EFFECT OF FIBROLYTIC ENZYMES ON LACTATIONAL PERFORMANCE, FEEDING BEHAVIOR AND DIGESTIBILITY IN LACTATING DAIRY COWS FED A WHOLE PLANT FABA BEAN SILAGE-BASED DIET

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By

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ABSTRACT

Faba beans (*Vicia faba* L.) is one of the most widespread and oldest grain legumes in the temperate regions due to its considerable content of protein and starch. The exogenous fibrolytic enzyme for ruminants was applied in order to improve feed efficiency and animal performance. The aim of this project was to evaluate the effects of pre-treating whole plant faba bean silage based- diet with exogenous fibrolytic enzyme in lactational dairy cows and develop an efficiently feeding strategy of whole plant faba bean silage for dairy cows. Statistical analyses were performed using PROC MIXED of SAS 9.4 with significance declared at $P< 0.05$. Orthogonal polynomial contrast was used to detect linear, quadratic and cubic effect when increased enzyme dosage to treat whole plant faba bean silage. The results obtained from *in situ* method show that fibrolytic enzyme cubically ($P<0.05$) affected in situ DMD and quadratically ($P=0.01$) affected in situ NDFD with increasing level of enzyme application. Both *in vitro* DM and NDF degradability were quadratically ($P<0.01$) affected by the increasing dosage of enzyme. Correlation analysis between in situ assay-biological approach and in vitro Daisy^{II} approach showed a strong correlation ($R=0.98$, $P<0.01$) on overall DMD and also a satisfactory relationship ($R=0.84$, $P<0.01$) on overall NDFD. The washable and potential degradable (W+D) fraction of NDF was linearly ($P=0.05$) increased by the enzyme treatments. In contrast, undegradable fraction was linearly decreased ($P=0.05$) with increasing dosage of enzyme. The K_d of NDF in whole plan faba bean silage was cubically ($P<0.05$) affected by the enzyme. Both rumen bypass (B) and effective degradable (ED) NDF were cubically ($P=0.05$) affected by fibrolytic enzyme. The response of NDF digestibility and digestible NDF to the increasing level of FETR was linear ($P<0.05$), where lower enzyme group (0.5 mL of enzyme/kg of TMR DM) exhibited the highest NDF digestibility (48.54%). The

enzyme application effects on percentage of milk fat and milk fat yield were linearly ($P<0.05$) affected by enzyme treatment, with the highest (4.35%, 1.82 kg/d) in low dosage group. The control milk averaged 41.2 kg/d with 4.35 percent fat. Both energy (ECM, $P = 0.018<0.05$) and fat corrected milk yield (FCM, $P=0.058<0.10$) were linearly affected or tended to be affected by fibrolytic enezyme dose level. The ECM and FCM production efficiency (kg of ECM or FCM/kg of DMI) cubically ($P<0.05$) and linearly affected by the enzyme application. Based on the results, it was suggested that the low dosage of enzyme for whole plant faba bean silage at 0.50 mL of enzyme/kg of silage DM has the potential to enhance substrate fermentation thus provides additional energy for animals and improve animal performance.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
ADL	Acid detergent lignin
AMCP	Truly absorbed microbial protein in the small intestine
aNDF	Neutral detergent fiber
BDM	Rumen bypass dry matter
BHB	β-hydroxybutyrate
BNDF	Rumen bypass feed neutral detergent fiber
BW	Body weight
BWG	Body weight gain
CA1	Acetic, propionic and butyric acids
CA2	Lactic acid
CA3	Other organic acids
CA4	Sugars
CB1	Starch
CB2	Soluble fiber
CB3	Available NDF
CC	Indigestible fiber
CHO	Carbohydrate
CP	Crude protein
CT	Condensed tannin

CTRL	Control group
DE	Digestible Energy
DM	Dry matter
DMD	Dry Matter Degradability
DIM	Days in milk
DMI	Dry matter intake
ECM	Energy corrected milk
EDNDF	Effective degraded neutral detergent fiber
EE	Ether extracts
FCM	Fat corrected milk
FETR	Fibrolytic Enzyme derived from *Trichoderma reesei*
FMV	Feed milk value
iNDF	Indigestible neutral detergent fiber
ISDMD	In Situ Dry Matter Degradability
ISNDFD	In Situ Neutral Detergent Fiber Degradability
IVDMD	In Vitro Dry Matter Degradability
IVNDFD	In Vitro Neutral Detergent Fiber Degradability
K_d	Degradation rate of degradable fraction
K_p	Passage rate
ME	Metabolizable energy
MP	Metabolizable protein (NRC Dairy model)
MUN	Milk urea nitrogen

N	Nitrogen
NDF	Neutral Detergent Fiber
NDFD	Neutral Detergent Fiber Degradability
NDICP	Neutral Detergent Insoluble Crude Protein
NE_L	Net Energy for Lactation
NE_m	Net Energy for Maintenance
NFC	Non-Fiber Carbohydrate
NH_3	Ammonia
NPN	Non-protein Nitrogen
NSTC	Non-structural Carbohydrates
OM	Organic Natter
P	Phosphorus
PA1	Ammonia
PA2	Soluble true protein
PB2	Fiber bound protein
PC	Indigestible protein
pdNDF	Potentially Digestible Neutral Detergent Fiber
S	Soluble fraction (washable in NDF)
SCC	Somatic Cell Count
SCFA	Short Chain Fatty Acid
SCP	Soluble Crude Protein
SNF	Solids-Non-Fat

ST	Starch
TDN	Total Digestible Nutrient
TMR	Total Mixed Ration
Trt	Treatment group
U	Rumen Undegradable Fraction
uNDF	Undigested Neutral Detergent Fiber
VFA	Volatile Fatty Acids
WG	Weight gain

1. GENERAL INTRODUCTION

In Western Canada, dairy and beef cattle are usually fed with a mixture of grains and forages, such as barley silage (Nair et al. 2016). However, the present feeding of normal cereal grains to dairy and beef cattle is faced with increasing challenges in terms of animal production and health. The cost of feed grains is variable and has highly increased recently, threatening the economic competitiveness of livestock production in western Canada. In order to minimize feed cost and maximize dairy and beef production, producers and breeders are seeking an alternative feed resource. As a result, faba bean shows its potential as a protein source. Also, production of pulses in Canada has dramatically increased in the past few years, due to the rapid growth of international markets and its high market values (Clancey 2018b).

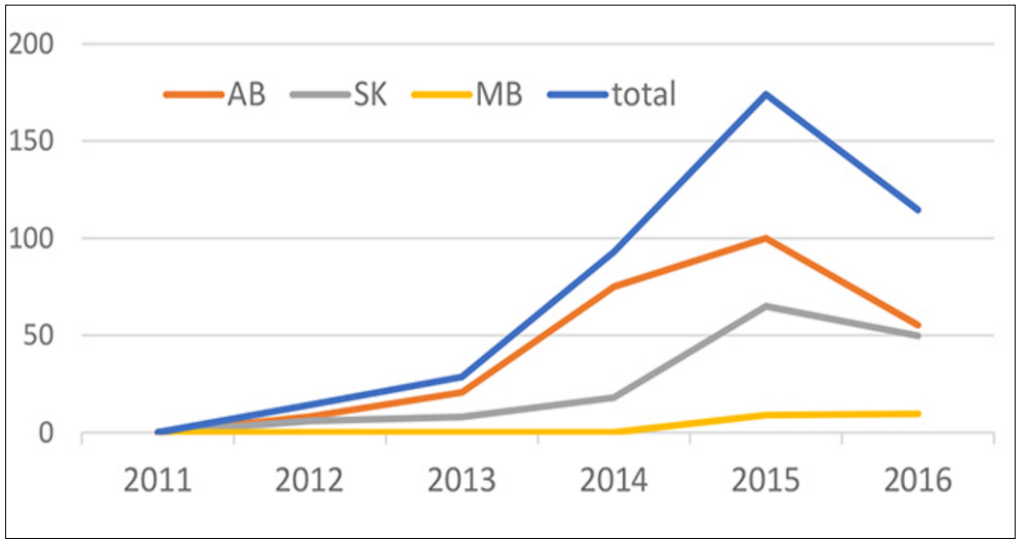


Figure 1.1. Seed acres of faba bean in western Canada. Adapted from Phelps 2017.

In 2013, there were 50,000 acres of faba bean seeded in Canada, especially in Alberta, Saskatchewan, and Manitoba. In 2015, faba bean production raised to approximately 62,000 acres in Saskatchewan from 19,000 in 2014 and 8,500 in 2013 (Phelps 2017) (Figures 1.1 and 1.2). Faba

bean has increased rapidly in acres in western Canada, because of its great resource of protein and energy. However, the use of faba bean as a source of silage is unknown and very limited. Therefore, the objective of this study was to develop an alternative feeding strategy to highly and efficiently utilize the emerging silage with innovative enzymatic solution for western Canadian dairy and beef producers in comparison with conventional silages in western Canada.

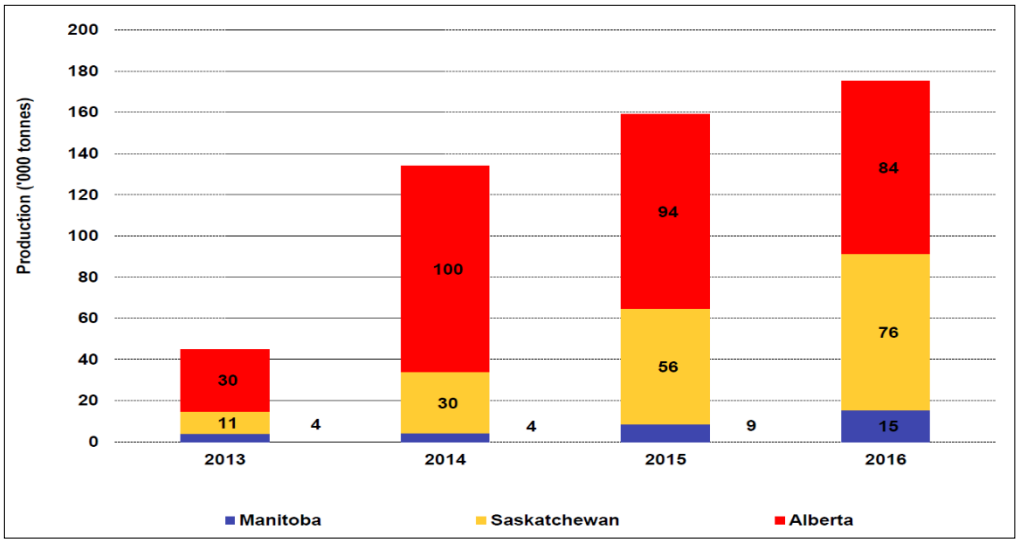


Figure 1.2. Faba bean production in Canada by province from year 2013 to 2016. Adapted from McGill 2016.

2. LITERATURE REVIEW

2.1. Introduction: Origin and History of Faba bean

Faba bean (*Vicia faba* L.) is an annual legume also known as Fava bean, Broad bean (large seed), Horse bean, Windsor bean, Tick bean (small seed), which is widely used worldwide (Singh and Bhatt 2012b). Faba bean is one of the most widespread and oldest grain legumes in the temperate regions and it is the third most important feed grain legumes after soybean (*Glycine max* L.) and pea (*Pisum sativum* L.) (Hanelt and Mettin 1989; Singh and Bhatt 2012b; Singh et al. 2013). Faba bean has been cultivated over thousands of years, although its origin is still unclear;

recent survey indicated that faba bean is originated in the Near East and then spread to the Mediterranean, Central Europe and North Africa (Zohary and Hopf 1973; Singh et al. 2013). Chinese have used faba bean as food for 5,000 years, and Egyptian cultivated faba bean for 3,000 years, and later Greeks and Romans started to use it as food resource (Singh and Bhatt 2012a; Singh et al. 2013). Nowadays, the most common use of faba bean is as human food in developing countries; on the other hand, the United States and Northern Europe primarily use it as livestock feed (Duc 1997; Singh et al. 2013).

2.2. Characteristic of Faba Bean

The *Vicia faba* is an annual legume crop which has thick stem that can keeps the plant up to 1.5 meters tall. This plant also has a thick long root that can reach into soil up to 1 meter long with nitrogen fixation (Singh et al. 2012). It has large pinnate leaves and they usually grows two to six leaflets (Singh et al. 2012). Faba bean can be planted in winter or spring, winter cultivars usually grow higher than summer cultivars (Singh et al. 2012). Faba bean has multiple colors of flowers such as white, brown or violet and flowers clutches can produce 1 to 4 pods per clutch. However, like other legumes, only 15% of flowers will develop pods (Singh et al. 2012). Pods of faba bean are long and green and seeds inside will turn dark brown black when they mature. Flowering usually occurs approximately 45-60 days following emergence, and full maturity is reached within 110-130 days (Alberta Faba bean Producers Manual 2013). Normally, faba bean can be classified by size of seeds in to three groups: large seeded *Vicia faba major* (broad bean), small seeded *Vicia faba minor* (field or horse bean) and medium seeded type *Vicia faba equine*. Sometimes, size and number of seeds can be difference among the plants. For instance, higher seed sets were found on the bottom podding nodes of a stem.

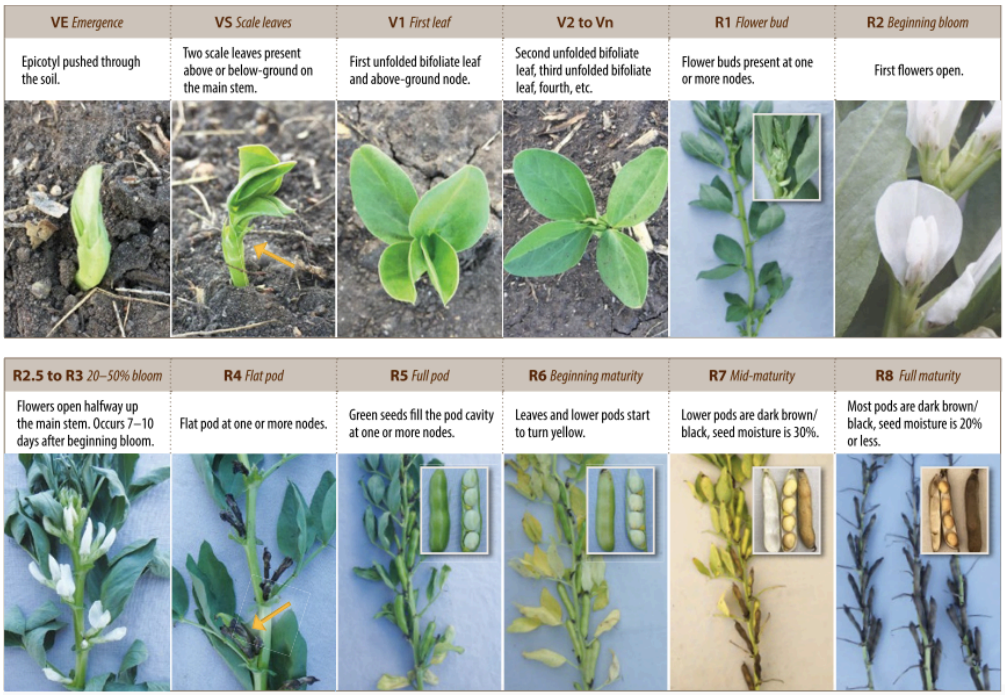


Figure 2.1. Faba bean growth staging guide. Adapted from Saskatchewan Pulse Growers (2019).

This crop is capable of growing under cool and moist conditions, which is suitable for growing in mid-west Canada. Moreover, it can tolerate winter temperature with -15 ℃ but hot, dry weather can be injurious to the plant, the optimal temperature for this plant is 4℃. The ideal rainfall is 650 to 1000 mm per year, and the adequate soil pH ranging from 6.5 to 8 (Singh et al. 2013). Although faba bean is primarily grown for its edible seeds, but it can also be used as a whole crop due to its considerable economic potential (Hanelt and Mettin 1989; Alberta Faba Bean Producer Manual 1.0, 2014; O'Kiely et al. 2018).

2.3. Nutrition Value

Faba bean is considered as a potential source of both protein and energy for human consumption and in animal feed. The crude protein content of faba bean can reach up to 30% of dry matter basis, approximately 37% of starch and with a good composition of amino acid (Yu 2005). Chemical composition of faba bean in comparison with other feed grains is present in Table

2.1. It shows that faba bean has the ability to compete with other conventional feeds. Faba bean has 866 g/kg in dry matter, 290 g/kg DM in crude protein, 159 g/kg DM in neutral detergent fiber and 80% N degradability (O'Kiely et al. 2018). Faba bean contains higher lysine and lower fat content in comparison with other common feeds. These make faba bean easily degraded in rumen and provide sufficient amino acid to dairy cows. The energy in faba bean is also as good as other cereal grains, because of its high content of starch (O'Kiely et al. 2018).

Table 2.1. Average and range in chemical composition of faba bean seeds (beans) and, for comparison, average values for barley grain, soybean meal and pea seed. Adapted from O'Kiely et al. (2018).

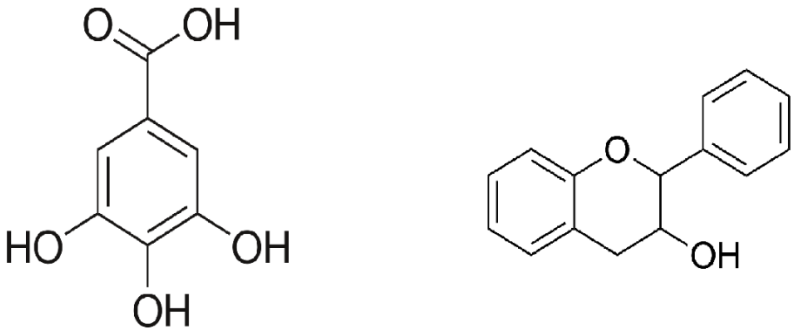
Constituents	Faba bean	Barley grain	Soybean meal	Pea seed
DM (%)	86.6	87.1	87.9	86.6
CP (% DM)	29.0	11.8	51.8	23.9
NDF (%DM)	15.9	21.7	13.7	14.2
ADF (%DM)	10.7	6.4	8.3	7.0
EE (%DM)	1.4	2.0	2.0	1.2
Starch (%DM)	44.7	59.7	5.0	51.3
Sugar (%DM)	3.6	2.0	9.4	4.9
Ash (%DM)	3.9	2.6	7.1	3.5
ME (Mcal/kg)	3.13	2.96	3.25	3.20
Ca (g/kg DM)	1.5	0.8	3.9	1.2
P (g/kg DM)	5.5	3.9	6.9	4.5
K (g/kg DM)	11.5	5.7	23.7	11.3
Na (g/kg DM)	0.1	0.1	0.1	0.0
Mg (g/kg DM)	1.8	1.3	3.1	1.7
Mn (mg/kg DM)	10.0	19.0	45.0	10.0
Zn (mg/kg DM)	34.0	30.0	54.0	37.0
Cu (mg/kg DM)	13.0	12.0	18.0	8.0
Fe (mg/kg DM)	75.0	184.0	346.0	107.0
Cysteine (g/kg protein)	12.0	22.0	15.0	14.0
Lysine (g/kg protein)	62.0	37.0	61.0	72.0
Methionine (g/kg protein)	8.0	17.0	14.0	10.0
Condensed tannin (g/kg DM)	4.8	-	-	0.1

2.2.1. Anti-nutritional Factors in Faba Bean

Tannins

Faba bean may be consider as a satisfactory feed for livestock. However, they contain a small amount of anti-nutritional factors, some of which may affect the animal health and performance, particularly for monogastric animals. Condensed tannins are one of the main anti-nutritional factors in faba bean. They are water-soluble polyphenolic compounds which can be commonly found in many plant species. Tannins play a crucial role in plant defense against pathogens, herbivores, and changing environmental conditions, you can easily find in leaves, flowers, seeds and tree barks. However, tannins bring their anti-nutritional factors influence by precipitate or binding proteins and form either soluble or insoluble complexes to animals which are not yet to be digested. Thus, this may largely affect animal performance.

Tannins can be classified into two main groups which is based on their chemical property: Hydrolysable Tannins (HT) and Condensed Tannin (CT) (Figure 2.2) (Frutos et al. 2004; Singh et al. 2012). Hydrolysable Tannins have a polyhdric alcohol and hydroxyl groups which can be esterified with either gallic or hexahydroxydiphenic acid (Caballero et al. 2003), when heating with weak acid will yield gallic acid or ellagic acid. Condensed tannins are flavonoid polymers and have individual monomers joined through carbon bonds and resist hydrolysis (Singh et al. 2012).



(a) Gallic acid (b) Flavan-3-ol

Figure 2.2. Base unit of two tannins group: (a) Hydrolysable Tannins; (b) Condensed Tannin. Adapted from "Tannin" (2020).

The main negative effect of tannins is reducing palatability and feed intake. The reduction in palatability is due to astringent property of tannins or could be caused through a reaction between the tannins and the salivary mucoproteins (Alokan and Aletor n.d.). Thus, reduction in palatability and feed intake will directly affect animal growth rate. Moreover, tannins will also affect other performance such as fibre digestibility and nitrogen utilization (Alokan and Aletor n.d.). Although tannins have been reported to be responsible for several negative effects on animal performance, but it can also carry out positive effect, depending on which and what types of tannins consuming (Frutos et al. 2004). Previous studies have reported that when ruminants consuming adequate condensed tannins (less than 50 g CT Kg/DM) will benefit livestock by reducing bloat and improve utilization of nitrogen (Barry and McNabb 1999; Frutos et al. 2004; Kelln et al. 2020; Min 2003).

Vicine and Covicine

Vicine and convicine are anti-nutritional factors that accumulated in cotyledons of faba bean. They are belonged to a group of pyrimidine glycosides and can cause a disease called favism in human who are deficient with glucose-6-phosphate dehydrogenase (G6PD) (Khamassi et al.

2013). Symptoms of favism including dark urine, headaches, fever, and abdominal pain, sometimes it can be very severe and life-threatening. Muduuli et al. 1982 have reported that laying hens fed with 10 g vicine kg/diet had lower feed intake, egg weight and hatchability of egg, in addition, this may lead to increased liver weights and liver glutathione levels.

Trypsin and Chymotrypsin Inhibitor

Trypsin and chymotrypsin inhibitor are part of a large group of protease inhibitors. They can be found in many plant seeds, but particularly in legumes. Its main function is a protect mechanism of plant and regulation of endogenous proteinases. However, their ability to inhibit activity of trypsin and other pancreatic enzymes has been widely reported to enlarged pancreas, depressed protein digestion and reduction in growth (Hove and King 1979; Singh et al. 2012). The concentration level of trypsin and chymotrypsin inhibitors is depending on genetic, variety and environment.

Lectin (haemagglutinin)

Lectins are carbohydrate binding proteins which can be found in viruses and plants mostly in legume seeds. They also have the ability to agglutinate animal cells and precipitate multivalent carbohydrates (Hamid and Masood n.d.). Dietary lectins act as protein antigens which adhere to surface glycoproteins in small intestine, thus inhibiting animal growth. It is well evidenced that lectins are toxic to animals. In order to eliminate lectin activity in legume products, heat processing such as dry roasting or toasting, autoclaving, microwaving, and infrared heating treatments can be applied (Pusztai and Grant 1997).

Phytate

Phytate is one of anti-nutritional factors that presents in most of plants and inhibiting mineral absorption. Generally, it is present as a storage form of phosphorus in legume seeds and

the extent of 1% to 5% of dry weight of legume seeds (Huisman et al. 1989). Phytate has mineral binding capacity which may result in reducing mineral absorption through forming complexes with calcium and magnesium. It also affects protein digestibility and inhibits digestive enzymes such as alpha-amylase, lipase, or proteinase (Caballero et al. 2003). Soaking, germination, and fermentation have been demonstrated to reduce the phytate content of feeds (Sandberg 1991; Singh et al. 2012).

2.2.2. Environmental Impact

As well as the growth of global human population, food productions are required doubly to meet demand. Nitrogen is an essential nutrient for plants growth and development (Sulieman 2011; Hossain et al. 2016), and it is dependent with agricultural production and environment (Figure 2.3). However, current cropping system are heavily relied on high input of inorganic N-fertilizer. In order to minimize the use of N-fertilizer and pollution to environment, introducing pulse crops into cropping system can be one of effective methods due to their biological nitrogen fixation ability.

Faba bean is one of the highest nitrogen fixing grain crop. Barker and Ag (2019) found that faba bean inoculated with the proper bacteria *Rhizobium leguminosarum* bv. *Viciae* can fix approximate 90 % nitrogen of their requirement. In western Canada, faba bean was reported to have highest N-fixed (45.35 ± 10.9 kg/ha) and highest seed yield (1948 ± 264 kg/ha) in comparison with other pulse crops such as chick pea, dry bean, field pea, and lentil (Hossain et al. 2016). Consequently, introducing faba bean into cropping system shows a number of benefits. The main function of faba bean is to contribute great source of protein and energy, besides it can also supply nitrogen to the system and improve microbial diversity (Jensen et al. 2010).

