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Series on the Safety of Novel Foods and Feeds, No. 13

CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF ALFALFA AND OTHER TEMPERATE FORAGE LEGUMES: KEY FEED NUTRIENTS, ANTI-NUTRIENTS AND SECONDARY PLANT METABOLITES

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No. 1, Consensus Document on Key Nutrients and Key Toxicants in Low Erucic Acid Rapeseed (Canola) (2001)

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Environment Directorate

Organisation for Economic Co-operation and Development

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FOREWORD

The OECD's Task Force for the Safety of Novel Foods and Feeds decided at its first session, in 1999, to focus its work on the development of science-based ***consensus documents,*** which are mutually acceptable among member countries. These consensus documents contain information for use during the regulatory assessment of a particular food/feed product. In the area of food and feed safety, consensus documents are being published on the nutrients, anti-nutrients or toxicants, information of its use as a food/feed and other relevant information.

This consensus document addresses compositional considerations for new varieties of *Alfalfa and other Temperate Forage Legumes: Key Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites*. A general description of these components is provided. As well, there is background material on the production, processing and uses of Alfalfa and other Temperate Forage Legumes and considerations to be taken when assessing new Alfalfa and other Temperate Forage Legumes varieties.

Canada and the United Kingdom served as lead countries in the preparation of this document.

This document is published on the responsibility of the Joint Meeting of the Chemicals Group and Management Committee of the Special Programme on the Control of Chemicals of the OECD.

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PREAMBLE

Food and feed products of modern biotechnology are being commercialised and marketed in OECD Member countries. The need has been identified for detailed technical work aimed at establishing appropriate approaches to the safety assessment of these products.

At a Workshop held in Aussois, France (OECD, 1997), it was recognised that a consistent approach to the establishment of substantial equivalence might be improved through consensus on the appropriate components (e.g., key nutrients, key toxicants and anti-nutritional compounds) on a crop-by-crop basis, which should be considered in the comparison. It is recognised that the components may differ from crop to crop. The Task Force therefore decided to develop consensus documents on phenotypic characteristics and compositional data. These data are used to identify similarities and differences following a comparative approach as part of a food and feed safety assessment. They should be useful to the development of guidelines, both national and international and to encourage information sharing among OECD Member countries.

These documents are a compilation of current information that is important in food and feed safety assessment. They provide a technical tool for regulatory officials as a general guide and reference source, and also for industry and other interested parties and will complement those of the Working Group on Harmonization of Regulatory Oversight in Biotechnology. They are mutually acceptable to, but not legally binding on, Member countries. They are not intended to be a comprehensive description of all issues considered to be necessary for a safety assessment, but a base set for an individual product that supports the comparative approach. In assessing an individual product, additional components may be required depending on the specific case in question.

In order to ensure that scientific and technical developments are taken into account, Member countries have agreed that these consensus documents will be reviewed periodically and updated as necessary. Users of these documents are invited to provide the OECD with new scientific and technical information, and to make proposals for additional areas to be considered.

THE ROLE OF COMPARATIVE APPROACH AS PART OF A SAFETY ASSESSMENT

In 1990, a joint consultation of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) established that the comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment (WHO, 1991).

In 1993 the Organisation for Economic Co-operation and Development (OECD) further elaborated this concept and advocated the approach to safety assessment based on substantial equivalence as being the most practical approach to addressing the safety of foods and food components derived through modern biotechnology (as well as other methods of modifying a host genome including tissue culture methods and chemical or radiation induced mutation). In 2000 the Task Force concluded in its report to the G8 that the concept of substantial equivalence will need to be kept under review.

The Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology in 2000 concluded that the safety assessment of genetically modified foods requires an integrated and stepwise, case-by-case approach, which can be aided by a structured series of questions. A comparative approach focusing on the determination of similarities and differences between the genetically modified food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of genetically modified foods. The concept of substantial equivalence was developed as a practical approach to the safety assessment of genetically modified foods. It should be seen as a key step in the safety assessment process although it is not a safety assessment in itself; it does not characterise hazard, rather it is used to structure the safety assessment of a genetically modified food relative to a conventional counterpart. The Consultation concluded that the application of the concept of substantial equivalence contributes to a robust safety assessment framework.

A previous Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety (1996) elaborated on compositional comparison as an important element in the determination of substantial equivalence. A comparison of critical components can be carried out at the level of the food source (i.e., species) or the specific food product. Critical components are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question. The comparison of key nutrients should be between the modified variety and non-modified comparators with an appropriate history of safe use. The data for the non-modified comparator can be the natural ranges published in the literature for commercial varieties or those measured levels in parental or other edible varieties of the species (FAO and WHO, 1996). The comparator used to detect unintended effects should ideally be the near isogenic parental line grown under identical conditions. While the comparative approach is useful as part of the safety assessment of foods derived from plants developed using recombinant DNA technology, the approach could, in general, be applied to foods derived from new plant varieties that have been bred by other techniques.

INTRODUCTION TO ALFALFA AND OTHER TEMPERATE LEGUMES USED AS ANIMAL FEED

Forage legumes are an essential component of agricultural systems in temperate regions of the world. The benefits of forage legumes include providing top quality animal feed, suitable ground cover, and a valuable source of nitrogen. The nitrogen fixing ability of the legume occurs through inoculation with rhizobia. The root nodules contain the rhizobia, which have a symbiotic relationship with the legume allowing for the fixation of nitrogen for the plant. In return, the legumes supply the rhizobium bacteria with a source of fixed carbon derived from the photosynthetic process. This allows the legumes to survive and grow with little or no nitrogen added to the soil. When legumes are used as cover crops they contribute large amounts of nitrogen to the soil for uptake by the subsequent crop.

Leguminosae is one of the largest plant families in the world. The genera *Trifolium* and *Medicago* are prominent in sustainable farming systems within temperate regions. In Canada, for example, more than 26 million hectares are devoted annually for livestock grazing and forage production. Of this, 4 million hectares are tame or seeded pasture and 6.5 million hectares are cultivated tame hay and fodder crops.

Legumes are favoured by ruminants, whether for grazing or as well preserved silage or hay. The lower content of structural fibre and the higher protein content of legumes when compared to grasses results in an improved voluntary intake and digestion process as well as a more efficient absorption of nutrients (Ulyatt *et al*., 1977, Beever and Thorp, 1996). By feeding legumes, animal production response is also improved mainly due to the high concentration of protein and minerals within legumes. Legumes are generally grown in combination with grasses to reduce the persistent, high-viscosity foam (bloating hazard) that occurs with low fibre, high protein legume species (Howarth *et al*., 1991; Popp *et al*., 2000). Although there is contradictory evidence (Majak *et al*., 1980, Clark and Reid, 1974), saponins have also been implicated in bloat (Klita *et al*., 1996). The presence of condensed tannins in legume forages disrupts the foam and prevents bloat (Tanner *et al*., 1995; Lees, 1992).

This document will review alfalfa, the most common forage legume grown in the temperate regions, and will introduce the other prominent legumes.

SECTION I – ALFALFA (*MEDICAGO SATIVA* L.)

A. PRODUCTION

Alfalfa, also known as lucerne, is a herbaceous perennial legume that grows throughout the world in a variety of climates. It is widely distributed in temperate zones including, USA, southern Canada, Europe, China, southern Latin America and South Africa. More than 33 million hectares of alfalfa are cultivated throughout the world. This was one of the first forages to be domesticated and with its high yield potential, it soon became a popular choice for livestock feeding.

Alfalfa breeders recognise three types of cultivated alfalfa (lucerne) as members of a single species, *M. sativa.* The three subspecies are ssp *medicago (*purple alfalfa), ssp *falcata* (yellow alfalfa) and spp *varia* (variegated alfalfa). Ssp *varia* is a probable hybrid between ssp *medicago,*and ssp *falcata.* Common purple alfalfa is a high yielding, early maturing yet less hardy species. Yellow alfalfa has a lower yield but a higher level of hardiness than common purple alfalfa. Cultivars used in drier, cooler regions of Canada have a higher proportion of *M. falcata* germplasm, conferring winter dormancy and hence winter hardiness and a higher tolerance to grazing than those bred from mainly ssp *medicago* germplasm (Frame *et al*., 1998).

Alfalfa is believed to have originated in Iran, however related plants are found throughout central Asia and Siberia. Its cultivation around Lake Lucerne in Switzerland is thought to have resulted in the crop taking the name, lucerne. Until the early 1900's, alfalfa was not grown successfully in the Northern hemisphere due to the lack of cold hardiness. Wendelin Grimm introduced the hybrid variegated lucerne from Germany which formed the basis of cultivars capable of surviving the cold winters in the northern US and Canada (Frame *et al*., 1998). Alfalfa is the world's most important forage crop (Michaud *et al*., 1988).

A wide range of soil and climatic conditions are suitable for alfalfa production, however, well-drained soil with a neutral pH and good fertility produce an optimum forage. This long-lived perennial is more drought-tolerant than most other temperate forage legumes, including birdsfoot trefoil and red clover (Peterson *et al*., 1992), which become dormant under severe drought conditions. Alfalfa also has a tolerance for alkaline soils and a high salt content. However, it is intolerant of acidic soils with a pH below 6, poor drainage, or water logging (Sheaffer *et al*., 1988).

Unlike other forage legumes, alfalfa is generally grown in monoculture, although it can be mixed with other legumes and/or grasses. A grass/alfalfa sward may reduce weed invasion, provide a more balanced nutrient composition for successful ensiling, or the grass may utilize transferable nitrogen from alfalfa (Chamblee and Collins, 1988). However, mixtures may not always improve dry matter yields relative to alfalfa monoculture.

Alfalfa stands decline in yielding ability with age under irrigation and “optimum” management conditions (Hayman and McBride, 1984). Progressive annual decline in alfalfa dry matter yields has been attributed to many factors -competition from companion grasses, weed pressure, injury or loss of plants from pests and/or diseases, winter damage, poor drainage, or management factors such as uncontrolled grazing, over- frequent cutting, or inadequate fertilization.

It has a poor persistence if continuously stocked; sufficient regrowth between defoliations is critical to ensure stand survival. Alfalfa is best used in a rotational grazing system.

One of the important functions of alfalfa is its ability to fix nitrogen from the atmosphere and enhance the nitrogen balance of the soil, which the plant utilises in turn. This eliminates the need for nitrogen fertilizer. *Rhizobium meliloti* is one of the main bacterial groups that infects and induces nitrogen-fixing nodules on the roots of alfalfa plants. Estimates of nitrogen fixation by alfalfa vary widely but are generally higher than for other temperate forage legumes (Frame *et al.*, 1998). Soil mineral nitrogen or fertilizer nitrogen imposes a restriction on nitrogen fixation. The deficiency of certain minerals such as potassium, calcium or magnesium or excessive soil acidity may also limit nitrogen fixation (Frame *et al.*, 1998).

B. PROCESSING

In addition to its use in grazing systems, alfalfa is primarily used for hay, silage, artificially dried forage, and pelleted meal. Cutting alfalfa at the 10% bloom stage and then at 5-7 week intervals was shown to maximize dry matter (DM) production, provide forage of reasonable nutritive value and helped to maintain sward longevity in Minnesota (Sheaffer *et al.*, 1988). In certain regions under ideal conditions, six to nine cuttings per year may be achieved, whereas in other regions two to five cuttings may be the maximum. To ensure plant survival through the winter, the last harvest should be early enough to allow plants to build up carbohydrate and nitrogen reserves before cessation of growth, but not allow a heavy canopy to develop prior to winter. Studies demonstrated that three cuts per year yielded more than four cuts, although the forage was of lower nutritive value (Brink and Marten, 1989).

Before processing, alfalfa is cut and allowed to dry to varying moisture content in the field. Hay should be dried to approximately 85% dry matter. The optimum dry matter content for chopped silage is 30% when stored in bunker silos, 35% in concrete tower silos and 45% in oxygen limiting silos. Composition of alfalfa hay compared with silage is shown in Table 1. To produce dehydrated alfalfa, a regular supply of forage with a high protein content is required, as well as cutting before the growth reaches the bud stage. Proper dehydration of alfalfa can increase the utilization of forage protein by ruminants.

Alfalfa meal or alfalfa leaf meal is hay that has been dried (either naturally or artificially) and ground. Alfalfa leaf meal is of better quality, and contains not more than 18% crude fibre. Alfalfa meal includes stem fractions and therefore higher fibre. Alfalfa leaf meal and alfalfa meal are good sources of carotene. When processing alfalfa, it is important to retain the nutritious leaf fraction as much as possible during handling.

Table 1. Quality of alfalfa hay or silage made from the same second cut crop (Broderick, 1995).

Component	*Silage*	*Hay*
DryMatter(DM) gm/kg	413	850
NDF gm/kg DM	354	352
ADF gm/kg DM	265	257
CP gm/kg DM	212	197
NPNgm/100gm of totalN	49.4	7.7

Within the alfalfa plant, the leaves have a higher concentration of nutrients than stems, with the exception of potassium. Magnesium concentrations decline with crop maturity. In late-cut hay, Mg may be restricted to levels well below the minimum for animal requirements if soil potassium levels are high due to preferential uptake of potassium by the plants (Frame *et al*., 1998). Alfalfa has a low concentration of sodium, therefore, salt supplementation of cattle and sheep on alfalfa pasture has been beneficial to their health and production (Jagusch, 1982).

C. TRADITIONAL CHARACTERISTICS SCREENED BY ALFALFA DEVELOPERS

For registration/public release of new varieties of alfalfa in the US and Canada, only phenotypic characteristics are required to be considered. Main indicators of alfalfa quality for livestock feeding include the proximates, acid detergent fibre, neutral detergent fibre, lignin, and minerals (summarized in Table 2, Forage Genetics Inc., 2003). Published values of these components vary widely in the literature, depending on geographical location, environmental conditions, variety, time of harvest, and storage conditions. Therefore, it is important to make comparisons only with appropriate comparators, e.g., near isogenic lines, reference cultivars, or commercial varieties grown at the same time under similar conditions and locations.

Table 2. Constituents typically monitored in forages for livestock feeding (Forage Genetics, 2003, personal communication).

Constituent	***Importance***
Moisture	Feeding value
Proximates: Protein Fat Ash	Nutrition/feeding value
Acid Detergent Fibre	Digestibility
Neutral Detergent Fibre	Digestibility
Lignin	Digestibility/anti-quality factor
Minerals: Ca Cu Fe Mg Mn P K Na Zn	Nutrition

SECTION II – NUTRIENTS IN ALFALFA

Tables 3-6 summarize proximate, amino acid, fatty acid and mineral composition of alfalfa from a variety of databases.

Table 3. Proximate, lignin, acid detergent fibre (ADF), and neutral detergent fibre (NDF) composition of late vegetative/early bloom alfalfa. Data except for dry matter presented on a % dry matter basis.

%	NRC71[1]	NRC82[2]	Ensminger[3]	NRC96[4]	Monsanto[5]	Range
Dry matter	90.1	23.0	91.0	19.0	17.9 – 29.2	17.9 – 91.0
Crude Protein	19.7	19.0	17.9	25.0	15.3 – 25.8	15.3 – 25.8
Crude fat	2.2	3.1	2.6	2.9	1.3 – 3.2	1.3 – 3.2
Crude fibre	29.8	25.0	25.8	-	-	25.0 – 25.8
NDF	-	40.0	36.8	39.3	26.5 – 35.7	26.5 – 40.0
ADF	-	31.0	29.0	-	23.1 -33.4	23.1 – 33.4
Lignin	7.7	7.0	5.8	7.9	3.9–9.7	3.9 – 9.7
Ash	8.7	9.5	8.4	9.2	8.8 – 15.3	8.4 – 15.3

[1] NRC, 1971
[2] NRC, 1982
[3] Ensminger *et al.*, 1990
[4] NRC, 1996
[5] Monsanto, 2003

Table 4. Amino Acid Composition of Alfalfa. Data presented on a % of dry matter basis.

