviewed within this report, articles reviewed report that lupins contain negligible amounts of phyto-oestrogen.

1.4.2 Effect of high phyto-oestrogen levels in lupins on ewes

As stated above, the levels of phyto-oestrogen found within lupins is very low or negligible. Although the focus of this review is on phyto-oestrogens in forage legumes, the only research on the effects of lupins on fertility was with respect to feeding lupin grain. A number of articles cited feeding lupins to ewes pre tupping as a factor which can improve fertility. However, this was seen in the main as a result of an increase in nutritional intake of the ewes, improving the level of ovarian activity, as opposed to a hormonal consequence. For example, Somchita *et al.* (2007), conducted some research on cyclic Welsh mountain ewes

and concluded that lupin grain supplement increased the number of follicles, but this increase was due to the increased state of nutrition in the animals.

1.4.3 Effect of high phyto-oestrogen levels in lupins in cows

Following a review of the available literature, no evidence of research into the effect of lupins on fertility in cows was evidenced. Research carried out at IBERS (Marley *et al.,* 2009) on lupin grain investigated the effects of feeding concentrates containing either yellow lupins or soya bean meal to mature dairy cows from weeks 5- 12 of lactation. Results indicated that yellow lupins could play a role in replacing imported soya in the diet of dairy cows in the UK, but that further research was required to determine their effects on fertility and animal health over a longer experimental period.

1.4.4 Effect of high phyto-oestrogen levels in lupins on rams and bulls

Following a review of the available literature, there were no reports of research into the effects of lupins on fertility on rams or bulls.

1.5 Other agricultural important legumes

1.5.1 Birdsfoot trefoil

Birdsfoot trefoil (*Lotus corniculatus*) has being recognised as a forage legume in the UK for over 200 years (Ellis, 1774) but its use is limited due to poor agronomic performance (Frame *et al*., 1998). However, it is native to areas with temperate climates (Jones and Turkington, 1986) and has several attributes that are potentially advantageous in comparison to more widely grown forage legumes, such as red and white clover. Firstly, *Lotus* species are more resilient on soils of acid pH and low fertility than white and red clover and are drought-tolerant (Duke, 1981). Secondly, grazing studies in the UK have shown that lambs feeding on *L. corniculatus* have higher liveweight gains compared to lambs grazing other legumes (Speijers *et al*., 2000) and reduced total nematode parasite intensities compared to lambs grazing ryegrass / white clover swards (Marley *et al*., 2003).

Research conducted back in 1955, involving a grazing study conducted in the USA, showed that ewes grazing on birdsfoot trefoil had a lower rate of conception compared to ewes grazing grass swards, with 43% and 31% of ewes on birdsfoot trefoil conceiving in the fourth or fifth period of six successive 17-day heat periods (Engle *et al*., 1955). However, in contrast, more recent studies in New Zealand found that mating ewes on birdsfoot trefoil compared to ryegrass swards increased the number of lambs born, and linearly increasing the numbers of days of grazing birdsfoot trefoil before ovulation can increase ovulation rate in ewes ($P < 0.05$) (Ramírez-Restrepo, 2004).

1.5.2 Alsike clover

Alsike clover (*Trifolium* hybridum), although not classed as a major agricultural forage legumes, is widespread in the UK and is commonly found in pastures, on roadsides and waste areas. Research has shown that it contains the same 10 isoflavone aglycones found in red and white clover: daidzein, formononetin, genistein, pseudobaptigenin, glycitein, calycosin, prunetin, biochanin A, irilone and pratensein in (Wu *et al*., 2003).

2. Legumes and livestock fertility – lessons from other countries

2.1 Subterranean clover

Subterranean clover (*T. subterraneum*) is not commonly used in sowing mixtures in UK agriculture but it is native to northwest Europe and can be found in many pastures that have not been improved in Australia and New Zealand. Research in these countries highlighted that grazing subterranean clover had negative effects on fertility in ewes (Davies *et al*., 1970; Obst and Seamark, 1970). The studies showed that temporary and, in some cases, permanent infertility was induced by prolonged feeding on a subterranean clover pasture. It was identified that subterranean clover with high levels of the phyto-oestrogen formononetin caused the infertility. Later work with low formononetin varieties of subterranean clover showed reproductive characteristics were not affected and it could induce significant improvement in animal weight gain and, in males, good carcass and meat characteristics (Pace *et al*., 2006). The work on this species shows that the phyto-oestrogen content of forage legumes can differ across the season (Bennetts and Underwood, 1951).

The subterranean clover cultivar Yarloop was reported to have a detrimental effect on the reproductive cycle of ewes grazing it (Obst and Seamark, 1970). It was this work that showed that the reason for reduced fertility, despite oestrus cycle lengths being normal in ewes grazing high oestrogen pastures, was due to the reduced period of corpus luteum function as shown by changes in plasma progesterone concentrations.

