# PERFORMANCE OF DAIRY COWS FED SOYBEAN SILAGE

By

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## ABSTRACT

Master of Science Animal Science (Nutrition)

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**PERFORMANCE OF DAIRY COWS FED SOYBEAN SILAGE**

The objective of this research was to determine the feeding values of forage soybean (cv. Kodiak, full pod stage) silage to lactating dairy cows. Two diets with a 50:50 forage:concentrate ratio were formulated to meet nutrient requirements of dairy cows in early lactation. Soybean silage (SS) or a fourth cut alfalfa silage (AS) comprised 72% of the forage in each diet, with corn silage comprising the remaining 28%. Twenty Holsteins cows in early lactation were used in a Switchback design to determine the effects of dietary treatments on milk yield and milk composition. Four lactating cows fitted with ruminal cannulae were used in a Switchback design to determine effects of dietary treatments on ruminal fermentation and total tract nutrient digestibilities. Cows fed SS consumed less ($P < 0.05$) feed and produced less ($P < 0.05$) milk than cows fed AS. However, energy-corrected milk and milk efficiency were similar for both dietary treatments. Milk fat percentage and milk urea nitrogen were higher ($P < 0.05$) in milk of cows fed SS than in milk of cows fed AS. However, milk protein and lactose concentrations were similar for both dietary treatments. Ruminal pH and $NH_3$ N were lower ($P < 0.05$) in cows fed AS than in cows fed SS. However, total and molar proportions of volatile fatty acids were not influenced by dietary treatments. Total tract digestibility of DM, OM, CP, NDF, and GE were similar for dietary treatments. Results of the present study revealed the potential of forage

soybean silage as a forage source of dairy cows. More research is needed to determine the optimum stage of development at harvest to improve the nutritive value of soybean silage for dairy cows.

**RÉSUMÉ**

Maîtrise en science — Science animale (Nutrition)

Einar Vargas Bello Pérez

**ÉVALUATION DE LA PERFORMANCE DE VACHES LAITIÈRES ALIMENTÉES AVEC DE L'ENSILAGE DU SOYA**

Le but de cette recherche était de déterminer la valeur nutritive de l'ensilage de soya (cv. Kodiak, Gousses remplies) lorsque servit à des vaches laitières en lactation. Deux diètes avec un ratio fourrage : concentré 50 : 50 ont été formulées afin de rencontrer les besoins nutritifs de vaches laitières en début de lactation. De l'ensilage de soya (ES) ou de l'ensilage de quatrième coupe de luzerne (EL) représentait 72% du fourrage dans chacune de ces diètes, alors que le 28% résiduel était comblé par de l'ensilage de mais. Vingt vaches de race Holstein en début de lactation ont été utilisées sous un plan de permutation de traitements afin de déterminer les effets des deux différentes diètes sur la production et la composition du lait. Toujours à l'aide d'un plan de permutation de traitements, quatre vaches munies d'une fistule ruminale permanente ont été utilisées afin de déterminer les effets des deux différentes diètes sur la fermentation ruminale et la digestibilité totale des nutriments. Les vaches recevant une diète ES avaient une consommation volontaire moindre ($P < 0.05$) et produisaient moins de lait ($P < 0.05$) que celles recevant une diète EL. Toutefois, les valeurs du lait corrigé et d'efficacité laitière étaient comparables pour les deux diètes. Le pourcentage de gras du lait et l'urée du lait des vaches recevant une diète ES étaient plus élevés ($P < 0.05$) que ceux des vaches recevant une diète EL. Toutefois, les pourcentages de protéine et de lactose du lait étaient similaires pour les deux diètes. Le pH et le $NH_3$ N au niveau du rumen étaient plus bas ($P < 0.05$) pour les vaches recevant une diète EL que celles recevant une diète ES. Le type de diète n'a pas eu d'influence sur les proportions totales et molaires des acides gras volatils. Des valeurs semblables ont été observées en ce qui concerne la digestibilité totale de la M.S, de la M.O, de la P.B, de la fibre NDF et de l'E.T. Les résultats de cette étude démontrent le potentiel possible d'utiliser l'ensilage de soya comme source de fourrage dans l'alimentation des vaches laitières. D'autres recherches sont nécessaires afin de déterminer le stade de maturité optimal de la fève de soya au moment de l'ensilage pour améliorer la valeur nutritive de celle-ci lorsque servit sous forme d'ensilage aux vaches laitières.

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## Contribution of Authors

In accordance to McGill thesis submission guidelines, this is a manuscrip-based thesis and includes a table of contents, a brief abstract in both English and French, an introduction, a comprehensive review of literature, a final conclusion, and a summary, a thorough bibliography and appendices where appropriate.

This manuscript was co-authored by Dr. Arif Mustafa who contributed to experimental design of the study and proof reading of the manuscript.

A mi familia,

Sin su continuo apoyo y amor jamás hubiera llegado a ésta meta

To my parents,

Without your love and support, I would have never reached this goal

## TABLE OF CONTENTS



**SECTIONS**

## LIST OF TABLES

## LIST OF FIGURES

SECTION 1

# INTRODUCTION

## 1. Introduction

Annual legumes can be used as emergency crops when stands of perennial legumes have been winterkilled (Carrauthers et al., 2000; Sheaffer et al., 2001). However, their use as forages is currently very limited and in most cases restricted to situations where climatic conditions may have compromised grain production. Previous studies have shown that annual legumes, such as peas, soybeans and faba beans, can be used to produce good quality silages (Mustafa et al., 2000, 2002, 2003a; Fraser et al., 2004).

Soybean is an annual legume that can be grown as either a cold- or a warm-season crop (Martin et al., 1990; Mislevy et al., 2005). When using management practices for forage production, quality of soybean as forage was equivalent to that of alfalfa (Hintz et al., 1992). Moreover, forage soybean also contains higher levels of protein than many other types of forage and possesses nitrogen fixation capability (Redfearn et al., 1999). Another advantage of using forage soybean is the flexibility of harvest dates, since its quality is good over a long period (Blount et al., 2006). New high-yielding forage cultivars of soybean have recently been developed (Devine and Hatley, 1998; Devine et al., 1998 a,b; Undersander, 2001). Forage soybean could be potentially of higher forage quality if it is grown to R6 (full seed) or R7 (beginning maturity) when crude protein increases and acid and neutral detergent fiber concentrations decrease due to the nutrient accumulation in pods and beans (Seiter et al., 2004). Higher CP and lower fiber levels would be desirable to achieve a nutritional value comparable to alfalfa cut at bud or early bloom growth stage (Frame et al., 1998). However, data regarding their feeding values for dairy cows are not available.

Therefore, the major objectives of this research were:

- To determine the effects of feeding forage soybean silage to dairy cows on:
    - Milk yield and milk composition
    - Ruminal fermentation parameters
    - Total tract nutrient utilization

The hypothesis of this research was:

- Soybean silage can replace alfalfa silage as the major legume forage in dairy cow diets.

SECTION 2

# LITERATURE REVIEW

## 2. Literature Review

### 2.1 Importance of legume forages

Generally, forages can be divided into legume and grass forages. In Canada, corn and barley silages are the most common grass/cereal silages while alfalfa and red clover are the main legume silages (Reid et al., 1990; Khorasani et al., 1993; Mustafa et al., 2000, 2003). A major advantage of legume over grass forages is their ability to fix atmospheric nitrogen. Legumes are also higher in CP content than grasses (Albrecht and Beauchemin, 2003; Al-Mabruk et al., 2004). Moreover, it has been shown that legumes have higher ruminal DM and CP degradabilities and relativelyfaster degradation rate of slowly degradable NDF fraction than grasses (Hoffman et al., 1993; Andrighetto et al., 1993).

### 2.2 Legumes versus grasses

Several studies have compared the performance of dairy cows fed legume forages with that of cows fed perennial grass forages and showed that legumes are superior to grasses. This is mainly due to a greater DM intake of legume silages than grass silages (Jorgensen, 1985; Waldo, 1985; Van Soest, 1995; Hoffman et al., 1998; Albrecht and Beauchemin, 2003) (Table 2.1). Dewhurst et al. (2003) compared grass (i.e. ryegrass) and legume silages (i.e. red clover and white clover) for milk production; they found that legume silages increased DM intake and milk production in comparison with grass silage. They also found a significant increase in rumen ammonia concentration with the legume silages, reflecting their higher protein content. Rumen fill was lower, and rumen passage rates were higher for cows offered legume silages than those fed grass silages.

Al-Mabruk et al. (2004) reported that cows fed red clover silage consumed more forage and produced more milk than cows fed grass silage (i.e. ryegrass). It is important to mention that milk protein percentage was higher for cows fed legume silage than for those fed grass silage (Table 2.1).

**Table 2.1-** Lactation performance of cows fed legume and grass silages

| | Silages | | Reference |
|---|---|---|---|
| | Timothy | Alfalfa | |
| Dry matter intake, kg $d^{-1}$ | 17.1 | 18.0 | |
| Milk yield, kg $d^{-1}$ | 23.9 | 23.3 | Orozco-Hernández et al. (1997) |
| Fat, % | 4.59 | 4.39 | |
| Protein, % | 3.56 | 3.54 | |
| Lactose, % | 4.56 | 4.63 | |
| | Ryegrass | Alfalfa | |
| Dry matter intake, kg $d^{-1}$ | 20.3b | 22.5a | |
| Milk yield, kg $d^{-1}$ | 30.2b | 31.8a | Hoffman et al. (1998) |
| Fat, % | 3.76 | 3.61 | |
| Protein, % | 2.93 | 2.96 | |
| | Ryegrass | Alfalfa | |
| Dry matter intake, kg $d^{-1}$ | 16.8b | 25.2a | |
| Milk yield, kg $d^{-1}$ | 35.6 | 41.1 | Broderick et al. (2002) |
| Fat, % | 2.80 | 3.08 | |
| Protein, % | 3.16 | 3.31 | |
| Lactose, % | 4.76b | 4.93a | |
| | Ryegrass | White clover | |
| Dry matter intake, kg $d^{-1}$ | 18.2b | 19.8a | |
| Milk yield, kg $d^{-1}$ | 24.9b | 31.5a | Dewhurst et al. (2003) |
| Fat, % | 4.45 | 4.39 | |
| Protein, % | 3.26 | 3.20 | |
| Lactose, % | 4.71 | 4.71 | |
| | Orchardgrass | Alfalfa | |
| Dry matter intake, kg $d^{-1}$ | 27.0 | 25.5 | |
| Milk yield, kg $d^{-1}$ | 40.4 | 40.5 | Cherney et al. (2004) |
| Fat, % | 3.41 | 3.70 | |
| Protein, % | 3.15 | 3.06 | |
| Lactose, % | 4.78 | 4.81 | |
| | Ryegrass | Red clover | |
| Dry matter intake, kg $d^{-1}$ | 15.9b | 19.4a | |
| Milk yield, kg $d^{-1}$ | 23.1b | 24.9a | Al-Mabruk et al. (2004) |
| Fat, % | 3.68 | 3.78 | |
| Protein, % | 2.90b | 2.94a | |
| Lactose, % | 4.56 | 4.55 | |

a, b Means within row followed by different letters are different (*$P<0.05$*)

There are some nutritional differences between grass and legume silages. This can be attributed to the higher CP and often lower NDF contents that legume silages have compared with grass silages (Christensen, 1991; Khorasani et al., 1993; Mustafa et al., 2000).