	Hay NRC82[1]	Hay NRC01[2]	Hay Literature[3]	Hay Monsanto[4]	Hay Range	Silage Range[5]
Ala	-	-	.70	.79-1.59	.70 – 1.59	.69-.94
Arg	1.14	1.18	.62	.71–1.54	.62 - 1.54	.27-.51
Asp	-	-	1.40	1.75-3.52	1.40 – 3.52	1.83-1.95
Cys	-	.32	.20	.18-.35	.18 - .35	-
Glu	-	-	1.20	1.52-3.03	1.20 – 3.03	1.27-1.48
Gly	1.03	-	.60	.71-1.47	.60-1.47	.67-.76
His	.50	.44	.28	.37-.74	.28 - .74	.14-.28
Ile	.96	.97	.50	.66-1.26	.50 – 1.26	.55-.76
Leu	1.64	1.68	.90	1.11-2.25	.90 – 2.25	.90-1.23
Lys	1.27	1.17	.59	.99-1.81	.59 – 1.81	.32-.74
Met	.36	.36	.18	.24-.48	.18 - .48	.06-.21
Phe	1.07	1.09	.65	.72-1.59	.72 – 1.59	.53-.79
Pro	-	-	.70	.75-1.34	.70 – 1.34	.89-1.14
Ser	.97	-	.60	.75-1.36	.60 – 1.36	.57-.67
Thr	1.08	1.00	.60	.61-1.15	.60 – 1.15	.63-.72
Trp	-	.35	-	.16-.31	.16 - .35	-
Tyr	.74	-	.50	.50-1.16	.50 – 1.16	.25-.41
Val	1.22	1.20	.60	.79-1.55	.60 – 1.55	.76-.94

[1] NRC, 1982.
[2] NRC, 2001.
[3] Cunningham *et al.*, 1994; Phuntsok *et al.* 1998.
[4] Monsanto, 2003.
[5] Christensen, 2004a; Phuntsok *et al.*, 1998.

Table 5. Fatty Acid Composition of Alfalfa.

	Hay gm/100 gm of FA[1]	Silage gm/100 gm of DM[2]
C12:0	0.70	0.01-0.03
C14:0	2.90	0.01-0.02
C16.0	27.6	0.41-0.47
C16.1	0.20	0.04-0.05
C17:0	2.15	0.01-0.11
C18:0	36.5	0.06-0.07
C18:1	4.11	0.06-0.07
C18:2	0.75	0.34-0.42
C18:3	-	0.14-0.63
Other	24.90	0.35-0.92
Total	100	2.09-2.10

[1] gm/100gm fatty acids; Bas *et al.*, 2003
[2] gm /100 gm dry matter; Christensen , 2004b

Table 6. Mineral Composition of late vegetation to early bloom alfalfa (expressed on dry matter basis).

	NRC71[1]	NRC82[2]	Ensminger[3]	NRC00[4]	Preston[5]	NRC01[6]	Monsanto[7]	Range
Na g/100gm	.15	.19	.15	.12	-	.03	.02-.21	.02 - .21
K g/100gm	2.08	2.09	2.56	2.51	2.50	2.56	1.39-4.31	1.39-4.31
Ca g/100gm	1.40	1.96	1.63	1.41	1.41	1.56	.90-1.53	.90-1.96
P g/100gm	.21	.30	.22	.22	.26	.31	.22-.45	.22-.45
Mg g/100gm	.30	.27	.34	.34	-	.33	.11-.45	.11-.45
Fe mg/100gm	.02	.03	.02	.02	-	.021	.02-1.54	.02 -1.54
S g/100gm	.30	.37	.30	.30	.27	.33		.27-.37
Cu mg/kg	13.4	10.0	12.6	12.7	-	10.0	5.3-10.2	5.3-13.4
Co mg/kg	.01	.13	.29	.29	-	.65		.01-.65
Mn mg/kg	31.5	43.0	36.2	36.0	-	49.0	34.6-109.5	31.5-109.5
Zn mg/kg	-	18.0	30.2	30.0	22.0	26.0	18.1-36.0	18.0-36.0
Se mg/kg	-	-	.55	.55	-	.20		.20-.55
Cl g/100gm	.38	.47	.38	.34	.38	.55		.34-.55

[1] NRC, 1971
[2] NRC, 1982
[3] Ensminger *et al*., 1990
[4] NRC, 2000
[5] Preston, 2003.
[6] NRC, 2001
[7] Monsanto, 2003

SECTION III –ANTINUTRIENTS AND SECONDARY METABOLITES IN ALFALFA

Bloat potential in ruminants

Legumes are unusual in that the very characteristics that make them valuable as ruminant feed (a high content of readily digestible protein and carbohydrate), can predispose animals to bloating, a potentially serious condition that can result in death. The etiology of bloat and plant and animal risk factors are reviewed in Clark and Reid (1974), Colvin and Backus (1988), Howarth *et al*. (1991), and Popp *et al*. (2000).

The condition and its incidence

Primary bloat or frothy bloat (tympanites) is the over-distension of the rumen caused by the accumulation of fermentation gases in a stable protein foam or froth (Tanner *et al*., 1995), and usually occurs as an outbreak in several animals on pasture that contains high levels of leguminous plants. Primary bloat can also occur in feedlot cattle. When an animal is experiencing pasture bloat, the stable froth is produced in the rumen in a "layer" on top of the ruminal contents (mostly liquid), and prevents the gas bubbles from rising to the top and dispersing their contents. Once the froth has formed and natural eructation is prevented, the rumen motility is initially increased, causing further frothing. Finally there is a loss of muscle tone and rumen motility. Death is a result of several factors, including the depressive effect of rumen distension on the heart and lungs and absorption of toxins from the rumen.

The main risk factor in pasture bloat is the rapid ingestion of immature/fast-growing legumes in pre-flowering stages. Alfalfa, red clover, and white clover have similar bloat potential. Other forage legumes are considered to be of low risk.

Ingestion of only the most succulent parts of the plant is an important risk factor, in addition to the sward type. Frost and growth of alfalfa at low temperatures have been shown to increase bloat risk by increasing the leaf cell constituents (soluble protein, pectic polysaccharides) implicated in pasture bloat (MacAdam and Whitesides, 1996). Wetness of the pasture has also been suspected to be a risk factor for bloat. It is, however, more likely that the real risk is the fast growth brought on by wet and favourable weather.

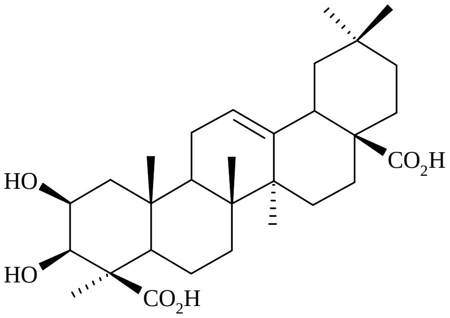
Several animal factors contribute to bloat (Mendel and Boda, 1961, Howarth *et al*., 1991). Young animals are considered more susceptible to acute and severe bloat than older animals, and it is suspected that animals can adapt to eating bloating pastures and are less susceptible after exposure. Fasting has also been shown to predispose animals to pasture bloat, but the mechanism is not established. Since there are individual differences in the ability of cattle to tolerate rumen distension and the presence of contributory factors in any given situation, some animals only suffer sub-clinical or mild bloating. While the toleration of mild bloat allows adaptation to new pastures, sub-clinical and mild bloat have been recognised as causing major losses on clover dominant pastures in the form of reduced feed intake and subsequent lower weight gains (Latimori *et al*., 1992; Rossi *et al*., 1997)

Bloat is a common problem in all areas in which temperate legumes are used as ruminant feed and has long been recognised as a major problem in countries like New Zealand, where clover forms an important part of the pastures (Carruthers *et al.*, 1987). Due to its association with clover, bloat has been considered a risk factor on organic farms, where clover often constitutes more than 50% of the sward content. However, research both in the UK and elsewhere in Europe on organic farms suggests that the incidence of clinical bloat is not higher than on conventional farms (Weller *et al.*, 1996; Frankow-Lindberg, and Danielsson, 1997).

Although there is no widely recognised test for bloating potential, selection for a low initial rate of digestion (to four hours) has been successfully used as the criterion in developing a "bloat-reduced" cultivar of alfalfa AC Grazeland Br (Coulman *et al.*, 2000). In addition to the initial rate of digestion, a number of other factors known to influence the bloat potential of forages can be measured, such as leaf venation pattern, fibre content and digestibility, cell wall thickness, ease of nucleation of rumen bacteria, and preferential synthesis of protein and reduction in lipids in chloroplasts (Lees *et al.*, 1982; Howarth *et al.*, 1979; Fay *et al.*, 1981; Lees, 1984; Stifel *et al.*, 1968).

Saponins

Saponins are divided into two groups, including the steroidal saponins, which occur as glycosides in some pasture grasses, and the triterpenoid saponins that occur in many temperate legumes, particularly alfalfa. Because saponins have a distinct foaming characteristic (Marston *et al.*, 2000), historically they have been considered a primary cause of bloat in animals grazing temperate forages. The development of low saponin varieties of alfalfa that still caused bloat suggests the importance of other factors as the main causal agent(s) (Majak *et al.*, 1980).



The monodesmodosidic medicagenic acid

A total of some 24 saponins have been identified in alfalfa (Bialy *et al.*, 1999) but the soyasapogenols, zanhic acid glycosides and medicagenic acid are quantitatively the most important (Table 7; Oleszek *et al.*, 1992; Massiot *et al.*, 1988; 1991). Saponins can have a positive or a negative role in plants. Supplementation with saponins has been shown to decrease ammonia production and protozoal count, and improve growth rates in lambs (Makkar and Becker, 2000). The toxicity of the various saponins to animals differs (Hostettmann and Marston, 1995). Triterpenoid saponins may reduce feed palatability and feed degradation in the rumen and their presence greatly limits the use of alfalfa in some non-ruminant diets (Lu and Jorgensen, 1987; reviewed in Oleszek, 1996). Poultry rations containing 10% alfalfa meal depress chick growth and egg production due to saponins (Birk, 1969; Bondi *et al.*, 1973; Pedersen *et al.*, 1972). Saponins are highly toxic to fish and amphibians (Cheeke, 1971; Khalil and El Adawy, 1994; Makkar and Becker, 2000)), but not to ruminants and swine (Bins and Pedersen, 1964). Symptoms of saponin toxicity, believed largely due to the medicagenic and zanhic acid content in alfalfa, include irritation to mouth and digestive tract, increased membrane permeability and, in acute cases, haemolysis (Oleszek, 1996). Zanhic acid glycosides may also cause production of intestinal gases. Ensiling of alfalfa can reduce the total saponin and medicagenic acid content (Kalac *et al.*, 1996).

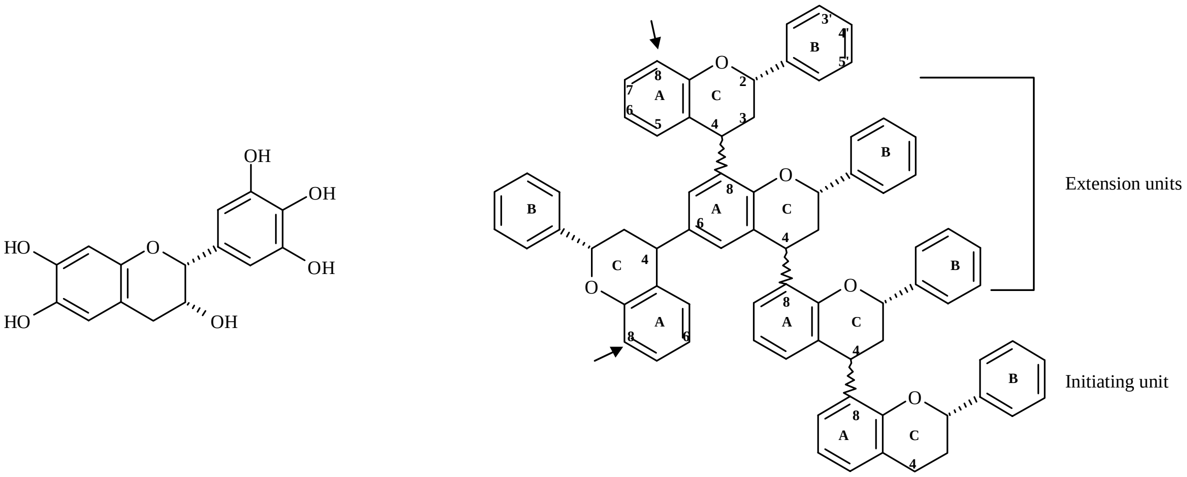
Table 7. Crude saponin and medicagenic acid content in various cultivars of alfalfa grown in Mexico (data from Pérez *et al*., 1997).

Cultivar	Crude saponin (g/kg dry matter)	Medicagenic acid (g/kg dry matter)
Sundor	17.7	0.023
Maxidor	11.7	0.027
Valenciana	8.8	0.165
Condor	8.5	0.024
Puebla 76	8.3	0.097
Inia 76	6.8	0.115
NK-819	5.9	0.013
Pierce	4.9	0.031

A number of analytical methods for various saponins have been used with varying success. Biological methods have been used but are dependent on the inhibition of the growth of the fungus, *Trichoderma viride*; these methods measure exclusively medicagenic acid glycosides. A high pressure liquid chromotography method was developed by Oleszek (2004), but the method has not been sufficiently modified to make it a practical routine procedure. There does not yet appear to be enough reliable data in the literature for meaningful comparisons with database values. It is important that analysis of appropriate comparators be conducted if saponin analysis is to be undertaken.

Condensed tannins

Condensed tannins (proanthocyanidins) derive from the flavonoid biosynthesis pathway and are essentially oligomers of flavan-3-ols of varying size and complexity. The chemistry, biochemistry and molecular regulation of these plant metabolites have been reviewed recently in Marles *et al*. (2003). They are widespread in the plant kingdom. A universal characteristic of condensed tannins is their ability to bind reversibly or irreversibly to proteins in feed, saliva and microbial cells, with microbial enzymes, and with endogenous proteins or other feed components and to inhibit ruminant microorganism activity (Bae *et al*., 1993; Hagerman *et al*., 1993; Jones *et al*., 1994; Tanner *et al*., 1994; Molan *et al*., 2001). The protein binding capacities among oligomers from different plant species and developmental stages differ with variations in proanthocyanidin and protein structure (Hagerman and Butler, 1981; Butler *et al*., 1984). Condensed tannins are also metal chelators and strong antioxidants (Muir, 1997; Stoutjeskijk *et al*., 2001; Slabbert, 1992). They have the potential to eliminate pasture bloat, improve the efficiency of conversion from plant to animal protein (ruminal bypass protein), reduce greenhouse gases, reduce gastrointestinal parasites, and inhibit insect feeding (Waghorn, 1990, Waghorn and Shelton, 1992; Neizen *et al*., 1995; 1998; Broderick and Albrecht, 1997; Aerts *et al*., 1999; Muir *et al*., 1999; McMahon *et al*., 2000; Butter *et al*., 2001; McSweeney *et al*., 2001). Tannins and saponins can act in an additive fashion in the rumen (Makkar *et al*., 1995).



A flavan-3-ol (epigallocatechin) monomer and a model proanthocyanidin oligomer showing the mechanism of extension through additional 4-8 and 4-6 inter-flavonoid linkages.

Condensed tannin levels exceeding 40-50 g kg^{-1} dry matter in forages may reduce protein and DM digestibility of the forages by ruminants and, consequently at high concentrations condensed tannins may be regarded as "antinutritional" compounds (Barry, 1989). However at low to moderate levels (20-40g kg^{-1} dry matter) tannins can increase the quantity of dietary protein, especially essential amino acids, flowing to the small intestine increasing production without any effect on feed intake (Aerts *et al.*, 1999).