2.2 Ladino clover

Ladino clover is a giant white clover (*T. repens lodigense*), and is typically grown in the USA although allegedly it originated in the Lombardy province of Italy, and was introduced to the United States in 1891. In the same study investigating the effects of birdsfoot trefoil on fertility in ewes, Engle *et al*. (1955) showed that this type of white clover slowed the rate of conception in grazing ewes during mating, with 24% and 22% of ewes taking until the fourth or fifth 17-day heat period to conceive, respectively.

3. To explain how the level of legume content and variety may affect the impact on fertility and to determine implications for future legume breeding

3.1 Breeding for modified phyto-oestrogen content

Selection for reduced phyto-oestrogen content in red clover began at what was then the Welsh Plant Breeding Station (later IGER, now IBERS) in the early 1980s (Gosden *et al*., 1984). It focused on formononetin with a rationale based on studies in Australia that showed the effects on infertility; temporary effects with reduced lamb yield but also permanent infertility with prolonged feeding on a high formononetin diet (Morley *et al.,* 1969; Neil *et al.,* 1969). However, where formononetin was kept below 0.3%, only a small reduction in fertility was seen (Neil *et al.,* 1969). At this stage two methods were used to estimate formononetin content: a rapid simple method applied to seedlings (Gosden and Jones, 1978) and a more accurate measure using HPLC which was applied to mature plants. Four red clover varieties (Sabtoron, Norseman, Robusta and Astra) were selected for low formononetin content over several generations. The reductions in formononetin demonstrated were from 0.60-0.12% in cv. Norseman and 0.30-0.12% in cv. Sabtoron, based on random samplings of mature plants grown in the same environment. High levels of formononetin were also selected for and gave levels of 1.27% in Norseman and 0.73% in Sabtoron. At this stage (1984) selection was for high levels of formononetin in the absence of any evidence for a beneficial (anabolic) effect, which has since been shown for lamb production, with induced a significant improvement in animal liveweight gain and, in males, good carcass and meat characteristics when phyto-oestrogens from clovers are fed to lambs (Moorby *et al*., 2004; Pace *et al*., 2006). However, such an effect had been demonstrated for coumestrol concentrates from lucerne (Johnston *et al*., 1965) and the effect of coumestrol on ewes is similar to that of formononetin.

In 1986, the results of a grazing trial studying the effects of phyto-oestrogen content on ovulation rate and lambing performance were reported (Jones, 1986). The low and high selection lines from Astra and Sabtoron were used with a perennial ryegrass/white clover control under rotational grazing of ewes for a period of 25 days prior to mating. Little difference was observed between the low phyto-oestrogen red clover and control in terms of their effects on reproduction. However, significant reductions in mean ovulation rate (13%) and return to service (15%) were seen with the high phyto-oestrogen content red clover in comparison to both control and low phyto-oestrogen lines. Subsequent development of the low oestrogen selection line derived originally from Sabtoron resulted in the variety AberRuby, which gained status on the UK Descriptive List in 2005.

Red clover selections with low formononetin content were also made as part of breeding programmes in Switzerland (Boller, 1994). In this work the heritability of formononetin content was shown to be high. Similar work in New Zealand resulted in the red clover variety Grasslands G7, derived from Grasslands Pawera but with a formononetin content in

leaflets of 0.23% compared to 1.04% in Pawera (Keogh, 1995; Rumball *et al.,* 1997). A very high formononetin red clover line was also selected from Pawera (Gerard *et al*., 2005).

Considerable progress has been made, detailed above, in the breeding of red clover with both enhanced and reduced levels of the main phyto-oestrogens. There is no reason to assume that further improvement is unfeasible. However, the major requirement is for clear, definitive and quantitative breeding targets from animal studies: what is the minimum level at which phyto-oestrogens have no effect on fertility and , conversely, what is the optimum level for the effects of elevated phyto-oestrogen levels on liveweight gain. This information, coupled with the major advances in red clover genomics now underway would allow the development of varieties of red clover with phyto-oestrogen content tailored to particular uses on farm.

With regard to other species, progress has been more limited due largely to lower breeding activity for this trait in these crops. However, it is likely that the same considerations would apply and that concerted action from breeders and animal scientists would lead to rapid progress.

4. To identify and analyse case studies, highlighting key areas for development into management guidelines

In this section, information is provided on specific case studies from farms covering all sectors; dairy is included where there is a relevant link between legumes and cattle fertility. Further to this, an analysis of all farmer enquiries to the Grassland Development Centre regarding legumes and the proportion of these which were relating to fertility concerns with using legumes is recorded.