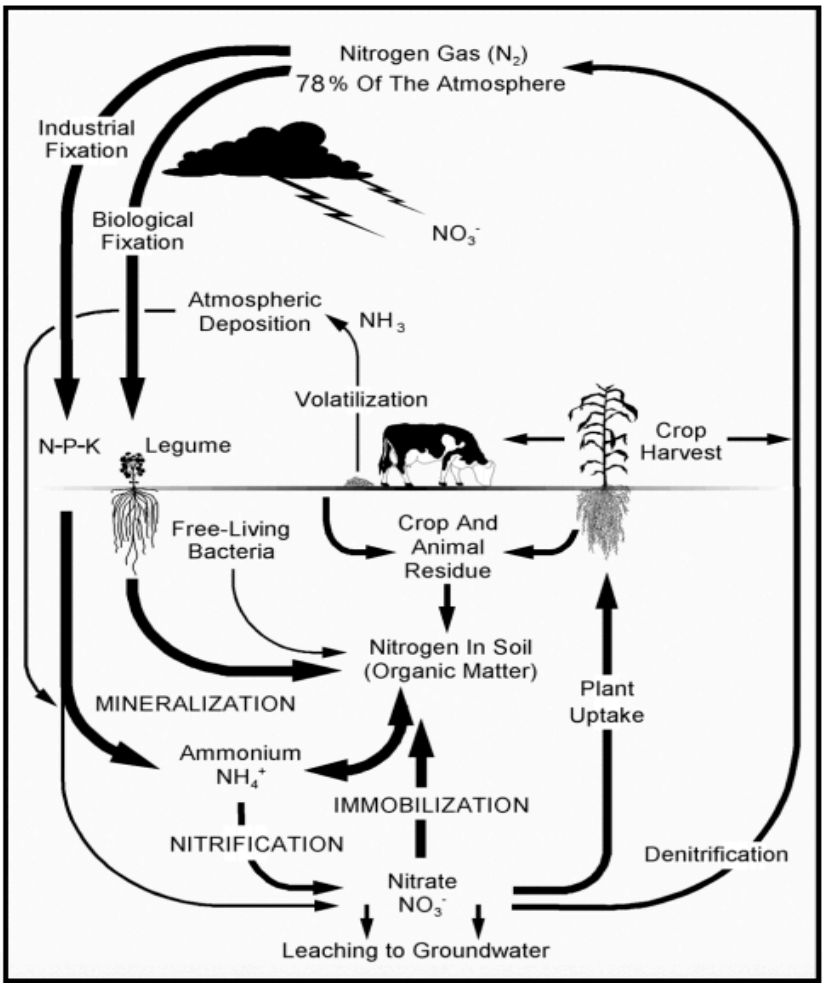


Figure 2.3. Nitrogen forms and pathways within an agricultural production system. Adapted from McKague et al. (2005).

2.3. Faba Bean as Animal Feed

2.3.1. Faba Bean as Animal Feeds for Monogastric

Faba bean is considered as an alternative source of protein and energy in pigs feed. Faba bean seeds have relatively high protein content (about 300 g/kg DM) and with similar amino acid content except methionine when compare to soybean (Emiola and Gous 2011). However, the use of faba bean in pig feeds has not been widely applied due to faba bean its anti-nutritional factors such as trypsin, lectin, phytate, and tannins. Because of these reasons, faba bean has been reported

not suitable for growing-finishing pigs as a sole protein source (Thacker and Kirkwood 1992). Nonetheless, several studies have demonstrated that faba bean has a significant impact on animal performance and carcass quality in pigs.

Aherne et al. (1977) has reported that when dietary faba bean level exceed 200 g/kg of feed reduced feed intake and growth performance and increased feed conversion ratios. A later study with growing-finishing pigs showed similar results (Landry 2014). Result from another study showed there was a quadratic effect on daily weight gain when pigs fed with increasing substitution of rapeseed meal with faba bean (Partanen et al. 2003). In both studies, the reduction in growth performance may be due to unbalanced amino acid of faba bean and tannins. Furthermore, another study examined the effect of zero-tannins faba bean on finisher pigs. The results showed that an inclusion rate up to 30% of faba bean does not alter average daily gain and feed efficiency in finisher pigs (Zijlstra et al. 2008).

2.3.2. Faba Bean as Animal Feeds for Poultry

Faba bean is also considered as a protein supplement for poultry. However, the anti-nutritional factors of faba bean has a significant negative impact on protein digestibility and growth performance, as shown by Wareham et al. (1993) and Lacassagne et al. (1991). Studies in Europe reported that growth rate of young chickens (0 to 4 weeks age) fed with faba bean has no difference with the chickens fed with the control diet (Blair et al. 1970). Similar results were found in Canada. The experiment was conducted with male Leghorn chicks fed heat treated faba bean diet (Marquardt and Campbell 1973). The optimum inclusion of faba bean for broilers is up to 250 g/kg diets (Gous 2011). It was suggested that a diet containing faba beans supplemented with methionine will promote the growth rate in young chickens which is similar to the diet containing

soybean meal. Since most broiler feeds are pelleted, it would appear that processed faba beans could be used successfully as an alternative protein source in feeds for broilers.

2.3.3. Faba Bean as Animal Feeds for Ruminants

The major difference between ruminants and monogastric is that for ruminant protein quality is dependent on the availability of amino acid leaving rumen instead of ingested diet. Ruminants only digest and absorb when protein access to the small intestine. The rumen degradability and soluble fraction of faba beans are relatively high, so do other grain legumes. The nitrogen degradability of faba bean, soy bean, and pea can reach up to 80% (Mustafa and Seguin 2003). Fortunately, heat-based treatments such as dry roasting, pressure toasting and extrusion, seem to be effectively lower the protease inhibitors and anti-nutritional factors and increase the protein fraction escaping the rumen degradation (Volpelli et al. 2010).

Faba bean, with such high proportion of protein, can be successfully used as sole diet for ruminants. Not only has the high biological value, faba bean is also palatable to cattle. There are a few studies that investigated faba bean as protein and energy sources in ruminants in order to seek an alternative feed for soybean meal. For instance, a study showed that the use of 30% of ground faba bean in the concentrate feed for dairy cows does not alter the feed consumption, the milk production or the milk composition (Brunschwig and Lamy 2002). When substituted faba bean silage for corn-alfalfa silage had no negative effect on daily dry matter intake, average daily gain or feed conversion.

2.3.4. Whole Plant Faba Bean Silage

Traditionally, legumes are less competitive than cereal species because of mineral soil nitrogen uptake and anti-nutritional factors in legumes (Carton et al. 2018). However, since 2002 tannin-free faba bean production has increased due to its lack of anti-nutritional factors, high

energy, high protein, high yields, and similar production costs relative to field pea (Strydhorst et al. 2008). This made faba bean a candidate as an alternative feed for animals. Faba bean (*Vicia faba* L.) can be used in different ways, such as whole plant fresh forage, silage, seeds, and other heat-treated product which may be considered as concentration for animals. Besides, use of faba bean as a forage source in dairy is still limited. The maximum dry matter yields and crude protein of faba bean forage has been reported as 7.8 t/ha and 180 g/kg dry matter in United Kingdom (Fraser et al. 2001). Silage is a type of fodder made from green forage crops which have been preserved by acidification. Silage achieved through fermentation with high moisture end product.

During fermentative process, rumen microbes will act on the cellulose and carbohydrates in the forage to produce volatile fatty acids such as acetic, propionic, lactic, and butyric acids, which are the main energy source for ruminants. Nonetheless, this process requires consideration of a wide variety of factors including plant growth, harvest, storage, and feeding practices. There are numerous advantages in silage including greater nutritive value, more stable nutrients preserved, longer preserved period and less affect by the climate. Figure 2.4 shows the changes in protein fractions of three different legumes.

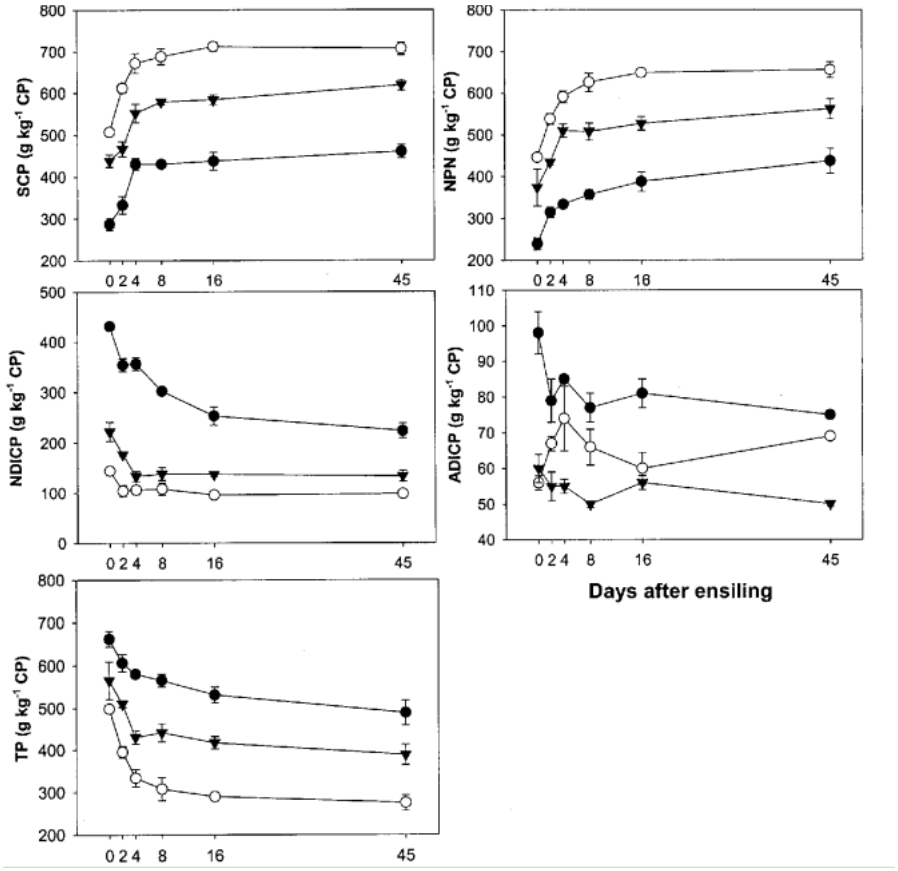


Figure 2.4. Changes in soluble protein (SCP), non-protein nitrogen (NPN), neutral detergent insoluble protein (NDICP), acid detergent insoluble protein (ADICP), and true protein (TP) of ensiled whole-plant forage faba bean (●), pea (○), and soybean (▼). Vertical bars represent ± SD. Adapted from Mustafa and Seguin (2003).

We can see there was an increase in soluble protein and non-protein nitrogen, and a reduction in neutral detergent insoluble protein and true protein. This is due to the proteolysis of legume silage (Heron et al. 1986). Table 2.2 shows the chemical composition between faba bean silage and other legume silages.

Table 2.2. Chemical composition of whole-plant legume forages after 45 d of ensiling. Adapted from Mustafa and Seguin (2003).

	Legume silage			SEM[z]
	Faba bean	Soybean	Pea	
DM (g/kg)	261	257	250	7.4
Ash (g/kg DM)	106a	102a	81b	2.1
NDF (g/kg DM)	428	420	416	7.4
ADF (g/kg DM)	313a	292b	312a	3.6
ADL (g/kg DM)	110	113	111	3.1
Starch (g/kg DM)	44b	38c	79a	1.5
CP (g/kg DM)	222a	197b	178c	3.6
SCP (g/kg CP)	460c	619b	706a	8.4
NPN (g/kg CP)	437c	562b	656a	14.2
NDICP (g/kg CP)	224a	133b	98c	6.5
ADICP (g/kg CP)	75a	50c	69b	6
True protein				
Total (g/kg CP)	488a	388b	275c	14
Rapidly degradable protein (g/kg CP)	23	57	50	15.1
Intermediately degradable protein (g/kg CP)	316a	249b	196c	10.6
Slowly degradable protein (g/kg CP)	149a	83b	29c	6.6
DM recovery (mini silo, %)	97.9	96.7	96.9	0.35

A study reported the effect of faba bean silage as sole forage in lactating cows and comparison between chemical composition of faba bean silage and grass-legume (Table 2.3). Faba bean silage was higher in protein (20.1 vs. 16.l%) and lower in crude fibre (25.0 vs. 29.6%), ether extract (1.8 vs.3.2%), ash (7.1 vs. 8.0%), calcium (0.32 vs. 0.85 %) and phosphorus (0.39 vs. 0.43%) than grass-legume silage (McKnight and MacLeod 1977). Nonetheless, faba bean silage had relatively high NDF and lower indigestible fibre content (lignin) in comparison with different legume forages. Digestibility of neutral detergent fiber (NDF) is positively related to dry matter intake and milk yield in dairy cows (Oba and Allen 1999). Mustafa and Seguin (2003) reported low effective degradability of NDF of faba bean silage. Thus, it is important to improve faba bean silage digestibility to maximize milk production in dairy cows. As shown, faba bean silage has the potential as a sole forage source that could compete with other traditional silage.

Table 2.3. Daily intake, body weight change and milk composition from cows fed faba bean silage and grass-legume silage. Adapted and modified from McKnight and MacLeod (1977).

	Faba bean Silage	Grass-Legume Silage	
Intake (kg/day)			SEM
Silage (as fed)	32.5	33.3	0.65
Concentrate (as fed)	8.5	8.5	
Total (DM)	17.9 b	18.8 a	0.27
Body Wt Change (kg/30days)	10.4	3.4	2.11
Milk Composition			
Milk yield (kg/day)	22.4	22.8	0.17
Milk Protein (%)	4.09	3.77	0.12
Milk Fat (%)	3.36	3.42	0.03
Total Solid (%)	12.86	12.6	0.15

*a, b Means within columns within differing letters differ ($P< 0.05$).

2.4. Forage Fiber

2.4.1. Fiber Concentration

Forage is extensively used in most of ruminant industries worldwide. The most abundant composition in the forage is fiber which refers to the cell wall constituents of hemicelluloses, cellulose, and lignin. Understanding fiber digestibility and content is important in ruminant ration because it is the primary factor that determining feed intake and animal performance. Also, digestibility of NDF can be used as an important parameter identify forage quality because of its variability among forages and consistent effect on animal performance (Oba and Allen 1999). Hence, if a greater utilization of forage was applied to ruminant, it would bring a tremendous impact to industries and animal production.

In general, high NDF content indicates that there is a high fiber content in forage, whereas the lower NDF value refers to higher available energy in forage. Based on previous research, cows fed with lower level of dietary NDF diet has increased significantly in milk yield and dry matter intake (Oba and Allen 2000). Grass forage usually contains higher NDF content in comparison with legume forage. Allen (2000) reported that higher NDF content diet often brings a negative

impact to animal, such as reduces in milk production. For legume forage which contains less than 40% of NDF may be considered as high quality, whereas for grass forage should be lower than 50%. The reason why dairy cows require large amount of NDF from forage because it's closely related to chewing activity and rumen pH (Allen 1997). Table 2.4 showed the recommend NDF concentration for lactating cows when fed with a Total Mixed Ration (NRC 2001). However, the concentration of NDF may widely differentiate among the forage species, growing environment, and forage maturity stages. NRC (2001) have clearly stated that the adequate NDF concentration should be formulated on the actual composition of feedstuff, instead of the provided table.

Table 2.4. Recommended Minimum Concentrations (% of DM) of Total and Forage NDF and Recommended Maximum Concentrations (% of DM) of NFC for Diets of Lactating Cows. Adapted from NRC (2001).

Minimum Forage NDF	Minimum Dietary NDF	Minimum Dietary NFC[1]	Minimum Dietary ADF
19	25	44	17
18	27	42	18
17	29	40	19
16	31	38	20
15	33	36	21

[1] Non-fiber carbohydrate is calculated by difference 100 - (%NDF + %CP + %Fat +%Ash)

2.4.2. Fiber Digestibility

Although the NDF content of forage is the dominant factor that affecting feed intake, however, digestibility of fiber also plays a critical role in animal performance. According to NRC (2001), ruminal digestibility of forage NDF can range from less than 25% to over 75% for different forage types (NRC 2001). Figure 2.5 presents the differences in NDF digestibility of common forage species. Legumes usually have greater lignification as compared with grass and corn silage, thus it makes legume silage to have lower NDF digestibility. There are numerous factors that attribute to digestibility of NDF, primary is the plant maturity stage. As plant mature, it developes

xylem tissue for structural support, water transport and accumulation of complex carbohydrates, and this process is known as lignification which result in depressing NDF digestibility (Hoffman et al. n.d.). Other factors such as plant genetic, growing environment, and management may also influence NDF digestibility.

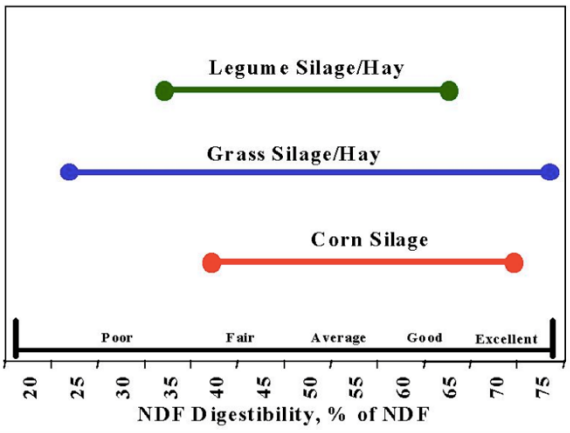


Figure 2.5. Differences in NDF digestibility between common forage species. Adapted from Hoffman et al. (2001).

Several researchers have investigated the interactions between NDF digestibility and animal performance. For instance, in animal trials where cows fed with a higher in vitro digestibility of NDF, significantly increased in DMI and milk production (Grant et al. 1995; Oba and Allen 1999; Kendall et al. 2009). In addition, enhanced NDF digestibility of forage may result in a faster disappearance of NDF from rumen, thus reducing physical rumen fill, and increase voluntary feed intake (Raffrenato 2011). Oba and Allen (1999) have also reported that marginal effect of NDF digestibility on animal performance which 1 unit of increase in in vitro or in situ NDF digestibility positively associated with 0.17 and 0.25 kg/day increase in DMI and 4% FCM yield, respectively.

2.4.3. Evaluation of Fiber Digestibility

Fibre digestion is a complex process that can be affected by many different factors, such as plant characteristics, microbial population and animals. In order to predict the dynamic nutrient

flow and feed energy value, passage rate of fiber digestion and the potentially digestible fiber are required to be mathematical described. Once fiber enters the rumen, it can be split into two fractions: potentially digestible fraction (pdNDF) and indigestible fraction (iNDF) (Waldo et al. 1972). When fiber first enters rumen, pdNDF fractions will not be digested by the rumen protozoa and bacteria immediately. The lag period at time zero is known as lag phase, which can be various among forage composition (Figure 2.6).

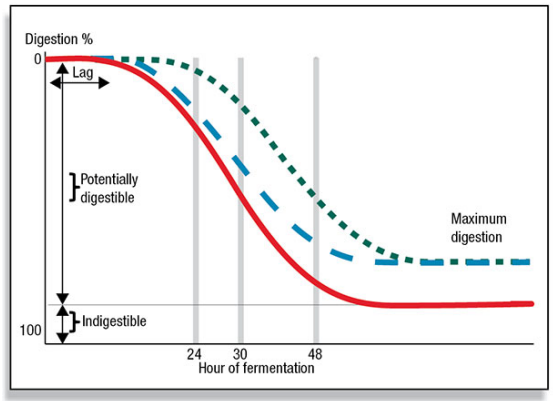


Figure 2.6. Ruminal fiber digestion of three example forages. Adapted from Siciliano-Jones (n.d.).

The iNDF fraction is an estimation of long-term incubation of fiber in in situ or in vitro digestions (Waldo et al. 1972). When NDF residue remaining after 240 h of incubation (uNDF240), which refers to the functional component of fiber and highly related to physical effectiveness, gut fill, digestion and passage rate (Van Amburgh et al. 2015). Another more recently procedure to obtain uNDF is using the measurement of lignin and multiply by 2.4 (Van Amburgh et al. 2015). However, several studies have reported that the results were inconsistence. The pdNDF is calculated by subtracting the uNDF fraction from total NDF:

[1]

The iNDF fraction can only disappear by the passage rate because of its indigestible fraction. While the disappearance of pdNDF fraction is affected by both digestion and passage.

Aside from the digestible and indigestible fiber pools, there are two important parameters need to be considered for prediction of NDF digestibility: the rate of digestion (K_d) and the rate of passage (K_p). The rate of digestion can be various among forage species, for example, potentially digestible fibre in alfalfa (4–6% per hour) is twice faster digested that than corn silage (2–3% per hour) (Combs 2016). Once potentially digestible fiber enters the gastrointestinal tract, the amount of the fiber that is digested per unit time is refers to a constant fraction (K_d) (Smith et al. 1971). The rate of digestion can be measured by in vitro or in situ method or by measuring the volume of gas production produced by fermentation. The rate of passage (K_p) is described as retention times of feed and can be measured by using Cr-mordanted fiber as K_p markers (NRC 2001a; Dijkstra et al. 2005). Rate of passage is highly correlated to rumen capacity and feed intake, yet, rumen capacity have been reported to have a linear function of body weight (Allen and Mertens 1988).

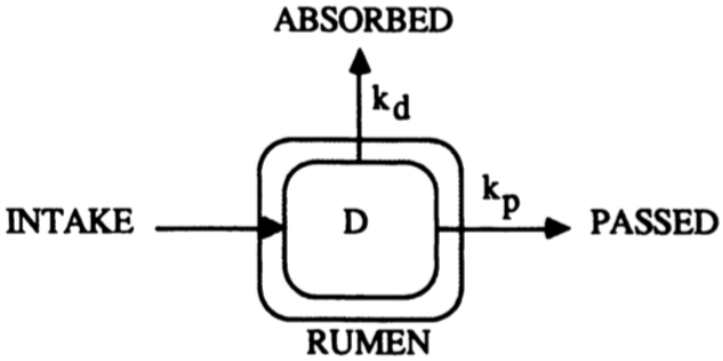


Figure 2.7. Diagrammatic representation of a simple kinetic model of fiber disappearance from the rumen. Potentially digestible fiber is represented as D, fractional rate of digestion as K_d and fractional rate of passage as K_p. Adapted from Allen and Mertens (1988).

Most of forage fiber is primarily digested in the rumen (90-95%) (Huhtanen et al. 2010). However, to accurately calculate the digestibility of NDF, the digestions that occur beyond rumen must be accounted. Apparent diet digestibility in ruminant is usually lower than true digestibility. This is because feces contain endogenous protein coming from salivary mucoproteins, sloughed epithelial cells (Figure 2.8) (Mertens 2009).

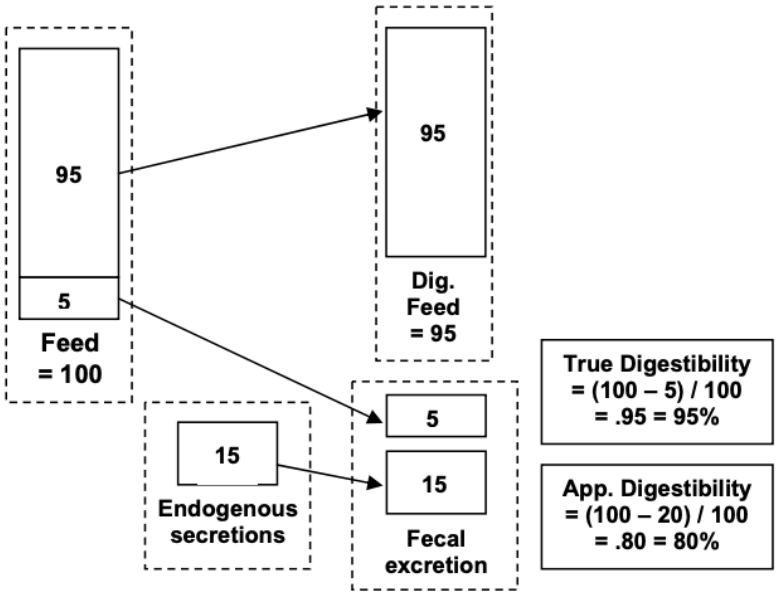


Figure 2.8. The relationship between true and apparent digestibility. Adapted from Mertens (2009).

Thus, the equation of forage fiber digestibility developed by Allen and Mertens (1988) is presented below:

[2]

Where pdNDF is refers to potentially digestible NDF, K_d is the rate of digestion and K_p is the rate of passage of potentially digestible NDF from the rumen. A recent study (Combs 2014) developed a new lab assay to evaluate Total Tract NDF Digestion (TTNDFD) in ruminants, which is shown as follow:

Total tract NDF digestibility = rumen digested NDF + hindgut digested NDF [3]

Understanding NDF content and digestibility is the most important due to its great impact on voluntary feed intake and milk production. Moreover, fiber is also the least digestible component in feed, this makes it as a perfect parameter for determining forage quality.

2.4.4. Fiber Digestion: Plant Cell Wall

The biggest anatomical difference between ruminant and non-ruminant animals is that ruminant has multi-compartment digestive tract which can effectively digest plant material. Plant material contains a large amount of cell wall carbohydrate, which is the major factor that limiting feed intake and energy availability of forage crops in beef and dairy production (Hatfield 1989). Several studies (Mertens, 1973; Reid et al., 1988; Jung & Allen, 1995) also reported negative correlations between NDF and voluntary intake, indicating that reduction in forage NDF will allow higher intake for ruminants.

The cell wall of plants is a complex matrix composed of polysaccharides, proteins, phenolics, water, and minerals, but mainly composed by carbohydrate (up to 90%) (Caffall and Mohnen 2009). Polysaccharides are the major group that made up cell walls and represent as energy source for ruminant. Polysaccharides can be classified by their Physico-chemical properties into three groups, including (1) pectic, (2) hemicellulose, and (3) cellulose fraction. Pectic polysaccharides contain galacturonans and rhamnogalacturonan-I. Galacturonans have a backbone that consists of α-1,4-linked galacturonic acid (Caffall and Mohnen 2009). Its function is involved with the cell growth and cell differentiation.

Cellulose is the most abundant structural polysaccharide in plants which contains 40 to 45% in plant cell wall. It consists of linear chain of glucose linked by β-1,4-glycosidic bond (Figure 2.9) and having simple primary and complex tertiary structures. Hemicellulose are generally classified according to the main sugar residue in the backbone such as xylan, mannans, and glucans (Wyman et al. n.d.). It accounts for 30 to 35% in plant cell wall and can be extracted by alkali. Arabinoxylans are a hemicellulose which can be found in cereal endosperm and lignified tissues.

It contains a large amount of acetic (ferulic acid) and phenolic acids (*p*-coumaric) which are covalently linked (Bedford and Partridge 2010).