Although having a potential detrimental effect on protein digestibility, generally, legumes that contain condensed tannins in excess of 50 g kg^{-1} DM do not cause bloat (Table 8). Dietary condensed tannins may provide a means to beneficially manipulate protein digestion and/or prevent pasture bloat in ruminants. Research efforts are currently being directed to genetically modify alfalfa to derepress its anthocyanidin biosynthetic pathway, or to isolate genes encoding steps of this pathway and introduce them into alfalfa and clover from other plant species (reviewed in Marles *et al.*, 2003). Within the past two years, a host of new condensed tannin biosynthetic and regulatory genes have been discovered to contribute to these strategies (reviewed in Marles *et al.*,2003). In addition, the *Lc* anthocyanin regulatory gene from maize induces small amounts of condensed tannin in alfalfa forage (Ray *et al.*, 2003), and the forage has a reduced initial rate of digestion and reduced gas production *in vitro* (Wang *et al.*, 2003).

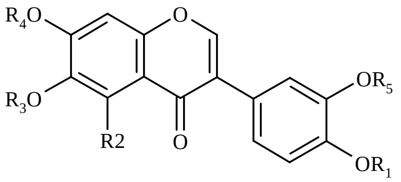
Table 8. Extractable and bound condensed tannin in bloating and bloat-safe temperate legumes measures by the butanol-HCl method (from Barry and McNabb, 1999).

Forage	Condensed tannin (g kg^{-1} dry matter)		
	Extractable	Bound	Total
Bloat safe			
Big trefoil (*Lotus pedunculatus*)	61	15	77
Birdsfoot trefoil (*Lotus corniculatus*)	36	11	47
Sulla (*Hedysarum coronarium*)	33	12	45
Sainfoin (*Onobrychis vicifolia*)	29	-	-
Potentially bloating			
Red clover (*Trifolium pratense*)	0.4	1.3	1.7
Alfalfa (*Medicago sativa*)	0.0	0.5	0.5

Oestrogen agonists and antagonists

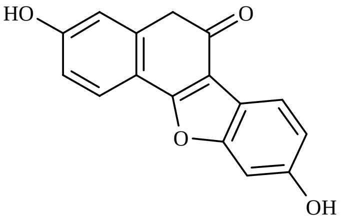
Adverse effects on reproductive health of farm animals grazing legumes have been recognised since the early 1940s when there was a substantial outbreak of infertility in Australian sheep grazing subterranean clover. This was subsequently shown to result from the presence of a variety of naturally occurring oestrogen mimetics, the so-called "phytoestrogens". Two types of phytoestrogens are now recognised; the coumesterols (coumestrol) related to the coumarins and quantitatively more important in alfalfa, and the isoflavonoids more widely distributed in *Trifolium* spp (Livingston, 1978). These compounds can also be induced in alfalfa with pathogen stress (Latunde-Dada and Lucas, 1985). Levels of coumestrol in alfalfa forage range from 2.99 – 104.37 ppm (Monsanto, 2003). The structure of the more important isoflavonoids recognised in red clover are shown in Table 9. Other isoflavone conjugates have been identified (Klejdus *et al.*, 2001). Phytoestrogen infertility appears to be species-specific, and ruminants such as cattle and sheep are more susceptible than other animals (Stob, 1983; Moule *et al.*, 1963; reviewed in Howarth, 1988).

Table 9. Structure of the isoflavonoids identified in red clover (from He *et al.*, 1996)



Compound	R_1	R_2	R_3	R_4	R_5
Daidzein	H	H	H	H	H
Daidzin	H	H	H	Glucose	H
Genistein	H	H	OH	H	H
Genistin	H	H	OH	Glucose	H
Formononetin	CH_3	H	H	H	H
Ononin	CH_3	H	H	Glucose	H
Biochainin A	CH_3	H	OH	H	H
Sissotrin	CH_3	H	OH	Glucose	H
Trifoside	Glucose	H	H	CH_3	H
Calycosin	CH_3	H	H	H	OH
Pectolinarigenin	CH_3	OH	OCH_3	H	H
Pratensein	CH_3	OH	H	H	OH
Pseudobaptigenin	$-CH_2^-$	H	H	H	$-O^-$

Formononetin and biochainin A are the two isoflavones found in the greatest amounts in forage legumes (Smolenski *et al.*, 1981) and together can reach 15g kg^{-1} dry matter in some red clover cultivars. Concentrations in white clover are usually substantially lower (0.5g kg^{-1} dry matter). The major metabolic transformation of the isoflavones occurs in the rumen. Biochainin A is demethylated to genistein and via ring cleavage to 4-ethylphenol and organic acids with the loss of all oestrogenic activity. Formononetin is mainly demethylated to daidzein and then to equol by hydrogenation and ring cleavage (Lundh, 1995). However, unlike the end products of biochainin A metabolism, equol is a more potent oestrogen mimetic than either of its parent compounds.

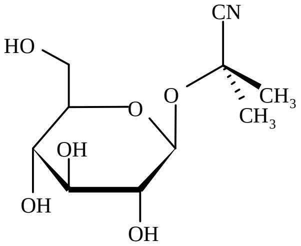


Coumesterol (coumestrol)

Coumesterol, present as the major phytoestrogen in alfalfa but also occurring in white clover, is not metabolised by the rumen flora and is absorbed in its original form. Coumesterol is known to be approximately 30-fold more effective than genistein in mice and to cause oestrogen-related disorders in animals. Concentrations in healthy plants rarely exceed 30-60 mg kg^{-1} dry matter, but coumesterols can accumulate in plants subject to fungal attack. Significant genetic variation exists in alfalfa for coumesterol (Hanson *et al*., 1965). In addition, doses showing no effects in the short term, may induce hormonal effects if consumed over a longer period.

Cyanogenic glycosides

The cyanogenic glycosides are composed of an α-hydrozynitrile type aglycone and a sugar moiety which is usually D-glucose. They are widely distributed in the plant kingdom but, within the temperate forage legumes, are considered a cause for concern only in some cultivars of white clover and *Lotus corniculatus* (Vetter, 2000). In populations of white clover, plants that produce HCN following damage of the leaves, and plants that fail to do so, co-exist within the same population. The difference in cyanogenic glucoside content is caused by variation in two genes: *Ac* regulating the production of the cyanogenic glucosides linamarin and lotaustralin, and *Li* regulating the production of the hydrolysing enzyme linamarase. Only plants that contain at least one active allele of each of the genes *Ac* and *Li* are cyanogenic. White clover and *Lotus corniculatus* contain varying amounts of both kinds of metabolites (reviewed in Smolenski *et al*.,1981).



Linamarin a cyanogenic glucoside from white clover

Ruminant animals are more susceptible to HCN poisoning than non-ruminants due to fast microbial breakdown of cyanogenic glycosides (Smolenski *et al*., 1981). Hydrocyanic acid released from linamarin and lotaustralin is further metabolised within the grazing animal to inorganic thiocyanate, which is goitrogenic. North American cultivars of white clover have a notably lower HCN-generating potential than most European cultivars although there are considerable differences among cultivars bred within countries (Wheeler, 1989). In Switzerland, cultivars with a mean HCN content above 370 mg HCN kg^{-1} dry matter are excluded from the national list. The HCN potential is also greatly affected by environmental factors and is increased by moisture stress, predation, low light intensity cool grazing conditions and low soil phosphorus supply (Vickery *et al*., 1986).

Other secondary metabolites

The occurrence of a wide range of secondary metabolites in temperate legumes has been documented in various natural product databases and resources (see for example http://www.ars.usda.gov) (Duke, 1992; Chapman and Hall, 1982-1998). This is particularly true for alfalfa and for *Trifolium pratense*, both of which have elicited interest as herbal products with claimed health benefits. However, in most instances, quantitative data is not available and would have to be generated as part of a comparative assessment. Measurement of known secondary metabolites would only be justified if there were reason to suspect some change to the metabolic pathway involved in their generation or if they were of known toxicity. Canavanine is a potentially toxic structural analogue of L-arginine that is a stored by many legumes including alfalfa (Rosenthal and Nkomo, 2000). Under normal conditions L-canavanine is found in seeds, cotyledons and the emerging shoots and only in very low amounts in older vegetative tissues. However, its cytotoxicity might warrant its inclusion in a comparative analysis.

SECTION IV – FEED USE OF ALFALFA AND ALFALFA PRODUCTS

Alfalfa is rich in protein, vitamins and minerals and is a main component in livestock rations. The natural phenolic defence compounds of alfalfa include a simple alkaloid, saponins, coumestans (which are increased by exposure to the pea aphid), isoflavones (pterocarpans and medicarpan, both of which are induced in response to fungal infection) and flavones (Massiot *et al*., 1988; 1991; Oleszek *et al*., 1992; Stochmal *et al*., 2001; Ray *et al*., 2003; reviewed in Howarth, 1988). Other natural components can be found in natural product databases (eg. Duke, 1992). Alfalfa accumulates only trace amounts of condensed tannins in forage (Goplen *et al*, 1980; Ray *et al*., 2003).

Throughout the world, alfalfa is recognised as a premium forage for feeding to dairy cattle and horses. It can also be a valuable feed for beef cattle, sheep and other livestock with high nutrient requirements such as lactating ewes and dairy goats or backgrounded calves. However, alfalfa is a forage legume with high bloat potential (Howarth *et al*., 1991; Popp *et al*., 2000). AC Grazeland Br is the world's first bloat-reduced variety (60-80% bloat-reduced), and was selected for a lower initial rate of digestion (Coulman *et al*., 2000). Plants of this variety have a thicker cell wall and fast regrowth (Goplen *et al*., 1993; Najda, 2002).

Alfalfa meal is not a suitable feedstuff for use at high dietary levels by non-ruminant animals except horses, rabbits and gestating sows. The problems associated with alfalfa use by monogastric animals include low protein digestibility, low digestible energy, moderately high fibre, saponins and phenolics content, and low palatability.

The feeding value of alfalfa is largely determined by the stage of growth as the nutritive value decreases as the plant matures. The leaves of the alfalfa plant are abundant in nutrients including protein, vitamin E and K, calcium, magnesium, potassium and carotene. The dry matter yield of alfalfa increases with advancing maturity but the nutritive value is reduced. Plant maturity results in a decline of the leaf:stem ratio, an increase in lignin content of the stem and leaf loss through leaf shatter.

In ruminants, the utilisation of alfalfa protein is inefficient and causes problems because of rapid turnover in the rumen and a high proportion of protein nitrogen which is lost as ammonia, although ruminal protein degradability declines with plant maturity (Amrane and Michaeletdoreau, 1993). The crude protein content is generally higher in ensiled alfalfa than in hay, due mainly to greater leaf loss in hay making; however, much more of the nitrogen comprises non-protein nitrogen (NPN) in silage (Broderick, 1995).

Compared with grass, alfalfa has higher intake characteristics and a higher animal production response per unit of DM ingested. The potential reasons are the rapid passage of digesta out of the rumen (which stimulates appetite), high concentration of soluble protein (which assists in microbial synthesis in the rumen), the stimulation of cellulose digestion, a low concentration of cell wall in the dry matter, and an adequate supply of minerals and vitamins (Conrad and Klopfenstein, 1988).

Specialty protein extracts of alfalfa are also used in livestock feeding; for example, xanthophyll is sometimes used to impart a yellowish colour to poultry eggs and flesh.

SECTION V – FOOD USES OF ALFALFA

The use of sprouted seeds as food has a very long history. Currently, in North America, sprouted mung bean and alfalfa seeds are often available in the fruit and vegetable sections of grocery stores and a wider range of sprouted seeds or seeds for sprouting, including mung beans and alfalfa as well as adzuki bean, Chinese cabbage, clover, lentil, onion and radish, are sold in natural and health food stores. Although only sprouts of alfalfa and clover from the list of forage legumes discussed in this document were identified as used for food in a brief search of the internet, it should not be assumed that other forage legumes might not at some stage be considered for sprouting for human food use. In addition to sprouts, protein extracts of alfalfa (e.g. rubisco) are receiving attention for possible use in various food applications.

Most people in a North American dietary context would be expected to consume minor quantities of these foods, roughly 60 mL (8-20 g serving, depending on the type of sprout) and only on an occasional basis. Among the small segment of committed users amounts of 1-2 cups per day may, however, be common.

A decision regarding the importance of assessing the nutrient composition of forage legumes used as sprouted seeds in human diets should be guided by the frequency and quantity of such sprouts in a given country and their contribution to nutrient intake. The fact that they are often promoted as being highly nutritious may also be a consideration in requesting data.

Table 10 shown below, showing composition of alfalfa sprouts, is extracted from the USDA National Nutrient Database for Standard Reference, Release # 16. This database provides data for sprouted alfalfa, kidney beans, mung beans, navy beans, pinto beans, lentils, peas, radish seeds, soybeans and wheat.

A comparison of the nutrient composition of one cup of alfalfa sprouts to recommended intakes of these nutrients suggests that the contribution is minor. A suggested minimum compositional analysis where alfalfa is likely to be sold for food use would be the analysis of fresh forage or sprouted alfalfa seed for the parameters listed in Table 11 with the addition of vitamin C, beta-carotene, folate and phytoestrogens to provide a basis for assessment of potential unintended effects with relevance to human food use.

Table 10. Composition of raw sprouted alfalfa seeds (Source: USDA National Nutrient Database for Standard Reference, Release 16, 2003).

NUTRIENT	UNIT	Value per 100 g	Value per 240 mL (33 g)	Sample Count	Standard Error	Value per 100 g dry matter
Water	g	91.140	30.076	10	1.226	
Energy (calculated)	kcal	29.000	9.570	0		327.314
Protein	g	3.990	1.317	10	0.563	45.034
Total lipid (fat)	g	0.690	0.228	10	0.141	7.788
Ash		0.400	0.132	10	0.044	4.515
"Carbohydrate,by difference"	g	3.780	1.247	0		42.664
"Fibre, total dietary"	g	2.500	0.825			28.217
"Sugars, total"	g	0.180	0.059	3	0.012	2.032
Calcium	mg	32.000	10.560	10	4.659	361.174
Iron	mg	0.960	0.317	10	0.114	10.835
Magnesium	mg	27.000	8.910	10	3.978	304.740
Phosphorus	mg	70.000	23.100	10	7.914	790.068
Potassium	mg	79.000	26.070	10	9.79	891.648
Sodium	mg	6.000	1.980	10	1.094	67.720
Zinc	mg	0.920	0.304	10	0.273	10.384
Copper	mg	0.157	0.052	10	0.017	1.772
Manganese	mg	0.188	0.062	10	0.019	2.122
Selenium	mcg	0.600	0.198	0		6.772
Vitamin C	mg	8.200	2.706	10	0.678	92.551
Thiamin	mg	0.076	0.025	10	0.005	0.858
Riboflavin	mg	0.126	0.042	10	0.017	1.422
Niacin	mg	0.481	0.159	10	0.044	5.429
Pantothenic Acid	mg	0.563	0.186	10	0.069	6.354
Vitamin B-6	mg	0.034	0.011	10	0.005	0.384
"Folate, total"	mcg	36.000	11.880	10	0.8	406.321
Vitamin B12	mcg	0.000	0.000	0		0.000
Vitamin A (carotenoids)	IU	155.000	51.150	0		1749.436
Vitamin E	mg	0.020	0.007	0		0.226
Vitamin K	mcg	30.500	10.065	0		344.244
Threonine	g	0.134	0.044	1		1.512
Isoleucine	g	0.143	0.047	1		1.614
Leucine	g	0.267	0.088	1		3.014
Lysine	g	0.214	0.071	1		2.415
Valine	g	0.145	0.048	1		1.637

SECTION VI – IDENTIFICATION OF KEY PRODUCTS AND SUGGESTED ANALYSIS FOR NEW FORAGE VARIETIES

Forage legumes are an essential component of the livestock feed industry. They also provide several environmental benefits including a reduction in soil erosion and the ability to fix nitrogen from the atmosphere. Forages are an excellent source of crude protein, carbohydrates, vitamins, calcium, magnesium, potassium, iron, cobalt and carotene. It is important that these nutrients are considered when evaluating novel legumes.