4.1 Individual farm Case Studies

4.1.1 Mixed beef/arable/sheep farm (coastal)

The sheep flock is comprised of improved Lleyns and lambs in March. For several years the farmer has grazed ewe lambs on red clover leys containing AberRuby at pre-, post- and during mating with no perceived detrimental effects on lambing percentage. The farmer aims for 150% lambing in this group and regularly achieves this. He does not however mate his ewes on the red clover leys since, despite the experience with the ewe lambs, he feels that as he is aware of a risk it is too big a risk to take with the main flock. The red clover leys on this farm are used as a fertility-build for arable (cereal) crops, finishing lambs and for high quality bale silage and remain an integral part of his farming system. The farm has no plans to change his current strategy of using AberRuby leys for grazing ewe lambs around tupping but would not do so if using other varieties of red clover unless he was assured that they were ‘low oestrogen varieties’. He is in the process of altering his sheep breed type but this is unrelated to the use of red clover leys.

4.1.2 Dairy/beef /sheep/arable farm

This farm runs a large sheep flock and regularly finishes lambs on red clover leys in the late summer and uses red clover silage for winter feeding ewes and has not noted any adverse effects on ewes. He has never used these leys for mating ewes or ewe lambs, and has always followed the standard advice given. The farm system aims to capitalise on the value of red clover leys for its quality grazing in late summer/ autumn, high quality silage for productive stock, be they beef, sheep or dairy cows, and as important, as a soil fertility build for subsequent arable cropping. As a biochemist he understands better than most the reasons why a crop with an oestrogen pre-cursor would not be appropriate for mating sheep but would be interested in trialling low oestrogen varieties with ewes/ ewe lambs on farm as part of a structured study linked to research.

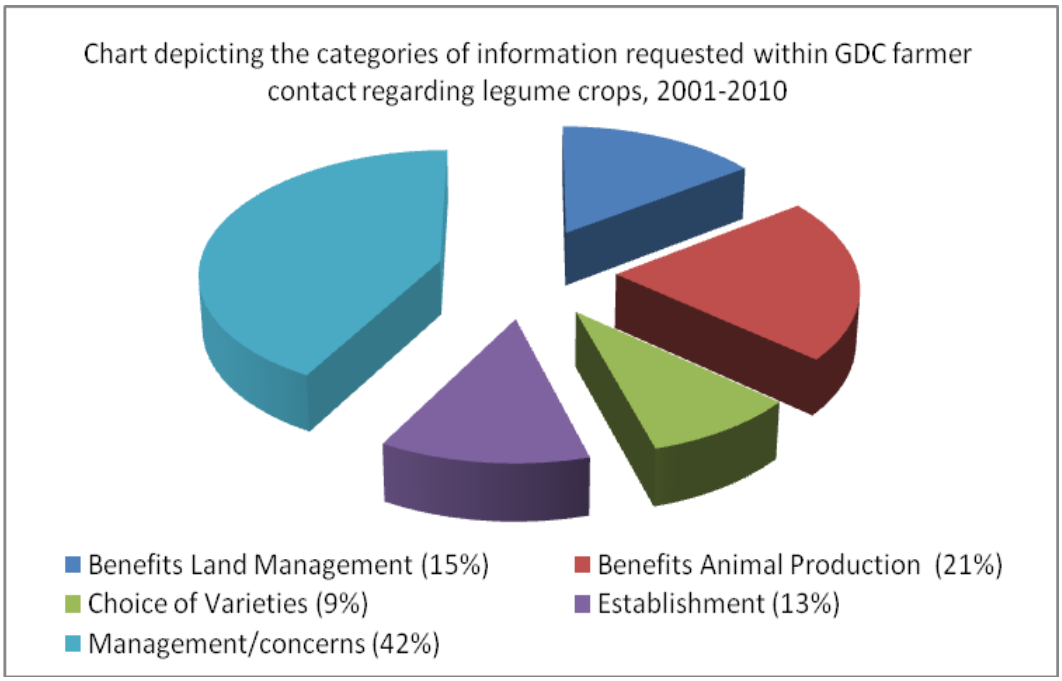
4.1.3 Dairy farmer (all grassland)

