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THE POTENTIAL OF FENUGREEK (*Trigonella foenum-graecum)* AS A FORAGE FOR DAIRY HERDS IN CENTRAL ALBERTA

by

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Dedicated my Mom and Dad, and Janus

Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is a single-cut, annual legume typically grown for seed. Fenugreek has potential as a forage because it maintains high quality throughout the growing season, and offers the benefits of a legume in a crop rotation. This work aimed to evaluate the growth of two fenugreek genotypes, AAFC F70 and CDC Quatro, in the central AB area over two growing seasons, and to evaluate fenugreek haylage degradation and digestion in dairy cows. In general, the two genotypes were similar in their growth patterns and fenugreek biomass yield was comparable to alfalfa in the same area. Plant quality was sufficient to be used for lactating dairy cows. The digestion studies revealed that while Quatro haylage was comparable to alfalfa haylage, F70 haylage was of lower quality and was not utilized to the same extent by dairy cows as the other two forage types.

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List of Abbreviations

a	Soluble fraction of a feed during degradation in the rumen
AARD	Alberta Agriculture and Rural Development
AAFC	Agriculture and Agri-Food Canada
ADF	Acid Detergent Fibre
ADG	Average Daily Gain
b	Potentially degradable fraction of a feed during degradation in the rumen
c	Insoluble fraction of a feed during degradation in the rumen
CDC	Crop Diversification Centre
cm^2	Centimeter squared
CP	Crude Protein
d	Day(s)
DDM	Digestible Dry Matter
DM	Dry Matter
DMD	Dry Matter Digestibility
DMI	Dry Matter Intake
DRTC	Dairy Research and Technology Centre
ERD	Effective Ruminal Degradability
ERS	Edmonton Research Station
H	Harvest
ha	hectare
IVDMD	In Vitro Dry Matter Digestibility
K_d	Rate of Degradation of a feed in the rumen

kg	Kilogram
K_p	Rate of Passage of material from the rumen
LAI	Leaf Area Index
LAR	Leaf Area Ratio
LRC	Lethbridge Research Centre
LWR	Leaf Weight Ratio
m^2	Metre squared
N	North
NDF	Neutral Detergent Fibre
NRC	National Research Council
NS	Not Significant
O	Oxygen
P	Phosphorus
PAI	Pod Area Index
QT	Quatro
r^2	Regression coefficient
RFV	Relative Feed Value
RGR	Relative Growth Rate
SEM	Standard Error of the Mean
SLA	Specific Leaf Area
t	Tonne
t	Time
t/ha	Tonne/Hectare

Var	Variety
W	West
W240	West 240
WSC	Water Soluble Carbohydrate

Prologue

Recent research in southern Alberta and Saskatchewan has identified fenugreek (*Trigonella foenum-graecum* L.) as a crop with potential for use as a forage crop. Fenugreek offers benefits in crop rotations such as nitrogen fixation, flexibility of harvest time, yield comparable to alfalfa, and high quality which does not decline during the growing season (Acharya et al. 2008). Fenugreek also has potential as a feed for high producing dairy animals due to its high nutrient value and non-bloat characteristics.

Fenugreek has not previously been grown in central Alberta, and we have been unable to find any published studies that give a detailed description of the growth and development of fenugreek. Fenugreek feeding studies have indicated that fenugreek is palatable to dairy animals but detailed studies of fenugreek utilization have not been published.

This research thus focuses on addressing these two research gaps: documenting the basic growth characteristics of fenugreek over the growing season including plant biomass, resource partitioning, and nutrient content; and using the rumen mobile bag technique to assess the degradation and digestion of fenugreek forage in dairy animals.

The information provided by this research will lay a foundation for fenugreek production and use in Alberta and identify further areas of enquiry.

Chapter 1: Literature Review

1.1. Introduction

The production of forages for dairy animals combines several aspects of agricultural knowledge as both crop production and animal nutritional requirements must be considered. Forage production must occur in an economical and sustainable fashion in order to optimize yield, quality, and crop rotation benefits while satisfying the requirement of dairy animals for high-quality and highly palatable forages that will provide the dietary base for high milk production. Having a wide variety of forage crops available allows dairy producers to optimize their crop rotation in order to decrease the prevalence of diseases and pests and to improve soil quality. Research into alternative forage crops in the central Alberta area may lead to an increase in forage crop options and the development of best management practices for the production of these crops.

Fenugreek is a crop grown in warm temperate and tropical regions of the Mediterranean, Europe, and Asia, where it is used for human and animal consumption. Though mainly produced for seed, interest in fenugreek in Western Canada has also extended to the development of forage-type genotypes (Acharya et al. 2007, 2008; Basu et al. 2008). The reported high quality of fenugreek forage lends itself to use in dairy diets.

This thesis will examine the growth characteristics in central Alberta of varieties of fenugreek developed on the Canadian Prairies, as well as the potential nutrient value of fenugreek forage in dairy diets. While previous research indicates the success of fenugreek in dairy (Shah and Mir 2004) and beef (Mir et al. 1998) rations, research on fenugreek forage use in dairy animals and growth has not been conducted in central Alberta, and producers in this area stand to benefit from the availability of a high-quality crop which is easily incorporated into crop rotation systems.

1.2. Fenugreek Production

1.2.1. Origins of fenugreek

Fenugreek (*Trigonella foenum-graecum* L.), also known as Greek Hay (Sinskaya 1961), is a single-cut, annual legume (Acharya et al. 2008, Slinkard et al. 2006). The species component of the Latin name indicates its historical use as a forage (Acharya et al. 2008, Petropoulos 2002). The two origins of fenugreek are reported as the Indian subcontinent and Eastern Mediterranean (Acharya et al. 2006, 2008, Sinskaya 1961), but indigenous species have been reported in Asia, Europe, Africa and Australia (Petropoulos 2002). Fenugreek is widely cultivated in the warm temperate and tropical areas of the Mediterranean in Europe and Northern Africa, as well as on the Indian subcontinent, in West and South Asia, in North and South America, and in Australia (Acharya et al. 2006, 2008). Major seed producing countries include India, Ethiopia, Egypt, and Turkey (Slinkard et al. 2006). Varieties originating from Mediterranean regions have greater potential for use in Western Canada because of similarity in day length in these regions (Slinkard et al. 2006) and are also taller, larger, and bear more leaves (Sinskaya 1961). Fenugreek is also suited to Western Canada because of its ability to offer consistent seed yield under short growing season conditions (Sinskaya 1961) and adaptation to dry-land conditions (Acharya et al. 2008).

1.2.2. Fenugreek characteristics

Fenugreek is an annual dicot belonging to the subfamily Papilionaceae of the Leguminaceae family (Acharya et al. 2006). Fenugreek has an indeterminate growth habit (Acharya et al. 2008), and plant growth will continue until heavy frost or dessication (Acharya et al. 2006). Fenugreek plants in North America typically grow 40 – 60 cm high (Acharya et al. 2006, Slinkard et al. 2006). The plant has been described as ‘malodorous’ (Lust 1974) due to its distinct smell. It is trifoliate with branched stems, and has white or yellow flowers (Acharya et al. 2008). The pods are similar in appearance to canola pods; they are typically 7-15 cm long, slender and green to brown, glabrous, and contain up to 20 seeds (Acharya et al. 2006).

The seeds are golden in colour (Acharya et al. 2006) and rectangular in shape, averaging 4 mm in length, 2 mm in width, and 1.5 mm in thickness (Altuntaş et al. 2005). The 1000-seed weight varies from 15.5 – 16.4 g (Altuntaş et al. 2005) to 18-22 g (Slinkard et al. 2006), and bushel weight is 25-27 kg. Slinkard et al. (2006) described the chemical composition of fenugreek seeds as 32% insoluble dietary fibre, 13% soluble dietary fibre, 36% protein, 6% oil, 3% ash, 1.6% starch, and 0.4% sugar. The seeds also contain calcium, iron, and beta-carotene (Sauvaire et al. 1976). Diosgenin is found at 0.5% DM in fenugreek seeds (Shah and Mir 2004). The seeds of fenugreek may contribute to the relatively high protein content of the plant (Mir et al. 1993).

The root system consists of a tap root with branching side roots that are capable of nodulation (Acharya et al. 2006). Flowers are mainly closed (cleistogamous), but can be open (aneictogamous) (Petropoulos, 1973, Acharya et al. 2006). Closed flowers account for the mainly self-pollinating habit of fenugreek (Acharya et al. 2006, 2008, Petropoulos 1973). Because of the self-pollinating tendency of fenugreek, artificial crossing is difficult (Fehr, 1993), therefore breeding selection mainly occurs from world accessions and through mutation breeding (Petropoulos 2002, Fehr 1993, Raghuvanshi and Singh 1981, Basu et al. 2008, Jain and Agarwal 1994), and hybridization (Saleh 1996, Cornish et al. 1983). High genetic variability (McCormick et al. 2006) and polymorphism (Sinskaya 1961) in fenugreek allow for goal-oriented selections. Genetic variability and environmental factors interact to alter seed and forage yield, and chemical composition of the seeds (Acharya et al. 2008). While members of the genus *Trigonella* can differ in chromosome number, *Trigonella foenum-graecum* is a diploid where 2n = 16 (Acharya et al. 2006, 2008, Sinskaya 1961).

Fenugreek grown in western Canada does not exhibit great variation in flower colour or morphology, but does show variability in seed and forage yield across years and differing environmental conditions (Acharya et al. 2008, Basu et al. 2004). Varieties from around the world differ in seed yield when grown in Canada (Acharya et al. 2006, 2008, Basu et al. 2004, 2007). International

varieties also vary in the chemical composition of the seed, notably the diosgenin content (Taylor et al. 2002).

Fenugreek is mainly cultivated for its seeds, which are used as a spice (Acharya et al. 2006, 2008) or dye (Slinkard et al. 2006). The world market for seeds is roughly 30,000 to 50,000 t (McCormick et al. 2006). According to Slinkard et al. (2006), the indeterminate growth habit, and requirement for a long growing season, make fenugreek grown for seed production best adapted for southern Saskatchewan. Fenugreek can benefit from the naturally dessicating conditions of drought which commonly occur in that area, especially in the late summer and early fall. In southern Saskatchewan and Alberta, fenugreek plants mature in 105-140 days for seed production (Slinkard et al. 2006, Basu et al. 2008). Growth is slower under cooler and wetter conditions, and long periods of these conditions may cause a failure of plants to mature for seed harvest. The growth rate of fenugreek is slow at the beginning of the growing season, and leaf development is temperature-dependent (McCormick et al. 2006). Fenugreek is easier to harvest than lentils and field peas, but similar to faba bean and chickpea as it is of sufficient height for mechanical harvesting, and does not easily shatter, though taller varieties can lodge (McCormick et al. 2006).

In Turkey, 700 hectares of fenugreek are grown annually for seed production, averaging 670 tonnes, or 957 kg/ha of seed (Anonymous 2000, cited in Altuntaş et al. 2005). Australian yields of fenugreek seed are similar to lentils, field peas, faba beans, and chickpeas (McCormick et al. 2006). Average seed yield under dryland conditions in Saskatchewan is 1500 kg/ha, though the range is wide (150 – 2800 kg/ha) depending on environmental conditions (Slinkard et al. 2006). As of 2006, between 140 ha (Slinkard et al. 2006) and 500 ha (Thomas et al. 2006) of fenugreek were grown in Western Canada, mainly for seed production.

The 15-year average dry-land fenugreek forage yield in southern Alberta is 5.8 t/ha dry matter (DM), while the long-term yield under irrigation is 6.0 t/ha (Acharya et al. 2008), though yields up to 10 t/ha under irrigation have been

achieved (Mir et al. 1997). Dry matter biomass of fenugreek in Australia was 3.8 t/ha and 5.6 t/ha when harvested at late flowering in two growing seasons.

Fenugreek is a crop gaining popularity in countries that are not traditionally large producers of the legume, such as Canada and Australia. In Australia, because of its adaptation to alkaline grey vertisol soil zones, fenugreek is generating interest both as a grain crop and as a green manure in increasingly diverse and intense crop rotations (McCormick et al. 2006). Fenugreek has a greater gross margin than any other pulse in Australia except lentils, and a relatively lower risk because of lower input costs and lower break-even yield, making it a financially viable alternative crop (McCormick et al. 2006). When used in a crop-rotation, wheat grown after fenugreek did not have a different incidence of root disease or levels of nitrogen uptake than wheat grown after other legumes (McCormick et al. 2006). Interest in fenugreek as a forage crop has been generated in southern Alberta at the Agriculture and Agri-Food Canada (AAFC) Lethbridge Research Centre (LRC) due to its ability to maintain a high protein content regardless of advancing maturity (Slinkard et al. 2006).

1.2.3. Alternative fenugreek uses

While the main market for fenugreek in many countries is as a food ingredient for humans or animals, European and North American consumers are more familiar with fenugreek as a component in artificial flavourings such as butterscotch or maple, and more predominantly, as a health food or neutraceutical product (Slinkard et al. 2006). Increased interest in fenugreek in the health-food market has been generated due to medicinal compounds in the leaves and seeds (Petropoulos 2002), including steroidal sapogenins, isoleucine, and galactomannans (Acharya et al. 2006, 2008). The medicinal properties of fenugreek were recorded by the Egyptians and Hippocrates (Lust 1974), making it one of the oldest recorded plants used in medicine (Acharya et al. 2008, Sinskaya 1961). It is referred to in Indian Ayurvedic (Sur et al. 2001), Greek, Chinese and Arabian medicines (Evidente et al. 2007), and has also been used for veterinary purposes (Sinskaya 1961). The medicinal uses of fenugreek are various and

include wound-healing, bust enhancement, aphrodisiac (Tiran 2003, Acharya et al. 2006), galactogogue (Tiran 2003), and expectorant. It is used in the treatment of bronchial ailments, sore throats, sciatica, wounds and sores, irritation of the skin (Lust 1974), tumours (Sur et al. 2001), head lice (El-Bashier and Fouad 2002), and sickness caused by air pollution, and to reduce UVA/UVB radiation damage to skin cells (Singh et al. 2004). Seeds are also used in the treatment of hypertension (Balaraman et al. 2006), hyperglycemia, which can help in the regulation of Type 2 diabetes (Raghuram et al. 1994), hypocholesterolemia (McAnuff et al. 2002), and as an anti-inflammatory.

Fenugreek is a source of diosgenin that could be used in the production of pharmaceutical hormones (Petropoulos 1973, Hardman 1969, Raghuram et al. 1994). Steroidal sapogenins, such as disogenin, can be used to treat hypercholesterolemia, often associated with diabetes (McAnuff et al.2002). Diosgenin, not present in other legumes (Mir et al. 1997), also acts as a natural growth promoting substance (Basu et al. 2008) which may increase the growth efficiency of cattle. Saponins, from which sapogenins are derived, are found in fenugreek and have anti-microbial properties that suppress acetate-producing bacteria, but leave propionate-producing bacteria unchanged (Devant et al. 2007). The anti-microbial properties of these compounds are of concern when considering fenugreek for ruminants. However, Thomas et al. (2006) found no inhibition of common enteric bacteria by extracts of *Trigonella* accessions.

Isoleucine, present in fenugreek, can be used metabolically as a precursor of 4-hydroxyisoleucine that regulates insulin secretion in animals (Broca et al. 2000, Sauvaire et al. 1998). This, in combination with the gastro-intestinal effects of dietary fibre found in the fenugreek seed, accounts for most of the hypoglycaemic and anti-hyperglycaemic effects of fenugreek (Sauvaire et al. 1996).

Fenugreek may be a viable source of galactomannan gum (McCormick et al. 2006), which can be used as a thickening agent in foods (Slinkard et al. 2006), or as a food emulsifier (Garti et al. 1997). Galactomannans, often found in the endosperm cell wall (Meier and Reid 1977), are the main polysaccharide in

fenugreek seeds, and can comprise up to about 50% of seed weight (Raghuram et al. 1994).

Production of fenugreek allows for the recycling of waste material (Pandya et al. 1991). The use of farm-produced manure as a fertilizer for fenugreek can reduce the cost of off-farm manure disposal, while decreasing purchased inputs for fenugreek production, and promote more environmentally friendly farm practices such as manure re-use (Acharya et al. 2008, Acharya et al. 2006, Khiriya and Singh 2003). Petropoulos (2002) found that fenugreek can grow under organic soil enrichment conditions, where kitchen compost, field waste, or manure were incorporated into the soil.

Fenugreek has been used for its nemacitidal (Zia et al. 2001) and molluscicidal effects (Singh et al. 1997). Fenugreek can also be used as a tool in land reclamation because of its ability to grow on marginal lands, and can be used as habitat for ground-nesting birds and small animals. It also has the potential as a high quality late-fall or winter feed source for wild ungulates (Acharya et al. 2006).

Fenugreek extract has been included in a blended plant extract as an alternative to monensin, a growth promoter, in European markets where the use of monensin is prohibited (Devant et al. 2007). The final body weight of Holstein bulls fed a high-concentrate diet plus the fenugreek plant extract was intermediate between the control diet and monensin diet. Feed consumption and feed efficiency were not affected by the plant extract or monensin. Fenugreek can be used as a flavouring agent in ruminant and swine feed (Fotopoulos 2002). Goodwin et al. (2005) found that stabled horses prefer concentrate meals flavoured with fenugreek over concentrate flavoured with other agents or not flavoured at all.

1.2.4. Benefits of fenugreek

Interest in fenugreek production in western Canada arose from the potential benefits of fenugreek to forage producers. Increased diversity of plants in a cropping system can diversify sources of income, interrupt plant disease, pest

and weed cycles, and improve the soil condition (Forbes and Watson 1999). Fenugreek, as an annual, lends itself to increasing diversity in short-term crop rotations (Acharya et al. 2006, 2007, Moyer et al. 2003, Mir et al. 1997). Fenugreek can be used as a green manure crop because of its ability to produce a large amount of biomass with high nitrogen content (Acharya et al. 2006, Sinskaya 1961). Legumes can reduce the need for nitrogen fertilizers, and thus reduce the input costs of crop production and the potential environmental impact of chemical fertilizer use (Acharya et al. 2004, 2006, 2008). The amount of nitrogen fixed by fenugreek in Australia is similar to that of lentils and medic, ranging from 76 to 85 hg/ha (McCormick et al. 2006).

Fenugreek is reported to be frost-tolerant. McCormick et al. (2006) found that fenugreek had the highest late-season frost-tolerance of field peas, lentils, and faba beans in Australia. Fenugreek sustained the lowest level of frost damage at 5%, versus 10% for faba beans, 20% for field peas, and 50% for lentils. Fenugreek is adapted for dryland production, meaning increased potential to tolerate the dry growing conditions of the prairies in Western Canada (Sinskaya 1961, Acharya et al. 2006, 2007 and Basu et al. 2004).

As a forage, fenugreek is desirable because of its ability to provide high quality forage at all stages of growth (Acharya et al. 2008). It does not show a marked decline in quality once reproductive growth has been initiated. This allows producers increased flexibility of harvest time, especially being able to fit fenugreek harvest around the harvest of other crops that have a more limited harvest window (Slinkard et al. 2006). Studies by Mir (1997, 1998) have shown that fenugreek forage at various stages has comparable nutritive value to early bloom alfalfa. Acharya et al. (2006) found that there is high nutritional value in the leaves and small stems of fenugreek. Gupta et al. (1998) also found fenugreek leaves to have high nutritional quality. High quality feed can increase feed efficiency and decrease the feed requirements of animals (Acharya et al. 2006, 2008).

The diosgenin content in fenugreek may increase the growth of cattle through its natural steroidal properties (Mir et al. 1998, Acharya et al. 2008).

Decreased reliance on synthetic steroids for efficient cattle production could decrease input costs for beef producers (Acharya et al. 2006, 2008). Inclusion of fenugreek plants, seeds or seed meal in animal diets is considered desirable for animal health, as well as improving the flavour of animal diets (Sinskaya 1961). Fenugreek is considered to be a bloat-free legume (Acharya et al. 2006, Mir et al. 1997, 1998, Basu et al. 2008), eliminating a major concern associated with feeding highly nutritious and palatable legumes to ruminants.

1.2.5. Growing conditions

1.2.5.1. Seeding conditions

In Western Canada, seeding of fenugreek in late-April to mid-May has been successful Acharya et al. (2008). Slinkard et al. (2006) found that seeding is optimum in early May. In India, optimal productivity of fenugreek occurs at a row spacing of 20 – 30 cm (Korla and Saini 2003, Gill et al. 2001, Baswana and Pandita 1989, Bhatt 1988). On well-drained soil, 15 – 30 cm row spacing resulted in the highest yield, with optimum plant density at 135 plants/m^2, or 18 plants per meter of row (Slinkard et al. 2006). Seeding rate should be 27-33 kg/ha (25-30 lbs/ac) at a depth of 2-4 cm (1-1.5 inches) (Slinkard et al. 2006). In previous studies fenugreek has been seeded at 38 kg/ha (Moyer et al. 2003), 30 kg/ha using 17.5 cm spacing at a depth of 2.5 cm (Mir et al. 1998), and 26 kg/ha using 18 cm row spacing (Mir et al. 1997). The range of seeding rates is varied (Petropoulos 2002). The presence of stubble helps to promote early maturity, and denser stands have a more uniform time of maturation (Slinkard et al. 2006).

Fenugreek performs best on well-drained and fertile soils that have a pH of 5.3-8.2, and at environmental temperatures between 8 and 27^{o}C (Slinkard et al. 2006). Heavy and wet soils limit fenugreek growth (Petropoulos 1973).

Inoculation of seeds with proper *Rhizobium* species should occur in order to take advantage of the nitrogen-fixing ability of the legume (Acharya et al. 2008), as well as to increase seed yield and quality when seed production is the objective (Abd-Ala and Omar 1997). Bacteria commonly used on fenugreek are aerobic, non-sporulating Gram-negative bacillus *Rhizobium meliloti* (Subba Rao

and Sharma 1968). Fenugreek varieties from different origins have specific strains of *Rhizobium*, one for the group of Indian origin, and a Russian strain for the Mediterranean group. The Russian strain is RGFU1, produced on a small scale by Becker Underwood (Saskatoon, SK). However, inoculant for alfalfa or sweet clover can also be used on fenugreek (Slinkard et al. 2006).

1.2.5.2. Nutrient requirements

Organic and inorganic sources of nitrogen and phosphorus (i.e. fertilizer) increase fenugreek yield, as can manure (Khiriya and Singh 2003, Detoroja et al. 1995). Nitrogen requirement of fenugreek in Saskatchewan is similar to that of lentils (Slinkard et al. 2006). A significant amount of fenugreek's nitrogen requirement can be fixed by the plant provided the seed has been effectively inoculated. If provided with excess nitrogen fertilizer, vegetative growth will exceed reproductive growth, resulting in decreased seed production and a delay in seed maturity (Slinkard et al. 2006), which may be an advantage for forage production purposes. Phosphorus is essential to optimize nodulation, for flower and seed production, and to ensure plant maturation. If required as indicated on a soil test, addition of phosphorus fertilizer (P_2O_5) increases yield (Slinkard et al. 2006).

1.2.5.3. Weed control

No herbicides are currently registered for use on fenugreek in Canada (Moyer et al. 2003, Slinkard et al. 2006). Fenugreek does not compete well with weeds, as fenugreek plants grow more slowly after emergence than other legumes (Slinkard et al. 2006, McCormick et al. 2006, Moyer et al. 2003). Moyer et al. (2003) found that initial fenugreek growth is slower following a barley, oat or wheat crop as opposed to summer fallow (Moyer et al. 2003). A clean field prior to seeding is essential (Slinkard et al. 2006), as pre-emergent herbicide choices are limited (McCormick et al. 2006). Contamination of fenugreek fields with flax, wheat, or canaryseed should be avoided because of the difficulty separating the seed after harvest (Slinkard et al. 2006). Inter-row cultivation of fenugreek as

a method of weed control was less effective than herbicide (Slinkard et al. 2006), though inter-row cultivation may be an alternative for organic producers.

Without herbicidal control of weeds in fenugreek, weeds can represent 37% to 86% of dry matter (Moyer et al. 2003). When irrigated AC Amber fenugreek was treated with imazamox/imazethapyr or mixes of imazethapyr or imazamox/imazethapyr with ethalfluralin, annual weeds (mainly seeded green foxtail [*Setaria viridis* L.] and wild oats [*Avena fatua* L.], and red-root pigweed [*Amaranthus retroflexus* L.]) were controlled to 5% of dry matter production without fenugreek injury or yield loss (Moyer et al. 2003). 2,4-D resulted in 15-20% fenugreek injury, and is therefore not recommended for use on fenugreek (Moyer et al. 2003). Using imazamox/imazethapyr, or mixes of imazethapyr or imazamox/imazethapyr with ethalfluralin, fenugreek yields were similar to the yield of two cuts of hand-weeded alfalfa, regardless of seeding method, previous crop, or tillage management (Moyer et al. 2003). The DM yield of irrigated AC Amber in Lethbridge, AB, was 4.35 t/ha and 3.69 t/ha in plots without herbicide application in 1998 and 1999 and 7.20 t/ha and 9.02 t/ha with herbicide application for the same two years (Moyer et al. 2003). In the same study, it was found that ensiled fenugreek forage with low weed content also had a lower neutral detergent fibre (NDF) and acid detergent fibre (ADF) and higher crude protein (CP) content than samples with higher weed content. The difference in quality was likely due to the advanced maturity of weeds at the time of fenugreek harvest.

1.2.5.4. Disease

Physiological conditions related to mineral deficiency, disease or insect problems have not been observed in fenugreek over 20 years of testing in western Canada (Acharya et al. 2006, 2008). However, they have been observed in other parts of the world. Yellowing of plants can be caused by deficiencies in magnesium, manganese, or potassium (Sinskaya 1961), and can result in decreased yield of forage and seed (Petropoulos 2002). Acharya et al. (2008)

concluded that reduced height, yellowing of leaves and leaf loss can be caused by very hot dry periods. If conditions improve, plants may recover.

The two most common fenugreek diseases are *Cercospora* leaf spot and powdery mildew caused by *Erysiphe polygoni* (Alberta Agriculture and Rural Development 1998, Prakash and Saharan 2000). *Cercospora* leaf spot can result in serious defoliation and pod and stem damage; however, this disease is favoured by warm and humid growing conditions, which are not prevalent on the prairies (Slinkard et al. 2006). Powdery mildew can reduce yield (Jongebloed 2004, Basu et al. 2006b, Slinkard et al. 2006).

No fungicides are registered for use on fenugreek in Canada (Slinkard et al. 2006), though fenugreek is not usually affected by fungal disease (Sinskaya 1961). *Cercospora traversiana* blight disease and *Fusarium oxsysporum* and *Rhizoctonia solani* wilt are a problem in Australia (Jongebloed 2004). Leaf spot caused by *Pseudomonas syringae* has been reported in the United States (Fogg et al. 2000). Other potential diseases include collar rot, pod spot, and infection by *Xanthomonas alfalfa* (Petropoulos 2002). In Australia, fenugreek is less susceptible to foliar fungal pathogens than lentils, faba beans and field peas, decreasing its requirement for foliar fungicides. Fenugreek is, however, susceptible to bacterial blight (McCormick et al. 2006). It is also susceptible to a variety of viral infections, all of which can cause loss of seed and forage yield (Petropoulos 2002).

1.2.5.5. Pests

Fenugreek is considered an insect tolerant crop during growth (Edison 1995). In Australia, insect pests of fenugreek include thrips, pod-borers, and heliothis, all of which can decrease forage yield (Lucy 2004), while the soil nematode *Meloidogyne incognita* causes root rot and death in immature plants (Jongebloed 2004). In southern Alberta, non-damaging infestations of *Lygus* bugs and alfalfa plant bugs as well as aphids have occurred (Acharya et al. 2008). Black aphids (*Aphis cracivora*) are a pest of fenugreek in India (Singh et al. 2006). Malathion applied at 625 ml/ha has been found to effectively control black

aphid populations in fenugreek without negative impacts on plants, or on food safety 5 days after application (Singh et al. 2006). The presence of beneficial ladybird beetles and parasitoid wasps in fenugreek has been noted in southern Alberta (Basu et al.2006a).

Wireworms and cutworms can damage fenugreek seedlings. Grasshoppers are considered a pest in years of heavy infestations, as well as aphids and blister beetles. Dipel 2X (Bacillus thuringiensis, subsp. kurstaki; Valent BioSciences Corp. Libertyville, IL, USA) is registered as an insecticide for use on fenugreek (Slinkard et al. 2006).

1.2.6. Fenugreek varieties

The only forage-type fenugreek cultivar released in North America is AC Tristar, released in 2004 by Agriculture and Agri-Food Canada (AAFC). It was selected for high biomass production in the western Canadian (temperate) climate, and was designed as a high-yielding, dryland-adapted, disease-resistant cultivar (Acharya et al. 2006). Seed supply is currently limited. Varieties released in Western Canada for seed production include AC Amber, registered in 1992 by AAFC Research Station Morden (Manitoba). Crop Diversification Centre (CDC) Saskatoon registered CDC Quatro in 1995, and CDC Canagreen and CDC Canafen in 2002 (Slinkard et al. 2006).

CDC Quatro is a double-podded variety, with increased seed yield, height, and vigour. CDC Canagreen is similar to CDC Quatro, except that it has 28% higher seed yield (Slinkard et al. 2006). CDC Canafen is a white-flowered variety of fenugreek with a slightly higher seed yield than CDC Quatro. White-flowered fenugreek varieties have less taste and odour, increasing their potential for use in food markets. CDC Quatro, Canagreen and Canafen are sold by Emerald Seed Products Ltd. in Saskatchewan, and CDC Amber is licensed exclusively to G.H. Schweitzer Enterprises (Saskatchewan) (Slinkard et al. 2006).

1.2.7. Ensiling of fenugreek

Excellent quality legume forage should contain at least 19% CP, less than 31% ADF, and 40-50% leaves (AARD 1988). Quality is lower when CP is lower, ADF is higher, and the percentage of leaves declines. Moisture content for materials ensiled in horizontal silos should be 60-70%, and have a final pH below 4.8 (AARD 1988). For silages above 35% DM, the typical fermentation profile is: pH 4.2-5.4, 1.6-5.5% lactic acid, 0.4-2.6% acetic acid, 0.1-0.5% propionic acid, 0.1-0.6% iso-butyric acid, 0.0-1.0% butyric acid, and 0.5-2.1% ammonia (CP equivalents) on a DM basis (Jones et al. 2004).

In a study by Mir et al. (1998) in Lethbridge, AB, silage was made in plastic tube silos from fenugreek harvested at 17 weeks after seeding. Dry matter yield was 14.1 t/ha, at 38.4% DM. Ensiling occurred for 40 days. Silage nutrient content was 17.2% CP, 38.2% NDF, 35.1% ADF, 8.2% lignin, 9.5% ash, 2.0% water soluble carbohydrate, 4.49 mg/g ammonia, and pH was 4.4. Protein content, NDF, ash, water-soluble carbohydrates (WSC), ammonia and pH were all lower for fenugreek silage than for mid-bloom alfalfa (var. Beaver) silage. Fenugreek silage had greater total bacteria, lactic acid producing bacteria, and yeast than alfalfa silage; mould was not detectable in either silage, both at the time the bag was opened and after 16 days of exposure to air. Fenugreek silage was higher in acetic, propionic, butyric, and isobutyric acids than alfalfa silage.

1.2.8. Comparison of fenugreek to other legumes

Alfalfa (*Medicago sativa* L.) is a commonly grown forage legume crop in Alberta, widely adapted to growing conditions and soil zones throughout the province (AARD 1988). It requires well-drained soil with a pH above 6.0. Its ability to grow in combination with grasses makes it attractive for mixed silages. The annual average dry matter yield of alfalfa hay (var. Beaver) in the Edmonton, AB area is 7.15 t/ha (AARD 2008). Plant material is of high quality, with a CP content of 17-19% when cut at 10% bloom (AARD 1988, NRC 2001), which is higher protein production per unit area than other crops (NRC 2001, Jones et al. 2004). Alfalfa is the most important and extensively fed type of hay for dairy

cows in the United States due to its high yield with high protein content, mineral and carotene content, high palatability, and adaptation to various climates (Foley et al. 1972). While alfalfa forage quality is highest when cut pre-bloom, stands last longer when harvesting occurs at around 10% bloom, while quality is still relatively high (Foley et al. 1972).

Alfalfa silage grown in Alberta contains, on average, 55.4% moisture, 18.2% CP, 36.1% ADF, 1.77% calcium, and 0.25% phosphorus on a DM basis (AARD 1988). In a study by Kennelly et al. (1993), alfalfa silage had relatively higher DM content (50.8%), CP (19.8%), and ADF (34.3%) when compared to barley, oat, triticale, and combined barley/triticale silage. Alfalfa silage NDF content (45.3%) was lowest of the silage types.

Mir et al. (1997) conducted three experiments to compare fenugreek and alfalfa forage, using small samples of silage made in laboratory silos. Initially, fenugreek grown for 9 weeks in a greenhouse was compared to early-bloom alfalfa. In the second experiment, fenugreek grown in the greenhouse and harvested at 15 weeks and 19 weeks was compared to early-bloom alfalfa. In the third experiment, field-grown fenugreek harvested at 9, 15 and 19 weeks was compared to early bloom alfalfa. Table 1.1. is a summation of the relevant results, including CP, ADF, NDF, and in-vitro dry matter digestibility (IVDMD).

Table 1.1. Neutral detergent fibre, acid detergent fibre, crude protein (% DM) and *in vitro* dry matter digestibility (IVDMD) (%) compared between fenugreek and early-bloom alfalfa forages from three experiments conducted in Lethbridge, AB, grown under greenhouse and field conditions (adapted from Mir et al. 1997).

	Exp.1 Greenhouse		**Exp.2** Greenhouse			**Exp.3** Field			
	9-week fenugreek	Alfalfa	15-week fenugreek	19-week fenugreek	Alfalfa	9-week fenugreek	15-week fenugreek	19-week fenugreek	Alfalfa
CP	21.7a	17.8b	13.5d	12.9d	18.7c	24.8e	19.8ef	15.7f	18.2f
ADF	29.4	28.8	34.8	36.7	35.9	25.2g	30.6f	33.7ef	35.9e
NDF	32.6b	40.4a	42.0d	47.3c	43.9d	29.1g	37.1f	38.7f	43.9e
IVDMD	59.5a	47.7b	52.8c	53.9c	47.9d	64.1e	66.5e	66.2e	59.2f

Different letters within a row in an experiment are significantly different ($P < 0.05$).

In the greenhouse experiments, fenugreek at 9 weeks had higher CP than early-bloom alfalfa, but fenugreek at 15 and 19 weeks had lower CP. In the field, fenugreek at all ages had comparable CP to early-bloom alfalfa. Fenugreek at all harvest dates in the greenhouse had NDF content comparable to early-bloom alfalfa, while in the field the NDF of alfalfa was greater than that of fenugreek. The ADF content of 9 week greenhouse and field fenugreek was lower than early bloom alfalfa, while greenhouse fenugreek at 15 weeks was comparable to and 19 week fenugreek had higher ADF than early bloom alfalfa. In vitro DMD of fenugreek at all harvest dates in all experiments was greater than that of alfalfa. The higher fenugreek IVDMD of fenugreek relative to alfalfa in all three experiments indicates that fenugreek may be utilized more efficiently by ruminants. The overall conclusion of Mir et al. (1997) was that irrigated fenugreek, regardless of advancing maturity, is a viable alternative to alfalfa.