Grasses are characterized by greater concentrations of cell wall materials than legumes. Grasses also have greater extents but slower rates of wall digestion compared with legumes (Mertens and Ely, 1979; Harrison et al., 2003). Perennial forage legumes usually have greater CP concentrations than annual and perennial grasses. Alfalfa silage has higher CP concentration than barley silage (199 vs 124 g $kg^{-1}$ respectively); alfalfa rarely contains more than 460 g $kg^{-1}$ NDF whereas barley silage NDF ranges from 520 g $kg^{-1}$ to 580 g $kg^{-1}$ (Khorasani et al., 1993). These differences usually affect ruminal and total tract digestibility and may alter DM intake and cow performance (Orozco-Hernández et al., 1997; Broderick et al., 2002).

When dairy cattle are fed both legume and grass silage diets, apparent total tract digestibility of NDF from legumes is lower than that from grasses (Glenn et al., 1989; Holden et al., 1994). Thus the contribution of digestible NDF to digestible DM is often lower for legumes than for grasses (Glenn et al., 1993). It has been shown that fiber digestibility by dairy cows is lower for diets based on alfalfa than on grass silages (Weiss and Shockey, 1991). Similarly, Broderick et al. (2002) reported lower NDF (436 vs 662 g $kg^{-1}$ respectively) and ADF (426 vs 647 g $kg^{-1}$ respectively) digestibilities for cows fed alfalfa silage compared with those fed rye grass silage. However, apparent digestibility of the digestible fraction of ADF was higher for those cows fed alfalfa silages than those fed rye grass silage (961 vs 914 g $kg^{-1}$ respectively). The authors indicated that this is likely due to a faster microbial attack of digestible fiber in the rumen despite higher intakes and presumably greater rates of passage. Possibly the greater DM intake for those cows fed alfalfa silage was mainly due to a more rapid fiber and particle breakdown in the rumen than for those cows fed rye grass silage.

## 2.3 Performance of animals fed legume silages

Limited studies have been conducted to compare the feeding value of legume silages to that of cereal silages.

### 2.3.1 Studies with alfalfa silage

Performance of dairy cows fed alfalfa or orchardgrass silage was investigated by Weiss and Shockey (1991), and Holden et al. (1994). In both studies, cows fed alfalfa silage had a greater DM intake (average = 21.5 vs 18.9 kg $d^{-1}$ respectively) and milk yield (average = 29.3 vs 26.0 kg $d^{-1}$ respectively) than those fed orchardgrass silage. Milk composition was not affected by dietary treatments in both studies. Broderick et al. (2002) reported higher DM intake and milk yield and greater concentrations of milk fat, protein and lactose for cows fed alfalfa silage compared with those fed ryegrass silage. However, Orozco-Hernández et al. (1997) observed similar DM intake for those cows fed alfalfa silage compare with those fed timothy grass silage.

In a recent performance study Cherney et al., (2004) compared grass (i.e. orchardgrass and fescue grass silages) and legume (i.e. alfalfa silage) silages. Cows fed alfalfa silage had similar DM intake (25.5 vs 27.0 and 26.8 kg $d^{-1}$ respectively) and milk production (40.5 vs 40.4 and 40.4 kg $d^{-1}$ respectively) as those fed first-cutting grass silages. However, cows fed second-cutting grass-based TMR, had lower DM intake (25.5 vs 22.3 and 22.7 kg $d^{-1}$ respectively) and milk production (40.5 vs 34.4 and 36.9 kg $d^{-1}$ respectively) than cows fed first-cutting grass. The differences in DM intake and milk production were attributed to differences in fiber digestibility and indigestible residue, resulting from lignin differences, as well as to differences in non-structural carbohydrates and to higher NDF of grass. The study demonstrated that dairy cows fed either fescue or orchardgrass silage would perform as well as those cows fed alfalfa silage, provided that the forage is of adequate CP, NDF, and maturity stage.

### 2.3.2 Alfalfa versus corn silage

In North America, alfalfa silage and corn silage are the most common ensiled forages fed to ruminants (Allen et al., 2003). Similar milk production usually results from cows fed alfalfa or corn silages when diets are properly balanced

(Broderick, 1985; Colenbrander et al., 1986; Dhiman and Satter, 1994, 1997; Albrecht and Beauchemin, 2003; Buxton et al., 2003).

Borton et al. (1997) found that alfalfa as the sole forage source for dairy diets produced large amounts of N excreted in manure, whereas diets including corn silage dramatically decreased the amount of N excreted. Alfalfa and corn silages are complementary forages due to their characteristics; alfalfa silage is high in both CP and rumen degradable protein and low in rumen undegradable protein, on the other hand, corn silage is a good source of ruminally fermentable carbohydrates but is low in CP (Brito and Broderick, 2006). Technically speaking, alfalfa and corn silages are also complementary due to the fact that both fit well into crop rotations for managing N, and each has different harvest schedule, which reduces labor and equipment costs (Dhiman and Satter, 1997). Combinations of both forages as one-third to two-thirds of the total forage are recommended to improve management of crops, manure disposal, and distribution of labor requirements (Dhiman and Satter, 1997; Allen et al., 2003).

Several studies have reported similar milk yield when alfalfa and corn silages were fed (Table 2.2) (Broderick, 1985; Onetti et al., 2002; Krause and Combs, 2003; Ruppert et al., 2003). However, Broderick (1985) reported a significant reduction in milk yield from 30.3 to 28.0 kg $d^{-1}$ when corn silage was increased from 60 to 76% of DM. Conversely, Wattiaux and Karg (2004) reported greater milk yield for those cows fed higher corn silage diets (49.0 kg $d^{-1}$) than those fed higher alfalfa diets (46.4 kg $d^{-1}$). Regarding to DM intake, various studies (Onetti et al., 2002; Krause and Combs, 2003; Ruppert et al., 2003; Wattiaux and Karg, 2004; Brito and Broderick, 2006) reported that intake of DM decreases as corn silage ratio increases in the diets compared to that of alfalfa silage diets.

**Table 2.2 -** Lactation performance of cows fed alfalfa and corn silages

| | Silage | | References |
|---|---|---|---|
| | Corn | Alfalfa | |
| Dry matter intake, kg $d^{-1}$ | 23.1 | 24.7 | Onetti et al. (2000) |
| Milk, kg $d^{-1}$ | 35.2 | 36.2 | |
| Fat, % | 3.11 | 3.32 | |
| Protein, % | 3.34 | 3.30 | |
| Lactose, % | ND[1] | ND | |
| Dry matter intake, kg $d^{-1}$ | 22.6 | 24.8 | Ruppert et al. (2003) |
| Milk, kg $d^{-1}$ | 32.3 | 33.3 | |
| Fat, % | 3.18 | 3.39 | |
| Protein, % | 3.11 | 3.14 | |
| Lactose, % | ND | ND | |
| Dry matter intake, kg $d^{-1}$ | 24.2 | 24.4 | Wattiaux and Karg (2004) |
| Milk, kg $d^{-1}$ | 49.3 | 46.1 | |
| Fat, % | 3.03 | 3.59 | |
| Protein, % | ND | ND | |
| Lactose, % | 4.11 | 4.11 | |
| Dry matter intake, kg $d^{-1}$ | 22.0 | 22.6 | Krause and Combs (2003) |
| Milk, kg $d^{-1}$ | 42.0 | 42.7 | |
| Fat, % | 2.89 | 3.38 | |
| Protein, % | 2.89 | 2.83 | |
| Lactose, % | 4.89 | 4.88 | |
| Dry matter intake, kg $d^{-1}$ | 23.7b | 26.8a | Brito and Broderick (2006) |
| Milk, kg $d^{-1}$ | 39.5b | 41.5a | |
| Fat, % | 3.34b | 3.81a | |
| Protein, % | 3.17a | 3.07b | |
| Lactose, % | 4.84 | 4.88 | |

[1]Not detected. a,b Means within row followed by different letters are different (*$P<0.05$*)

## 2.4 Importance of annual legumes

Annual legumes can offer several advantages such as being a potential alternative when stands of perennial legumes have been winterkilled. They can also be intercropped with annual cereals such as corn or barley to increase forage yield and CP content and reduce the use of chemical fertilizers (Carrauthers et al., 2000; Sheaffer et al., 2001). Furthermore, some annual legumes such as peas and faba beans are characterized by their unique nutrient composition as they can serve as sources of both starch and CP (Mustafa et al., 2000, 2002, 2003a; Fraser et al., 2004). Annual legumes can also provide more diversified agro-ecosystems and flexibility in crop rotations (Mustafa et al., 2003a).