These plants are introduced into the growing environment as seeds and if the conditions are favourable, growth begins. An important stage of plant growth is the initiation of flowering or inflorescence. It is recommended that the forage legume be cut or grazed at this time, due to its optimum nutritive value and yield. After this point, the proportion of lignin increases and digestibility decreases. Forages are well adapted to the environments in which they grow. Plants have been selected to withstand frost and winter damage, drought, salinity, acidity or alkalinity.

Forage legumes can be processed in a variety of ways including hay, silage, pelleted meal or dehydrated cubes or simply remain as pasture. With the exception of pasture legumes, forages are processed to preserve nutrients and assist with handling of the product. These processes may influence the structure of the plant and the nutritive value for the animal.

The chemical composition of forages varies with physiological age and therefore forage quality analyses are essential. Analyses listed in Table 11 should be considered for new varieties. Additional analyses to be further considered, on a crop by crop basis are listed in Table 12. When evaluating a novel forage, the compositional analysis should be conducted on material sampled at the late vegetative/early bloom stage of growth, when hay and silage cuts are normally taken.

The risk of bloat and the presence of saponins within forage legumes are the main factors that limit the use of these plants.

Table 11. Suggested minimum compositional parameters to be analysed in hay or fresh forage legumes used for animal feed.

Parameter	Fresh Forage/Hay
Crude protein	X
NDF	X
ADF	X
Lignin (ADL or other)	X
Crude Fat	X
Ash	X
Minerals (Ca, P)	X
Amino acids	X

Table 12. Additional compositional parameters to be considered for analysis in hay, silage or fresh forage for legumes used for animal feed, on a crop by crop basis.

Parameter	Fresh Forage/Hay	Silage
Total condensed tannins	X	X
Total saponins	X	X
-Medicagenic acid	X	-
-Zanhic acids	X	-
Phytoestrogens		
-Coumesterol (and its methyl derivatives)	X	X
-Formononetin	X	X
-Daidzein	X	X
Cyanogenic glycosides		
-Liminarin	X	-
-Lotaustralian	X	-
Canavanine	X	-

SECTION VII – FORAGE LEGUMES OTHER THAN ALFALFA

The information presented in the review of alfalfa in this paper is largely applicable to all temperate legumes. The remainder of this paper serves to introduce other important forage legumes used in livestock feeding. Key components to be analysed in new forage varieties are identified in Table 11.

Red Clover (*Trifolium pratense* L.)

The *Trifolium* species are widely distributed. Within this genus there are 240 species found in most temperate regions. The total area where they can be found in the United States is believed to exceed that of alfalfa (Smith *et al*., 1985). Alfalfa and clovers collectively meet the legume pasture, hay and silage production requirements of temperate, humid and subhumid regions (Rumbaugh, 1990).

Red clover (*Trifolium pratense* L.) is an important forage legume grown in northern temperate areas of the world, especially Europe and North America. In the 1980's, 7 million hectares of red clover were grown in North America out of a world total of 20 million hectares (Smith *et al*., 1985). Red clover is thought to have originated in southeastern Europe and Asia Minor.

Physiology

Red clover is adapted to a wide range of soil and environmental conditions, especially poorly drained soils and is relatively tolerant of lower soil pH and lower soil fertility. A deep tap root allows red clover a high degree of resistance to drought although not as much tolerance as alfalfa or sainfoin. The optimum temperature for growth is 20-25°C and the optimum pH is 6-7.5. Although generally considered a short-lived perennial, improved U.S. types of red clover are relatively productive for three and sometimes four years.

Carbohydrates are important in red clover for the plant's survival overwinter. The polysaccharide starch is the principal storage carbohydrate, which accumulates in the roots during the growing season and is depleted during winter. Taking more than one autumn harvest reduces carbohydrate accumulation, and therefore reduces yield at the first harvest in the following year.

Red clover grown in monoculture or in combination with grasses is a major hay crop in several regions of the world. In North America, it is grown in the humid northeast and in the Pacific northwest of the US under irrigation and used as an annual in southeast U.S. (Taylor and Smith, 1995). For red clover, the yield potential is high; red clover varieties tend to have slightly lower forage yields than alfalfa in the area south of the U.S. - Canada border (Undersander *et al*, 2002). Red clover plants survive better in severe winters when sown with a grass, rather than as monoculture (Belzile, 1987). It is commonly grown for silage and pasture, and not commonly harvested for dry hay due to its slow drying rate. A common production practice in parts of North America is to harvest the second-cut crop for seed, following a first cut-crop for forage or silage. Silage management for red clover-dominant swards includes a first cut at the early flowering stage and a second cut 6-8 weeks later. Traditionally, red clover was regarded as a "difficult" crop for silage making due to low dry matter, low water soluble carbohydrate contents, and a high buffering capacity, which slowed the attainment of low pH for good fermentation. A satisfactory silage fermentation is more likely to result from red clover/grass mixtures because of higher dry matter and water soluble carbohydrate concentrations and lower nitrogen contents (Frame *et al*., 1998). Red clover has high bloat potential (Howarth *et al.*, 1991).

Nitrogen fixation

Rhizobial inoculation is not usually carried out in European countries, since most soils contain *R. leguminosarum* bv. *trifolii*. If red clover use is extended to soils without a previous history of clover growth, rhizobial inoculation of the seed is essential.

The total amount of nitrogen fixed by red clover and the contribution it makes to the nitrogen content of the soil can vary. The factors for variation in nitrogen fixation include, climatic and soil conditions, presence and efficacy of *Rhizobium*, companion species, and stage of plant development. Nitrogen fixation can contribute up to 80% of total nitrogen assimilation in red clover (Heichel *et al*., 1985). However, the rate of N_2 fixation may be greatly reduced due to drought, accumulation of inorganic nitrogen in soil, soil acidity, or plant defoliation (Maag and Nosberger, 1980).

Feed

Red clover has a high nutritive value for ruminants. It can improve the quality of autumn-saved forage for out-wintered livestock where the climate allows this practice. The digestibility of red clover’s primary growth declines with advancing maturity in a linear fashion and is related to the declining leaf:stem ratio. The decline in digestibility is associated with increasing lignin content and a reduction in degradability of polysaccharides other than starch (Taylor and Quesenberry, 1996).

Nutrient composition of red clover is shown in Tables 13 and 14. Compared with grasses, red clover is usually higher in concentrations of pectin, lignin, nitrogen, calcium, magnesium, iron and cobalt (Frame *et al*., 1998). Alfalfa and red clover have similar nutrient content. One of the main differences is that red clover contains polyphenol oxidases, which are enzymes that play a role in inhibiting plant proteases (protein degrading enzymes) and proteolysis (protein breakdown) in the silo. As a result of the polyphenol oxidase action, red clover protein is not broken down during silage fermentation to the same extent as alfalfa protein. Therefore, red clover has more undegradable protein (bypass protein 25-35%) than alfalfa (15-25%). Additional research has shown that when red clover and alfalfa are of similar fibre content, red clover may be more digestible than alfalfa, providing a more energy-dense forage to the diets of lactating dairy cows (Hoffman and Broderick, 2001). Unfortunately, red clover does not stand up to continuous stocking, but works well in a rotational stocking system. Red clover does not accumulate condensed tannins in forage (Sarkar *et al.*, 1976). As discussed in the antinutritional factors of alfalfa section, isoflavonoids are more common in clover species than alfalfa.

Table 13. Proximate, lignin, acid detergent fibre (ADF), and neutral detergent fibre (NDF) composition of Red Clover (*Trifolium pratense L.)* Harvested at Early Bloom Stage (expressed on dry matter basis).

%	Hay NRC71[1]	Hay NRC82[2]	Hay Ensminger[3]	Hay NRC96[4]	Hay Hoffman[5]	Range for Hay	Silage Lit Range[6]
Dry matter	87.3	89.0	87.0	89.0		.87 - .89-	21.1-53.5
Crude protein	21.4	16.0	21.4	20.8	18.4	16.0 – 21.4	14.9-22.5
Crude fat	3.9	2.8	3.9	3.0	-	2.8 – 3.9	4.3
Crude fibre	20.4	28.8	20.4	-	-	20.4 - 28.8	-
NDF	-	-	-	48.0	34.9	34.9 – 48.0	31.7-50.5
ADF	-	-	-	-	24.4	24.4	24.9-37.0
Lignin	-	10.0	-	16.67	4.3	4.3 – 10.0	4.2-4.3
Ash	9.7	8.5	9.7	7.0	-	7.0 – 9.7	1.9-11.5

[1] NRC, 1971
[2] NRC, 1982
[3] Ensminger *et al*. 1990.
[5] Hoffman *et al*., 1993
[6] Dewhurst *et al*., 2003; Broderick *et al*., 2001; Coblentz *et al*., 1998; Hoffman *et al*., 1997; Hoffman *et al*., 1993; Al-Mabruk *et al*., 2004

Table 14. Mineral Composition of late vegetation to early bloom Red Clover (*Trifolium pratense L.)* (expressed on dry matter basis).

	NRC71[1]	NRC82[2]	Ensminger[3]	NRC01[4]	Range
Na, mg/100 g	-	.19	-	.18	.18 - .19
K, mg/100 g	2.57	1.62	3.24	1.81	1.62 – 3.24
Ca, mg/100 g	1.77	1.53	1.55	1.38	1.38 – 1.77
P, mg/100 g	.31	.25	.37	.24	.24 - .37
Mg, mg/100 g	.51	.43	.39	.38	.38 - .51
Fe, mg/100 g	-	.018	.073	.024	.018 - .073
S, mg/100 g	-	.17	-	.16	.16 - .17
Cu, mg/kg	-	11.0	21.1	11.0	11.0 – 21.1
Co, mg/kg	-	.16	.23	.16	.16 - .23
Mn, mg/kg	-	73.0	86.7	108.0	73.0 – 108.0
Zn, mg/kg	-	17.0	52.0	17.0	17.0 – 52.0
C1, mg/100 g	-	.32	-	.32	.32

[1] NRC, 1971
[2] NRC, 1982
[3] Ensminger *et al*., 1990
[4] NRC, 2001

White Clover (*Trifolium repens* L.)

On a world basis, white clover (*Trifolium repens* L.) is the most important true clover species for grazed swards within the genus *Trifolium*. White clover is used primarily in Western Europe and North America, New Zealand and Australia. There are approximately 15 million hectares of pasture with white clover in Australasia and 5 million hectares in the United States. This legume is thought to have originated in the Mediterranean area (Taylor *et al*., 1980).

Production

White clover is usually grown in association with suitable grass species such as perennial ryegrass, or in the United States, Kentucky bluegrass (*Poa pratensis*). Grass/clover swards may be utilised successfully by a range of grazing systems for both intermittent (rotational), continuous grazing, or a blend of both types in the same season. In Atlantic Canada, white clover is allowed to stockpile from late summer for use in late autumn thus extending the grazing season (Fraser *et al*., 1993; Kunelius and Narasimhalu, 1993). White clover has high bloat potential (Howarth *et al*., 1991), likely due to the large amount of foliage.

White clover plays an important role in arable cropping particularly in sustaining or building up soil fertility whether as a green manure or as a legume-rich phase within a crop rotation (Barney, 1987; Ten Holte and Van Keulen, 1989). It also has a role, when undersown in arable crops such as corn (maize), in protecting the soil from erosion and minimising damage from harvesting operations (Lampkin, 1990). In monoculture or in combination with grass, white clover acts as a protective ground cover or soil-stabilization plant (Parente and Frame, 1993). There is increased interest in the use of white clover as an understorey to supply the nitrogen requirements of a cereal crop.

Physiology

White clover is capable of spreading and establishing itself in suitable niche situations in grazed pastures. It can tolerate severe defoliation better than other types of legumes, is more persistent, and has the ability to colonise bare spaces (Burdon, 1983). White clover is adapted to a wide range of soils but it does not thrive in poorly drained soils (McAdam, 1983), shallow drought-prone soils (Foulds, 1978, Thomas, 1984) or saturated, unamended peat (Burdon, 1983). Unlike red clover and alfalfa, white clover has a continual generation of new leaves.

Nitrogen fixation

For nitrogen fixation, rhizobial populations of the strain *Rhizobium leguminosarum* bv. *trifolii* infect the roots of white clover and are highest in soils in which Trifolium species have been or are currently prevalent. Otherwise, white clover needs to be inoculated with effective and competitive strains of Rhizobia (Newbould *et al*., 1982).

Feed

Nutrient composition of white clover is shown in 15 and 16. The digestibility of white clover is higher than that of other temperate forage legumes. White clover is almost always grown in association with grasses; approximately 10 - 20% white clover allows for optimal animal productivity (Curll, 1982; Stewart, 1984). Dry matter intake by a variety of livestock has been shown to be higher for white clover than for grass, regardless of feed form (fresh, dried, hay or silage) (Thomson, 1984). The physical, chemical and plant anatomical features all contribute to the superior intake quality of white clover. Sheep spend less time masticating white clover, and the weight per bite is heavier due to a greater bulk density (Edwards *et al*., 1995). Heifers spend a longer time grazing and ruminating on grass than clover (Orr *et al*., 1996). The rate of particle degradation in the rumen is faster with white clover than with ryegrass (Moseley and Jones, 1984, Ulyatt *et al*., 1986) and there is enhanced ruminal digestion with the legume (Beever and Thorp, 1996). In addition to a faster rate of intake for white clover than for grass at comparable digestibility levels, ingested nutrients in white clover may be utilised more efficiently (Beever *et al*., 1985) and more efficient use made of metabolizable energy (ME) for animal production (Rattray and Joyce, 1974). White clover does not accumulate condensed tannin in forage, but accumulates these polymers in flowers (Sarkar *et al.*, 1976; Foo *et al.*, 1982). Some white clover cultivars can contain cyanogenic glycosides.

Table 15. Proximate, lignin, acid detergent fibre (ADF), and neutral detergent fibre (NDF) composition of White Clover *(Trifolium repens L.)* Harvested at Late Vegetative / Early Bloom Stage (expressed on a dry matter basis).

%	NRC71[1]	NRC82[2]	Ensminger[3]	NRC96[4]	Range	Dewhurst[5]
	Hay	Hay	Hay	Hay	Hay	Silage
Dry matter	17.7	90.0	89.0	89.0	17.7 – 90.0	24.2
Crude protein	28.2	22.0	22.4	22.4	22.0 – 28.2	26.1
Crude fat	3.3	2.7	2.7	2.7	2.7 – 3.3	-
Crude fibre	15.7	21.2	20.8	-	15.7 – 21.2	-
NDF	-	-	36.0	36.0	36.0	26.9
ADF	-	32.0	32.0	-	32.0	27.4
Lignin	-	7.0	6.6	7.0	6.6 – 7.0	-
Ash	11.9	10.1	9.4	9.4	9.4 – 11.9	10.0

[1] NRC, 1971
[2] NRC, 1982
[3] Ensminger *et al.*, 1990
[4] NRC, 1996
[5] Dewhurst *et al.*, 2003

Table 16. Mineral Composition of late vegetation to early bloom White Clover *(Trifolium repens L.)* (expressed on DM basis).