In a study comparing fenugreek and alfalfa hay, Mir et al. (1993) found that 12-week fenugreek hay was similar in nutrient composition and had higher IVDMD than early cut (10% bloom) alfalfa. In a subsequent study, Mir et al. (1998) examined the effects of feeding alfalfa or fenugreek silage, supplemented with barley grain, on the growth of backgrounding beef steers. This study was undertaken in part to determine if the disogenin content of fenugreek contributes to improving the growth rate, feed efficiency and animal health of growing steers. No significant difference was found between the two silage treatments on the steers' final weight, average daily gain, dry matter intake or feed:gain ratio. Rumen pH, rumen ammonia, and total rumen volatile fatty acid production were similar for both forages. There seemed to be no additional positive effect of diosgenin in fenugreek. Fenugreek silage as a forage was comparable to alfalfa silage for growing beef animals in terms of intake and utilization.

Peas are another crop commonly grown in central Alberta as a legume forage. Roughly 610,000 acres of peas were grown in Alberta in 2007 (AARD 2009). Because peas are an annual with an indeterminate growth habit, they are a crop for which fenugreek could be considered an alternative. Peas are sensitive to very hot weather and to drought, especially during flowering (AARD 1988).

Fenugreek is less affected by those conditions. Dry matter forage yield of peas in central Alberta is 7.2 tonnes/ha, and the CP content is around 13% (AARD 2004). In the Peace River region of Alberta, pea dry matter biomass ranges from 3.7 tonnes/ha to 4.2 tonnes/ha, depending on method of inoculation (Clayton et al. 2004).

1.2.9. Growth characteristics of crops

The growth characteristics of a crop are the meaningful representation of relatively simple quantitative data, which provide information on the way in which a plant grows and allocates resources. The quantitative growth characteristics can then be used to compare the growth of one plant to another, or the growth of a plant grown in two or more locations. They can also be used to examine the growth of a new crop (Hunt 1990), such as fenugreek. The following growth characteristic equations are taken from Hunt (1990).

Relative growth rate (RGR) is the rate of increase of plant dry weight compared to the plant's initial dry weight over the time interval from one harvest to the next. It can be calculated using: $RGR = (\log W_2 - \log W_1) / (t_2 - t_1)$, where W is plant weight and t is time. RGR is expressed as weight/weight/time, or simply as a number per unit time. The RGR per day of several herbaceous dicotyledons between 7 and 21 days after seeding ranged from 0.16 for *Lotus corniculatus* L. (bird's-foot trefoil) and *Galium aparine* L. (cleavers), to 0.17 for *Helianthus annuus* L. (sunflower), to 0.25 for *Chenopodium album* L. (lambsquarters) (Hunt and Cornelissen 1997). RGR is strongly correlated to leaf area ratio and specific leaf area (Cornelissen et al. 1996).

The leaf area ratio (LAR) is used to measure the 'leafiness' of a plant, calculated by dividing the total leaf area of a plant over the total plant weight. This is a measure of the amount of photosynthetically contributing tissues compared to the tissues that require the products of photosynthesis. LAR can be calculated from one harvest to the next using the formula $LAR = (A_1/W_1 + A_2/W_2) / 2$, where A is leaf area and W is plant dry weight. LAR is expressed as area/weight. The LAR in mm^2/mg of *L. corniculatus* was 11.1, *H. annuus* was

12.3, *G. aparine* was 14.5, and *C. album* 16.4 between 7 and 21 days after seeding (Hunt and Cornelissen 1997).

The leaf weight ratio (LWR) compares the total leaf weight to the total plant weight. This can be used as an indicator of the dry weight investiture that a plant puts towards its photosynthetic tissues. LWR is calculated over the time interval from one harvest to the next as: $LWR = ((W_{L1}/W_1) + (W_{L2}/W_2))/2$, where W_L is leaf weight and W is plant weight. LWR is expressed as weight/weight, or more simply without units. The LWR between 7 and 21 days after seeding of *L. corniculatus* and *G. aparine* was 0.6, *H. annuus* was 0.5, and *C. album* was 0.7 in a study by Hunt and Cornelissen (1997).

Specific leaf area (SLA) is the ratio of total leaf area to total leaf weight, and represents the thickness of the leaves. Specific leaf area over the time interval from harvest to harvest is calculated as: $SLA = ((A_1/W_{L1}) + (A_2/W_{L2})) / 2$, where A is leaf area, and W_L is dry weight of leaves. SLA is expressed as area/weight. SLA and LWR can be used to calculate LAR by the formula LAR = LWR * SLA. Between 7 and 21 days after seeding, the SLA in mm^2/mg of *L. corniculatus* was 18.4, *G. aparine* and *H. annuus* was 24.4, and *C. album* was 23.7 in a study by Hunt and Cornelissen (1997).

Leaf area index (LAI) is a measure of the leafiness of a crop on the ground in which it is growing. It is calculated as LAI = A/P, where A is leaf area, and P is the area of ground on which A was measured. Leaf area index is most commonly expressed without units. The LAI of oats grown in the Edmonton area by Ross et al. (2005) was between 7 and 8 at its maximum, between 60 and 70 days after planting. The LAI of alfalfa in St. Paul, Minnesota, at three harvests over the growing season was 5.1, 5.4, and 3.8 (Sharratt and Baker 1986). The LAI of peas ranged from 0.74 – 1.06 in a study by Mahon (1990), increasing with increasing planting density of peas.

1.3. Ruminant nutrition and fenugreek

1.3.1. Fenugreek in dairy rations

It has been reported that when livestock are first offered fenugreek they may be initially discouraged from eating by its odour (Slinkard et al. 2006). Of concern in dairy production is that the odour may come through in the milk, giving it an off-odour and making it less appealing to the consumer (Shah and Mir 2004). However, a study conducted by Shah and Mir (2004) demonstrated that this is not the case. In that study, fenugreek seed was included at 20% of dietary DM in order to test the effect of fenugreek on dairy cow performance and milk characteristics. Dry matter and energy intake were similar for cows on the fenugreek and control diets. Milk production and composition were similar across diets. No adverse effects on organoleptic characteristics of the milk were found. Milk cholesterol content was lower from cows on the fenugreek diet compared to the alfalfa diet, and functional fatty acid (linoleic, linolenic, and conjugated linoleic acids) concentration was increased for cows on the fenugreek diet.

1.3.2. Legumes in ruminant nutrition

High quality forage is of particular importance to lactating dairy cows as it is central to supporting high levels of milk production. Nutrient intake can be limited by total feed intake, especially during early lactation. Therefore, the feed that cows consume must be palatable and nutritious to support both milk production and gestation during late lactation. High quality forages have high digestibility and efficiency of use (Juskiw et al. 2000). Legume forages are generally consumed by cattle in greater quantities compared to grass forages at a comparable digestibility (Kennelly et al. 1993). Legumes also have higher nutritional quality than grasses (Barnes et al. 2003, Forbes and Watson 1999). Legumes have lower cell wall content than grasses, providing faster rates of digestion. However, while ruminants digest 60 – 70% of grass fibre, only 40 – 50% of legume fibre is digested (Buxton and Redfearn 1997). Energy availability of forages is limited by the fibre component because it is relatively slowly and

incompletely digested. Therefore, forages with lower fibre content, or more digestible fibre increase the energy available, and thus nutritional value (Buxton and Redfearn 1997). Forage quality has a large impact on the nutritional status of dairy cows as well as their productive ability because NDF, which is relatively high in forages, significantly influences rumen fill, rate of digestion and passage rate of feed from the rumen (Khorasani et al. 2001). Neutral detergent fibre is required in dairy rations to ensure proper rumen function (Oba and Allen 1999). Ration NDF should come from forages, and rations should contain at least 25% NDF. However, high NDF increases rumen fill resulting in lower intake (Oba and Allen 1999). Increased digestibility of the NDF fraction of feeds results in higher dry matter intake and milk yield (Oba and Allen 1999). In a study by Kennelly et al. (1993), alfalfa silage quality was higher than that of barley, triticale, or oat silages, based on the NDF content. In that study, cows fed alfalfa and barley silage based diets had higher DMI than those on oat or triticale silage based diets, but there was no difference in milk production or milk fat.

Intake of nitrogen is higher in alfalfa-based diets than grass-based diets because of the higher CP content of the legume (Khorasani et al. 2001). According to Broderick (1995), the CP of forage-based rations should include 35% of CP content that is not digested in the rumen but is passed through to be digested in the intestines.

1.3.3. *In situ* rumen bag studies in ruminant nutrition

The mobile bag technique can be used to assess the potential utilization of feed by ruminants. *In situ* methods of feed analysis, such as mobile nylon bags, are less expensive and can be used to measure more types of feed than *in vivo* methods (Van Straalen and Dooper 1993). Nylon bags placed in the rumen for a variety of time intervals provide information about the timing and degree to which a feed is degraded in the rumen. Bags that are put through the intestines of a ruminant provide information on the digestion of feed after degradation in the rumen. Taken together, the ruminal microbial fermentation and enzymatic

digestion in the intestines of a feed in the mobile bags provides information on the whole digestive tract utilization of a feed (Van Straalen and Dorper 1993).

In a 1991 study, Kennelly et al. determined that the DM and CP disappearance of alfalfa in the rumen increased with increasing time in the rumen, and that the amount of protein degraded in the rumen determined the amount going to, and therefore potentially digested in, the intestine. This finding was corroborated by Koenig and Rode (2001). Lower disappearance in the rumen is compensated for by increased disappearance in the intestine (Van Straalen and Dooper 1993). Kennelly et al. (1991) stated that when nylon bags are rinsed during the mobile bag procedure, digestibility can be considered a true, rather than apparent measurement, because rinsing removes microbes and endogenous materials.

In the 1991 study by Kennelly et al., 87% of alfalfa CP was digested in the rumen over 24 hours. After ruminal incubation, 8% of the CP content disappeared in the intestines. The total tract alfalfa CP digestion after 24 hours ruminal incubation was 96%. Alfalfa dry matter was degraded to 68% in the rumen after 24 hours, and 71% over the whole tract. In another study by Kennelly (1999), the mobile bag technique was used to compare high and low quality alfalfa silage to barley silage. After 144 hours of incubation of high quality alfalfa silage in the rumen, the DM was degraded to 81%, while the DM of low quality alfalfa was degraded to 78%. The CP fraction of the high and low quality alfalfa silages was degraded to 96% and 95%, respectively, over the same time period (Kennelly et al. 1999).

In a study by Kennelly et al. (1993) which compared alfalfa silage to cereal silages, 74% of the alfalfa silage dry matter was degraded, 89% of CP was degraded, and 33% of NDF was degraded after 24 hours of incubation in the rumen. Dry matter and NDF were digested to the same extent as the barley, oat and triticale silages to which alfalfa was being compared. However, alfalfa had the highest residual % CP of the four silage types. A study by Khorasani et al. (2001) showed the ruminal degradation and whole tract digestion of alfalfa silage to be 51% and 73% for DM, 38% and 71% for CP, 45% and 53% for NDF, and

43% and 52% for ADF. In a study by Van Straalen and Dooper (1993), disappearance of DM and CP of clover samples was 55% and 58% in the rumen, 69% and 91% in the intestine, and 86% and 96% over the whole tract.

While the ruminal degradation of a feed can be plotted as percent disappearance over time, the disappearance can also be used to calculate several parameters related to rumen kinetics. These include *a*, *b*, and *c* fractions of the feed, which are the rapidly degradable, potentially degradable, and undegradable fractions, respectively. The rate of digestion, or K_d, in %/h, can also be calculated. The effective ruminal degradability (ERD) can be calculated using the following formula, given an assumed and constant rate of passage K_p.

$ERD = a + (b * K_d) / (K_d + K_p)$

Kennelly (1999) found that the rate of digestion and ERD of high quality alfalfa silage dry matter were 6.77%/hr and 60%. The same parameters for CP of high quality alfalfa silage were 11%/hr, and 88%, assuming a rate of passage of 5%/hr. In a study by Khorasani et al. (2001), the rumen kinetics of alfalfa silage were compared to those of bromegrass silage. The *a* fractions of alfalfa silage and bromegrass silage were not significantly different at 35% and 32% respectively. The *b* fraction of alfalfa silage was 39%, significantly lower than 56% in bromegrass silage. The rate of degradation of alfalfa silage, 4.7%/h, was significantly higher than bromegrass silage, 2.5%/h. The ERD, assuming a 7%/h rate of passage, was 50.6% for alfalfa silage, significantly higher than the bromegrass silage at 42.2%. The *a* fraction of the CP content of alfalfa silage was significantly higher than bromegrass silage, 70.5% and 50.5% respectively, while the *b* fraction was significantly lower in the alfalfa silage than the bromegrass silage, 19.9% and 39.7% respectively. Rate of degradation of the CP fraction was significantly higher for alfalfa silage, 7.66%/h, than the bromegrass silage, 3.39%. The ERD was 80.5% for alfalfa silage, which was significantly higher than 62.7% for bromegrass silage.

Mustafa et al. (1996) conducted a study in which the rumen kinetics of fenugreek and alfalfa hay were determined. Fenugreek and alfalfa hays did not have significantly different *a* and *b* fractions for DM and NDF (30% and 32%

respectively for the DM *a* fractions for fenugreek and alfalfa hays, 39% and 41% respectively for the DM *b* fractions, 12% for both varieties for the NDF *a* fraction, and 44% and 47% respectively for the NDF *b* fractions). While the *b* fraction of CP was not significantly different between the forages types at 49%, the CP *a* fraction of fenugreek hay at 41% was significantly higher than that of alfalfa at 34%. The *a* fraction of the ADF content was not significantly different between forages at 10%, but the *b* fraction was significantly higher in alfalfa hay at 49% than fenugreek hay at 36%. The rates of degradation of DM, CP, and ADF were significantly higher for fenugreek hay at 8%, 20% and 6% respectively than for alfalfa hay at 5%, 10% and 3% respectively. There was no significant difference in rate of degradation for the NDF content at 3% and 4% respectively. Mir et al. (1993) also compared the *in situ* degradation of DM and CP in fenugreek and 10% bloom alfalfa hay. The *a* fractions of DM and of CP were not significantly different at 39% and 32% for DM and 41% and 45% for CP for fenugreek and alfalfa respectively, nor was the *b* fraction of DM at 36% and 34% respectively. However, fenugreek hay had a higher CP *b* fraction at 50% than alfalfa hay at 38%. The degradation rate was not significantly different between fenugreek and alfalfa hays for DM at 14% and 13% respectively, or CP at 20% and 16% respectively. The ERD of fenugreek hay was higher than that of alfalfa hay for DM at 65% and 58% respectively, and CP at 80% and 75% respectively.

1.4. Conclusion

Fenugreek has great potential for wider use in western Canada. Fenugreek is a marketable resource for humans or animals. However, before this can occur, research must be performed to determine the ability of fenugreek to grow in the more northern climate of central Alberta in order to determine if this crop can be produced in an economical, sustainable, and agronomically–sound fashion.

Given the potential yield and quality of fenugreek shown in previous studies, the goal of the research in this thesis was to determine if fenugreek can be grown in the Edmonton area to yield forage of sufficient quantity and quality for use by lactating dairy cows.

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Chapter 2: Fenugreek Development and Growth Characteristics

Null hypotheses

CDC Quatro and AAFC F70 will exhibit no significant differences in growth characteristics such as plant height, biomass, stage of development, resource partitioning between plant components, and growth analysis indicators.

The quality of whole plants and of plant components will not be significantly different between fenugreek genotypes.

2.1. Introduction

Over one million acres are planted to silage crops in Alberta every year (Helm and Salmon 2002). A high concentration of dairy producers in central Alberta means that there is particular emphasis on the production of high quality silage in this area. Legume forages are an important source of high-quality forage, and in Alberta alfalfa and peas are commonly used as sources of legume silage. Increased diversification of plants in cropping rotations which include high-quality forages would benefit producers in the central Alberta area. Annual legumes have many of the same rotation benefits as alfalfa, but increase crop rotation flexibility as they occupy cropping land for only one growing season (Mir et al. 1997).

Fenugreek (*Trigonella foenum-graecum* L.), a relatively new legume to be grown in AB, is being considered as another source of high quality legume forage. Fenugreek is an annual, single-cut legume originating in the Mediterranean region and on the Indian subcontinent. It is primarily grown for seed production, for use as a spice. Fenugreek has potential as a forage in Alberta because of the high and sustained quality of the plant (Acharya et al. 2008, Basu et al. 2008), in addition to its nitrogen fixation, and drought and frost tolerance (Sinskaya 1961, Acharya et al. 2006, 2008; McCormick et al. 2006). Fenugreek has an indeterminate growth habit, without a decline in quality at the onset of reproductive growth which allows greater flexibility of harvest timing (Slinkard et al. 2006). Fenugreek also provides the same rotation benefits as other legume crops, mainly nitrogen fixation (Acharya et al. 2008) for improved soil quality and reduced nitrogen inputs in subsequent crops. The high feed quality of fenugreek may make it suitable for forage production for dairy herds, which have high nutritional requirements.

Mir et al. (1998) recorded DM silage yield of fenugreek of 14.1 t/ha, though the long-term yield in Lethbridge, AB, under dryland conditions is 5.8 tonnes/ha (Acharya et al. 2008). Mir et al. (1997) noted that under field conditions, the crude protein (CP) content of fenugreek was 24.8% at 9 weeks post seeding, 19.8% at 15 weeks, and 15.7% at 19 weeks. Neutral detergent fibre

(NDF) increased over the growing season from 29.1% at 9 weeks, to 37.1% at 15 weeks, and 38.7% at 19 weeks, while acid detergent fibre (ADF) increased from 25.2%, to 30.6%, to 33.7% at 19 weeks. In this study by Mir et al. (1997), fenugreek was compared to early-bloom alfalfa, which contained 18.2% CP, 43.9% NDF, and 33.7% ADF. Mature fenugreek had lower fibre content than alfalfa, and CP was not different between fenugreek and alfalfa. A comparison of 17-week fenugreek and mid-bloom alfalfa silage fed to steers was carried out by Mir et al. (1998). Fenugreek silage had a lower NDF content than alfalfa. There was no difference in the dry matter intake, average daily gain, or feed efficiency of steers fed either alfalfa or fenugreek silage. Mustafa et al. (1996) concluded that fenugreek hay CP, NDF, and ADF were not different than late-cut (full-bloom) alfalfa hay. Mir et al. (1993) showed fenugreek had higher CP and lower NDF and ADF than late-bloom alfalfa, but lower CP and higher NDF and ADF than 10%-bloom alfalfa.

The potential benefits of fenugreek, demonstrated in southern Alberta and in Saskatoon, Saskatchewan, could be of use in the central Alberta region where there are many dairy producers interested in high quality forage options. Fenugreek has not previously been tested under the cooler growing conditions of central Alberta. Knowledge of fenugreek growth, yield, and quality is required in order to determine if it is an economically viable production choice in central Alberta. Information on the partitioning of plant resources between photosynthetic, structural, and reproductive tissues over time could provide insight into the way fenugreek grows.

Objectives of this study were to: 1) observe basic growth characteristics of fenugreek during sequential harvests over the growing season in the Edmonton area; these characteristics included plant height, crop biomass, and plant stage. 2) observe the partitioning of resources between root, stem, leaf, and pod+seed tissues in fenugreek, in terms of absolute weight per plant as well as percent of total plant weight. 3) determine the quality of morphological components as well as intact forage samples to verify that fenugreek is indeed a high quality forage, and that the quality profile is maintained over the duration of the growing season.

4) use growth analysis to gain further insight into the mechanisms of fenugreek growth and development.

2.2. Materials and Methods

2.2.1. Treatments and measurements

Two genotypes of fenugreek (*Trigonella foenum-graecum* L.) were grown on the University of Alberta Edmonton Research Station (53°25′N, 113°33′W), AB, Canada, in the growing seasons of 2006 and 2007, on an Orthic Black Chernozemic Malmo silty clay loam. The two genotypes used were AAFC F70, a breeding line from the forage-fenugreek breeding program at AAFC Lethbridge, and CDC Quatro, released commercially in 1992 by the Crop Diversification Centre (CDC) Saskatoon and the University of Saskatchewan. F70 seeds were obtained directly from LRC, while Quatro seeds came from Emerald Seed Products (Avonlea, Saskatchewan).

The research design was a randomized complete block with nine harvest dates randomly assigned to plots within genotype (see Appendix 1). Individual plots were 4 m long by 2.4 m wide containing 8 rows at 30 cm spacing.

The fall prior to seeding, Edge (ethalfluralin, Dow AgroSciences Canada Inc.), a pre-emergent herbicide was applied to the research plot area and cultivated into the soil during seed-bed preparation. Edge was re-applied in early May of the growing season, and weed post-emergent herbicides were also applied (Roundup: glyphosate, Monsanto Canada Inc., and Target: MCPA+mecoprop+dicamba, Syngenta Crop Protection Canada Inc.) for control of existing weeds prior to crop seeding. Phosphorus 11-52-0 fertilizer was applied to the test area at the rate recommended according to soil tests taken earlier in the month: 38 kg/ha in 2006, and 37 kg/ha in 2007.

Seed from both varieties was inoculated in mid-May, using a 10% solution of corn syrup in water as an adherent. The inoculant used in 2006 was rhizobia specific for alfalfa, provided by the LRC. Four grams of inoculant was used per kg of seed from each variety. Because of lack of nodulation in the 2006 growing season, a different inoculant was used in 2007. A tag-team mix, typically used on

soybeans, of *Sinorhizobium meliloti* for nitrogen-fixation and *Penicillium billaii* for phosphorus-solubilization, was provided by Philom Bios (currently Novozyme, Saskatoon, Saskatchewan). It was applied to the fenugreek seed at a minimum rate of 3.15g/kg of seed.

Seeding occurred on 29 May 2006, and 28 May 2007, at a rate of 22 lbs/ac (24.7 kg/ha), at a depth of 2 cm, using a 4-row custom plot seeder (Fabro Enterprises Ltd., Swift Current, SK, Canada). The seeding rate was similar to that used by Mir et al. (1997), which was 26 kg/ha. Emergence counts were conducted on 19 June 2006 and 2007 at 3 weeks post-seeding.

Harvests were conducted approximately every two weeks starting on 22 June 2006 and 5 July 2007 (Table 2.1.). At each harvest, plant height was recorded at 3 points within each plot prior to sample collection. Biomass samples consisted of two centre rows (rows 3 and 4) in each plot. The number of plants in rows 3 and 4 was counted as they were cut. Plants were cut 1" above the ground, placed in paper bags, dried in a forced air oven for 72h at 65^{o}C, and weighed. A sub-sample of 10 plants including roots were taken from the two rows adjacent to the biomass sample, 5 plants from each of rows 5 and 6 (Appendix 1.). These plants were measured for individual height and staged for the following criteria: the number of internodes, the number of shoots on the main stem, the number of true leaves on the main stem, the stage of flowering, and the development of pods (Stauss 1994). Each plant was then broken down into component parts – roots, stem, leaves and pods. These four components were pooled for all 10 sub-sample plants from each plot. Leaf area and pod area (when pods were present) were measured in cm^2 using a leaf area metre (LI-3100 Area Meter, Li-Cor Inc., Lincoln, Nebraska, USA) for each plot. Leaf and pod area were converted to leaf area index (LAI) and pod area index (PAI) by dividing the leaf or pod area per plant over the area occupied by each individual plant, as calculated by the number of plants cut in rows 3 and 4, given the area occupied by two rows of plants. Root, stem, pod and leaf samples were then dried in a forced air oven at 65^{o}C and weighed when dry. As the plants grew bigger the number of plants in a subsample was reduced per plot, such that only three plants per plot were taken

for sub-sampling in Harvests 8 and 9 in 2006. Harvests continued until mid-October in 2006 in order to determine a complete record of fenugreek growth, including the period of senescence. Plots at the east end of the trial area in 2007, with the numbers *26 and *27, were not harvested as they were proximate to a line of trees and were not subject to the same light and nutrient regime as the other plots.

2.2.2. Quality (nutrient content) analysis

Biomass, leaf, pod, and stem samples from each harvest were ground through a 2 mm screen in preparation for quality analysis. Roots were not ground, being of insufficient sample size. Plant component samples from Harvest 1 in 2006 were not ground for the same reason. To overcome this problem in early 2007, all plants in rows 5 and 6 were collected in order to provide a sufficiently large sample for grinding, though only 10 plants were selected for staging. Samples from two reps were used for quality analysis of each harvest.

ADF, NDF, and CP analysis were carried out at the University of AB, in the Department of Agricultural, Food and Nutritional Science. Neutral detergent fibre and ADF were determined using the Ankom^{200} Fibre Analyzer (Ankom Technology, Macedon, New York, USA), and CP was determined using a Leco TruSpec® C/N Elemental Determinator (Leco, St. Joseph, Michigan, USA).

Relative feed value (RFV) was calculated using the formula from Jeranyama and Garcia (2004):

RFV = (DDM*DMI)/1.29

where DDM = digestible dry matter = 88.9 – (0.779 * %ADF)

and DMI = dry matter intake as a percent of body weight = 120 / %NDF

2.2.3. Growth parameters

Growth parameters including relative growth rate, leaf area ratio, leaf weight fraction, and specific leaf area over time intervals were calculated using an online tool (Hunt et al. 2002). Formulas can be found in Hunt (1990).

- Relative growth rate (RGR) is calculated using
  $RGR = (\log_e W_2 - \log_e W_1) / t_2 - t_1$
  where W is plant weight, and t is time.
- Leaf weight fraction (LWF) is calculated using
  $LWF = ([L_{W1}/W_1] + [L_{W2}/W_2]) / 2$
  where W is plant weight, and L_W is the leaf weight.
- Leaf area ratio (LAR) is calculated using
  $LAR = ([L_{A1}/W_1] + [L_{A2}/W_2]) / 2$
  Where W is plant weight, and L_A is leaf area.
- Specific leaf area (SLA) is calculated using
  $SLA = ([L_{A1}/L_{W1}] + [L_{A2}/L_{W2}]) / 2$
  where L_A is leaf area, and L_W is leaf weight.

2.2.4. Statistical analysis

Fenugreek plots were arranged in a randomized complete block design (see Appendix 1). All data were tested for normality and homogeneity, and were found to meet these criteria. The GLM procedure in SAS (version 9.1, SAS Institute Inc., Cary, NC, USA) was used to determine if there was a significant difference ($P < 0.05$) in characteristics measured between years. The GLM procedure was used to test the effect of year in a fixed effect model. When testing the significance of year, comparisons were made between harvests of similar days from seeding in 2006 and 2007 (Table 2.2.). Therefore, Harvests 1 and 9 from 2006 were not used as there was no comparable days of growth harvested in 2007. Variety, year and interaction of variety and year were the fixed terms, and the random term was rep nested within year. Year was considered a fixed term because we were specifically interested in the differences between 2006 and 2007. There were sufficient differences between years to warrant examining the data from 2006 and 2007 separately. Therefore, the Mixed procedure in SAS was used to determine the significance ($P < 0.05$) of variety on criteria within year. Random terms were rep, and the rep*variety interaction, and the fixed term was variety. Characteristics tested included percent emergence, plant height, biomass

yield, weight per plant, weight of root per plant, weight of stem per plant, weight of leaves per plant, weight of pod+seed per plant, the percent of plant weight made up by root, stem, leaves, and pod+seed, leaf to stem ratio, leaf area index, and pod area index. For NDF, ADF and CP content of stems, leaves, pods and whole plants, and relative feed value of whole plants, it was statistically feasible to pool years 2006 and 2007, based on analysis using the GLM procedure. The Mixed procedure was then used to test for differences between genotypes. Tables showing the level of significance for the effect of year and genotype within year are in Appendix 2.

Regression equations were calculated using Proc reg in SAS to relate stand and plant characteristics to days after planting in each growing season. Characteristics examined were stand height, stand biomass, LAI, PAI, and NDF, ADF, CP, and RFV of whole plant samples. Linear and quadratic relationships were investigated.

Statistics were not conducted on the growth analyses characteristics, as there was only one data point per genotype per time interval.

2.3. Results and Discussion

2.3.1. Environmental conditions

The total rainfall (mm) over the growing season (May – October) in 2006 and 2007 was lower than the 30-year average (1971 – 2000) (Table 2.3. Agriculture, Life and Environmental Science website, National Climate Data and Information Archive). May precipitation in both years was higher than the 30-year average but all other months in 2006 and 2007 (June to October) were below the 30-year average. In 2006, only 8.6 mm of precipitation were received in August and September, compared to 60.2 mm over the same two months in 2007, and the 30-year average of 111.2 mm.

The overall growing season temperature average (^{o}C) for both years was similar to the 30-year average. The monthly average daily temperature from May to October was also similar to the long-term averages, with the exception of July, which was higher than the 30-year average in both years. The daily average

temperatures in 2006 (Figure 2.1.) show a spike in temperature in the middle of July where the average daily temperature was greater than 25°C. This coincided with the beginning of flowering of fenugreek. Average daily temperatures in July of 2007 also reached 25°C, and remained in the high 20°Cs for a sustained period of time (Figure 2.2.).

In 9 out of 10 years, the first fall frost in Edmonton occurs before October 6th, and the latest recorded first fall frost is October 8th (Dzikowski and Heywood 1990). In one out of three years, the growing season in the Edmonton area will be 121 days or less (Dzikowski and Heywood 1990). Because the last two harvests in 2006 represented fenugreek growth at 122 and 126 days after seeding, the final harvests in 2006 provided valuable information regarding the post-growing season state of fenugreek.

2.3.2. Growth characteristics

Data were tested for significant differences between years 2006 and 2007. Since the majority of growth characteristics were significantly different for year in more than 50% of the tests, years are presented separately.

2.3.2.1. Emergence

In both 2006 and 2007, F70 had significantly higher emergence than Quatro at three weeks after planting (Figure 2.3.). In 2006, emergence was 64 and 34 plants per m^2 for F70 and Quatro respectively, while in 2007 emergence was lower at 43 and 27 plants per m^2 respectively. It is possible that the emergence in 2007 was lower because of decreased soil moisture, as May 2007 precipitation was lower than in 2006. et al. (2006) recommended 135 plants/m^2, which was higher than the observed density in this study.

2.3.2.2. Plant height

At the beginning of the growing season, plant height in 2007 was lower than 2006, but was similar by 65 days after planting (Figure 2.4.). The increase in plant height in both years fit well to quadratic equations ($P < 0.001$ for both years)

(r^2 = 0.92 and 0.94 for F70 and Quatro in 2006, Table 2.6.; r^2 = 0.97 and 0.91 for F70 and Quatro in 2007, Table 2.7.).

In 2006, F70 and Quatro grew from 6 cm and 5 cm respectively at 24 days, to achieve maximum height of 60 cm and 63 cm at 122 days (Figure 2.4.). By 136 days, senescence led to a decrease in height to 51 cm and 58 cm respectively. In 2007, both genotypes were 9 cm at 38 days, and grew to 55 cm and 52 cm for F70 and Quatro respectively at 107 days (Figure 2.4.). The height of fenugreek plants in this study are similar to those reported by Duke (1981), Acharya et al. 2006 in Lethbridge, AB, and Slinkard et al. (2006) in Saskatchewan, in the range of 40 – 60 cm.

2.3.2.3. Biomass

Initially, biomass increased in a similar fashion for the 2006 and 2007 growing seasons (Figure 2.5.). However, while biomass levelled off in 2006 after 94 days, biomass in 2007 continued to increase later into the growing season. Biomass accumulation of Quatro in 2006 was best described by a linear function ($P < 0.001$, $r^2 = 0.76$), while F70 biomass accumulation had improved fit to a quadratic regression equation ($P < 0.001$, $r^2 = 0.77$) (Table 2.6.). Both genotypes in 2007 accumulated biomass in a linear fashion ($P < 0.001$, $r^2 = 0.88$ and 0.92 for F70 and Quatro respectively) (Table 2.7.).

In 2006, F70 had significantly higher biomass than Quatro up to 80 days, after which the two genotypes were not significantly different (Figure 2.5.). Both genotypes had accumulated 4.5 tonnes/ha DM by 136 days. In 2007, F70 and Quatro were not significantly different at the beginning and end of the growing season, but were different from 65 to 100 days, when F70 had greater biomass (Figure 2.5.). F70 achieved maximum biomass production at 100 days at 7.2 tonnes/ha. Maximum Quatro yield was 5.7 tonnes/ha DM at 107 days.

In 2006, the higher biomass of F70 than Quatro could be attributed to its higher plant density, as shown by its significantly higher emergence. However, after 80 days in 2006, the individual plant weight of the Quatro plants increased relative to that of the F70 plants in order to match the biomass production of F70.

In 2007, F70 and Quatro began the growing season with comparable biomass, indicating that Quatro plants had greater dry weight than F70 plants at the beginning of the season to make up the difference in plant density. However, from 65 to 100 days, the higher plant density of F70 provided a biomass advantage.

It appears that F70 senesces earlier than Quatro, as exhibited by the quadratic regression equation which describes F70 biomass in 2006, compared to Quatro and both varieties in 2007, which all follow a linear equation. It is unlikely that a producer would harvest fenugreek as late in the growing season as the final harvests in 2006, so the earlier decrease of F70 biomass likely won't be observed by producers.

Ross et al. (2005) found that the biomass yield in Edmonton of berseem clover (*Trifolium alexandrium* L.), an annual clover, was 6.7 to 8.1 tonnes/ha DM at approximately 82 days after planting. By 80 days, the yield of fenugreek was 3 – 4 DM tonnes/ha. Berseem and red clover (*Trifolium pratense* L.) achieved maximum DM yields of 5.47 and 4.07 tonnes/ha at 61 days of growth in a study by Brink and Fairbrother (1992). The maximum DM production of fenugreek was higher than berseem or red clover, but the maximum was achieved later in the growing season than the clovers, at or after 100 days.

Cereal DM silage yields in Lacombe, AB, averaged 14 tonnes/ha for triticale (*X Triticosecale* Wittmack) and 13 tonnes/ha for barley (*Hordeum vulgare* L.) (Helm and Salmon 2002). Silage yield of cereal and pea (*Pisum sativum* L.) mixes ranged from 6.6 tonnes/ha for triticale+peas to 12 tonnes/ha for oats (*Avena sativa* L.) +peas (Alberta Agriculture 2006). While the yield of fenugreek is lower than that of forage cereals, fenugreek forage quality is generally higher.

2.3.2.4. Individual plant weight

In general, individual plant weight was higher in 2007 than in 2006, and in both years Quatro had higher individual plant weight than F70 (Figure 2.6.). In 2006, there were significant genotype differences at 24, 80, 122 and 136 days.

While F70 was higher at 24 days, Quatro had higher plant weight at 80, 122, and 136 days. At 136 days, plant weight of Quatro at 23.8 g/plant was almost double that of F70 at 12.8 g/plant, explaining why biomass between the two genotypes was comparable at that time. This demonstrates the plasticity of fenugreek plants, as lower plant density was compensated for by increased individual plant weight.

In 2007, there was no significant effect of genotype (Figure 2.6.). F70 and Quatro were 23.4 g/plant and 21.6 g/plant respectively at 107 days. F70 was nearly double the weight of the previous year. Given that the plant weight of the two genotypes was not significantly different, it is likely that the increased F70 plant density accounted for the higher biomass yield from 65 to 100 days.

2.3.2.5. Root weight per plant

The root weight increased at a greater rate initially in 2007 than in 2006; however, root weight in 2006 continued to increase while in 2007 root weight levelled off around 80 days after planting. In 2006, root weight was significantly higher for F70 than Quatro at 24 days (Figure 2.7*a*.). Root weight increased to about 0.50 g at 122 days. In 2007, root weight was only significantly higher for Quatro at 100 days. Throughout the rest of the growing season, the two genotypes were not significantly different, and ended the growing season at 0.36 g (Figure 2.7*a*.).

Fenugreek roots were not excavated for this study. Plants were pulled up by hand, and therefore the root weight represented here is an underestimation of the true root weight due to fibrous roots that were not recovered with this method. There was no evidence of nodulation in either year, which could be due to the fact that there was sufficient nitrogen in the soil at the beginning of the growing season.