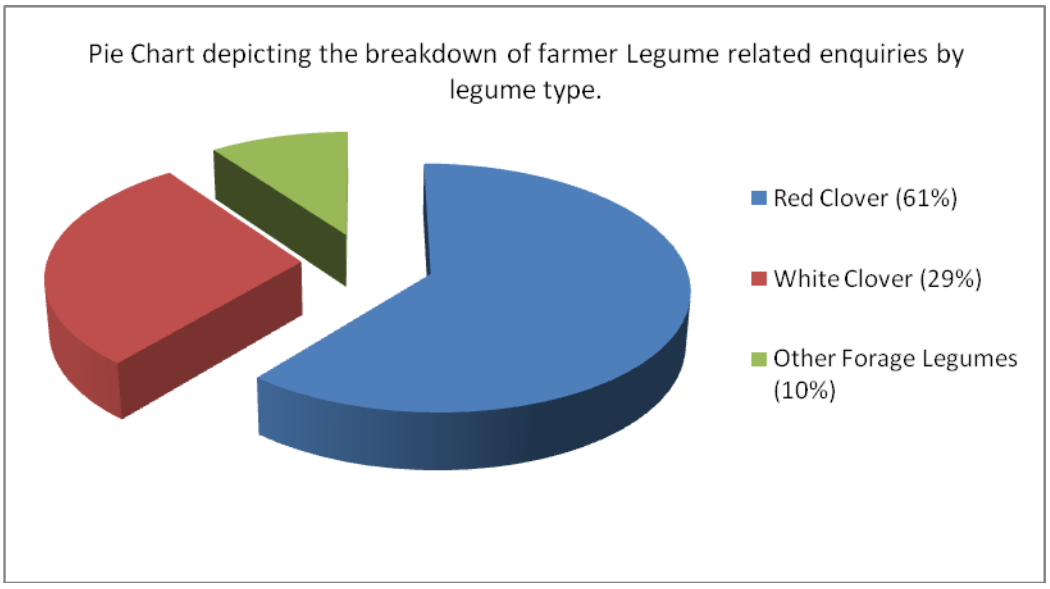
This farm has for many years grown red clover leys for silage for milking dairy cows and been pleased with the results from a feed rationing and nutritional value perspective. However the farmer approached GDC very recently asking if there was evidence of red clover silage influencing fertility in the herd. He is concerned that difficulties in getting cows back into calf, despite being in good condition, may be related to the red clover silage rations but has not been able to confirm this to be the case. As a precaution he is gradually switching the leys when reseeding to a broad leaved white clover ley to replace the red clover reseeding policy as a result of his misgivings about red clover.

4.1.4 Mixed beef/sheep/arable farm (inland)

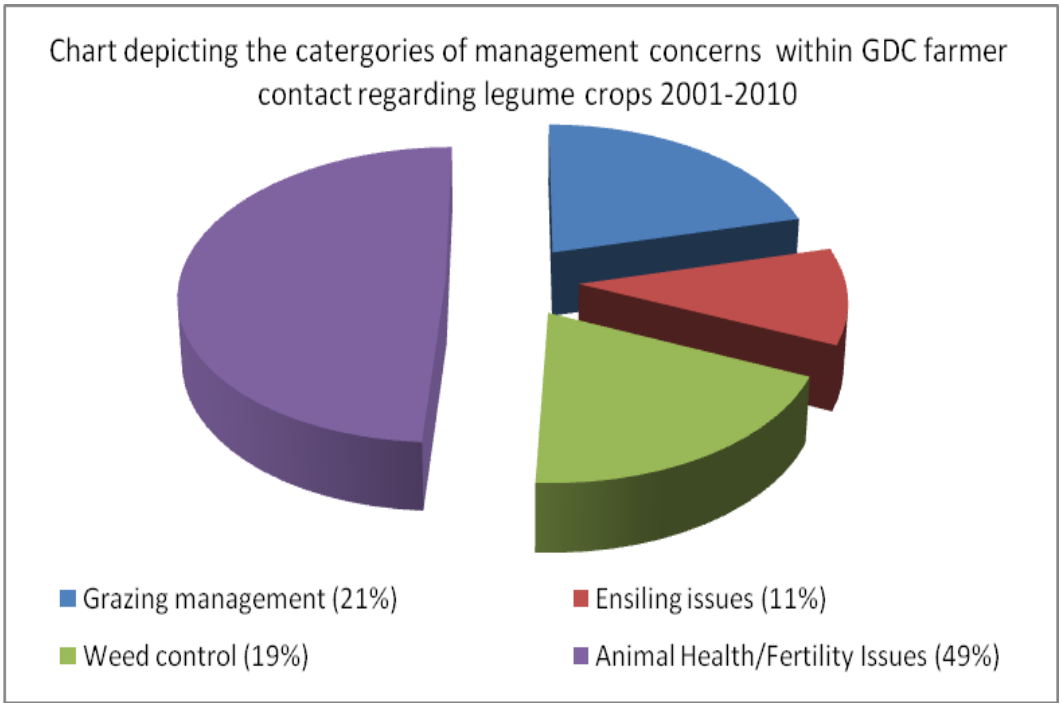
The farm has grown red clover for silage leys and finishing lambs in a rotation with cereals for over 10 years and usually establishes the red clover by under-sowing. Mating or bulling animals are never grazed on the red clover, not only because of the concerns for livestock fertility, but because the farm has developed a system where maximising the value of the red clover leys rests with quality silage and late summer lamb finishing.