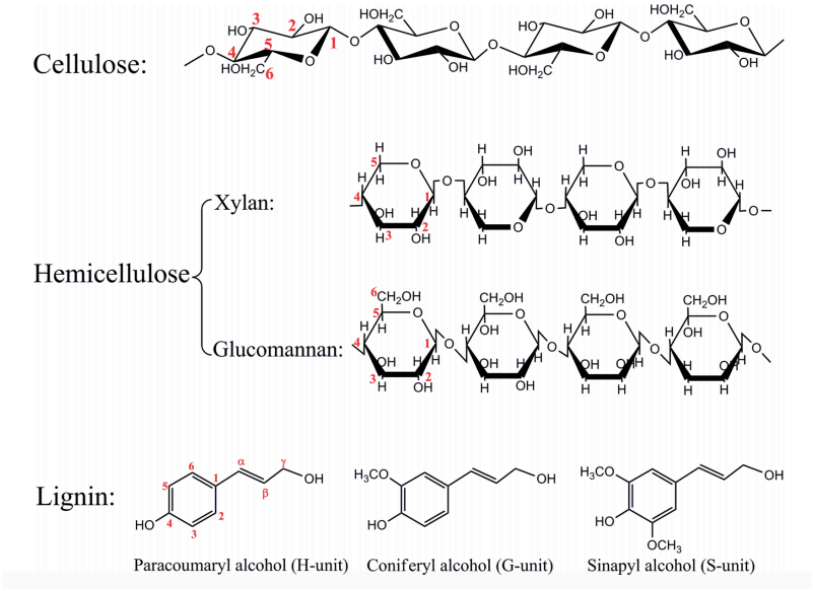


Figure 2.9. Structures of cellulose, hemicellulose, and lignin. Adapted from Zhang et al. (2011).

Lignin is a complex, hydrophobic, and indigestible polymer that presents in most of plants. As one of major polymer of cell walls, its main function is supporting plant tissues and provides waterproofing property to plant cell wall. However, lignin has been reported that is has a negative impact on animal productivity. For instance, previous research indicated that it reduced the quality and digestibility of forage crops such as alfalfa (Humphreys and Chapple 2002). Lignin is made up of phenylpropenyl randomly branched units which are connected through carbon-carbon and carbon-oxygen (ether) bonds (Cherubini and Strømman 2011). The three originators of lignin are *β*-coumaryl alcohols, coniferyl, and sinapyl (Figure 2.9). These originators will then involve in three major biosynthetic pathways: the shikimate, general phenylpropanoid, and lignin branch pathways (Barber and Mitchell 1997), which will lead them to the synthesis of lignin intermediates such as *p*-coumaric acid, ferulic acid, and diferulic acid.

2.5. The Use of Exogenous Enzyme

2.5.1. Introduction of Exogenous Enzymes

Over the decades, world population grew to six billion and the population growth is still continuing. It can be expected that the demand of animal protein will increase at an even greater rate. Further, if a greater utilization of forage was applied to ruminant, it would have a vast impact on cost reduction. Exogenous enzyme has been used extensively since 1990s (McAllister et al. 2000), the purposes of using exogenous enzyme are to (1) prevent the anti-nutritional factors in animal feed, (2) improve availability of nutrient in digestive system, (3) break down specific chemical bonds in raw materials, (4) act as a supplement for young animals that lack a mature digestive system (Bedford and Partridge 2010). Exogenous enzyme can be grouped into four types based on their functions which are used to break down fibre, protein, starch, and phytic acid (Bedford and Partridge 2010). Several previous research teams begun to study the efficacy of adding enzyme as an additive in animal feed (Burroughs et al. 1960; Perry et al. 1966), and found that enzyme supplementation has a positive response in different aspect of animal performance.

Most of enzyme are proteins that naturally act as biological catalysts, and they are mostly derived from four bacterial (*Bacillus subtilis*, *Lactobacillus acidophilus*, *L. plantarum* and *Streptococcus faecium*) and three fungal (*Aspergillus oryzae*, *Trichoderma reesei* and *Saccharomyces cerevisiae*) species (Bedford and Partridge 2010). The enzymatic solutions used in ruminants are primarily dependent on their capacity of breaking down plant cell walls, which are often referred to cellulases or xylanases (McAllister et al. 2000).

Cellulases and xylanases are the enzyme whose major function is to hydrolyse the β-1,4-glycosidic linkages in plant structural polysaccharides and convert into monosaccharides, known

as cellulose and xylan. The three main types of cellulase components are β-1,4-endoglucanase, which cleaves internal β-1,4-glycosidic bonds; exoglucanase which releases sugar from the cellulose and β-glucosidase, which is responsible for cellulose hydrolysis (Gilbert and Hazlewood 1993).

The main component in hemicellulose are xylan and mannan, which comprise a backbone of xylose residues linked by β-1,4-glycosidic bonds. In contrast to cellulose, they require a more complex repertoire of enzymes to hydrolyse xylan or mannan to soluble sugar. The two main types of enzymes that attach the back-bone structure are endoxylanases (xylanases) and endomannanases (mannanases). Other hemicellulases, including β-xylosidase, β-mannosidase, α-L-arabinofuranosidase, α-D-glucuronidase, α-galactosidase, acetyl and phenyl esterases, are responsible for removing side chains and substituents (Bedford and Partridge 2010). Figure 2.10 shows the pits in the cell walls of barley straw that were caused by fibrolytic enzyme. However, this is only observed when applying high concentration of fibrolytic enzyme.

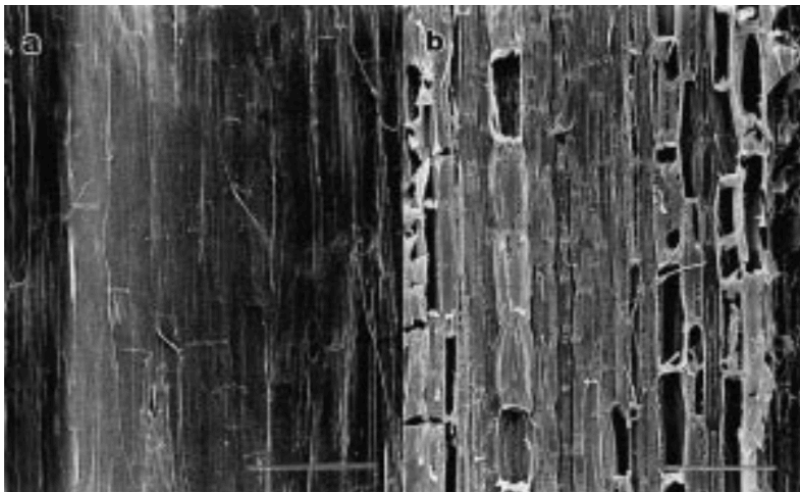


Figure 2.10. Scanning electron micrographs of barley straw, (a) untreated or (b) incubated in a 1:10 dilution of concentrated enzyme product containing cellulases and xylanases. Adapted from Bedford and Partridge (2010).

2.5.2. Factors Affecting Enzyme Action

In the literature, there are numerous studies that reported of evaluations of effect of exogenous enzymatic solution, however the responses are in a wide range with variation. This variance may be attributed to the following reasons: (1) enzyme product composition; (2) time of enzyme delivery; (3) enzyme activity; (4) types of substrates; (5) level of enzyme application; and (6) stage of lactation of dairy cows (Adesogan 2005). Commercial and experimental enzymatic solution are products of microbial fermentation process, the most common types of enzyme mixture for ruminant are cellulases and/or xylanases. However, even with similar level of enzyme mixture and identical labels, the effect of enzymes may differ on ruminal fiber digestion.

Enzyme activity is dictated on several factors including the type and stability of enzyme, animal species, ruminal pH, temperature, moisture, gastrointestinal condition and other factors that related to ruminal environment (Mendoza et al. 2014). These factors may cause an overestimated or underestimated of measuring enzyme activity because the enzyme activities are measured on model substrate which cannot represent the complexity of plant cell wall.

2.5.3. Exogenous Enzyme Responses in Ruminant Diet

Fibrolytic enzymes (β-glucanases and xylanases) were first used in swine and poultry diet in order to improve feed utilization and to remove anti-nutritional factors in the feed (Mendoza et al. 2014). It was not used in ruminant ration because the enzyme will be affected by ruminal proteolysis and rumen microbes (Adesogan 2005; Mendoza et al. 2014). However, these concerns have been opposed to more recent studies that suggested the addition of enzymes could benefit animal performance when ruminal digestibility of NDF is less than 50%.

Beef Cattle

The use of exogenous fiber degrading enzyme additives for ruminants was first examined in the 1960s (Burroughs et al. 1960). A number of evidences have showed that exogenous enzymes fed with forage could improve average daily gain and feed efficiency in beef cattle (Salem et al. 2013). When adding fibrolytic enzymes to grass hay before feeding beef steers increased DM intake, particulate passage rate, and DM, NDF, and ADF digestibility (Feng et al. 1996). Table 2.5 shows the effect of exogenous enzyme that fed to beef cattle. Despite these studies provided the potential benefits of using exogenous enzyme, but they showed the inconsistency result on animal performance. This might possibly due to these commercial enzymes haven't been widely evaluated under a range of dietary conditions and different forage sources.

Table 2.5. Effect of exogenous enzyme on beef cattle performance.

Enzyme	Forage Source	Feed Intake	ADG	Digestibility	Reference
Cellulases +Xylanases	Wheat straw	Not reported	Increased	Not affected	(Balci et al. 2007)
Cellulases +Xylanases	Barley silage/barley straw	Not affected	Not reported	ADF increased	(Krause et al. 1998)
Cellulases +Xylanases	Barley silage	Increased	Not affected	DM, NDF increased	(McAllister et al. 1999)
Cellulases +Xylanases	Grass hay+ barley	Not affected	Not reported	DM, ADF & NDF increased	(Lewis et al. 1999)
Cellulases +Xylanases	Grass hay	Increased	Not affected	DM, NDF &ADF increased	(Krueger et al. 2008)
Endoglucanase+ exoglucanase+ xylanase+ α-amylase	Alfalfa hay+ corn silage	Not affected	Not affected	Not reported	(Eun et al. 2009)

Dairy Cattle

The use of exogenous fiber degrading enzyme additives for ruminants was first examined in the 1960s (Burroughs et al. 1960). In order to improve forage fiber digestibility and lactational performance of dairy cows, the use of fibrolytic enzyme has been rising in the past decades. A number of previous studies involving dairy cows reported that fibrolytic enzymes could substantially improve animal performance and milk production (McAllister et al. 1999; Kung et al. 2002). However, similar to beef cattle, responses by dairy cattle to exogenous enzymes have also been variable (See Table 2.6). Some of the results showed a great impact on milk performance. For instance, Yang et al. (1999) reported that increasing the amount of enzyme applied to alfalfa cubes from 1 g/kg to 2 g/kg increased milk production from 23.7 kg/d (control) to 24.6 kg/d and 25.6 kg/d, respectively (Table 2.6; Yang et al. 1999). Moreover, another study also showed that cows consuming alfalfa based-diet with enzymes (cellulase and xylanase mixed) had greater milk production than the control diet (27.2 kg/d vs. 25.9 kg/d), also digested more DM per day (Table 2.6; Lewis et al. 1999). On the contrary, there was no response in milk composition when dairy cows fed with the same enzyme and same forage source (Rode et al. 1999).

It is clear that adding exogenous enzymes to dairy cattle can positively improve milk production and fibre utilization, but it is important to determine the conditions that are most likely to result in positive responses. In addition, the use of fibrolytic enzyme on innovative feed stuff such as whole plant faba bean silage is still unclear. Further studies are needed for a more comprehensive evaluation of fibrolytic enzyme on innovative feed source.

Table 2.6. Effect of exogenous enzyme on dairy cattle performance.

Enzyme	Forage source	Feed intake	Milk yield	Milk Production	Digestibility	Reference
Cellulases +Xylanases	Corn silage+ alfalfa hay	Not affected	Increased	Not affected	DM, OM, ADF, NDF and CP increased	(Rode et al. 1999)
Cellulases +Xylanases	Barley silage+ alfalfa silage	Not affected	Increased	Milk lactose increased	OM & NDF increased	(Yang et al. 1999)
Cellulases	Alfalfa silage+ Corn silage	Not affected	Increased	Not affected	DM increased	(Knowlton et al. 2002)
Xylanase+ Endoglucanase+ Endoglucanase	Corn silage+ alfalfa hay	Not affected	Not affected	Not affected	CP increased	(Arriola et al. 2011)
Xylanases	Corn silage+ alfalfa hay	Not affected	Increased	Milk fat, protein and lactose increased	Not reported	(Mohamed et al. 2013)
Xylanases	Corn silage	Increased	Not affected	Milk protein increased	Not reported	(Silva et al. 2016)

2.6. Feed Evaluation Methods

2.6.1. Chemical Evaluation of Feeds

In order to systematically determine chemical characterizations and nutrient profiles of the pre-treating silages for dairy cattle and compare with the conventional silages. The following parameters of feed component will be analyzed using standard methods described by AOAC 2000; Dry matter, Ash, Ether Extract, Crude Protein, Starch etc. The detergent system which refers to neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin will be determined using the methods described by Van Soest et al. (1991). Neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) will be determined

using the methods provided by Licitra et al. (1996). Soluble crude protein (SCP) will be measured using the methods provided by Roe (1990).

2.6.2. Application of Cornell Net Carbohydrate and Protein System V6.5 in Feed Evaluation

The protein and carbohydrate subtractions were partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS), which was first introduced in 1990's (Van Amburgh et al. 2015a). Cornell Net Carbohydrate and Protein System (CNCPS) was used to determine the protein and carbohydrate factions. The CP fractions are determined using the following formulas: PA1 = ammonia × (SP/100) × (CP/100) which refers ammonia; PA2 = SP × CP/100 − PA1 which is soluble true protein fraction; PB1 = CP − (PA1 − PA2 − PB2 − PC) refers to insoluble true protein fraction; PB2 = (NDICP − ADICP) × CP/100) which is fiber-bound protein, and PC = ADICP × CP/100 which is indigestible protein. The K_d values for CP fractions are: PA1, K_d is 200 %/h; PA2, K_d range is 10-40 %/h; PB1, K_d range is 3-20 %/h; PB2, K_d range is 1-18 %/h. The carbohydrate fractions are determined using the following formulas: CA1 = Acetic + Propionic + (Butyric + Isobutyric) indicates volatile fatty acids; CA2 is lactic acid, CA3 refers to other organic acids; CA4 refers to water soluble carbohydrates; CB1 starch; CB2 = NFC − CA1 − CA2− CA3 − CA4 − CB1 which refers to soluble fiber; CB3 = aNDFom – CC which is digestible fiber, and CC = (aNDFom × (Lignin × aNDFom) × 2.4)/100 or, aNDFom × uNDFom, refers to indigestible fiber. The K_d values for CHO fractions are: CA1, K_d value is 0 %/h; CA2, K_d value is 7 %/h; CA3, K_d is 5 %/h; CA4, K_d range is 40-60 %/h; CB1, K_d range is 20-40 %/h; CB2, K_d range is 20-40 %/h; CB3, K_d is 1-18 %/h. (Higgs et al. 2015; Van Amburgh et al. 2015). The undegradable neural detergent fiber (uNDF) is determined using samples bags (3 grams) which are incubated in the rumen of a cannulated cow for 288 hours. After complete incubation, the bags are washed then dried for 48 h at 55º C (Huhtanen et al. 1994).

2.6.3. Energy Evaluation in Feeds and Animal Diet

To determine energy values of faba bean silage for dairy cattle, the NRC-2001 summary approach will be applied (Abeysekara et al. 2013). The energy contents of total digestible CP (tdCP), fatty acids (tdFA), NDF(tdNDF), NFC (tdNFC), total digestible nutrients at 1× maintenance ($TDN_{1\times}$), digestible energy (DE) at the production level of intake ($DE_{3\times}$), metabolizable energy (ME) at the production level of intake ($ME_{3\times}$) and net energy for lactation at the production level of intake ($NEL_{3\times}$) will be evaluated using the summative chemical approach from the NRC (2001) and ME, NE_m, and NE_g will be evaluated from the NRC beef (1996).

2.6.4. Using In situ Technique for Estimation of Rumen Degradation Kinetics

The rumen degradation kinetics of DM and NDF are determined using *in situ* technique as described in detail by (Yu et al. 2003). Rumen degradation parameters of each component are estimated according to the first order kinetics model described by (Ørskov and McDonald 1979; Robinson et al. 1986). The results are calculated using the non-linear (NLIN) procedure of SAS and iterative least-squares regression (Gauss Newton method).

The model used for measuring rumen degradation kinetics of DM is as follows: $R(t) = U + (100 - S - U) \times e^{-Kd \times (t - T0)}$ where R(t) is the residue at the time of incubation (%), S is the soluble fraction (%), U is the undegradable fraction (%), T0 is lag time (h), and K_d is degradation rate (%/h). Rumen degradable DM (EDDM) is calculated according to the NRC-2001 standard using the non-linear parameters predicted by the above equation (S, U, and K_d) as $EDDM = S + D \times (K_d / K_d + K_p)$. The rumen undegradable DM (RUDM)) is calculated as $RUDM = U + D \times (K_p / K_d + K_p)$ where, K_p is the estimated passage rate from the rumen (%/h).

The descendent model (the residue decreases as time increases) used for measuring rumen NDF kinetics is: $R(t) = U + (100 - U) \times e^{-Kd \times (t - T0)}$ where R(t) is the residue at the time of incubation

(%), U is the undegradable fraction (%), T0 is lag time (h), and K_d is degradation rate (%/h). Then rumen undegradable NDF (RUNDF) is calculated according to the NRC-2001: RUNDF = U + D× (K_p/ K_p + K_d).

2.6.5. Using In vitro Technique for Estimation of DM and NDF Degradability

In vitro studies will be performed by using Daisy II incubator (Ankom®, Tech. Co., Fairport, NY, USA). This methodology allows simultaneous incubation of a large number of samples. The detail procedure is following by Goering and Soest (1970). The Daisy II incubator is designed to efficiently and accurately analyze up to 100 samples individually enclosed in filter bags. The Daisy II incubator can be also used to perform digestibility studies using enzymes or rumen inoculum. This incubator maintains an incubation temperature of 39.5° C, while providing agitation ("ANKOM Technology" 2017).

2.7. Literature Review Summary, Research Objectives and Hypotheses

2.7.1 Summary

Faba bean (*Vicia faba* L.) is an annual legume that widely used worldwide as animal feed and human consumption. The production of faba bean has dramatically increased in the past few years in western Canada due to rapid growth of international market opportunities. In western Canada, dairy and beef cattle are usually fed with a mixed of grains and forages, such as barley or corn silage. The present feeding of normal cereal grains to dairy and beef cattle is faced with increasing challenges such concern of animal health as well as feed cost. Faba bean has shown its potential of high protein content and energy density as an alternative feed source for livestock. However, there is limited study on the use of whole plant faba bean silage for ruminant.

Fibrolytic enzymes have been developed for many years and been proved that has positive response in different aspects of animal performance such as increase in digestibility of nutrient and feed intake. Even though, there is no study regarding to its effect on whole plant faba bean silage. As a result, the study is required to reveal the effect of fibrolytic enzyme on dairy cows fed with whole plant faba bean silage based-diet.

2.7.2. Research Hypotheses

1. It was hypothesized that the *in vitro* Daisy^{II} procedure could predict with accuracy DM and NDF degradability when compare with traditional *in situ* technique.
2. Rumen degradation characteristics of whole plant faba bean silage could be improved by the exogenous fibrolytic enzyme.
3. It was hypothesized that whole plant faba bean silage-based diet treated with fibrolytic enzymes would improve nutrient intake, feed utilization efficiency, lactational performance and feeding behavior in dairy cows.
4. Faba bean silage could be used as alternative protein and energy sources to compete with conventional feed in dairy industries in western Canada with a superior performance.

2.7.3. Research Objectives

Long-term:

1. To utilize enzymatic solutions to facilitate the efficient utilization of different silages for dairy cattle in western Canada.
2. To establish a new and alternative feeding strategy to improve the feeding value of different silages through adding fibrolytic enzyme to improve lactational performance in dairy cows.

3. To increase the basic knowledge about the role of the enzymatic solutions for sustainable livestock production to maximize benefit to Saskatchewan crop, feed and dairy industries.

Short-term:

1. To evaluate the effect of pre-treating silage with fibrolytic enzyme on chemical composition, nutritional characteristics and feed quality in dairy cows using various standard lab such as in vitro and in situ methods.
2. To examine the impact of enzymatic solution on lactation performance of dairy cows fed with whole plant faba bean silage-based diet.
3. To determine the optimum dose level applied on whole plant faba bean silage based-diet for dairy cows.
4. To evaluate the effect of applying enzymatic solutions to the silages on feeding behavior of dairy cows.

3. EFFECTS OF FIBROLYTIC ENZYME ON RUMEN DEGRADATION CHARACTERISTICS AND DEGRADABILITY OF DRY MATTER (DM) AND NEUTRAL DETERGENT FIBRE (NDF) OF WHOLE PLANT FABA BEAN SILAGE

3.1. Determined Ruminal Degradability of DM and NDF of Whole Plant Faba Bean Silage Using Both In Situ and In Vitro Techniques: Effect of Fibrolytic Enzyme

3.1.1. Abstract

The objectives of this study were to (1) determine the effect of fibrolytic enzyme (FE) on dry matter (DM) and neutral detergent fibre (NDF) degradability of whole plant faba bean silage (Snowbird) and (2) compare the difference between in vitro approach (Daisy^{II} incubation method) and in situ assay-biological approach (nylon bag technique) in the determination of degradability of dry matter (DMD) and neutral detergent fibre (NDFD). The whole plant faba bean samples were treated with seven doses of fibrolytic enzyme, which 0 as control, 0.25, 0.5, 0.75, 1, 1.25 and 1.5 mL of FETR /kg DM of silage. The results obtained from *in situ* method show that fibrolytic enzyme cubically ($P<0.05$) affected DMD and quadratically ($P<0.01$) affected NDFD with increasing level of enzyme application. *In vitro* DM and NDF degradability were quadratically and cubically ($P<0.01$) affected by the increasing dosage of enzyme. Correlation analysis between in situ assay-biological approach and in vitro Daisy^{II} approach showed a strong correlation ($r=0.98$, $P<0.01$) on overall DMD and also a satisfactory relationship ($r=0.84$, $P<0.01$) was found on overall NDFD. These results indicated that fibrolytic enzyme had a great impact on DMD and NDFD of whole plant faba bean silage determined using both in situ and in vitro techniques. Although *in vitro* Daisy^{II} method showed a relatively larger variation and inconsistent result compared with in situ method, it still remains to be a useful tool to estimate DM and NDF degradability with less cost, time and labour.

3.1.2 Introduction

Forage is extensively used in most of ruminant industries around the world. The most abundant composition in the forage is fiber which refers to the cell wall constituents of hemicelluloses, cellulose, and lignin. Understanding fiber digestibility and content is important in ruminant ration, especially the content and digestibility of neutral detergent fiber (NDF). The NDF degradability of forage is not only closely related to feed intake and lactational performance but also can be used for estimation of physical rumen fill and energy prediction of forage (NRC 2001; Hoffman et al. 2001).

The exogenous fibrolytic enzyme have been used in dairy industries in order to improve feed efficiency and animal performance. There are numerous studies that have demonstrated the effect of fibrolytic enzyme on total tract digestibility in dairy cows, most of studies have reported with a positive response in DM, OM, NDF and/or ADF (Yang et al. 1999; Rode et al. 1999; Beauchemin et al. 2003). Moreover, the fibrolytic enzymes were also examined the positive response in either in vitro or in situ experiments (Colombatto et al. 2003; Eun et al. 2007; Refat et al. 2018). Although these results were shown with variance, generally showed increase in digestibility of nutrient. There are many factors that may cause these inconsistent responses of fibrolytic enzyme, such as enzyme activity, rumen condition, substrate specificity and animals.

In situ nylon bag technique has been widely used for measuring rumen degradability in the past decades. This method is known as effective recurrence rate and its highly correlation to *in vivo* method (Wang et al. 2015). However, this method requires animals to be cannulated on rumen or duodenum and fed with standardized diets which is time consuming and costly. Recently, ANKOM Technology Corporation (Fairport, NY, USA) developed *in vitro* Daisy^{II} technique for analyzing forage *in vitro* dry matter (DM) and neutral detergent fiber (NDF) degradability. This

incubator was designed to improve labor efficiency and allows a large number of samples incubated at the same time. The objective of this study was to use both *in situ* and *in vitro* techniques to evaluate the effect of fibrolytic enzyme derived from *Trichoderma reesei* on DM and NDF degradability of whole plant faba bean silage.

3.1.3. Materials and Methods

3.1.3.1. Silage Sampling

The variety of faba bean used in this study is Snowbird which has very low condensed tannin content (0.4% of condensed tannin on DM basis). It was seeded on May 12th, 2018 and harvested on August 19th, 2018 at late pod stage (97 days after seeding) in Melfort, SK, Canada (crop location NW 16 44 21 W2). Fresh material (226 tonnes) was wilted to a targeted 45 %DM and chopped into 2.5 cm length on August 20th and August 21st, 2018. Silage piles were constructed and covered with plastic on August 22nd and fermentation process took 150 days for completion. The chemical composition of faba bean silage is presented in Table 3.1.

The samples of whole plant faba bean silage, Snowbird, were oven dried at 55°C and ground through a 3 mm screen using the 8 inches Laboratory Mill (Christy & Norris LTD, Ipswich, England). The enzyme solution was diluted with distilled water (0.1% of FETR) and prepared in a hand sprayer. Faba bean silage samples were treated with seven doses of fibrolytic enzyme, which 0 as control, 0.25, 0.5, 0.75, 1, 1.25 and 1.5 mL of FETR /kg DM of silage. Therefore, approximately 7.5 g of whole plant faba bean silage sample was weighed into 10 x 20 cm nylon bags with a size pore of 41 μm. Incubation time in the rumen was completed at 0, 3, 6, 12, 24, 48 and 72 hours. After 48 hours incubation, bags were removed, washed and dried. Finally, the sample will be analyzed for DM and NDF degradability.