2.3.2.6. Stem weight per plant

Stem weight increased steadily over the 2006 growing season. Stem weight increased at a greater rate in 2007 than in 2006, but F70 levelled off at 80 days while Quatro continued to increase. Stem weight was significantly higher

for F70 at 24 days in 2006, but higher for Quatro at 80 and 136 days. Stem weight at 136 days was 9.06g for Quatro, and only 5.63g for F70 (Figure 2.7*b*.). Quatro had significantly higher stem weight in 2007 at 80 and 100 days (Figure 2.7*b*.). Both genotypes ended the season with approximately 6.5g of stem at 107 days.

The maximum stem DM weight was 316 and 205 g/m^2 for berseem and red clover (Brink and Fairbrother 1992). In 2006, F70 and Quatro stem weight were 371 and 309 g/m^2, while in 2007 stem weight was 183 and 348 g/m^2. Except for F70 in 2007, the stem weight per m^2 of fenugreek was similar to that of berseem and red clover.

Brink and Fairbrother (1992) found that maximum DM production coincided with maximum stem weight accumulation in erect berseem and red clover species, at 61 days of growth. While the fenugreek absolute stem weight was at its maximum after 100 days, as was biomass, the percent stem was maximized at 52 or 65 days (Figure 2.9.).

2.3.2.7. Leaf weight per plant

The rate of leaf weight increase was greater at the beginning of 2007 than 2006. However, F70 leaf weight in 2007 levelled off at 65 days and was similar to the genotype leaf weight from 2006 while Quatro continued to increase until the end of the growing season. At 24 and 80 days in 2006, Quatro had greater leaf dry weight than F70 (Figure 2.7*c*.). Both genotypes achieved maximum leaf weight of 3.3 g by 122 days, and lost leaf weight during senescence to 136 days. In 2007, Quatro had significantly higher leaf weight than F70 at 80 and 100 days. Both genotypes accumulated about 3.5 g of leaf dry matter per plant by 107 days (Figure 2.7*c*.).

Brink and Fairbrother (1992) stated that new leaf growth is offset by leaf senescence, and that senescence in erect species occurred primarily in the lower canopy. While lower leaves did senesce on the fenugreek plants, the leaf dry weight continued to increase over the growing season, indicating that new leaf tissue was still greater than senescing leaf tissue.

Root, stem and leaf weight in 2007 followed a similar trend to leaf weight, where Quatro stem and leaf weight continued to increase while F70 stem and leaf weight remained relatively constant at 80 and 100 days. However, F70 stem and leaf weight increased at 107 days, eliminating the significant difference between the two varieties. In 2007, the higher individual plant weight of Quatro at 100 days was likely due to significantly higher stem weight and leaf weight in Quatro plants than F70 plants at that time.

2.3.2.8. Pod+seed weight per plant

Pod+seed weight in 2007 increased at a greater rate over the growing season than in 2006. In 2006, pod+seed weight was significantly higher for F70 at 52 and 65 days, after which point Quatro had greater pod+seed weight (Figure 2.7*d*.). By 136 days, the pod+seed weight of Quatro, at 11.50g, was almost three times that of F70, at 4.15 g. There was no significant difference of pod+seed weight between genotypes in 2007. F70 and Quatro pod+seed weight increased to 12.85 g and 11.92 g respectively by 107 days.

In 2006, F70 pod+seed weight showed little change after 94 days, while the pod+seed weight of Quatro continued to increase. The F70 plants may have experienced some decrease in pod production as a result of the spike in temperature that occurred between 55 and 64 days, while the plants were flowering. However, Quatro plants were flowering at approximately the same time, so perhaps F70 plants were more susceptible to damage by hot conditions than Quatro plants. In 2007, the pod+seed weight for both genotypes was greater than in 2006, perhaps due to the lack of high temperature spikes during initial flowering.

While Quatro plants had significantly higher stem and leaf weight than F70 plants in 2007 at 100 days, the pod+seed weight was not different between the two genotypes. This may indicate that F70 was putting more growth resources into reproductive structures, as opposed to structural or primarily photosynthetic structures compared to Quatro.

2.3.2.9. Leaf to stem weight ratio

The curves of leaf to stem weight ratio decline were very similar between 2006 and 2007. In 2006, Quatro had a significantly higher leaf to stem ratio than F70 at 24 and 38 days, after which the genotypes were not significantly different until 136 days, when F70 was higher than Quatro (Figure 2.8.). The leaf to stem ratio decreased from 5.66 and 4.86 for Quatro and F70 respectively at 24 days, to 0.29 and 0.46 at 136 days. There was no genotype difference in leaf to stem ratio in 2007 (Figure 2.8.). F70 and Quatro leaf to stem ratio decreased from 1.86 and 1.79, respectively, at 38 days, to 0.54 and 0.52, respectively, at 107 days.

Though Quatro stem and leaf weights were significantly higher than F70 at 80 days in 2006 and 80 and 100 days in 2007, the stem and leaf weights were proportionally higher such that the leaf to stem ratio was not significantly different between genotypes at those time.

The maximum leaf to stem weight ratio reported by Brink and Fairbrother (1992) for berseem clover was 1.42. In white clover, which has a prostrate growth habit versus berseem clover and fenugreek which are more erect, the maximum leaf to stem weight ratio was 3.04. Both of these species' maximum leaf to stem weight ratio is less than that of fenugreek, which was maximum at 24 days, between 5 and 6.

2.3.2.10. Percent root per plant

The percent root per plant decreased to a similar extent over the growing season in both years. In 2006, the percent root was significantly different between genotypes at 80, 122, and 136 days, when F70 had significantly higher percent root (Figure 2.9*a*.). Percent root decreased from 13.2% and 12.1% for F70 and Quatro respectively at 24 days, to 3.1% and 2.1% at 136 days. In 2007, only at 80 days was there a significant genotype difference, when F70 was significantly higher than Quatro (Figure 2.9*a*.). Over the growing season, the percent root per plant decreased from 5.3% and 4.9% for F70 and Quatro respectively at 38 days, to 1.7% and 1.8% at 107 days.

The significant differences that are present between genotypes for percent root weight may reveal that F70 was investing more resources in root tissues than Quatro. However, the root collection method was relatively crude, and so differences between genotypes may not represent any true differences in root resource allocation.

2.3.2.11. Percent stem per plant

While the percent stem increased in a similar fashion in 2006 and 2007 at the beginning of the growing season, the 2006 percent stem decreased after 94 days while the percent stem in 2007 continued to increase. The percent stem per plant was significantly higher in 2006 for F70 at 24, 38, and 136 days, but higher for Quatro at 65 and 80 days (Figure 2.9*b*.). The percent stem increased until 52 days, decreased slightly from 65 to 94 days, and increased again from 94 to 136 days. By 136 days, the percent stem per plant was 44.4% and 37.7% for F70 and Quatro, respectively.

In 2007, the Quatro percent stem was significantly higher than F70 for 65, 80 and 100 days (Figure 2.9*b*.). The maximum percent stem was 44.3% for both genotypes, achieved by F70 at 52 days and Quatro at 65 days. By 107 days, the percent stem was 28.6% and 29.2% for F70 and Quatro respectively.

In both years between 65 and 100 days, it appears that Quatro placed more of a structural emphasis on growth than F70. The difference in stem weight and percent stem was not reflected in increased plant height. Therefore, Quatro stem was increasing in width or structural integrity rather than only in height.

The maximum percent stem of red and berseem clover occurred at 51 and 61 days of growth, respectively, at 60% and 49% (Brink and Fairbrother 1992). The maximum percent stem in fenugreek occurred around the same days of growth as these two erect clovers, but the maximum percent stem approached only 45 %. The percent stem in barley grown in Lacombe, AB is between 20 and 30% (Aasen 2000), which is generally lower than the percent stem in legumes, and fenugreek specifically.

2.3.2.12. Percent leaf per plant

The percent leaf in 2006 and 2007 decreased in a similar fashion, until 80 days when both genotypes in both years levelled off for the remainder of the growing season. In 2006, Quatro initially had significantly higher percent leaf than F70 (Figure 2.9*c*.). F70 had significantly higher percent leaf at 136 days. Both genotypes were more than 70% leaf at the beginning of the growing season and then decreased to less than 20% by 136 days. In 2007, the only significant difference was at 100 days when Quatro was greater than F70 (Figure 2.9*c*.). Percent leaf decreased from 61% at 38 days, to 15% at 107 days.

The trend of decrease of percent leaf of berseem, red, subterranean and white clovers over the growing season (Brink and Fairbrother 1992) was also shown in fenugreek plants. Fenugreek had higher percent leaf than the four clover varieties until 80 days of growth. After 80 days of growth, the percent leaf of fenugreek was similar to that of berseem clover, but lower than the other three clover species. The percent leaf of fenugreek decreases more rapidly than for the four clover species. This may be due to the increased resource partitioning to pod+seed in fenugreek later in the growing season.

2.3.2.13. Percent pod+seed per plant

While the percent pod+seed increased until 94 days in 2006, followed by a decrease in both genotypes, in 2007 the percent pod+seed continued to increase through the growing season. At 52 to 80 days and 136 days in 2006, Quatro had significantly higher percent pod+seed than F70 (Figure 2.9*d*.). By the end of the growing season, Quatro was 49.0% and F70 was 32.1% pod+seed. In 2007, F70 had significantly higher percent pod+seed in the middle of the growing season (Figure 2.9*d*.). The percent pod at 107 days was 54.5% and 53.7% for F70 and Quatro respectively. While Quatro put more emphasis on structural growth, F70 put more emphasis on reproductive growth.

Of berseem, red, subterranean (*Trifolium subterraneum* L.) and white clovers (*Trifolium repens* L.), maximum percentage of the reproductive fraction of the total forage was achieved by berseem, at 22% at 71 days of growth. The

other three species had 8% or less of total weight represented by the reproductive fraction (Brink and Fairbrother, 1992). It is clear that the reproductive (pod+seed) fraction of fenugreek contributes much more to overall plant weight than in the clovers. This is likely due to selection on fenugreek for seed production, since the crop is traditionally grown for seed.

Distribution of plant weight between plant components over time is demonstrated in Figure 2.10*a, b, c* and *d*. Overall plant percent composition change is represented in Figures 2.11. and 2.12. These graphs clarify the difference in resource allocation between genotypes and years.

2.3.2.14. Leaf area index (LAI)

In 2006, LAI increased steadily to 122 days, after which it decreased, while in 2007, LAI increased more rapidly until 80 days, after which it fluctuated until the end of the growing season (Figure 2.13.). The greater rate of increase of LAI in 2007 could be explained by the sustained increase in average daily temperature which occurred around 50 days in 2007, promoting photosynthetic tissue development in both genotypes.

The LAI of both genotypes in both years was best described by quadratic functions ($P < 0.001$) ($r^2 = 0.44$ for both genotypes in 2006, Table 2.6.; $r^2 = 0.56$ and 0.65 for F70 and Quatro respectively in 2007, Table 2.7.). The LAI in 2006 was significantly higher for F70 than Quatro from 24 to 80 days and at 136 days (Figure 2.13.). The highest leaf area index was 1.2, achieved by F70 at 122 days. In 2007, the LAI of F70 and Quatro was not significantly different throughout the growing season (Figure 2.13.). By 107 days, F70 had achieved maximum LAI at 1.2. The maximum LAI reached by Quatro was 1.05 at 100 days. Despite Quatro having significantly higher percent leaf dry weight than F70 at the beginning of the 2006 growing season, F70 had significantly higher LAI over these harvest dates. This may indicate that Quatro leaves were denser, while F70 leaves were larger but thinner.

In crambe (*Crambe abyssinica* Hochst.), the maximum LAI coincides with onset of flowering (Kmec et al. 1998). This is not the case in fenugreek, where

the maximum LAI is achieved between 100 and 122 days, approximately 50 days after the start of flowering. While the maximum LAI of oats is between 7 and 8 (Ross et al. 2005), the maximum LAI of peas was 1.14 in a study by Mahon (1990).

2.3.2.15. Pod area index (PAI)

While the PAI increased more rapidly in 2007 than 2006, both genotypes experienced a decrease in PAI at the end of both growing seasons due to senescence. PAI in both years followed a quadratic regression equation with increasing time ($P < 0.001$) (r^2 = 0.46 and 0.59 for F70 in 2006 and 2007, respectively, and r^2 = 0.59 and 0.72 for Quatro in 2006 and 2007, respectively) (Tables 2.6. and 2.7.).

In 2006, the only significant difference of PAI was on 52 and 65 days, when F70 was significantly higher than Quatro (Figure 2.14.). The PAI in 2006 did not exceed 0.4, achieved by both genotypes at 122 days. In 2007, the maximum PAI achieved by both genotypes, at 100 days, was 0.6, approximately half of the area index achieved by the leaves (Figure 2.14.). The only significant difference between the two genotypes occurred at 65 days, where F70 had a higher index than Quatro. As it is F70 which has the higher PAI versus Quatro, it seems that F70 puts more resources into reproductive structures, in terms of weight and area, than Quatro. Clarke and Simpson (1978) found that yield was more highly correlated to leaf area than to pod area in oilseed rape (*Brassica napus* L.). This is likely the case for fenugreek as well, as the PAI of fenugreek is one-third to one-half of the LAI.

2.3.3. Growth analyses

2.3.3.1. Relative growth rate (RGR)

The RGR of F70 and Quatro in 2006 decreased over the growing season from 0.14 for both genotypes at 24 – 38 days, to -0.003 and 0.010 for F70 and Quatro respectively at 122 – 136 days (Table 2.4.). The RGR over the span of the whole growing season was 0.044 and 0.053 for F70 and Quatro, respectively.

In 2007, RGR of both genotypes decreased from 0.136 and 0.149 for F70 and Quatro respectively at 38 – 51 days, to 0.039 and 0.024 at 100 – 107 days (Table 2.5.). The RGR over the whole season from 38 days to 107 days was 0.061 and 0.058 for F70 and Quatro respectively.

Whaley et al. (2000) determined that increased RGR of plants at a lower density could aid in maintaining dry matter production. Therefore, the higher overall RGR for Quatro in 2007 could have helped that genotype to match the biomass production of F70.

2.3.3.2. Leaf weight fraction (LWF)

The LWF of F70 decreased from 0.6669 to 0.02186 from the 24 – 38 day interval to the 122 – 136 day interval in 2006 (Table 2.4.). The LWF of Quatro decreased from 0.7395 to 0.1664 over the same time period. The LWF over the whole season was 0.4800 and 0.4468 for F70 and Quatro, respectively. In 2007, the LWF of F70 decreased from 0.6047 to 0.1565 from the 38 – 51 day interval to the 100 – 107 day interval (Table 2.5.). Over the same time period, the LWF of Quatro decreased from 0.5694 to 0.1837. The overall seasonal LWF was 0.4125 and 0.3924 for F70 and Quatro respectively. LWF was the only characteristic for which F70 had a higher overall seasonal value than Quatro, both in 2006 and 2007.

2.3.3.3. Leaf area ratio (LAR)

The LAR of F70 in 2006 decreased from 0.0103 cm^2/g at the interval of 24 – 38 days, to 0.0014 cm^2/g at the interval of 122 – 136 days (Table 2.4.). Similarly, the LAR of Quatro decreased from 0.0109 to 0.0013 cm^2/g over the same time intervals as above. The overall seasonal LAR for F70 and Quatro were 0.0068 and 0.0067 cm^2/g, respectively. The LAR of both genotypes spiked towards the middle of the growing season; F70 increased to 0.0116 cm^2/g for the interval 65 – 80 days, while Quatro increased to 0.0130 cm^2/g at 80 – 94 days. In 2007, F70 LAR decreased from 0.0098 to 0.0012 cm^2/g from the 38 - 51 day interval to the 100 - 107 day interval (Table 2.5.). Quatro LAR decreased from

0.0092 to 0.0016 cm^2/g over the same time period. The overall seasonal LAR were 0.0056 and 0.0052 for F70 and Quatro, respectively.

2.3.3.4. Specific leaf area (SLA)

The SLA of F70 decreased from 0.0157 to 0.0069 cm^2/g from the 24 – 38 day interval to the 122 to 136 day interval in 2006. Similarly, Quatro SLA decreased from 0.0158 to 0.0079 over the same time period (Table 2.4.). The overall SLA from 22 to 136 days was 0.0115 and 0.0112 for F70 and Quatro respectively. As for LAR, both genotypes experienced a spike in SLA towards the middle of the growing season. F70 increased to 0.0470 at the 65 – 80 day interval, while Quatro increased to 0.0614 at the 80 – 94 day interval. SLA of F70 decreased from 0.0177 at 38 – 51 days to 0.0080 cm^2/g at 100 – 107 days in 2007, while SLA of Quatro decreased from 0.0168 to 0.0091 cm^2/g over the same time (Table 2.5.). The seasonal overall SLA of the two genotypes was very similar: 0.0124 and 0.0123 for F70 and Quatro, respectively. The higher LAI of F70 in 2006 is not reflected in a difference in SLA.

2.3.4. Quality characteristics

There were few significant differences between years in the measured quality parameters, and results are therefore presented pooled across 2006 and 2007.

2.3.4.1. Neutral detergent fibre (NDF)

NDF content in F70 whole plants followed a quadratic regression equation relating it to increasing days from seeding, whereas Quatro NDF content was better described by a linear relationship ($P < 0.001$) ($r^2 = 0.56$ and 0.71 for F70 and Quatro, respectively) (Table 2.8.). In general, the NDF content of each component stabilized at about 97 days. There were no significant differences due to genotype for stem NDF content, which increased from 43% at 52 days to 63% at 115 days (Figure 2.15*a.*). The NDF content of leaves was 15% at 52 days, then decreased to 11% before increasing again (Figure 2.15*b.*). Quatro leaf NDF

increased more quickly, and was significantly higher than F70 at 80 days. Both genotypes had 14% leaf NDF at 115 days. The NDF content of pod+seed was significantly higher at 65 days for F70 at 30.5% than Quatro at 28.3% (Figure 2.15*c*). At 115 days pod+seed NDF was 36.6% and 35.5% for F70 and Quatro respectively. No significant genotype differences occurred in the NDF content of the whole plant. F70 and Quatro whole plant NDF increased from 19.5% and 18.3% at 38 days to 34.7% and 38.2% at 115 days for F70 and Quatro respectively (Figure 2.15*d*.).

Stem had the highest NDF, being that the role of stem is mainly structural. The NDF of pod+seed was also relatively high, but because the percent pod+seed was in general the greatest contributor to plant weight, the whole plant level of NDF was closer to that of pod+seed than to stem. The low NDF value at 38 days is likely due to the greater percent contribution of leaves. After 38 days, the percent leaf decreased and the other plant components with higher NDF content represented more of the plant weight. Brink and Fairbrother (1992) found that because the quality of stems is lower than, and declines at a greater rate than that of leaves, the accumulation of stem is the primary determinant to overall plant quality.

The NDF content of berseem clover over the growing season in the Edmonton area was 35% (Ross et al. 2005), which is slightly higher than the fenugreek in this study. The NDF content of whole fenugreek plants was lower than the 55% threshold described by Baron et al. (1992) citing Van Soest (1965), above which the intake of plant matter by ruminants is decreased. The NDF values of alfalfa (*Medicago sativa* L.), corn (*Zea mays* L.) silage, and timothy (*Phleum pratense* L.) are 49%, 51%, and 66% (Collins 1988 in Jeranyama and Garcia 2004). Given that the maximum NDF value of fenugreek is between 35% and 40%, NDF quality of fenugreek is superior to the three forages listed. Fenugreek grown in Lethbridge by Mir et al. (1997) had comparable NDF values to the fenugreek grown in Edmonton for the current study, at 29.1% at 9 weeks of growth and 37.1% at 15 weeks.

2.3.4.2. Acid detergent fibre (ADF)

ADF of whole F70 and Quatro plants was best described by a quadratic regression equation when related to increasing days from seeding ($P < 0.001$) ($r^2 = 0.51$ and 0.70 for F70 and Quatro, respectively) (Table 2.8.). There were no significant genotype differences for the ADF content of stem, leaves or the whole plant. The ADF content of pod+seed was significantly higher for Quatro at 97 days. In general, the ADF content of leaves, pod+seed, and the whole plant levelled off after 65 days, and after 97 days for stems. From 52 to 115 days, the ADF content of stems increased from 34% to 49%, and the ADF content of leaves decreased from 10% to 7%. From 65 days to 115 days, the ADF content of pods remained relatively constant at 20%. The ADF content of the whole plant increased from 38 to 115 days, from 13% to 22% (Figures 2.16*a – d*.).

Trends in ADF content were similar to NDF trends. The major contributors to ADF content were pod+seed and stem, and the whole plant ADF content was closer to that of the pod+seed. ADF content at 38 days was relatively low due to the increased percent contribution of leaves, which had the lowest ADF content of all components.

The ADF content of berseem clover over the growing season in the Edmonton area was 24.5% (Ross et al. 2005), comparable to the fenugreek values in this study. Fenugreek grown in Lethbridge by Mir et al. (1997) had slightly higher ADF content than the fenugreek grown in Edmonton, increasing from 25% at 9 weeks to 34% at 19 weeks. The ADF values of alfalfa, corn silage, and timothy are 34%, 28%, and 34% (Collins 1988 in Jeranyama and Garcia 2004). Given that the maximum ADF value of whole fenugreek plants is between 20% and 25%, ADF quality of fenugreek is superior to these three forages.

2.3.4.3. Crude Protein (CP)

Crude protein content of whole plants for both genotypes fit reasonably well to quadratic regression equations relating it to increasing age ($P < 0.001$) ($r^2 = 0.77$ and 0.81 for F70 and Quatro, respectively) (Table 2.8.). The CP content of leaves, pod+seed, and the whole plant was not significantly different between

genotypes. At 52 days, the CP content of stems was significantly higher for Quatro than for F70. From 38 to 115 days, the CP content of stems decreased from 28% to 7%, while the CP content of leaves decreased from 35% to 20%. The CP content of pod+seed stayed relatively stable, only changing from 25% to 23% from 65 to 115 days. The CP content of the whole plant decreased from 32% to 19% from 38 to 115 days (Figure 2.17*a* – *d*.).

The CP content of leaves was the highest of any of the measured components, while stem was the lowest. The CP content of pod+seed was greater than 20%. Since pod+seed was one of the greatest contributors to plant weight, pod+seed CP contributed substantially to the relatively high CP content of the whole plant.

Berseem clover grown in the Edmonton area had 18% CP at 88 days after planting (Ross et al. 2005), which is comparable to the values obtained in this study for fenugreek. The overall CP mean for berseem clover, across harvest dates, was 24.5% (Ross et al. 2005), which is slightly higher than the CP value for fenugreek over the whole growing season. The CP of berseem clover leaves was 27% and 12.5% for stems (Iannucci et al. 1996). These values are comparable to the CP content of fenugreek leaves and stems in this study. The CP content of whole plants was similar to fenugreek values found by Mir et al. (1997).

In berseem and red clover, whole-plant CP decreased from 28.2% to 13.9% at 71 days, and stem CP decreased from 18.5% to 7.8% (Brink and Fairbrother 1992). While the whole plant CP content of fenugreek was consistently higher than the two clover varieties, stem CP of fenugreek approached the same level by 80 days. The CP values of alfalfa, corn silage, and timothy are 16%, 10% and 10% (Collins 1988 in Jeranyama and Garcia 2004). Therefore, fenugreek is comparable to alfalfa, and both legumes have superior CP content to grass forage sources. The NDF, ADF and CP values over the growing season demonstrate that quality does not deteriorate rapidly after a 65 days, as there was little change in the fibre and protein content of F70 and Quatro.

2.3.4.4. Relative Feed Value (RFV)

The RFV calculation was only carried out using the data of whole-plant samples. The RFV was not significantly different between genotypes. At 38 days, the RFV was 420, and then decreased to end the season at 185 at 115 days (Figure 2.18.). RFV for both genotypes was described by quadratic regression equations relating it to increasing harvest date (r^2 = 0.47 and 0.64, $p<0.01$ and $p<0.001$ for F70 and Quatro respectively) (Table 2.8.).

The RFV of fenugreek plants was consistently higher than that of full-bloom alfalfa (100). The RFV of pre-bud alfalfa is 164; therefore, even at its most mature, the RFV of fenugreek was greater than high quality alfalfa. The highest RFV requirement by dairy animals is for cows in their first three months of lactation, who require forages with an RFV of 140 – 160. Fenugreek is therefore a suitable forage source for dairy cows, even those with the highest nutrient requirement.

Because the RFV index was developed in the United States using alfalfa, it may not be an accurate measure against which to compare fenugreek grown in Alberta. Given the results in Chapter 3 that indicate fenugreek was degraded and digested to a similar extent as alfalfa, the fenugreek RFV may be an over-estimation of the feed potential of this plant.

2.3.5. Staging and development

The outcome of plant staging at sequential harvests during the growing seasons of 2006 and 2007 is shown in Figure 2.19. Pictures of plant development over the 2007 growing season are found in Figures 2.20 and 2.21.

At 24 days, fenugreek plants were a single branch with a single leaf. Branching commenced by 38 days, and flowering occurred around and after 52 days. Pod development began between 52 and 65 days. Even though the number of internodes increased to 26, the maximum number of shoots was 5. There was little variation in the measured traits between the two genotypes at any specific harvest, or between years. Staging and development information can provide a useful tool for determining the correct timing for herbicide application.

Developmental information is also useful for producers, in order to mark the progress of their crop through the growing season. This is especially true for fenugreek, novel to the central Alberta area, where producers may wish for extra information on crop growth during initial growing seasons.

2.4. Conclusion

The description of fenugreek growth in the Edmonton area is important to determine if this crop can successfully be incorporated into growing systems in that region. Fenugreek is especially suited to dairy producers who have an emphasis on high quality forage.

Sequential harvests over two growing seasons described the growth of fenugreek. In general, there were few differences between the two genotypes studied within year, indicating that in many respects F70 and Quatro grew similarly under the conditions in Edmonton, AB. Plant height, biomass production, and plant stage and development were similar between genotypes. Plant heights were within the range of previously recorded fenugreek height, and while fenugreek biomass production was generally lower than alfalfa in the same region, it was similar to long-term fenugreek averages in the Lethbridge, AB, area, and to pea+cereal silage mixes. Fenugreek's plasticity was demonstrated by Quatro's ability to achieve the same biomass as F70 in 2006, despite having a lower plant density. In general, F70 and Quatro did not partition resources differently between components. Percent pod+seed was a major contributor to plant weight in the middle and late growing season, and was therefore a determinant of whole-plant quality. This resulted in relatively high whole-plant quality. Quality was not significantly different in 2006 and 2007, and there were very few genotype differences. RFV was between 150 and 250 for most of the growing season, so even at its minimum, the RFV of fenugreek makes it suitable for dairy cows during lactation. Pod area index was about one-third to one-half of leaf area index. RGR, LAR, LWF, and SLA all decreased in general over the growing season for both genotypes in both years, and both genotypes were similar over the whole growing season.

The results of this experiment indicate that fenugreek is a potential source of high quality legume forage for dairy producers in the central Alberta area. Yield is comparable to that of other legume forages, and quality is high enough to be used in dairy rations to support high levels of milk production. Further research is needed on specific agronomic practices to optimize fenugreek quality and biomass production in central Alberta.

Table 2.1. Date of harvest and days from seeding for sequential fenugreek harvests conducted in 2006 and 2007 on the Edmonton Research Station, Edmonton, AB.

Year			
2006		2007	
Harvest date	Days from seeding	Harvest date	Days from seeding
1: 22 June	24		
2: 6 July	38	1: 5 July	38
3: 20 July	52	2: 18 July	51
4: 2 August	65	3: 1 August	65
5: 17 August	80	4: 15 August	79
6: 21 August	94	5: 5 September	100
7: Did not occur (adverse weather)		6: 12 September	107
8: 28 September	122		
9: 12 October	136		

Table 2.2. Harvests compared to determine if the effect of year was significant on growth characteristics and plant quality of fenugreek grown on the Edmonton Research Station, Edmonton, AB, in 2006 and 2007.

Harvest in 2006	Harvest in 2007	Days from seeding
Compared to		
H2	H1	38
H3	H2	51
H4	H3	65
H5	H4	80
H6	H5	97
H7	H6	115

Table 2.3. Total monthly and seasonal rainfall (mm) and average monthly and seasonal temperature (^{o}C) for the growing seasons in 2006 and 2007 on the Edmonton Research Station, Edmonton, AB.

	Rainfall (mm)			Average temperature (^{o}C)		
	2006	2007	Long-term average*	2006	2007	Long-term average*
May	70.6	58.2	45.1	12.5	11.4	11.7
June	63.0	61.2	87.1	17.0	15.9	15.5
July	28.2	51.8	91.7	19.7	20.9	17.5
August	6.1	32.5	68.9	16.8	14.7	16.6
September	2.54	27.7	42.3	12.5	10.5	11.3
October	35.0	2.8	10.5	2.9	6.3	5.6
Total rainfall	205.5	234.2	345.6			
Average temperature				13.6	13.3	13.0

*Indicates the average calculated from 1971 to 2000 at the Edmonton Municipal Airport (National Climate Data and Information Archive 2009.)

Table 2.4. Mean relative growth rate (g/g/day), leaf area ratio (cm^2/g), leaf weight fraction (g/g), and specific leaf area (cm^2/g) per plant for two fenugreek genotypes (F70 and Quatro) for harvest intervals and over the whole growing season of 2006 on the Edmonton Research Station, Edmonton, AB.

Growth interval (days)	Relative growth rate		Leaf area ratio		Leaf weight fraction		Specific leaf area	
	F70	Quatro	F70	Quatro	F70	Quatro	F70	Quatro
24-38	0.1377	0.1424	0.0103	0.0109	0.6669	0.7395	0.0157	0.0158
38-52	0.0765	0.0982	0.0062	0.0070	0.5036	0.6149	0.0122	0.0123
52-65	0.0721	0.0455	0.0035	0.0039	0.3494	0.4091	0.0102	0.0099
65-80	0.0212	0.0735	0.0116	0.0027	0.2475	0.2713	0.0470	0.0103
80-94	0.0404	0.0170	0.0017	0.0130	0.2108	0.2169	0.0084	0.0614
94-122	0.0072	0.0190	0.0018	0.0020	0.2192	0.1990	0.0084	0.0107
122-136	-0.0029	0.0101	0.0014	0.0013	0.2186	0.1664	0.0069	0.0079
24-136	0.0441	0.0533	0.0068	0.0067	0.4800	0.4468	0.0115	0.0112

Table 2.5. Mean relative growth rate (g/g/day), leaf area ratio (cm^2/g), leaf weight fraction (g/g), and specific leaf area (cm^2/g) per plant for two fenugreek genotypes (F70 and Quatro) for harvest intervals and over the whole growing season of 2007 on the Edmonton Research Station, Edmonton, AB.

Growth interval (days)	Relative growth rate		Leaf area ratio		Leaf weight fraction		Specific leaf area	
	F70	Quatro	F70	Quatro	F70	Quatro	F70	Quatro
38-51	0.1360	0.1490	0.0098	0.0092	0.6047	0.5694	0.0177	0.0168
51-65	0.0970	0.0760	0.0070	0.0070	0.4258	0.4339	0.0164	0.0161
65-79	0.0304	0.0558	0.0033	0.0037	0.2651	0.2937	0.0125	0.0125
79-100	0.0172	0.0180	0.0018	0.0022	0.1880	0.2205	0.0093	0.0100
100-107	0.0385	-0.0239	0.0012	0.0016	0.1565	0.1837	0.0080	0.0091
38-107	0.0606	0.0579	0.0056	0.0052	0.4125	0.3924	0.0124	0.0123

Table 2.6. Regression equations for two fenugreek genotypes (F70 and Quatro) relating plant height (cm), biomass (t/ha), leaf area index per plant and pod area index per plant to days after planting over the 2006 growing season, where *d*=days after planting minus 24 (time of first harvest). Standard errors are in parentheses.

Characteristic	Variety	Regression equation	r^2
Stand height (cm)	F70	$Y = 6.06(1.66) + 1.02(0.07)d - 0.005(0.0006)d^2$	0.92***
	Quatro	$Y = 2.66(1.76) + 1.14(0.07)d - 0.006(0.0006)d^2$	0.94***
Stand biomass (t/ha)	F70	$Y = -0.44(0.32) + 0.09(0.01)d - 0.0004(0.0001)d^2$	0.77***
	Quatro	$Y = -0.58(0.28) + 0.05(0.004)d$	0.76***
Leaf area index per plant	F70	$Y = 0.02(0.11) + 0.02(0.005)d - 0.0001(0.00004)d^2$	0.44***
	Quatro	$Y = -0.07(0.10) + 0.02(0.004)d - 0.00008(0.00004)d^2$	0.44***
Pod area index per plant	F70	$Y = -0.21(0.11) + 0.01(0.004)d - 0.00008(0.00003)d^2$	0.46***
	Quatro	$Y = -0.31(0.13) + 0.01(0.004)d - 0.00006\ (0.00003)d^2$	0.55***

NS = not significant $P > 0.05$; * = significant at $P < 0.05$; ** = significant at $P < 0.01$; *** = significant at $P < 0.001$

Table 2.7. Regression equations for two fenugreek genotypes (F70 and Quatro) relating plant height (cm), biomass (t/ha), leaf area index per plant and pod area index per plant to days after planting over the 2007 growing season, where *d*=days after planting minus 24 (time of first harvest). Standard errors are in parentheses.

Characteristic	Variety	Regression equation	r^2
Stand height (cm)	F70	$Y = 9.53(1.04) + 1.10(0.07)d - 0.006(0.001)d^2$	0.97***
	Quatro	$Y = 9.22(2.01) + 1.28(0.14)d - 0.01(0.002)d^2$	0.91***
Stand biomass (t/ha)	F70	$Y = -0.29(0.30) + 0.11(0.007)d$	0.88***
	Quatro	$Y = -0.38(0.19) + 0.09(0.005)d$	0.92***
Leaf area index per plant	F70	$Y = 0.23(0.11) + 0.03(0.008)d - 0.0003(0.0001)d^2$	0.56***
	Quatro	$Y = 0.1(0.1) + 0.04(0.007)d - 0.0004(0.00009)d^2$	0.65***
Pod area index per plant	F70	$Y = -0.34(0.17) + 0.03(0.009)d - 0.0002(0.0001)d^2$	0.59***
	Quatro	$Y = -0.34(0.12) + 0.03(0.007)d - 0.0002(0.00008)d^2$	0.72***

NS = not significant $P > 0.05$; * = significant at $P < 0.05$; ** = significant at $P < 0.01$; *** = significant at $P < 0.001$

Table 2.8. Regression equations for two fenugreek genotypes (F70 and Quatro) relating neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP) and relative feed value (RFV) of whole F70 and Quatro fenugreek plants to days after planting over the 2006 and 2007 growing seasons, where *d*=days after planting minus 38 (time of first harvest). Standard errors are in parentheses.