In general annual legumes are high in CP content. However, some annual legumes such as peas and faba bean also contain moderate level of starch (Mustafa et al., 2000; 2003a). Examples of annual legumes in the literature include peas, faba bean, red clover, berseem clover, chickpea, black lentil, chickling vetch, winter vetch, hairy vetch, Persian clover, crimson clover and arrowleaf clover (Kunelius and Narasimhalu, 1983; Bjorge and Bjorge, 1988; Westcott et al., 1991; Martin, 1993; Panciera and Sparrow, 1995; Biederbeck et al., 1996; Thompson and Stout, 1997; Altinok et al., 1997; Ross et al., 2001; Fraser et al., 2004; McCartney et al., 2004).

### 2.4.1 Ensiling characteristics of annual legumes

Several studies have shown that annual legumes can easily be ensiled (Mustafa et al., 2000, 2002, 2005; Mustafa and Seguin, 2003a, b). This was evident by the rapid drop in pH and the sharp increase in lactic acid production within a few days of ensiling.

### 2.4.2 Chemical composition of annual legumes

In general, the drop in pH during ensiling is faster in grass than legume silages. This is mainly due to a higher buffering capacity of legume than grass forages (Broderick et al., 2002; Dewhurst et al., 2003; Cherney et al., 2004). The annual legume silages are a good source of CP and NDF (Table 2.3). Fraser et al. (2004) evaluated the forage quality of annual legumes in southern Alberta and northeast Saskatchewan. They found that in general, CP, ADF and

NDF content for most annual legumes was >15 g $kg^{-1}$, <35 g $kg^{-1}$ and <45 g $kg^{-1}$ respectively.

**Table 2.3 -** Chemical composition of annual legumes (g $kg^{-1}$)

| | Silages | | | | |
|---|---|---|---|---|---|
| | Pea | Faba bean | Soybean | Berseem clover | Red clover |
| Dry matter | 250 | 261 | 257 | 290 | 211 |
| Crude protein | 178 | 222 | 197 | 217 | 202 |
| Neutral detergent fiber | 416 | 428 | 420 | 507 | 505 |
| Acid detergent fiber | 312 | 313 | 292 | 505 | 370 |

Adapted from Mustafa and Seguin (2003a, b); Al-Mabruk et al. (2004)

### 2.4.3 *In situ* ruminal degradability of annual legumes

Ruminal degradability of annual legumes is affected by their chemical composition, type of cultivar, preservation method and the interactions between those factors (Seguin and Mustafa, 2003). The *in situ* degradability will vary between different annual legume silages depending on their soluble fraction, slowly degradable fraction and rate of degradation in the rumen (Mustafa et al., 2000; Mustafa and Seguin, 2003a, b) (Table 2.4). An earlier study demonstrated that annual legumes have higher ruminal nutrient degradabilities when compared with grass silages (Table 2.5). Hoffman et al. (1993) also showed that legumes (i.e. alfalfa hay) have higher ruminal DM degradability than grasses. Generally, ensiled forages have high ruminal CP degradability (Wattiaux et al., 1994; Makoni et al., 1994; Mustafa et al., 2000). According to Griffin et al. (1994) forage maturity at harvest may also influence CP degradability. Likewise, Hoffman et al. (1993) found that ruminal degradability of protein decreased with advancing maturity of alfalfa, red clover (annual legume) and birdsfoot trefoil. Higher rates of ruminal NDF degradability have been reported for legumes compared with grasses (Andrighetto et al., 1993, Mustafa et al., 2000). Similarly, Varga and Hoover (1983) indicated that NDF of legumes is degraded at a faster rate than grasses NDF. Varga et al. (1998) reported that the slowly degradable NDF fraction of alfalfa is more degradable than that of grasses by lactating cows in early lactation.

### 2.4.4 Digestibility of annual legumes

Relative to conventional legume silages (i.e. alfalfa silage), annual legume silages (i.e. berseem clover) have lower total tract DM digestibility for dairy cows (Table 2.6). Whole-tract CP digestibility of annual legumes will depend on the complete utilization of the dietary protein and the level of ruminal undegraded protein that passes to the small intestine (Mustafa and Seguin, 2003b). Ruminal undegraded true protein that is not digested in the small intestine will be excreted in the feces (Sniffen et al., 1992). The digestibility of NDF of annual legumes is negatively correlated with ADL content (Jung et al., 1997; Mustafa and Seguin, 2003b). Unlike the fibrous fractions that are fermented more slowly and retained in the rumen longer, forages with high NDF digestibility are degraded more rapidly in the rumen which will allow for faster passage and disappearance from the rumen (Oba and Allen, 2000). In addition, the improvement of NDF digestibility is correlated with the increase in milk yield (Oba and Allen, 1999).

**Table 2.4 -** Ruminal nutrient kinetic parameters and effective degradability of annual legumes

| | Annual legume silages | | | |
|---|---|---|---|---|
| | Faba bean | Soybean | Pea | Berseem clover |
| Dry matter, DM | | | | |
| Soluble fraction, g $kg^{-1}$ of DM | 448 | 450 | 478 | 328 |
| Degradable fraction, g $kg^{-1}$ of DM | 368 | 373 | 318 | 477 |
| Degradation rate, % $h^{-1}$ | 7.0 | 8.7 | 8.9 | 5.9 |
| Lag time, h | 0.1 | 1.1 | 1.2 | 0.4 |
| Effective degradability, g $kg^{-1}$ | 662 | 685 | 691 | 588 |
| Crude protein, CP | | | | |
| Soluble fraction, g $kg^{-1}$ of CP | 494 | 594 | 703 | 461 |
| Degradable fraction, g $kg^{-1}$ of CP | 415 | 320 | 220 | 455 |
| Degradation rate, % $h^{-1}$ | 13.2 | 12.6 | 7.7 | 6.8 |
| Lag time, h | 0.4 | 0.2 | 0.6 | 0.8 |
| Effective degradability, g $kg^{-1}$ | 794 | 822 | 836 | 723 |
| Neutral detergent fiber, NDF | | | | |
| Soluble fraction, g $kg^{-1}$ of NDF | 104 | 101 | 99 | 27 |
| Degradable fraction, g $kg^{-1}$ of NDF | 472 | 470 | 533 | 681 |
| Degradation rate, % $h^{-1}$ | 5.1 | 5.1 | 5.5 | 3.9 |
| Lag time, h | 0.6 | 0.1 | 0.7 | 0.2 |
| Effective degradability, g $kg^{-1}$ | 342 | 355 | 345 | 323 |

Adapted from Mustafa and Seguin (2003a, b)

### 2.4.5 Performance of animals fed annual legumes

#### 2.4.5.1 Dry matter intake

Several studies have shown that feeding annual legume silages improved DM intake by ruminants. Sheep fed berseem clover silage consumed more DM than those fed alfalfa silage (Mustafa and Seguin, 2003b) (Table 2.6). Similar findings were also reported for dairy cows fed pea silage relative to those fed alfalfa silage (28.6 vs 27.5 kg $d^{-1}$ respectively) (Mustafa et al., 2000). Dairy cows fed red clover also consumed more DM than cows fed grass silage

(Dewhurst et al., 2003; Al-Mabrulk et al., 2004). The DM intake from those studies ranged between 19.4 and 20.3 kg $d^{-1}$.

**2.4.5.2 Effects of annual legumes on milk yield and composition**

Earlier studies have established the milk production potential of annual legume silages in comparison with grass silages (Castle et al., 1983; Thomas et al., 1985; Hazard et al., 2001; Dewhurst et al., 2003; Al-Mabruk et al., 2004). Annual legume silages can replace conventional forages in dairy diets. When cows are fed with a diet containing peas, the milk fat percentage increases for early, mid and late lactation cows (Corbett et al., 1995; Mustafa et al., 2000; Khorasani et al., 2001). Also, pea silage can replace alfalfa silage without a detrimental effect on the milk yield and milk composition when fed to dairy cows (Mustafa et al., 2000) (Table 2.7). Dewhurst et al. (2003) reported higher milk yield for cows fed red clover silage than those fed grass silage. Milk fat, protein and lactose concentrations were higher in cows fed red clover than those fed grass silage. It was also shown that red clover can lead to some improvement in the saturated fatty acid content of milk. Previous studies have also highlighted the superiority of red clover silage over alfalfa silage in relation to milk yield (Hoffman et al., 1997; Broderick et al., 2001). Al-Mabruk et al. (2004) also demonstrated that cows fed red clover silage had higher milk yield as well higher concentrations of milk protein and fat when compared with grass silage. It was also observed that milk from cows fed legume silages, generally contains higher levels of polyunsaturated fatty acids (linoleic acid, conjugated linoleic acid and α-linoleic acid) that are regarded as beneficial for human health (Dewhurst et al., 2003).

**Table 2.5 -** Effective degradability of pea, alfalfa and barley silages (g $kg^{-1}$)

| | Silage | | |
|---|---|---|---|
| | Pea | Alfalfa | Barley |
| Dry matter | 670a | 664a | 539b |
| Crude protein | 889a | 879b | 821c |
| Neutral detergent fiber | 235b | 270a | 219c |
| Starch | 863b | ND[1] | 974a |

[1]Not determined. a, b, c Means in the same row with different superscripts are different (*P*< *0.05*). Adapted from Mustafa et al. (2000)

**Table 2.6 -** Intake and nutrient utilization of berseem clover silage by sheep

| | Silage | |
|---|---|---|
| | Berseem clover | Alfalfa |
| Dry matter intake, g $d^{-1}$ | 812a | 653b |
| Digestibility coefficient, g $kg^{-1}$ | | |
| Dry matter | 707a | 660b |
| Organic matter | 715a | 659b |
| Crude protein | 692b | 759a |
| Neutral detergent fiber | 656a | 545b |
| Acid detergent fiber | 646a | 546b |
| Gross energy | 696a | 640b |

a, b Means within row followed by different letters are different (*P*< *0.05*). Adapted from Mustafa and Seguin (2003b)

**Table 2.7 -** Effect of feeding pea, alfalfa or barley silage on milk yield and milk composition of dairy cows

| | Silage | | |
|---|---|---|---|
| | Pea | Alfalfa | Barley |
| Milk Yield | | | |
| Total, kg $d^{-1}$ | 45.2 | 45.3 | 43.2 |
| Milk composition | | | |
| Fat, % | 3.65 | 3.36 | 3.65 |
| Protein, % | 3.03 | 3.16 | 3.08 |
| Lactose, % | 4.50 | 4.48 | 4.51 |
| Total solids, % | 12.24 | 12.14 | 12.34 |

Adapted from Mustafa et al. (2000)

## 2.5 History of soybean

Soybean (*Glycine* max) has been an important feed ingredient for ruminants not only as a grain but also as a forage crop. Soybean was introduced in North America in the 1800s and was initially promoted as a forage crop (Arny, 1926; Good, 1942). The interest in soybean has shifted through the last century; from forage to grain production. However, in some areas, soybean continue to be used as an alternative forage source when there is a shortage of forage from other sources or when frost damage limits grain harvest (Sheaffer et al., 2001). In the 1960s and 1970s when the value of oilseed increased, soybean production shifted almost exclusively to grain cultivars. In Ontario, Canada soybean was first introduced as a hay crop in 1893 but since World War II most of its production shifted to oil extraction and meal production (Upfold and Olechowski, 1988).