Table 3.1. Chemical composition of whole plant faba bean silage

Items[1]	Whole plant faba bean silage
DM	45.7
OM, % DM	92.19
Ash, % DM	7.81
Ether Extract, % DM	1.09
Protein Profile	
CP, % DM	21.9
SCP, % DM	7.5
SCP, % CP	34.1
ADICP, % DM	1.50
ADICP, % CP	6.9
NDICP, % DM	2.17
NDICP, % CP	9.9
Carbohydrate profile	
Starch, % DM	23.7
aNDF, % DM	39.2
ADF, % DM	34.7
Lignin, % DM	5.2
NFC, % DM	32.11
NSC, % DM	24.3

[1]DM: dry matter; OM: organic matter; EE: ether extract (crude fat); CP: crude protein; SCP: soluble crude protein; ADICP: acid detergent insoluble crude protein; NDICP: neutral detergent insoluble crude protein; aNDF: neutral detergent fiber analyzed with amylase; ADF: acid detergent fiber; NFC: non-fiber carbohydrate; NSC: non-soluble carbohydrate.

3.1.3.2. Chemical Analysis

The samples of whole plant faba bean were oven dried at 55° C for 48 h and ground through a 1 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) for chemical analyses. Dry matter (method 930.15) was analyzed according to AOAC standard (2000). Neutral detergent fiber (NDF) were analyzed using ANKOM F57 filter bags (ANKOM Technology Corp., Fairport, NY) according to Van Soest et al. (1991).

3.1.3.3. Enzyme Product

The enzymatic solution used in this study was fibrolytic enzyme which derived from *Trichoderma reesei* (a mixture of xylanase and cellulase; AB Vista, UK). The product is a mixture

of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute).

3.1.3.4. In Situ Study

Four rumen cannulated Holstein dairy cows were used for *in situ* study at Rayner Dairy Teaching and Research Facility (University of Saskatchewan, Saskatoon, Canada). The cows were kept in tie stalls during the period of sampling and were milked three times a day. Cows were fed a total mixed ration (TMR) with 47.2 % of barley silage, 19.4% of barley/corn grain and 9.9% of grass, detailed ingredient composition is presented in Table 3.2. The cows used for this study were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1993) and the protocols were approved by the Animal Research 125 Ethics Board (AREB) at the University of Saskatchewan, Canada, with Animal Use Approval Protocol #19910012.

Rumen incubation timepoint was performed at 0, 6, 24 and 48 h; four polyester mesh bags were used to hold the nylon bags in the rumen (maximum 30 bags per cow). At the final timepoint, the bags were removed at the same time. After incubation, bags were removed, and hand rinsed in a bucket with cold tap water in order to eliminate excess ruminal content and stop microbial activity; therefore bags were washed 6 times in a "grab-released" motion; after washing, the excess water was drained, and the bags were dried at 55° C for 48 h in a forced-air drying oven. Dried bags were weighed, and the residues were pooled according to treatments, incubation time and run. Residues were ground through a 1 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) later, for further chemical analyses of dry matter and neutral detergent fiber.

Table 3.2. Ingredient composition of total mixed ration for *in situ* dairy cows

Item	Tie-stall/Parlor TMR Amount
Ingredient, % of DM	
Barley Silage	47.16
Grass Hay	9.94
Barley/Corn Grain	19.40
Protein Supplement	16.68
Lactating Supplement	3.82
Enervive[1]	1.70
Essentium[2]	1.28
Water	0.01

[1]Palm fat supplement; [2]Essential fatty acid supplement

3.1.3.5. In Vitro Study

In vitro study was performed by using Daisy^{II} Incubators (Ankom®, Tech. Co., Fairport, NY, USA). Two Daisy^{II} incubators were used in this study with two experimental runs. Whole plant faba bean silage samples used for in vitro incubation were oven dried at 55°C for 48 h and finely ground through 1mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany). The whole plant faba bean silage samples were treated with seven doses of fibrolytic enzyme, which 0 as control, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mL of FETR /kg DM of silage. Therefore, approximately 0.5 g was weighed into each filter bag (F57, Ankom Technology, Macedon, NY) and sealed. Each incubator contains three digestion jars, each jar signified at 0, 6, 24 and 48 hours and heated at 39°C. Each digestion jar was filled with pre-warmed (39°C) 1500 ml of McDougall's buffer solution (described in detail by (Goering and Soest 1970)) and 500 ml of strained ruminal fluid while flushed with CO_2. Rumen fluid was collected from two rumen-cannulated Holstein cows fed the same TMR with 47.2 % of barley silage, 19.4% of barley grain, 9.9% of grass hay and 20% of lactation concentrate. After 48 hours of incubation, bags were removed and rinsed thoroughly with cold tap water and dried at 105°C overnight, later analyzed for DM and NDF degradability.

3.1.3.6. Statistical Analysis

In situ and *in vitro* degradability of DM and NDF data were analyzed using a randomized complete block design. The model used for this design was as follow: $Y_{ijk} = \mu + T_i + B_j + e_{ijk}$, where Y_{ijk} was an observation of the dependent variable ij; μ was the population mean for the variable; T_i was the treatment effect, as a fixed effect, B_j was a block effect with *in situ* animals, as a random effect, and e_{ijk} was the random error associated with the observation ij. The difference among treatments was evaluated with a multiple comparison analysis using the Tukey method. Orthogonal polynomial contrast was used to determine the linear, quadratic and cubic effect of increasing enzyme application. The model assumptions were checked using Residual Analysis in SAS. The normality test was preformed using Proc Univariate. Comparison of *in vitro* Daisy^{II} with *in situ* biological approach in DM and NDF degradability of faba bean silage, the paired t test procedure of SAS (SAS 2005) and Pearson correlation analysis were performed to establish the relationship between the *in vitro* Daisy^{II} procedure and the *in situ* biological approach. For all statistical analyses, significance was declared at $P \leq 0.05$ and a trend at $0.05 < P < 0.10$ unless otherwise stated.

3.1.4 Results and Discussion

3.1.4.1. In Situ Degradability of DM and NDF

Table 3.3 shows the effect of adding different FE levels on *in situ* DM and NDF degradability of whole plant faba bean silage. The *in situ* DM degradability was improved cubically ($P<0.05$) with increasing FETR levels application. There is a quadratic effect ($P<0.01$) shows on *in situ* NDF degradability. These results are aligned with previous studies which reported that fibrolytic enzyme improved DM degradability *in vitro* or *in situ* experiment (Feng et al. 1996; Yang et al. 1999; Elwakeel et al. 2007). However, some other studies did not observe significant

response of fibrolytci enzyme on DM degradability (ZoBell et al. 2000). This discrepancy may be due to several factors which mentioned in the previous section, such as composition of enzyme, substrate, and/or interactions of the enzyme with substrates and environment condition. The highest degradability of DM was observed at the intermediate levels of FETR, which were the groups treated with 0.75 and 1 ml of FETR /kg DM of silage. The control group degraded 53% of DM degradability. The DMD was decreased at the groups of 0.25 and 0.50 ml of FETR, and then increased and reached the plateau at the groups of 0.75 and 1 ml of FETR /kg DM of silage. Additionally, the highest NDF degradability was also observed at the group treated with 0.75 ml of FE /kg DM of silage and increased from 13% (control) to 27% (0.75 mil of FE/ kg DM of silage). The Control (no FETR) and the lowest FETR level group (0.25 ml of FETR /kg DM) shows the lowest NDF degradability. The most effective enzyme dosage for whole plant faba bean silage, based on both in situ DM and NDF degradability was 0.75 ml of FETR /kg DM with the maximum effect of NDF on the group treated with 0.75 ml of FETR /kg DM.

Table 3.3. Effect of different dosage of fibrolytic enzymes[1] derived from *Trichoderma reesei* on in situ NDF and DM degradability of whole plant faba bean silages at different incubation time.

Item	In situ DM degradability of whole plant faba bean silage (%)	In situ NDF degradability of whole plant faba bean silage (%)
Dose level of fibrolytic enzymes (ml/kg)		
Control (0)	53.49	12.59
0.25	51.83	13.98
0.50	53.37	18.85
0.75	56.45	27.16
1.00	56.54	23.78
1.25	55.97	20.15
1.50	56.47	20.84
SEM	0.797	2.000
In situ incubation time (Time)		
0 h	30.23	5.45
6 h	43.58	9.63
24 h	67.45	19.89
48 h	78.24	43.52
SEM	0.631	1.632
Statistical Analysis	P value	P value
Dose level	0.0002	<0.0001
Time	<0.0001	<0.0001
Dose level × Time Interaction	0.1033	0.3905
Orthogonal Polynomial Contrast for FETR dose level	P value	P value
Linear	<0.0001	0.0001
Quadratic	0.4049	0.0002
Cubic	0.0245	0.5151
Orthogonal Polynomial Contrast for incubation time	P value	P value
Linear	<0.0001	<0.0001
Quadratic	<0.0001	0.0107
Cubic	0.0382	0.4071

SEM: Standard Error of Mean.

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose. The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

3.1.4.2. In Vitro Degradability of DM and NDF

Table 3.4. shows the *in vitro* DM and NDF degradability obtained from Daisy^{II} incubators. The DM and NDF degradability were quadratically and cubically ($P<0.01$) affected by the increasing dosage of enzyme. The *in vitro* DM degradability ranged from 52 to 58 % and in vitro NDF degradability ranged from 16 to 30% with different levels of enzyme application. The highest in vitro DMD was observed at 1.5 ml of FETR which is confirmed by other studies that claimed fibrolytic enzyme tended to increase microbial colonization of feed particles and assumed that exogenous enzyme may act similarly to primary bacterial colonization (Lewis et al. 1996; Yang et al. 1999). The highest in vitro NDFD was at the group treated with 0.5 ml of FETR which was up to 30%. Both results from *in situ* and *in vitro* techniques suggest that FETR has positive impacts on DM and NDF degradability of pre-treated faba bean silage.

Table 3.4. Effect of different dosage of fibrolytic enzymes[1] derived from *Trichoderma reesei* on in vitro NDF and DM degradability of whole plant faba bean silage at different incubation time.

Item	In vitro DM degradability of whole plant faba bean silage (%)	In vitro NDF degradability of whole plant faba bean silage (%)
Dose level of fibrolytic enzymes (ml/kg)		
Control	55.52	16.75
0.25	52.79	17.05
0.50	53.12	30.26
0.75	55.50	16.75
1.00	56.02	22.48
1.25	54.62	17.05
1.50	57.69	21.33
SEM	0.768	1.583
In situ incubation time (Time)		
0 h	32.71	3.81
6 h	44.23	5.82
24 h	68.00	28.31
48 h	75.22	43.90
SEM	0.664	1.296
Statistical Analysis	P value	P value
Dose level	<0.0001	0.6695
Time	<0.0001	0.0058
Dose level × Time Interaction	0.9429	<0.0001
Orthogonal Polynomial Contrast for dose level	P value	P value
Linear	<0.0001	0.6695
Quadratic	0.0029	0.0058
Cubic	0.0799	<0.0001
Orthogonal Polynomial Contrast for incubation time	P value	P value
Linear	<0.0001	<0.0001
Quadratic	<0.0001	0.0031
Cubic	0.9133	0.0005

SEM: Standard Error of Mean.

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

3.1.4.3. Comparison of In Situ and In Vitro Method

Comparison and correlation analysis between *in vitro* approach (Daisy-II incubation method) and *in situ* assay-biological approach (nylon bag technique) are shown in Table 3.5. The difference between in situ and in vitro DM degradability was significant ($P<0.01$) at 0 and 24 h incubation time. The correlation of overall DM degradability between two approaches was strong with $r=0.98$ ($P<0.01$). This is in an agreement with the study that performed with the same comparison (*in situ* versus Daisy^{II}). Trujillo et al. (2010) reported a good correlation on in situ and in vitro disappearance of DM and NDF that exceeded 0.80.

On the other hand, the greatest difference (8.42%, $P<0.01$) of NDF degradability was found at the 24 h incubation time between two approaches. The correlation of overall NDF degradability between in situ and in vitro was in a satisfactory relationship ($r=0.84$, $P<0.01$). This relationship is in line with a previous study which reported a highly correlation ($r=0.94$, $P<0.01$) between *in situ* and *in vitro* Daisy^{II} techniques on NDF degradability (Spanghero et al. 2003).

The overall DM and NDF degradability between two approaches are non-statistically significant. However, the results obtained from *in vitro* Daisy^{II} incubators were slightly higher than in situ method and also having a higher variability. This may attribute to overestimation of the *in vitro* procedure because ruminal motility may put a greater pressure in bags than Daisy^{II} incubators This finding is in agreement with other studies which used the same methods (*in situ* versus *in vitro* Daisy^{II}) to compare the NDF degradability of different cutting frequencies of hay (Spanghero et al. 2003). Robinson et al. (1999) reported that the in vitro digestion of NDF at 48 hours was higher than in situ method because of higher continuous fluid flow in *in vitro* bags. Another reason

for this could be attributed to growth and death of the microorganisms in *in situ* bags that affecting nutrient disappearance (Meyer and Mackie 1986).

Table 3.5. Comparison and correlation analysis between *in vitro* approach (Daisy^{II} incubation method) and *in situ* assay-biological approach (nylon bag technique) in the determination of degradability of dry matter (DMD) and neutral detergent fibre (NDFD) at different incubation times for whole plant faba bean silage pre-treated fibrolytic enzyme[1] derived from *Trichoderma reesei* at different dose levels[2].

Items	Comparison In vitro (Daisy-II) vs. In situ assay-biological approach					Correlation analysis In vitro (Daisy-II) vs. In situ biological approach	
	Mean $^{In\ vitro}$	Mean^{biological}	Difference	SED	P value	r	P value
Degradability of dry matter (DMD)							
Individual incubation time							
DMD at 0 h incubation (%, n=14)	32.71	30.23	2.48	0.632	0.0017	0.31	0.2762
DMD at 6 h incubation (%, n=14)	44.23	43.58	0.65	1.071	0.5538	0.20	0.5013
DMD at 24 h incubation (%, n=14)	68.00	67.45	0.55	0.858	0.5322	0.28	0.3381
DMD at 48 h incubation (%, n=14)	75.22	78.24	-3.02	0.986	0.0077	0.15	0.6055
Overall (n=56)							
DMD (%)	55.04	54.87	0.17	0.511	0.7476	0.98	<0.0001
Degradability of neutral detergent fibre (NDFD)							
Individual incubation time							
NDFD at 0 h incubation (%, n=14)	3.81	5.45	-1.64	2.415	0.5093	0.06	0.8343
NDFD at 6 h incubation (%, n=14)	5.82	9.63	-3.82	2.757	0.1896	-0.24	0.4166
NDFD at 24 h incubation (%, n=14)	28.31	19.89	8.42	2.155	0.0018	0.14	0.6250
NDFD at 48 h incubation (%, n=14)	43.90	43.52	0.38	2.503	0.8808	0.12	0.6912
Overall (n=56)							
DNDF (%)	20.46	19.62	0.84	1.352	0.5382	0.82	<0.0001

SED= standard error of the difference. R = Pearson correlation coefficient.

[1] Fibrolytic enzyme derived from Trichoderma reesei (FETR, mixture of xylanase and cellulose. The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

[2] Dose levels of fibrolytic enzyme included Control, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 ml/kg on DM basis

3.1.5. Conclusion

This present study has demonstrated that fibrolytic enzyme has a positive impact on DM and NDF degradability of whole plant faba bean silage revealed by both in situ nylon bag and in vitro Daisy^{II} techniques. The result of conventional *in situ* nylon bag method shows a more consistent result with less variation when compared with *in vitro* Daisy^{II} incubator. Although *in vitro* Daisy^{II} may underestimate the nutrient disappearance, two procedures have showed in a good relationship. To conclude, *in vitro* Daisy^{II} still appears to be a useful tool because of its advantages as fast, simple, and efficiency.

3.2. Effect of Fibrolytic Enzymes Derived from *Trichoderma Reesei* on Rumen DM and NDF Degradation Kinetics of Whole Plant Faba Bean Silage Revealed Using In Situ Techniques

3.2.1. Abstract

An *in situ* assay was carried out to evaluate the effects of fibrolytic enzyme (FETR) on DM and NDF degradation kinetics of whole plant faba bean silage. The *in situ* trial was performed using two rumen cannulated Holstein cows and seven dosage of enzyme (0, 0.25, 0.5, 0.75, 1, 1.25 and 1.5 mL of FETR /kg DM of silage) were used in this study. *In situ* rumen degradation residues and kinetics were determined. Enzyme application linearly decreased ($P<0.05$) DM degradation residue at 0 hour. There were significant quadratic effects ($P<0.05$) observed at 3 and 24hours incubation. However, there were no differences on degradation residues found in other time point. NDF degradation residue at 0, 6 and 24 hours were quadratically ($P<0.01$) affected with enzyme addition and a cubic effect ($P<0.05$) was found at 48 hours incubation. The soluble fraction of DM (S_DM) in whole plant faba bean silage was increased linearly ($P<0.05$) as the dose of enzyme increased. The enzyme application had a great impact on NDF rumen degradation kinetics by decreasing undegradable fraction, increasing potential degradable fraction and effective degradable content of fiber. W+D fraction were linearly ($P=0.05$) increased by the enzyme treatments. Therefore, undegradable fraction was linearly decreased ($P=0.05$) with increasing dosage of enzyme. Both bypass (BNDF) and effective degradable NDF (EDNDF) were cubically ($P=0.05$) affected by fibrolytic enzyme. There results suggested that the enzyme significantly increased fibre degradation for whole plant faba bean silage. Further study is needed to evaluate the effect of pre-treatment of fibrolytic enzyme derived from *trichoderma reesei* on lactational performance, rumen fermentation parameters and nutrient digestibility in lactating dairy cows fed whole plant faba bean silage as a main source of forage.

3.2.2. Introduction

The cell wall of plants is a complex matrix composed of polysaccharides, proteins, phenolics, water, and minerals, but mainly composed by carbohydrate which consist of cellulose and hemicellulose (Caffall and Mohnen 2009). Forage fibre is indigestible to most of animals but can be hydrolyzed and fermented by a number of microorganisms in the rumen and therefore, utilized by the ruminants as volatile fatty acid. Fibre digestibility and content are important in ruminant ration because it is the primary factor that determine feed intake and animal performance. Moreover, digestibility of NDF can be used as an important parameter for identify forage quality because of its variability among forages and consistent effect on animal performance (Oba and Allen 1999). According to NRC (2001), ruminal digestibility of forage NDF can range from less than 25% to over 75% for different forage types. However, the degradation of fibre in rumen is not optimal, as is supported by the fact that fibre recovered from feces is fermentable (Krause et al. 2003).

The effect of exogenous fibrolytic enzyme have been investigated by a number of researchers, which reported to increase *in vitro* or *in situ* fibre degradation (Feng et al. 1996; Oba and Allen 1999; Adesogan 2005). Although the responses to fibrolytic enzyme are inconsistent depending on the type of substrates, enzyme composition, enzyme activity etc, generally it shows positive impact on fibre digestion. For instance, Hatfield et al. (1999) reported that in the U.S. dairy industry, a 10% increase in cell wall digestion would result in an increasement in milk and meat sales while reducing manure waste and use of grains in diet. The objective of the present study was to determine the effect of fibrolytic enzyme on rumen DM and NDF degradation characteristics of whole plant faba bean silage.

3.2.3. Materials and Methods

3.2.3.1. Silage Sampling

The variety of faba bean used in this study is Snowbird which has very low tannin content (0.4% of condensed tannin on DM basis). It was seeded on May 12th, 2018 and harvested on August 19th, 2018 at late pod stage (97 days after seening) in Melfort, SK, Canada (crop location NW 16 44 21 W2). Fresh material (226 tonnes) was wilted to a targeted 45 %DM and chopped into 2.5 cm length on August 20th and August 21st, 2018. Silage piles were constructed and covered with plastic on August 22nd and fermentation process took 150 days for completion. The chemical composition of faba bean silage is presented in Table 3.1.

The samples of whole plant faba bean silage, Snowbird, were oven dried at 55°C and ground through a 3 mm screen using the 8 inches Laboratory Mill (Christy & Norris LTD, Ipswich, England). The enzyme solution was diluted with distilled water (0.1% of FETR) and prepared in a hand sprayer. The faba bean samples were treated with seven doses of fibrolytic enzyme, which 0 as control, 0.25, 0.5, 0.75, 1, 1.25 and 1.5 mL of FETR /kg DM of silage. Therefore, approximately 7.5 g of whole plant faba bean silage sample was weighed into 10 x 20 cm nylon bags with a size pore of 41 μm. Incubation time in the rumen was completed at 0, 3, 6, 12, 24, 48 and 72 hours. After 48 hours incubation, bags were removed, washed and dried. Finally, the sample will be analyzed for DM and NDF degradability.

3.2.3.2. Chemical Analysis

The samples of whole plant faba bean silage were oven dried at 55° C for 48 h and ground through a 1 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) for chemical analysis. Dry matter (method 930.15) was analyzed according to AOAC standards (2000). Neutral detergent

fiber (NDF) were analyzed using ANKOM F57 filter bags (ANKOM Technology Corp., Fairport, NY) according to Van Soest et al. (1991).

3.2.3.2. Enzyme Product

The enzymatic solution used in this study is fibrolytic enzyme which derived from *Trichoderma reesei* (a mixture of xylanase and cellulase; AB Vista, UK). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute).

3.2.3.3. In Situ Study

Two rumen cannulated Holstein dairy cows were used for *in situ* study at Rayner Dairy Teaching and Research Facility (University of Saskatchewan, Saskatoon, Canada). The cows were kept in tie stalls during the period of sampling and were milked three times a day. Cows were fed a total mixed ration (TMR) with 47.2 % of barley silage, 19.4% of barley/corn grain, 9.9% of grass hay, detailed ingredient composition is presented in Table 3.2. The cows used for this study were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1993) and the protocols were approved by the Animal Research 125 Ethics Board (AREB) at the University of Saskatchewan, Canada with Animal Use Approval Protocol #19910012. Whole plant faba bean silage, CDC Snowbird, were oven dried at 55°C and ground through a 1 mm screen using the Retsch ZM 200 grinder (Retsch ZM 200, Retsch Inc, Haan, Germany). The whole faba bean silage samples were treated with seven doses of fibrolytic enzyme, which 0 as control, 0.25, 0.5, 0.75, 1, 1.25 and 1.5 mL of FETR /kg DM of silage. Therefore, in each bag, approximately

0.5 g of faba bean sample was weighed into 5 x 10 cm Ankom bags with 6 μm pore size. Incubation time in the rumen was completed at 0, 3, 6, 9, 12, 24, 48 and 72 hours; two polyester mesh bags were used to hold the nylon bags in the rumen (maximum 30 bags per cow). After 72 hours incubation, all bags were removed and washed, later analyzed for DM and NDF degradability.

3.2.3.4. Statistical Analysis

In situ degradation residue of DM and NDF data were analyzed using a randomized complete block design (RCBD). The model used for this design was as follow: $Y_{ijk} = \mu + T_i + B_j + e_{ijk}$, where Y_{ijk} was an observation of the dependent variable ij; μ was the population mean for the variable; T_i was the treatment effect, as a fixed effect, B_j was a block effect with *in situ* animals, as a random effect, and e_{ijk} was the random error associated with the observation ij. The difference among treatments was evaluated with a multiple comparison analysis using the Tukey method. Orthogonal polynomial contrast was used to determine the linear, quadratic and cubic effect of increasing enzyme application. The RCBD model assumptions was checked using Residual Analysis in SAS. The normality test was preformed using Proc Univariate with Normal and Plot options. For all statistical analyses, significance was declared at $P \leq 0.05$ and a trend at $0.05 < P < 0.10$ unless otherwise stated.

The rumen degradation characteristics was performed using the first-order degradation kinetics model described by Ørskov and McDonald (1979) and Tamminga et al. (1994). Degradation parameters for dry matter and NDF were calculated using the following formula: $R(t) = U + D \times e^{-Kd \times (t - T0)}$, where, R(t) = residue percentage at t hours of incubation in the rumen (%), U = undegradable fraction (%), D = potentially degradable fraction (%), K_d = degradation rate (%/h), and T0 = lag time (h).

The effectively degradable fractions (ED) or extent of degradation in the rumen, as well as the ruminally undegradable fractions (RU) of the nutrients were determined with equations described in NRC (2001) and Yu et al. (2002) ; $ED = S + D \times K_d / (K_p + K_d)$; $RU = U + D \times K_p / (K_p + K_d)$ where, S represents the soluble fraction (%), K_p is the flow of degraded feed from the rumen, which was assumed to be equal to 6%/h (Tamminga et al. 1994).

3.2.4. Results and Discussion

3.2.4.1. In situ DM and NDF Rumen Degradation Residue

Degradation residues (%) of DM of whole plant faba bean silage are shown in Table 3.6. Enzyme application linearly decreased ($P<0.05$) DM degradation residue at 0 hour. In addition, a significant quadratic effect ($P<0.05$) were observed at 3 and 24 hours. However, there is no difference found in other time points. This finding is in line with our previous study which found that *in situ* DM degradability was linearly affected by the enzyme. Control group in each time point tended to remain the higher percentage of residue.

Table 3.7 shows the NDF degradation residue of faba bean silage. NDF degradation residue at 0, 6 and 24 hours were quadratically ($P<0.01$) affected with enzyme addition. Cubic effect ($P<0.05$) was found at 48 hours. The NDF degradation residue was rapidly decreased at the first 12 h of incubation. The lowest NDF residue at each time point was found in a range of medium dosage (0.5 to 1.00 ml of FETR) of fibrolytic enzyme. Our previous *in situ* study also demonstrated that medium range of fibrolytic enzyme had a greater effect on NDF degradability.