Characteristic	Variety	Regression equation	r^2
NDF of whole plant (%)	F70	$Y = 21.41(2.11) + 0.47(0.13)d - 0.004(0.002)d^2$	0.56***
	Quatro	$Y = 22.17(1.39) + 0.23(0.03)d$	0.71***
ADF of whole plant (%)	F70	$Y = 14.20(1.55) + 0.41(0.10)d - 0.004(0.001)d^2$	0.51***
	Quatro	$Y = 13.99(1.16) + 0.37(0.07)d - 0.003(0.0009)d^2$	0.70***
CP of whole plant (%)	F70	$Y = 31.35(1.32) - 0.55(0.08)d + 0.005(0.001)d^2$	0.77***
	Quatro	$Y = 33.41(1.37) - 0.61(0.08)d + 0.006(0.001)d^2$	0.81***
Relative Feed Value	F70	$Y = 375.28(35.30) - 7.51(2.19)d + 0.07(0.03)d^2$	0.47**
	Quatro	$Y = 389.10(27.69) - 7.57(1.72)d + 0.06(0.02)d^2$	0.64***

NS = not significant $P > 0.05$; * = significant at $P < 0.05$; ** = significant at $P < 0.01$; *** = significant at $P < 0.001$

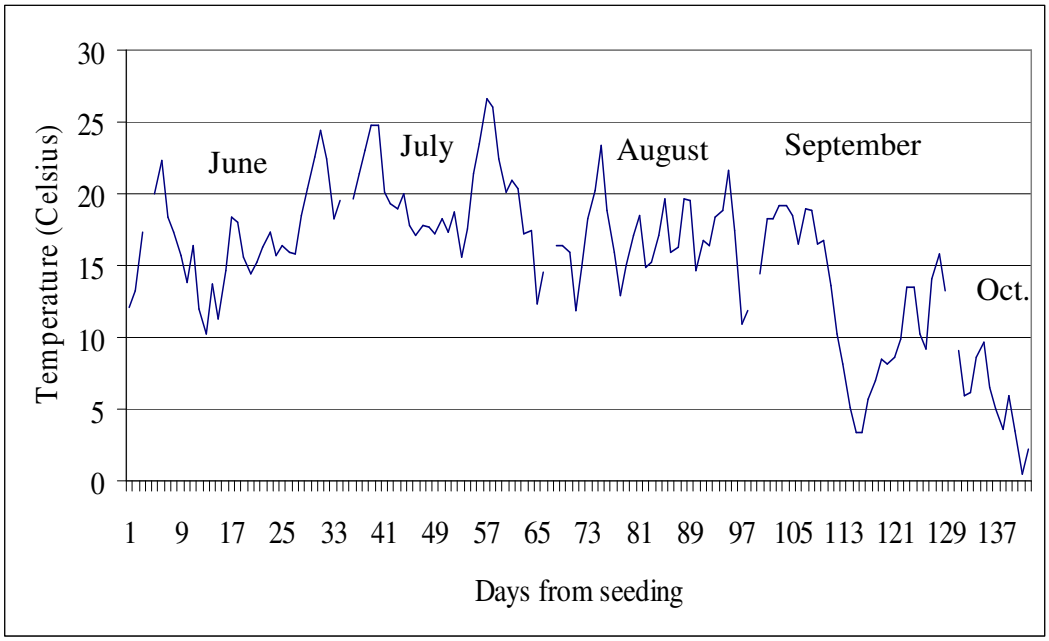


Figure 2.1. Average daily air temperature (°C) for the growing season in 2006 on the Edmonton Research Station, Edmonton, AB, shown from the date of seeding, 29 May. (Anonymous 2008)

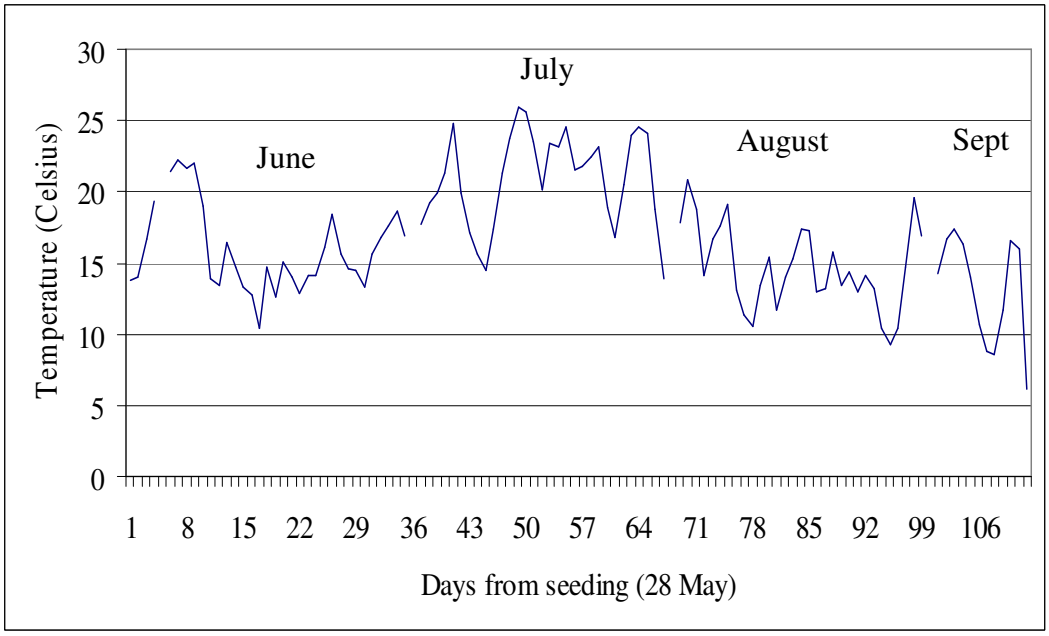


Figure 2.2. Average daily air temperature (°C) for the growing season in 2007 on the Edmonton Research Station, Edmonton, AB, shown from the date of seeding, 28 May. (Anonymous 2008)

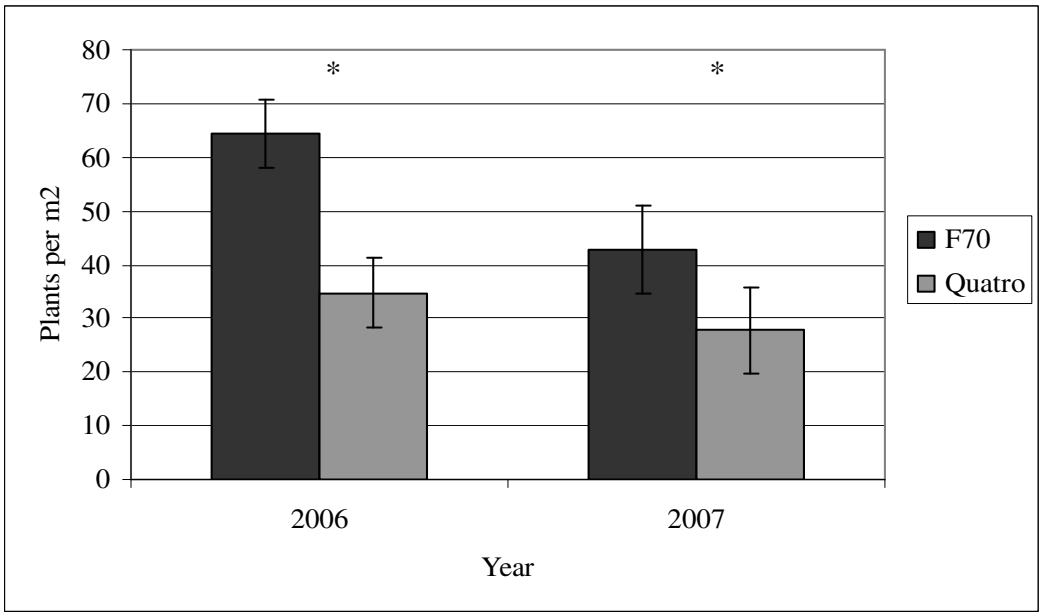


Figure 2.3. Mean emergence (plants per m^2) for two fenugreek genotypes (F70 and Quatro) three weeks after seeding in 2006 and 2007, grown on the Edmonton Research Station, Edmonton, AB.

* significant difference ($P < 0.05$) between varieties within year.

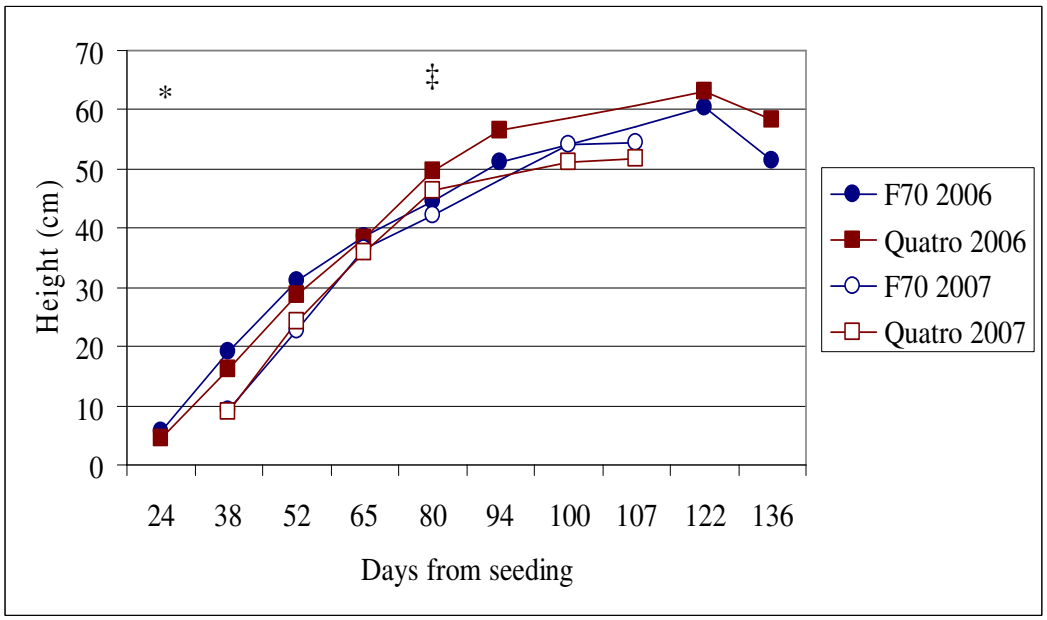


Figure 2.4. Mean plant height (cm) for two fenugreek genotypes (F70 and Quatro) taken over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.

Lines without markers indicate data was not collected at that time.

* significant difference ($P < 0.05$) between varieties in 2006.

‡ significant difference ($P < 0.05$) between varieties in 2007.

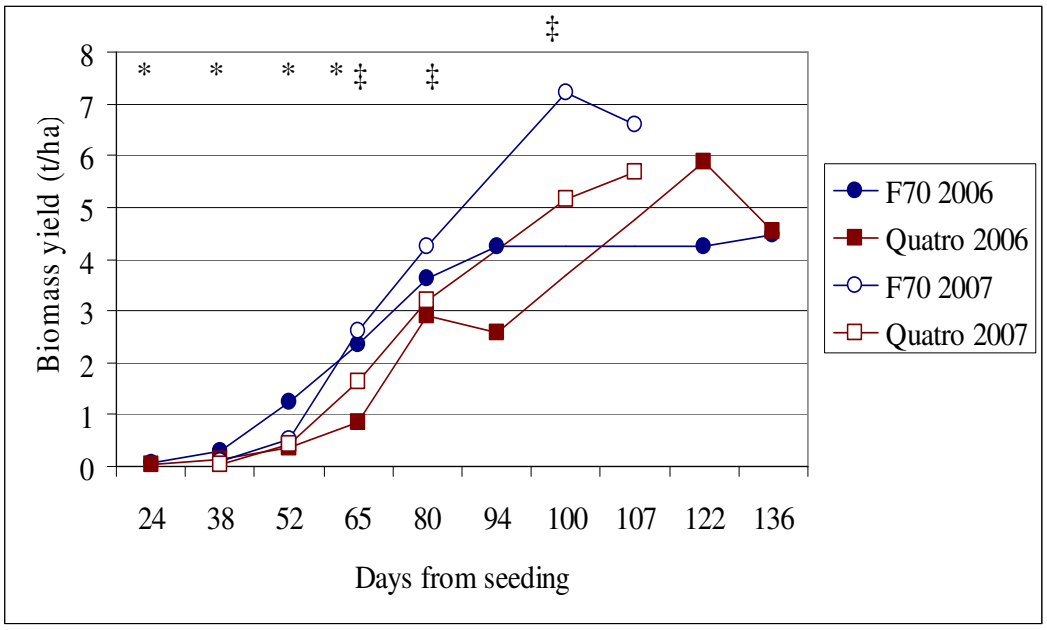


Figure 2.5. Mean above-ground biomass yield (tonnes/ha) for two fenugreek genotypes (F70 and Quatro) grown in 2006 and 2007 on the Edmonton Research Station, Edmonton, AB.

Lines without markers indicate data was not collected at that time.
* significant difference ($P < 0.05$) between varieties in 2006.
‡ significant difference ($P < 0.05$) between varieties in 2007.

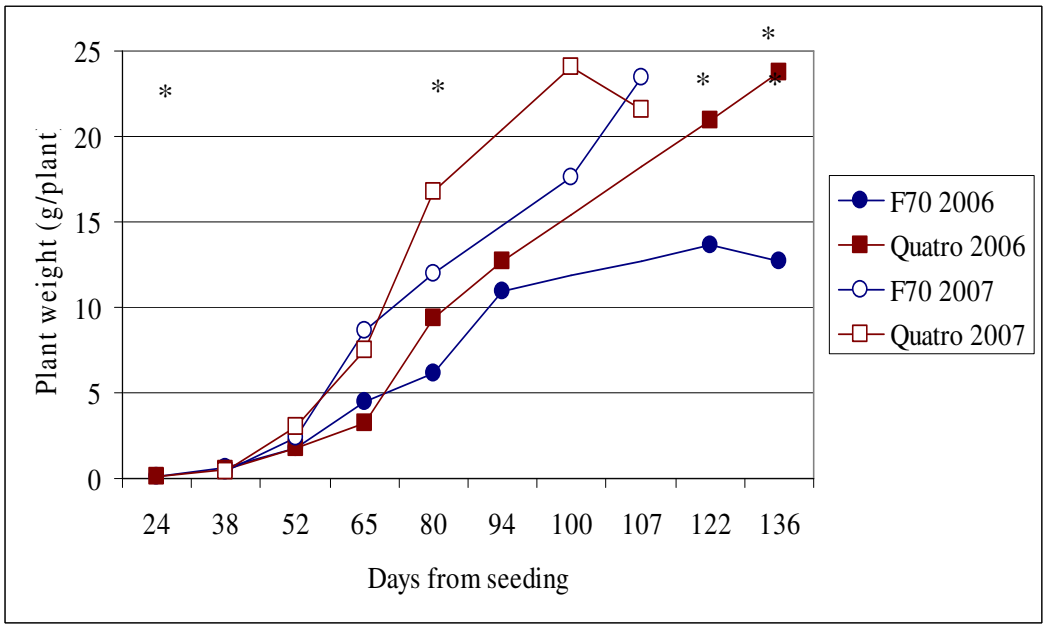
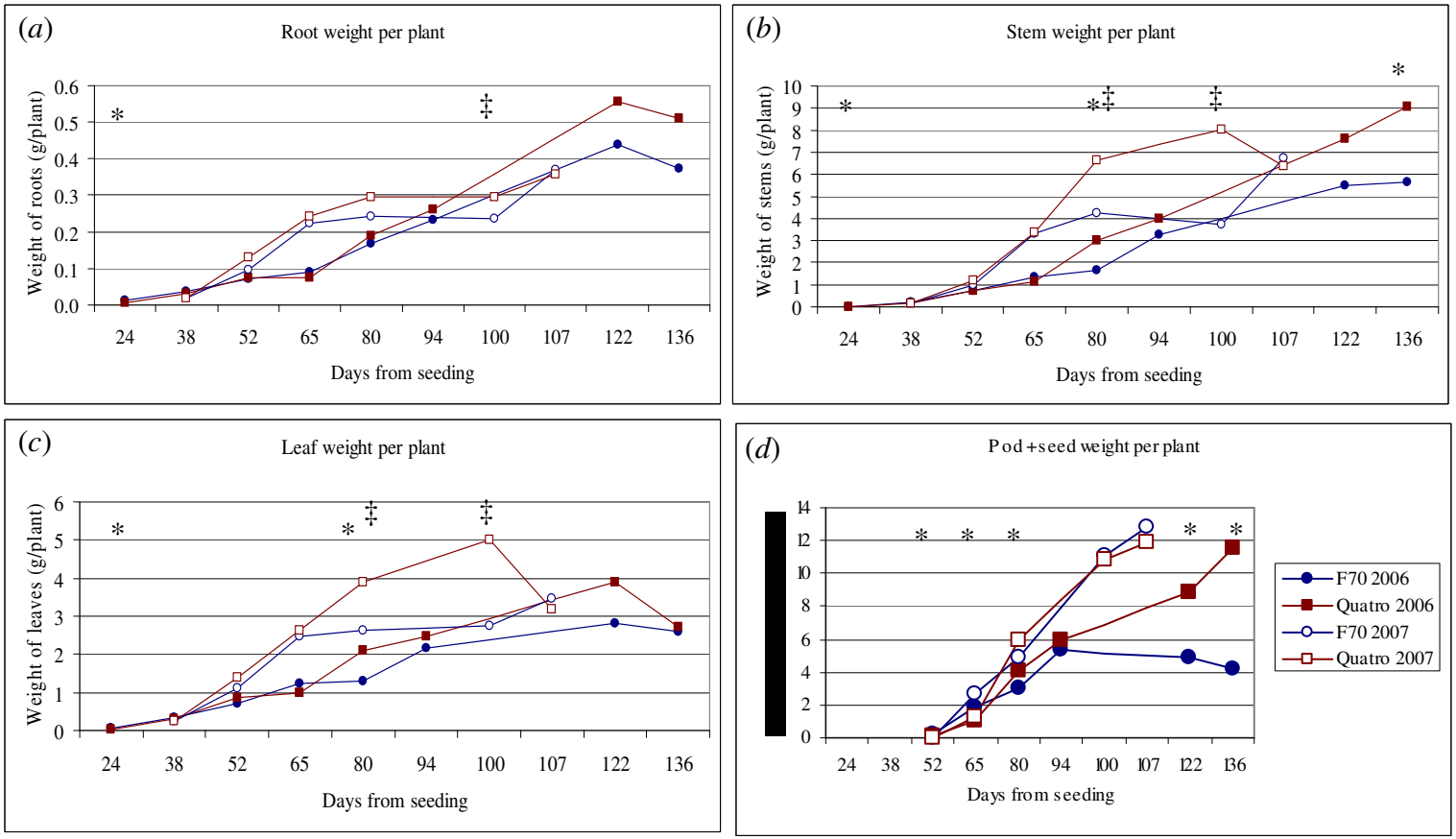


Figure 2.6. Mean dry weight per plant (g) for two fenugreek genotypes (F70 and Quatro) over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.

Lines without markers indicate data was not collected at that time.
* significant difference ($P < 0.05$) between varieties in 2006.
‡ significant difference ($P < 0.05$) between varieties in 2007.



Figures 2.7. *a, b, c* and *d*. Mean dry weight of root (*a*), stem (*b*), leaf (*c*), and pod+seed (*d*) components per plant (g) of two fenugreek genotypes (F70 and Quatro) over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.
Lines without markers indicate data was not collected at that time.
* significant difference ($P < 0.05$) between varieties in 2006.
‡ significant difference ($P < 0.05$) between varieties in 2007.

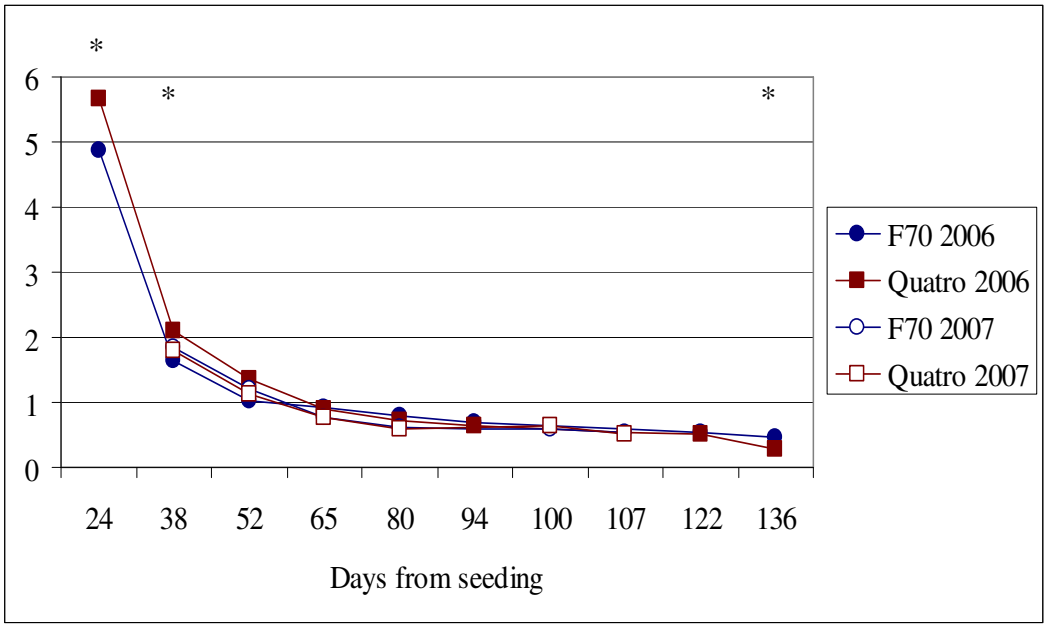
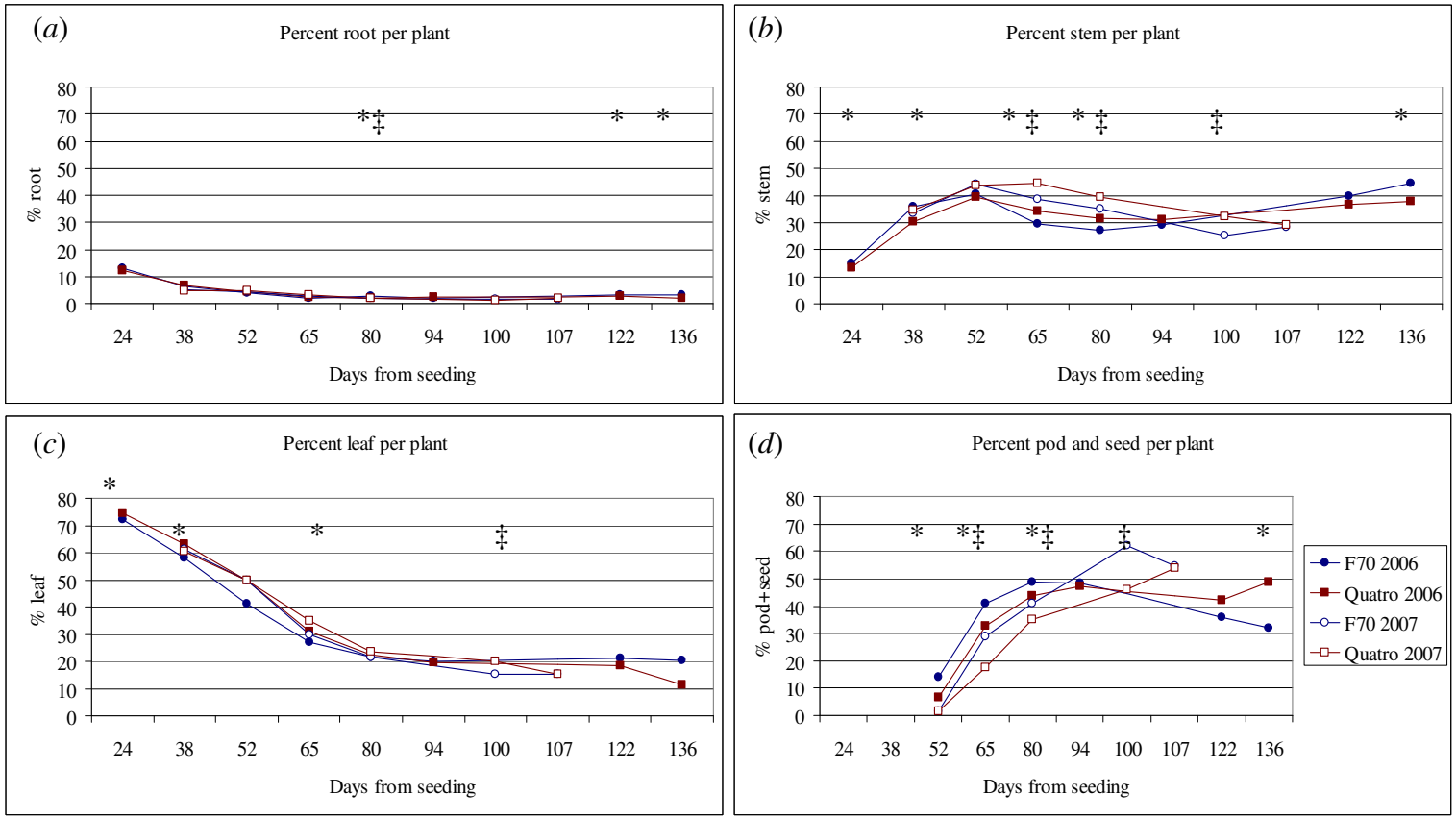


Figure 2.8. Mean leaf to stem weight ratio per plant for two fenugreek genotypes (F70 and Quatro) over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.

Lines without markers indicate data was not collected at that time.

* significant difference ($P < 0.05$) between varieties in 2006.

‡ significant difference ($P < 0.05$) between varieties in 2007.



Figures 2.9. *a, b, c,* and *d*. Mean percent contribution of root (*a*), stem (*b*), leaf (*c*), and pod+seed (*d*) components to total dry weight per plant of two fenugreek genotypes (F70 and Quatro) over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.

Lines without markers indicate data was not collected at that time.

* significant difference ($P < 0.05$) between varieties in 2006.

‡ significant difference ($P < 0.05$) between varieties in 2007.

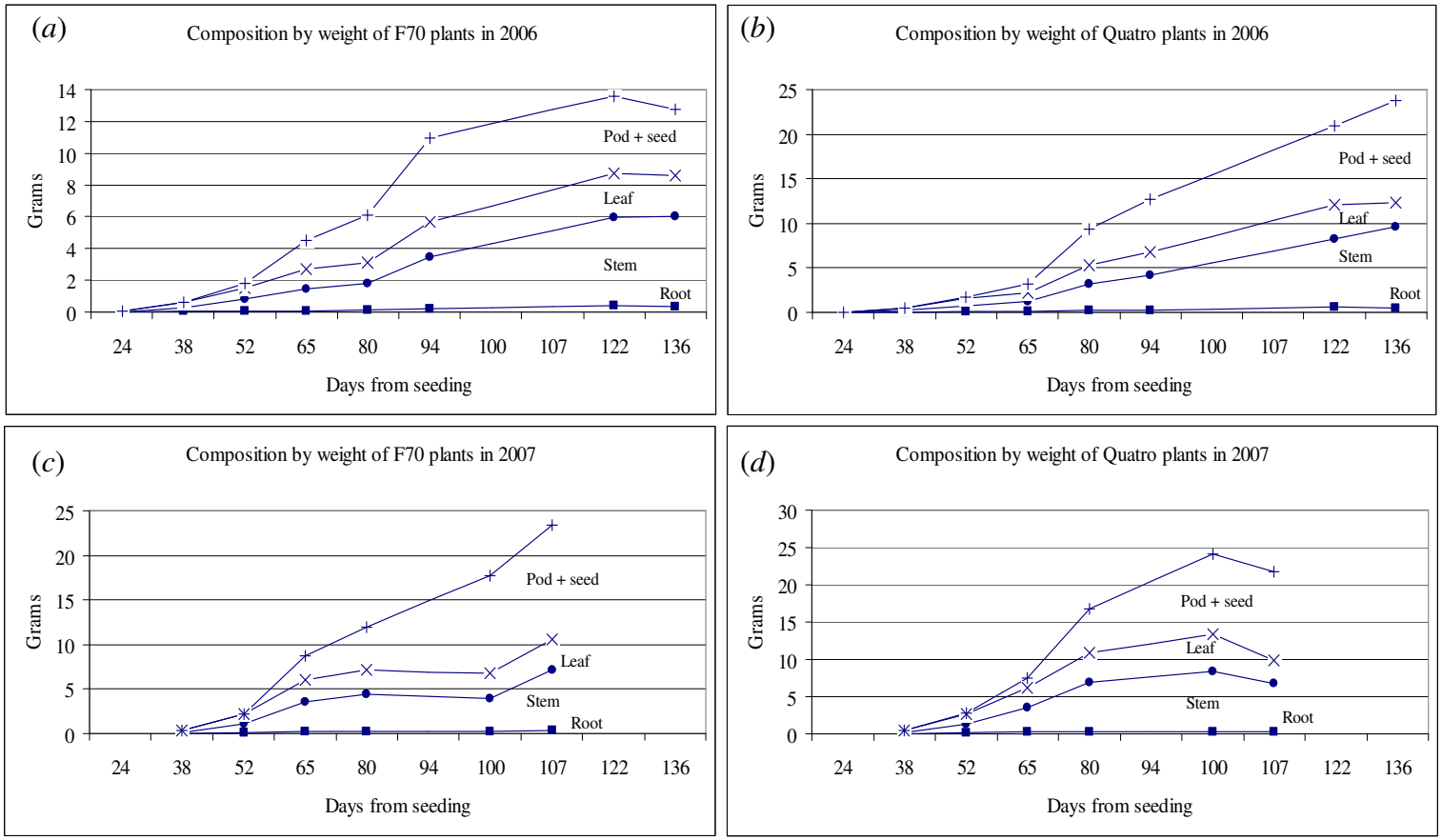


Figure 2.10. *a, b, c,* and *d*. Cumulative contribution of root, stem, leaf, and pod+seed components (g) to the total dry plant weight of two fenugreek genotypes (F70 and Quatro) over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.

Lines without markers indicate data was not collected at that time.

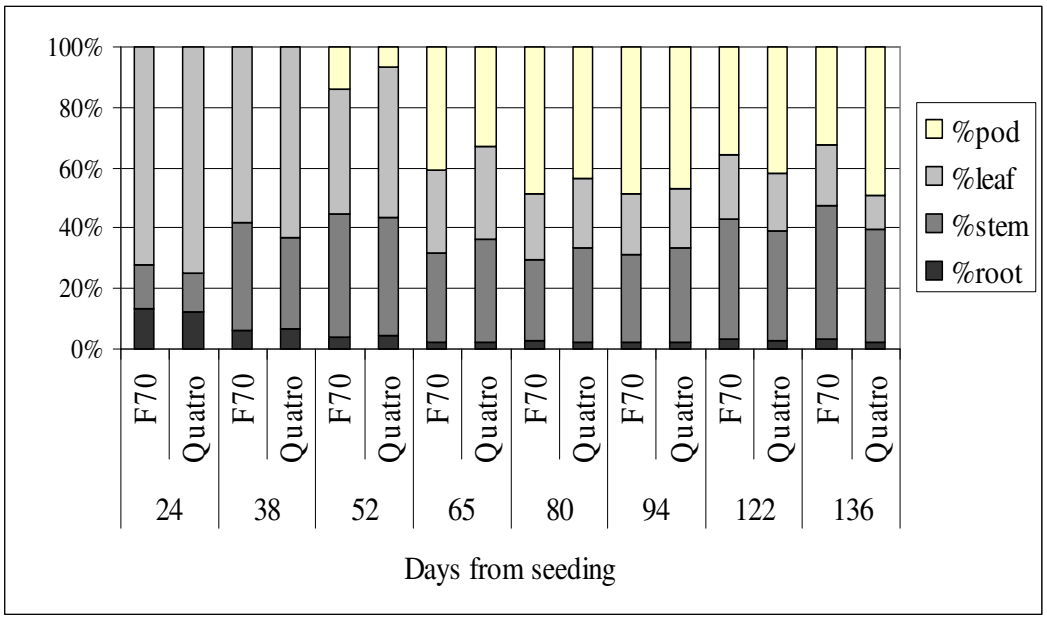


Figure 2.11. Mean percent composition per plant, made up of root, stem, leaf, and pod+seed components, for two fenugreek genotypes (F70 and Quatro) over the 2006 growing season on the Edmonton Research Station, Edmonton, AB.

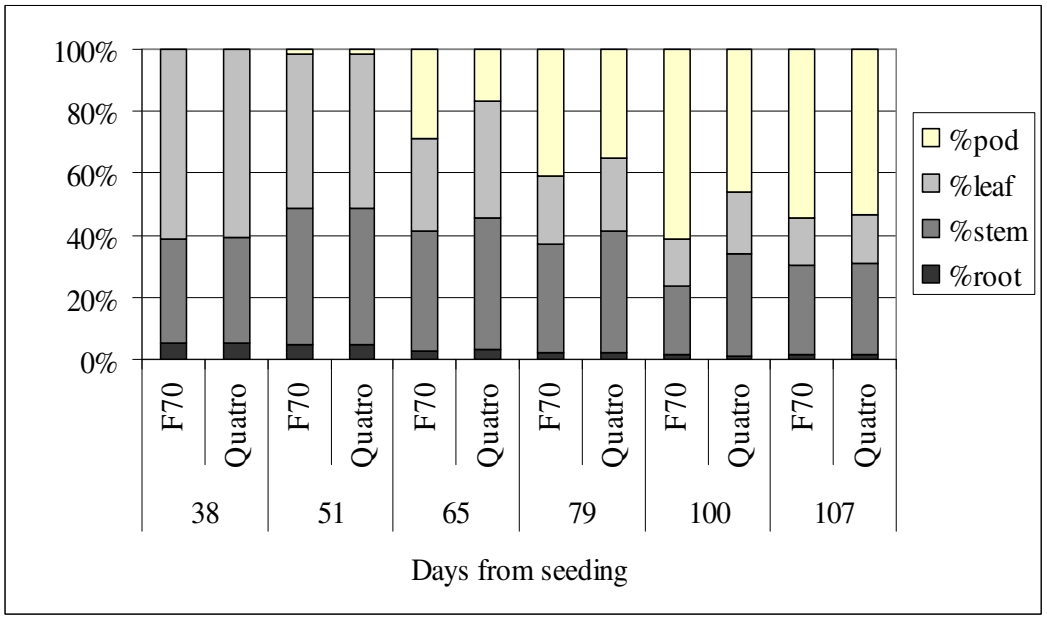


Figure 2.12. Mean percent composition per plant, made up of root, stem, leaf, and pod+seed components, for two fenugreek genotypes (F70 and Quatro) over the 2007 growing season on the Edmonton Research Station, Edmonton, AB.

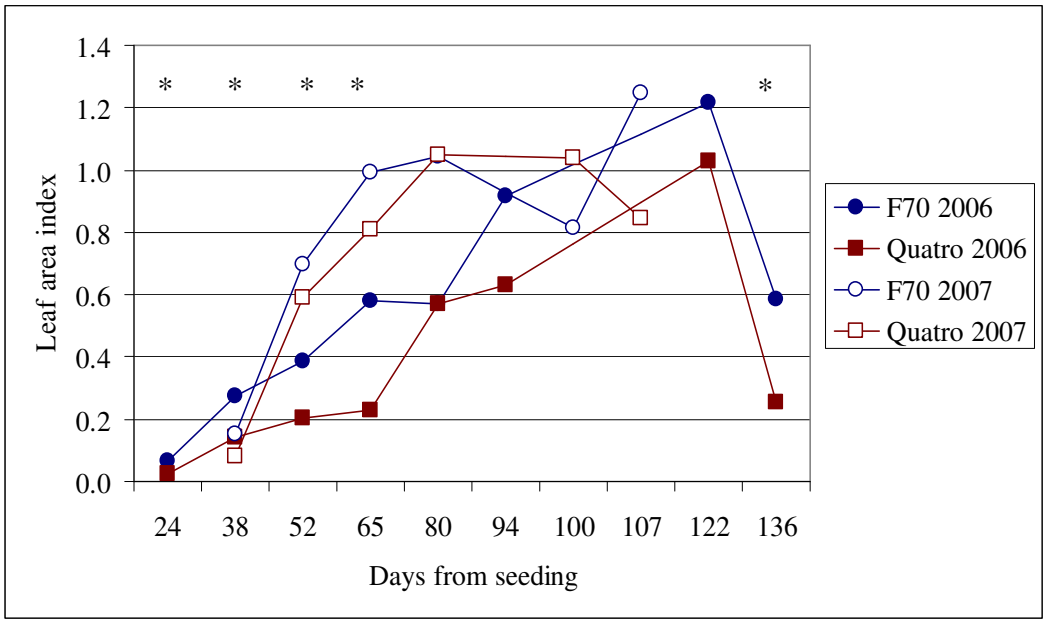


Figure 2.13. Mean leaf area index per plant (cm^2 of leaf area/cm^2 of area occupied by the plant) for two fenugreek genotypes (F70 and Quatro) over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.
Lines without markers indicate data was not collected at that time.
* significant difference ($P < 0.05$) between varieties in 2006.
‡ significant difference ($P < 0.05$) between varieties in 2007.