## 2.6 Forage soybean

Due to several agronomical features, soybean has a great potential as a forage crop. Soybean can be grown as either as a cold- or a warm-season crop (Martin et al., 1990; Mislevy et al., 2005). Soybean can tolerate high temperatures and various moisture soil conditions. Furthermore, soybean can be a good substitute for cereals such as sorghum crops under stressful climatic conditions (Mislevy et al., 2005). Forage soybean also contains higher levels of protein than many other types of forage and possesses nitrogen fixation capability (Redfearn et al., 1999). Moreover, when using management practices for forage production, quality of soybean as forage is equivalent to that of alfalfa (Hintz et al., 1992). Another advantage of using forage soybean is the flexibility of harvest dates, since its quality is good over a long period (Blount et al., 2006).

Stage of maturity at harvest has a significant effect on the chemical composition of soybean plants. Hintz and Albrecht (1994) demonstrated that as leaves and stems mature the concentrations of NDF, ADF and ADL increase while CP concentration declines. However, the pod component showed an opposite trend with a decrease in the concentrations of NDF, ADF and ADL, and an increase in CP concentration. Hintz et al., (1992) found that despite similar CP, NDF, ADF and ADL to alfalfa at the same stage of maturity (i.e. R7).

Differences in digestibility of forage soybean in relation to stage of development are minimal. In vitro DM digestibility of forage soybean at 50% flowering was 590 g $kg^{-1}$ while the corresponding values at 90% pod fill was 610 g $kg^{-1}$ (Minson et al., 1993; Sollenberger et al., 2003). Forage soybean cultivars selected for late maturity could have 590 to 640 g $kg^{-1}$ in vitro OM digestibility and 150 to 180 g $kg^{-1}$ CP (Sollenberger et al., 2003). Similar results were reported for forage soybean (cv. Tyrone) where in vitro OM digestibility ranged from 470 g $kg^{-1}$ to 600 g $kg^{-1}$ and CP concentration from 114 to 189 g $kg^{-1}$ (Mislevy et al., 2005).

Highest quality of forage soybean can be achieved when forages are harvested at R6 (full seed) or R7 (beginning maturity) when CP increases and ADF and NDF concentrations decrease due to the nutrient accumulation in pods and beans (Seiter et al., 2004). Higher CP and lower fiber levels would be desirable to achieve a nutritional value comparable to alfalfa cut at the bud or early bloom growth stage (Frame et al., 1998).

### 2.6.1 Differences in composition between forage and seed soybean cultivars

Recently, several cultivars of forage soybean have been developed. Examples of these cultivars include Tyrone, Derry and Donegal (Table 2.8) (Darmosarkoro et al., 2001). Forage soybean cultivars produce more leaf and stem and less pod yield than grain cultivars; therefore forage cultivars yield greater vegetative growth and higher total DM than grain cultivars (Darmosarkoro et al., 2001). Relative to grain cultivars, forage cultivars Derry, Donegal and Tyrone have greater leaf and stem biomass. However, among those cultivars, Tyrone has greater leaf and stem yields (Rao et al., 2005). Research data regarding morphological and agronomical characteristics, yield and quality are needed to help increasing forage soybean production (Redfearn et al., 1999; Altinok et al., 2004).

Factors affecting quality of soybean as a forage include maturity stage and changes in the proportions of leaf, stem and pod of the forage as well as the seed proportion of the pod fraction (Sheaffer et al., 2001). Usually, leaves from forage soybean cultivars have higher CP concentrations than grain cultivars. This has been attributed to the fact that forage soybean does not have significant seed production (Sheaffer et al., 2001). According to Hanway and Weber (1971), the N is translocated from leaves to grain during grain formation. Pods from forage soybean cultivars have lower CP and higher ADF and NDF concentrations than pods from grain cultivars, likely due to a greater proportion of high quality seeds in the more mature grain cultivars (Sheaffer et al., 2001).

Sheaffer et al. (2001) reported higher CP (218 vs 146 g $kg^{-1}$) and lower NDF (400 vs 523 g $kg^{-1}$) concentrations for grain soybean than forage cultivars. The authors attributed this effect to the fact that grain soybean was more mature and had greater pod proportion than forage soybean. Recently, Seiter et al. (2004) observed that ADF, NDF and CP increased between R3 (beginning pod) and R5.5 growth stages. They reported values of 155 g $kg^{-1}$ for CP, 362 g $kg^{-1}$ for ADF and 469 g $kg^{-1}$ for NDF, for forage soybean at the beginning seed stage (R5.5). The authors also observed that the forage quality was comparable to previous studies (Devine et al., 1999; Koivisto et al., 2002; Nayigihugu et al., 2000) with Donegal forage cultivar.

**Table 2.8 -** Chemical composition of forage soybean cultivars (g $kg^{-1}$)

| | Forage soybean cultivar | | | | | |
|---|---|---|---|---|---|---|
| | Derry | Donegal | Tyrone | Corsoy | Pella | Williams |
| Crude protein | 153 | 162 | 165 | 205 | 190 | 187 |
| Neutral detergent fiber | ND[1] | 442 | 449 | 405 | 395 | 421 |
| Acid detergent fiber | ND | ND | ND | 287 | 285 | 306 |

[1]ND: Not determined. Adapted from Devine and Hatley (1998); Devine et al. (1998 a, b); Undersander (2001)

### 2.7 Soybean intercropping

Soybean has been intercropped with cereals such as corn, oat, barley, sorghum and wheat (Anil et al., 1998). It has also been intercropped with other crops such as cassava and cow pea (Quainoo et al., 2000; Li et al., 2001; Chabi-Olaye et al., 2002).

Relative to mono-cropping, intercropping has several benefits including improved DM yield and CP content. Forage yield of soybean and corn intercrop was comparable with that of corn monoculture (Sheaffer et al., 2001). When corn growth is limited to a poor establishment, soybean is able to grow well and produce yields similar to those of monocrop soybean (Carruthers et al., 2000). However, relative to corn alone, intercropping with soybean improved CP content by 110 to 150 g $kg^{-1}$ (Putnam et al., 1986; Toniolo et al., 1987; Carruthers et al., 2000). Similar results were also obtained when soybean was intercropped with ryegrass or cereal rye. Both had crude protein concentrations greater than 170 g $kg^{-1}$, NDF and ADF contents lower than 560 g $kg^{-1}$ and 230 g $kg^{-1}$ respectively (Smith and Kallenbach, 2006). A tendency of lower stem NDF concentration has also been reported when soybean was intercropped with sorghum compared with soybean monocrop (645 vs 681 g $kg^{-1}$) and consequently *in vitro* DM digestibility was 3.3% greater for intercropped than monocropped soybean (Redfearn et al., 1999).

### 2.8 Soybean as hay

Soybean can be also used in dry form as hay. Although prices of grain and hay can vary to a great extend, Heitholt et al. (2004) showed that hay production can be more profitable than that of grain. Soybean can be harvested for hay at any stage of growth. However, the stem is the least desirable plant component, since it is considered to be course and fibrous (Blount et al., 2006). It has been reported that coarse stems of soybean hay provoke a large wastage (from 10 to 20%) during feeding. Nevertheless, the part that is eaten can be equal to average quality alfalfa in feeding value (Barnes, 2006).

According to the NRC (2001), soybean hay (mid maturity) contains 208 g $kg^{-1}$ CP, 429 g $kg^{-1}$ NDF and 591 g $kg^{-1}$ total digestible nutrients. Protein content of the different plant parts of the soybean hay was determined by Miller et al (1973). Depending on stage of development, CP ranged between 120 and 140 g $kg^{-1}$ for stems, 190 and 200 g $kg^{-1}$ for leaves, and 120 and 270 g $kg^{-1}$ for pods. The average concentrations of NDF, ADF, and ADL of stems were 681, 588 and 115 g $kg^{-1}$, respectively. The corresponding values for leaves were 339, 247 and 66 g $kg^{-1}$, respectively (Redfearn et al., 1999).

Contrary to other legume crops used for hay, soybean provides digestible protein from its foliage and pods (Blount et al., 2006). Unlike many other forages, the nutritive value of soybean hay is not greatly influenced by stage of maturity between pod formation until the beans are almost fully developed and the lower leaves are yellow (Vagts, 2005; Kallenbach et al., 2003). It worth’s mentioning that when soybean is harvested at full pod stage, the dry out is often very slow in the pods, and are apt to mold when hay is stored. Soybean hay that contains a high proportion of beans can result in a diet too high in fat resulting in scouring, depressed appetite, and digestive problems (Barnes, 2006).

### 2.9 Soybean silage

Soybean can also be grown as a silage crop in pure culture or intercropped with corn or sorghum. The best stage to harvest soybeans for silage is near full-pod stage and before any leaf loss. However, at this point, the concentration of water-soluble carbohydrates for proper ensiling is low.