Table 3.6. Effect of different dosage of fibrolytic enzymes [1] derived from *Trichoderma Reesei* on in situ rumen DM degradation residue (RDMD, %) of whole plant faba bean silage at individual incubation time using both in situ nylon bag and in situ ANKOM bag methods

Item	In situ rumen DM degradation residue (%)						
	0 h (%)	3 h (%)	6 h (%)	12 h (%)	24 h (%)	48 h (%)	72 h (%)
Dose level of fibrolytic enzymes (mL/kg)							
Control (0)	74.91	74.72	62.62	59.23	39.63	25.96	30.04
0.25	74.68	69.03	63.73	53.74	42.37	24.65	30.72
0.50	71.21	65.63	62.60	53.76	35.65	26.02	26.87
0.75	71.87	70.57	60.57	44.78	37.26	26.32	25.14
1.00	72.78	66.26	60.75	53.31	37.53	27.92	26.78
1.25	71.81	66.26	61.76	57.31	37.90	28.68	26.78
1.50	70.77	72.31	61.30	49.59	40.07	24.63	26.11
SEM	1.643	1.791	1.918	4.735	1.411	2.215	2.930
In situ methods							
In situ Nylon bag	73.45	-	59.33 b	-	34.31 b	22.91 b	-
In situ ANKOM	71.70	69.25	64.48 a	53.67	42.95 a	29.71 a	27.49
Orthogonal Polynomial Contrast for dose levels (P value)							
Linear	0.028	0.240	0.347	0.491	0.451	0.616	0.230
Quadratic	0.468	0.010	0.685	0.488	0.031	0.492	0.481
Cubic	0.387	0.952	0.599	0.192	0.391	0.196	0.989
Statistical Analysis (P value)							
Dose level	0.213	0.054	0.898	0.429	0.059	0.818	0.757
In situ methods	0.094	-	0.002	-	<0.001	<0.001	-

SEM: standard error of mean; [a-b] Means with the different letters in the same column are significantly different ($P < 0.05$).

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

Table 3.7. Effect of dose level of fibrolytic enzymes [1] derived from *Trichoderma Reesei* on in situ rumen NDF degradation residue (RNDFD, %) of whole plant faba bean silage using both in situ nylon bag and in situ ANKOM methods.

Item	In situ rumen NDF degradation residue (%)						
	0 h (%)	3 h (%)	6 h (%)	12 h (%)	24 h (%)	48 h (%)	72 h (%)
Dose level of fibrolytic enzymes (mL/kg)							
Control	84.79	68.85	78.13	52.56	60.62	39.86	13.04
0.25	77.00	67.52	72.38	48.82	55.07	29.78	20.37
0.50	69.52	60.92	64.40	47.66	42.50	27.10	12.54
0.75	75.04	65.89	67.87	36.17	54.14	35.50	9.82
1.00	76.93	63.66	69.95	50.18	50.19	35.83	12.00
1.25	79.29	63.66	68.94	50.18	54.68	37.16	12.00
1.50	80.42	69.68	75.80	44.88	57.91	33.26	11.79
SEM	3.844	2.873	2.611	6.044	2.810	4.421	4.660
In situ methods							
In situ Nylon bag	87.01 a	-	82.24 a	-	72.31 a	51.16 a	-
In situ ANKOM	68.12 b	65.74	59.89 b	42.21	34.87 b	16.98 b	13.08
Orthogonal Polynomial Contrast for dose level (P value)							
Linear	0.945	0.874	0.553	0.596	0.936	0.876	0.398
Quadratic	0.004	0.074	<0.001	0.406	0.001	0.401	0.788
Cubic	0.061	0.791	0.495	0.461	0.162	0.049	0.500
Statistical Analysis (P value)							
Dose level	0.045	0.397	0.020	0.596	0.006	0.460	0.728
In situ methods	<0.001	-	<0.001	-	<0.001	<0.001	-

SEM: standard error of mean; [a-b] Means with the different letters in the same column are significantly different ($P < 0.05$).

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

3.2.4.2. In situ DM Degradation Kinetics

The *in situ* DM degradation kinetics of whole plant faba bean silage is presented in Table 3.8. The degradation rate (K_d), time lag (T0), degradable and undegradable fractions of DM were not significantly affected (P>0.05) by the enzyme application. Numerically, the Control group showed a higher K_d and undegradable fraction than treatments, and lowest in degradable fractions. However, soluble fraction of faba bean silage was increased linearly (P<0.05) as the dose of enzyme increased, and the highest values were obtained with the highest dosage (1.50 ml of FETR /kg DM of silage). This finding is agree with a previous study which reported higher dosage of enzyme had a higher soluble fraction of DM degradation for alfalfa cubes (Yang et al. 1999). However, another study claimed that there was no effect of fibrolytic enzyme on soluble fraction of DM in alfalfa hay (Gallardo et al. 2010). These inconsistent results may be due to different enzyme composition, activity and specificity of substrates that were used in experiments.

When applying firbrolytic enzyme solutions on whole faba bean silage, there were no differences found in rumen degradation characteristics of BDM and EDDM. This may be explained by specificity of substrates. Straalen (1995) has indicated that different chemical composition of forage could influence enzyme efficiency, which may be related to several factors such as cell wall structures, complexity and components (Yu et al. 2011).

3.2.4.3. In situ NDF Degradation Kinetics

The effect of fibrolytic enzyme on in situ NDF degradation kinetics of faba bean silage is presented in Table 3.9. In this study, the application of fibrolytic enzyme showed a great impact on NDF degradation kinetic. The W+D fraction which refers to washable plus potential degradable fractions was linearly (P=0.05) increased by the enzyme treatments. In contrast, undegradable fraction was linearly decreased (P=0.05) with increasing dosage of enzyme. These results are in

line with the published study which reported a higher potential degradable fraction and degradation rate on alfalfa hay when treated with enzyme (Gallardo et al. 2010); however in that study, corn silage, corn stover, elephant grass, Guinea grass and oat straw were not affected by the exogenous fibrolytic enzyme. The bypass (B) and effective degradable (ED) NDF were both cubically (P=0.05) affected by fibrolytic enzyme. The BNDF in the Control group was much higher than treatments (54.3 vs. 37.3-42.8% of NDF). Contrarily, EDNDF in the Control group showed the lowest value (45.7 % of NDF or 127 g/kg DM of silage), whereas treatment groups ranged from 57 to 63% of NDF or 160 to 175 g/kg DM of silage).

These positive results of enzyme application on in situ rumen degradation characteristics of NDF were in consist with other studies (Colombatto et al. 2007; Elwakeel et al. 2007). In this study, exogenous fibrolytic enzyme increased 26% the washable and potential degradable fraction of NDF and 14% of effective degradable NDF when compared the untreated control group with the treated intermediate group (0.75 ml of FETR /kg DM). These improvements on NDF degradation kinetic could result in a potentially greater dry matter intakes by reducing physical rumen fill and therefore increase energy density from diets (Allen 1996; Oba and Allen 2000). Although the mode of action by fibre degrading enzyme is still unclear, it would be related to the enhancement of rumen enzyme activity that caused by fibrolytic enzyme (Yang et al. 1999). Moreover, fibrolytic enzyme could also induce the release of soluble carbohydrates from feed particles (Hristov et al. 2000), thus provide additional energy for microbial growth and shortening lag time for microbial colonization (Wang et al. 2001).

Table 3.8. Effect of dose level of fibrolytic enzymes [1] derived from Trichoderma reesei on in situ rumen DM degradation kinetics of whole plant faba bean silage using both in situ nylon bag and in situ ANKOM methods.

Item	K_d_DM (%/h)	T0_DM (h)	S_DM (%)	D_DM (%)	U_DM (%)	BDM (g/kg DM)	EDDM (g/kg DM)
Dose level of fibrolytic enzymes (mL/kg)							
Control	6.99	1.89	25.10	49.11	25.80	545.1	454.9
0.25	4.69	1.08	25.32	56.26	18.42	602.1	397.9
0.50	5.58	2.20	28.80	49.28	21.93	531.7	468.4
0.75	6.23	1.42	28.14	48.90	22.97	516.4	483.6
1.00	4.94	0.98	28.15	50.90	20.95	552.7	447.3
1.25	4.93	1.33	28.19	50.79	21.03	562.1	437.9
1.50	5.35	2.14	29.24	50.74	22.03	538.1	461.9
SEM	0.895	0.796	1.414	2.688	2.753	24.28	24.28
In situ methods							
In situ Nylon bag	6.19	2.09	26.55 b	55.21 a	18.24 b	528.7 b	471.3 a
In situ ANKOM	4.87	1.06	28.57 a	46.50 b	24.93 a	570.7 a	429.2 b
Orthogonal Polynomial Contrast for dose level (P value)							
Linear	0.258	0.990	0.017	0.759	0.380	0.542	0.542
Quadratic	0.497	0.505	0.326	0.901	0.737	0.666	0.666
Cubic	0.548	0.539	0.543	0.415	0.286	0.843	0.843
Statistical Analysis (P value)							
Dose level	0.454	0.875	0.172	0.520	0.636	0.313	0.314
In situ methods (trial)	0.047	0.101	0.049	0.0004	0.0043	0.033	0.033

SEM: standard error of mean; [a-b] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison; Kd: the degradation rate of D fraction (%/h); T0: lag time; S: soluble fraction in the in situ incubation; D: degradable fraction; U: rumen undegradable fraction; BDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter.

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

Table 3.9. Effect of dose level of fibrolytic enzymes [1] derived from *Trichoderma reesei* on in situ rumen NDF degradation kinetic of whole plant faba bean silage using both in situ nylon bag and in situ ANKOM methods.

Item	K_d_NDF (%/h)	T0_NDF (h)	W+D (%)	U_NDF (%)	BNDF (%)	BNDF (g/kg DM)	EDNDF (%)	EDNDF (g/kg DM)
Dose level of fibrolytic enzymes (mL/kg)								
Control	3.58	1.55	67.52	32.48	54.3	169.1	45.67	127.3
0.25	3.03	5.09	94.40	5.60	40.0	124.2	60.02	167.7
0.50	3.65	4.49	87.40	12.60	37.3	115.7	62.76	175.3
0.75	2.99	3.08	94.49	5.52	39.5	122.6	60.54	169.1
1.00	2.56	2.15	95.16	4.84	40.0	124.1	60.04	167.8
1.25	2.50	0.88	93.40	6.60	42.8	132.9	57.22	159.8
1.50	3.62	6.86	94.55	5.45	40.2	125.1	59.77	166.9
SEM	0.689	1.966	7.870	7.870	3.88	12.11	3.884	10.91
In situ methods								
In situ Nylon bag	1.79 b	4.49	88.33	11.67	51.94 a	162.2 a	48.06 b	135.2 b
In situ ANKOM	4.47 a	2.39	90.80	9.21	32.06 b	98.9 b	67.94 a	188.8 a
Orthogonal Polynomial Contrast for dose level (P value)								
Linear	0.485	0.625	0.050	0.050	0.109	0.109	0.109	0.109
Quadratic	0.285	0.593	0.126	0.126	0.028	0.028	0.028	0.028
Cubic	0.221	0.023	0.306	0.306	0.050	0.050	0.050	0.050
Statistical Analysis (P value)								
Dose level	0.547	0.356	0.188	0.188	0.088	0.088	0.088	0.088
In situ methods (trial)	<0.001	0.172	0.683	0.683	<0.001	<0.001	<0.001	<0.001

SEM: standard error of mean; [a-b] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison; Kd: the degradation rate of D fraction (%/h); T0: lag time; W+D: washable and potential degradable fractions; U: rumen undegradable fraction; BNDF: rumen bypass or undegraded feed neutral detergent fiber; EDNDF: effective degraded neutral detergent fiber.

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

3.2.5. Conclusion

In conclusion, fibrolytic enzyme showed its highly positive impact on NDF rumen degradation kinetics/characteristic by decreasing undegradable fraction, increasing potential degradable fraction and effective degradable content of fiber. There was less impact on DM rumen degradation characteristics. In this study, low to medium dosage range (eg. 0.50 to 1.00 ml of FE /kg DM) of fibolytic enzyme was selected for further in vivo study to determine the effects of fibrolytic enzyme on lactational performance, rumen fermentation parameters and nutrient digestibility in lactating dairy cows fed with whole plant faba bean silage as a main source of forage in TMR ration.

4.EFFECT OF EXOGENOUS FIBROLYTIC ENZYME DERIVED FROM TRICHODERMA REESEI ON LACTATIONAL PERFORMANCE, FEEDING BEHAVIOR, RUMEN FERMENTATION AND NUTRIENT DIGESTIBLITY IN DAIRY COWS FED WHOLE PLANT FABA BEAN SILAGE-BASED DIET

4.1. Abstract

The objectives of this study were to evaluate the effects of pre-treating whole plant faba bean silage based-diet with exogenous fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulase) on lactational performance, digestibility, rumen fermentation characteristics, and feeding behavior in lactational dairy cows. The animal trial was conducted using eight Holstein dairy cows (BW = 710 ± 44 kg and DIM = 121 ± 17 days) with four enzyme treatments (0, 0.5, 0.75, and 1.0 ml of FETR /kg dry matter of silage) in a double Latin square design. The enzyme treatments used in this trial were selected from previous *in situ* and *in vitro* studies which showed positive responses on whole plant faba bean silage. The response of NDF digestibility and digestible NDF to the increasing level of FE was linear ($P<0.05$), where the enzyme group (0.5 mL of enzyme/kg of silage DM) exhibited the highest NDF digestibility (48.5%). The enzyme application significantly affected on percentage of milk fat and milk fat yield which was linearly ($P<0.05$) differed among treatments, being highest (4.35%, 1.82 kg/d) for low dosage groups. The milk yield in Control averaged 41.2 kg/d with 4.35 percent fat. Both energy (ECM, $P = 0.018<0.05$) and fat corrected milk yield (FCM, $P=0.058<0.10$) were linearly affected or tended to be affected by fibrolytic enezyme dose level. The ECM production efficiency (kg of ECM/kg of DMI) was cubically ($P<0.05$) affected by the enzyme application. The FCM production efficiency (kg of FCM/kg of DMI) was linearly ($P<0.05$) affected by the enzyme application. This study demonstrated positive effects of pre-treating whole plant faba bean silage with lower dose

level (0.5 1 ml of FETR /kg dry matter of TMR) of fibrolytic enzyme to dairy cows which could benefit the development of a new and alternative feeding strategy in western Canada.

4.2. Introduction

Barley and corn silage are commonly fed to ruminants in western Canada. However, the present feeding of normal cereal grains to dairy and beef cattle is faced with increasing challenges in terms of relatively low starch content and higher feed cost when compare to legume silage such as soybean and alfalfa (Guevara Oquendo 2020). In order to minimize feed cost and maximize animal production, producers and breeders are seeking an alternative feed resource. Faba beans (*Vicia faba* L.) as a great source of protein and starch has led to an interest in ruminant diets. Furthermore, the presence of tannin-free genotypes resulting from the improvement of plant breeding also makes faba bean more adequate in animal industries. The crude protein content of faba bean seed can reach up to 30% of dry matter basis, approximately 37% of starch and with a good composition of amino acid (Yu 2005). Another advantage of faba bean is its capacity of nitrogen fixation, higher nitrogen fixation capacity indicates an increased in yields and nutritional values. However, there are limited studies of whole plant faba bean silage on dairy cows. A few studies reported the use of faba bean silage for lactating cows with favorable results (McKnight and MacLeod 1977; Berkenkamp and Meeres 1986). Recently, a study in our laboratory has also suggested that replacing barley and corn silages with whole crop faba bean silage in high producing dairy cows is comparable and positively affected animal performance (Guevara Oquendo 2020).

The use of exogenous fiber degrading enzyme additives for ruminants has been examined since 1960s (Burroughs et al. 1960). The purposes of using enzyme supplements are to (1) prevent the anti-nutritional factors in animal feed, (2) improve availability of nutrient in digestive system, (3) break down specific chemical bonds in raw materials, and (4) act as a supplement for animals

that are lack of mature digestive system (Bedford and Partridge 2010). Previous studies involving dairy cows reported that fibrolytic enzymes could substantially improve animal performance (McAllister et al. 1999; Kung et al. 2002; Refat 2019). Adding fibrolytic enzyme to ruminant diets improved milk production in some studies (Yang et al. 1999; Rode et al. 1999; Arriola et al. 2011; Refat 2019), but others reported no difference (Kung et al. 2002). The positive responses of fibrolytic enzyme application showed the increase in fibre digestibility, feed efficiency and chewing activity (Lewis et al. 1999; Beauchemin et al. 2000, 2011; Arriola et al. 2011). However, the responses of fibrolytic enzyme on ruminants have been reported to be inconsistent. These discrepancies may be attributed to many factors such as differences in enzyme activity, application rate, method of application, substrate specificity, and stage of lactation of dairy cows (Beauchemin et al. 2003; Adesogan 2005).

To our knowledge, there has been limited study on pre-treating fibrolytic enzyme on whole plant-faba bean silage. It was hypothesized that adding fibrolytic enzyme on whole plant-faba bean silage would improve lactational performance, feeding behavior, rumen fermentation and nutrient digestibility in high producing dairy cows. The objectives of this study were to evaluate the effects of pre-treating whole plant faba bean silage based- diet with exogenous fibrolytic enzyme in lactational dairy cows on and develop a feeding strategy of whole-plant faba bean silage for dairy cows.

4.3. Materials and Methods

All cows used in the present trial were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1993) and were approved (Animal Use Approval Protocol #19910012) by Animal Research 125 Ethics Board (AREB) at the University of Saskatchewan (Saskatoon, SK, Canada).

4.3.1. Silage Sampling

The variety of faba bean used in this study is Snowbird which has very low tannin content (0.4% of condensed tannin on DM basis). It was seeded on May 12th, 2018 and harvested on August 19th, 2018 at late pod stage (97 days after seeding) in Melfort, SK, Canada (crop location NW 16 44 21 W2). Fresh material (226 tonnes) was wilted to a targeted 45 %DM and chopped into 2.5cm length on August 20th and August 21st, 2018. Silage piles were constructed and covered with plastic on August 22nd and fermentation process took 150 days for completion. The chemical composition of faba bean silage is presented in Table 3.1.

4.3.2. Enzyme Product

The enzymatic solution used in this study was fibrolytic enzyme which derived from *Trichoderma reesei* (a mixture of xylanase and cellulase; AB Vista, UK). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute). The enzyme mixture was prepared twice a week and stored in 4°C. Every morning, enzyme liquid was sprayed onto whole plant faba bean silage one hour before feeding in order to increase enzyme efficacy, and later FETR pre-treated whole plant faba bean silage was mixed with other ingredients in a mixer at Rayner Dairy Research and Teaching Facility (University of Saskatchewan, Saskatoon, SK, Canada).

4.3.3. Animals, Diets and Experimental Design

Eight mid-lactating Holstein cows with average BW = 710 ± 44 kg, DIM = 121 ± 17 and parity = 2.25 ± 0.43 were kept in individual tie stalls (4 cannulated cows and 4 non-cannulated

cows) at the Rayner Dairy Research and Teaching Facility (University of Saskatchewan, Saskatoon, SK, Canada). The experimental design of this animal trial was a double 4 × 4 Latin square design with four periods, each experimental period consisted of 21 days, 14 days of diet adaptation, and 7 days of sample collection. Cows were allowed to adapt to the tie stalls 7 days prior the beginning of the first experimental period. Diets were formulated using the NDS Professional software (Version 3, RUM&N - NDS Professional, Reggio Nell'Emilia, Emilia-Romagna, Italy) based on adequate metabolizable energy and metabolizable protein for mid-lactating cows at 120 days in milk. The forage in the diet was formulated with 31% whole plant faba bean silage, 14% of grass hay and 3.6% of straw on DM basis. The detailed TMR composition is presented in Table 4.1.

Three doses (0.5, 0.75 and 1.0 ml of fibrolytic enzyme /kg dry matter of whole plant faba bean silage) selected from the previous studies were used in this dairy production and metabolic trial. Four dietary treatments were T1=Control, T2=TMR with 0.5, T3=TMR with 0.75, and T4=TMR with 1.0 ml of fibrolytic enzyme/kg dry matter of whole plant faba bean silage). Cows were provided with ad libitum water and a total mixed ration (TMR) once a day at 1030 h. The offered feed was monitored constantly to keep refusals at 5 to 7% (on as fed basis). Approximately 1 kg of silage and forage samples were taken directly from the piles and dried in a forced-air oven at 55 ºC for 48 h for further determination of DM content to maintain the adequate forage to concentrate ratio in the diet during all the trial. Before the first trial started, there was 5 days for diet change over, cows were fed 25% of the new TMR + 75% of the old TMR the first day, 50% of the new TMR + 50% of the old TMR the second and third days, and 75% of the new TMR + 25% of the old TMR at the fourth day and last day.

Table 4. 1. Ingredient, chemical composition and energy values of the TMR and whole plant faba bean silage used for exogenous fibrolytic enzyme-supplemented diet treatment

	Whole plant faba bean silage based TMR
Item	Amount
Ingredient, % of DM	
Faba Bean Silage	31.41
Rayner Grass Hay	14.41
Rayner Straw	3.58
Dried Molasses	4.77
RP10 Palmitic[1]	1.76
Megalac	1.32
Rayner Barley/Corn	30.04
Flaked Barley	21.32
Rolled Corn	8.72
Rayner Protein Mix	8.75
Canola Meal	5.98
Soybean Meal	2.77
Lactating Supplement	3.94
Water	0.01
Chemical composition of diet	
DM	53.7
CP (% DM)	16.1
aNDF (% DM)	30.1
Starch (% DM)	25.9
Ash (% DM)	9.02
Ca (% DM)	1.01
P (% DM)	0.37
Energy values of diet	
TDN, %	72.1
DE, Mcal/kg	3.18
ME, Mcal/kg	2.61
Chemical composition of silage, n=	
DM	45.0
CP (% DM)	21.9
Starch (% DM)	23.7
aNDF (% DM)	39.2
Ash (% DM)	7.81
Ca (% DM)	0.67
P (% DM)	0.28

F: C = 49.4: 50.6; [1]Palmitic acid (C16–0)

4.3.4. Milk Sampling

Cows were milked three times a day at 0700, 1500, and 2300 at a milking parlor and yield was recorded from d 10 to d 21 at each milking time per each experimental period. Milk samples were collected on d 19, d 20, and d 21 and preserved with potassium dichromate and refrigerated until sent for chemical analyses at the Central Milk Testing Lab, CanWest DHI (Edmonton, Alberta, Canada). Milk samples were analyzed for somatic cells (SCC), total solids, milk urea nitrogen (MUN), protein (%), lactose (%), and milk fat (%) using infrared spectroscopy (MilkoScan 605, Foss Electric, Hillerød, Denmark; AOAC, 2000; method 972.16). Solid-non-fat, fat corrected milk (FCM), energy corrected milk (ECM), and yields of milk protein, lactose, and fat were calculated according to Refat et al. (2018).

4.3.5. Chewing Activity

Chewing activity was recorded with cameras and a digital video recorder (QSEE, QC908 HD DVR, China). Cameras were set up at the tie stall area to monitor the chewing activity of cows on d 14 to d 15 (24h) in each period. Feeding behavior were monitored visually for cows, chewing activities were noted at 5 min intervals and each activity was assumed to persist for the entire 5 min interval. The mean intake for the period for that cow was used to estimate time spent on chewing activities (Mugerwa et al. 1973; Beauchemin et al. 2000).

4.3.6. Total Collection

All the feed sample were collected in the morning before cows were fed. Refusals were removed from the bunks and weighed. Feed TMR and orts were collected daily from d 16 to d 21 of each period and stored at −20°C for further analyses. At the end of the trial (last experimental period), samples were thawed at room temperature and pooled by cow and period. Then collected in paper bags and dried in a forced-air oven at 55ºC for 48 h.

Urine and faeces sample were collected for 3 d every 6 h on d 19, d 20, and d 21 of each period. Urine catheters (Foley bladder catheters, 26 Fr, 75-mL ribbed balloon, lubricious- coated; C. R. Bard Inc., Covington, GA) were inserted in all cows on d 18. On d 22 at 0900 h, large metal containers for total faeces collection were placed in each tie stall and catheters were connected through a hose into individual collection containers. On d 22 at 0900 h, large metal containers were placed in each tie stall and catheters were connected through a hose into individual collection containers. Urine and faeces containers were weighed, collected and recorded every 6 hours from d 19 to d 21. 90 ml of urine samples were collected in tamper evident specimen containers with screw caps (120 ml) and stored at 4°C until the end of the day. After each period, the urine samples were pooled by day, cow, and period based on the proportion of total urine collected on each time point and stored at −20°C for further analyses. A fecal grab sample (300 to 500 g fresh basis) was collected immediately and weighed. All fecal samples were mixed and pooled per cow, and a subsample stored at −20°C for later analysis. Fecal samples were dried in an air oven at 55ºC for 48 h (AOAC, 930.15) (AOAC, 2000).

4.3.7. Rumen Fermentation Parameters

Rumen fluid samples were collected from the 4 cannulated cows on the last day of each period. Collection started at 0900 h and continued collected at 3 hours intervals till the next day at 0900 h. Approximately 1,000 ml of rumen fluid was collected from each cannulated cow. Fluid and solid material were taken from the cranial ventral, rumen ventral, and caudal ventral sections of the rumen of each cow and strained through 4 layers of cheesecloth into individual plastic buckets.

The ruminal pH was measured using a pH meter (Accumet AP110, Thermo Fisher Scientific, Waltham, MA) and results were recorded for later analysis. 10 ml of rumen fluid were collected

into conical-bottom centrifuge plastic tubes (15 ml) per cow for further analysis of ammonia and volatile fatty acids. All tubes were stored at −20°C until chemical analyses were performed.

4.3.8. Chemical Analysis

All frozen TMR, refusals and fecal samples were thawed overnight at room temperature and dried at 55°C for 48 h. Dried samples were ground to 1 mm with a Retsch ZM 200 grinder (Retsch Inc, Haan, Germany) and sent to Cumberland Valley Analytical Services (CVAS, Hagerstown, MD) for use in chemical analyses: DM (AOAC, 930.15), ash (AOAC, 942.05), crude fat (AOAC, 2003.05), CP (AOAC, 990.03), NDF (Van Soest et al. 1991), and starch (Hall 2009). Dried faeces samples were ground through a 1-mm using a Retsch ZM 200 grinder (Retsch Inc, Haan, Germany) for analysis of DM (AOAC, 930.15), ash (AOAC, 942.05), crude fat (AOAC, 2003.05), CP (AOAC, 984.13), NDF (Van Soest et al. 1991), and starch (Hall 2009) at Cumberland Valley Analytical Services (CVAS, Hagerstown, MD).