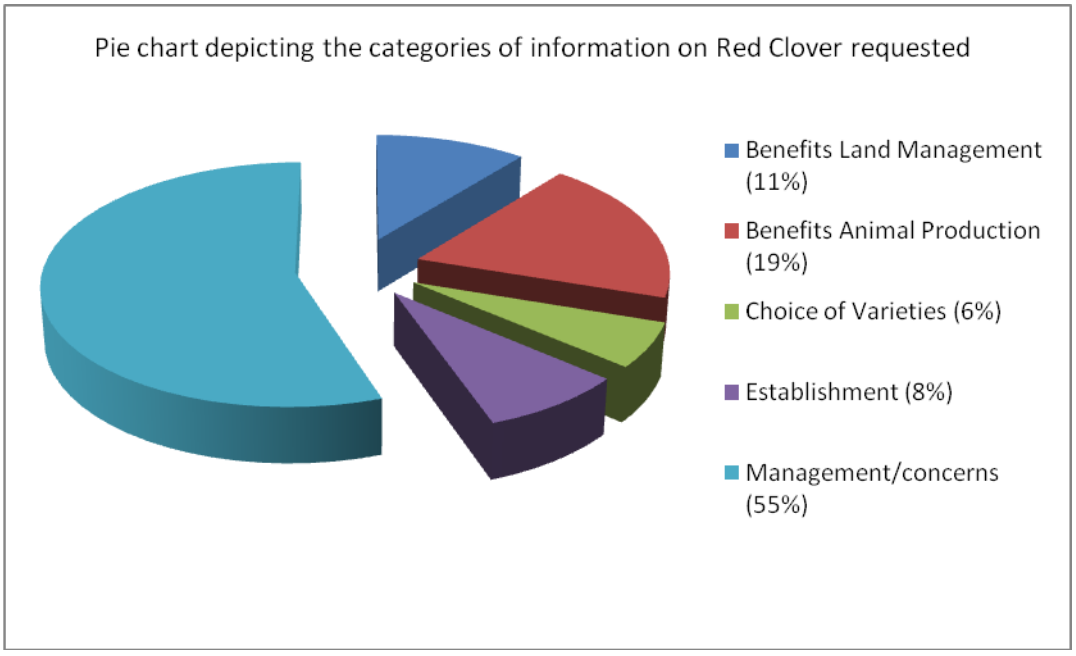
4.2 Overview of the enquiries regarding legumes in beef and sheep systems – indicating the key areas for development into management guidelines





The pie chart above gives a breakdown of enquiry records by forage legume type, with enquiries about red clover dominating, and representing 61% of all the contacts.