Furthermore, mature soybean forage may contain high oil content which reduces its ensilability (Undersander 2001; Blount et al., 2006). Soybean harvested for silage should be harvested when seeds completely fill the pods and the lower leaves of the plant are just beginning to turn yellow (just before R7 stage) (Undersander, 2001). Whole-plant soybean at 50% pod fill contains 740 to 780 g $kg^{-1}$ of moisture concentration (Wolfe and Kipps, 1959). Thus forage soybean must be wilted to 300 to 500 g DM $kg^{-1}$ before ensiling. Wilting forage soybean will decrease storage losses associated with seepage of effluent and Clostridial spoilage compare with a direct-cut silage (Pitt, 1990). Muck et al. (1996) reported that soybean silage pH decreases and lactic:acetic acid ratio increases as growth stage advances. They also found that the fermentation characteristics were similar to those observed for alfalfa; concluding that wilting to 650 g $H_2O$ $kg^{-1}$ was important to prevent Clostridial fermentation.

### 2.9.1 Ensiling characteristics of soybean

Recent studies have shown that forage and grain soybean cultivars can be well preserved and fermented as silages (Mustafa and Seguin, 2003a, Mustafa et al., 2005). That was evident by a rapid decline in pH and sharp increase in lactic acid concentration within few days of ensiling (Figure 1). Soybean silage reached pH of 4.5 after 45 days of ensiling suggesting a well-fermented legume silage (Mustafa and Seguin, 2003a). The decline in pH of soybean silage was slower than that observed for other legumes such as faba bean and pea silages, mainly due to its lower water-soluble carbohydrate content relative to those legumes (Figure 1). Ensiling characteristics of forage soybean can also be influenced by cultivar. Mustafa et al. (2005) reported differences in pH, and lactic acid concentration between two forage soybean cultivars (i.e. Kodiak and Mammouth) during a 45-d ensiling period. However, differences in other fermentation characteristics were minimal.

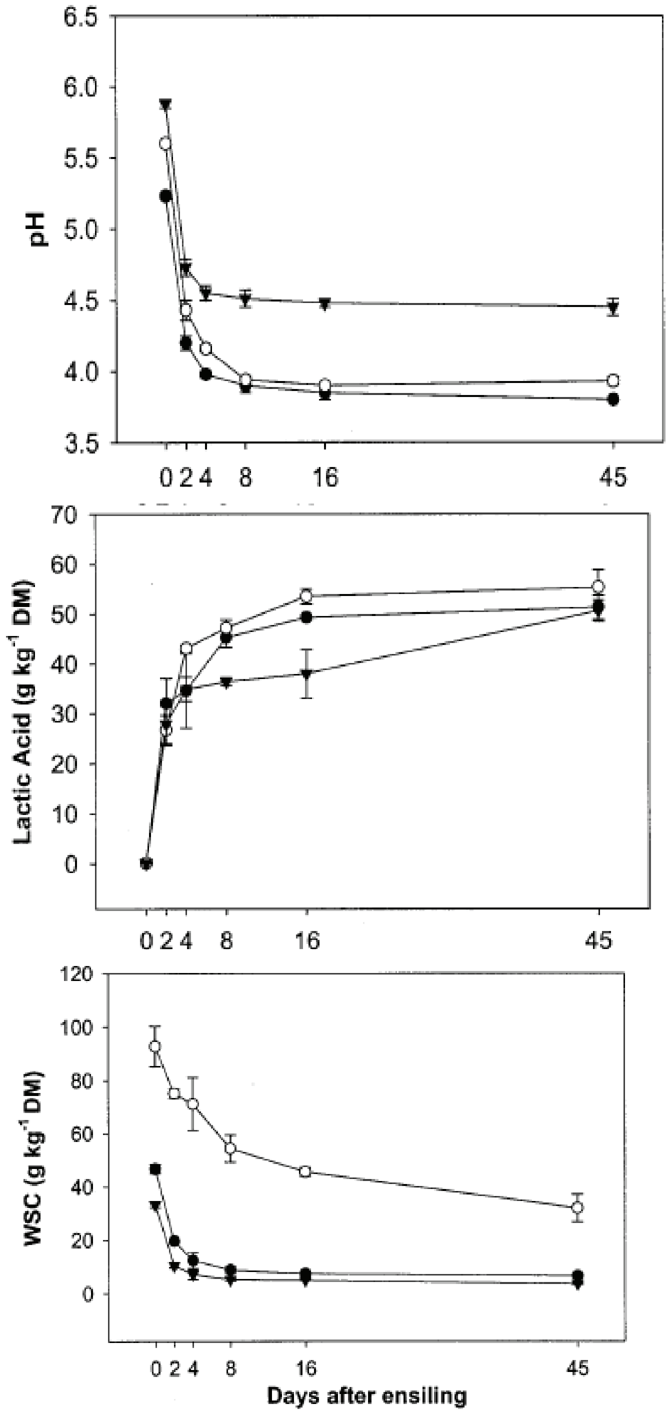


**Figure 1.** Ensiling characteristics of soybean silage relative to other legume silages (pea (○), faba bean (●), and soybean (▼). Vertical bars represent ± SD). Mustafa and Seguin, (2003a)

### 2.9.2 Chemical composition of soybean silage

Data on chemical composition of soybean silage are limited. Chemical composition of grain soybean (cv. Golden) silage was compared with other legumes silages (Table 2.9). Relative to pea and faba bean silages, soybean silage had lower ADF content and intermediate NDF content. Crude protein, non-protein nitrogen and soluble protein concentrations were also intermediate for soybean silage relative to faba bean and pea silages. In a recent study, Mustafa et al. (2005) determined the effects of cultivar (i.e. Kodiac and Mammouth) on chemical composition of forage soybean silages. The authors found that Kodiak cultivar had lower NDF (444 vs 490 g $kg^{-1}$) and ADF (353 vs 371 g $kg^{-1}$) but higher CP (208 vs 149 g $kg^{-1}$) concentration than Mammouth cultivar. However, soluble protein and non-protein nitrogen concentrations were similar for the two cultivars (averaging 615 and 587 g $kg^{-1}$, respectively). The CP and NDF concentrations reported for the forage soybean silages (Mustafa et al., 2005) are in good agreement with those reported for grain soybean silage (Mustafa and Seguin, 2003a).

### 2.9.3 Ruminal degradability of soybean silage

Mustafa and Seguin (2003a) determined ruminal degradability of soybean silage relative to other legume silages. Soybean silage had higher ruminal DM and CP degradability than faba bean but lower than pea silages (Table 2.10). However, ruminal NDF degradability was similar for the three silages and averaged 347 g $kg^{-1}$. In another study Mustafa et al. (2005) determine the effects of cultivar of ruminal nutrient degradability of forage soybean silage. The authors found that Kodiac cultivar had higher ruminal nutrient degradabilities than Mammouth cultivar (Table 2.11). The DM, CP and NDF degradabilitites reported by Mustafa and Seguin (2003a) for the grain soybean silage (822, 685 and 355 g $kg^{-1}$ respectively) agree with the values reported for forage soybean silage (Mustafa et al., 2005).

**Table 2.9 -** Chemical composition of soybean silage relative to other legume silages (after 45 d of ensiling)

| | Silages | | |
|---|---|---|---|
| | Faba bean | Soybean | Pea |
| Dry matter, g $kg^{-1}$ | 261 | 257 | 250 |
| Neutral detergent fiber, g $kg^{-1}$ | 428 | 420 | 416 |
| Acid detergent fiber, g $kg^{-1}$ | 313a | 292b | 312a |
| Crude protein, CP, g $kg^{-1}$ | 222a | 197b | 178c |
| Soluble crude protein, g $kg^{-1}$ of CP | 460c | 619b | 706a |
| Non-protein nitrogen, g $kg^{-1}$ of CP | 437c | 562b | 656a |

a, b, c Means within row followed by different letters are different ($P < 0.05$).
Adapted from Mustafa and Seguin (2003a)

**Table 2.10 -** *In situ* effective degradability of soybean silage relative to other legume silages (g $kg^{-1}$)

| | Silages | | |
|---|---|---|---|
| | Faba bean | Soybean | Pea |
| Dry matter | 662b | 685a | 691a |
| Crude protein | 794b | 822a | 836a |
| Neutral detergent fiber | 342 | 355 | 345 |

a, b Means within row followed by different letters are different ($P < 0.05$).
Adapted from Mustafa and Seguin (2003a)

**Table 2.11 -** Effects of cultivars on *in situ* ruminal degradability of soybean silage (g $kg^{-1}$)

| | Soybean cultivar | |
|---|---|---|
| | Kodiak | Mammouth |
| Dry matter | 606a | 549b |
| Crude protein | 828a | 752b |
| Neutral detergent fiber | 272a | 227b |

a, b Means within row followed by different letters are different ($P < 0.05$).
Adapted from Mustafa et al. (2005)

SECTION 3

# MATERIALS AND METHODS

## 3. Materials and Methods

### 3.1 Soybean silage

Forage soybean (variety Kodiac) was seeded in the last week of May 2005 in Ste-Anne-de-Bellevue, Québec, Canada, on a Chicot fine sandy loam (seeding rate of 70 kg $ha^{-1}$, 7,980 seeds $kg^{-1}$, 558, 600 seeds $ha^{-1}$). The soybean forage was harvested on the $15^{th}$ of August at the full-pod stage (R6, Fehr et al., 1971). A fourth cut alfalfa crop was harvested at the early bloom stage. All forages were chopped using a forage harvester (New Holland 900) to obtain a theoretical cut length of 10 mm. The harvested forages were wilted to a targeted 30% DM content and then ensiled in tower silos for three months.

### 3.2 Animals and Diets

#### 3.2.1 Production study

Twenty Holstein cows of mixed parties (13 multiparous and 7 primiparous) in early to mid lactation (BW 634 ± 65; DIM 69 ± 38d) were used. The cows were housed in tie stalls with free access to water. Two iso-nitrogenous diets were formulated with 50:50 forage:concentrate ratio to meet nutrient requirements of dairy cows in early lactation (NRC, 2001). The forage part of the diets consisted of 72% soybean or alfalfa silage. The remaining 28% consisted of corn silage in both diets (Table 3.1). Diets were offered *ad-libitum* as TMR twice daily at 0800 and 1600 h.