	NRC71[1]	NRC82[2]	Ensminger[3]	NRC01[4]	Range
Na, g/100 g	.39	.13	.13	.13	.13 - .39
K, g/100 g	2.13	2.62	2.44	2.44	2.13 – 2.44
Ca, g/100 g	1.40	1.35	1.45	1.45	1.35 – 1.45
P, g/100 g	.51	.31	.34	.33	.31 - .51
Mg, g/100 g	.45	.48	.47	.47	.45 - .48
Fe, g/100 g	.034	.041	.047	.047	.034 - .047
S, g/100 g	.33	.21	.21	.21	.21 - .43
Cu, mg/kg	-	10.0	9.40	9.41	9.40 – 10.0
Co, mg/kg	-	.16	.16	.16	.16
Mn, mg/kg	307.2	95.0	123.1	123.0	95.0 – 307.2
Zn, mg/kg	-	17.0	17.0	17.9	17.0 – 17.9
C1, g/100 g	.61	.30	.30	.30	.30 - .61

[1] NRC, 1971
[2] NRC, 1982
[3] Ensminger *et al.*, 1990
[4] NRC, 2001

Alsike Clover (*Trifolium hybridum* L.)

Alsike clover (*Trifolium hybridum* L.) is grown in temperate and subartic areas of Europe, Asia, North and South America and some regions of Australasia. This legume tends to yield and grow better in cooler climates. It is thought to have originated in northern Europe. This short-lived perennial has similar persistence to red clover.

Production

This legume grows best in cool temperate conditions, but is adaptable to wet, infertile or acid soils that are unsuitable for red clover or alfalfa (Townsend, 1995). However, it is intolerant of drought or salinity. Alsike clover tends to be very tolerant of cold and frost and therefore, allows for its establishment and growth in cooler climate areas. The majority of the world's alsike clover seed is produced in North America, including Alberta, Idaho and Oregon.

This forage legume is usually grown in combination with grasses and other legumes. In North America, it is recommended to grow alsike in a mixture with red clover and a grass such as timothy (Townsend, 1995). The agronomic and management requirements of alsike clover are similar to those of red clover, and the forage causes bloat (Howarth *et al*., 1991).

Feed

As with other legumes, alsike clover is rich in protein and minerals although it declines in digestibility as the plant matures. It is very palatable for livestock and continues to bloom throughout the season. It is used for pasture and hay although the high moisture content makes it difficult to dry for hay production. The regrowth after taking a cut of hay is excellent for use in a fall grazing system. It is important to note that hay or pasture containing more than 5% alsike clover is not recommended for horses; it is associated with alsike clover poisoning characterized by liver damage and photosensitization in horses. The causal toxin in not known, and may originate with an associated fungus rather than the clover itself (Knight and Walter, 2003). Alsike clover composition is shown in Tables 17 and 18.

Table 17. Proximate Analysis of Alsike Clover (*Trifolium hybridum L.)* Harvested at Late Vegetative / Early Bloom Stage (expressed on a dry matter basis).

	NRC71[1]	Ensminger[2]	NRC82[3]	Range
Dry matter	87.4	88.0	19.0	19.0 – 88.0
Crude protein	14.2	14.2	24.1	14.2 – 24.1
Crude fat	2.7	2.8	3.2	2.7 – 3.2
Crude fibre	30.1	29.9	17.5	17.5 – 30.1
Ash	8.7	8.7	12.8	8.7 – 12.8

[1] NRC, 1971
[2] Ensminger *et al*., 1990
[3] NRC, 1982

Table 18. Mineral Composition of late vegetation to early bloom Alsike Clover (*Trifolium hybridum L.)* (expressed on a dry matter basis).

	NRC71[1]	Ensminger[2]	NRC82[3]	Range
Na, g/100 g	.46	.46	.46	.46
K, g/100 g	2.74	2.22	2.62	2.22 – 2.74
Ca, g/100 g	1.29	1.30	1.32	1.29 – 1.32
P, g/100 g	.26	.25	.28	.25 - .28
Mg, g/100 g	.32	.45	.31	.31 - .45
Fe, g/100 g	.045	.026	.046	.026 - .046
S, g/100 g	.21	.19	.17	.17 - .21
C1, g/100 g	.78	.78	.77	.77 - .78
Cu, mg/kg	6.0	6.0	6.0	6.0
Mn, mg/kg	117.0	69.0	117.0	69.0 – 117.0

[1] NRC, 1971
[2] Ensminger *et al*., 1990
[3] NRC, 1982

Subterranean clover (*Trifolium subterraneum* L.)

Subterranean clover (*Trifolium subterraneum* L., also known as subclover) is a winter annual that is very important in the drylands of Australia. This legume is thought to have originated in the Mediterranean region and was developed for pastoral use and soil improvement especially in Australia, where it is used in rotation with cereal cropping. It is also used in the northwest United States, southern Europe, Latin America and New Zealand to a lesser degree. It is adapted to regions with hot dry summers and moist winters with mild temperatures (6-14^{O} C) and abundant rainfall.

Production

Subterranean clover germinates rapidly in the moist autumn, grows during winter and spring, flowering and seeding occur in late winter/early spring and then survives the dry summer as a dormant seed. This efficient system is designed to escape the damaging summer drought. This legume grows best when soil fertility levels are relatively high especially with high phosphorus and sulfur, regardless, it is valued for its ability to grow in less fertile, acidic soils (Frame *et al*., 1998).

Along with its use in grazing, this legume is used for erosion control, hydro-seeding road side banks and as a green manure or weed smothering cover in horticultural and orchard situations (Caporali *et al*., 1993).

Nitrogen fixation

If a pasture is being renewed by sowing with subclover or in a mixture with grass, inoculation with a rhizobium strain is advisable unless there has been a long history of satisfactory subclover growth. Using the correct strain of *Rhizobium leguminosarum* bv.*trifolii* has a positive impact on establishment and performance of subclover.

Feed

Subclover is outstanding among annual forage legumes for its tolerance to grazing (Caporali *et al*., 1993). An annual seed crop is essential for subclover persistence in pasture. Therefore, it is important that the sward's potential to produce a seed crop is not jeopardized by overgrazing. In common with other legume species, subclover is rich in crude protein compared to grasses. The protein concentration declines steadily with advancing plant maturity, as does the digestibility.

Grazed subclover in irrigated swards has high digestibility and nitrogen content and low NDF, ADF and lignin content (Frame *et al*., 1998). Effective rumen-degradable protein in the leaf can be so low that microbial protein synthesis in the rumen is limited, adversely affecting animal production (Mulholland *et al*., 1996). Table 19 shows proximate composition of Subterranean clover.

Table 19. Proximate Analysis of Subterranean Clover (*Trifolium subterraneum L.)* Harvested at Early Bloom Stage (reported on dry matter basis).

%	NRC71[1]
DM	90
CP	30.5
Fat	3.7
CF	10.1
Ash	11.1

[1] NRC, 1971

Birdsfoot Trefoil (*Lotus corniculatus* L.) and Greater Lotus (*Lotus* spp.)

The species within the *Lotus* genus are referred to as pioneer legumes because they are suitable for developing pastures on acidic, infertile soils in cool, moist areas of the world (Frame *et al*., 1998). Both perennials and annuals are components of this genus. There are a large number of species of *Lotus* Zandstra and Grant, 1968; USDA Plants Database, 2003). Three examples used for forage include birdsfoot trefoil (*Lotus corniculatus L.*), marsh birdsfoot trefoil (big trefoil or lotus) (*Lotus uliginosus* Schkuhr. also called *L. pedunculatus*) and narrow-leaf birdsfoot trefoil (*Lotus tenuis*).

Birdsfoot trefoil was not introduced to North America until the early 1900's, however, it was very common in Europe, Africa and Asia. The majority of species are found in the Mediterranean region and this is thought to be their area of origin. Approximately 1.2 million hectares are grown in northeastern North America on acidic, infertile and low-input management systems (Beuselinck and Grant, 1995). Greater lotus can be found in Britain, France and Germany as well as the northwestern United States.

Production

Birdsfoot trefoil is suited to clay soils which are too wet or too acidic for alfalfa. Birdsfoot trefoil is drought tolerant, even more so than alfalfa (Peterson *et al*., 1992). It also persists in poorly drained soil more than alfalfa or red clover (Barta, 1986) and is highly tolerant of saline soils (Schachtman and Kelman, 1991). Narrow-leaf birdsfoot trefoil is adapted to poorly drained soils and sown in central Europe and northern United States especially on saline and alkaline soils. The *Lotus* species are slow to become popular due to their slow establishment, slow growth rate and poor competitive ability (McKersie *et al*., 1981). The greater lotus species is not winter hardy. Birdsfoot trefoil is very winter hardy once established, although less than alfalfa, but it does not survive in harsh Canadian prairie conditions. Unlike alfalfa, which has a significant period of flower-free growth, lotus plants have a short non-flowering period.

Birdsfoot trefoil is very useful on marginal land, and is a non-bloating legume (Howarth *et al.*, 1991) due to the presence of forage condensed tannins (Foo *et al*., 1982; Sarkar *et al*., 1976). Big trefoil also contains tannins (Foo *et al*., 1982). A number of reports from different areas of the world confirm the use of lotus species, especially birdsfoot trefoil, for pasture renovation in a variety of situations, ranging from lowland grazing to alpine pastures (Frame *et al*., 1998). If this legume is used for a combination of hay and pasture, the hay crop should be taken at the early bloom stage and the subsequent regrowth grazed at the first flower. Weed control is very important, especially in the establishment year, since birdsfoot trefoil is not competitive in a weedy stand (Beuselink and Grant, 1995). This legume produces less forage with hay yields of 25-30% less than alfalfa. It is recommended that birdsfoot trefoil be used only in areas that are not suitable for alfalfa production because of soil acidity, poor drainage or low fertility.

Feed

There is little information available on the chemical composition of Lotus trefoil forage, but birdsfoot trefoil nutritive value is similar to that of alfalfa (Marten and Jordan, 1979). Composition of birdsfoot trefoil is shown in Tables 20 and 21. The lignin content of birdsfoot trefoil is lower than in other legumes such as white clover, red clover or alfalfa. The *Lotus* species contain varying amounts of floral and forage condensed tannins (Sarkar *et al*., 1976; Foo *et al*., 1982; Muir *et al*., 1999; Muir, unpublished), as well as varying amounts of flavonols (Harney and Grant, 1964; 1965) and cyanogenic glycosides (Grant and Sidhu, 1967). *Lotus uliginosis* has a moderate condensed tannin content ranging from 40-245 $mg.g^{-1}$ dry weight (Lees *et al*., 1994; Muir *et al*., 1999). *Lotus corniculatus* produces small-to-moderate amounts (Muir *et al*., 1999). Some *Lotus corniculatus* plants have very high levels of cyanogenic glycosides (Zandstra and Grant, 1968).

The more upright types of birdsfoot trefoil are suited to hay and silage production with a possibility of 2-3 cuts per season. This legume is of major importance for hay, silage and grazing in the northern United States and eastern Canada (Beuselinck and Grant, 1995). Birdsfoot trefoil is highly palatable to livestock, even though it accumulates condensed tannins. Therefore, these pastures are best used in a rotational stocking system (Van Keuren and Davis, 1968, Van Keuren *et al*., 1969). Early spring grazing or continuous stocking will weaken and eliminate a stand of birdsfoot trefoil. Birdsfoot trefoil is persistent, and will last for several years if managed properly.

Table 20. Proximate, lignin, acid detergent fibre (ADF), and neutral detergent fibre (NDF) composition of Birdsfoot Trefoil *(Lotus corniculatus L.)* Harvested at Late Vegetative / Early Bloom Stage (expressed on a dry matter basis).

%	NRC71[1]	NRC82[2]	Ensminger[3]	NRC96[4]	Hoffman[5]	Range
DM	89.0	92.0	91.0	91.0	100	89.0 - 100
Crude protein	16.0	16.3	15.3	15.9	17.0	15.3 – 16.3
Crude fat	2.2	2.5	2.1	2.1		2.1 – 2.5
Crude fibre	29.6	30.7	32.3			29.6 – 32.3
NDF			47.0	47.5	44.4	44.4 47.5
ADF		36.0	36.0		35.8	35.8 – 36.0
Lignin		9.0		9.1	9.8	9.1 – 9.8
Ash	7.6	7.0	7.4	7.4		7.0 – 7.6

[1] NRC, 1971
[2] NRC, 1982
[3] Ensminger *et al*., 1990
[4] NRC, 1996
[5] Hoffman *et al*., 1993

Table 21. Mineral Composition of early bloom Birdsfoot Trefoil *(Lotus corniculatus L.)* (expressed on DM basis).

	NRC71[1]	Ensminger[2]	Range
Na, g/100 g	.07	.07	.07
K, g/100 g	1.92	1.92	1.92
Ca, g/100 g	1.70	1.7	1.70
P, g/100 g	.27	.23	.23 - .27
Mg, g/100 g	.51	.51	.51
Fe, g/100 g	.023	.023	.023
S, g/100 g	.25	.25	.25
Cu, mg/kg	9.0	9.3	9.0 – 9.3
Co, mg/kg	.11	.11	.11
Mn, mg/kg	29.0	28.7	28.7 – 29.0
Zn, mg/kg		77.2	77.2

[1] NRC, 1971
[2] Ensminger *et al*., 1990

Sainfoin (*Onobrychis viciifolia* Scop.)

Sainfoin (*Onobrychis viciifolia scop*.) is also known as St. Foin, cock’s head, esparcet, holy clover or holy grass. In French, sainfoin, is interpreted to mean “healthy hay”, which is probably referring to its non-bloat characteristics. This perennial legume is indigenous to temperate western Asia and southern Europe. It can be found on dry calcareous soils of the western United States and Canada (Miller and Hoveland, 1995), although its lack of genetic variability has prevented it from becoming agriculturally important in either country.

Production

Sainfoin grows well on calcareous soils having a pH of 6 or higher, which tend to be too dry or too barren for clover or alfalfa. It is even more drought-resistant than alfalfa; however, it yields less (Rogers, 1976). Sainfoin yields best on deep, well-drained soils, and will not withstand wet soils or high water tables. It is somewhat intolerant of saline soils and tends to grow well on soils that are low in phosphorus. Sainfoin requires soil rich in lime and can withstand cold temperatures. It is not as winter hardy as the locally-recommended cultivars of alfalfa, and tends to be very susceptible to invasion from weeds because of its slow growth during the establishment year.

Grown in monoculture or in combination with grasses such as fescue or cocksfoot, this legume competes poorly with creeping, rooted grasses. The stage of growth at the time of cutting determines the quality of the hay or silage, cutting at mid-flowering for hay and early flowering for silage. Growth after the first harvest is nutritious and preferred by livestock. However, overgrazing should be avoided since re-growth will be limited, especially if grazing is intensive. Sainfoin is very palatable and is grazed by livestock in preference to alfalfa. Forage dry-matter yields of sainfoin are about 20% lower under dryland conditions compared with alfalfa, and may be 30% or more lower in irrigated areas.

Unlike alfalfa, sainfoin does not drop its lower leaves; stems remain succulent as the plant matures so that quality does not decrease as rapidly. Unfortunately, use of sainfoin has been limited by the cost and availability of seed. Seed supplies have been inadequate, primarily because reliance on native insect pollinators provides inconsistent seed yields. Also, with the increase in cheap sources of N fertilizer, this legume’s popularity has declined.

This legume is recommended only for short term rotations in pure stands or for planting in grass legume mixtures (along with alfalfa, birdsfoot trefoil, meadow bromegrass or orchard grass) that persist after sainfoin declines. The seeding of sainfoin with a noncompetitive grass may help to boost yields and reduce weed pressure. The advantages of sainfoin for pasture use include excellent quality and palatability that give superior animal performance without the danger of bloat (Howarth *et al*., 1991). Addition of 10-20% sainfoin to an alfalfa diet also suppressed most of the bloating in steers (McMahon *et al*., 1999).

Nitrogen Fixation

The nitrogen fixation abilities in sainfoin are poor in comparison with alfalfa and clovers. For good establishment and growth, sainfoin must be inoculated with a special rhizobium prior to planting. Nitrogen-fixing bacteria may be short-lived or ineffective so that nitrogen fertilisation may be required for this legume.