Nitrogen balance and total tract digestibility were determined by the analysis of urine and faeces samples. Total nitrogen was determined using the method according to Kirk (1950). Nitrogen retained was calculated as intake N − milk N – manure N (fecal and urine N), where milk N was obtained as milk CP/6.38.

Rumen fluid samples were thawed overnight at 4°C and analyzed for ammonia and VFA. Ammonia was measured based on the procedure from Broderick and Kang (1980), and VFA were determined using a gas chromatography-flame ionization detector (GC-FID) system at the University of Saskatchewan (Saskatoon, SK, Canada). Sample preparation was according to an internal standard method as follows: 1) Vortexed thawed sample, 2) Pipetted 1 ml of solution into high speed centrifuge tubes and centrifuged at 16000 g for 10 min at 4°C, 3) 0.4 ml of supernatant were pipetted into GC vials, 4) 0.1 ml of 25% phosphoric acid + 0.1 ml of internal STD (isocaproic

acid) were added to the vials, 5) The solution was diluted with 0.75 ml of double distilled water to reduce the concentration and viscosity of the rumen fluid.

4.3.9. Energy Partitioning

Energy partitioning was calculated by using milk yield, milk composition, and BW gain data. The equation of energy value of feeds was calculated as following:

$$DE\ (Mcal/kg) = 0.04409 \times TDN\ (\%);$$

$$ME\ (Mcal/kg) = 0.82 \times DE\ (Mcal/kg);$$

$$NE_L = 0.0245 \times TDN\ (\%) - 0.12$$

The net energy for maintenance was calculated as described by (NRC 2001) according to the equation as below:

$$BW^{0.75} \times 0.08.$$

Estimation of energy of BW gain was assuming BW×4.924 Mcal/kg for body weight loss and BW×5.114 Mcal/kg for body weight gain (NRC 2001a). The net energy of milk production was calculated using the following equation:

$$\text{Milk NEL (Mcal/d)} = \text{Milk yield (kg)} \times [0.0929 \times (\text{Fat \%}) + 0.0563 \times (\text{True Protein \%}) + 0.0395 \times (\text{Lactose \%})].$$

The predicted NE_L was calculated by summation of net energy (maintenance, BW gain, and milk) divided by DMI (Neal et al. 2014).

4.3.10. Statistical Analysis

All the results obtained from the Latin Square Design (LSD) were analyzed using the Mixed model procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC, US). One model was analyzed for rumen fermentation characteristics using single square data with cannulated cows only. The single Latin square data were analyzed using Proc Mixed with the following model:

$$Y_{ijk} = \mu + T_i + P_j + C_k + e_{ijk},$$

where, Y_{ijk} was the observation of the dependent variable; μ was the overall mean; T_i was the fixed effect of treatment i; P_j was the fixed effect of the period j; C_k was the random effect of the cow k; e_{ijk} was the random error associated with the observation.

The double Latin square data were analyzed with all cows for milk production and composition, chewing activity, total tract digestibility, nitrogen balance and energy partitioning using Proc Mixed with the following model:

$$Y_{ijkL} = \mu + S_l + T_i + P_j + C(S)_{kL} + e_{ijk},$$

where, Y_{ijkL} was the observation of the dependent variable; μ was the overall mean; S_l was the square L; T_i was the fixed effect of treatment i; P_j was the fixed effect of the period j; $C(S)_{kl}$ was the random effect of the cow k nested within square; e_{ijkl} was the random error associated with the observation.

The final variance and covariance structure models were selected based on AIC and BIC values. Orthogonal polynomial contrast was used in this study to determine the linear, quadratic and cubic effect of increasing enzyme application. Comparison between the Control versus treatments all faba bean silages-based diets was carried out using a contrast procedure of SAS. For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$.

4.4. Results and Discussion

4.4.1. Feed Intake and Body Weight Change

The effect of fibrolytic enzyme application on nutrient intake and body weight change is shown in Table 4.2. Intake of DM and other nutrients was not affected by enzyme, and there was no significant difference among the treatment and control groups except for CP in which the intake of CP was linearly ($P<0.05$) decreased from 4.54 to 4.36 kg/d. This lack effect of fibrolytic enzyme

on nutrient intake was consistent with most studies that involved with enzyme supplementation (Rode et al. 1999; Holtshausen et al. 2011).

Some studies reported a decrease in DMI after adding the enzyme and this may be due to the positive effect of fibrolytic enzyme that enhanced the available energy from diets to animals (Holtshausen et al. 2011; Refat et al. 2018). In contrast, other studies reported that adding enzyme to diet increased or tended to increase feed intake (Gado et al. 2009). However, the improvement of enzyme supplement on DMI may lead increased milk yield in early lactation cows which are in negative energy balance (Rode et al. 1999). There was no difference observed in body weight change. This finding is reasonable because body weight was reported to be a critical factor that explained variation in DMI (Kertz et al. 1991). Since there was no significant difference in DMI, it was expected that that body weight would not change.

4.4.2. Digestibility of Primary Nutrient

The digestibility of primary nutrient in dairy cows fed whole plant faba bean silage with different levels of fibrolytic enzyme is present in Table 4.3. The response of NDF digestibility and digestible NDF to the increasing level of FETR was linear ($P<0.05$), where lower enzyme group (0.5 mL of enzyme/kg of silage DM) exhibited the highest NDF digestibility (48.54%) in numerical. However, there was no high beneficial effect when adding higher level of enzyme. Beauchemin et al. (1995, 2000) also reported quadratic effects of enzyme on fiber digestibility, suggesting that higher concentration of enzyme may compete with ruminal microbes for binding sites of feed particle. Consequently, it resulted in reducing the overall endogenous bacterial activity. The highest NDF digestibility at lower dose level of enzyme (0.5 treatment group) is in line with our previous *in situ* and *in vitro* studies, which revealed that FETR had highly positive effect on fibre digestion by decreasing undegradable fraction and increasing potential degradable fractions.

It is speculated that the positive responses in NDF digestibility may be attributed to the enhancement of microbial colonization of feed particles (Yang et al. 1999) or by direct cell wall hydrolysis. Starch digestibility was cubically ($P<0.01$) affected by the application of enzyme. However, most studies in the field of enzyme supplements did not report similar effects on starch digestibility. One of study from our laboratory indicated that the majority of starch content was contained in faba bean pods (Yan 2020). Thus, fibrolytic enzymes are believed to function on the hulls of faba bean, thereby inducing more available starch for dairy cows.

The digestible OM, CP and ST were not affected by the application of fibrolytic enzyme. But digestible DM and ash were cubically ($P<0.05$) affected by the application of fibrolytic enzyme with the highest (18.17, 1.54 kg/d, respectively) observed in intermediate level group (0.75 mL of enzyme/kg of silage DM). The digestible NDF was linearly ($P<0.05$) decreased with increasing enzyme level. Decrease in digestible NDF may resulted from the negative effect of increasing enzyme level on NDF digestibility.

Table 4.2. Effect of exogenous fibrolytic enzyme[1] derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulase) levels on intake and body weight in lactating dairy cows

Item	Enzyme level (mL FETR / kg silage DM)					Contrast, *P*-value			
	Control	0.50	0.75	1.00	SEM[1]	Control vs. Treatments	Linear	Quadratic	Cubic
Intake (kg/d)									
DM	26.60	26.48	26.34	26.19	1.313	0.365	0.327	0.800	0.500
OM	23.95	23.84	23.62	23.76	1.189	0.446	0.435	0.764	0.576
Ash	2.65	2.64	2.58	2.56	0.132	0.225	0.115	0.862	0.278
CP	4.54	4.47	4.37	4.36	0.221	0.003	0.004	0.800	0.303
NDF	8.76	8.91	8.70	8.80	0.473	0.867	0.964	0.778	0.496
ST	6.24	6.09	6.13	6.09	0.380	0.529	0.575	0.812	0.798
Weight									
BW (kg)	712.00	710.75	707.13	707.00	13.332	0.392	0.279	0.905	0.672
WG (kg/d)	0.15	0.21	0.38	0.13	0.216	0.730	0.836	0.598	0.561

[1]SEM = standard error of mean

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

Table 4.3. Digestibility of primary nutrients in dairy cows fed whole plant faba bean silage based TMRs with different level of exogenous fibrolytic enzyme[1] derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose)

	Enzyme level (mL FETR / kg silage DM)					Contrast, *P*-value			
Item	Control	0.50	0.75	1.00	SEM	Control vs. Treatments	Linear	Quadratic	Cubic
Digestibility (%)									
DM	65.36	64.69	66.40	66.79	1.801	0.587	0.232	0.351	0.423
OM	65.20	64.88	64.86	65.10	1.507	0.651	0.810	0.589	0.919
Ash	48.18	51.16	55.02	50.72	2.897	0.060	0.110	0.146	0.134
CP	61.33	60.37	61.45	61.05	1.658	0.613	0.937	0.505	0.266
NDF	48.25	48.54	44.78	45.19	2.437	0.151	0.038	0.518	0.125
ST	92.43	89.63	92.28	91.17	1.000	0.029	0.291	0.038	0.002
Digestible (kg/d)									
DM	17.21	16.72	18.17	17.78	0.970	0.442	0.173	0.367	0.024
OM	15.71	15.53	15.72	15.50	0.935	0.718	0.726	0.986	0.582
Ash	1.27	1.29	1.54	1.35	0.117	0.099	0.083	0.365	0.014
CP	2.74	2.66	2.81	2.74	0.173	0.953	0.688	0.612	0.127
NDF	4.68	4.50	3.93	3.75	0.398	0.008	0.003	0.300	0.216
ST	5.85	5.23	5.70	5.69	0.341	0.232	0.741	0.105	0.162

SEM: standard error of mean

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

4.4.3. Milk Yield and Composition

Table 4.4 shows the effect of fibrolytic enzyme on milk production, composition and feed efficiency in lactating dairy cows fed with whole plant faba bean silage based-TMR. The enzyme application did not significantly affect milk yield, although it numerically increased in low enzyme level group (0.50 mL of enzyme/kg of silage DM). However, after milk yield was corrected with energy and fat, it was found that there was linear effect ($P<0.05$) on FCM, being highest for low enzyme level group (0.50 mL of enzyme/kg of silage DM). Likewise, the highest ECM was observed in low enzyme group (0.50 mL of enzyme/kg of silage DM). Some studies reported positive responses on milk yield (Yang et al. 1999; Lewis et al. 1999; Rode et al. 1999), but some did not find the positive effect (Beauchemin et al. 2000; Elwakeel et al. 2007; Arriola et al. 2011). This inconsistency may be due to differences in enzyme activity, mixture and types of substrates. One explanation to this lack high effect on milk yield in this study might be because of lactation stage of dairy cows which were in mid-lactation. Rode et al. (1999) indicated that improvement of FETR on feed intake may lead to increase on milk yield which are likely to be more beneficial for cows in early lactation.

The ECM and FCM production efficiencies were cubically ($P<0.05$) and linearly ($P<0.05$) affected by the enzyme application, respectively. The ECM and FCM production efficiencies were greater in cows fed with low enzyme dosage (0.50 mL of enzyme/kg of silage DM) with 1.78 for ECM/DMI and 1.83 for FCM/DMI. Instead of changes in milk yield, the improvements in feed conversion efficiency were due to relatively lower DMI that was observed in this study. This finding partly supported our hypothesis that fibrolytic enzyme would improve feed efficiency when cows were fed with whole plant faba bean silage based-TMR. It is speculated that positive

responses of fibrolytic enzyme in feed efficiency might be attributed to the increased in NDF digestibility (Holtshausen et al. 2011).

Pre-treating whole plant faba bean silage based-TMR with fibrolytic enzyme did not affect percentage of milk protein, lactose, and SNF ($P>0.05$; Table 4.4). Milk urea nitrogen reflects the amount of urea found in milk and can be used to monitor the protein nutritional status of dairy cows (Amaral-Phillips n.d.). The MUN concentration in current study was averaged 15.34 mg/dl, which seems to be slightly above the ideal range (less than 15 mg/dl). This may be due to excessive amount of RDP in whole plant faba bean silage based-diet which resulted in higher nitrogen excretion. it is possible that the utilization of the whole plant faba bean silage based-TMR could be further improved.

Some researchers reported that milk protein and lactose were decreased when the cows fed with enzyme-treated feed or high concentration enzyme (Rode et al. 1999; Beauchemin et al. 2000; Refat et al. 2018). However, this effect of fibrolytic enzyme did not show in this study (CTRL vs. treatments; $P>0.05$). The enzyme application linearly affected ($P<0.05$) on both percentage of milk fat and milk fat yield with highest milk fat (1.82 kg/d) in lower dosage group (0.5 mL FETR /kg silage DM). The improvement in milk fat yield for low enzyme group may be resulting from the increased NDF digestibility as we mentioned. On the other hand, highest concentration enzyme group exhibited lower milk fat percentage (4.35 vs. 4.14 %) and milk fat yield (1.80 vs. 1.69 kg/d) when compared with the Control group. This finding is in agreement with other studies that used different levels of fibrolytic enzyme in dairy cows (Yang et al. 1999; Elwakeel et al. 2007). One of explanation to this lack effect may be due to a lower acetic acid concentration that was observed in the group treated with high enzyme dosage, compared with the control (64.41 vs. 61.87 mmol/l; Table 4.8). Acetate is a major precursor that provides carbon

sources in *De novo* lipogenesis in the mammary gland. In addition, the reduction in ruminal acetate concentration may result in milk fat depression in lactating dairy cows (Urrutia and Harvatine 2017).

Moreover, adding fibrolytic enzyme linearly ($P<0.05$) decreased ruminal β-hydroxybutyrate concentration, being lowest (0.088 mmol/L) for highest dosage enzyme group (1.50 mL of enzyme/kg of silage DM). The decrease of BHB in current study may also explain the reduction of milk fat in high dosage enzyme group. β-hydroxybutyrate derived from rumen microbial also plays a crucial role in *De novo* lipogenesis in the mammary gland (Le and Gj 2018). This finding is in line with other studies (Adesogan et al. 2007; Holtshausen et al. 2011; Dean et al. 2013) that the use of fibrolytic enzymes improved the energy status of cows by increasing plasma insulin and reducing plasma concentrations of β-hydroxybutyrate. Results from these experiments indicated that the use of enzymes reduced mobilization of fat from adipose tissue in lactating dairy cows.

Table 4.4. Effect of exogenous fibrolytic enzyme[1] derived from *Trichoderma reesei* (FETR; mixture of xylanase and cellulase) levels on milk yield, milk composition, and feed efficiency in lactating dairy cows fed with faba bean silage based-TMR.

Item	Enzyme level (mL FETR / kg silage DM)					*P* value	Polynomial Contrast. *P* value		
	Control (Ctrl)	0.50	0.75	1.00	SEM[1]	Contrast Ctrl vs. Trt	Linear	Quadratic	Cubic
Yield, kg/d [2]									
Milk	41.21	41.46	40.40	40.91	1.843	0.597	0.390	0.879	0.156
FCM	47.24	47.55	45.63	45.61	1.067	0.131	0.018	0.268	0.094
ECM	45.55	46.00	44.41	44.00	1.603	0.306	0.058	0.207	0.276
Milk composition [3]									
Fat, %	4.35	4.34	4.33	4.14	0.125	0.249	0.047	0.104	0.371
Protein, %	3.15	3.15	3.14	3.13	0.056	0.791	0.456	0.427	0.849
Lactose, %	4.53	4.53	4.51	4.51	0.022	0.398	0.312	0.787	0.578
Total solids, %	12.95	12.95	12.90	12.69	0.163	0.224	0.031	0.078	0.626
SNF, %	8.65	8.66	8.62	8.62	0.062	0.575	0.438	0.706	0.545
SCC, 10^3 cells/ml	61.05	75.91	53.08	44.09	28.59	0.888	0.520	0.425	0.637
MUN, mg/dL	15.10	15.38	15.53	15.52	0.400	0.202	0.320	0.417	0.703
BHB (mmol/L)	0.103	0.091	0.088	0.088	0.008	0.002	0.004	0.139	0.185
Milk component yield									
Fat, kg/d	1.80	1.82	1.74	1.69	0.062	0.260	0.037	0.122	0.483
CP, kg/d	1.23	1.30	1.28	1.27	0.045	0.625	0.374	0.737	0.202
Lactose, kg/d	1.87	1.88	1.82	1.85	0.085	0.442	0.281	0.962	0.142
SNF, kg/d	3.56	3.58	3.52	3.48	0.146	0.557	0.333	0.807	0.162
Efficiency									
ECM/DMI	1.76	1.78	1.69	1.68	0.033	0.130	0.021	0.120	0.045
FCM/DMI	1.81	1.83	1.74	1.72	0.038	0.146	0.024	0.102	0.076

SEM: standard error of mean

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

4.4.4. Nitrogen Balance and Utilization

The predicted and actual nitrogen balance and nitrogen utilization are presented in Table 4.5. The results showed that fibrolytic enzyme has no significant effect on nitrogen utilization among the treatments. The predicted values in nitrogen intake was similar to real nitrogen intake (avg 706 vs. 709 g/d) and but predicted values overestimated nitrogen excretion (505 vs. 450 g/d). A previous study from our laboratory that replaced barley and corn silage with whole plant faba bean silage reported 711 g/d of nitrogen intake and 236 g/d of productive nitrogen with 2.96% milk protein and 4.02% milk fat. In contrast to this current study, whole plant faba bean silage based-diet showed a higher productive nitrogen (259 g/d) with lower nitrogen intake (707 g/d) and performed higher milk protein and fat percentage (3.14 and 4.29%, respectively). This indicated enzyme pre-treating whole plant faba bean silage based-TMR in this study is well balanced within adequate nitrogen and energy supply.

Table 4.5. Nitrogen balance and utilization in dairy cows fed whole plant faba bean silage based TMRs with different levels of exogenous fibrolytic enzyme[1] derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose)

	Enzyme level (mL FETR / kg silage DM)					Contrast, *P*-value			
Item	Control	0.50	0.75	1.00	SEM	Control vs. Treatments	Linear	Quadratic	Cubic
Predicted N utilization (g/d)									
N intake	717.71	704.35	698.97	702.34	16.426	0.158	0.189	0.527	0.810
Milk N	202.13	203.68	198.44	200.29	7.059	0.648	0.396	0.736	0.219
Urinary N	296.56	283.93	284.70	285.65	8.351	0.146	0.227	0.397	0.848
Fecal N	219.01	216.74	215.82	216.40	2.792	0.158	0.189	0.526	0.810
Manure N	515.58	500.66	500.53	502.05	10.940	0.145	0.216	0.416	0.912
Productive N	202.13	203.68	198.44	200.29	7.059	0.648	0.396	0.736	0.219
N utilization efficiency (g in milk/g intake)	28.21	28.93	28.35	28.49	0.547	0.355	0.690	0.329	0.281
Real N utilization (g/d)									
N intake	705.85	698.29	721.63	711.18	27.965	0.685	0.423	0.772	0.137
Milk N	202.13	203.68	198.44	200.29	7.059	0.648	0.396	0.736	0.219
Urinary N	200.46	185.30	197.99	188.20	8.300	0.283	0.438	0.642	0.180
Fecal N	259.64	269.65	252.25	246.47	12.732	0.654	0.188	0.087	0.225
Manure N	458.46	451.68	453.77	436.06	15.690	0.355	0.251	0.496	0.480
Productive N	249.37	245.94	270.08	271.60	26.451	0.457	0.230	0.597	0.457
Retained N	39.62	36.82	75.55	80.44	26.101	0.203	0.085	0.395	0.250
N utilization efficiency (g in milk/g intake)	29.02	29.45	27.94	28.38	1.246	0.504	0.221	0.589	0.121

SEM: standard error of mean

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

4.4.5. Energy Partitioning

Table 4.6 shows the energy values and energy partitioning. The fibrolytic enzyme application did not affect (P>0.05) energy for maintenance, energy for body weight gain plus milk production and total energy. However, the energy for total milk production of milk per day (P=0.06) and energy for 1 kg of milk production (P=0.054) tended to be linearly affected by the enzyme application. These findings may be resulting from the increase in milk fat yield that was improved by fibrolytic enzyme. The NE_L was averaged at 1.65 Mcal/ kg DMI, indicating that whole plant faba bean silage-based TMR provided sufficient energy for lactating dairy cows. There was no difference in percentage of net energy partitioning among the treatments. Dairy cows fed with enzyme pre-treated whole plant faba bean silage-based diet performed an average of 26.07% for maintenance and 72.26% for milk production, suggesting that fibrolytic enzyme did not alter energy from maintenance towards milk production. Similar results were demonstrated by Refat (2018), who reported that fibrolytic enzyme had no significant effect on energy partitioning.

Table 4.6. Energy Partitioning in dairy cows fed whole plant faba bean silage based TMRs with different levels of exogenous fibrolytic enzyme[1] derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose)

	Enzyme level (mL FETR / kg silage DM)					Contrast, *P*-value			
Item	Control	0.50	0.75	1.00	SEM	Control vs. Treatments	Linear	Quadratic	Cubic
Calculated Energy (Mcal/d)									
Maintenance	11.07	11.09	11.04	10.99	0.176	0.589	0.299	0.245	0.915
BWG	0.15	0.25	0.35	0.13	0.215	0.712	0.886	0.527	0.630
1kg milk	0.76	0.76	0.76	0.74	0.013	0.339	0.054	0.064	0.488
kg milk/day	31.21	31.31	30.49	30.18	1.061	0.245	0.060	0.306	0.439
BW gain + milk	32.04	32.62	32.34	30.88	1.519	0.951	0.594	0.363	0.854
Total energy	43.10	43.69	43.40	41.88	1.645	0.942	0.583	0.360	0.853
NE_L/d (Mcal/kg DMI)	1.67	1.67	1.65	1.60	0.056	0.735	0.462	0.580	0.998
Net energy partitioning (%Total energy)									
Maintenance	26.31	25.46	26.01	26.51	1.034	0.794	0.917	0.486	0.849
BWG	0.04	2.50	2.96	1.18	2.917	0.525	0.682	0.524	0.867
Milk	73.65	72.05	71.03	72.31	2.242	0.481	0.561	0.622	0.760
BW gain + milk	73.69	74.55	74.00	73.49	1.005	0.766	0.901	0.400	0.824

SEM: standard error of mean

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

4.4.6. Feeding Behavior

The results of chewing behavior are shown in Table 4.7. The length of laying, standing and first meal duration were not significantly affected by the enzyme treatment, with average at 801.0, 639.0 and 110.8 min/d, respectively. Total eating time, eating time per kilogram of dry matter and eating time per kilogram of NDF were also similar among the treatments (averaged at 384.6 min/d, 14.8 min/kg DM and 44.8 min/kg NDF). However, the length of ruminating was significantly differed ($P<0.05$) between control and treatment groups (CTRL 428.8 vs. Treatments 452.7 min/d). A trend ($P=0.06$) for higher ruminating time per kilogram of NDF was observed among treatments, being highest (55.5 min/kg of NDF) for intermediate dosage enzyme group (0.75 mL of enzyme/kg of silage DM). Pre-treating fibrolytic enzyme in whole plant faba bean silage diet tended to have a quadratic effect ($P=0.054$) on total chewing time (eating and ruminating time) and approached the borderline of significance ($P=0.058$) on total chewing time per kilogram of NDF between control and treatments. The longer chewing time observed in treatment groups must be related to an increase in NDF intake that improved by enzyme application.

In theory, pre-treating fibrolytic enzyme to feed may decrease chewing time and saliva output which may increase the risk of acidosis (Arriola et al. 2011). Bowman et al. (2002) noted that adding enzyme before feeding may induce a modification on plant cell wall structure which could cause a reduction in the physical effectiveness of the fiber. The effect of fibrolytic enzyme on chewing activity have been reported differently. Some studies reported an increase in eating when cows fed with enzyme pre-treated diet (He et al. 2015; Gandra et al. 2017). Silva et al. (2016) pointed out that total chewing activity was increased with fibrolytic enzyme supplementation. On the contrary, other studies found that fibrolytic enzyme showed no effect on total chewing activity (Beauchemin et al. 2000; Refat et al. 2018). Although this discrepancy is still unclear, it is assumed

to be related to differences in types of substrate, enzyme activity and application of enzyme. Further study is needed to clarify these inconsistent effects of fibrolytic enzyme in feeding behavior.

Table 4.7. Feeding behavior in dairy cows fed TMRs with different level of exogenous fibrolytic enzyme[1] derived from Trichoderma reesei (FETR, mixture of xylanase and cellulose)

Item	Enzyme level (mL FETR / kg silage DM)				SEM	Contrast, *P*-value			
	Control	0.50	0.75	1.00		Control vs. treatments	Linear	Quadratic	Cubic
Laying (min/d)	807.68	780.59	792.80	823.06	39.630	0.787	0.812	0.261	0.992
Standing (min/d)	632.32	659.41	647.20	616.94	39.630	0.787	0.812	0.261	0.992
First Meal (min/d)	102.54	133.39	100.15	107.18	16.819	0.444	0.973	0.175	0.080
Eating									
Min/d	374.76	412.97	372.90	377.87	20.869	0.554	0.929	0.246	0.189
Min/kg DM	14.64	16.05	14.12	14.51	1.047	0.798	0.729	0.325	0.147
Min/kg NDF	41.87	47.12	44.87	45.32	3.271	0.223	0.440	0.360	0.495
Ruminating									
Min/d	428.75	451.35	461.42	445.37	9.791	0.028	0.103	0.081	0.381
Min/kg DM	16.59	17.53	17.49	17.12	0.841	0.219	0.456	0.255	0.992
Min/kg NDF	48.00	50.97	55.48	53.56	2.862	0.051	0.061	0.575	0.242
Total chewing activity									
Min/d	802.29	863.45	835.23	824.40	22.904	0.091	0.445	0.054	0.358
Min/kg DM	31.170	33.591	31.620	31.659	1.739	0.372	0.871	0.172	0.234
Min/kg NDF	89.597	98.066	100.480	99.047	5.693	0.058	0.115	0.356	0.840

SEM: standard error of mean

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

4.4.7. Rumen Fermentation Parameters

The rumen fermentation parameters are presented in Table 4.8. There was no effect of FETR on ruminal pH (averaged at 6.13) and ruminal ammonia concentration (averaged at 12.62 mg/dl). Eun and Beauchemin (2005) stated that applying fibrolytic enzyme to high concentrate diet may indirectly depress ruminal pH, thus could cause ruminal acidosis in dairy cows. However, this negative effect of fibrolytic enzyme did not show in this current experiment.