Experimental periods (n = 3) consisted of 7 d of diet adaptation and 14 d of data collection. Feed intake and milk yield were measured on d 8 to 21 of each period. Cows were milked twice daily at approximately 0900 and 1930 and daily milk samples were pooled by proportion according to milk yield at each milking. Diets were sampled daily during each collection period and composited by period. The composited samples were oven-dried at 60 °C for 48 h, ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA), and stored at room temperature for later analysis. Orts were measured daily to determine daily intake for each cow.

**Table 3.1 -** Ingredients and chemical composition of dietary treatments

| | Dietary treatment | |
|---|---|---|
| | Soybean silage | Alfalfa silage |
| Soybean silage | 36.0 | 0 |
| Alfalfa silage | 0 | 36.0 |
| Corn silage | 12.2 | 12.2 |
| Corn grain | 36.9 | 37.1 |
| Canola meal | 2.2 | 2.2 |
| Corn gluten meal | 1.7 | 1.7 |
| Corn distiller's grains | 4.4 | 4.4 |
| Trituro | 4.3 | 4.4 |
| Sodium bicarbonate | 0.8 | 0.8 |
| Mineral mix[1] | 1.1 | 1.1 |
| Urea | 0.5 | 0 |
| Chemical composition | | |
| Dry matter, g $kg^{-1}$ | 540 ± 39.3 | 569 ± 15.4 |
| Ash, g $kg^{-1}$ of DM | 85±5.2 | 80±6.7 |
| Ether extract, g $kg^{-1}$ of DM | 31±1.6 | 34±1.3 |
| Neutral detergent fiber, g $kg^{-1}$ of DM | 369±12.4 | 350±15.1 |
| Acid detergent fiber, g $kg^{-1}$ of DM | 254±8.7 | 233±11.2 |
| Acid detergent lignin, g $kg^{-1}$ of DM | 54±6.5 | 50±1.2 |
| Crude protein, g $kg^{-1}$ | 187±4.8 | 206±0.1 |
| Soluble protein, g $kg^{-1}$ of CP | 507±28.4 | 545±7.1 |
| Non-protein nitrogen, g $kg^{-1}$ of CP | 495±9.2 | 514±5.5 |
| Neutral detergent insoluble protein, g $kg^{-1}$ of CP | 232±23.5 | 210±18.0 |
| Acid detergent insoluble protein, g $kg^{-1}$ of CP | 101±5.7 | 105±4.4 |
| Non structural carbohydrate, (g $kg^{-1}$ of DM)[2] | 328±9.0 | 330±17.6 |
| Net energy of lactation, (MJ $kg^{-1}$)[3] | 6.66±0.06 | 6.83±0.12 |
| Total digestible nutrients, (g $kg^{-1}$)[3] | 662±72.8 | 679±11.3 |

[1]Contained: 45 g $kg^{-1}$ Ca, 25 g $kg^{-1}$ P, 66 g $kg^{-1}$ Na, 15 g $kg^{-1}$ Mg, 12 g $kg^{-1}$ K, 11 g $kg^{-1}$ S, 1372 mg $kg^{-1}$ Fe, 1032 mg $kg^{-1}$ Mn, 1500 mg $kg^{-1}$ Zn, 247 mg $kg^{-1}$ Cu, 16 mg $kg^{-1}$ I, 16 mg $kg^{-1}$ Co, 10 mg $kg^{-1}$ Se, 185,000 IU $kg^{-1}$ Vit. A, 32,500 IU $kg^{-1}$ Vit. $D_3$, 900 IU $kg^{-1}$ Vit. E.
[2]Non structural carbohydrates were calculated according to Beauchemain et al. (1997)
[3]Calculated using the equation of Weiss et al. (1992)

### 3.3 Ruminal fermentation parameters and total tract nutrient utilization

Four multiparous lactating Holstein cows (BW 710 ± 81; DMI 148 ± 69) fitted with ruminal cannulae were used in a Switchback design with three experimental periods (n = 3) consisting of 14 d of diet adaptation and 7 d of data collection. Cows were housed in tie stalls and had continuous access to water. Dietary treatments were the same as in the production study.

Chromic oxide was used as an inert external marker to determine total fecal output. Gelatin capsules containing 8 g of $Cr_2O_3$ were inserted into the rumen of each cow twice daily in equal intervals starting on d 12 of each period. Grabbed fecal samples were collected 4 times daily during the last 3 d of each period. Samples were then dried at 60 °C in a forced-air oven for 48 h and pooled by cow within each period. Feed samples were collected during the fecal collection period and were dried as previously described and pooled by treatment within each period.

Samples of ruminal fluid were collected with a syringe equipped with a filtering device (ANKOM Technology, Macedon, NY, USA.) from various parts of the rumen on d 19 of each period prior to morning feeding (0 h) and at 2, 4, 6, 8, 10 and 12 h post-feeding. Ruminal pH was determined immediately using an Accumet pH meter (AB15 Plus, Fischer Scientific, Pittsburgh, PA). Following pH determination, 10 mL of ruminal fluid was preserved by adding 1 mL of 25% metaphosphoric acid to determine volatile fatty acids, and 10 mL of ruminal fluid was preserved by adding 1 mL of 0.1 N HCl to determine $NH_3$ N. Samples were immediately frozen (-20 °C) for later analysis.

### 3.4 *In situ* ruminal nutrient degradability

Equal portions (200 g) of the dry silages from the three different periods were composited to obtain a single sample for soybean and alfalfa silage. Duplicated samples weighing approximately 5 g (air basis) were placed into nylon bags (9 x 21 cm; 41µm pore size; ANKOM Technology, Macedon, NY). The nylon bags were then incubated in the rumen of two lactating Holstein cows (two bags per treatment per time period per cow) fitted with flexible rumen cannulas for 0, 3, 6, 12, 24, 48, 72 and 96 h. The cows were fed ad-libitum a 50:50 forage:concentrate total mixed diet which contained 69 g $kg^{-1}$ ash, 178$g^{-1}$ CP, 148 $g^{-1}$ ADF, and 286 g $kg^{-1}$.

At the end of the incubations, bags were removed from each cow and washed under cold tap water until rinsing water was clear. Zero-hour disappearance was estimated by washing duplicated bags containing samples. Washed bags were dried at 60 °C for a constant weight. Residues from the nylon bags were analyzed for DM, CP and NDF as previously described. Ruminal nutrient disappearance was calculated from concentrations of these nutrients in the original samples and the nylon bag residues. Ruminal nutrient disappearance data were then used to determine nutrient kinetic parameters using the equation of Dhanoa (1988):

$$p = a + b * ( 1 - e^{-ct} )$$

where $p$ is ruminal nutrient disappearance at time $t$, $a$ is soluble fraction (%), $b$ is potentially degradable fraction (%), $c$ is rate of degradation of the $b$ fraction (%/h). Effective degradability (ED) of DM, CP, and NDF was then calculated according to the equation of Orskov and McDonald (1979):

$$\mathrm{ED} = a + bc / (c + k)$$

where $k$ is the ruminal outflow rate (5%/h) and $a$, $b$ and $c$ are as described above.

**3.5 Chemical analyses**

Ground feed and silage samples were analyzed for DM, ash, and ether extract using standard procedures (AOAC, 1990). Neutral detergent fiber and ADF were determined using an Ankom Fiber Analyzer (Ankom Technology Corporation, Fairport, NY). Analysis of NDF was conducted without the inclusion of sodium sulfite and with the use of heat-stable α-amylaze. Crude protein (N x 6.25) was measured using a Leco Nitrogen Analyzer (FP-428 Nitrogen Determinator, LECO Corporation, St-Joseph, MI). Soluble protein (SCP) and NPN were determined according to the procedures of Licitra et al. (1996) and Roe et al. (1990) while neutral (NDICP) and acid (ADICP) detergent insoluble protein were determined by analyzing NDF and ADF residues for total N. Gross energy (GE) of feed samples were determined using an oxygen bomb calorimeter (Parr Instrument Company, Moline, IL).

Ground fecal samples were analyzed for DM, ash, CP, NDF, and GE as previously described. Chromic oxide in fecal samples was determined according to the procedure of Fenton and Fenton (1979).

Milk samples for milk fat, protein, lactose and milk urea nitrogen by infrared analysis (VALACTA, Ste-Anne-de-Bellevue, Québec, Canada). Milk total solids were determined according to AOAC (1990).

Samples of ruminal fluid preserved for volatile fatty acids analysis were centrifuged for 15 min at 11,000 x g and analyzed for acetic, propionic and butyric acids by gas chromatrography (Hewlett Packard, Palo Alto. CA) equipped with 15 m Nukol fused capillary column (Supelco Inc., Bellefonte, PA). Isocaproic acid was used as an internal standard. Column temperature was fixed at 150 °C for a run time of 8 min. Injector and detector temperatures were 180 and 200°C, respectively. Gas flows were 30, 300, and 30 mL $min^{-1}$ for He, air, and $H_2$, respectively. Ruminal $NH_3$-N was determined by colorimetry using a multichannel Lachat autoanalyzer (Lachat Instruments, Milwaukee, WI).