Feed

Sainfoin is rich in protein similar to other legumes, however, its digestibility is limiting. It has lower crude protein and digestibility than alfalfa (Karnezos *et al*., 1994). Sainfoin is rich in minerals compared to grasses, but its calcium and sodium contents are much lower than in other forage legumes (Spedding and Diekmahns, 1972).Sainfoin is higher in carbohydrates than alfalfa, and lower in crude protein, fibre and ash. Sainfoin forage and flowers contain moderate levels of condensed tannin ranging from 27-75 mg.g-1 dry weight (Koupai-Abyazani *et al*., 1993; Marais *et al*., 2000). Substantially lowered beef production costs occur when cattle are raised in alfalfa mixed pastures that include sainfoin as a source of condensed tannin (Popp *et al.*, 2000.).

Cicer milkvetch (*Astragalus cicer*)

Cicer milkvetch (*Astragalus cicer*) is a long-lived perennial that is native to the European continent. This legume is grown in a wide variety of environments, since it performs well on poor, infertile soil. Cicer milkvetch is grown in Canada on a small scale.

Production

This pasture legume is a hardy forage plant with deep roots and a creeping growth habit. It is tolerant to drought, slight acidity and alkalinity, but is intolerant to waterlogged soils. Cicer milkvetch is more accepting of late spring and early frosts than alfalfa.

Two years are required after establishment to produce any hay or pasture. Cicer milkvetch tolerates grazing and grows well throughout the season. An advantage to this legume is its bloat-safe property, which occurs because of its reticulate leaf veins and epidermal thickness (Howarth *et al*., 1979; Lees *et al*., 1982). Yields for this legume are comparable to alfalfa in a longer growing season area. Due to its slow spring growth and slow recovery after harvest, it may only be harvested 2 or 3 times per season. It is competitive in combination with grasses and therefore requires an equally competitive grass if the legume is to be equally maintained. These grasses include creeping foxtail, meadow bromegrass, orchard grass and tall fescue.

Feed

The protein content of cicer milkvetch equals or exceeds that of other legumes. This high protein level is due to the leaf:stem ratio, which is 40% higher than alfalfa as well as its ability to hold its leaves during the drying and baling processes. The moisture content, when harvested, is on average 4-8% higher than alfalfa or sainfoin. This results in an extended drying time that is approximately three days longer than other legumes. It is especially well suited for use in a pasture environment and resists damage from overgrazing. Cicer milkvetch tends to be readily consumed by all classes of livestock either in the form of hay or pasture. No cases of bloat have been reported for cicer milkvetch.

Sweet clover (*Melilotus officinalis*)

Sweet clover (*Melilotus officinalis*) is a hardy, drought-tolerant biennial that has adapted to a wide range of soils. This legume is tolerant of alkalinity but not acidity (Gorz and Smith, 1978). The yellow type of sweet clover is more drought-tolerant, shorter in stature, and earlier maturing than the white type of sweet clover (*Melilotus alba*).

Production

This legume is used in Canada and the United States cornbelt, in areas with alkaline soils, for both hay and pasture, as well as an aid for erosion control with its deep root system. Sweet clover should be cut prior to the bud stage for good quality hay. For grazing, regrowth will occur if a 30 cm stubble is maintained.

Feed

As with other clover species, this legume has the potential to cause problems with bloat, although the potential is not as high as alfalfa and clovers (Howarth *et al.*, 1991). Sweet clover produces coumarins, a sweet-smelling phenolic that develops into dicoumarol under sub-optimal hay-curing conditions (wet, mouldy). Dicoumarol is an anti-coagulant that causes livestock death from internal bleeding (sweet clover disease). Low-coumarin varieties have been developed (Goplen, 1971; 1981).

Serradella (*Ornithopus* spp)

Serradella is a summer annual which is native to south-western Europe. This legume is a winter or cool season annual when it is grown in mild regions, such as southern Australasia. There are two species, Pink or French serradella (*Ornithopus sativus* Brot.) which is cultivated for forage in some parts of Europe, Australia, high altitudes in Kenya and South Africa; and Yellow serradella (*Ornithopus compressus* L.) which occurs widely in natural pastures in countries surrounding the Mediterranean on non-calcareous soils.

Production

It grows on all soil types on which subterranean clover is grown but also on sandy, gritty soils where clover cannot grow (Gladstones and McKeown, 1977). The yellow type of serradella is confined to areas that receive at least 500 mm of rainfall per year.

Feed

Similar to most legumes, the crude protein and digestibility decline with advancing plant maturity, although the rates of decline are slower than for alfalfa or red clover (Iglesias and Lloveras, 2000).

Once established, serradella can be grazed in systems similar to those for subclover with similar stocking rates but it can also be cut for silage (Taylor and Hughes, 1978). The dry matter yields for this legume are quite variable and are dependent on several factors. The pink serradella variety tends to have a high nutritive value (Gladstones and Barrett-Lennard, 1964). In north-western Spain, pink serradella, planted in early fall, can be used alongside corn in a double-cropping system (Iglesias and Lloveras, 1998).

Serradella is used as an understorey for grazing in agroforestry situations in New Zealand due to its nitrogen fixing ability. It can also be used as an understorey in vineyards, growing while the vines are dormant, controlling weeds and supplying nitrogen to the vines (Lloveras, 1987).

Sulla (*Hedysarum coronarium* L)

Sulla (*Hedysarum coronarium* L.) is also known as Italian sainfoin, French honeysuckle or sweet vetch. This short-lived perennial is thought to have originated in the western Mediterranean region and North Africa (Duke, 1981).

Production

Sulla is the main legume in southern Italy with approximately 250 000 hectares used for grazing and hay (Martiniello and Ciola, 1994). It has been evaluated for use in North America but at the present time occupies few acres of commercial production (Allen and Allen, 1981). Sulla is mainly sown alone, but can be grown with a cereal or in a mixture with other legumes and on soils with a pH greater than 6-6.5.

Feed

The forage is of high nutritive value (especially the leaflets), and therefore it is important that sulla is cut prior to the onset of flowers for an optimal hay product. With respect to grazing this legume is best utilised in a rotational grazing system. *Hedysarum* species contain floral and forage condensed tannins which eliminate the risk of bloat (Skadhauge *et al*., 1997)

SECTION VIII – REFERENCES

Aerts, J.R., Barry, T.N. and McNabb, W.C. 1999. Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. Agric. Ecosyst. Environ. 75: 1-12.

Allen, O.N. and Allen, E.K. 1981. *The Leguminosae, a source book of characteristics, uses and nodulation*. Univ. Wisconsin Press, Madison, WI, USA.

Al-Mabruk, R.M., Beck, N.F.G. and Dewhurst, R.J. 2004. Effects of silage species and supplemental vitamin E on the oxidative stability of milk. J. Dairy Sci. 87:406-412.

Amrane, R. and Michaeletdoreau, B. 1993. Effect of maturity stage of Italian ryegrass and lucerne on ruminal nitrogen degradability. Ann. Zootech. 42: 31-37.

Bae, H.E., McAllister, T.A., Yanke, J., Cheng, K.-J. and Muir, A.D. 1993. Effects of condensed tannins on endoglucanase activity and filter paper digestion by Fibrobacter succinogenes S85. Appl. Environ. Microbiol. 59: 2132-2138.

Barney, P.A. 1987. The use of *Trifolium repens*, *Trifolium subterraneum* and *Medicago lupulina* as overwintering leguminous green manures. Biol. Agric. Hort. 4, 225-234.

Barry, T.N. 1989. Condensed tannins: Their role in ruminant protein and carbohydrate digestion and possible effects upon the rumen ecosystem. In: Nolan, J.V., Leng, R.A. and Demeyer, D.I. 1988. The roles of Protozoa and Fungi in Ruminant Digestion. Penambul Books, Armidale, NSW Australia.pp. 153-169.

Barry, TN. and McNabb, W.C. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Br. J. Nutr. 81: 263-272.

Barta, A.L. 1986. Metabolic response of *Medicago sativa* L. and *Lotus corniculatus* L. roots to anoxia. Plant Cell Env. 9: 127-131.

Bas, P., Archimede, H., Rouzeau, A. and Sauvant, D. 2003. Fatty acid composition of mixed-rumen bacteria: Effect of concentration and type of forage. J. Dairy Sci. 86:2940-2948.

Beever, D.E. and Thorp, C. 1996. Advances in the understanding of factors influencing the nutritive value of legumes. In: Younie, D. (ed.) *Legumes in Sustainable Farming Systems*. Occasional Symposium No. 30, British Grassland Society, Reading, pp. 194-207.

Beever, D.E., Thomson, D.J., Ulyatt, M.J., Cammell, S.B. and Spooner, M.C. 1985. The digestion of fresh perennial ryegrass (*Lolium perenne* L. cv. Melle) and white clover (*Trifolium repens* L. cv. Blanca) by growing cattle fed indoors. Br. J. Nutr. 54: 763-775.

Belzile, L. 1987. Effect of companion timothy on winter survival of red clover. Can. J. Plant Sci. 67: 1101-1103.

Beuselinck, P.R. and Grant, W.F. 1995. Birdsfoot trefoil. In: Barnes, R.F. Miller, D.A. and Nelson, C.J. (eds) *Forages*, 5th edn, Vol. 1, An Introduction to Grassland Agriculture. Iowa State University Press, Ames, Iowa pp.237-248.

Bialy, Z., Jurzysta, M., Oleszek, W., Piacente, S. and Pizza, C. 1999. Saponins in alfalfa (*Medicago sativa* L.) root and their structural elucidation. J. Agric. Food Chem. 47: 3185-3192.

Bins, W. and Pedersen, M.W. 1964. Effecting of feeding high saponin hay to four-month-old Holstein-Friesian bulls. Proc. 7th Conf. Rumen Function, Chicago, IL.

Birk, Y. 1969. Saponins. Pp. 169-210. In: Liener, E.I. (ed.) Toxic Constituents of Plant Foodstuffs. Academic Press, New York.

Bondi, A., Birk, Y. and Gestetner, B. 1973. Forage saponins. Ppg. 511-528. In: Butler, G.W. and Bailey R.W. (eds.). Chemistry and Biochemistry of Herbage. Academic Press, New York.

Brink, G.E. and Marten, G.C. 1989. Harvest management of alfalfa - nutrient yield vs. forage quality and relationship to persistence. J. Prod. Agric. 2: 32-36.

Broderick, G.A. 1995. Performance of lactating dairy cows fed either alfalfa silage or alfalfa hay as the sole forage. J. Dairy Sci. 78: 320-329.

Broderick, G.A. and Albrecht, K.A. 1997. Crop quality and utilization. Ruminal in vitro degradation of protein in tannin-free and tannin-containing forage legume species. Crop Sci. 37: 1884-1891.

Broderick, G.A., Walgenbach, R.P. and Maignan, S. 2001. Production of lactating dairy cows fed alfalfa or red clover silage at equal dry matter or crude protein contents in the diet. J. Dairy Sci. 84:1728.

Burdon, J.J. 1983. Biological flora of the British Isles: *Trifolium repens.* J. Ecol. 71: 307-330.

Butler, L.G., Riedl, D.J., Lebryk, D.G. and Blytt, H.J. 1984. Interaction of proteins with sorghum tannin: mechanism, specificity and significance. JAOCS 61: 916-920.

Butter, N.L., Dawson, J.M., Wakelin, D. and Buttery, P.J. 2001. Effect of dietary condensed tannins on gastrointestinal nematodes. J. Agric. Sci. 137: 461-469.

Caporali, F., Campiglia, E. and Paolini, R. 1993. Prospects for more sustainable cropping systems in central Italy based on subterranean clover as a cover crop. In: Baker, M.J. (ed.) Proceedings of the XVII International Grassland, Congress, New Zealand and Australia, Vol. III. New Zealand Grassland Association., Palmerston North pp.2197-2198.

Carruthers, V. R. O'Connor, M. B. Feyter, C. Upsdell, M. P. and Ledgard, S. F. 1987. The 1986 MAF bloat survey. Proceedings of the 4th Seminar of the Dairy Cattle Society of the New Zealand Veterinary Association held at Rotorua, New Zealand, 18-20 November 1987. Massey University, Palmerston North, New Zealand: pp 11-16.

Chamblee, D.S. and Collins, M. 1988. Relationships with other species in a mixture. In: Hanson, A.A., Barnes, D.K. and Hill, R.R., Jr. (eds) Alfalfa and Alfalfa Improvement. Agronomy Monograph No. 29, ASA/CSSA/SSSA, Madison, Wisconsin, pp. 439-461.

Chapman and Hall., 1982-98. The Combined Chemical Dictionary on CD-ROM. HeadFAST/CD Software © Head Software Int. PST. Release 2.2.

Christensen, R.A. 2004a. unpublished data from the following experiments: Christensen, R.A., Drackley, J.K., LaCount, D.W. and Clark, J.H. 1994. Infusion of four long-chain fatty acid mixtures into the abomasum of lactating dairy cows. J. Dairy Sci. 77:1052-1069;., Overton, T.R.,, J.H., Drackley, J.K., Nelson, D.R., and Blum, S.A. 1996. Effects of dietary fat with or without nicotinic acid on nutrient flow to the duodenum of dairy cows. J. Dairy Sci. 79:1410-1424; and R.A. Christensen and J.H. Clark, personal communication.

Christensen, R.A. 2004b. unpublished data from the following experiments: Christensen, R.A., Clark, J.H., Drackley, J.K. and Blum, S.A. 1998. Fatty acid flow to the duodenum and in milk from cows fed diets that contained fat and nicotinic acid. J. Dairy Sci. 81:1078; Christensen, R.A., Cameron, M.R., Clark, J.H., Drackley, J.K., Lynch, J.M. and Barbano, D.M. 1994. Effects of amount of protein and ruminally protected amino acids in the diet of dairy cows fed supplemental fat. J. Dairy Sci. 77:1618-1629; and Christensen, R.A., Drackley, J.K., LaCount, D.W. and Clark, J.H. . 1994. Infusion of four long-chain fatty acid mixtures into the abomasum of lactating dairy cows. J. Dairy Sci. 77:1052-1069.

Cheeke, P.R. 1971. Nutritional and physiological implications of saponins. A review. Can. J. Anim. Sci. 51: 621-632.

Clark, R.T.J. and Reid, C.S.W. 1974. Foamy bloat of cattle. A review. J. Dairy Sci. 57: 753-785.

Coblentz, W.K., Fritz, J.O., Fick, W.H., Cochran, R.C. and Shirley, J.E. 1998. In situ dry matter, nitrogen, and fiber degradation of alfalfa, red clover, and eastern gamagrass at four maturities. J. Dairy Sci. 81: 150-161.

Colvin, H.W. and Backus, R.C. 1988. Bloat in sheep (Ovis aries). Mini Review. Comp. Biochem. Physiol. 91A: 635-644.

Conrad, H.R. and Klopfenstein, T.J. 1988. Role in livestock feeding - greenchop, silage, hay and dehy. In: Hanson, A.A., Barnes, D.K. and Hill, R.R., Jr. (eds) *Alfalfa and Alfalfa Improvement*. Agronomy Monograph No. 29, ASA/CSSA/SSSA, Madison, Wisconsin, pp. 539-566.

Coulman, B., Golen, B., Majak, W., McAllister, T., Cheng K.-J,, Berg, B., Hall, J., McCartney, D. and Acharya, S. 2000. A review of the development of a bloat-reduced alfalfa cultivar. Can J. Plant Sci. 80: 487-491.

Cunningham, K.D., Cecava, M.J.., and Johnson, T.R. 1994. Flows of nitrogen and amino acids in dairy cows fed diets containing supplemental feather meal and blood meal. J.Dairy Sci. 77:3666-3675.