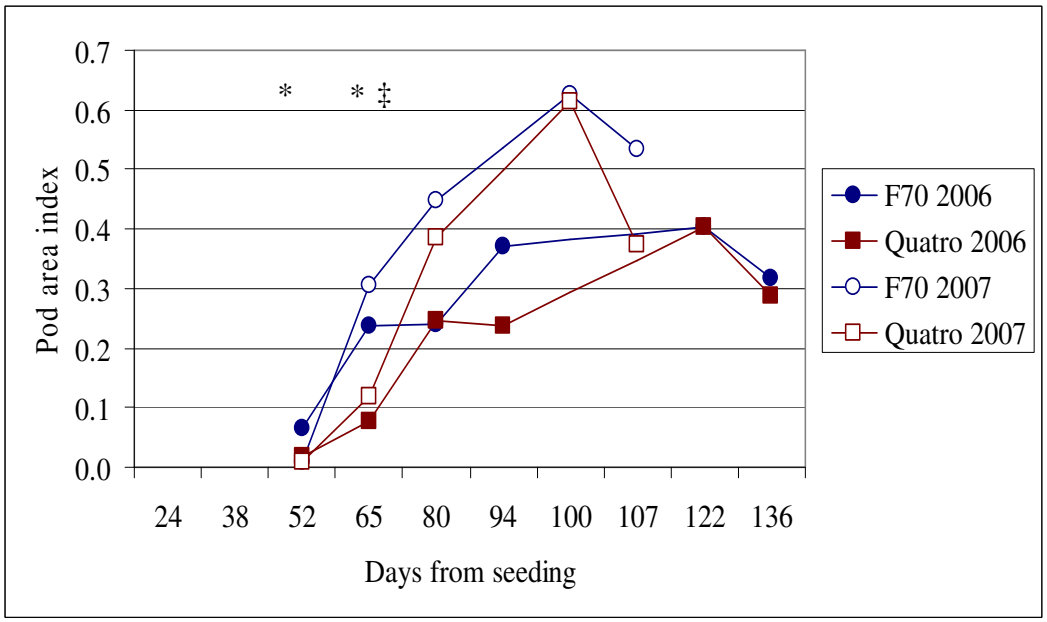
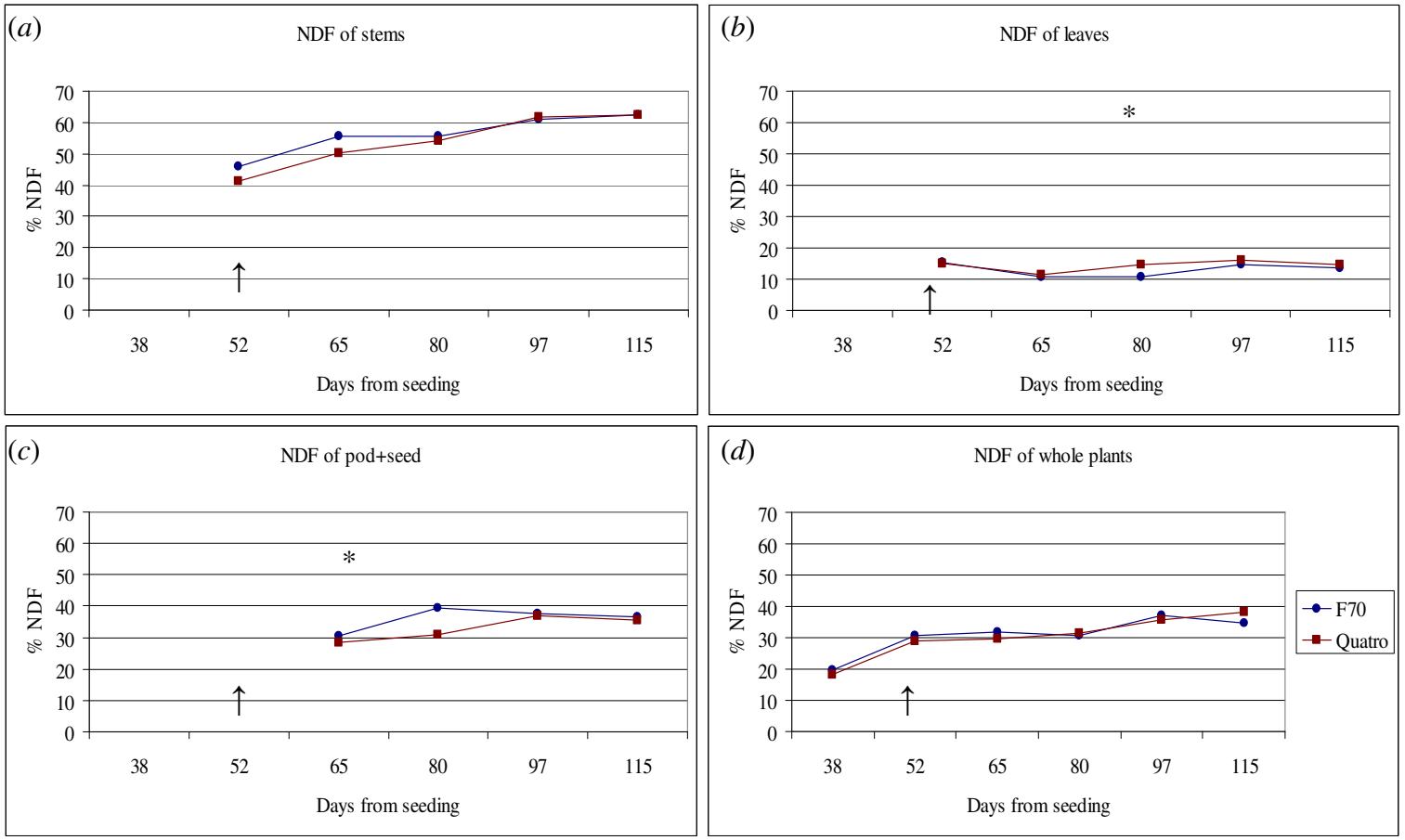
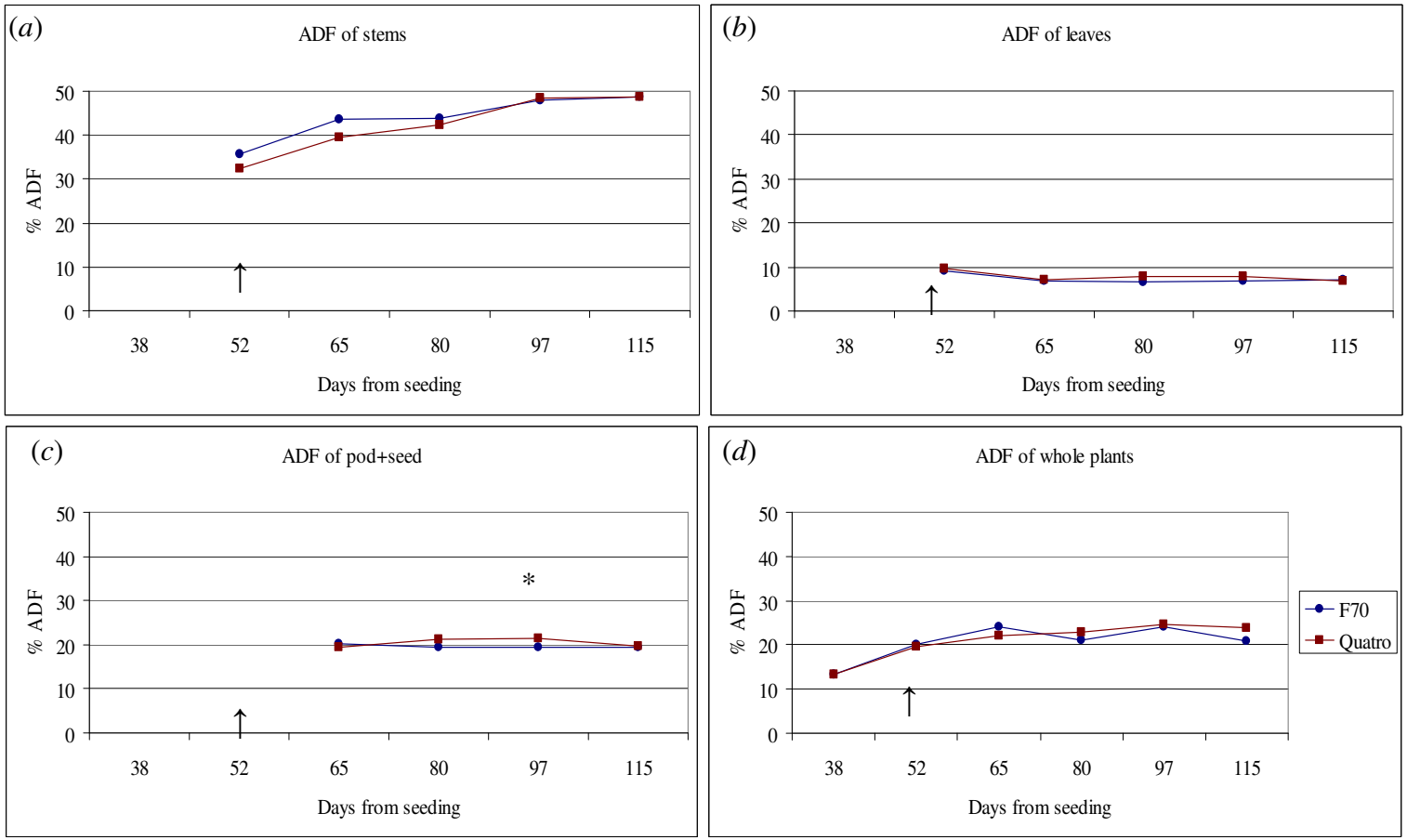


Figure 2.14. Mean pod area index per plant (cm^2 of pod area/cm^2 of area occupied by the plant) for two fenugreek genotypes (F70 and Quatro) over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.
Lines without markers indicate data was not collected at that time.
* significant difference ($P < 0.05$) between varieties in 2006.
‡ significant difference ($P < 0.05$) between varieties in 2007.



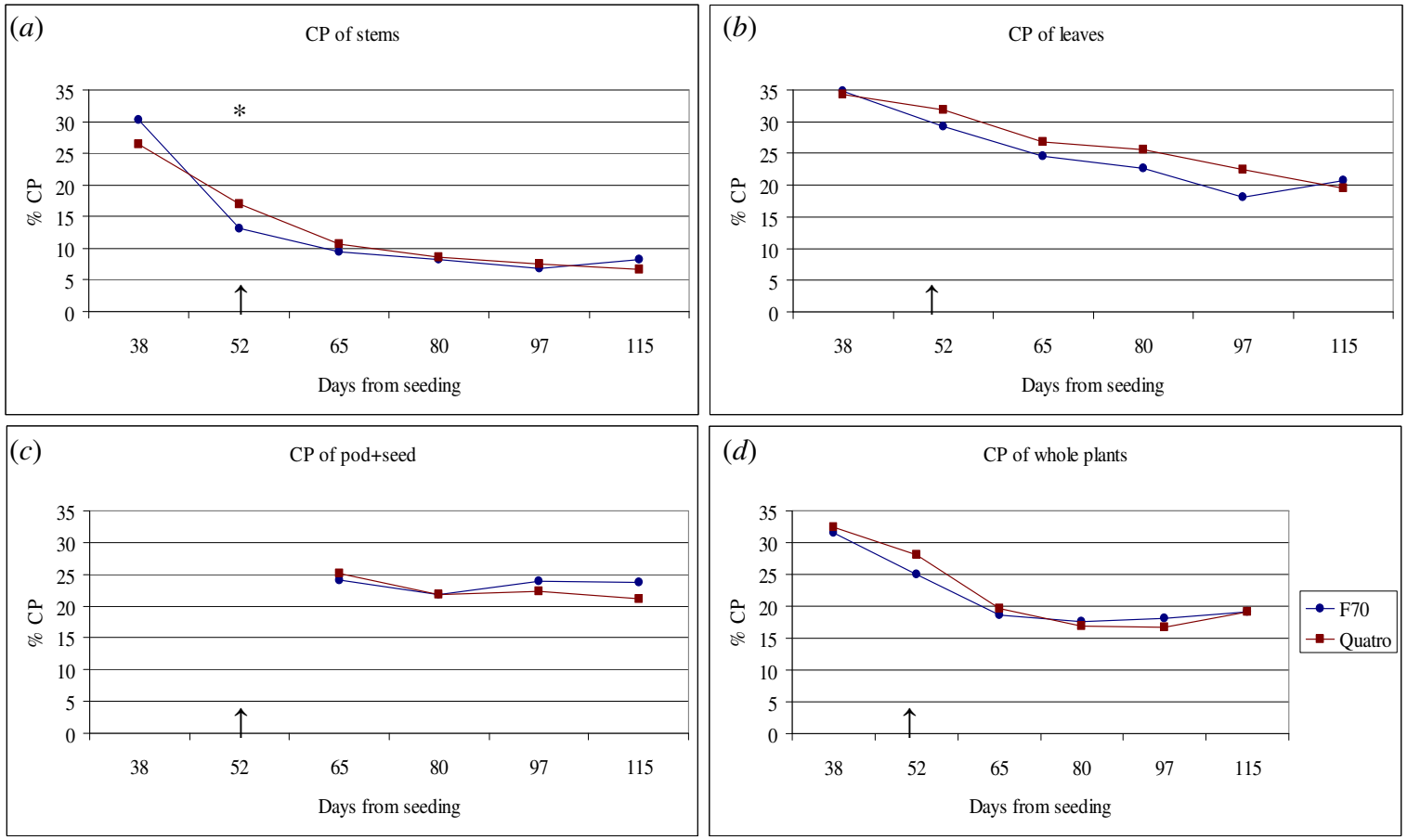
Figures 2.15. *a, b, c,* and *d*. Mean neutral detergent fibre content of stem (*a*), leaf (*b*) and pod+seed (*c*) components and of whole (*d*) F70 and Quatro fenugreek plants over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.
* significant difference ($P < 0.05$) between varieties.
↑ indicates initialization of flowering



Figures 2.16. *a, b, c,* and *d*. Mean acid detergent fibre content of stem (*a*), leaf (*b*) and pod+seed (*c*) components and of whole (*d*) F70 and Quatro fenugreek plants over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.

* significant difference ($P < 0.05$) between varieties.

↑ indicates initialization of flowering



Figures 2.17. *a, b, c,* and *d*. Mean crude protein content of stem (*a*), leaf (*b*) and pod+seed (*c*) components and of whole (*d*) F70 and Quatro fenugreek plants over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.

* significant difference ($P < 0.05$) between varieties.

↑ indicates initialization of flowering

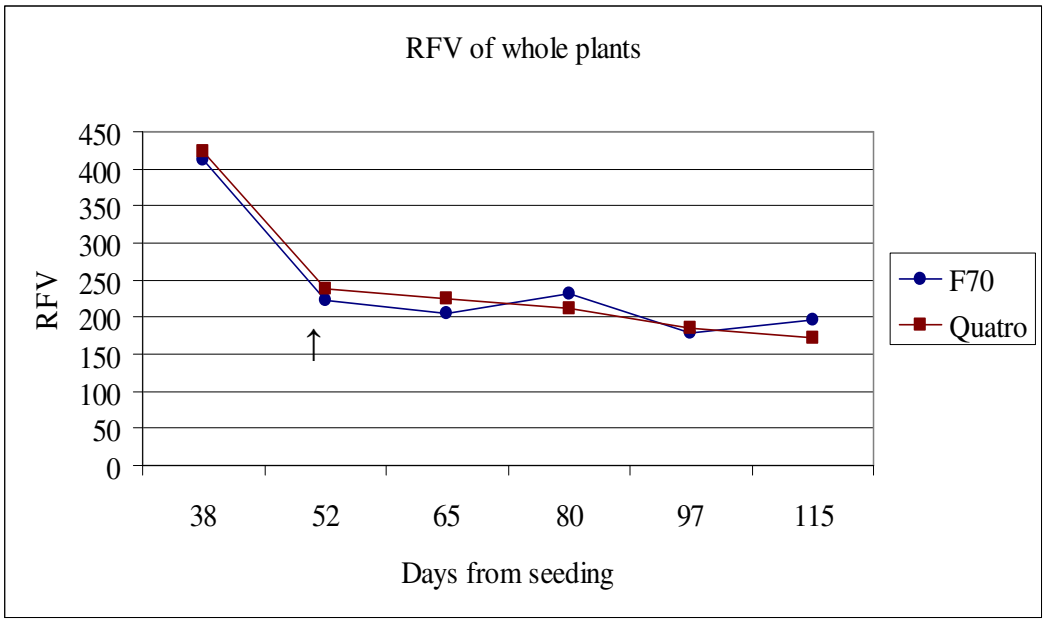


Figure 2.18. Mean relative feed value for whole F70 and Quatro fenugreek plants over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.

* significant difference ($P < 0.05$) between varieties.

↑ indicates initialization of flowering

Figure 2.19. Visual representation of the change in height, true leaves unfolded on the main stem, shoots on the main stem, and number of internodes, as well as the initialization of flowering and pod development for F70 and Quatro fenugreek plants over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.

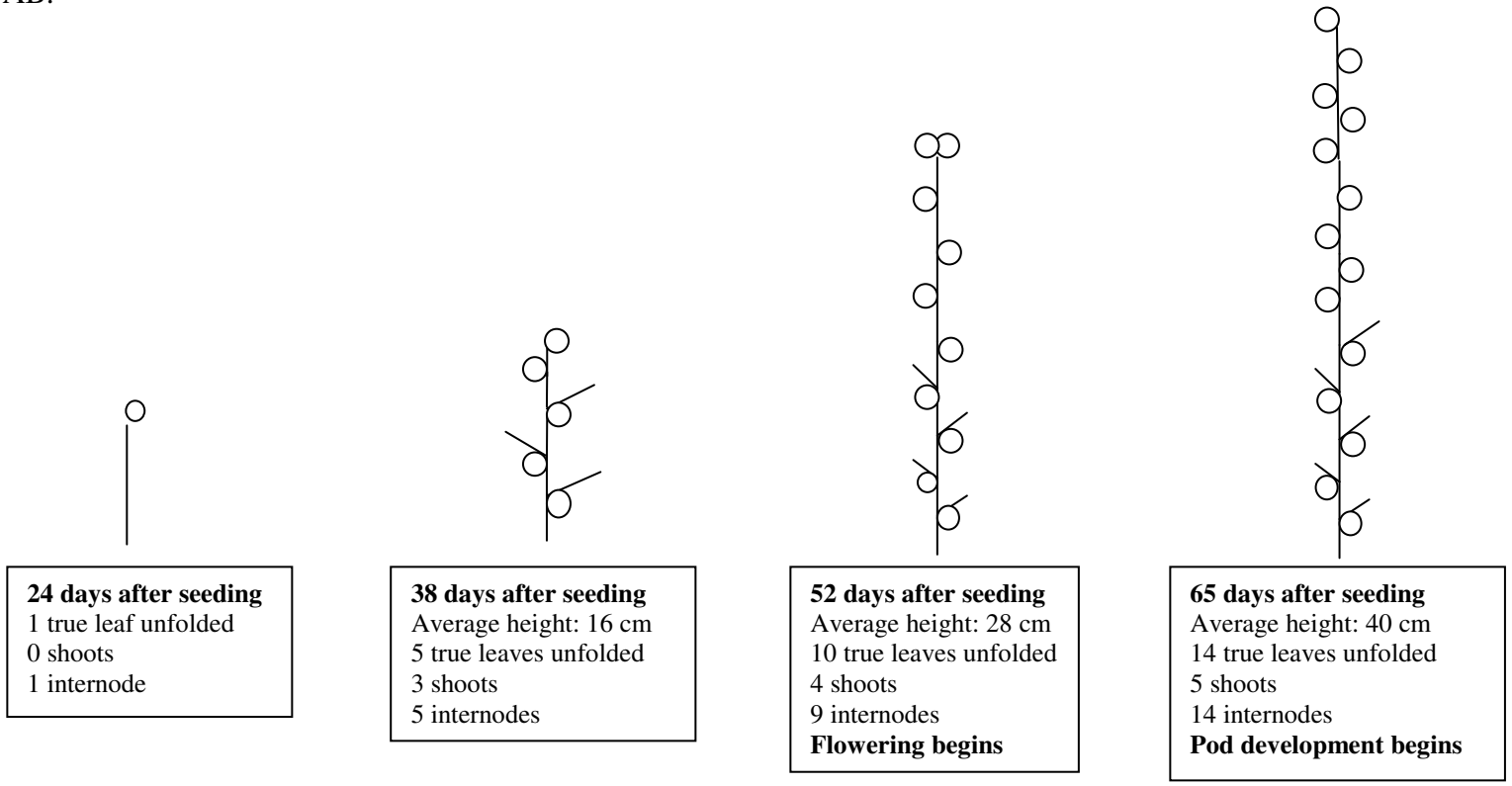


Figure 2.19. Visual representation of the change in height, true leaves unfolded on the main stem, shoots on the main stem, and number of internodes, as well as the initialization of flowering and pod development for F70 and Quatro fenugreek plants over the 2006 and 2007 growing seasons on the Edmonton Research Station, Edmonton, AB.

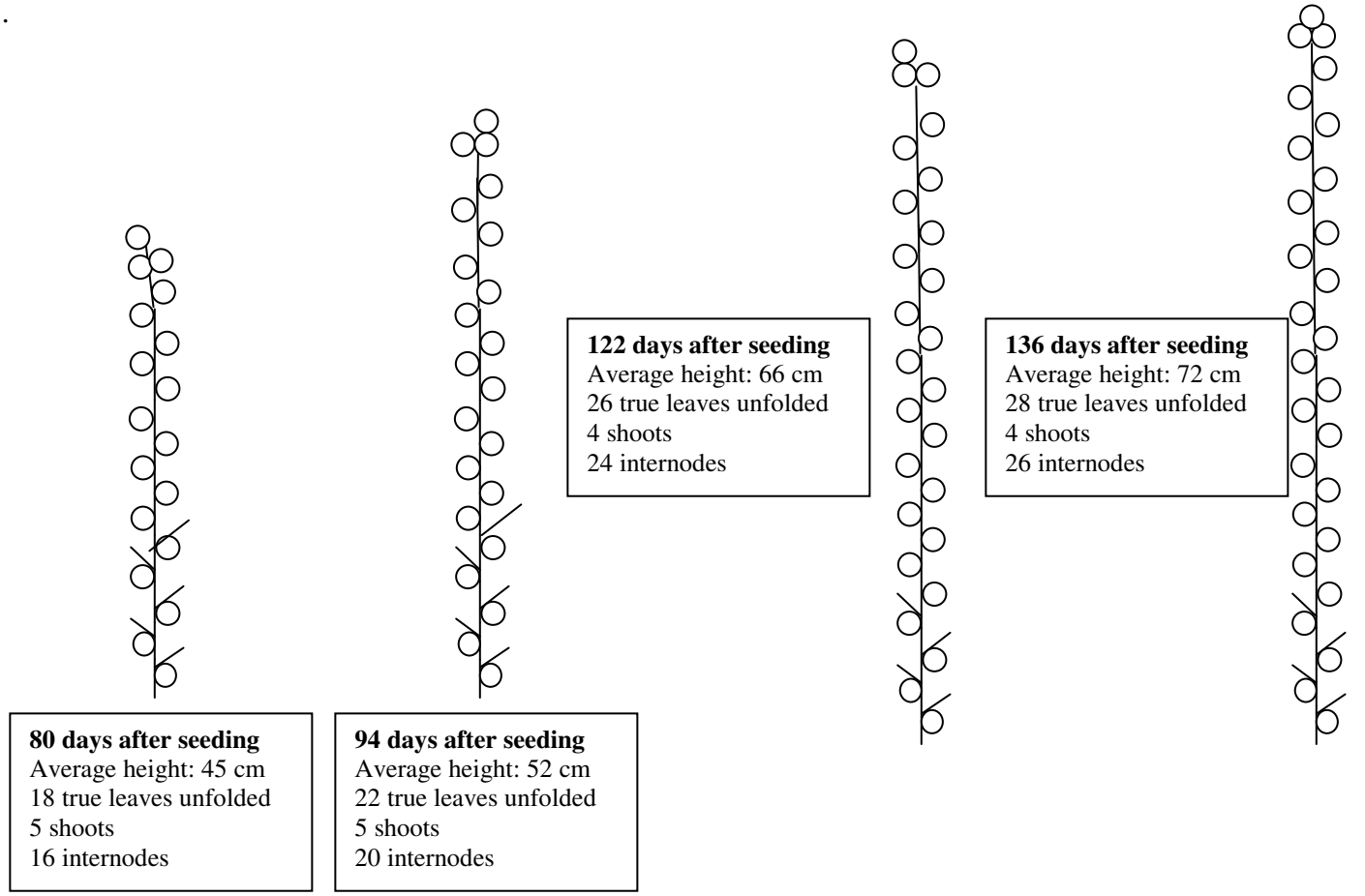


Figure 2.20. Harvest subsamples in 2007. A: 38 days. B: 52 days. C: 65 days. D: 80 days. E: 100 days. F: 107 days.

Figure 2.21. Plants at 80 days in 2007.

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Chapter 3: Assessment of ruminal degradation and whole-tract digestion of fenugreek forage in dairy cows.

Null hypotheses

AAFC F70 and CDC Quatro haylages will be degraded and digested to the same extent in the rumen and whole gastro-intestinal tract of dairy cows. Both fenugreek genotype haylages will be digested to the same extent as alfalfa haylage.

3.1. Introduction

Legume forages are an important source of high-quality feed on dairy operations. In Alberta, alfalfa (*Medicago sativa* L.) is the primary forage legume on dairy operations. However, there is increased interest in diversification of forage in cropping rotations. Annual legumes have many of the same benefits as alfalfa, such as nitrogen fixation and high feed quality, but increase crop rotation flexibility as they occupy cropping land for only one growing season (Mir et al. 1997). Fenugreek (*Trigonella foenum-graecum* L.) is a single-cut, annual legume originating around the Mediterranean and on the Indian subcontinent. It is primarily grown for seed production as the seeds are used as a spice. Interest has extended to the use of fenugreek as a forage in Alberta because of the high and sustained quality of the plant (Acharya et al. 2008, Basu et al. 2008), in addition to nitrogen fixation, and drought- and frost tolerance (Sinskaya 1961, Acharya et al. 2008, McCormick et al. 2006).

The inclusion of high quality forage is an essential part of dairy ration formulation. The *in situ* nylon bag technique is a relatively inexpensive and simple method to determine ruminal and total-tract utilization of forages in ruminant animals (Kennelly and Ha 1983) in order to evaluate the potential nutritional value of a forage. Ørskov and McDonald (1979) and Mir et. al (1991a) emphasized the importance of estimating ruminal degradability as degradation affects the availability of nutrients to rumen microbes and the portion that is available directly to the host animal for digestion in the intestines after exiting the rumen. The extent to which feed is digested in the rumen or passes through to the intestine for further digestion is partially determined by the rate of passage of that feed material. Degradation kinetics that can be determined using the nylon bag technique include the rapidly soluble fraction of a feed '*a*', the potentially degradable fraction of a feed '*b*', the undegradable fraction of the feed '*c*', the rate at which the *b* fraction is degraded (percent per hour) 'K_d', and the effective ruminal degradation (ERD) of a feed, given a theoretical passage rate of material from the rumen (K_p).

Mir et al. (1997) found that fenugreek quality does not deteriorate with age, and that it is comparable to that of alfalfa. Mustafa et al. (1996) concluded that fenugreek hay nutrient content was similar to that of late-cut (full-bloom) alfalfa hay. There was no difference in the CP, NDF, ADF or IVDMD between the two hay types, nor were the ERD of the DM and CP. Mir et al. (1993) compared fenugreek to 10%-bloom and late-bloom alfalfa forages and found that fenugreek nutrient content was intermediate between 10%-bloom alfalfa and full-bloom alfalfa. However, the IVDMD of fenugreek was higher than that of 10%- or late- bloom alfalfa. The DM *a* and *b* fractions and K_d of 10%-bloom and fenugreek were not different. At an assumed passage rate of 3.3%/h (from Mir et al. 1991b), fenugreek DM ERD (65.2%) was higher than alfalfa (57.6%). The CP *a* fraction was not different for the two forages, but the *b* fraction was higher for fenugreek. K_d was not different, but CP ERD was higher for fenugreek at 79.8% vs. 75.1%. The rumen kinetic parameters indicate that fenugreek is at least comparable to alfalfa in terms of ruminant utilization, and is superior for DM and CP ERD.

Research on the use of fenugreek forage for ruminants is scarce. Steers fed diets based on alfalfa silage or fenugreek silage did not have significantly different dry matter intake (DMI), average daily gain (ADG), or feed efficiency (Mir et al. 1998). While there have been no studies on dairy rations that included fenugreek forage, Shah and Mir (2004) conducted a study where fenugreek seed was included at 20% DM of a dairy ration. There were no differences in cow performance or milk production and milk composition between cows on the fenugreek diet and cows on the control diet.

The main objective of the research outlined here was to establish whether fenugreek may be a suitable alternative to alfalfa for use in dairy rations. This was done by conducting two experiments, the goals of which were to: compare ruminal degradation and kinetic parameters of AAFC F70 and CDC Quatro fenugreek haylage to alfalfa haylage, and to compare the ruminal, intestinal, and whole-tract digestion of these forages.

3.2. Materials and Methods

3.2.1. Haylage production

Two genotypes of fenugreek, CDC Quatro and AAFC F70 (a breeding line from the AAFC Lethbridge Research Centre (LRC)), were seeded on 5 May 2006 at the University of Alberta Edmonton Research Station (ERS). Five acres were seeded on the main ERS block (denoted as the ERS treatment), and five acres on the West 240 block (denoted as the W240 treatment). Each five acre plot was divided into two 2.5-acre plots, one plot of Quatro and one plot of F70. The plots were grown for silage production to be used in a dairy cow rumen metabolic and production study.

Edge (ethalfluralin; Dow AgroSciences Canada Inc.) was applied at the recommended rate to the plot area in fall 2005 as a pre-emergent measure of weed control. Seeding occurred in spring 2006 at a rate of 23.6 kg/ha, using a row spacing of 18 cm and a depth of 5 cm. Two seeding passes were conducted on each field with harrowing in between passes. Fenugreek seed was inoculated the same day as seeding using an alfalfa *Rhizobium* species obtained from the LRC. The inoculant was applied at a rate of 104 g per 50 kg of seed. As per recommendations based on soil tests, no additional fertilizer was applied to plots on the ERS block, and a 22% phosphorus blend fertilizer was applied on W240 at the time of seeding (nitrogen 9 kg/ha, phosphorus 26 kg/ha, potassium 9 kg/ha, and sulfur 7 kg/ha). Odyssey (imazamox + imazethapyr; BASF Canada) was applied at the recommended rate on 14 June 2006 as an in-crop measure of weed control.

By mid-August 2006, the deteriorating state of the fenugreek and the apparent lack of biomass to fill a silage bag led to the decision to produce wrapped haylage bales instead of silage. Swathing and baling of W240 Quatro and F70 and ERS Quatro occurred on 22 August 2006 at 15.5 weeks of growth. ERS F70 was swathed but not baled as it was too wet. Baling was done using a John Deere 535 large round baler and bales were wrapped using a Tug-Line TL5500 Automatic. The wrapped bales were stored at the University of Alberta Dairy Research and Technology Centre (DRTC) located on ERS. All bales were

labeled on the wrap indicating location, variety, and bale number. ERS F70 was baled on 24 August. Initial samples were taken from each bale of all types before wrapping using a Star Uni-Forage Sampler (Star Quality Samples, Edmonton, AB). On 25 August, second-cut alfalfa at ERS was swathed for haylage to use as a comparison to fenugreek forage, and was baled and wrapped on 28 August.

After initial sampling at harvest, bales from each location and variety were sampled with a core sampler using the following schedule (time post-baling): 24 hours and 3, 5, 7, 14, 21, 28, 42, and 56 days. At each sampling, multiple cores from one bale of each variety and location were taken and combined to provide adequate sample volume. After sampling, blue stock salt was rubbed on the exposed part of the bale to prevent spoilage before re-taping the bale wrap. One-half of each sample by volume from the initial, 24 hour, and 5, 14, and 28 day samples for each variety and location were sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD, USA) for analysis of DM content, % CP, % ADF, % NDF, pH, % ammonia, % lactic acid, % acetic acid, % propionic acid, % butyric acid, and % isobutyric acid.

3.2.2. Rumen degradation of fenugreek and alfalfa haylages

On 29 November 2006, alfalfa haylage samples were collected for use in whole-tract digestion and rumen degradation experiments; ERS Quatro and F70 samples were collected on 2 December 2006 and all samples were kept frozen until use. Samples consisted of cores from several bales combined. Each feed was dried at 55^{o}C for 72 hours to ascertain % DM and then ground through the 6mm screen of a Thomas Scientific Wiley Mill Model 4 (Thomas Scientific, Swedesboro, NJ, USA).

Six ruminally-cannulated, lactating dairy cows at the DRTC were used to determine the ruminal degradation of the haylages in March – April of 2007. Each feed type was incubated in two cows that were on a diet of the corresponding feed type for a study on fenugreek rumen metabolism. Rumen bags were prepared using Ankom nitrogen-free polyester (Ankom Technology, Macedon, New York, USA), sewn on three sides with nylon thread. Bag size

before filling was 6 cm long by 4 cm wide. Polyester pore size was 50 microns. Five grams (+/- 0.1g) of dried and ground feed was placed in each bag, which was then sealed by folding the upper edge of the bag over and holding it in place with an elastic band. Two rumen bags were put in each cow at each time point and were kept in the rumen in mesh bags which had weights sewn into them. The mesh bags were kept closed using cordlocks.

Rumen bags were put into the rumen at the following time intervals such that they could all be removed and processed at the same time: 168, 120, 96, 72, 48, 36, 24, 18, 12, 8, 6, 4, 2, and 1 hours. Zero-hour bags were washed under cold water until water ran clear. All bags were removed from the rumen and put immediately into ice water to halt microbial activity, then rinsed in cold water until run-off was clear. The bags were put into a freezer for 72 hours to separate microbes from the forage material, and then thawed in cool water. They were then rinsed by plunging and agitating with very cold water in a large plastic bucket to remove microbial material, gently squeezed to remove extra moisture, and dried in a 55^{o}C oven. Dry weights were taken to assess percent DM disappearance, and each two bags that were the same for feed type, cow, and time of incubation were mixed and sub-sampled for NDF, ADF, and CP analysis.

Acid detergent fibre and neutral detergent fibre were determined using the Ankom^{200} Fibre Analyzer, following the Acid and Neutral Detergent Fiber in Feeds Filter Bag Techniques (Ankom Technology, Macedon, New York, USA), and CP was determined using a Leco TruSpec® C/N Elemental Determinator (Leco, St. Joseph, Michigan, USA) with CP calculated as N x 6.25. ADF, NDF, and CP analysis were carried out at the University of AB, in the Department of Agricultural, Food and Nutritional Science.