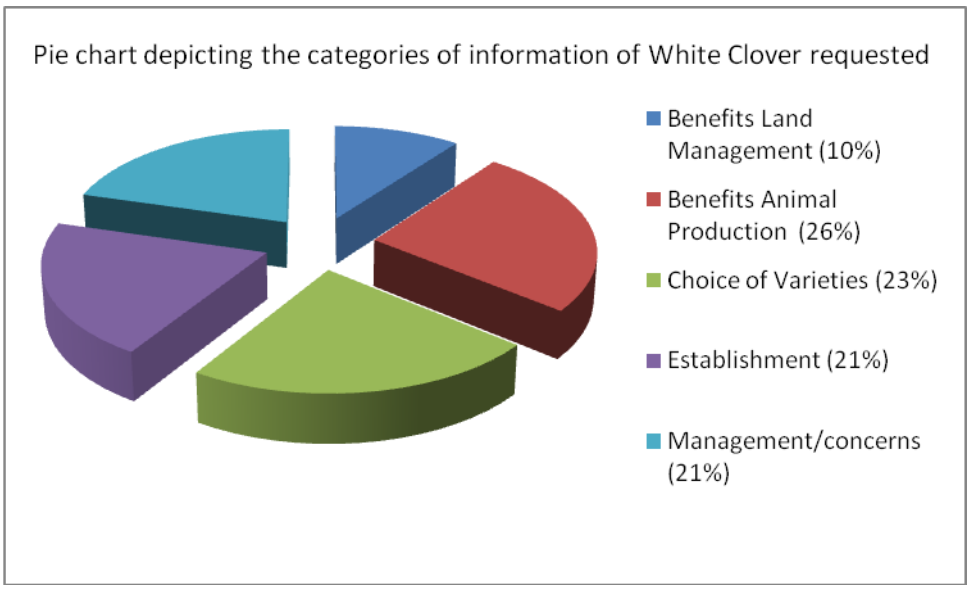
4.2.1 Red clover

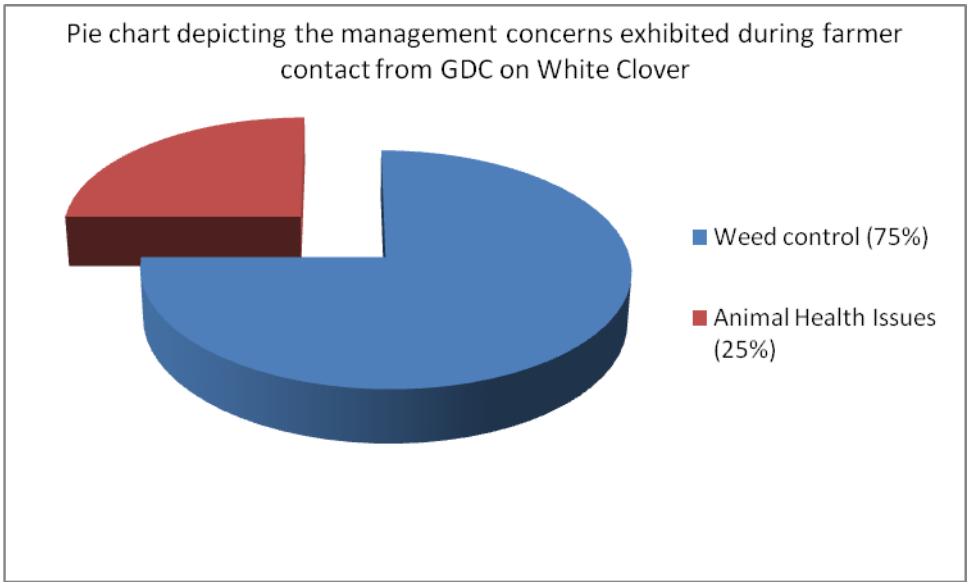
Of the red clover management concerns, by far the largest proportion was with regard to animal health with 52% of the calls asking about infertility in ewes pre- and post tupping, and feeding red clover silage to pregnant ewes and cows. Over 50% percent of the red clover enquiries were about management concerns and 19% of the enquiries regarding red clover related to livestock production and nutritional benefits. There have been no comments from

farmers to GDC about any damaging effects of growing ewe lambs on red clover ley, if, for example, a batch of lambs are finished on red clover and the ewe lambs are subsequently retained for breeding. However, one farmer did mention that he felt that oestroegenic properties of the red clover must be having a growth promoting effect on his wether (male castrated) lambs. Another farmer reported that he grazed ewe lambs on red clover swards for up to three weeks pre-mating without any adverse effects on conception rates.

4.2.2 White clover

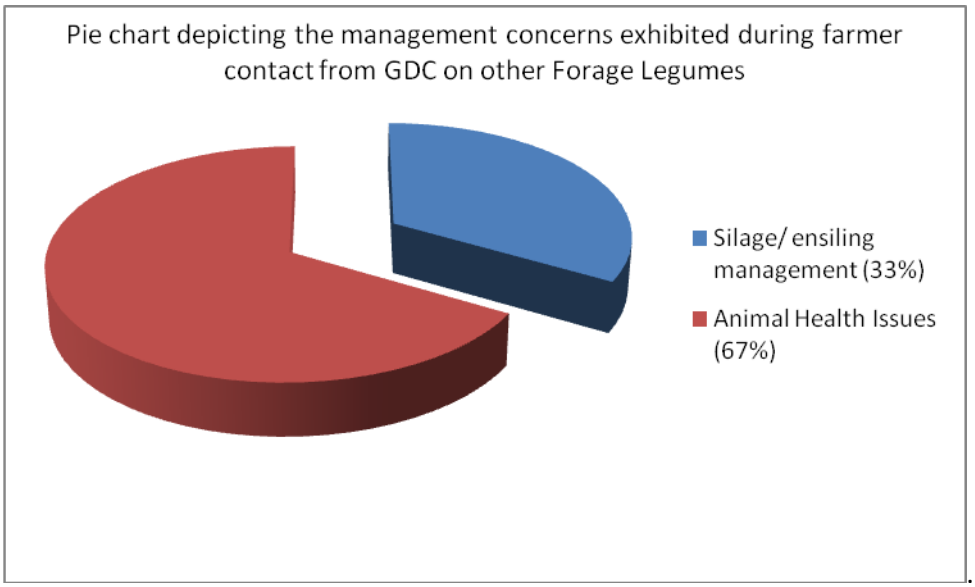
The most common topic was the benefit of white clover to animal production, and how a white clover ley can improve the proportion of home grown protein. The choice of varieties, and effective over-sowing methods were key management issues, as well as many weed control queries, particularly thistles and docks, in established clover leys. Animal health queries were largely regarding concerns about bloat when livestock are grazing white clover.