### 3.6 Animal care

The study was approved by the Macdonald Campus Animal Care Committee and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

### 3.7 Statistical analysis

Data from the production study and total tract nutrient utilization were analyzed using a Switch-back design using PROC MIXED of SAS (1999) with the following model:

$$Y_{ijkl} = \mu + T_{ij} + P_{ij}C_k + \pi_k + \tau_h + e_{ijk}$$

where:

$Y_{ijkl}$ = observation, μ = population mean, $T_{ij}$ = true effect of the $j^{th}$ cow in the $i^{th}$ sequence group, being a random effect, normally and independently distributed with a mean of zero and common variance, $\sigma^2_{cow,}$ $P_{ij}$ = linear time trend of the $j^{th}$ cow in the $i^{th}$ sequence group, $C_k$ = units of time (X = 1 in first period, 0 in second period and -1 in third period), $\pi_k$ = true effect of the $k^{th}$ period ($\Sigma_k{}^{\pi k} = 0$), $\tau_h$ = true effect of the $h^{th}$ treatment ($\Sigma_k{}^{\tau h} = 0$) (h = 1,2; being a function of i and k), $e_{ijk}$ = random error, normally and independently distributed with zero mean and common variance, $\sigma^2_e$

Data of volatile fatty acids, ruminal pH and $NH_3$ N were analyzed as repeated measurements across time using the Mix Procedure of SAS (1999) with the following model:

$$Y_{ijkl} = \mu + T_i + P_j + C_k + S_l + T_i{*}S_l + e_{ijkl}$$

Where:

$Y_{ijkl}$ = observation, μ = population mean, $T_i$ = treatment (i = 1 or 2), $P_j$ = period (j = 1,2 or 3), $C_k$ = random effect of cow (k = 1, 2, 3 or 4), $C_k \sim N(0, \sigma^2 \text{ cow})$, $S_l$ = sampling time (l = 0,2,4,6,8,10 or 12 hours), $T_i{*}S_l$ = treatment by interaction time, and $e_{ijkl}$ = residual error , $e_{ijkl} \sim N(0, \sigma^2 \text{ cow})$

Data from the *in situ* trial were analyzed as a randomized complete block design using cows as blocks. Significance was declared at $P<0.05$:

$$X_{ij} = \mu + a_i + B_j + e_{ij}$$

Where:

μ = population mean, $a_i$ = effect of the treatment *i*, $B_j$ = effect of the block *j*, Random $B_j \sim N(0, \sigma^2_B)$, and $e_{ij}$ = experimental error, Random $e_{ij} \sim N(0, \sigma^2_e)$

SECTION 4

# RESULTS

## 4. Results

### 4.1 Chemical composition of soybean silage

Chemical composition of SS and AS is shown in Table 4.1. Relative to AS, SS contained 10% more NDF, 45% more ADL and 24% less CP.

**Table 4.1 -** Chemical composition of silages[z]

| | Silages | |
|---|---|---|
| | Soybean | Alfalfa |
| Dry matter, g $kg^{-1}$ | 409 ± 51.7 | 445 ± 16.9 |
| pH | 5.29±0.57 | 4.89±0.09 |
| Ash, g $kg^{-1}$ of DM | 126±16.2 | 100±7.2 |
| Ether extract, g $kg^{-1}$ of DM | 15±3.7 | 26±1.9 |
| Neutral detergent fiber, g $kg^{-1}$ of DM | 469±10.0 | 425±3.14 |
| Acid detergent fiber, g $kg^{-1}$ of DM | 377±14.3 | 324±37.3 |
| Acid detergent lignin, g $kg^{-1}$ of DM | 110±6.9 | 76±4.8 |
| Crude protein, CP, g $kg^{-1}$ | 189±25.9 | 249±6.1 |
| Soluble protein, g $kg^{-1}$ of CP | 620±36.2 | 685±27.6 |
| Non-protein nitrogen, g $kg^{-1}$ of CP | 604±27.7 | 656±48.8 |
| Neutral detergent insoluble protein, g $kg^{-1}$ of CP | 191±26.8 | 186±18.2 |
| Acid detergent insoluble protein, g $kg^{-1}$ of CP | 123±35.7 | 88±16.4 |
| Non structural carbohydrate, (g $kg^{-1}$ of DM)[1] | 202±42.6 | 201±37.2 |
| Net energy of lactation, (MJ $kg^{-1}$)[2] | 4.96±0.08 | 5.95±0.23 |
| Total digestible nutrients, (g $kg^{-1}$)[2] | 497±65.6 | 593±23.0 |

[1]Non structural carbohydrates were calculated according to Beauchemain et al. (1997)
[2]Calculated using the equation of Weiss et al. (1992).

### 4.2 Ruminal degradability of soybean silage

*In situ* soluble DM and NDF fractions were higher ($P$<0.05) for AS than SS while *in situ* soluble CP fraction was similar for both silages (Table 4.2). Slowly degradable DM, CP and NDF fractions were not affected by silage type and averaged 386, 308, and 445 g kg$^{-1}$, respectively. Relative to alfalfa silage, SS had slower ($P$<0.05) rates of degradation of DM, CP and NDF fractions. Effective ruminal degradability of DM, CP and NDF were all higher ($P$ < 0.05) for AS than SS (Table 4.1).

**Table 4.2 -** *In situ* ruminal nutrient degradabilities of soybean silage relative to alfalfa silage

| | Silage | | SEM | *P* value |
|---|---|---|---|---|
| | Soybean | Alfalfa | | |
| Dry matter, DM | | | | |
| Soluble fraction, g kg$^{-1}$ of DM | 338 | 400 | 0.32 | < 0.05 |
| Slowly degradable fraction, g kg$^{-1}$ of DM | 395 | 377 | 0.81 | 0.16 |
| Degradation rate, % h$^{-1}$ | 6.0 | 8.0 | 0.56 | < 0.05 |
| Effective degradability, g kg$^{-1}$ | 533 | 639 | 0.52 | < 0.05 |
| Crude protein, CP | | | | |
| Soluble fraction, g kg$^{-1}$ of CP | 601 | 596 | 1.52 | 0.81 |
| Slowly degradable fraction, g kg$^{-1}$ of CP | 304 | 312 | 1.48 | 0.73 |
| Degradation rate, % h$^{-1}$ | 6.5 | 9.4 | 0.83 | 0.05 |
| Effective degradability, g kg$^{-1}$ | 769 | 799 | 0.42 | < 0.05 |
| Neutral detergent fiber, NDF | | | | |
| Soluble fraction, g kg$^{-1}$ of NDF | 100 | 153 | 0.47 | < 0.05 |
| Slowly degradable fraction, g kg$^{-1}$ of NDF | 439 | 451 | 1.60 | 0.62 |
| Degradation rate, % h$^{-1}$ | 4.8 | 6.1 | 0.35 | < 0.05 |
| Effective degradability, g kg$^{-1}$ | 312 | 406 | 0.53 | < 0.05 |

### 4.3 Production study

#### 4.3.1 Dry matter intake

Differences in the NDF and ADL concentrations between SS and AS were reflected in the composition of the dietary treatments (Table 3.1). Cows fed SS consumed less ($P$ < 0.05) DM, CP, NDF and OM than cows fed AS (Table 4.3).

**Table 4.3 -** Effects of feeding soybean silage on performance of dairy cows

| | Dietary treatment | | SEM | *P* value |
|---|---|---|---|---|
| | Soybean silage | Alfalfa silage | | |
| Intake, kg $d^{-1}$ | | | | |
| Dry matter | 22.7 | 23.8 | 0.46 | < 0.05 |
| Crude protein | 4.0 | 4.9 | 0.15 | < 0.05 |
| Neutral detergent fiber | 7.4 | 9.3 | 0.25 | < 0.05 |
| Organic matter | 19.2 | 23.5 | 0.63 | < 0.05 |
| Yield | | | | |
| Milk production, kg $d^{-1}$ | 35.5 | 37.2 | 0.32 | < 0.05 |
| Energy corrected milk[1] | 32.1 | 32.9 | 1.33 | 0.33 |
| Milk efficiency, kg $kg^{-1}$ | 1.56 | 1.52 | 0.012 | 0.34 |
| Fat, kg $d^{-1}$ | 1.35 | 1.33 | 0.019 | 0.39 |
| Protein, kg $d^{-1}$ | 1.09 | 1.16 | 0.006 | < 0.05 |
| Lactose, kg $d^{-1}$ | 1.67 | 1.74 | 0.006 | < 0.05 |
| Total solids, kg $d^{-1}$ | 4.49 | 4.71 | 0.045 | < 0.05 |
| Milk composition | | | | |
| Fat, % | 3.78 | 3.58 | 0.051 | < 0.05 |
| Protein, % | 3.17 | 3.18 | 0.022 | 0.76 |
| Lactose, % | 4.69 | 4.69 | 0.012 | 0.89 |
| Total solids, % | 12.65 | 12.61 | 0.079 | 0.73 |
| Milk urea nitrogen, mg $dl^{-1}$ | 15.67 | 15.03 | 0.164 | < 0.05 |

[1]ECM=Energy corrected milk (Tyrrell and Reid, 1965)

### 4.3.2 Milk yield and composition

Milk yield was 4.7% lower ($P < 0.05$) for cows fed SS than for cows fed AS. However, energy-corrected milk and milk efficiency were not affected by dietary treatments. Milk fat percentage and milk urea nitrogen were higher ($P < 0.05$) in milk of cows fed SS than in milk of cows fed AS (Table 4.3). However, milk protein and lactose concentrations were similar for both dietary treatments.

## 4.4 Ruminal fermentation parameters and total tract nutrient utilization

No time by silage type interactions were observed for ruminal fermentation parameters and therefore only main effects were reported (Table 4.4). Ruminal pH and $NH_3$ N were lower ($P<0.05$) in cows fed AS than in cows fed SS. However, total and molar proportions of volatile fatty acids were not influenced by dietary treatments. Total tract digestibility of DM, OM, CP, NDF, and GE were similar for dietary treatments and averaged 710, 717, 687, 537, and 698 g kg$^{-1}$, respectively (Table 4.5).