Molar concentration of volatile fatty acid (acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, caproic acid) was similar ($P>0.05$) among the treatments (averaged at 62.98, 25.64, 0.8, 13.00, 2.07, 1.52 and 0.53 mmol/l). The ratio of acetic acid to propionic acid was linearly increased ($P<0.05$) by the enzyme application which was unexpected. The higher ratio of acetate-to-propionate in high enzyme dosage group could partly explain the decrease in milk fat. The ratio of acetate-to-propionate is an indicator of milk fat synthesis and reflects animal performance. The lower ratio of acetate-to-propionate implied an improvement in ruminal energetic efficiency which suggesting that increase delivery of glucogenic precursors to the mammary gland (Yang et al. 1999; Arriola et al. 2011). Although some researchers reported that enzyme application decreased the acetate-to-propionate ratio (Yang et al. 1999; Rode et al. 1999; Arriola et al. 2011), but not others (Kung et al. 2000; Bowman et al. 2002).

Table 4.8. Rumen fermentation characteristics of dairy cows fed TMRs with different level of inclusion of exogenous fibrolytic enzyme[1] derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose)

	Enzyme level (mL FETR / kg silage DM)					Contrast, *P*-value			
Item	Control	50	75	100	SEM	Control vs. Treatments	Linear	Quadratic	Cubic
Ruminal pH	6.07	6.08	6.11	6.27	0.077	0.388	0.138	0.258	0.742
NH_3 (mg/dl)	13.50	13.01	11.24	12.74	1.299	0.456	0.470	0.647	0.371
VFA (mmol/l)									
Acetic Acid	64.41	61.05	64.57	61.87	2.183	0.466	0.602	0.762	0.236
Propionic Acid	27.41	25.92	26.11	23.12	1.433	0.187	0.088	0.488	0.467
Isobutyric Acid	0.81	0.80	0.80	0.79	0.046	0.821	0.760	0.909	0.916
Butyric Acid	13.53	12.46	13.07	12.93	0.344	0.109	0.298	0.184	0.243
Isovaleric Acid	1.98	2.18	2.11	2.01	0.139	0.488	0.816	0.316	0.870
Valeric Acid	1.53	1.48	1.56	1.50	0.066	0.774	0.879	0.917	0.353
Caproic Acid	0.52	0.46	0.58	0.57	0.053	0.769	0.356	0.391	0.256
Ratio									
Acetic: Propionic	2.39	2.45	2.56	2.72	0.076	0.066	0.014	0.250	1.000

SEM: standard error of mean

[1] Fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulose). The product is a mixture of xylanase (EC 3.2.1.8; xylanase activity = 350,000 BXU/g, where one BXU is the amount of enzyme that is able to release 0.06 micromole of xylose equivalent from birchwood xylan per minute) and cellulase (EC 3.2.1.8; endoglucanase activity = 10000 ECU/g, where one ECU is the amount of enzyme is able to release 0.06 micromole of glucose from medium-viscosity carboxymethylcellulose per minute.

4.5. Conclusion

To conclude, dairy cows fed pre-treated whole plant faba bean silage with different level of fibrolytic enzyme affected nutrient digestibility, feed efficiency and milk performance in linear or quadratic pattern. Numerically, the group treated with 0.5 ml of fibrolytic enzyme /kg dry matter of whole plant faba bean silage showed highest result among treatments. However, when increased to higher dose levels of fibrolytic enzyme, it showed a potentially negative effect of fibrolytic enzyme on milk performance. Overall, further study with a larger number of lactational dairy cows are needed to confirm these findings.

5. GENERAL DISCUSSION AND OVERALL CONCLUSION

Whole plant faba bean silage (Snowbird) is a newly developed alternative feed source for dairy cows in western Canada. The detailed nutrition values of whole plant faba bean silage affected by cutting stages, variety and/or tannin level have been reported by Yan (2020) and Guevara Oquendo (2020). Whole plant faba bean silages are rich in starch (176 g/kg DM), protein (212 g/kg DM), and relatively high NDF (328 g/kg DM) content (Yan 2020). The use of fbirolytic enzyme was first examined in 1960s in order to improve animal performance (Burroughs et al. 1960). The enhancement of fibrolytic enzyme on animal performance mainly result from the increase of fibre digestibility. This project investigated the effects of fibrolytic enzyme (derived from *Trichoderma reesei*; a mixture of xylanase and cellulase) on whole plant faba bean silage, revealed with *in situ, in vitro* and *in vivo* assay.

Results from the first study in Chapter 3 showed that fibrolytic enzyme had positive impacts on DM and NDF degradability of whole plant faba bean silage in both *in situ* and *in vitro* methods. We observed a cubic effect on ISDMD and a quadratic effect on ISNDFD. On the other hand, a quadratic effect was showed in IVDMD and a cubic effect in IVNDFD when applied fibrolytic enzyme to whole plant faba bean silage. The *in vitro* DM degradability ranged from 52 to 57 % and NDF degradability ranged from 16 to 30% with different level of enzyme application. Besides, *in situ* DM degradability ranged from 51 to 56% and NDF degradability was observed between 12 to 27%. Both techniques showed that DM and NDF degradability of faba bean silage were enhanced by the enzyme application. The degradability of neutral detergent fibre is a function of the potentially digestible fraction, digestion rate and passage rate (Oba and Allen 1999). Improvements of fibrolytic enzymes may allow greater voluntary feed intake by reducing physical fill in the rumen and increases energy density in the diets (Eun et al. 2007). It is expected that

increased in *in situ* and *in vitro* NDF degradability would increase milk production and animal performance.

Comparison and correlation analysis between *in vitro* approach (Daisy^{II} incubation method) and *in situ* assay-biological approach (nylon bag technique) in dairy cows was also explored in this study. The results from traditional *in situ* method presented a more consistent result with less variation in comparison with *in vitro* Daisy^{II} incubator. The higher values obtained from the Daisy^{II} incubator may be attributed to the differences in pore size of bags, incubation condition and microbial ability to degrade substrate. The rumen contractions during digestion could give a higher pressure on *in situ* bags that may cause a faster rates of rumen fluid flow through the bags. Factors such as ruminal pH, composition of buffer solution and rumen fluid sampling time may also affect the results. Regardless of differences between two techniques, low to medium dosage (0.50 to 1.00 mL of FETR /kg DM of silage) of enzyme application also showed the better responses on dry matter and neutral detergent fibre degradability. The overall DM and NDF degradability between two approaches are non-statistically significant ($P>0.05$), indicating *in vitro* Daisy-II incubation method appears to be a useful tool when compared to traditional method due to its advantages as fast, simple and efficiency.

The second study in Chapter 3 focused on effects of fibrolytic enzyme on DM and NDF degradation residue and degradation kinetics of whole plant faba bean silage. The enzyme application also showed its highly positive effects on both DM and NDF degradability. The DM degradation residue of all treatments rapidly decreased at the first 12 h of incubation and reached a plateau around 24 h of incubation time. Additionally, the 48 h *in situ* NDF residues showed lower value when compared with control group (treatments averaged at 33.11 vs. control 39.86 %). The dry matter soluble fraction of faba bean silage was increased linearly ($P<0.05$) as the dose of

enzyme increased. Moreover, applying firborlytic enzyme showed a great impact on NDF degradation kinetic. Potential degradable and undegradable fractions were linearly (P=0.05) affected by the enzyme application and a cubic effect (P<0.05) on lag phase of neutral detergent fiber. This indicates that fibrolytic enzymes attached to the substrate before the native enzymes degrading which allows more potentially degradable fraction released. The DM and NDF degradation residue and kinetics were improved with low to medium dosage of enzyme application by decreasing undegradable fraction, increasing potential degradable fraction and effective degradable content of fiber, whereas high levels of enzyme addition seemed to affect adversely.

The aim of Chapter 4 was to evaluate the effects of pre-treating whole plant faba bean silage based-diet with exogenous fibrolytic enzyme derived from *Trichoderma reesei* (FETR, mixture of xylanase and cellulase) on lactational performance, digestibility, rumen fermentation characteristics and feeding behavior in lactational dairy cows. The enzyme dose levels used in this study were selected based on the results of Chapter 3, which low to medium dosage enzyme (0.50 to 1.00 mL of FETR /kg DM of silage) presented the best responses. The data from our study showed that pre-treating whole plant faba bean silage diet with fibrolytic enzyme linearly affected FCM and ECM production without altering DMI. Moreover, there was no difference found in milk production between control and treatments, total milk production among all treatments was maintained at a high level (averaged at 41.0 kg/d). However, when milk yield was corrected with energy and fat, the results showed that both energy (ECM, P = 0.018<0.05) and fat corrected milk yield (FCM, P=0.058<0.10) were linearly declined or tended to be affected by fibrolytic enzyme dose level. The ECM (kg of ECM/kg of DMI) and FCM (kg of FCM/kg of DMI) production efficiency were also affected by the enzyme application, whereas low enzyme dosage (0.50 mL of enzyme/kg of TMR DM) preformed the highest (1.78, 1.83 respectively) in both feed conversion

ratio. Adding fibrolytic enzyme to the whole plant faba bean silage diet also affected percentage of milk fat and milk fat yield in a linear pattern, being highest for the low enzyme level group. However, applying with increasing levels of enzymes on faba bean based-TMR tended to depress lactation performance. Pre-treating fibrolytic enzyme in whole plant faba bean silage diet tended to affect ruminating time and total chewing activity. However, these findings are still unclear. Despite the effects on digestibility and chewing activities, enzyme supplementation had no effect on ruminal pH (averaged at 6.13) and short chain fatty acids concentration.

In conclusion, this study showed that treating whole plant faba bean silage with different levels fibrolytic enzyme did not affect DMI but reduced animal performance, linearly affected nutrient digestibility, chewing activities and feed efficiency. The inconsistent results observed in this study may be attributed to the method of enzyme application, the lactation stage of animals and the substrate specificity. Typically, exogenous enzymes could be an option to improve digestibility when cows fed with low quality forage source. However, faba bean silage used in this study is consider as high quality feed source which may not be the most appropriate to treated with fibrolytic enzyme. Based on the current study, it was suggested that the optimum dosage of enzyme for whole plant faba bean silage was at 0.50 mL of enzyme/kg of silage DM. Enzyme addition with this level has the potential to maximally enhance substrate fermentation thus provides additional energy for animals and improve animal performance, whereas high levels of enzyme addition seemed to affect adversely. Therefore, further studies are needed to confirm the effect of fibrolytic enzyme on other types of feedstuffs and to determine the specific, optimal enzyme combination.

6.REFERENCE

Abeysekara, S., Christensen, D.A., Niu, Z., Theodoridou, K., and Yu, P. 2013. Molecular structure, chemical and nutrient profiles, and metabolic characteristics of the proteins and energy in new cool-season corn varieties harvested as fresh forage for dairy cattle. J. Dairy Sci. **96**: 6631–6643. doi:10.3168/jds.2013-6841.

Adesogan, A. T. 2005. Improving forage quality and animal performance with fibrolytic EFEs. Pages 91–109 in Proc. 16th Florida ruminant nutrition symposium, University of Florida, Gainesville, FL.

Adesogan, A. T., S. C. Kim, K. G. Arriola, D. B. Dean, and C. R. Staples. 2007. Strategic addition of dietary fibrolytic enzymes for improved performance of lactating dairy cows. Pages 92–110 in Proc. Florida ruminant nutrition symposium. 18th Annual Mtg., Gainesville, FL. Univ. Florida, Gainesville.

Aherne, F.X., Lewis, A.J., and Hardin, R.T. 1977. An evaluation of faba beans (vicia Faba) as a protein supplement for wwine. Can. J. Anim. Sci. 57: 321–328. doi:10.4141/cjas77-040.

Alberta Faba Bean Producer Manual 1.0 2014.February 9. [Online] Available: https://albertapulse.com/2014/02/alberta-faba-bean-producer-manual-1-0/ [2019 Jun. 13].

Allen, M.S. 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74: 3063–3075. doi:10.2527/1996.74123063x.

Allen, M.S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. **80**: 1447–1462. doi:10.3168/jds.S0022-0302(97)76074-0.

Allen, M.S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. **83**: 1598–1624. doi:10.3168/jds.S0022-0302(00)75030-2.

Allen, M.S., and Mertens, D.R. 1988. Evaluating constraints on fiber digestion by rumen microbes. J. Nutr. **118**: 261–270. doi:10.1093/jn/118.2.261.

Alokan, J.A., and Aletor, V.A. 1997. Plant tannins-Their role in forage legume quality.[Online] Available: http://www.international grasslands.org/files/igc/publications/1997/1-08-025.pdf. [2020 Nov. 16]

Amaral-Phillips, D. (n.d.). Milk Urea Nitrogen - A Nutritional Evaluation Tool. [Online] Available: https://afs.ca.uky.edu/files/milk_urea_nitrogen.pdf [2020 Dec. 10].

AOAC. 2000. Official methods of analysis. 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

ANKOM Technology 2017. [Online] Available: https://www.ankom.com/sites/default/files/document-files/D200_D200I_Manual.pdf [2020 Dec. 18].

Arriola, K.G., Kim, S.C., Staples, C.R., and Adesogan, A.T. 2011. Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle. J. Dairy Sci. **94**: 832–841. doi:10.3168/jds.2010-3424.

Balci, F., Dikmen, S., Gencoglu, H., Orman, A., Turkmen, I.I., and Biricik, H. 2007. The effect of fibrolytic exogenous enzyme on fattening performance of steers. Bulg. J. Vet. Med., 10(2), 113-118.

Barber, M.S., and Mitchell, H.J. 1997. Regulation of Phenylpropanoid Metabolism in Relation to Lignin Biosynthesis in Plants. Pages 243–293 *in* K.W. Jeon, ed. International Review of Cytology. Academic Press. doi:10.1016/S0074-7696(08)62362-1.

Barker, B., and Ag, P. 2019. Inoculant Options for Pulse Crops. [Online] Available: https://saskpulse.com/files/technical_documents/190408_Inoculant_Options_for_Pulse_Crops.pdf [2020 Nov 20].

Barry, T.N., and McNabb, W.C. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Br. J. Nutr. **81**: 263–272. doi:10.1017/S0007114599000501.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., and Yang, W.Z. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. **81**: E37–E47. doi:10.2527/2003.8114_suppl_2E37x.

Beauchemin, K.A., Rode, L.M., Maekawa, M., Morgavi, D.P., and Kampen, R. 2000. Evaluation of a nonstarch polysaccharidase feed enzyme in dairy cow diets. J. Dairy Sci. **83**: 543–553. doi:10.3168/jds.S0022-0302(00)74914-9.

Beauchemin, K.A., Rode, L.M., and Sewalt, V.J.H. 1995. Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. Can. J. Anim. Sci. **75**: 641-644. doi:10.4141/cjas95-096.

Beauchemin, K.A., Rode, L.M., and Sewalt, V.J.H. 2011. Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. Can. J. Anim. Sci. **75**: 641-644. doi:10.4141/cjas95-096.

Bedford, M.R., and Partridge, G.G. 2001. Enzymes in Farm Animal Nutrition. CABI Publishing. Wallingford, UK.

Berkenkamp, B., and Meeres, J. 1986. Faba bean as forage in the parklands of Alberta. Can. J. Plant Sci. doi:10.4141/cjps86-022.

Bhat, M.K., and Hazlewood, G.P. 2001. Enzymology and other characteristics of cellulases and xylanases. Enzym. Farm Anim. Nutr.: 11–60. CABI Publishing. Wallingford, UK.

Blair, R., Wilson, B.J., and Bolton, W. 1970. Growth of broilers given diets containing field beans (*vicia faba* l.) during period the 0 to 4 week. Br. Poult. Sci. **11**: 387–398. doi:10.1080/00071667008415828.

Bowman, G.R., Beauchemin, K.A., and Shelford, J.A. 2002. The proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. J. Dairy Sci. **85**: 3420–3429. doi:10.3168/jds.S0022-0302(02)74430-5.

Broderick, G.A., and Kang, J.H. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. **63**: 64–75. doi:10.3168/jds.S0022-0302(80)82888-8.

Brunschwig, P., and Lamy, J.-M. 2002. Utilisation de féverole ou de tourteau de tournesol comme sources protéiques (...) - 3R - Rencontres autour des Recherches sur les Ruminants. [Online] Available: http://www.journees3r.fr/spip.php?article985 [2020 May 23].

Burroughs, W., Woods, W., Ewing, S.A., Greig, J., and Theurer, B. 1960. Enzyme additions to fattening cattle rations. J. Anim. Sci. **19**: 458–464. doi:10.2527/jas1960.192458x.

Caballero, B., Trugo, L.C., and Finglas, P.M. 2003. Encyclopedia of food sciences and nutrition. Academic. [Online] Available: https://agris.fao.org/agris-search/search.do?recordID=US201300082710 [2020 May 16].

Carton, N., Naudin, C., Piva, G., Baccar, R., and Corre-Hellou, G. 2018. Differences for traits associated with early N acquisition in a grain legume and early complementarity in grain legume–triticale mixtures. AoB PLANTS **10**. doi:10.1093/aobpla/ply001.

Caffall, K.H., and Mohnen, D. 2009. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydr. Res. **344**: 1879–1900. doi:10.1016/j.carres.2009.05.021.

Canadian Council on Animal Care. 1993. Guide to the care and use of experimental animals. Vol.1, 2nd ed. CCAC, Ottawa, ON.

Cherubini, F., and Strømman, A.H. 2011. Chapter 1 - Principles of Biorefining. Pages 3–24 *in* A. Pandey, C. Larroche, S.C. Ricke, C.-G. Dussap, and E. Gnansounou, eds. Biofuels. Academic Press, Amsterdam. doi:10.1016/B978-0-12-385099-7.00001-2.

Clancey, B. 2018a. Chickpea and Faba Bean Markets. [Online] Available: https://saskpulse.com/files/report/180927_PMR_Oct_2018_Clancey-compressed.pdf [2019 Jun 13.].

Clancey, B. 2018b. Market Reports. [Online] Available: https://saskpulse.com/resources/market-reports/P16 [2019 Jun. 13].

Colombatto, D., Morgavi, D.P., Furtado, A.F., and Beauchemin, K.A. 2003. Screening of exogenous enzymes for ruminant diets: Relationship between biochemical characteristics and in vitro ruminal degradation. J. Anim. Sci. **81**: 2628–2638. doi:10.2527/2003.81102628x.

Colombatto, D., Mould, F.L., Bhat, M.K., and Owen, E. 2007. Influence of exogenous fibrolytic enzyme level and incubation pH on the in vitro ruminal fermentation of alfalfa stems. Anim. Feed Sci. Technol. **137**: 150–162. doi:10.1016/j.anifeedsci.2006.10.001.

Combs, D.K. 2016. Relationship Between NDF Digestibility and Animal Performance. [Online] Available: https://wcds.ualberta.ca/wcds/wp-content/uploads/sites/57/wcds_archive/Archive/2016/Manuscripts/p%20083%20-%20098%20Combs.pdf [2020 Nov. 14]

Dean, D. B., Staples, C. R., Littell, R. C., Kim, S. & Adesogan, A. T. 2013. Effect of method of adding a fibrolytic enzyme to dairy cow diets on feed intake digestibility, milk production, ruminal fermentation, and blood metabolites. Anim. Nutr. Feed Technol. 13, 337-357.

Dijkstra, J., Forbes, J.M., and France, J. 2005. Quantitative Aspects of Ruminant Digestion and Metabolism. CABI. Wallingford, UK.

Duc, G. 1997. Faba bean (Vicia faba L.). Field Crops Res. **53**: 99–109. doi:10.1016/S0378-4290(97)00025-7.

Elwakeel, E.A., Titgemeyer, E.C., Johnson, B.J., Armendariz, C.K., and Shirley, J.E. 2007. Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. J. Dairy Sci. **90**: 5226–5236. doi:10.3168/jds.2007-0305.

Emiola, I.A., and Gous, R.M. 2011. Nutritional evaluation of dehulled faba bean (Vicia faba cv. Fiord) in feeds for weaner pigs. S. Afr. J. Anim. Sci., **41**: 19-86.

Eun, J.-S., and Beauchemin, K.A. 2005. Effects of a proteolytic feed enzyme on intake, digestion, ruminal fermentation, and milk production. J. Dairy Sci. **88**: 2140–2153. doi:10.3168/jds.S0022-0302(05)72890-3.

Eun, J.-S., Beauchemin, K.A., and Schulze, H. 2007. Use of exogenous fibrolytic enzymes to enhance in vitro fermentation of alfalfa hay and corn silage. J. Dairy Sci. **90**: 1440–1451. doi:10.3168/jds.S0022-0302(07)71629-6.

Eun, J.-S., ZoBell, D.R., Dschaak, C.M., Diaz, D.E., and Tricarico, J.M. 2009. Effects of supplementing a fibrolytic feed enzyme on the growth performance and carcass

characteristics of beef steers. Prof. Anim. Sci. **25**: 382–387. doi:10.15232/S1080-7446(15)30729-4.
Feng, P., Hunt, C.W., Pritchard, G.T., and Julien, W.E. 1996. Effect of enzyme preparations on in situ and in vitro degradation and in vivo digestive characteristics of mature cool-season grass forage in beef steers. J. Anim. Sci. **74**: 1349–1357. doi:10.2527/1996.7461349x.
Fraser, M.D., Fychan, R., and Jones, R. 2001. The effect of harvest date and inoculation on the yield, fermentation characteristics and feeding value of forage pea and field bean silages. Grass Forage Sci. **56**: 218–230. doi:10.1046/j.1365-2494.2001.00268.x.
Frutos, P., Hervás, G., Giráldez, F.J., and Mantecón, A.R. 2004. Review. Tannins and ruminant nutrition. Span. J. Agric. Res. **2**: 191. doi:10.5424/sjar/2004022-73.
Gado, H.M., Salem, A.Z.M., Robinson, P.H., and Hassan, M. 2009. Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Anim. Feed Sci. Technol. **154**: 36–46. doi:10.1016/j.anifeedsci.2009.07.006.
Gallardo, I., Bárcena, R., Pinos-Rodríguez, J.M., Cobos, M., Carreón, L., and Ortega, M.E. 2010. Influence of exogenous fibrolytic enzymes on in vitro and in sacco degradation of forages for ruminants. Ital. J. Anim. Sci. **9**: e8. doi:10.4081/ijas.2010.e8.
Gandra, J.R., Miranda, G.A., Goes, R.H.T.B., Takiya, C.S., Del Valle, T.A., Oliveira, E.R., Freitas Junior, J.E., Gandra, E.R.S., Araki, H.M.C., and Santos, A.L.A.V. 2017. Fibrolytic enzyme supplementation through ruminal bolus on eating behavior, nutrient digestibility and ruminal fermentation in Jersey heifers fed either corn silage- or sugarcane silage-based diets. Anim. Feed Sci. Technol. **231**: 29–37. doi:10.1016/j.anifeedsci.2017.06.009.
Gilbert, H.J., and Hazlewood, G.P. 1993. Bacterial cellulases and xylanases: Microbiology. **139**: 187-194.
Goering, H.K., and Soest, P.J.V. 1970. Forage Fiber Analyses: Apparatus, Reagents, Procedures, and Some Applications. Agricultural Research Service, U.S. Department of Agriculture. Washington, DC, USA.
Gous, R.M. 2011. Evaluation of faba bean (*Vicia faba* cv. Fiord) as a protein source for broilers. South Afr. J. Anim. Sci. **41**: 71–78. doi:10.4314/sajas.v41i2.71009.
Grant, R.J., Haddad, S.G., Moore, K.J., and Pedersen, J.F. 1995. Brown midrib sorghum silage for midlactation dairy cows. J. Dairy Sci. **78**: 1970–1980. doi:10.3168/jds.S0022-0302(95)76823-0.
Guevara Oquendo, V. 2020.July. Molecular Structural and Nutritional Evaluation of Faba Bean Plants as Hay and Silage For Ruminants: Effect of Tannin Concentration, Cutting Stage, and Frost-Damage. PhD Thesis, University of Saskatchewan. [Online] Available: https://harvest.usask.ca/handle/10388/12956 [2021 Jan. 17].
Hall, M.B. 2009. Determination of starch, including maltooligosaccharides, in animal feeds: comparison of methods and a method recommended for AOAC collaborative study. J. AOAC Int. **92**: 42–49.
Hamid, R., and Masood, A. 2009. Dietary Lectins as Disease Causing Toxicants. Pakistan Journal of Nutrition, **8**(3), 293-303.
Hanelt, P., and Mettin, D. 1989. Biosystematics of the Genus VICIA L. (Leguminosae). Annu. Rev. Ecol. Syst. **20**: 199–223. doi:10.1146/annurev.es.20.110189.001215.
Hatfield, R.D. 1989. Structural polysaccharides in forages and their degradability. Agron. J. **81**: 39–46. doi:10.2134/agronj1989.00021962008100010007x.

Hatfield, R.D., Ralph, J., and Grabber, J.H. 1999. Cell wall structural foundations: Molecular basis for improving forage digestibilities. Crop Sci. **39:** 27-37. doi: https://doi.org/10.2135/cropsci1999.0011183X003900010005x

He, Z.X., Walker, N.D., McAllister, T.A., and Yang, W.Z. 2015. Effect of wheat dried distillers grains with solubles and fibrolytic enzymes on ruminal fermentation, digestibility, growth performance, and feeding behavior of beef cattle1. J. Anim. Sci. **93**: 1218–1228. doi:10.2527/jas.2014-8412.