3.2.3. Whole-tract digestion of fenugreek and alfalfa haylages

The whole-tract digestion study was conducted at the Dairy Research Centre at the LRC in December 2006. Two cows were used for the study. Both cows were born in 2002 and had completed 2 lactations; they had been dried off approximately 6 months before this study. The cows were put on a diet of alfalfa

hay four days prior to the beginning of the digestion experiment and were fed this diet *ad libitum* for the duration of the experiment. Both cows were previously fitted with ruminal and duodenal cannulae.

Two-hundred and ten mobile bags were constructed using PeCap monofilament polyester fabric with a 51 μm pore size (Sefar., St.-Laurent, Quebec, Canada), heat-sealed on three sides, the fourth side already being sealed as the fabric was folded in half length-wise. The bags were a finished size of 3 cm x 5 cm and contained 1g (+/- 0.05g) of feed in order to fit through the duodenal cannula.

Six bags per feed type (total of 18 bags) were used as zero-hour bags; these were soaked in warm water for 20 minutes, then rinsed until the water running off the bags was clear, and dried in a 55^{o}C oven. Sixteen bags were used for each feed in each cow for each incubation time. Bags were incubated in the rumen for either 18 or 30 hours (total 192 bags) inside mesh laundry-style bags that were fitted with a weight to keep the bags immersed in rumen content. When removed from the rumen, all nylon bags were immersed in ice water to halt microbial action. Of the sixteen bags in each category, 6 bags were washed and dried as above immediately upon removal from the rumen, in order to represent the digestion of the feeds that occurred in the rumen only. The other 10 bags were immersed for one hour in a pepsin-HCl solution to mimic passage through the omasum and abomasum and then put into the small intestine via the duodenal cannula at a rate of two bags every 20 minutes so as not to cause a blockage in the digestive system. Duodenal bags were then collected from feces using mesh trays placed behind the cows for up to 24 hours after the insertion of the last bag into the duodenal cannula (Figure 3.1). All bags were rinsed upon collection and dried as above.

Dry weights of mobile bags from the beginning and end of the study were compared to calculate percent dry matter disappearance (% DMD) of feed material from the bags. Bags were retained in order to perform nutrient content analysis on the feed material. Samples of the same feed type, in the same cow, for the same time period and the same treatment (either rumen-only or duodenum)

were pooled to obtain sufficient sample volume for nutrient analysis. Chemical analysis of CP, ADF, and NDF was performed as for the ruminal degradation experiment.

Ruminal disappearance was calculated as the difference in rumen bag content from the original samples and the samples collected after 18 hours or 30 hours in the rumen. Whole-tract disappearance was calculated as the difference in content of the original samples and the samples collected from the feces after ruminal incubation, mock-passage through the true stomach, and passage through the intestine. Intestinal disappearance was calculated as the difference between whole-tract and ruminal disappearance. The disappearance that occurred in the intestine was expressed both as a percentage of the total material that was initially put in the cow, as well as a percentage of the material put into the duodenum after incubation in the rumen.

3.2.4. Statistical analysis

The haylage nutrient content data were not analyzed statistically as there was only one data point per haylage type per time.

For the rumen degradation study, the non-linear procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC, USA) was used to estimate the rumen kinetic parameters, fitting by iterative least squares to the model developed by Ørskov and McDonald (1979):

$$y = a + b(1-e^{-Kd*t})$$

where y is the percent DM, NDF, ADF or CP that has disappeared at time point t, *a* is the percent of each component which is rapidly soluble, *b* is the percent of each component that is potentially degradable given sufficient time, and K_d is the rate of disappearance of the *b* fraction. The remainder of 100-(*a*+*b*) is the *c* fraction, or percent of each component which is not digestible, regardless of length of time in the rumen.

Effective ruminal degradability was calculated using the following formula:

$ERD = a + (b * K_d) / (K_d + K_p)$

where K_p represents the theoretical rate of passage of material from the rumen. For this study, 3%/h, 5%/h, and 7%/h were used.

Each of the rumen kinetic factors was then analyzed using the Mixed procedure of SAS to determine significant differences between feed types, using cow nested within feed as the random term and feed as the fixed term.

Dry matter, NDF, ADF, and CP disappearance were also depicted graphically to assess differences in degradation over time between feed types. Confidence limits of 95% were determined using the Repeated Measures option in the Mixed procedure of SAS. Unstructured, compound symmetry, heterogeneous compound, and ante-dependence covariance structures were examined because they allow for sampling at unequal time intervals, and comparison of the Bayesian Information Criteria was used to determine the appropriate covariance structure for each model. In addition, the values of the zero-hour bags were tested as a covariate in the model, and for DM and CP degradation were found to be significant and were therefore included in the model. For DM and NDF degradation the ante-dependence covariance structure was used, while for ADF the compound symmetry structure was used, and for CP the heterogeneous compound structure was used. The 95% confidence limits allow for significance to be visually assessed on the graphs representing DM, NDF, ADF, and CP disappearance over time in the rumen.

For the whole-tract digestion study, the DM, NDF, ADF, and CP disappearance in the rumen, intestine, and total tract of the three feed types was analyzed using the Repeated Measures option in the Mixed procedure of SAS to determine the effect of feed and time in the rumen. Time was the repeated factor, and cow was the random term and feed was the fixed term. Upon comparison of Bayesian Information Criteria for covariance structures that did not require equal spacing of sampling time (unstructured, compound symmetry, heterogeneous

compound, and ante-dependence), the covariance structure with the best fit to the model was found to be compound symmetry.

For all parameters, differences are considered significant at $P < 0.05$.

3.3. Results and Discussion

3.3.1. Haylage production

Quatro DM biomass was 3.4 tonnes/hectare (t/ha) at ERS, and 2.4 t/ha at W240 (Figure 3.2). F70 produced 3.0 t/ha of DM biomass at ERS, and 1.9 t/ha of biomass at W240. The 15-year average fenugreek forage DM yield in southern Alberta was 5.8 t/ha, while the long-term yield under irrigation was 6.0 t/ha (Acharya et al. 2008). These long-term average yields are higher than those in the current study. The Canadian Climate Normals for 1971 – 2000 indicate that the average daily temperature in Lethbridge is higher in July and August than in Edmonton (Table 2.3, National Climate Data and Information Archive 2009), which may encourage increased biomass yield there. While the long term average rainfall is higher in Edmonton than in Lethbridge, it has been noted in Chapter 2 that the rainfall in June, July and August of 2006 was far below the 3-year average (Table 2.3). Also, spikes in the average daily temperature at the end of July and August (Figure 2.4) may have contributed to the senescence of fenugreek plants, decreasing biomass.

The DM content of all haylage types stayed relatively constant over the first 28 days of fermentation in the wrapped bales (Figure 3.3). Alfalfa haylage numerically had the greatest DM content, between 54% - 61%. F70 from both locations was similar for DM content at 45% - 54%. Quatro from W240 contained 37% - 41% DM, and ERS Quatro had the numerically highest moisture content with only 33% - 34% DM.

Minimal change occurred in the CP, ADF and NDF content of the haylage varieties from initial sampling over the first 28 days of ensiling (Figure 3.4). On average, 10%-bloom alfalfa silage in Alberta contains 55% moisture, 18.2 % CP, and 36% ADF (AARD 2004). Quatro haylage grown at both locations, as well as alfalfa haylage, all had greater moisture content, while F70 haylage grown at both

locations had lower moisture content than the AARD average. Quatro and F70 haylage from ERS and alfalfa haylage had CP content comparable to the AARD average at 21.5%, 19.6% and 24.7% respectively, while the Quatro and F70 haylage produced on W240 was lower at 15.8% and 11.0% respectively. In a study by Mir et al. (1998) fenugreek silage cut at 17 weeks had a CP content of 17.2%, which is lower than that of the ERS fenugreek haylages and alfalfa haylage produced in the current study. Both fenugreek haylage types produced on W240 had CP lower than the fenugreek silage of Mir et al. (1998). All haylages except W240 F70 had lower ADF content than the AARD average, ranging from 25% to 36%; W240 F70 had 41% ADF. The NDF content of the five haylage types was between 28% and 50%. Dairy rations should source NDF from forages and have at least 25% NDF in the diet in order to balance rumen function and rumen fill; however, excessive NDF must also be avoided as this can limit voluntary feed intake (Oba and Allen 1999).

The haylage pH showed an initial numerical decrease in the first 24 hours of fermentation for ERS F70, W240 F70, and W240 Quatro genotypes, after which the pH remained relatively constant (Figure 3.5). The pH of ERS Quatro and alfalfa did not show an initial decrease, and remained constant throughout the 28 d sampling period. W240 Quatro had the lowest pH by day 28 at 5.6, while the other four feed types remained close to a pH of 6. Haylage moisture content is usually 45% - 55%, and pH normally ranges from 4.7 to 5.8 (AARD 2003). Fermentation in haylage is slower and progresses to a lesser degree than chopped silage because of the lower moisture content and the increased length of plant material. Lower moisture will result in decreased fermentation and increased pH (Macaulay 2003). The haylage produced for this study all had pH levels close to or above 5.8 which is at the upper limits of the normal range. The haylage did not appear to ferment, as the pH did not change over the ensiling period. This could be due to the lack of leaf material present on the plants when the fenugreek was ensiled resulting in a lack of water soluble carbohydrates available to the microorganisms, or that the chop length was too long to allow for sufficient microbial access to plant material (AARD 2004).

For preserved forages that are predominantly legume and have greater than 35% DM, such as those in this study, a typical fermentation profile includes the average values of: pH of 4.2 to 5.4, 0.5% – 2.1% ammonia, 1.6% - 5.5% lactic acid, 0.4% - 2.6% acetic acid, 0.1% – 0.5% propionic acid, 0.0% – 0.1% butyric acid, and 0.1% - 0.6% isobutyric acid (Jones et al. 2004). The fermentation products of the 5 haylage types can be found in Figures 3.6 and 3.7. Ammonia content of the five haylage varieties was within the specified range, indicating that the protein of the haylage was not being broken down beyond an acceptable level (AARD 2004). Ammonia content in all haylage types increased over time, and both fenugreek varieties grown on ERS were numerically higher in ammonia content that the varieties grown on W240 by day 28. Lactic acid was within the specified range for both Quatro haylage types, but the other three haylage types were below the normal range. Lactic acid content influences the pH of silage (AARD 2004); the lack of lactic acid production during fermentation resulted in a pH that did not decrease. ERS Quatro was within the normal range for acetic acid, but all other feeds were below the range. Propionic acid in all feeds was below the specified range. Alfalfa was below the range for isobutyric acid. All the other feeds were normal for butyric and isobutyric acids, except ERS Quatro, which had butyric and isobutyric levels above the specified range. The production of butyric acid indicates the presence of undesirable microorganism species in the forage (AARD 2004); therefore, it seems as though these undesirable species were present in the ERS Quatro haylage, but were not operating in the other feed types. In general, it is likely that the feed types had low acid content profiles because of the apparent lack of fermentation, and therefore fermentation byproducts.

3.3.2. Rumen degradation of fenugreek and alfalfa haylages

3.3.2.1. Rumen kinetics

The rumen kinetic parameters for dry matter disappearance were all significantly influenced by forage type (Table 3.1). The soluble fraction, *a*, was significantly higher for alfalfa (44%) than Quatro (34%), which was higher than

F70 (25%). The potentially degradable fraction, *b*, of Quatro (38%) was significantly higher than alfalfa (32%), while F70 (35%) was intermediate to and not significantly different from Quatro or alfalfa. The undegradable fraction of the feed, *c*, was significantly higher for F70 (40%) than the other two forages, which were not different from each other. The rate of degradation, K_d, was highest for alfalfa at 8.6%/hr, while F70 was lowest at 3.5%/hr. Quatro was not significantly different than alfalfa or F70 at 6%. Effective ruminal degradability, at all three rates of passage, was significantly higher for alfalfa and Quatro, which were not different from each other, than for F70. While alfalfa ERD was above 60%, and Quatro ERD was above 50%, F70 ERD was below 44%. While the DM *a* fraction of alfalfa was the highest of the three feeds, the high *b* fraction of Quatro, combined with an intermediate K_d of the *b* fraction, resulted in the DM ERD of Quatro and alfalfa being similar. F70 had a significantly lower DM ERD because of a low *a* fraction, high *c* fraction, and a *b* fraction that was similar to the other feeds but was degraded at a lower K_d.

The *a* fraction of the NDF content was significantly lower for alfalfa at 1% than for Quatro at 12%, while F70 was intermediate to and not significantly different than the other forage types at 7% (Table 3.2). The *b* and *c* fractions for all three feeds were not significantly different from one another. The NDF content of all three feeds was primarily made up of the *c* fraction, comprising at least 50%. The rates of degradation were highest for alfalfa and Quatro (7%/hr and 5%/hr), which were not significantly different, and lowest for F70 (2%). There were no haylage differences for ERD at all three rates of passage.

The effect of forage type was not significant for the *a, b*, and *c* fractions of the ADF content (Table 3.3). All three feeds were primarily comprised of the *c* fraction, which was at least 52%. The rate of degradation was significantly higher for alfalfa (6%/hr) than F70 (2%/hr), while Quatro was intermediate and not significantly different from the other forages (4%/hr). The ERD at all rates of passage was not significantly affected by haylage type.

The *a* and *b* fractions of the CP content were not significantly different between feeds (Table 3.4). The CP of alfalfa was comprised mainly of the soluble

fraction *a* at 56%, while the CP of F70 and Quatro was comprised mainly the potentially degradable *b* fraction at 63% and 58% respectively. The undegradable *c* fraction of F70 (15%) was significantly higher than alfalfa and Quatro, which were not significantly different from each other at 8%. The rate of degradation was not significantly different between haylage types. At all three passage rates, the CP ERD of alfalfa was significantly higher than the other two feed types. At 3%/h and 5%/h, the ERD of F70 was significantly lower than that of Quatro, whereas at a passage rate of 7%/h, there was no significant difference. The lack of significant difference between feeds for the *a* and *b* fractions is likely due to the large standard error of the mean, as the values of alfalfa are numerically twice that of F70. Even though F70 had a large *b* fraction, it also had the lowest K_d, which in combination with the low *a* fraction, resulted in F70 having the lowest ERD values at all three rates of passage. Alfalfa had a large *a* fraction, a relatively low *b* fraction that was degraded significantly faster than the other two feeds, and a low *c* fraction, allowing the ERD of alfalfa to be highest at all of the passage rates. Quatro CP ERD was intermediate, given its intermediate *a* and *b* fractions and low *c* fraction.

Overall, F70 was the feed with poorest nutrient content due its consistently low rate of degradation, low *a* fraction, and high *c* fraction. Alfalfa and Quatro were more comparable in nutrient content. Rate of degradation and *a* fraction of Quatro DM were lower than those of alfalfa DM, which may aid in mitigating fluctuation of rumen conditions. This is corroborated by Mir et al. (1993), who found that fenugreek may have more of a sustained release of nutrients, especially nitrogen, in the rumen and therefore has the potential to be degraded to a greater extent. This could translate to greater feed utilization efficiency and therefore lower supplementation in ruminants.

Khorasani et al. (2001) determined the DM and CP rumen kinetics of alfalfa silage. The DM *a* fraction of alfalfa silage was 35%, which is comparable to the Quatro results in this study, but higher than F70, and lower than alfalfa haylage. The alfalfa silage DM *b* fraction was 39%, which was higher than the DM *b* fraction of the alfalfa and F70 haylage types, but comparable to Quatro

haylage. The rate of degradation of alfalfa silage DM was 4.7%/h and the ERD, at a 7%/h rate of passage, was 50.6%. The alfalfa silage DM rate of degradation was lower than that of alfalfa haylage, but comparable to that of the fenugreek haylage types, while alfalfa silage ERD was comparable to that of Quatro haylage, but higher than F70 haylage and lower than alfalfa haylage. The alfalfa silage CP *a* fraction was 70.5% and the CP *b* fraction was 19.9%. While the CP *a* fraction was lower in the three haylage types than for the alfalfa silage, the *b* fraction was higher for the haylage types. Rate of degradation of the CP fraction was 7.7%/h and the ERD was 80.5%. The CP rate of degradation was lower for alfalfa silage than for alfalfa haylage from the current study; however, both fenugreek haylage types had lower rates of degradation than the alfalfa silage. The CP ERD of all three haylage types was lower than that of the alfalfa silage.

The DM and CP kinetics of low and high quality alfalfa silage were determined by Kennelly (1999). The DM *a* fraction of low and high quality alfalfa silage was 37% and 36%, respectively, while the *b* fraction was 39% and 43%. The rate of digestion and ERD of high quality alfalfa silage dry matter were 6.8%/hr and 60% (assuming a passage rate of 5%/hr), while for low quality alfalfa silage these parameters were 4.5%/hr and 55%. In general, the rumen kinetics of low and high quality alfalfa silage were comparable to the rumen kinetics of Quatro haylage, and in general were lower than alfalfa haylage and higher than F70 haylage. The CP fractions of low and high quality alfalfa silage were not similar to the three haylage types from this study; the *a* fraction, at 77% and 76% for the low and high quality alfalfa silages respectively, was much higher than the three haylage types, while the *b* fraction, at 16% and 18% respectively, was lower than that of the three haylage types. Rate of degradation was 11% for both alfalfa silages, and ERD was 88% assuming a passage rate of 5%/hr. These values were comparable to the alfalfa haylage rate of degradation and ERD, but were higher than the values of either fenugreek haylage type.

Mir et al. (1993) compared *in situ* degradation of DM and CP of fenugreek and 10% bloom alfalfa hays. While the DM *a* and *b* fractions of the fenugreek and alfalfa hays were similar to those of Quatro haylage, around 35%, the rate of

degradation of the hays was much higher than the haylage types, at 13%/hr versus 3.5%/hr to 8.6%/hr. The rumen degradation kinetics of the CP content of the fenugreek and alfalfa hay was not similar to those of any of the three haylage types in this study.

Mustafa et al. (1996) determined the DM, NDF, ADF and CP rumen kinetics of fenugreek hay and alfalfa hay. The DM *a* fraction was 31% and 32% for fenugreek hay and alfalfa hay respectively, and the *b* fraction was 39% and 41%. While the alfalfa hay had a lower *a* fraction and higher *b* fraction than the alfalfa haylage of the current study, the fenugreek hay values were comparable to those of the Quatro haylage, and greater than those of the F70 haylage. DM rate of degradation was 7.8% for fenugreek hay and 5.2% for alfalfa hay, and DM ERD was 55% for fenugreek hay and 53% for alfalfa hay. Alfalfa haylage had a greater rate of degradation and ERD than alfalfa hay; however, rate of degradation of fenugreek hay was greater than either fenugreek haylage type, and while the ERD of fenugreek hay was comparable to that of Quatro haylage, it was greater than that of F70 haylage.

In the study of Mustafa et al. (1996) the NDF and ADF *a* and *b* fractions of alfalfa hay, as well as the ERD, were larger than those of alfalfa haylage in the present study. However, the NDF and ADF rate of degradation of the alfalfa haylage was greater than that of alfalfa hay. While the NDF and ADF *a* fractions of fenugreek hay were comparable to those of Quatro haylage, they were both greater than those of F70 haylage. The NDF *b* fraction of fenugreek hay was greater than that of either fenugreek haylage type, while the ADF *b* fraction of fenugreek hay was comparable to that of Quatro haylage, and greater than that of F70 haylage. The NDF rate of degradation of fenugreek hay was higher than that of F70 haylage, but lower than that of Quatro haylage, while the ADF rate of degradation for fenugreek hay was higher than either fenugreek haylage type. NDF and ADF ERD were comparable for fenugreek hay and Quatro haylage, both of which were higher than the ERD of F70.

The CP *a* fraction of fenugreek hay was 41%, while that of alfalfa hay was 34%. The CP *b* fraction for fenugreek and alfalfa hay was 49% (Mustafa et al.

1996). While the alfalfa hay *a* fraction was lower than that of alfalfa haylage, the *b* fraction was higher than the alfalfa haylage. The opposite was found in comparing the fenugreek forages: the *a* fraction of the fenugreek hay was higher than either fenugreek haylage, and the *b* fraction was lower than either fenugreek haylage. The fenugreek hay rate of degradation was 20%/hr while that of alfalfa hay was 10%/hr. While the alfalfa hay and haylage rate of degradation were comparable, the fenugreek hay rate of degradation was much higher than that of either fenugreek haylage. The ERD of fenugreek hay was 81%, and 67% for alfalfa hay. The alfalfa hay ERD was lower than that of the alfalfa haylage; however, the ERD of both fenugreek haylage types was lower than that of fenugreek hay.

3.3.2.2. Ruminal disappearance of fenugreek and alfalfa haylages

There was a significant interaction of feed by time for the ruminal degradation of feed DM, NDF, ADF and CP. The DM disappearance of F70 was significantly lower than that of alfalfa and Quatro at all time points (Figure 3.8). Quatro and alfalfa DM disappearance were only significantly different from each other at 2, 6, 8, 12, and 120 hours. All three forages showed little change in dry matter disappearance after 36 hours, and were degraded to 64% (F70), and 71% (Quatro and alfalfa).

Based on the curve of Figure 3.9, there was a lag time of approximately 6 hours for NDF degradation in Quatro and alfalfa, while the lag time for F70 was 4 hours. For alfalfa and Quatro, there was little change in NDF disappearance after 72 hours, while F70 NDF degradation continued to increase. While F70 started out intermediate to Quatro and alfalfa, after 12 hours F70 had the lowest degradation values until 168 hours, when it was not different from alfalfa. The NDF of all three feed types was not degraded to more than 50%.

Acid detergent fibre degradation occurred after a 4 hour lag period for alfalfa and Quatro, and 2 hours for F70, based on the curve of Figure 3.10. Alfalfa and F70 ADF disappearance was 41% and 42% respectively at 168 hours, while Quatro was 48%. Little change occurred for alfalfa and Quatro ADF

disappearance after 36 hours, while F70 ADF degradation continued to increase until 168 hours. As with NDF, F70 ADF degradation was initially intermediate to Quatro and alfalfa, but was then lowest at and after 12 hours.

For the first 12 hours, CP degradation of alfalfa was significantly higher than F70 and Quatro (Figure 3.11). From 8 to 12 hours, CP degradation of both fenugreek genotypes increased sharply. After 36 hours, alfalfa and Quatro were not different, while F70 continued to be lower. At 168 hours, the CP degradation of all three feeds was significantly different at 86%, 90% and 92% for F70, alfalfa, and Quatro respectively.

In a mobile bag study by Kennelly (1999), low and high quality alfalfa silage DM degradation increased from 38% and 39%, respectively, at 1 hour of ruminal incubation, to 78% and 81% degradation after 144 hours. While the alfalfa and Quatro haylage types were comparable to the alfalfa silage values at the beginning of degradation, F70 was lower. But the end of degradation, both alfalfa silages were degraded to a greater extent than any of the three haylage types. From 1 to 144 hours, the degradation of the CP fraction of low and high quality alfalfa silage increased from 77% and 78% to 95% and 96%. All three haylage types had lower initial rumen CP degradation than the alfalfa silages, but were comparable to the alfalfa silages by the end of degradation.

3.3.3. Whole-tract digestion of fenugreek and alfalfa haylages

Length of incubation time in the rumen, either 18 hours or 30 hours, did not have a significant effect on digestion of DM, NDF, ADF in the rumen, intestine, or the whole gastro-intestinal tract, or on CP digestion in the rumen or intestine (Tables 3.5, 3.6, 3.7, and 3.8).

For DM, NDF, and ADF digestion, the effect of forage type was significant for digestion in the rumen and through the whole-tract, but not for digestion in the intestine either as a percentage of feed DM put in the rumen or feed DM entering the intestine (Tables 3.5, 3.6, and 3.7). In the rumen, alfalfa and Quatro DM were digested to a similar extent, 75% and 74% respectively, both significantly higher than F70 at 60% (Table 3.5). This was also the pattern upon

digestion through the whole-tract, where alfalfa and Quatro at 77% were digested to a significantly higher level than F70 at 65%.

Neutral detergent fibre digestion in the rumen was highest for Quatro while alfalfa and F70 were lower, and not different from each other (Table 3.6). Upon passage through the whole-tract, Quatro NDF digestion was significantly greater than F70, which was in turn significantly higher than alfalfa.

The same pattern occurred for ADF digestion as for NDF digestion, with Quatro digested to a significantly higher extent in the rumen than the other two feeds at 62%, versus alfalfa at 47% and F70 at 51% (Table 3.7). Quatro had the highest digestion through the whole-tract, and alfalfa the lowest: 63%, 54%, and 48% for Quatro, F70 and alfalfa, respectively.

The three forages were not significantly different for CP digestion in the rumen or the intestine. However, for digestion though the whole-tract, the interaction between feed and time was significant (Table 3.8, Figure 3.12). Therefore, the relevant comparisons were 1) within each feed at 18 vs. 30 hours, and 2) between the three feeds at either 18 hours or 30 hours. There was no significant difference of digestion through the whole tract between 18 and 30 hours of rumen incubation for alfalfa or F70. However, whole-tract CP digestion of Quatro incubated for 18 hours was significantly lower than Quatro incubated for 30 hours. There was no difference in CP digestion between the three feeds at 18 hours, but there was a difference at 30 hours with Quatro the highest at 96%, alfalfa intermediate at 95%, and F70 lowest at 93%. Crude protein digested in the intestine contributed more to the whole-tract digestion of CP than NDF or ADF digested in the intestine contributed to whole-tract NDF or ADF digestion.

In a study by Kennelly et al. (1991), the CP of alfalfa degraded in the rumen after 24 hours of incubation was 87%, the CP digested in the intestine was 8%, and the CP digested through the whole tract was 96%. These values are comparable to those of the three haylage types. In the same study, the DM of alfalfa was 68% degraded in the rumen after 24 hours of incubation, while 2% was digested in the intestine, and 71% of DM was digested through the whole tract. These values are comparable to those obtained for the haylage in this study.

In a study comparing alfalfa silage to bromegrass silage in rations at two different forage:concentrate ratios, the DM ruminal digestibility of alfalfa silage was 51%, and 73% through the whole tract (Khorasani et al. 2001). Crude protein digestion was 38% in the rumen and 71% through the whole tract. The NDF fraction was digested to 45% in the rumen and 53% through the whole tract, and the ADF fraction was digested to 43% in the rumen and 52% through the whole tract. While the digestion of F70 was consistently lower than these values, the DM digestion of alfalfa and Quatro haylage from this study was higher than the alfalfa hay. NDF and ADF digestion values were similar between the alfalfa hay and the three haylage types of this study, but CP digestion was much higher for the haylage types than for the alfalfa hay.

3.4. Conclusion

The haylage produced in the 2006 growing season showed little evidence of fermentation during the 4-week ensiling period in which it was measured. In general, Quatro haylage was comparable to alfalfa haylage in degradation and digestion, and while alfalfa had increased protein degradation in the rumen, the fibre fraction of Quatro was more highly degradable in the rumen, and digestible through the whole-tract. F70 was generally lower in nutrient content than the other feeds in all regards.

For fenugreek varieties to become more appealing as a forage for dairy producers, further research is required for proper harvesting and ensiling methods specific to fenugreek. As well, development of best management practices for fenugreek silage production would aid in producers' understanding of fenugreek silage production, and help to ensure that fenugreek is a high nutrient content feed suitable for high-producing dairy cows.

Table 3.1. Rumen kinetics for the *in situ* dry matter degradation of F70 and Quatro fenugreek haylages and alfalfa haylage in lactating dairy cows.

DM[1]	Feed type			
Parameter	Alfalfa	F70	Quatro	SEM
a[2], %	44.2[a]	25.2[c]	33.5[b]	0.8
b[3], %	31.5[b]	35.2[ab]	37.8[a]	1.0
c[4], %	24.3[b]	39.6[a]	28.7[b]	1.3
K_d[5], /h	8.6[a]	3.5[b]	6.00[ab]	0.8
ERD[6]				
K_p=0.03/h	67.5[a]	43.6[b]	58.6[a]	2.9
K_p=0.05/h	64.1[a]	39.3[b]	54.1[a]	2.7
K_p=0.07/h	61.6[a]	36.7[b]	50.9[a]	2.5

[1]DM = dry matter
[2]*a* = soluble fraction of feed (%)
[3]*b* = slowly degradable fraction of feed (%)
[4]*c* = undegradable fraction of feed (%)
[5]K_d = rate of degradation (%/h)
Above parameters are calculated using the equation $y = a + b(1 - \exp(K_d*t))$
[6]ERD = Effective Ruminal Degradability, calculated using
$ERD=a+(b*K_d)/(K_d+K_p)$, where K_p is the theoretical rate of passage of material from the rumen per hour, 0.03, 0.05 or 0.07
[a,b,c]lsmeans within a row with different superscripts differ ($P < 0.05$)

Table 3.2. Rumen kinetics for the *in situ* neutral detergent fibre degradation of F70 and Quatro fenugreek haylages and alfalfa haylage in lactating dairy cows.

NDF[1]	Feed type			
Parameter	Alfalfa	F70	Quatro	SEM
a[2], %	0.6[b]	7.3[ab]	12.4[a]	1.9
b[3], %	38.1	32.5	35.9	1.9
c[4], %	61.3	60.2	51.7	2.1
K_d[5], /h	7.00[a]	2.3[b]	4.9[a]	0.6
ERD[6]				
K_p=0.03/h	27.2	21.2	34.6	3.3
K_p=0.05/h	22.8	17.5	30.1	3.0
K_p=0.07/h	19.6	15.3	27.1	2.8

[1]NDF = neutral detergent fibre
[2]*a* = soluble fraction of feed (%)
[3]*b* = slowly degradable fraction of feed (%)
[4]*c* = undegradable fraction of feed (%)
[5]K_d = rate of degradation (%/h)
Above parameters are calculated using the equation $y = a + b(1 - \exp(K_d*t))$
[6]ERD = Effective Ruminal Degradability, calculated using
$ERD=a+(b*K_d)/(K_d+K_p)$, where K_p is the theoretical rate of passage of material from the rumen per hour, 0.03, 0.05 or 0.07
[a,b,c]lsmeans within a row with different superscripts differ ($P < 0.05$)

Table 3.3. Rumen kinetics for the *in situ* acid detergent fibre degradation of F70 and Quatro fenugreek haylages and alfalfa haylage in lactating dairy cows.

ADF[1]	Feed type			
Parameter	Alfalfa	F70	Quatro	SEM
a[2], %	0.9	7.4	10.7	1.8
b[3], %	39.7	34.5	37.2	2.12
c[4], %	59.3	58.2	52.1	2.7
K_d[5], /h	5.9[a]	2.3[b]	4.4[ab]	0.5
ERD[6]				
K_p=0.03/h	27.3	22.1	32.8	3.7
K_p=0.05/h	22.5	18.2	28.1	3.4
K_p=0.07/h	19.1	15.9	25.1	3.1

[1]ADF = acid detergent fibre
[2]*a* = soluble fraction of feed (%)
[3]*b* = slowly degradable fraction of feed (%)
[4]*c* = undegradable fraction of feed (%)
[5]K_d = rate of degradation (%/h)
Above parameters are calculated using the equation $y = a + b(1 - \exp(K_d*t))$
[6]ERD = Effective Ruminal Degradability, calculated using
$ERD=a+(b*K_d)/(K_d+K_p)$, where K_p is the theoretical rate of passage of material from the rumen per hour, 0.03, 0.05 or 0.07
[a,b,c]lsmeans within a row with different superscripts differ ($P < 0.05$)

Table 3.4. Rumen kinetics for the *in situ* crude protein degradation of F70 and Quatro fenugreek haylages and alfalfa haylage in lactating dairy cows.

CP[1]	Feed type			
Parameter	Alfalfa	F70	Quatro	SEM
a[2], %	56.0	22.1	34.2	10.9
b[3], %	35.7	62.8	58.0	9.9
c[4], %	8.3[b]	15.2[a]	7.8[b]	1.1
K_d[5], /h	12.5	5.3	6.4	1.4
ERD[6]				
K_p=0.03/h	84.8[a]	62.1[c]	73.6[b]	1.0
K_p=0.05/h	81.5[a]	54.7[c]	66.7[b]	1.9
K_p=0.07/h	78.9[a]	49.6[b]	61.8[b]	2.8

[1]CP = crude protein
[2]*a* = soluble fraction of feed (%)
[3]*b* = slowly degradable fraction of feed (%)
[4]*c* = undegradable fraction of feed (%)
[5]K_d = rate of degradation (%/h)
Above parameters are calculated using the equation $y = a + b(1 - \exp(K_d*t))$
[6]ERD = Effective Ruminal Degradability, calculated using
$ERD=a+(b*K_d)/(K_d+K_p)$, where K_p is the theoretical rate of passage of material from the rumen per hour, 0.03, 0.05 or 0.07
[a,b,c]lsmeans within a row with different superscripts differ ($P < 0.05$)

Table 3.5. Mean percent dry matter (DM) disappearance for the *in situ* digestion of feeds after 18 hours or 30 hours of incubation in the rumen, and of alfalfa haylage and F70 and Quatro fenugreek haylages in the rumen, intestine and whole gastrointestinal tract of dry dairy cows.

% DM[1] disappearance		Feed				Time		
	GI location	Alfalfa	F70	Quatro	SEM	18 hours	30 hours	SEM
	Rumen	74.79[a]	60.41[b]	73.68[a]	0.85	65.89	73.36	0.63
	Intestine[2]	1.93	5.02	3.79	0.59	5.48	1.68	0.45
	Intestine[3]	7.28	12.04	13.11	2.06	15.51	6.10	1.63
	Whole tract	76.72[a]	65.43[b]	77.47[a]	0.40	71.37	75.04	0.29

[1]DM = Dry Matter
[2]percent disappearance in the intestine as a percentage of feed DM put in the rumen
[3]percent disappearance in the intestine as a percentage of feed DM entering the intestine
[a,b,c]lsmeans within a row for feed type with different superscripts differ ($P < 0.05$)

Table 3.6. Mean percent neutral detergent fibre (NDF) disappearance for the *in situ* digestion of feeds after 18 hours or 30 hours of incubation in the rumen, and of alfalfa haylage and F70 and Quatro fenugreek haylages in the rumen, intestine and whole gastrointestinal tract of dry dairy cows.

% NDF[1] disappearance		Feed				Time		
	GI location	Alfalfa	F70	Quatro	SEM	18 hours	30 hours	SEM
	Rumen	45.61[b]	48.42[b]	61.96[a]	1.04	47.52	56.46	0.75
	Intestine[2]	0.34	4.05	1.22	0.45	3.45	0.28	0.36
	Intestine[3]	0.52	7.35	2.83	1.06	6.54	0.59	0.86
	Whole tract	45.95[c]	52.46[b]	63.17[a]	0.82	50.97	56.75	0.65

[1]NDF = Neutral Detergent Fibre
[2]percent disappearance in the intestine as a percentage of feed DM put in the rumen
[3]percent disappearance in the intestine as a percentage of feed DM entering the intestine
[a,b,c]lsmeans within a row for feed type with different superscripts differ ($P < 0.05$)

Table 3.7. Mean percent acid detergent fibre (ADF) disappearance for the *in situ* digestion of feeds after 18 hours or 30 hours of incubation in the rumen, and of alfalfa haylage and F70 and Quatro fenugreek haylages in the rumen, intestine and whole gastrointestinal tract of dry dairy cows.