**Table 4.4 -** Effects of feeding soybean silage on ruminal fermentation parameters of dairy cows

| | Dietary treatment | | SEM | *P* value |
|---|---|---|---|---|
| | Soybean silage | Alfalfa silage | | |
| pH | 6.44 | 6.34 | 0.028 | < 0.05 |
| $NH_3$ N, mg dl$^{-1}$ | 22.6 | 18.6 | 0.79 | < 0.05 |
| Volatile fatty acids, mM | 78.6 | 81.6 | 2.11 | 0.34 |
| Molar proportion | | | | |
| Acetate | 66.2 | 63.9 | 1.65 | 0.34 |
| Propionate | 25.2 | 27.6 | 1.15 | 0.18 |
| Butyrate | 15.1 | 16.0 | 0.53 | 0.25 |

**Table 4.5 -** Total tract nutrient utilization of dairy cows fed soybean silage

| | Dietary treatment | | SEM | $P$ value |
|---|---|---|---|---|
| | Soybean silage | Alfalfa silage | | |
| Intake, kg $d^{-1}$ | 26.0 | 27.7 | 0.19 | < 0.05 |
| Digestibility, g $kg^{-1}$ | | | | |
| Dry matter | 711 | 709 | 0.9 | 0.88 |
| Organic matter | 718 | 717 | 1.0 | 0.95 |
| Crude protein | 690 | 684 | 1.0 | 0.74 |
| Neutral detergent fiber | 556 | 519 | 1.7 | 0.27 |
| Gross energy | 705 | 691 | 0.8 | 0.32 |
| Digestible energy, MJ $kg^{-1}$ | 13.0 | 12.6 | 0.03 | 0.17 |

SECTION 5

# DISCUSSION

## 5. Discussion

### 5.1 Chemical composition and ruminal degradability of soybean silage

Values of the various chemical components for SS and AS in the present study are in good agreement with those reported in the literature (Mustafa et al., 2000; Mustafa and Seguin, 2003a; Mustafa et al., 2005). Results of the *in situ* study indicate that differences in ruminal nutrient degradabilities between SS and AS were mainly due to differences in degradation rates particularly that of NDF (Table 4.2). The slower rate of degradation of the potentially degradable NDF fraction of SS relative to AS can be attributed to the higher ADL concentration of SS than AS (Table 3.1). Several studies have reported increase in rate of ruminal degradation of NDF when concentration of ADL is reduced as in the case of brown-mid rib trait (Aydin et al., 1999; Oliver et al., 2004; Mustafa et al., 2005).

The higher rate of degradation of the slowly degradable protein fraction of AS relative to SS is likely due to its lower ADICP concentration. A strong negative correlation between rate of degradation of slowly degradable protein fraction and concentration of ADICP has been reported for forages (Hoffman et al., 1999). Ruminal degradability values of SS and AS are in good agreement with our previous values (Mustafa et al., 2000; Mustafa and Seguin, 2003a).

### 5.2. Dry matter intake and milk yield

Because SS, replaced AS on weight basis, NDF of SS diet was 5% greater than that of AS diet. Due to lower dietary NDF concentration and higher NDF degradability, cows fed AS diet consumed more DM than cows fed SS diet. This probably reduced retention time and increase passage rate from the rumen and therefore may have resulted in reduced total tract nutrient digestibilities. Neutral detergent fiber content and digestibility are major factors affecting DM intake and milk yield of dairy cows particularly in early lactation where DM intake is often limited by rumen fill (Oba and Allen 1999; Allen 2000). The reduced DM intake and consequently milk yield for cows fed SS diet can be attributed at least in part to the higher (7.6% more) NDF content of SS diet relative to AS diet (Table 3.1). Neutral detergent fiber has been used as a chemical predictor of DM intake (Waldo 1986). For diets with more than 250 g $kg^{-1}$ NDF, DM intake decreased as the level of dietary NDF increased (Allen 2000). For high producing cows in early lactation, DM intake may be limited by gut fill when diets containing more than 320 g $kg^{-1}$ NDF are fed (Mertens 1994). In the present study, both dietary treatments contained more than 340 g $kg^{-1}$ NDF.

Neutral detergent fiber of SS was less digestible than that of AS (Table 4.2) which could also contribute to the reduced DM intake of cows fed SS diet relative to those fed AS. The effect of dietary NDF digestibility on DM intake of dairy cows is well documented and several authors have reported positive relationship between DM intake and NDF digestibility (Oba and Allen 1999; Oba and Allen 2000; Allen 2000). Oba and Allen (1999) indicated that one unit increased in NDF digestibility was associated with 0.17 kg increase in DM intake and 0.25 kg increase in milk yield.

Despite the negative effect of SS on DM intake and milk yield, energy corrected milk and milk efficiency were not affected by silage type suggesting efficient nutrient utilization of SS by dairy cows.

**5.3 Milk composition**

Except for milk fat concentration, silage type had no impact on milk composition (Table 4.3). Mustafa et al. (2000) also reported higher fat concentration from cows fed pea (annual legume) silage relative to alfalfa silage. Apparent milk fat depression might result from increased milk fluid yield relative to milk fat yield (i.e. dilution effect). Increased milk volume is generally associated with reduced milk fat concentration (Åkerlind et al., 1999; Murphy et al., 2000).

Milk urea N was significantly different among the two dietary treatments (Table 4.3). The excess of N supply to the rumen increases the urea concentrations in milk. Rumen degradable protein from the diet and milk urea N is correlated (Schepers and Meijer, 1998). Diets balanced for CP, rumen degradable protein and rumen undegradable protein with high quality protein sources results in milk urea concentrations of 15.1 mg $dL^{-1}$ (Baker et al., 1995). High concentrations of urea in body fluids (i.e. milk) of dairy cows reduce metabolic efficiency of milk yield (Tyrrell and Moe, 1975; Baker et al., 1995). Other studies (Hof et al., 1997; Jonker et al., 1999) have reported that milk urea N is more related to the ratio of CP intake to energy intake than the absolute CP intake.

### 5.4 Total tract nutrient utilization

In this study, cows fed SS had lower DM intake than those fed AS diets. Despite differences in DM intake between the two dietary treatments, total tract nutrient digestibilities were similar for cows fed SS and AS diets. The lack of differences in total tact nutrient utilization between SS and AS diets can be attributed, at least in part to differences in DM intake. High DM intake especially for diets containing more digestible fiber is usually associated with decreased total tract nutrient digestibilities due to a reduced ruminal retention time and therefore increased passage rate (Zinn et al., 1995; Oba and Allen, 1999). Oba and Allen (1999) indicated that forages with high in situ or in vitro digestibilities might have shorter retention time allowing greater DMI at the expense of total tract NDF digestibility.  It is also possible that cows fed SS diets had higher post-ruminal fermentation than cows fed AS diet to compensate for reduced ruminal fiber digestibility.

### 5.5 Ruminal fermentation

Effect of soybean silage on total and molar proportions of VFA was minimal. Furthermore, differences in pH are not expected to be biologically important. Differences in $NH_3$ N concentration between dietary treatments is likely due to differences in NDF concentration between dietary treatments. Soybean silage diet contained 0.2% less non-structural carbohydrate than AS treatment. Hristov et al. (2005) showed that provision of rapidly fermentable carbohydrate reduced ruminal $NH_3N$ concentration by decreasing $NH_3N$ production or through enhanced uptake for microbial synthesis to the inclusion of urea in the SS diet. The higher ruminal $NH_3$-N concentration for cows fed SS diet relative to those fed AS may help to explain their higher milk urea nitrogen content.

SECTION 6

# GENERAL CONCLUSIONS

## 6. General conclusions

Our study showed that SS at R6 (full-pod stage) was more lignified and therefore less digestible than a fourth-cut alfalfa silage. When fed at a similar proportion, SS had a lower feeding value than AS as indicated by lower feed intake and reduced milk yield. Differences in DMI and consequently milk production could be attributed to differences in fiber digestibility and higher NDF content of the SS diet relative to the AS diet. Despite differences in DM intake and milk yield, energy corrected milk and milk efficiency were similar for SS and AS diets suggesting efficient utilization of nutrient from soybean silage. This was evident by the lack of differences in total tract nutrient utilization between the two dietary treatments. Overall, our results suggest that although cows fed AS had higher DM intake and milk production. SS can be used to replace AS when stands for perennial legumes have been winterkilled. Moreover, apart from milk fat percentage, milk composition was not significantly affected by dietary treatments. More work is needed to determine the optimum stage of development at harvest to maximize dry matter intake and digestibility of forage soybean silage.

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Guidelines for completing the form are available at www.mcgill.ca/rgo/animal/

# McGill University
## Animal Use Protocol – Research

Protocol #: 5103
Investigator #: —
Approval End Date: Nov. 30, 2006
Facility Committee: AGR

**Title:** Performance of dairy cows fed soybean silage
*(must match the title of the funding source application)*

☒ **New Application** ☐ **Renewal of Protocol #** _____ ☐ **Pilot** **Category** *(see section 11)*: B

### 1. Investigator Data:

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### 2. Emergency Contacts: Two people must be designated to handle emergencies.

| Name | Work # | Emergency # |
|---|---|---|
| Arif Mustafa | 398-7506 | (514) 969-8019 |
| Einar Vargas Bello Perez | 398-810 | (514) 756-3001 |

### 3. Funding Source:

| External ☐ | Internal ☒ | For Office Use Only: |
|---|---|---|
| Source (s): __________ | Source (s): William Dawson | ACTION / CCs / DB ✓ Dec 21 '04 APPROVED |
| Peer Reviewed: ☐ YES ☐ NO** | Peer Reviewed: ☐ YES ☒ NO** | |
| Status: ☐ Awarded ☐ Pending | Status: ☒ Awarded ☐ Pending | |
| Funding period: _____ | Funding period: 2005-2010 | |

** All projects that have not been peer reviewed for scientific merit by the funding source require 2 Peer Review Forms to be completed e.g. Projects funded from industrial sources. Peer Review Forms are available at www.mcgill.ca/rgo/animal

| | | |
|---|---|---|
| **Proposed Start Date of Animal Use (d/m/y):** | October, 15, 2005 | **or ongoing** ☐ |
| **Expected Date of Completion of Animal Use (d/m/y):** | October, 15, 2006 | **or ongoing** ☐ |

**Investigator's Statement:** The information in this application is exact and complete. I assure that all care and use of animals in this proposal will be in accordance with the guidelines and policies of the Canadian Council on Animal Care and those of McGill University. I shall request the Animal Care Committee's approval prior to any deviations from this protocol as approved. I understand that this approval is valid for one year and must be approved on an annual basis.

| | | |
|---|---|---|
| **Principal Investigator's signature:** | Arif Mustafa | **Date:** October 07-2005 |

*Approved by:*

| | | |
|---|---|---|
| **Chair, Facility Animal Care Committee:** | [signature] | **Date:** Oct 20/05 |
| **University Veterinarian:** | [signature] | **Date:** Nov 10/05 |
| **Chair, Ethics Subcommittee** (as per UACC policy): | — | **Date:** — |
| **Approved Animal Use** | **Beginning:** Dec 1, 2005 | **Ending:** Nov. 30, 2006 |

☐ This protocol has been approved with the modifications noted in Section 13.

December 2003 20 DEC 2005