Curll, M.L. 1982. The grass and clover content of pastures grazed by sheep. Herbage Abstracts 52: 403-411.

Dewhurst, R.J., Fisher, W.J., Tweed, J.K.S., and Wilkins, R.J. 2003. Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. J. Dairy Sci. 86:2598-2611.

Duke, J.A. 1981. Handbook of Legumes of World Economic Importance. Plenum Press, New York and London.

Duke, J.A. 1992. Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants. Boca Raton, FL. CRC Press.

Edwards, G.R., Parsons, A.J., Penning, P.D. and Newman, J.A. 1995. Relationship between vegetation state and bite dimensions of sheep grazing contrasting plant species and its implications for intake rate and diet selection. Grass and Forage Sci. 50: 378-388.

Ensminger, M.E., J.E. Oldfield and W.W. Heinemann. 1990. Feeds & Nutrition. 2nd Edition. Ensminger Publishing Co., Clovis, CA, USA.

Fay, J.P., Cheng, K.-J., Hanna, M.R., Howarth, R.E. and Costerton, J.W. 1981. A scanning electron microscopy study of the invasion of leaflets of a bloat-safe and a bloat-casuing legume by rumen microorganisms. Can. J. Microbiol. 27: 390-399.

Foo, L.Y., Jones, W.T., Porter, L.J. and Williams, V.M. 1982. Proanthocyanidin polymers of fodder legumes. Phytochem. 21: 933-935.

Foulds, W. 1978. Response to soil moisture supply in three leguminous species. New Phytologist 80: 535-545.

Frame, J., Charlton, J.F.L. and Laidlaw, A.S. 1998. Temperate Forage Le*gumes*. CAB International, Wallingford, U.K.

Frankow-Lindberg, B. E. and Danielsson, D. A. 1997. Energy output and animal production from grazed grass/clover pastures in Sweden. Biol. Agric. Hort. 14: 279-290.

Fraser, J., Sutherland, K. and Martin, R.C. 1993. Effects of autumn harvest date on the performance of white clover/grass mixtures in Nova Scotia. J. Agric. Sci. 121:315-321.

Gladstones, J.S. and Barrett-Lennard, R.A. 1964. Serradella- a promising pasture legume in Western Australia. J. Australian Inst. Agric. Sci. 30: 258-262.

Gladstones, J.S. and McKeown, N.R. 1977. Serradella - a Pasture Legume for Sandy Soils. Bulletin 4030, Western Australia Department of Agriculture, Perth.

Goplen, B.P. 1981. Norgold – A low coumarin yellow blossom sweet clover. Can. J. Plant Sci. 61: 1029-1021.

Goplen, B.P. 1971. Polara, a low coumarin cultivar of sweet clover. Can. J. Plant Sci 51: 241-251.

Goplen, B.P., Howarth, R.E., Sarkar, S.K. and Lesin, K. 1980. A search for condensed tannins in annual and perennial species of Medicago, Trigonella, and Onobrychis. Crop Sci. 20: 801-804.

Goplen, B.P., Howarth, R.E. and Lees, G.L. 1993. Selection of alfalfa for a lower initial rate of digestion and corresponding changes in epidermal and mesophyll cell wall thickness. Can. J. Plant Sci. 73: 1111-122.

Gorz, H.J. and Smith, W.K. 1978. Sweetclover. In: Heath, M.E., Metcalfe, D.S., Barnes, R.F. (Eds) Forages:The Science of Grassland Agriculture 3rd edition, pp 159-166.

Grant, W.F. and Sidhu, B.S. 1967. Basic chromosome number, cyanogenic glycoside variation, and geographic distribution of *Lotus* species. Can. J. Bot. 45: 639-647.

Hagerman, A.E. and Butler, L.G. 1981. The specificity of proanthocyanidin-protein interactions. J. Biol. Chem. 256: 4494-4497.

Hagerman, A.E. and Robbins. C.T. 1993. Specificity of tannin-binding salivary proteins relative to diet selection by mammals. Can. J. Zool. 71: 628-633.

Hanson, C.H., Loper, G.M., Kohler, G.O., Bickoff, E.M.., Taylor, K.W.., Kehr, W.R.., Stanford, E.H., Dudley, J.W., Pedersen, M.W., Sorensen, E.L., Carnahan, H.L. and Wilsie, C.P. 1965. Variation in Coumestrol Content of Alfalfa as Related to Location, Variety, Cutting Year, Stage of Ggrowth, and Disease. UDSA Tech. Bull. 1333. U.S. Government Printing Office, Washington, DC.

Harney, P.M. and Grant, W.F. 1964. A chromatographic study of the phenolics of species of Lotus closely related to L. corniculatus and their taxonomic significance. Am. J. Bot. 51: 621-627.

Harney, P.M. and Grant, W.F. 1965. A polygonal presentation ofchromatographic investigations on the phenolic content of certain species of *Lotus*. Can. J. Genet. Cytol. 7: 40-51.

Hayman, J.M. and McBride, S.D. 1984. The Response of Pasture and Lucerne to Irrigation. Technical Report 17, Winchmore Irrigation Research Station, Ministry of Agriculture and Fisheries, p.79. (Cited in Douglas, 1986.)

He, X-G., Lin, L-Z. and Lian, L-Z. 1996. Analysis of flavonoids from red clover by liquid chromatography-elecrtospray mass spectrometry. J. Chromatography 755: 127-132.

Heichel, G.H., Vance, C.P., Barnes, D.K. and Henjum, K.I. 1985. Dinitrogen fixation and N and dry matter distribution during 4 year stands of birdsfoot-trefoil and red clover. Crop Sci. 25, 101-105.

Hoffman, P.C., Sievert, S.J., Shaver, R.D., Welch, D.A., and Combs, D.K. 1993. In situ dry matter, protein, and fiber degradation of perennial forages. J. Dairy Sci. 76: 2632-2643.

Hoffman, P.C., Combs, D.K., Brehm, N.M., and Welch, D.A. 1997. Performance of lactating dairy cows fed red clover or alfalfa silage. J. Dairy Sci. 80:3308-3315.

Hoffman, P. and Broderick, G. 2001. Red Clover Forages for Lactating Dairy Cows. Focus on Forage, University of Wisconsin.

Hostettmann, K. and Marston, A. 1995. Saponins. Cambridge University Press, Cambridge.

Howarth, R.E. 1988. Antiquality factors and non-nutritive chemical components. In: Alfalfa and Alfalfa Improvement. Agronomy monograph 29: 493-514.

Howarth, R.E., Goplen, B.P., Fay, J.P. and Cheng, K.-J. 1979. Digestion of bloat-causing and bloat-safe legumes. Ann. Rech. Vet. 10: 332-334.

Howarth, R.E., Chaplin, R.K., Cheng, K.-J., Goplen, B.P., Hall, J.W., Hironaka, R., Majak, W., Radostits, O.M. 1991. Bloat in Cattle. Agriculture Canada Publication 1858/E. Communications Branch. Agriculture and Agri-Food Canada, Ottawa.

Iglesias, I. and Lloveras, J. 1998. Annual cool-season legumes for forage production in mild winter areas. Grass and Forage Sci. 53(4): 318-325.

Iglesias, I. and Lloveras, J. 2000. Forage production and quality of serradella in mild winter areas in north-west Spain. New Zealand J. Agric. Res. 43: 35-40.

Jagusch, K.T. 1982. Nutrition of ruminants grazing lucerne. In: Wynn-Williams, R.B. (ed.) *Lucerne for the 1980s*. Special Publication No. 1, Agronomy Society of New Zealand, pp. 73-78.

Jones, G.A., McAllister, T.A., Muir, A.D. and Cheng, K.-J. 1994. Effects of sainfoin (*Onyobrychis viciifolia Scop*.) condensed tannins on growth and proteolysis by four strains of ruminal bacterial. Appl. Environ. Microbiol. 60: 1374-1378.

Kalac, P., Price, K.R. and Fenwick, G.R. 1996. Changes in saponin content and composition during ensilage of alfalfa (*Medicago sativa* L). Food Chemistry 56: 377-380.

Karnezos, T.P., Matches, A.G. and Brown, C.P. 1994. Spring lamb production on alfalfa, sainfoin and wheatgrass pastures. Agronomy Journal 86: 497-502.

Khalil, A. H. and El Adawy, T. A. 1994. Isolation, identification and toxicity of saponin from different legumes. Food Chem. 50:197-201

Klita, P.T., Mathison, G.W., Fenton, T.W. and Hardin, R.T. 1996. Effects of alfalfa root saponins on digestive function in sheep. J. Anim. Sci. 74: 1144-1156.

Klejdus, B., Vitamvasova-Sterbova, D. and Kuban, V. 2001. Identification of isoflavone conjugates in red clover (*Trifolium pratense*) by liquid chromatographic-mass spectrometry after two-dimensional solid-phase extraction. Analytical Chimica Acta 450: 81-97.

Knight, A.P. and Walter, R.G. 2003. Plants affecting the skin and liver. In: A Guide to Plant Poisoning of Animals in North America, A.P. Knight and R.G. Wlater (eds). Publisher: Teton NewMedia, Jackson WY. Internet Publisher: International Veterinary Information Service, New York, USA. (www.ivis.org)

Koupai-Abyazani, M.R., Muir, A.D., Bohm, B.A., Towers, G.H.N. and Gruber, M.Y. 1993. The proanthocyanidin polymers in some species of *Onobrychis*. Phytochemistry 34: 113-117

Kunelius, H.T. and Narasimhalu, P.R. 1993. Effect of autumn harvest date on herbage yield and composition of grasses and white clover. Field Crops Res. 31: 341-349.

Lampkin, N. 1990. Organic Farming. Farming Press Books, Ipswich.

Latimori, N. J. Kloster, A. M. Amigone, M. A. Cuerpo, L. and Pizzi, A. 1992. Treatment of pastures with paraquat in the control of bloat: effect on weight gain and residues in animal tissues. Revista Argentina de Produccion Animal 12: 217-222.

Latunde-Dada, A.O. and Lucas, J.A. 1985. Involvement of the phytoalexin medicarpin in the differential response of callus linesof Lucerne (Medicago sativa) to infection by Verticillium albo-atrum. Physiol. Plant Path. 26: 31-42.

Lees, G.L. 1984. Cuticle and cell wall thickness: Relations to mechanical strength of whole leaves and isolated cells from some forage legumes. Crop Sci. 24: 1077-1081.

Lees, G.L. 1992. Condensed tannins in some forage legumes: Their role in prevention of ruminant pasture bloat. In: Hemingway, R.W. and Laks, P.E. (eds.). Polant Polyphenols 1. pp. 915-935.

Lees, G.L., Howarth, R.E. and Goplen, B.P. 1982. Morphological characteristics of leaves from some legume forages: relation to digestion and mechanical strength. Can. J. Bot. 60: 2126-2132.

Lees, G.L., Hinks, C.F. and Suttill, N.H. 1994. Effect of high temperature on condensed tannin accumulation in leaf tissues of big trefoil (Lotus uliginosis Schkuhr). J. Sci. Food. Agric. 65: 415-421.

Livingston, A.L. 1978. Forage plant estrogens. J. Toxicol. Environ. Health 4: 301-324.

Lu, C.D. and Jorgensen, N.A. 1987. Alfalfa saponins affect site and extent of nutrient digestion in ruminants. J. Nutr. 117: 919-927.

Lloveras, J. 1987. Traditional cropping systems in northwestern Spain (Galicia). Agricultural Systems 23: 259-275.

Lundh, T. 1995. Metabolism of estrogenic isoflavones in domestic animals. Proc. Soc. Exp. Biol. Med. 208: 33-39.

Maag, H.P. and Nosberger, J. 1980. Photosynthetic rate, chlorophyll content and dry matter production of *Trifolium pratense* L. as influenced by nitrogen nutrition. Angewandte Botanik 54: 187-194.

MacAdam, J. W. and Whitesides, R. E. 1996. Growth at low temperatures increases alfalfa leaf cell constituents related to pasture bloat. Crop Sci. 36: 378-382.

Majak, W., Howarth, R.E., Fesser, A.C., Goplen, B.P. and Pedersen, M.W. 1980. Relationships between ruminant bloat and the composition of alfalfa herbage. II. Saponins. Can. J. Anim. Sci. 60: 699-708.

Makkar, h.P.S., Blummel, M. and Becker, K. 1995. In vitro effects of and interactions between tannins and saponins and fate of tannins in the rumen. J. Sci. Food. Agric. 69: 481-493.

Makkar, H.P.S. and Becker, K. 2000. Beneficial effects of saponins on animal production. In: Oleszek, W. and Marston, A. (eds.) Saponins in Food, Feedstuffs and Medicinal Plants. Kluwer Academic Publishers, Boston, MS. Pp. 281-286.

Marles, M.A.S., Ray, H. and Gruber, M.Y. 2003. Review. New perspectives on proanthocyanidin biochemistry and molecular regulation. Phytochemistry 64: 367-383.

Marais, J.P.J., Mueller-Harvey, I., Brandt, E.V. and Ferreira, D. 2000. Polyphenols, condensed tannins, and other natural products in Onyobrychis viciifolia (Sainfoin). J. Agric. Food Chem. 48: 3440-3447.

Marston, A., Wolfender, L.-L. and Hostettmann, K. 2000. Analysis and isolation of saponins from plant material. In: Oleszek, W. and Marston, A. (eds.). Saponins in Food, Feedstuffs and Medicinal Plants. Kluwer Academic Publishers, Boston, MS. Pp. 1-12.

Marten, G.C. and Jordan, R.M. 1979. Substitution value of birdsfoot trefoil for alfalfa-grass in pasture systems. Agronomy Journal 71: 55-59.

Martiniello, P. and Ciola, A. 1994. The effect of agronomic factors on seed and forage production in perennial legumes sainfoin (*Onobrychis viciifolia* Scop.) and French honeysuckle (*Hedysarum coronarium* L.) Grass and Forage Sci. 49: 121-129.

Massiot, G., Lavaud, C., Guillaume, D. and Le Men-Olivier, L. 1988. Reinvestigation of the sapogenins and prosapogenins from alfalfa (Medicago sativa). J. Agric. Food Chem. 36: 902-909.

Massiot, G., Lavaud, C., Besson, V., Le Men-Olivier, L. and Van Binst, G. 1991. Saponins from the aerial part of alfalfa (Medicago sativa). J. Agric. Food Chem. 39: 78-82.

McAdam, J.H. 1983. Characteristics of Grassland on Hill Farms in N. Ireland - Physical Features, Botanical Composition and Productivity. Report, Queens University of Belfast/ Department of Agriculture for Northern Ireland, Belfast.

McKersie, B.D., Tomes, D.T. and Yamamoto, S. 1981. Effect of seed size on germination, seedling vigour, electrolyte leakage, and establishment of birdsfoot trefoil (*Lotus corniculatus* L.). Can. J. Plant Sci. 61: 337-343.

McMahon, L.R., McAllister, T.A., Berg, B.P., Majak, W., Acharya, S.N., Popp, J.D., Coulman, B.E., Wang, Y. and Cheng K.-J. 2000. A review of the effects of forage condensed tannins on rumimal fermentation and bloat in grazing cattle. Can. J. Plant Sci. 80:469-485.

McMahon, L.R., Majak, W., McAllister, T.A., Hall, J.W., Jones, G.A., Popp, J.D. and Cheng K.-J. 1999. Effects of sainfoin on *in vitro* digestion of fresh alfalfa and bloat in steers. Can. J. Anim. Sci. 79:203-212.

McSweeney, C.S., Palmer, B., McNeill, D.M. and Krause, D.O. 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Animal Feed Sci. Tech. 91: 83-93.

Mendel, V.E. and Boda, J.M. 1961. Physiological studies of the rumen with emphasis on the animal factors associated with bloat. J. Dairy Sci. 44: 1881-1898.