4.2.3 Other forage legumes

A far more varied range of queries were presented when looking at other forage legume species, due to the relatively low level of these crops grown, but more than half the questions were about lucerne as a silage crop.



5. Guidelines to producers on the use of legumes in beef and sheep systems

There are a limited range of farmer guidelines focussed on minimising the adverse effects of fertility reduction through forage legumes. The majority of farmer guidelines in the UK (and elsewhere) are focussed on the general management of white clover, red clover and lucerne, with some limited guidelines on species like birdsfoot trefoil. These guidelines cover: agronomy, effective establishment, managing clover within a sward, variety choices, rotations to prevent plant diseases, production benefits, soil benefits and ensiling requirements. With respect to forage legumes, much of the literature is produced for organic farmers for whom management of forage legumes is pivotal to an effective system.

5.1 Guidelines on forage legumes and fertility

Most of the guidelines refer to the advice for farmers to avoid using red clover dominant swards around mating for ewes. However, there is a great deal of variation in the recommendations with respect to the percentage of red clover that is acceptable within the sward and the time period that ewes should avoid grazing these swards. There were no clear R & D or farmer guidelines found which specifically relate to replacement stock but some reports did highlight the potential 'cumulative' risks of grazing ewes on red clover (Croker *et al*. 2007). Guidelines on the effects of legumes and cattle include categorical statements such as 'red clover has no effect on cow fertility' (Alexander, 2007).

Here we highlight some of the key guidelines available from the UK and abroad:
In the UK, SAC (2010) report, 'Year round feeding the ewe for lifetime production', the advice states that "swards with over 30% red clover should not be fed to ewes or rams 45 days either side of tupping and silage made from red clover merely concentrates the oestrogens thus feeding red clover after tupping will result in barren ewes and undersized lambs. White clover as a grazed crop is safe''.

In Western Australia Dept of Agriculture literature, ''the concerns and guidelines highlight legumes with high levels of the phyto-oestroegen formononentin, including red clover, and some subterranean clover varieties' (Croker *et al*., 2007). These guidelines clearly identify annual legume species and varieties that are not high in formononentin, and describe times of year when levels are likely to be lower (i.e. when flowering) and which forage legumes do contain phyto-oestroegens. The problem is also now reduced in Western Australia as pastures are less clover dominant.

In New Zealand the guidelines are more tempered, with 'red clover sometimes contains enough oestrogen to lower sheep fertility when fed in the autumn, during mating; however recent selections contain much lower oestrogens'' (Charlton, 2009). The most innovative

guidelines are based on the work of Keogh *et al*. (1996): 'Because of the excellent positive attributes of red clover which include: high yields, later flowering and high stock preference, cultivars have been developed with low oestrogen levels so these advantages can be fully utilised in sheep. Such cultivars include Grasslands G27 and, to a lesser extent, Grasslands Colenso. Specific grazing management has also been shown to dramatically lower the effects of oestrogens on ewes around mating. This includes a system where ewes graze high oestrogen paddocks for a maximum of three days at a time. They must then be moved onto low oestrogen pasture for a minimum of four days before returning to the high oestrogen paddocks. This system is especially effective when red clover only makes up a 'small percentage' of the sward.'' (Clutha Vets,, 2011).

With respect to lucerne and fertility, guidelines from NZ are more positive, provided the crop is 'healthy' - 'ovulation rates will not be negatively affected by flushing ewes on healthy lucerne and includes anecdocal reports that 'tupping two-tooths on lucerne has lifted conception rates''. ''However oestrogenic compounds are produced by plants that are under disease stress (e.g. leaf spot or aphid) so tupping on these is not recommended. Also autumn is the time to let lucerne flower and recharge root reserves'' (Meat & Wool New Zealand, 2009). In contrast, guidelines from the New South Wales Government Board of Agriculture in Australia clearly defines that there may be health issues associated with sheep fertility, through a reduction in twinning rates - "The effects on livestock are short-lived, and the problem can be avoided by removing breeding animals from stressed lucerne pastures four weeks before and during joining" (NSW AGFACTS, 2003).