% ADF[1] disappearance		Feed				Time		
	GI location	Alfalfa	F70	Quatro	SEM	18 hours	30 hours	SEM
	Rumen	48.25[b]	50.73[b]	61.59[a]	0.97	49.12	57.93	0.69
	Intestine[2]	-0.59	3.20	0.96	0.44	2.73	-0.35	0.33
	Intestine[3]	-1.27	6.02	2.03	1.01	5.40	-0.88	0.78
	Whole tract	47.66[c]	53.93[b]	62.54[a]	0.64	51.85	57.57	0.45

[1]ADF = Acid Detergent Fibre
[2]percent disappearance in the intestine as a percentage of feed DM put in the rumen
[3]percent disappearance in the intestine as a percentage of feed DM entering the intestine
[a,b,c]lsmeans within a row for feed type with different superscripts differ ($P < 0.05$)

Table 3.8. Mean percent crude protein (CP) disappearance for the *in situ* digestion of feeds after 18 hours or 30 hours of incubation in the rumen, and of alfalfa haylage and F70 and Quatro fenugreek haylages in the rumen, intestine and whole gastrointestinal tract of dry dairy cows.

% CP[1] disappearance		Feed				Time		
	GI location	Alfalfa	F70	Quatro	SEM	18 hours	30 hours	SEM
	Rumen	87.28	80.28	83.41	1.78	77.80	89.51	1.32
	Intestine[2]	6.73	11.66	10.40	1.78	14.35	4.85	1.36
	Intestine[3]	50.99	53.80	58.11	4.48	62.31	46.29	3.68

	Alfalfa	F70	Quatro	SEM
Whole tract[4] 18 hours	93.45	91.36	91.63[B]	0.40
Whole tract[4] 30 hours	94.57[b]	92.53[c]	95.98[A/a]	0.22

[1]CP = Crude Protein
[2]percent disappearance in the intestine as a percentage of feed DM put in the rumen
[3]percent disappearance in the intestine as a percentage of feed DM entering the intestine
[4]Whole tract lsmeans are presented uniquely due to a significant interaction of feed and time
[A,B]lsmeans within a column for feed type with different superscripts differ ($P < 0.05$)
[a,b,c]lsmeans within a row for feed type with different superscripts differ ($P < 0.05$)

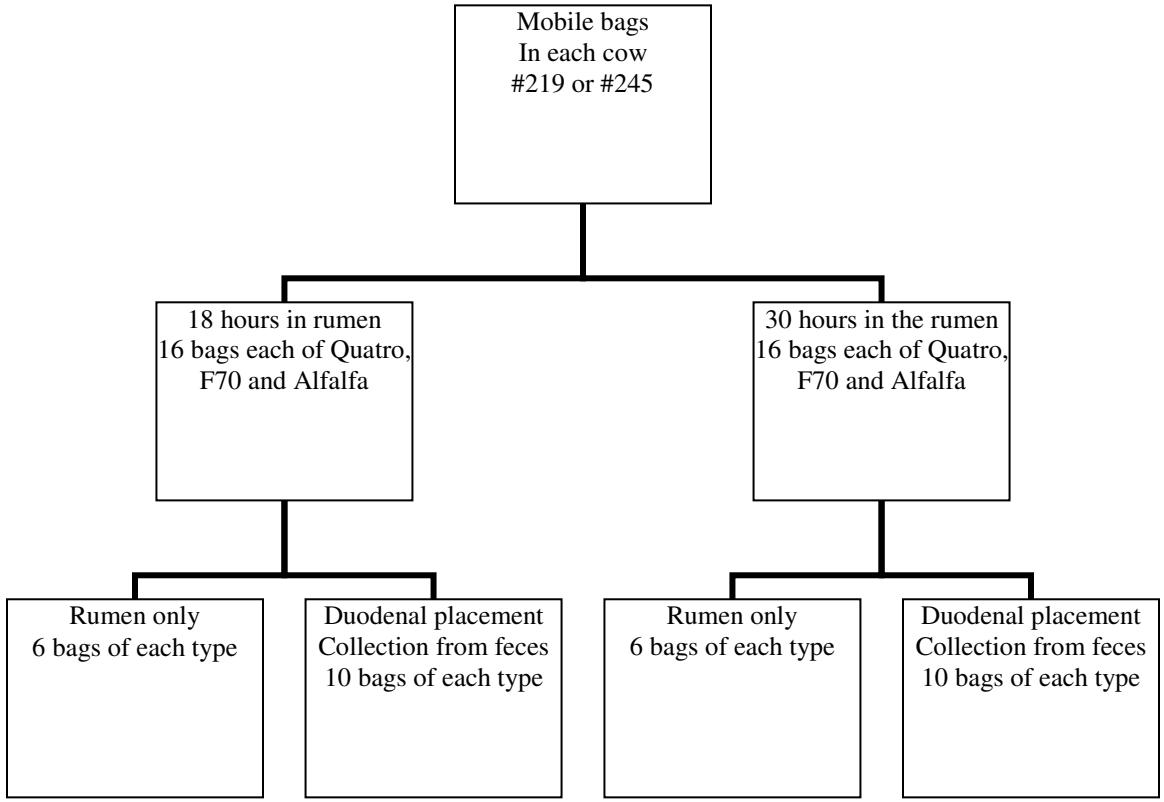

Figure 3.1. Representation of the experimental design for the rumen mobile nylon-bag study for assessment of whole-tract F70 and Quatro fenugreek haylages and alfalfa haylage digestion, conducted in December 2006 at the Lethbridge Research Centre, Lethbridge, AB, using two ruminally- and duodenally-cannulated dry cows fed *ad libitum* alfalfa hay.

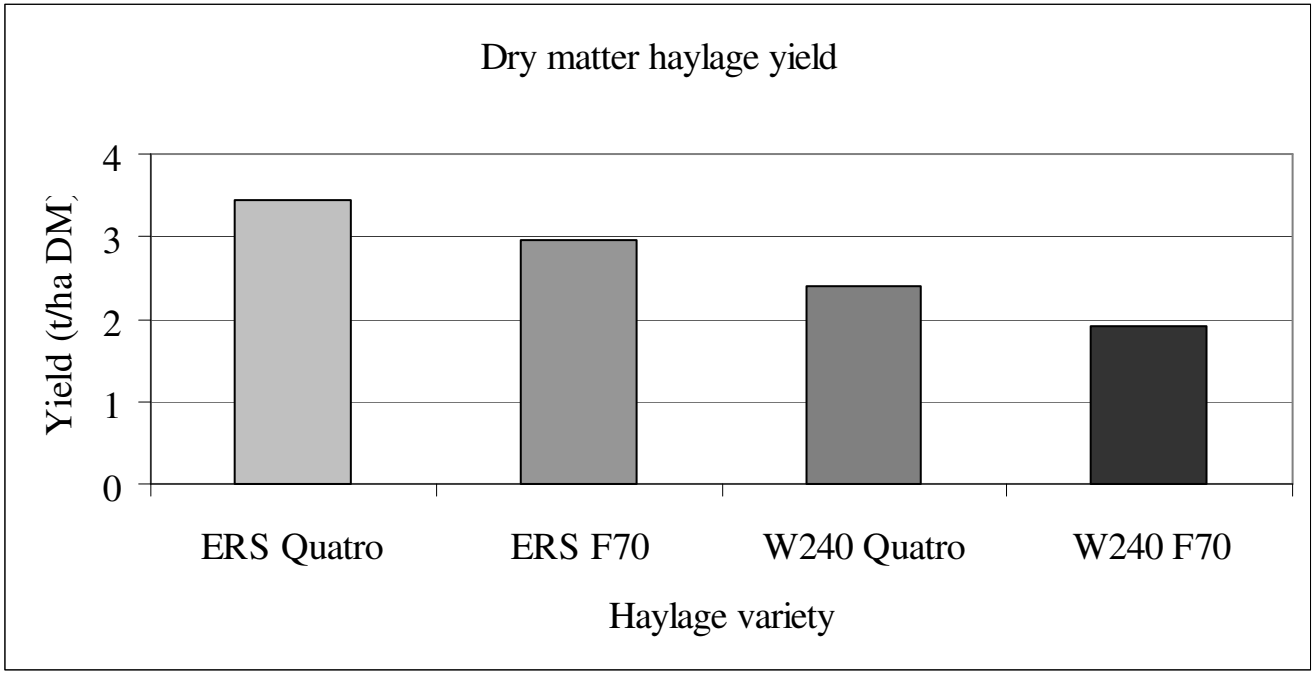

Figure 3.2. Dry matter yield (tonnes/ha) of Quatro and F70 fenugreek haylages grown in 2006 at two locations (ERS, W240) on the Edmonton Research Station, Edmonton, AB.

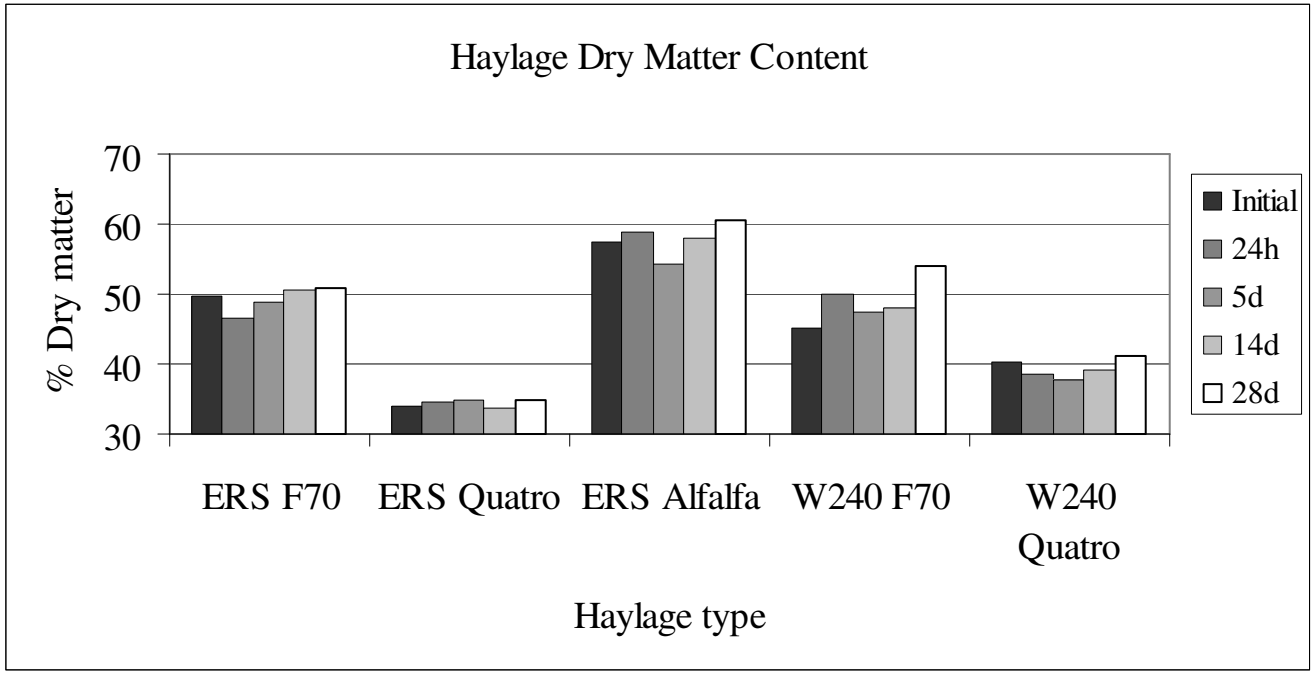

Figure 3.3. Dry matter content of 16 week Quatro and F70 fenugreek haylages grown in 2006 at two locations (ERS and W240) on the Edmonton Research Station, Edmonton, AB, and second-cut alfalfa haylage also grown on the Edmonton Research Station in 2006, taken before wrapping of bales (initial), and 24 hours, 5 days, 14 days, and 28 days after wrapping.

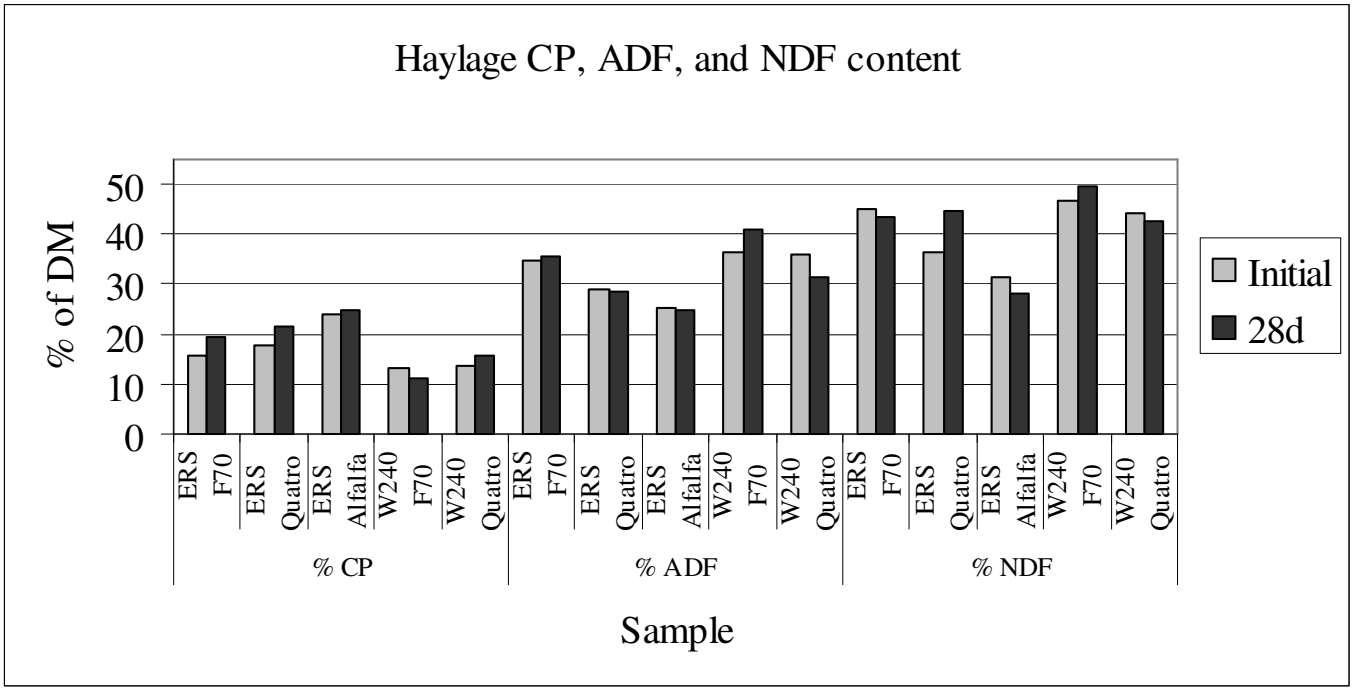

Figure 3.4. Mean crude protein (CP), acid detergent fibre (ADF), and neutral detergent fibre (NDF) content (% of DM) of 16 week Quatro and F70 fenugreek haylages grown in 2006 at two locations (ERS and W240) on the Edmonton Research Station, Edmonton, AB, and second-cut alfalfa haylage also grown on the Edmonton Research Station in 2006, at initial sampling before haylage bales were wrapped, and after 28 days of fermentation.

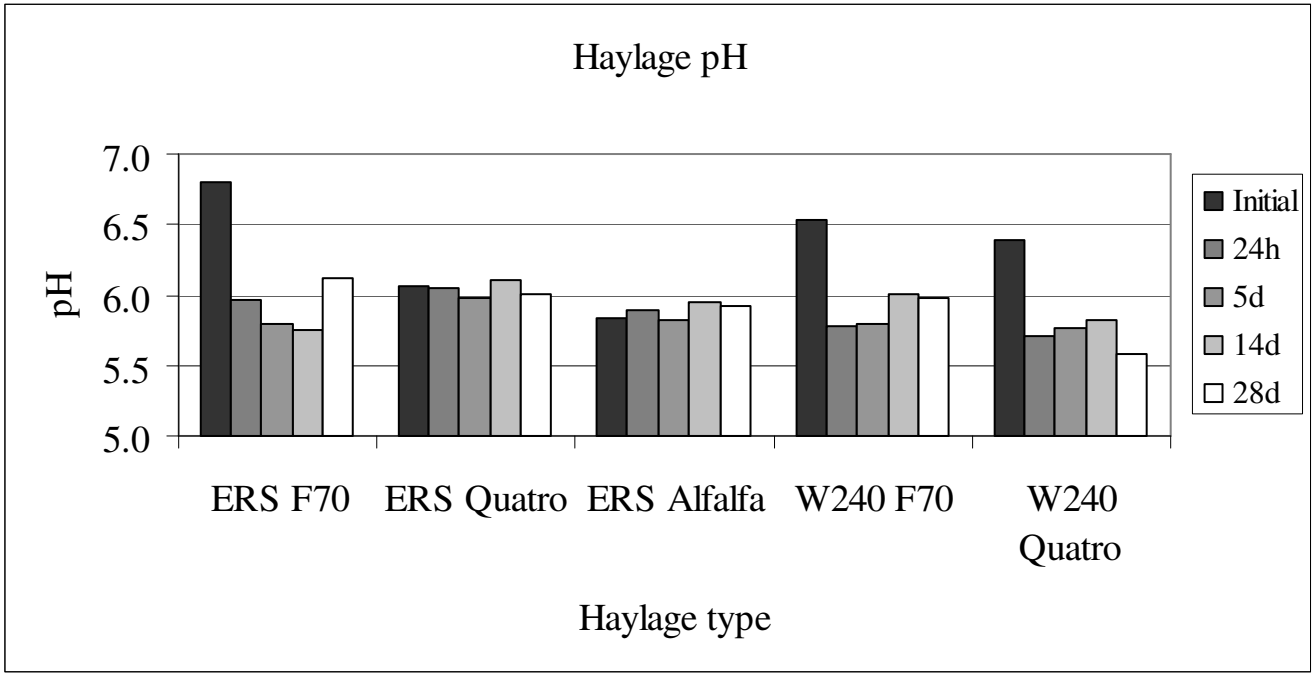

Figure 3.5. Mean pH of Quatro and F70 fenugreek haylages grown in 2006 at two locations (ERS and W240) on the Edmonton Research Station, Edmonton, AB, and second-cut alfalfa haylage also grown on the Edmonton Research station in 2006, taken before wrapping of bales (initial), and 24 hours, 5 days, 14 days, and 28 days after wrapping.

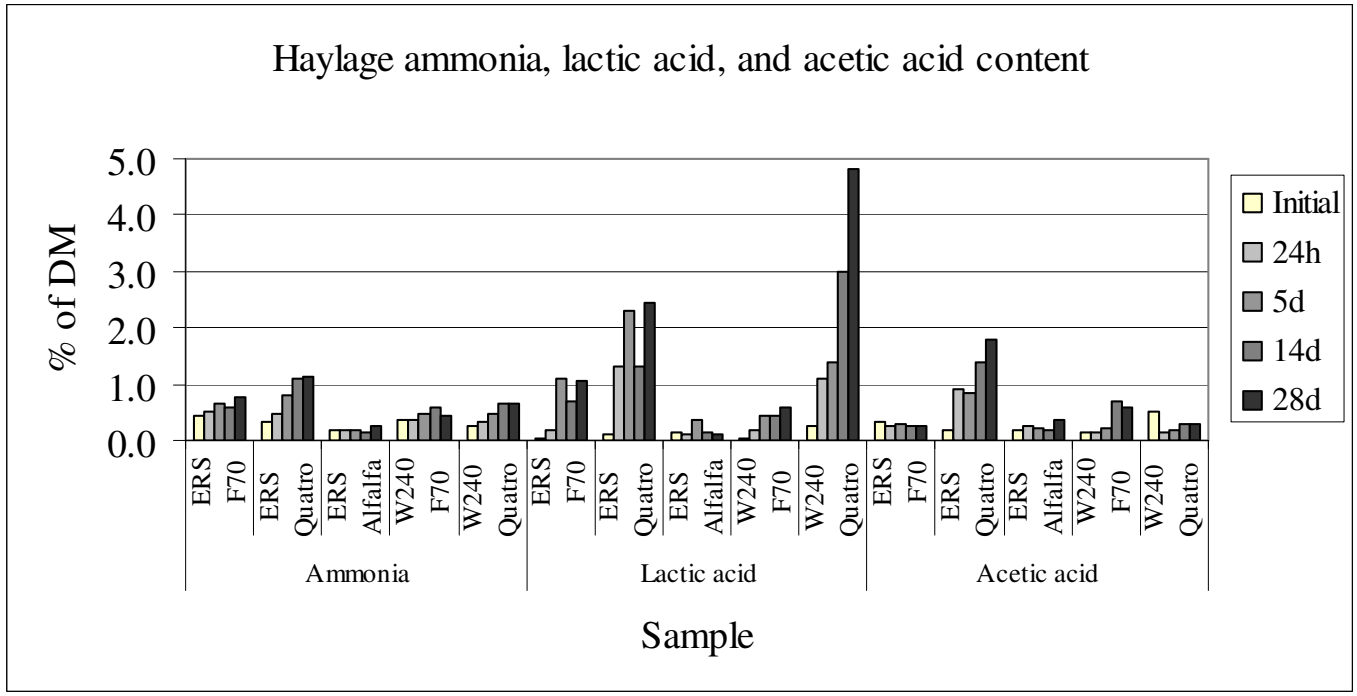

Figure 3.6. Mean ammonia, lactic acid, and acetic acid content (% of DM) of 16 week Quatro and F70 fenugreek haylages grown in 2006 at two locations (ERS and W240) on the Edmonton Research Station, Edmonton, AB, and second-cut alfalfa haylage also grown on the Edmonton Research Station in 2006, taken before wrapping of bales (initial), and 24 hours, 5 days, 14 days, and 28 days after wrapping.

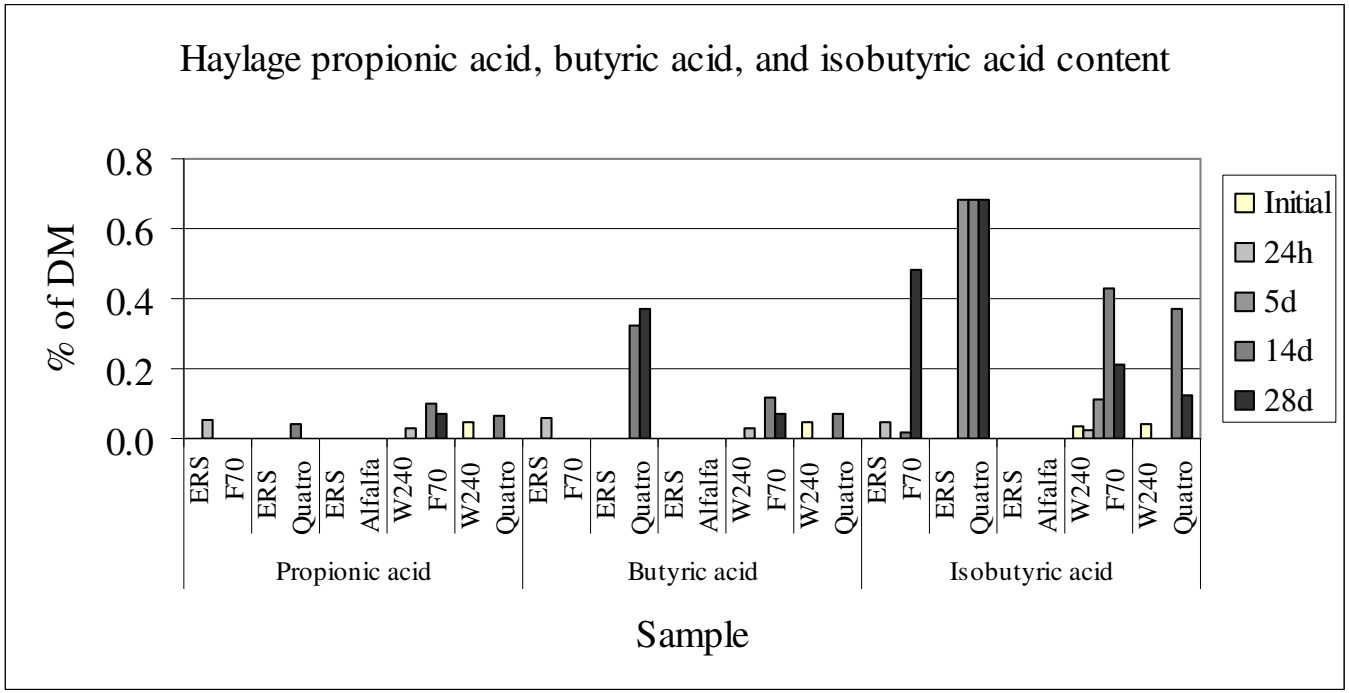

Figure 3.7. Mean propionic acid, butyric acid, and isobutyric acid content (% of DM) of 16 week Quatro and F70 fenugreek haylages grown in 2006 at two locations (ERS and W240) on the Edmonton Research Station, Edmonton, AB, and second-cut alfalfa haylage also grown on the Edmonton Research Station in 2006, taken before wrapping of bales (initial), and 24 hours, 5 days, 14 days, and 28 days after wrapping.

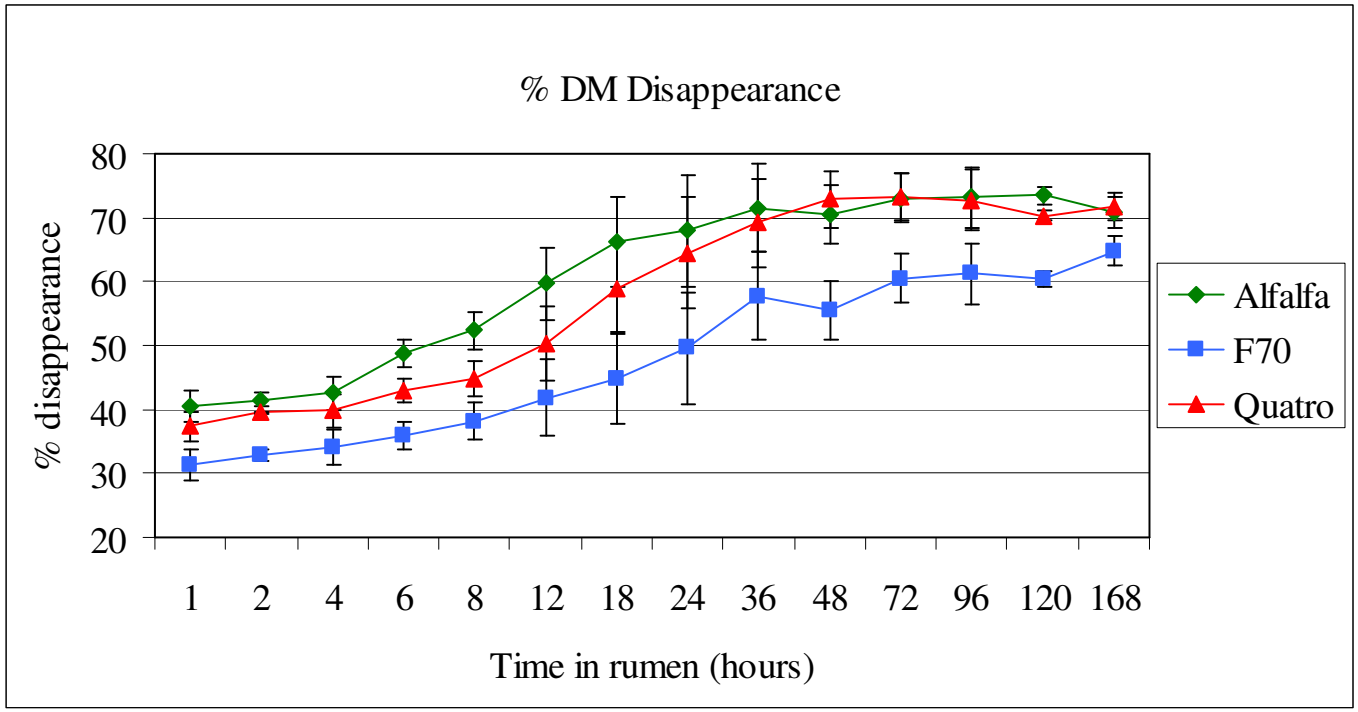

Figure 3.8. Mean ruminal percent dry matter (DM) disappearance from 1 to 168 hours for the *in situ* degradation of F70 and Quatro fenugreek haylages and alfalfa haylage in lactating dairy cows. Vertical bars represent 95% confidence limit; where bars do not overlap, values differ ($P < 0.05$).

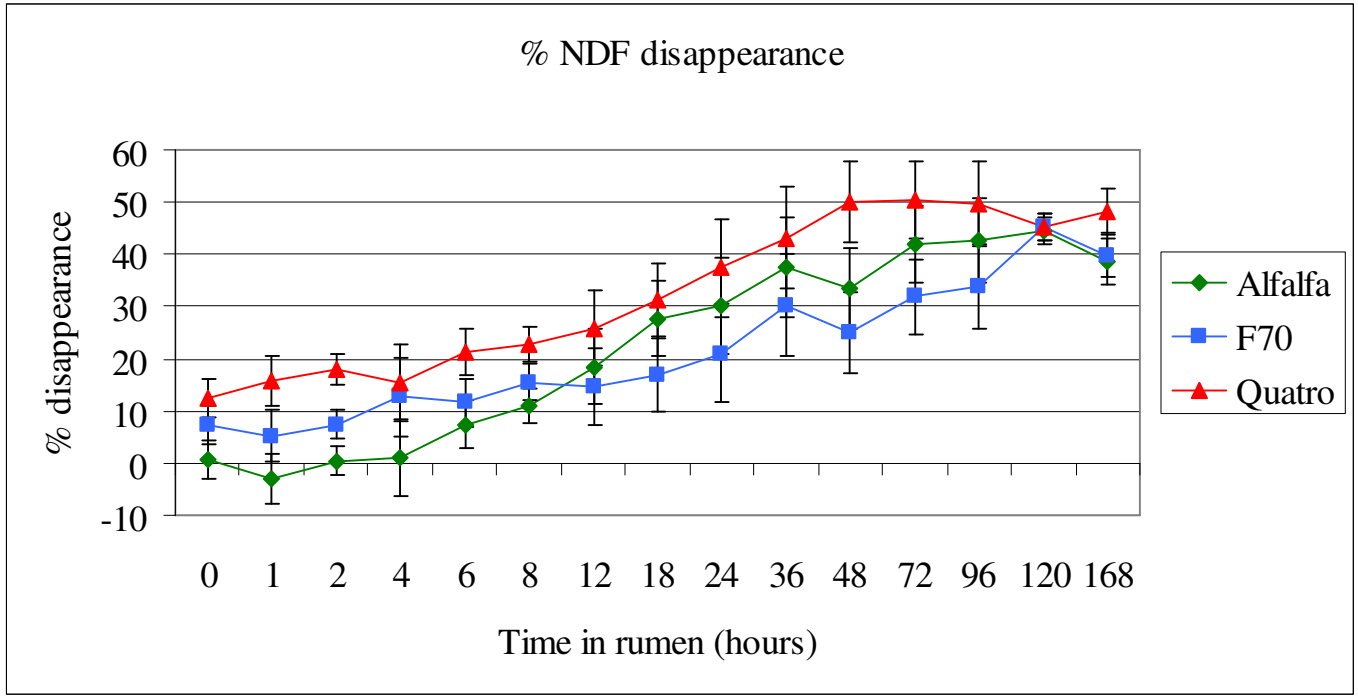

Figure 3.9. Mean ruminal percent neutral detergent fibre (NDF) disappearance from 1 to 168 hours for the *in situ* degradation of F70 and Quatro fenugreek haylages and alfalfa haylage in lactating dairy cows. Vertical bars represent 95% confidence limit; where bars do not overlap, values differ ($P < 0.05$).

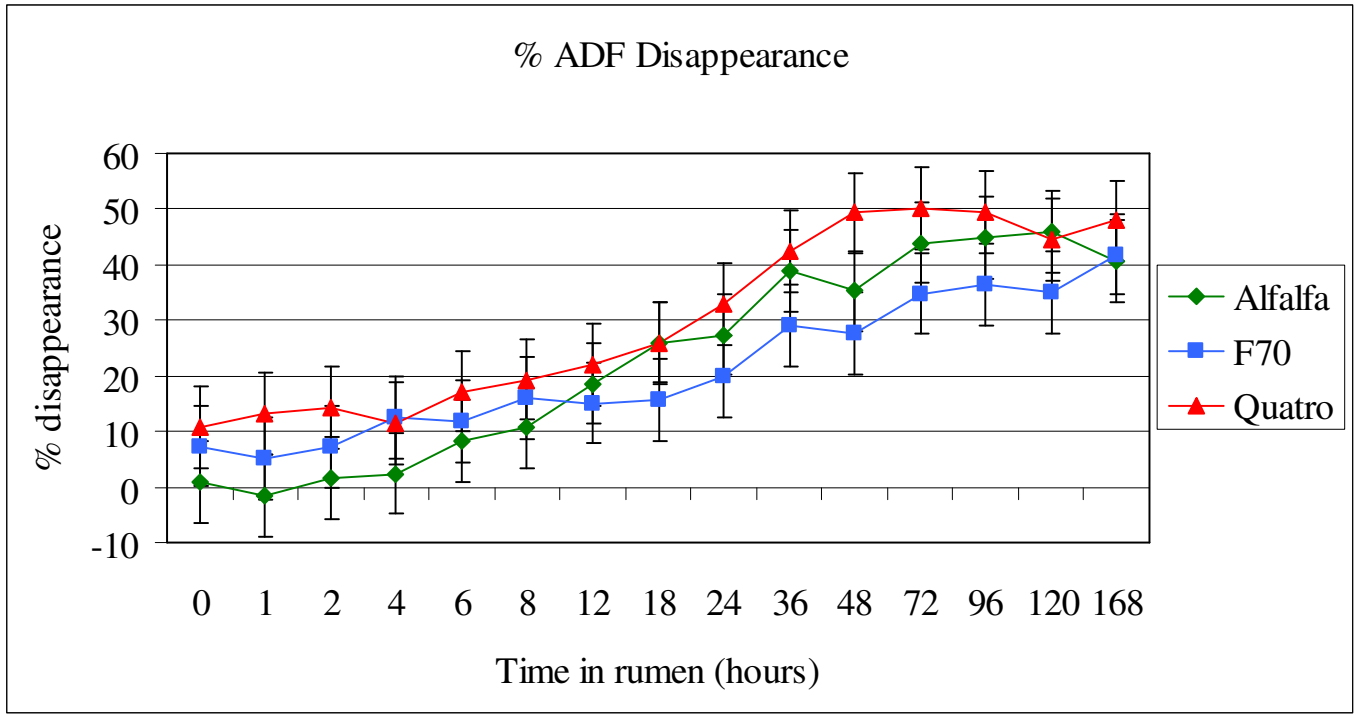

Figure 3.10. Mean ruminal percent acid detergent fibre (ADF) disappearance from 1 to 168 hours for the *in situ* degradation of F70 and Quatro fenugreek haylages and alfalfa haylage in lactating dairy cows. Vertical bars represent 95% confidence limit; where bars do not overlap, values differ ($P < 0.05$).