Michaud, R., Lehman, W.F., and Rumbaugh, M.D. 1988. World Distribution and historical development. In: A. Hanson, D.K. Barnes, and R.R. Hill (eds) Alfalfa and Alfalfa Improvement, American Society of Agronomy, Madison, WI., pp 259-25-91.

Miller, D.A. and Hoveland, C.S. 1995. Other temperate legumes. In: Barnes, R.F., Miller, D.A. and Nelson, C.J. (eds) *Forages*, 5th edn. Vol.1, *An Introduction to Grassland Agriculture*. Iowa State University Press, Ames, Iowa, pp.273-281.

Molan, A.L., Attwood, G.T., Min, B.R. and McNabb, W.C. 2001. The effect of condensed tannins from Lotus pedunculatus and Lotus corniculatus on the growth of protteolytic rumen bacteria in vitro and their possible mode of action. Can. J. Microbiol.47: 626-633.

Monsanto. 2003. Safety, Compositional and Nutritional Assessment of Roundup Ready Alfalfa Events J101 and J103. U.S. FDA/CFSAN. BNF84.

Moseley, G. and Jones, J.R. 1984. The physical digestion of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) in the foregut of sheep. Br. J. Nutr. 52, 381-390.

Moule, G.R., Braden, A.W.H. and Lamond, D.R. 1963. The significance of oestrogens in pasture platns in relation to animal production. Anim. Breed. Abstr. 31: 139-157.

Muir, A.D. 1997. Antioxidative activity of condensed tannins. In: Shahidi (ed.). Natural Antioxidants: Chemistry, Health Effects, and Applications. AOCS Press, Champaign, Illinois. Pp. 204-212.

Muir, A.D., Gruber, M.Y., Hinks, C.F., Lees, G.L. Ohyilagha, J., Hallett, R., Xia, F., Soroka, J. and Erlandson, M. 1999. The effects of condensed tanninsin the diets of major crop insects. In: Gross, G.G., Hemingway, R.W. and Yoshida, T. (eds.). Plant Polyphenols 2. Chemistry, Biology, Pharmacology, Ecology. Plenum Press. New York. Pp. 867-881.

Mulholland, J.G., Nandra, K.S., Scott, G.B., Jones, A.W. and Coombes, N.E. (1996) Nutritive value of subterranean clover in a temperate environment. Aust.J. Exp. Agric. 36, 803-814.

NRC (National Research Council) 1971. Atlas of Nutritional Data on United States and Canadian Feeds. National Academy of Science, Washington, D.C., USA.

NRC (National Research Council). 1982. United States – Canadian Tables of Feed Composition (Third Revision) National Academy Press, Washington D.C., USA.

NRC (National Research Council) 1996. Nutrient Requirements of Beef Cattle. 7th edition. National Academy PressResearch Council, Washington, D.C. USA.

NRC (National Research Council) 2000. Nutrient Requirements of Beef Cattle. Update 2000. National Academy Press, Washington, D.C., USA.

NRC (National Research Council). 2001. Nutrient Requirements of Dairy Cattle. 7th edition. National Academy Press, Washington, D.C., USA.

Najda, H. 2002. Standard Alfalfa. AC Grazeland Br. http://www1.agric.gov.ab.ca/general/cropvart.nsf/Varieties/AC+Grazeland+Br. Last revision. Dec. 12, 2002.

Neizen, K.E., Waghorn, T.S., Charleston, W.A.G. and Waghorn, G.C. 1995. Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. J. Agric. Sci. 125:281-285.

Neizen, J.H., Robertson, H.A., Waghorn, G.C. and Charleston, W.A.G. 1998. Production, faecal egg counts and worm burdens of ewe lambs which grazed six contrasting forages. Vet. Parasitol. 80: 15-27.

Newbould, P., Holding, A.J., Davies, G.J., Rangeley, A., Copeman, G.J.F., Davies, A., Frame, J., Haystead, A., Herriot, J.B.D., Holmes J.C., Lowe, J.F., Parker, J.W.G., Waterson, H.A., Wildig, J., Wray, J.P. and Younie D. 1982. The effect of *Rhizobium* inoculation on white clover in improved hill soils in the United Kingdom. J. Agric. Sci. 99: 591-610.

Oleszek, W., Jurzystra, M., Ploszynski, M., Colquhoun, I.J., Price, K.R. and Fenwick, G.R. 1992. Zanhic acid tridesmoside and other dominant saponins from alfalfa (*Medicago sativa* L) aerial parts. J. Agric. Food Chem. 40:191-196.

Oleszek, W. 1996. Alfalfa saponins: Structure, biological activity, and chemotaxonomy. In: Waller and Yamasaki (eds.). Saponins Used in Food and Agriculture. Plenum Press, New York, pp. 155-170.

Oleszek, W. 2004. Personal communication to W. D. Price, U.S. Food and Drug Administration, Rockville, MD

Orr, R.J., Rutter, S.M., Penning, P.D., Yarrow, N.H. and Champion, R.A. 1996. Grazing behaviour and herbage intake rate by Friesian dairy heifers grazing ryegrass or white clover. In: Younie, D. (ed.) *Legumes in Sustainable Farming Systems*. Occasional Symposium No. 30, British Grassland Society, Reading, pp.221-224.

Parente, G. and Frame, J. 1993. Alternative uses of white clover. FAO/REUR Technical Series 29: 30-36.

Pérez, N., Peña, S., Vega, S., Noa, M. and Enríquez, R. 1997. Medicagenic acid content in foliage of ten varieties of alfalfa (*Medicago sativa* L) cultivated in Mexico. J. Sci Food Agric. 75: 401-404.

Pedersen, M.W., Anderson, J.O., Street, J.C., Wang, L.C. and Baker, R. 1972. Growth response of chicks and rats fed alfalfa with saponin content modified by selection. Poult. Sci. 51: 458-464.

Peterson, P.R., Sheaffer, C.C. and Hall, M.H. 1992. Drought effects on perennial forage legume yields and quality. Agronomy Journal, 84 774-779.

Phuntsok, T., Froetschel, M.A., Amos, H.E., Zheng, M. and Huang, Y.W. 1998. Biogenic amines in silage, apparent postruminal passage, and the relationship between biogenic amines and digestive function and intake by steers. J.Dairy Sci. 81:2193-2203.

Popp, J.D., McCaughey, W.P., Cohen, R.D.H., McAllister, T.A. and Majak, W. 2000. Enhancing pasture productivity with alfalfa: A review. Can. J. Plant Sci. 80: 513-519.

Preston, R.L. 2003. Beef. March 2003, pages 40-48.

Rattray, P.V. and Joyce, J.P. 1974. Nutritive value of white clover and perennial ryegrass for young sheep. IV. Utilisation of dietary energy. New Zealand J. Agric. Res. 17, 401-406.

Ray, H., Yu, M., Auser, P., Blahut-Beatty, L., McKersie, B., Bowley, S., Westcott, N., Coulman, B., Lloyd, A. and Gruber, M.Y. 2003. Expression of anthocyanins and proanthocyanidins after transformation of alfalfa with maize Lc. Plant Physiol. 132: 1448-1463.

Rogers, H.H. 1976. Forage legumes. In: Plant Breeding Institute Annual Report, 1975. Plant Breeding Institute, Cambridge, pp.22-57.

Rosenthal, G.A. and Nkomo, P. 2000. The natural abundance of L-canavanine, an active anticancer agent, in alfalfa, *Medicago sativa* (L.). Pharmaceutical Biology 38, 1-6.

Rossi, D. M. Navarro, F. and Grivel, C. D. 1997. Effect of monensin on weight gain and prevention of bloat in steers feeding on lucerne pasture. Archivos de Medicina Veterinaria 29, 279-282.

Rumbaugh, M.D. 1990. Special purpose forage legumes. In: J.Janick and J.E. Simon (eds.). Advances in New Crops. Timber Press, Portland, Oregon.

Sarkar, S.K., Howarth, R.E. and Goplen, B.P. 1976. Condensed tannins in herbaceous legumes. Crop Sci. 16: 543-546.

Schachtman, D.P. and Kelman, W.M. 1991. Potential of *Lotus* germplasm for the development of salt, aluminum and manganese tolerant pasture plants. Aust. J. Agric. Res. 42: 139-149.

Sheaffer, C.C., Lacefield, G.D. and Marble, V.I. 1988. Cutting schedules and stands. In: Hanson, A.A., Barnes, D.K. and Hill, R.R., Jr (eds) Alfalfa and Alfalfa Improvement. Agronomy Monograph No. 29, ASA/CSSA/SSSA, Madison, WI, USA pp. 411-437.

Skadhauge, B., Gruber, M.Y., Thomsen, K.K. and von Wettstein, D. 1997. Leucocyanidin reductase and accumulation of proanthocyanidins in developing legume tissues. Am. J. Bot. 84: 494-503.

Slabbert, N. 1992. Complexation of condensed tannins with metal ions. In: Hemingway, R.W. and Laks, P.E. (eds.). Plant Polyphenols. Synthesis, Properties and Significance. Plenum Press, New York. Pp. 421-436.

Smith, R.R., Taylor, N.L. and Bowley, S.R. 1985. Red clover. In: Taylor, N.L. (ed.) Clover Science and Technology. ASA/CSSA/SSSA, Madison, WI, USA, pp. 457-470.

Smolenski, S.J., Kinghorn, A.D. and Balandrin, M.F. 1981. Toxic constituents of legume forage plants. Econ. Bot. 35: 321-355.

Spedding, C.R.W. and Diekmahns, E.C. 1972. Grasses and Legumes In British Agriculture. Bulletin No. 49, Commonwealth Bureau of Pastures and Field Crops, Farnham Royal, 511 pp.

Stewart, T.A. 1984. Utilising white clover in grass based animal production systems. In: Thomson, D.J. (ed.) Forage Legumes. Occasional Symposium No. 16, British Grasslands Society, Hurley, pp.93-103.

Stifel, F.B., Vetter, R.L., Allen, R.S. and Horner, H.T. Jr. 1968. Chemical and ultrastructural relationships between alfalfa leaf chloroplasts and bloat. Phytochem. 7: 335-364.

Stob, M. 1983. Naturally occurring food toxicants extorgens. Pages 81-100. In: Rechcigl, M., Jr. (ed.). CRC Handbook of Naturally Occurring Ffood Toxicants. CRC Press, Boca Raton.

Stochmal, A., Simonet, A.M., Macias, F.A., Oliveira, M.A., Abreu, J.M., Nash, R. and Oleszek, W. 2001. Acylated apigenin glycosides from alfalfa (*Medicago sativa L.*) var. Artal. Phytochemistry 57: 1223-1226.

Stoutjeskijk, P.A., Sale, P.W. and Larkin, P.J. 2001. Possible involvement of condensed tannins in aluminium tolerance of Lotus pedunculatus. Aust. J. Plant Physiol. 28: 1063-1074.

Tanner, G.J., Moore, A.E. and Larkin, P.J. 1994. Proanthocyanidins inhibit hydrolysis of leaf proteins by rumen microflora *in vitro*. Brit. J. Nutr. 71: 947-958.

Tanner, G.J., Moate, P., Dailey, L., Laby, R. and Larkin, P.J. 1995. Proanthocyanidins (condensed tannins) destabilise plant protein foams in a dose dependent manner. Aust. J. Agric. Res. 46:1101-1109.

Taylor, A.O. and Hughes, K.A. 1978. Conservation-based forage crop systems for major or complete replacement of pasture. Proceedings of the Agronomy Society of New Zealand 8, 161-166.

Taylor, N.L. and Quesenberry, K.H. 1996. Red Clover Science. Kluwer Academic Publishers, Dordrecht.

Taylor, N.L. and Smith, R.R. 1995. Red clover. In: Barnes, R.F., Miller, D.A. and Nelson, C.J. (eds) *Forages*. Vol. 1, An Introduction to Grassland Agriculture, 5th edn. Iowa State University Press, Ames, Iowa, pp.217-226.

Taylor, N.L., Quesenberry, K.H. and Anderson, M.K. 1980. Genetic system relationships in the genus *Trifolium.* Economic Botany 36, 431-441.

Ten Holte, L. and Van Keulen, H. 1989. Effects of white clover and red clover as a green crop on growth, yield and nitrogen of sugar beet and potatoes. Developments in Plant and Soil Sciences 37: 16-24.

Thomas, H. 1984. Effects of drought on growth and competitive ability of perennial ryegrass and white clover. J. Appl. Ecol. 21: 591-602.

Thomson, D.J. 1984. The nutritive value of white clover. In: Thomson, D.J. (ed.) *Forage Legumes*. Occasional Symposium No.16, British Grassland Society, Hurley, pp.78-92

Townsend, C.E. 1995. Registration of C-33, C-34 and C-35 genetic root stocks of diploid alsike clover. Crop Sci 35: 1519.

Ulyatt, M.J., Dellow, D.W., John, A., Reid, C.S.W. and Waghorn, G.C. 1986. Contribution of chewing during eating and rumination to the clearance of digesta from the reticulorumen. In: Milligan, L.P., Grovum, W.L. and Dobson, A. (eds) Control of Digestion and Metabolism in Ruminants. Proceedings of the VIth International Symposium on Ruminant Physiology, Banff, Canada. Prentice Hall, Englewood Cliffs, New Jersey, pp.498-515.

Ulyatt, M.J., Lancashire, J.A. and Jones, W.T. 1977. The nutritive value of legumes. Proceedings of the New Zealand Grassland Association 38: 107-118.

Undersander, D.J., Bertran, M.G., Crooks, A.E., Rankin, M.C., Silveira, K.G. and Wood, T.M. 2002. Forage variety update for Wisconsin, UWEX A 1525(revised).

USDA National Nutrient Database for Standard Reference, Release 16, 2003

USDA Plants Database, 2003. http://plants.usda.gov/

Van Keuren, R.W. and Davis, R.R. 1968. Persistence of birdsfoot trefoil, *Lotus corniculatus* L., as influenced by plant growth habit and grazing management. Agronomy Journal 60: 92-95.

Van Keuren, R.W., Davis, R.R., Bell, D.S. and Klosterman, E.W. 1969. Effect of grazing mangement on the animal production from birdsfoot trefoil pastures. Agronomy Journal 61: 422-425.

Vetter, J. 2000. Plant cyanogenic glycosides. Toxicon 38: 11-36.

Vickery, P.J., Wheeler, J.L. and Mulcahy C. 1986. Factors affecting the hydrogen cyanide potential of white clover (*Trifolium repens* L). Aust J. Agric. Res. 38, 1053-1059.

Waghorn, G.C. 1990. Beneficial effects of low concentrations of condensed tannins in forages fed to ruminants. In: Akin, D.E., G, L.L., Wilson, J.R.. and Harris, P.J. (eds.). Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants. Elsevier, New York. Pp. 137-147.

Waghorn, G.C. and Shelton, I.D. 1992. The nutritive value of Lotus for sheep. Proc. New Zealand Soc. Anim. Prod. 52: 89-92.

Wang, Y., Frutos, P., Gruber, M. Y., Ray, H. and McAllister, T. A. 2003. Comparison of *in vitro* digestibility of parental and anthocyanin-containing *Lc*-transgenic alfalfa. Can. J. Anim. Sci. 83: 639 (Abstract).

Weller, R. F. Cooper, A. and Wilkinson W. 1996. Animal Health. In: Conversion to Organic Milk Production (ed. Haggar and Padel), Technical Review No. 4, Institute of Grassland and Environmental Research, Aberystwyth, UK.

Wheeler, J.L. 1989. Variation in HCN potential among cultivars of white clover (*Trifolium repens* L). Grass and Forage Science 44: 107-109.

Zandstra, I.I. and Grant. W.F. 1968. The biosystematics of the genus Lotus (Leguminosae) in Canada. 1. Cytotaxonomy. Can. J. Botany 46: 557-583.

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