Given the variability of findings within the literature review and the current guidelines, most of which are based on work from abroad, it is currently not possible to extend and provide further clear guidelines to UK farmers on the effective use of forage legumes whilst guaranteeing to protect the fertility status of their livestock. This review has highlighted that there is a clear need for reliable, and UK-relevant, R&D on which to base up-to-date guidelines for farmers on the management and use of forage legumes whilst minimising or even eliminating the effects of phyto-oestrogens on cattle and sheep fertility.

6. To identify gaps in knowledge base and highlight where new research may be needed

There are three key areas that require further research to successfully select for future low-oestrogen forage legume varieties and to effectively develop the current management guidelines on the use of forage legumes without detrimental effects on livestock fertility. These are: grazing strategies around mating; guidance on diet inclusion rates; a better understanding of factors which affect the concentrations of oestrogens present in the plant

and on the plant (as myco-oestrogens) when offered fresh or conserved as hay or silage to livestock.

6.1 Grazing strategies

Research has shown that ewes grazing on low oestrogen red clover varieties in New Zealand did not have high levels of formononetin in their blood until day 5 after the start of grazing, and that levels returned to normal within two days after moving to ryegrass pasture. The work showed that implementing a short-term grazing strategy for high-oestrogen clovers (e.g. three days on red clover followed by four days on grass) allowed for low-oestrogen red clover to be used to flush ewes without affecting fertility (Keogh *et al*., 1996). There is a need to determine if these and other innovative grazing strategies can be employed to allow for the safe use of UK varieties of low-oestrogen red clovers for flushing ewes. For example, by using modern low-oestrogen varieties can we increase the time up to mating that ewes can safely graze red clover swards?

6.2 Diet inclusion rates – as proportion of DM intake and varieties used

There remains a shortage of evidence with respect to the levels of the major phyto-oestrogens below which red clover can be incorporated in the diet and have no or very low risk of effects on fertility. This is important since reductions in phyto-oestrogens have been achieved through breeding legume varieties with lower phyto-oestrogens, but the full benefit of this and possible future progress will only be felt by the farmer when the risk is eliminated and new options for red clover management can be recommended. A clear target for levels of the main phyto-oestrogens and acceptable variations around that target (e.g., with different managements, stress levels and climatic conditions) is needed for incorporation as a selection criterion in the development of new varieties. Ultimately, this will require feeding trials in ewes at mating with assessment of the effects on fertility of diets with different levels of phyto-oestrogen intake. Prior to this, forage plots containing different proportions of different UK varieties of red clover or other legumes could be used to determine the levels of phyto-oestrogens present in a sward environment on a DM / ha basis. This work should aim to develop guidelines on the risk of phyto-oestrogens levels present in swards according to their legume content and the variety of the legume used.

6.3 Further understanding of factors which affect phyto-oestrogen levels in and on plants when offered fresh and conserved to livestock

6.3.1 Agronomic management

The literature review highlighted that agronomic factors, such as sowing rates, could affect phyto-oestrogen concentrations. As phyto-oestrogen levels are related to factors which cause stress in plants, it is likely that different grazing and cutting strategies will influence and change a plants response to the production of these compounds. Small-scale plots investigating different cutting and grazing regimes are needed to determine the extent of these effects.

6.3.2 Pests and diseases

Reports showed that the highest incidence of fertility issues with white clover was due to the presence of fungal diseases on the plants, with stress resulting in increased levels of coumestrol in this species. However, it is also worth noting that soil-borne fungi that live on pasture plants can also produce oestrogenic compounds (called myco-oestrogens) (Markiewicz, 2008). These myco-oestrogens may be responsible for some of the effects observed for forage legumes, when fresh and ensiled, and warrants further investigation. Further understanding is needed to be able to separate and understand the potential role of these myco-oestrogens found on legume forages and their effects on the concentrations of phyto-oestrogens produced by the plant, especially as differing grazing or cutting regimes may influence the susceptibility of legumes to fungal infections. Overall, given the potential for fungal diseases to influence forage legume phyto-oestrogen concentrations, plant breeding programmes aiming to reduce these compounds need to select for plants with resistance to fungal infections as well as lower phyto-oestrogen concentrations. Future breeding research also needs to prepare for the impact of climate change on secondary plant metabolites, including phyto-oestrogens.

6.3.3 Conservation practices

There was contrary evidence as to whether conserving forage increased the levels of phyto-oestrogens offered in the diet to livestock, with factors such as mould-contamination from fungal diseases (Hawk *et al*., 1967) being implicated in the effects seen. Further studies showed that different silage additives could affect the levels of phyto-oestrogens in the resultant silage (Sarelli *et al*., 2003). Further studies on the effects of cutting, grazing and conservation practices on the phyto-oestrogen content of fresh and conserved forage legumes are required to provide guidelines to farmers to avoid the risk of these factors affecting fertility levels when forage legumes are offered to livestock.

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