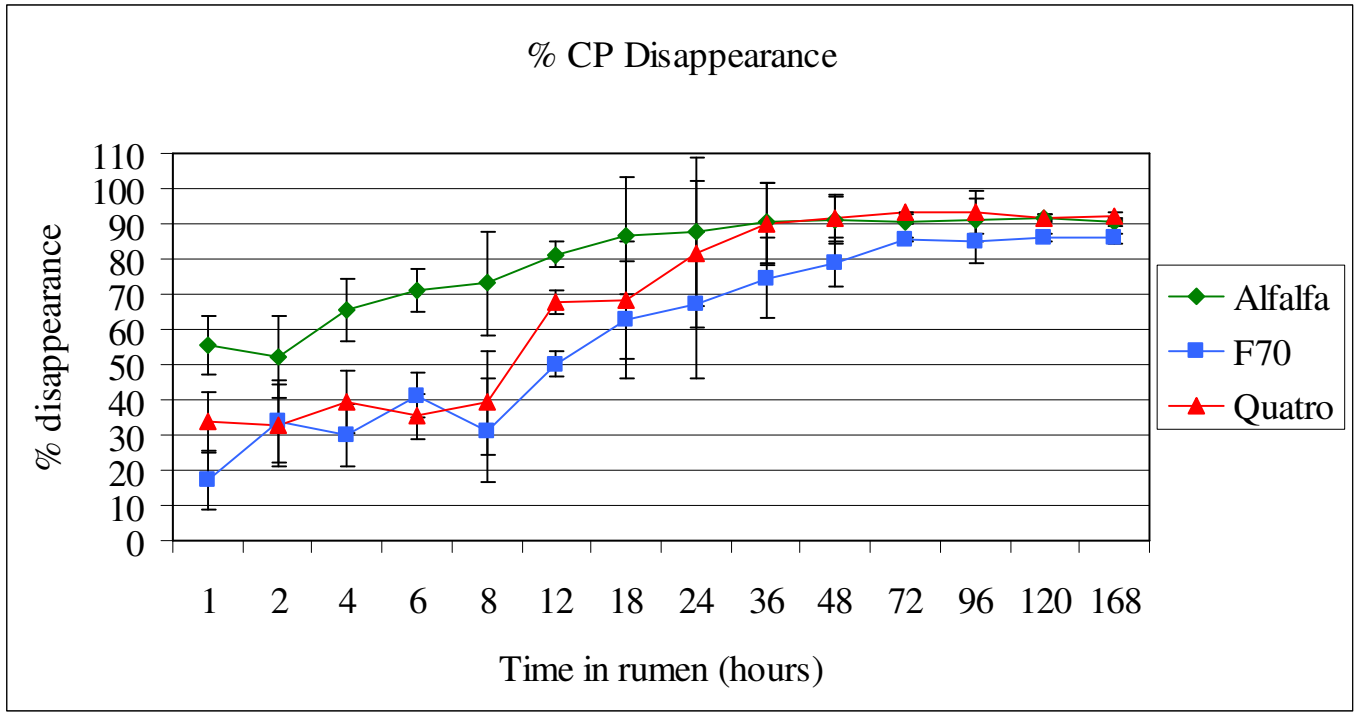

Figure 3.11. Mean ruminal percent crude protein (CP) disappearance from 1 to 168 hours for the *in situ* degradation of F70 and Quatro fenugreek haylages and alfalfa haylage in lactating dairy cows. Vertical bars represent 95% confidence limit; where bars do not overlap, values differ ($P < 0.05$).

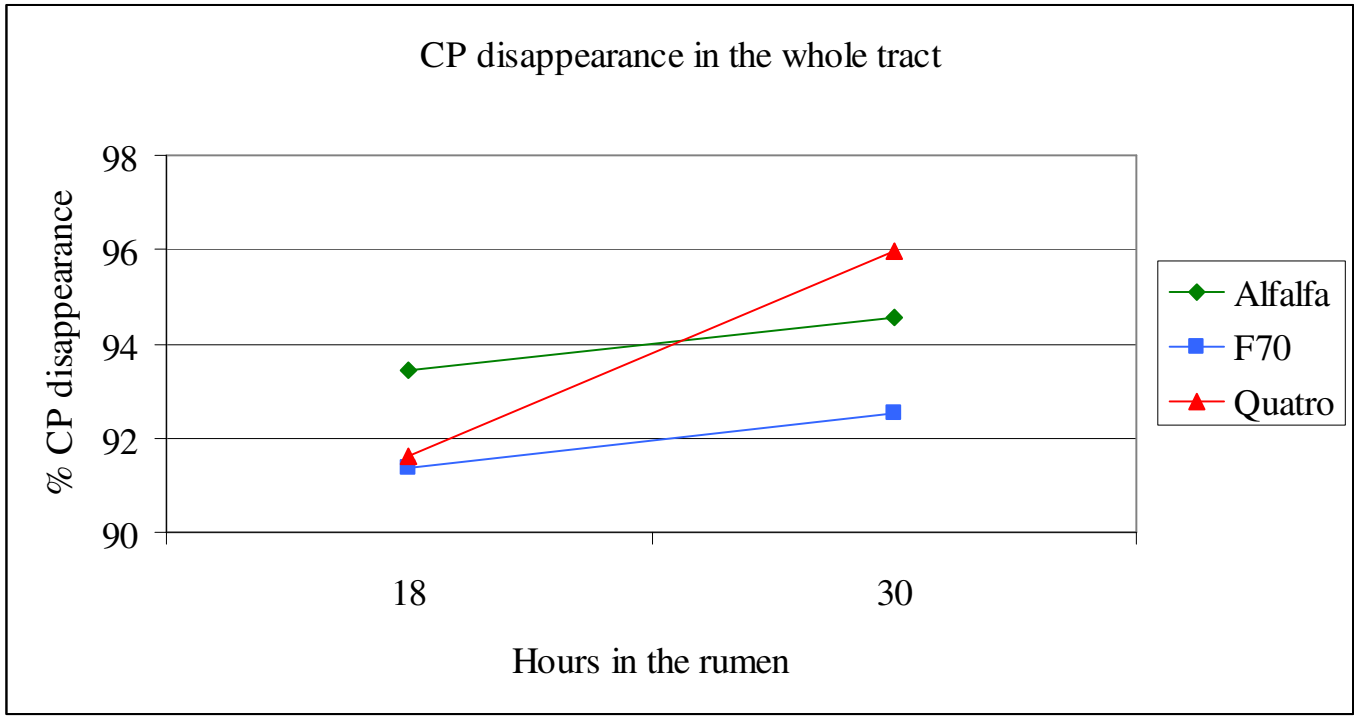

Figure 3.12. Percent crude protein (CP) disappearance for the *in situ* digestion of alfalfa haylage and F70 and Quatro fenugreek haylages through the whole gastrointestinal tract of dry dairy cows after 18 hours or 30 hours of incubation in the rumen. Graphical representation of the significant interaction of feed and time of rumen incubation for CP digestion through the whole gastrointestinal tract.

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Chapter 4: Synthesis

4.1. Background

Fenugreek (*Trigonella foenum-graecum* L.) is a single-cut, annual legume, traditionally grown for seed production (Acharya et al. 2008). Fenugreek has recently garnered interest as a forage source in Alberta because of its reported high and sustained quality, nitrogen fixation benefits, and adaptation to dry growing climates (Acharya et al. 2008). As a potential source of high-quality feed, specifically silage, fenugreek could be of particular value to dairy producers in Alberta. While previous research has been conducted on fenugreek in Lethbridge, AB, and Saskatchewan (Acharya et al. 2008, Slinkard et al. 2006), there have previously been no trials conducted in central AB, where there is a high concentration of dairy producers.

4.2. Objectives

The objectives of this study were:

1. To observe the growth of two fenugreek genotypes, AAFC F70 and CDC Quatro, under dry-land conditions in the Edmonton, Alberta area, including crop height, biomass, development, resource partitioning, and growth analysis.
2. To determine the quality of fenugreek plants and plant components, in order to verify if quality is of sufficient standard for feeding to livestock, and specifically to dairy herds, and to verify if the quality of fenugreek is maintained over the growing season.
3. To determine the ruminal degradation and kinetic parameters, and ruminal, intestinal, and whole-tract digestion of F70 and Quatro haylage.

4.3. Summary of Findings

4.3.1. Plant growth

- F70 had higher emergence than Quatro in both growing years, 2006 and 2007.
- Quatro and F70 did not differ significantly in crop height, and heights were similar to those previously recorded for fenugreek.

- By the end of the growing season in both years, Quatro biomass was not different than F70. Biomass production was generally lower than that of alfalfa in the Edmonton area, but similar to long-term dry-land fenugreek biomass production averages in the Lethbridge, AB, area.
- Quatro had higher plant weight than F70 in 2006, thereby making up the difference in plant density to achieve the same biomass as F70. The ability of fenugreek to compensate for decreased plant density with increased plant weight demonstrates the plasticity of the crop.
- Root weight and leaf weight were not different between genotypes by the end of the growing season in 2006 and 2007. Quatro had higher stem weight than F70 in 2006, but in 2007 the genotypes were not different.
- At the end of the 2006 growing season, the pod+seed weight of Quatro plants was threefold that of F70. However, in 2007, there was no genotype difference. Quatro may have increased tolerance for heat during flowering.
- In both years, the leaf to stem weight ratio of Quatro and F70 decreased in a similar fashion to 0.5.
- While leaf represented the greatest contribution to plant weight at the beginning of the growing season in both years, percent stem and pod+seed contributed the most to plant weight by and after 65 days.
- Leaf area index was about twice that of the pod area index.
- There was no significant difference of quality between years, and few genotype differences. Quality of fenugreek plant components and whole plants tended to stabilize around 65 days. Whole plant CP content was between 15 and 20%. RFV was between 150 and 250, so even at its minimum, the RFV of fenugreek makes it suitable for dairy cows during lactation.
- RGR, LAR, LWF, and SLA all decreased over the growing season for both genotypes in both years. The two genotypes were numerically similar for all four characteristics when considering the entire length of the growing season.

4.3.2. *In situ* degradation and digestion studies

- Haylage biomass production was lower than the long-term dry-land fenugreek production average for the Lethbridge, AB, area.
- Of the five haylage types produced, alfalfa haylage had the numerically highest CP content, and lower numerical NDF and ADF content. In general, ERS fenugreek haylage types had higher quality than the W240 types, and within location, Quatro haylage had higher quality than F70 haylage.
- The fenugreek and alfalfa haylage showed little evidence of fermentation in terms of pH change or fermentation acid production.
- Alfalfa haylage had superior dry matter degradation in the rumen. F70 haylage had lower dry matter degradation in the rumen compared to the other two haylage types.
- While F70 haylage had lower rates of NDF and ADF degradation than the other two haylage types, the fibre content of the Quatro and alfalfa haylages types were degraded in a similar fashion.
- The CP degradation of alfalfa haylage was higher than the fenugreek haylage types, despite few differences in the kinetic fractions of the CP content of the haylages.
- Dry matter disappearance in the rumen stabilized around 36 hours of incubation. The dry matter disappearance of alfalfa and Quatro was comparable, while that of F70 haylage was consistently lower.
- NDF and ADF disappearance in the rumen stabilized around 72 hours of incubation. After 12 hours of incubation, F70 haylage replaced alfalfa haylage as the haylage with the lowest degradation. By the end of the incubation period there was no difference between the fibre disappearance of the three haylage types.
- While the CP disappearance of Quatro and alfalfa haylages stabilized at 36 hours of incubation, F70 CP disappearance continued to increase until 72 hours. By the end of the incubation period the CP disappearance of Quatro and alfalfa was comparable, while that of F70 was lower.

- There were no significant differences between haylage types for DM, NDF, ADF or CP digestion in the intestine, or between 18 hour and 30 hour incubation in the rumen.
- DM digestion in the rumen and whole tract was comparable between Quatro and alfalfa haylage types, while F70 haylage was significantly lower.
- NDF and ADF digestion was significantly higher in the rumen and whole tract for Quatro haylage, while alfalfa and F70 were comparable.
- There were no significant differences between haylage types for CP digestion in the rumen. However, there was a significant interaction of time of incubation and haylage type for digestion in the whole tract. Quatro haylage was digested to a significantly greater extent in the whole tract after 30 hours of incubation in the rumen versus 18 hours. After 30 hours of incubation, Quatro had higher digestion than the other two haylage types, and F70 was lowest.

4.4. Synthesis

The objectives of this study were achieved in that we:

- established a detailed representation of fenugreek growth in the Edmonton area, including biomass accumulation, resource partitioning, and quality, and thus determined that fenugreek does have great potential for use in central Alberta;
- established that Quatro fenugreek haylage utilization was comparable to that of alfalfa haylage in dairy cows, but that F70 haylage was utilized to a lesser extent. Therefore, provided it is harvested at an appropriate stage and stored properly, fenugreek does represent a suitable forage alternative to alfalfa for use in dairy diets in Alberta.

The staging schematic that I developed as a tool to monitor the growth and development of fenugreek over the growing season has one limitation in that it is most applicable to fenugreek grown under similar conditions. This includes date

of seeding, photoperiod, and plant density, as all of these things could affect the timing of development and plant nutrient content.

The previously reported advantage of nitrogen fixation by fenugreek was not observed in this research. The lack of nodulation in our experiments was likely due to inherently high levels of nitrogen in the soil of the experimental area, however, this prevented us from determining the level to which fenugreek can contribute nitrogen to a crop rotation. If fenugreek is used on land where manure is spread, exporting nitrogen off the field at harvest may be an advantage to prevent nitrogen accumulation. Further research is required into the ability of fenugreek to fix nitrogen when grown on more nitrogen deficient soils.

Plants harvested from the small plot experiment had higher yield and quality than the haylage used for the rumen experiments. The haylage harvest procedure did not capture the full nutrient benefit of fenugreek. Determining the best conservation practices for fenugreek is thus one of the most critical areas for further research. Perhaps harvesting fenugreek for hay rather than silage would be an easier alternative in order to ensure capture of nutrients and yield. However, we observed significant leaf loss during harvest so the severity of leaf loss during haying would have to be investigated.

Our research also indicated certain advantages of fenugreek forage over alfalfa forage in terms of crude protein digestion. The rumen kinetics indicate that fenugreek crude protein is degraded to a lesser extent than alfalfa crude protein, which may translate into a higher level of rumen-bypass protein for digestion and absorption in the intestine of dairy cow. In addition, the slower initial rate of fenugreek crude protein disappearance from the rumen, compared to that of alfalfa, may result in a lower incidence of bloat in animals fed fenugreek.

4.5. Further research

This preliminary research has shown that F70 and Quatro fenugreek genotypes show positive potential for use in the central Alberta area as a forage source for dairy herds. The following research would be beneficial to improve the agronomic success of fenugreek production and the potential for more wide-spread use in the dairy industry.

- Research into agronomic practices which will optimize yield and quality of fenugreek crops, such as:
    - Seeding practices including depth, row spacing, soil preferences, and timing of seeding in the spring.
    - Herbicide use for pre-seeding and in-crop applications.
    - Optimal fertilizer and inoculant regimes.
    - Harvesting protocols including timing and equipment to optimize plant recovery from fields.
- Haying and silaging protocols are required in order to optimize the storage and feeding of high-quality forage. This includes use of silage additives, best storage practices, duration of storage, and best feeding practices.
- More information is required on the performance of dairy cows while being fed rations that are based on or that include fenugreek forage. This includes milk production and reproductive success as well as cow health,. Palatability of the forage and conveyance of an off-odour to the milk are of particular concern.
- Due to the neutraceutical properties of fenugreek, research into the possible health benefits to dairy cows and to humans who consume milk from dairy cows fed fenugreek-based diets could result in niche market production.

The findings of this research indicate that fenugreek could indeed be a potential source of high quality legume forage for dairy producers in the central Alberta area. Yield is comparable to that of other legume forages, and quality is high enough to be used in dairy rations to support high levels of milk production. It is not apparent that F70, as the forage-type fenugreek genotype, had any forage yield or quality advantage over Quatro, the seed-type genotype. Further research on the specific agronomic best management practices of fenugreek grown in central ALBERTA is required in order to refine the production of consistent, high quality yields.

4.6. References

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Appendix 1: Plot plans for F70 and Quatro fenugreek growth 2006 and 2007 on the Edmonton Research Station, Edmonton, AB.

601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618
H2	H4	H5	H2	H1	H4	H7	H3	H6	H1	H8	H5	H6	H9	H3	H9	H8	H7
F70	F70	F70	QT	F70	QT	F70	QT	F70	QT	QT	QT	QT	F70	F70	QT	F70	QT

501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518
H6	H4	H1	H1	H6	H8	H2	H9	H7	H8	H4	H7	H5	H3	H2	H3	H5	H9
QT	F70	F70	QT	F70	QT	F70	QT	QT	F70	QT	F70	QT	QT	QT	F70	F70	F70

401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418
H9	H1	H4	H5	H3	H4	H5	H2	H1	H6	H7	H8	H2	H3	H9	H7	H8	H6
QT	F70	QT	QT	F70	F70	F70	F70	QT	QT	F70	F70	QT	QT	F70	QT	QT	F70

301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318
H9	H5	H7	H3	H7	H4	H4	H1	H9	H5	H8	H1	H6	H8	H3	H2	H2	H6
F70	QT	QT	F70	F70	F70	QT	QT	QT	F70	QT	F70	F70	F70	QT	F70	QT	QT

201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218
H3	H1	H3	H4	H6	H6	H1	H7	H9	H9	H8	H4	H2	H2	H5	H5	H7	H8
QT	QT	F70	QT	QT	F70	F70	F70	QT	F70	QT	F70	QT	F70	QT	F70	QT	F70

101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118
H1	H9	H5	H5	H7	H2	H4	H8	H6	H3	H9	H7	H6	H1	H2	H4	H3	H8
F70	QT	F70	QT	F70	F70	F70	QT	F70	F70	F70	QT	QT	QT	QT	QT	QT	F70

Appendix 1.1. Randomized complete block design plot plan for the 2006 growing season, where H1 through H9 indicates Harvest number 1 through 9, and F70 and QT (Quatro) are fenugreek genotypes used in the trial.

601 4 F70	602 4 QT	603 4 CG	604 5 F70	605 2 QT	606 8 QT	607 7 QT	608 3 CG	609 9 F70	610 6 F70	611 6 CG	612 5 CG	613 2 F70	614 1 F70

501 6 F70	502 6 QT	503 8 CG	504 1 QT	505 3 QT	506 9 F70	507 5 CG	508 6 CG	509 2 F70	510 7 QT	511 1 CG	512 7 CG	513 9 QT	514 9 CG

401 7 QT	402 4 F70	403 1 F70	404 9 QT	405 6 F70	406 4 CG	407 5 QT	408 8 CG	409 8 F70	410 9 F70	411 8 QT	412 4 QT	413 3 CG	414 6 QT

… continued on next page

301 5 QT	302 1 F70	303 3 QT	304 6 F70	305 7 CG	306 2 CG	307 2 F70	308 9 CG	309 1 QT	310 3 F70	311 1 CG	312 9 QT	313 5 CG	314 7 F70

201 9 QT	202 6 CG	203 4 F70	204 2 F70	205 4 CG	206 2 QT	207 8 QT	208 3 CG	209 8 CG	210 3 F70	211 2 CG	212 9 CG	213 7 F70	214 6 QT

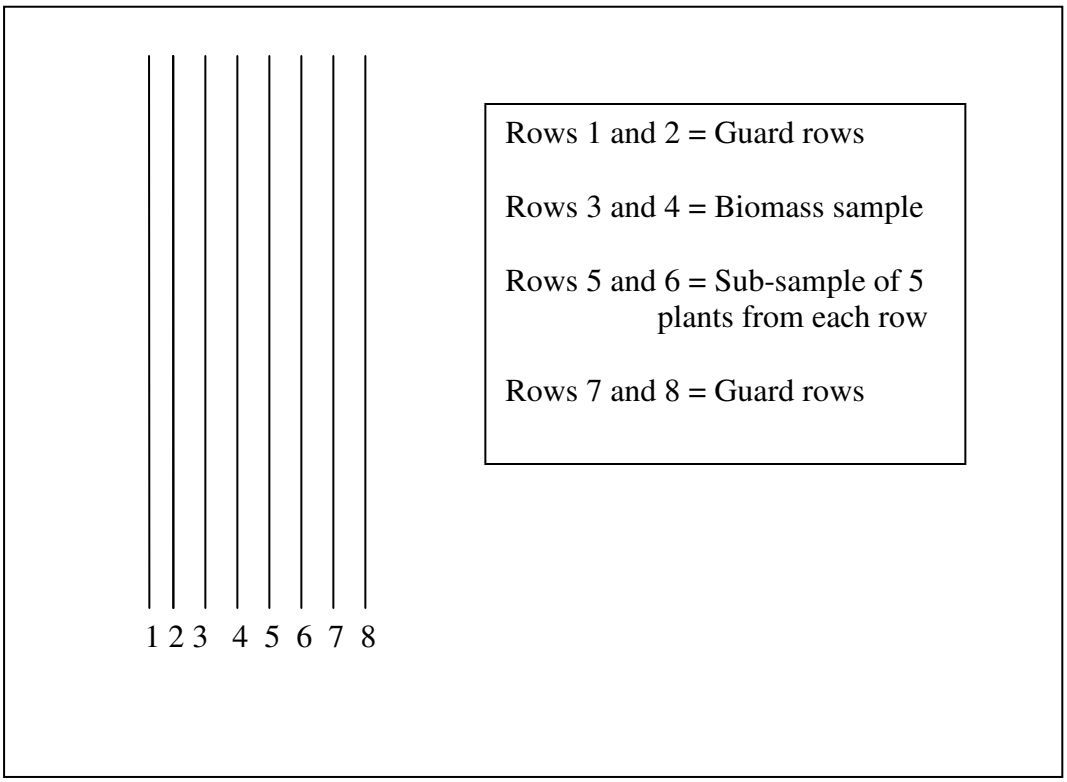
101 3 CG	102 8 CG	103 5 QT	104 9 F70	105 7 QT	106 2 F70	107 8 F70	108 1 F70	109 7 F70	110 3 QT	111 1 CG	112 9 CG	113 7 CG	114 6 CG

Appendix 1.2 – 1. Randomized complete block plot plan for the 2007 growing season, where H1 through H9 indicates Harvest number 1 through 9, and F70 and QT (Quatro) are fenugreek varieties used in the trial.

Continued from previous page …

615 3 QT	616 5 QT	617 7 F70	618 2 CG	619 6 QT	620 1 CG	621 8 F70	622 7 CG	623 9 CG	624 9 QT	625 8 CG	626 3 F70	627 1 QT
515 5 F70	516 3 F70	517 8 QT	518 7 F70	519 3 CG	520 4 F70	521 4 QT	522 4 CG	523 1 F70	524 2 CG	525 2 QT	526 8 F70	527 5 QT
415 2 QT	416 6 CG	417 5 F70	418 7 F70	419 5 CG	420 7 CG	421 1 QT	422 3 QT	423 9 CG	424 2 CG	425 3 F70	426 2 F70	427 1 CG
315 6 QT	316 5 F70	317 2 QT	318 6 CG	319 8 F70	320 8 QT	321 8 CG	322 4 CG	323 4 QT	324 3 CG	325 4 F70	326 7 QT	327 9 F70
215 5 CG	216 4 QT	217 6 F70	218 1 CG	219 1 QT	220 5 F70	221 7 CG	222 1 F70	223 7 QT	224 5 QT	225 3 QT	226 8 F70	227 9 F70
115 5 F70	116 3 F70	117 5 CG	118 4 F70	119 2 QT	120 6 F70	121 8 QT	122 2 CG	123 4 QT	124 4 CG	125 9 QT	126 1 QT	127 6 QT

Appendix 1.2 – 2. Randomized complete block plot plan for the 2007 growing season, where H1 through H9 indicates Harvest number 1 through 9, and F70 and QT (Quatro) are fenugreek varieties used in the trial.



Appendix 1.3. Representation of an individual fenugreek plot and the allocation of each row during the harvest procedure for fenugreek grown in 2006 and 2007 on the Edmonton Research Station, Edmonton, AB.

Appendix 2: Significance tables for effect of year and genotype on growth characteristics and plant quality of fenugreek grown in 2006 and 2007 on the Edmonton Research Station, Edmonton, AB

Appendix 2.1. Level of significance for the effect of year (2006 vs. 2007) on growth characteristics of fenugreek grown on the Edmonton Research Station, Edmonton, AB.

Days from seeding	Plant height	Biomass	Total plant weight	Root weight per plant	Stem weight per plant	Weight of leaves per plant	Weight of pod+seed per plant	Leaf to stem ratio
38	***	***	NS	*	NS	NS	--	NS
52	***	***	*	**	NS	*	**	NS
65	NS	*	**	***	***	***	NS	***
80	NS	NS	***	***	***	***	**	***
97	NS	***	***	NS	*	*	**	NS
115	**	NS	**	NS	NS	NS	*	NS

Days from seeding	% plant weight as root	% plant weight as stem	% plant weight as leaves	% plant weight as pod+seed	Leaf area index	Pod area index
38	*	NS	NS	--	*	--
52	NS	NS	NS	***	***	***
65	*	***	NS	***	***	NS
80	NS	***	NS	***	**	***
97	***	***	***	***	NS	***
115	***	***	***	**	NS	NS

NS = not significant $P > 0.05$; * = significant at $P < 0.05$; ** = significant at $P < 0.01$; *** = significant at $P < 0.001$; -- = data not taken at that time point.

Appendix 2.2. Level of significance for the effect of genotype (F70 vs. Quatro) on growth characteristics of fenugreek grown in 2006 on the Edmonton Research Station, Edmonton, AB.

Days from seeding	Plant height	Biomass	Total plant weight	Root weight per plant	Stem weight per plant	Weight of leaves per plant	Weight of pod+seed per plant	Leaf to stem ratio
24	*	**	*	*	*	*	--	**
38	NS	*	NS	NS	NS	NS	--	*
52	NS	**	NS	NS	NS	NS	*	NS
65	NS	**	NS	NS	NS	NS	**	NS
80	NS	NS	**	NS	**	***	*	NS
94	NS	NS	NS	NS	NS	NS	NS	NS
100	--	--	--	--	--	--	--	--
107	--	--	--	--	--	--	--	--
122	NS	NS	*	NS	NS	NS	*	NS
136	NS	NS	**	NS	*	NS	**	*

Days from seeding	% plant weight as root	% plant weight as stem	% plant weight as leaves	% plant weight as pod+seed	Leaf area index	Pod area index
24	NS	*	*	--	**	--
38	NS	*	**	--	**	--
52	NS	NS	NS	**	*	**
65	NS	*	**	**	**	**
80	*	**	NS	**	NS	NS
94	NS	NS	NS	NS	NS	NS
100	--	--	--	--	--	--
107	--	--	--	--	--	--
122	*	NS	NS	NS	NS	NS
136	*	*	*	**	*	NS

NS = not significant $P > 0.05$; * = significant at $P < 0.05$; ** = significant at $P < 0.01$; *** = significant at $P < 0.001$; -- = data not taken at that time point.

.Appendix 2.3. Level of significance for the effect of genotype (F70 vs. Quatro) on growth characteristics of fenugreek grown in 2007 on the Edmonton Research Station, Edmonton, AB.

Days from seeding	Plant height	Biomass	Total plant weight	Root weight per plant	Stem weight per plant	Weight of leaves per plant	Weight of pod+seed per plant	Leaf to stem ratio
24	--	--	--	--	--	--	--	--
38	NS	NS	NS	NS	NS	NS	--	NS
52	NS	NS	NS	NS	NS	NS	NS	NS
65	NS	*	NS	NS	NS	NS	NS	NS
80	*	*	NS	NS	*	*	NS	NS
94	--	--	--	--	--	--	--	--
100	NS	*	NS	*	*	*	NS	NS
107	NS	NS	NS	NS	NS	NS	NS	NS
122	--	--	--	--	--	--	--	--
136	--	--	--	--	--	--	--	--

Days from seeding	% plant weight as root	% plant weight as stem	% plant weight as leaves	% plant weight as pod+seed	Leaf area index	Pod area index
24	--	--	--	--	--	--
38	NS	NS	NS	--	NS	--
52	NS	NS	NS	NS	NS	NS
65	NS	**	NS	*	NS	*
80	*	*	NS	**	NS	NS
94	--	--	--	--	--	--
100	NS	*	*	*	NS	NS
107	NS	NS	NS	NS	NS	NS
122	--	--	--	--	--	--
136	--	--	--	--	--	-

NS = not significant $P > 0.05$; * = significant at $P < 0.05$; ** = significant at $P < 0.01$; *** = significant at $P < 0.001$; -- = data not taken at that time point.

Appendix 2.4. Level of significance for the effect of year (2006 vs. 2007) on quality parameters of fenugreek grown on the Edmonton Research Station, Edmonton, AB.

Days from seeding	NDF of stem	NDF of leaves	NDF of pod +seed	NDF of whole plant	ADF of stem	ADF of leaves	ADF of pod +seed	ADF of whole plant	CP of stem	CP of leaves	CP of pod +seed	CP of whole plant	RFV
38	--	--	--	*	--	--	--	*	NS	NS	--	NS	NS
52	**	NS	--	NS	*	*	--	NS	NS	NS	--	NS	NS
65	NS	*	*	NS	*	*	NS	NS	NS	*	NS	NS	NS
80	*	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
97	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
115	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*	*

NS = not significant $P > 0.05$; * = significant at $P < 0.05$; ** = significant at $P < 0.01$; *** = significant at $P < 0.001$; -- = data not taken at that time point.

Appendix 2.5. Level of significance for the effect of genotype (F70 vs. Quatro) on quality parameters of fenugreek grown on the Edmonton Research Station, Edmonton, AB, when data for both growing seasons (2006 and 2007) was pooled.

Days from seeding	NDF of stem	NDF of leaves	NDF of pod+seed	NDF of whole plant	ADF of stem	ADF of leaves	ADF of pod+seed	ADF of whole plant
38	--	--	--	NS	--	--	--	NS
52	NS	NS	--	NS	NS	NS	--	NS
65	NS	NS	*	NS	NS	NS	NS	NS
80	NS	*	NS	NS	NS	NS	NS	NS
97	NS	NS	NS	NS	NS	NS	*	NS
115	NS	NS	NS	NS	NS	NS	NS	NS

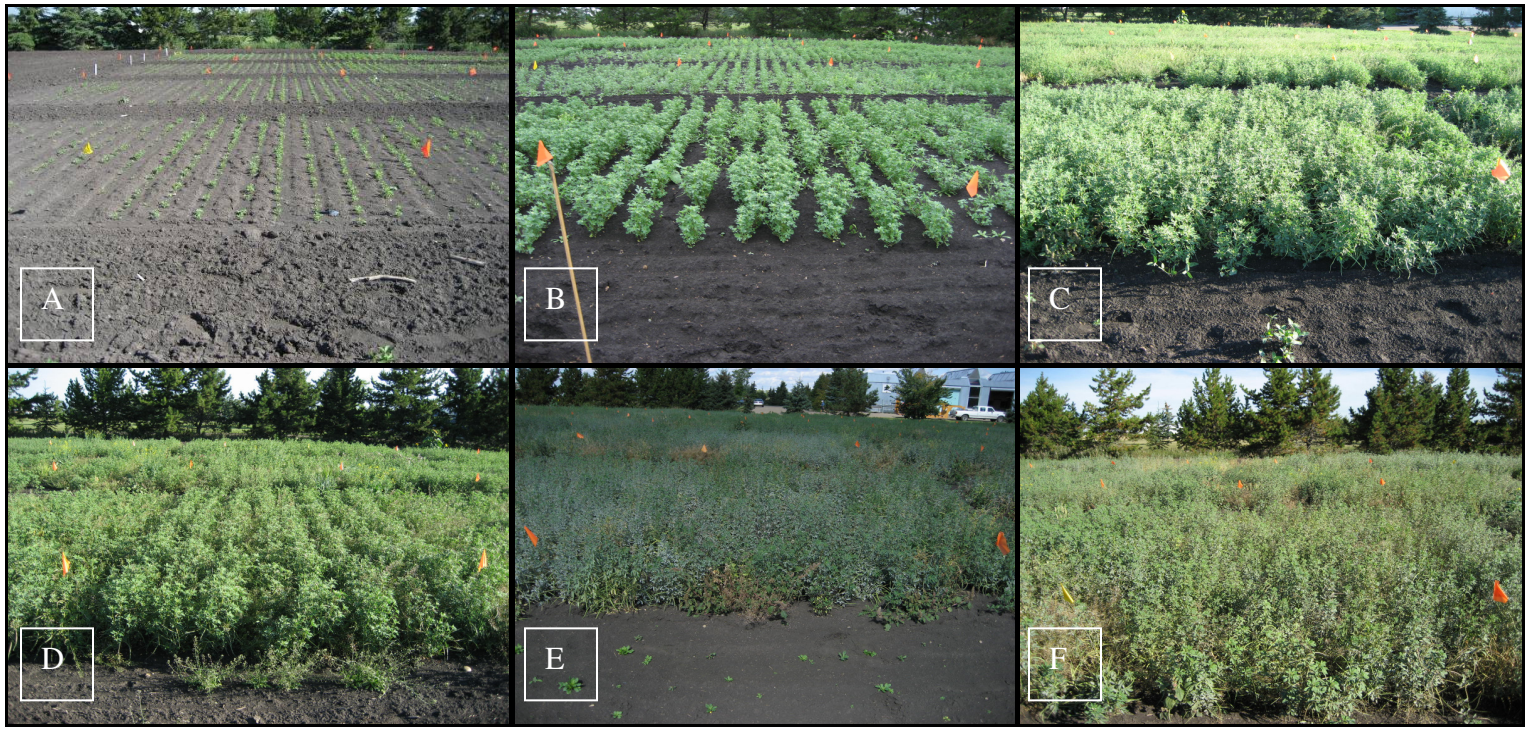
Days from seeding	CP of stem	CP of leaves	CP of pod+seed	CP of whole plant	RFV
38	NS	NS	--	NS	NS
52	*	NS	--	NS	NS
65	NS	NS	NS	NS	NS
80	NS	NS	NS	NS	NS
97	NS	NS	NS	NS	NS
115	NS	NS	NS	NS	NS

NS = not significant $P > 0.05$; * = significant at $P < 0.05$; ** = significant at $P < 0.01$; *** = significant at $P < 0.001$; -- = data not taken at that time point.

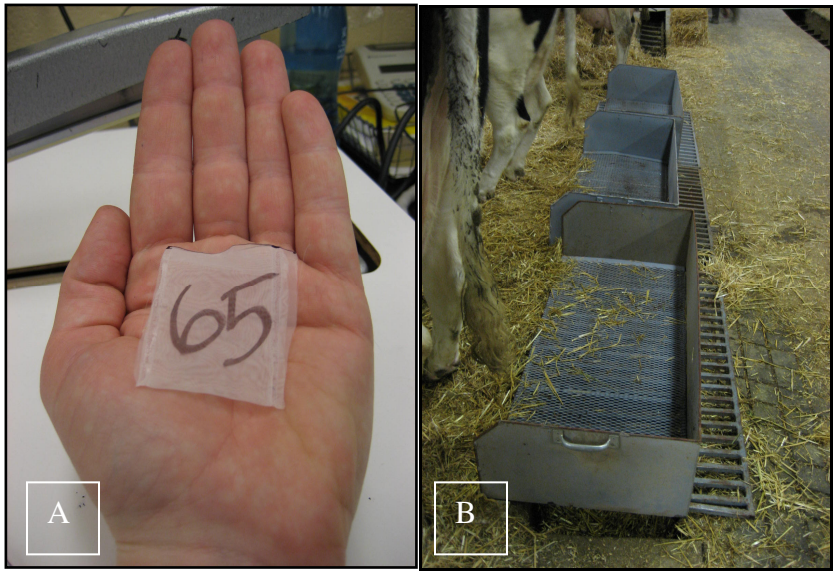
Appendix 3: Photographs of Research

Appendix 3.1. Quatro fenugreek grown for haylage on ERS in 2006.

Appendix 3.2. Fenugreek haylage harvest on W240 in 2006. A: Fenugreek swaths being baled. B: Fenugreek bale. C: Bales being wrapped.



Appendix 3.3. Photos of fenugreek growing in 2007. A: 38 days. B: 52 days. C: 65 days. D: 80 days. E: 100 days. F: 107 days.



Appendix 3.4. Materials used in the whole-tract digestion study. A: Rumen/duodenal nylon bag. B: Pans used to collect feces after putting nylon bags through the duodenal cannula.

Appendix 3.5. Materials used in the ruminal degradation study. A: Rumen bags with forage material. B: Mesh bags used to incubate nylon bags in the rumen. C: Bags being incubated in the rumen.