Heron, S.J.E., Edwards, R.A., and McDonald, P. 1986. Changes in the nitrogenous components of gamma-irradiated and inoculated ensiled ryegrass. J. Sci. Food Agric. **37**: 979–985. doi:10.1002/jsfa.2740371005.

Hoffman, P. C., Shaver, R. D., Combs, D. K., Undersander, D. J., Bauman, L. M., and Seeger, T. K. 2001. Understanding NDF digestibility of forages. Online. Focus on Forage, Vol 3, no. 10. UW Extension, Univ. of Wisconsin, Madison, WI.

Hoffman, P.C., Lundberg, K.M., Bauman, L.M., and Shaver, R.D. 2003. The Effect of Maturity on NDF Digestibility. Focus on Forage, 5(15), 1-3.

Holtshausen, L., Chung, Y.-H., Gerardo-Cuervo, H., Oba, M., and Beauchemin, K.A. 2011. Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. J. Dairy Sci. **94**: 899–907. doi:10.3168/jds.2010-3573.

Hossain, Z., Wang, X., Hamel, C., Knight, J.D., Morrison, M.J., and Gan, Y. 2016. Biological nitrogen fixation by pulse crops on the semiarid Canadian Prairie. Can. J. Plant Sci. **97**: 119-131. doi:10.1139/CJPS-2016-0185.

Hove, E.L., and King, S. 1979. Trypsin inhibitor contents of lupin seeds and other grain legumes. N. Z. J. Agric. Res. **22**: 41–42. doi:10.1080/00288233.1979.10420841.

Hristov, A.N., McAllister, T.A., and Cheng, K.-J. 2000. Intraruminal supplementation with increasing levels of exogenous polysaccharide-degrading enzymes: effects on nutrient digestion in cattle fed a barley grain diet. J. Anim. Sci. **78**: 477–487. doi:10.2527/2000.782477x.

Huhtanen, P., Ahvenjärvi, S., Broderick, G.A., Reynal, S.M., and Shingfield, K.J. 2010. Quantifying ruminal digestion of organic matter and neutral detergent fiber using the omasal sampling technique in cattle—A meta-analysis. J. Dairy Sci. **93**: 3203–3215. doi:10.3168/jds.2009-2988.

Huhtanen, P., Kaustell, K., and Jaakkola, S. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Anim. Feed Sci. Technol. **48**: 211–227. doi:10.1016/0377-8401(94)90173-2.

Huisman, J., Poel, T.F.B. van der, and International Workshop on Antinutritional Factors (ANF) in Legume Seeds (eds.) 1989. Recent advances of research in antinutritional factors in legume seeds: animal nutrition, feed technology, analytical methods. Proceedings of the First International Workshop on "Antinutritional Factors (ANF) in legume seeds", November 23-25, 1988, Wageningen, The Netherlands. Pudoc, Wageningen.

Humphreys, J.M., and Chapple, C. 2002. Rewriting the lignin roadmap. Curr. Opin. Plant Biol. **5**: 224–229. doi:10.1016/S1369-5266(02)00257-1.

Jalilvand, G., Odongo, N.E., López, S., Naserian, A., Valizadeh, R., Shahrodi, F.E., Kebreab, E., and France, J. 2008. Effects of different levels of an enzyme mixture on in vitro gas production parameters of contrasting forages. Anim. Feed Sci. Technol. **146**: 289–301. doi:10.1016/j.anifeedsci.2008.01.007.

Jensen, E.S., Peoples, M.B., and Hauggaard-Nielsen, H. 2010. Faba bean in cropping systems. Field Crops Res. **115**: 203–216. doi:10.1016/j.fcr.2009.10.008.

Jung, H.G., and Allen, M.S. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim. Sci. **73**: 2774–2790. doi:10.2527/1995.7392774x.

Kelln, B.M., Penner, G.B., Acharya, S.N., McAllister, T.A., and Lardner, H.A. 2020. Impact of condensed tannin-containing legumes on ruminal fermentation, nutrition, and performance in ruminants: a review. Can. J. Anim. Sci. doi:10.1139/cjas-2020-0096.

Kendall, C., Leonardi, C., Hoffman, P.C., and Combs, D.K. 2009. Intake and milk production of cows fed diets that differed in dietary neutral detergent fiber and neutral detergent fiber digestibility. J. Dairy Sci. **92**: 313–323. doi:10.3168/jds.2008-1482.

Kertz, A.F., Reutzel, L.F., and Thomson, G.M. 1991. Dry Matter Intake from Parturition to Midlactation. J. Dairy Sci. **74**: 2290–2295. doi:10.3168/jds.S0022-0302(91)78401-4.

Khamassi, K., Ben Jeddi, F., Hobbs, D., Irigoyen, J., Stoddard, F., O'Sullivan, D.M., and Jones, H. 2013. A baseline study of vicine–convicine levels in faba bean (*Vicia faba* L.) germplasm. Plant Genet. Resour. **11**: 250–257. doi:10.1017/S1479262113000105.

Kirk, P.L. 1950. Kjeldahl Method for Total Nitrogen. Anal. Chem. **22**: 354–358. doi:10.1021/ac60038a038.

Knowlton, K.F., McKinney, J.M., and Cobb, C. 2002. Effect of a direct-fed fibrolytic enzyme formulation on nutrient intake, partitioning, and excretion in early and late lactation holstein cows. J. Dairy Sci. **85**: 3328–3335. doi:10.3168/jds.S0022-0302(02)74421-4.

Krause, D.O., Denman, S.E., Mackie, R.I., Morrison, M., Rae, A.L., Attwood, G.T., and McSweeney, C.S. 2003. Opportunities to improve fiber degradation in the rumen: microbiology, ecology, and genomics. FEMS Microbiol. Rev. **27**: 663–693. doi:10.1016/S0168-6445(03)00072-X.

Krause, M., Beauchemin, K.A., Rode, L.M., Farr, B.I., and Nørgaard, P. 1998. Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle. J. Anim. Sci. **76**: 2912–2920. doi:10.2527/1998.76112912x.

Krueger, N.A., Adesogan, A.T., Staples, C.R., Krueger, W.K., Kim, S.C., Littell, R.C., and Sollenberger, L.E. 2008. Effect of method of applying fibrolytic enzymes or ammonia to Bermudagrass hay on feed intake, digestion, and growth of beef steers. J. Anim. Sci. **86**: 882–889. doi:10.2527/jas.2006-717.

Kung, L., Cohen, M.A., Rode, L.M., and Treacher, R.J. 2002. The effect of fibrolytic enzymes sprayed onto forages and fed in a total mixed ratio to lactating dairy cows. J. Dairy Sci. **85**: 2396–2402. doi:10.3168/jds.S0022-0302(02)74321-X.

Kung, L., Treacher, R.J., Nauman, G.A., Smagala, A.M., Endres, K.M., and Cohen, M.A. 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. J. Dairy Sci. **83**: 115–122. doi:10.3168/jds.S0022-0302(00)74862-4.

Lacassagne, L., Melcion, J.P., de Monredon, F., and Carré, B. 1991. The nutritional values of faba bean flours varying in their mean particle size in young chickens. Anim. Feed Sci. Technol. **34**: 11–19. doi:10.1016/0377-8401(94)90187-2.

Landry, E. J. 2014. Faba bean (*Vicia faba L.*) a promising new pulse crop for southeastern Washington. Ph.D. dissertation, Washington State University, Pullman, WA. USA. 247 pp.

Le, K., and Gj, L. 2018. Milk fat depression: etiology, theories, and soluble carbohydrate interactions. J. Anim. Res. Nutr. **03**. doi:10.21767/2572-5459.100046.

Lewis, G.E., Hunt, C.W., Sanchez, W.K., Treacher, R., Pritchard, G.T., and Feng, P. 1996. Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. J. Anim. Sci. **74**: 3020–3028. Oxford Academic. doi:10.2527/1996.74123020x.

Lewis, G.E., Sanchez, W.K., Hunt, C.W., Guy, M.A., Pritchard, G.T., Swanson, B.I., and Treacher, R.J. 1999. Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. J. Dairy Sci. **82**: 611–617. doi:10.3168/jds.S0022-0302(99)75274-4.

Licitra, G., Hernandez, T.M., and Van Soest, P.J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. **57**: 347–358. doi:10.1016/0377-8401(95)00837-3.

Marquardt, R.R., and Campbell, L.D. 1973. Raw and autoclaved faba beans in chick diets. Can. J. Anim. Sci. **53**: 741–746. doi:10.4141/cjas73-117.

McAllister, T.A., Hristov, A.N., Beauchemin, K.A., Rode, L.M., Cheng, K.J., Bedford, M.R., and Partridge, G. 2001. Enzymes in ruminant diets. Enzymes in farm animal nutrition, 273-298. doi:10.1079/9780851993935.0273.

McAllister, T.A., Oosting, S.J., Popp, J.D., Mir, Z., Yanke, L.J., Hristov, A.N., Treacher, R.J., and Cheng, K.-J. 1999. Effect of exogenous enzymes on digestibility of barley silage and growth performance of feedlot cattle. Can. J. Anim. Sci. **79**: 353–360. doi:10.4141/A98-099.

McGill, J., Finley, W., and Feyertag, J. 2016. Opportunities for Canadian Faba Beans. [Online] Available: https://static1.squarespace.com/static/5b2fcd533c3a53bd6c82384a/t/5b637fe7562fa7b55f24ae30/1533247529705/2017-Joe-Feyertag.pdf [2019 Jun. 1].

McKague, K., Reid, K., and Simpson, H. 2005. Environmental impacts of nitrogen use in agriculture. Ontario, Ministry of Agriculture, Food and Rural Affairs. Ontario, Canada.

McKnight, D.R., and MacLeod, G.K. 1977. Value of whole plant faba bean silage as the sole forage for lactating cows. Can. J. Anim. Sci. **57**: 601–603. doi:10.4141/cjas77-077.

Mendoza, G.D., Loera-Corral, O., Plata-Pérez, F.X., Hernández-García, P.A., and Ramírez-Mella, M. 2014.October 14. Considerations on the Use of Exogenous Fibrolytic Enzymes to Improve Forage Utilization. Review Article, Hindawi. doi:https://doi.org/10.1155/2014/247437.

Mertens, D.R. 1973. Application of theoretical mathematical models to cell wall digestion and forage intake in ruminants. Doctoral dissertation. Cornell Univeristy. Ithaca, NY, USA.

Mertens, D.R. 1994. Regulation of Forage Intake. Pages 450–493 *in* Forage Quality, Evaluation, and Utilization. John Wiley & Sons, Ltd. doi:10.2134/1994.foragequality.c11.

Mertens, D.R. 2009. Impact of NDF content and digestibility on dairy cow performance. [Online] Available: https://pubag.nal.usda.gov/catalog/30864 [2020 Jun. 30].

Meyer, J.H., and Mackie, R.I. 1986. Microbiological evaluation of the intraruminal in sacculus digestion technique. Appl. Environ. Microbiol. **51**: 622–629.

Min, B.R., Barry, T.N., Attwood, G.T., and McNabb, W.C. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Anim. Feed Sci. Technol. **106**: 3–19. doi:10.1016/S0377-8401(03)00041-5.

Mohamed, D.E.-D.A., Borhami, B.E., El-Shazly, K.A., and Sallam, S.M.A. 2013. Effect of dietary supplementation with fibrolytic enzymes on the productive performance of early lactating dairy cows. J. Agric. Sci. **5**: p146. doi:10.5539/jas.v5n6p146.

Muduuli, D.S., Marquardt, R.R., and Guenter, W. 1982. Effect of dietary vicine and vitamin E supplementation on the productive performance of growing and laying chickens. Br. J. Nutr. **47**: 53–60. doi:10.1079/BJN19820008.

Mugerwa, J.S., Christensen, D.A., and Ochetim, S. 1973. Grazing behaviour of exotic dairy cattle in Uganda. East Afr. Agric. For. J. **39**: 1–11. doi:10.1080/00128325.1973.11662610.

Mustafa, A.F., and Seguin, P. 2003. Characteristics and in situ degradability of whole crop faba bean, pea, and soybean silages. Can. J. Anim. Sci. **83**: 793–799. doi:10.4141/A03-065.

Nair, J., Christensen, D., Yu, P., Beattie, A.D., McAllister, T., Damiran, D., Preston, N., Fuhr, L., and McKinnon, J.J. 2016. A nutritional evaluation of common barley varieties grown for silage by beef and dairy producers in western Canada. Can. J. Anim. Sci. **96**: 598–608. doi:10.1139/cjas-2016-0032.

Neal, K., Eun, J.-S., Young, A.J., Mjoun, K., and Hall, J.O. 2014. Feeding protein supplements in alfalfa hay-based lactation diets improves nutrient utilization, lactational performance, and feed efficiency of dairy cows. J. Dairy Sci. **97**: 7716–7728. Elsevier. doi:10.3168/jds.2014-8033.

National Research Council (NRC). 2001. Nutrient Requirements of Dairy Cattle, Seventh Revised Edition. Nutrient Requirements of Dairy Cattle. doi:10.17226/9825. National Academy of Science, Washington, DC, USA.

National Research Council (NRC). 1996. Nutrient requirements of beef cattle. Seventh Revised Edition. Nutrient Requirements of Dairy Cattle. doi:10.17226/9791. National Academy of Science, Washington, DC, USA.

Oba, M., and Allen, M.S. 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci. **82**: 589–596. doi:10.3168/jds.S0022-0302(99)75271-9.

Oba, M., and Allen, M.S. 2000. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 1. feeding behavior and nutrient utilization. J. Dairy Sci. **83**: 1333–1341. doi:10.3168/jds.S0022-0302(00)75000-4.

O'Kiely, P., McGee, M., and Kavanagh, S. Undated. Faba beans as a feed for cattle and sheep. Teagasc, Carlow, Ireland. [Online] Available: https://www.teagasc.ie/media/website/crops/crops/Beans_for_ruminants.pdf [2019 Aug. 25].

Ørskov, E.R., and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. **92**: 499. doi:10.1017/S0021859600063048.

Partanen, K., Alaviuhkola, T., Siljander-Rasi, H., and Suomi, K. 2003. Faba beans in diets for growing-finishing pigs. Journal paper, MTT Agrifood Research Finland. [Online] Available: https://orgprints.org/16755/ [2020 May 22].

Perry, T.W., Purkhiser, E.D., and Beeson, W.M. 1966. Effects of supplemental enzymes on nitrogen balance, digestibility of energy and nutrients and on growth and feed efficiency of cattle. J. Anim. Sci. **25**: 760–764. doi:10.2527/jas1966.253760x.

Phelps, S. 2017. Saskatchewan Pulse Growers.pdf. [Online] Available: https://saskpulse.com/files/general/151222_Phelps_Fababean_webinar.pdf. [2019 Dec 14].

Pusztai, A., and Grant, G. 1997. Assessment of Lectin Inactivation by Heat and Digestion. Pages 505–514 *in* Lectin Methods and Protocols. Humana Press, New Jersey. doi:10.1385/0-89603-396-1:505.

Raffrenato, E. 2011. Physical, Chemical And Kinetic Factors Associated With Fiber Digestibility In Ruminants And Models Describing These Relationships. [Online] Available: https://ecommons.cornell.edu/handle/1813/33520 [2020 Jun. 28].

Refat, B. 1987- 2018.April. Molecular Structure Features and Nutrient Availability and Utilization Of Barley Silage Varieties With Varying Digestible Structural Carbohydrate In Comparison With A New Short-Season Corn Silage In High-Producing Dairy Cattle. PhD Thesis, University of Saskatchewan. [Online] Available: https://harvest.usask.ca/handle/10388/8499 [2019 Jun. 26].

Refat, B., Christensen, D.A., McKinnon, J.J., Yang, W., Beattie, A.D., McAllister, T.A., Eun, J.-S., Abdel-Rahman, G.A., and Yu, P. 2018. Effect of fibrolytic enzymes on lactational performance, feeding behavior, and digestibility in high-producing dairy cows fed a barley silage–based diet. J. Dairy Sci. **101**: 7971–7979. doi:10.3168/jds.2017-14203.

Reid, R.L., Jung, G.A., and Thayne, W.V. 1988. Relationships between nutritive quality and fiber components of cool season and warm season forages:a retrospective study. J. Anim. Sci. **66**: 1275–1291. doi:10.2527/jas1988.6651275x.

Robinson, P.H., Fadel, J.G., and Tamminga, S. 1986. Evaluation of mathematical models to describe neutral detergent residue in terms of its susceptibility to degradation in the rumen. Anim. Feed Sci. Technol. **15**: 249–271. doi:10.1016/0377-8401(86)90079-9.

Robinson, P.H., Mathews, M.C., and Fadel, J.G. 1999. Influence of storage time and temperature on in vitro digestion of neutral detergent fibre at 48h, and comparison to 48h in sacco neutral detergent fibre digestion. Anim. Feed Sci. Technol. **80**: 257–266. doi:10.1016/S0377-8401(99)00062-0.

Rode, L.M., Yang, W.Z., and Beauchemin, K.A. 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. J. Dairy Sci. **82**: 2121–2126. doi:10.3168/jds.S0022-0302(99)75455-X.

ROE, M. 1990. Techniques for measuring protein fractions in feedstuffs. Proceeding Cornell Nutr. Conf. Feed Manuf. Dep. Anim. Poult. Avian Sci. 1990: 81–88.

Salem, A.Z.M., Gado, H.M., Colombatto, D., and Elghandour, M.M.Y. 2013. Effects of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers. Livest. Sci. **154**: 69–73. doi:10.1016/j.livsci.2013.02.014.

Sandberg, A.-S. 1991. The Effect of Food Processing on Phytate Hydrolysis and Availability of Iron and Zinc. Pages 499–508 *in* M. Friedman, ed. Nutritional and Toxicological Consequences of Food Processing. Springer US, Boston, MA. doi:10.1007/978-1-4899-2626-5_33.

Saskatchewan Pulse Growers 2019. Faba Bean Growth Staging.pdf. [Online] Available: https://saskpulse.com/files/technical_documents/Faba_Bean_Growth_Staging_2019_Horz_v5_FINAL.pdf [2020 May 13].

Siciliano-Jones, L.R.J. and J. (n.d.). NDF digestibility: Are you confused? [Online] Available: https://www.progressivedairy.com/topics/feed-nutrition/ndf-digestibility-are-you-confused [2020 Jun. 30].

Silva, T.H., Takiya, C.S., Vendramini, T.H.A., Jesus, E.F. de, Zanferari, F., and Rennó, F.P. 2016. Effects of dietary fibrolytic enzymes on chewing time, ruminal fermentation, and

performance of mid-lactating dairy cows. Anim. Feed Sci. Technol. **221**: 35–43. doi:10.1016/j.anifeedsci.2016.08.013.
Singh, A., and Bhatt, B. 2012a. Faba Bean (*Vicia faba L.*): A potential leguminous crop of India. [Online] Available: https://www.researchgate.net/publication/239812685_Faba_bean_Vicia_faba_L_A_Potential_Leguminous_Crop_of_India [2020 May 20].
Singh, A., and Bhatt, B. 2012b. An introduction to faba bean (Vicia faba L.). [Online] Available: https;//researchgate.net/publication/237066249_An_introduction_to_faba_bean_Vicia_faba_L [2020 May 20].
Singh, A.K., Bharati, R.C., Manibhushan, N.C., and Pedpati, A. 2013. An assessment of faba bean (Vicia faba L.) current status and future prospect. African Journal of Agricultural Research. **8**: 6634-6641.

Singh, B.K., Amarjeet, G., and Lal, S. 2012. Improvement of faba bean (Vicia faba L.). [Online] Available: https://www.researchgate.net/publication/256982114_Improvement_of_faba_bean_Vicia_faba_L [2020 May 13].
Smith, L.W., Goering, H.K., Waldo, D.R., and Gordon, C.H. 1971. In vitro digestion rate of forage cell wall components. J. Dairy Sci. **54**: 71–76. doi:10.3168/jds.S0022-0302(71)85780-6.
Spanghero, M., Boccalon, S., Gracco, L., and Gruber, L. 2003. NDF degradability of hays measured in situ and in vitro. Anim. Feed Sci. Technol. **104**: 201–208. doi:10.1016/S0377-8401(02)00327-9.
Straalen, W. van 1995. Modelling of nitrogen flow and excretion in dairy cows. Landbouw Universiteit, Wageningen.
Strydhorst, S.M., King, J.R., Lopetinsky, K.J., and Harker, K.N. 2008. Forage potential of intercropping barley with faba bean, lupin, or field pea. Agron. J. **100**: 182–190. doi:10.2134/agronj2007.0197.
Sulieman, S. 2011. Does GABA increase the efficiency of symbiotic N_2 fixation in legumes? Plant Signal. Behav. **6**: 32–36. doi:10.4161/psb.6.1.14318.
Tamminga, S., Van Straalen, W.M., Subnel, A.P.J., Meijer, R.G.M., Steg, A., Wever, C.J.G., and Blok, M.C. 1994. The Dutch protein evaluation system: the DVE/OEB-system. Livest. Prod. Sci. **40**: 139–155. doi:10.1016/0301-6226(94)90043-4.
Tannin 2020.May 10. [Online] Available: https://en.wikipedia.org/w/index.php?title=Tannin&oldid=955930162 [2020 May 16].
Thacker, P.A., and Kirkwood, R.N. 1992. Non-Traditional Feeds for Use in Swine Production. CRC Press. Boca Raton, FL, USA.
Trujillo, A.I., Marichal, M. de J., and Carriquiry, M. 2010. Comparison of dry matter and neutral detergent fibre degradation of fibrous feedstuffs as determined with in situ and in vitro gravimetric procedures. Anim. Feed Sci. Technol. **161**: 49–57. doi:10.1016/j.anifeedsci.2010.08.001.
Urrutia, N., and Harvatine, K.J. 2017. Effect of conjugated linoleic acid and acetate on milk fat synthesis and adipose lipogenesis in lactating dairy cows. J. Dairy Sci. **100**: 5792–5804. doi:10.3168/jds.2016-12369.
Van Amburgh, M.E., Collao-Saenz, E., Higgs, R.J., Ross, D., Recktenwald, E.B., Raffrenato, E., Chase, L.E., Overton, T.R., Mills, J.K., and Foskolos, A. 2015a. The Cornell Net

Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. J. Dairy Sci. **98**: 6361–6380. doi:10.3168/jds.2015-9378.

Van Amburgh, M.E., Collao-Saenz, E.A., Higgs, R.J., Ross, D.A., Recktenwald, E.B., Raffrenato, E., Chase, L.E., Overton, T.R., Mills, J.K., and Foskolos, A. 2015b. The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. J. Dairy Sci. **98**: 6361–6380. doi:10.3168/jds.2015-9378.

Van Soest, P.J., Robertson, J.B., and Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. **74**: 3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Volpelli, L.A., Comellini, M., Masoero, F., Moschini, M., Fiego, D.P.L., and Scipioni, R. 2010. Faba beans (Vicia faba) in dairy cow diet: effect on milk production and quality. Ital. J. Anim. Sci. **9**: e27. doi:10.4081/10.4081/ijas.2010.e27.

Van Vuuren, A.M., Van Der Koelen, C.J., and Vroons-De Bruin, J. 1993. Ryegrass versus corn starch or beet pulp fiber diet effects on digestion and intestinal amino acids in dairy cows. J. Dairy Sci. **76**: 2692–2700. doi:10.3168/jds.S0022-0302(93)77605-5.

Waldo, D.R., Smith, L.W., and Cox, E.L. 1972. Model of cellulose disappearance from the rumen. J. Dairy Sci. **55**: 125–129. doi:10.3168/jds.S0022-0302(72)85442-0.

Wang, Y., McAllister, T.A., Rode, L.M., Beauchemin, K.A., Morgavi, D.P., Nsereko, V.L., Iwaasa, A.D., and Yang, W. 2001. Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the Rumen Simulation Technique (Rusitec). Br. J. Nutr. **85**: 325–332. doi:10.1079/BJN2000277.

Wang, Y., Zhang, Y.G., Liu, X., Kopparapu, N.K., Xin, H., Liu, J., and Guo, J. 2015. Measurement of the intestinal digestibility of rumen undegraded protein using different methods and correlation analysis. Asian-Australas. J. Anim. Sci. **28**: 1454–1464. doi:10.5713/ajas.15.0085.

Wareham, C.N., Wiseman, J., and Cole, D.J.A. 1993. Influence of faba bean tannins on male broiler chicks: evaluation of hulls from white- and coloured-flowered cultivars and of near-isogenic lines. J. Agric. Sci. **121**: 427–436. doi:10.1017/S0021859600085622.

Wyman, C.E., Decker, S.R., Himmel, M.E., Brady, J.W., Skopec, C.E., and Viikari, L. 2005. Hydrolysis of Cellulose and Hemicellulose. Polysaccharides: Structural diversity and functional versatility. CRC Press. **1**: 1023-1062. Boca Raton, FL, USA.

Yan, M. 2020.June. Interactive Association Between Molecular Structure Spectral Profiles and Nutrient Utilization and Availability of Leaf, Stem, Pods, and Whole Plant Faba Bean Forage in Ruminants Before and After Rumen Incubation. Thesis, University of Saskatchewan. [Online] Available: https://harvest.usask.ca/handle/10388/12900 [2021 Jan. 19].

Yang, W.Z., Beauchemin, K.A., and Rode, L.M. 1999. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. J. Dairy Sci. **82**: 391–403. doi:10.3168/jds.S0022-0302(99)75245-8.

Yu, P. 2005. Potential protein degradation balance and total metabolizable protein supply to dairy cows from heat-treated faba beans. J. Sci. Food Agric. **85**: 1268–1274. doi:10.1002/jsfa.2093.

Yu, P., Christensen, D.A., McKinnon, J.J., and Markert, J.D. 2011. Effect of variety and maturity stage on chemical composition, carbohydrate and protein subfractions, in vitro rumen degradability and energy values of timothy and alfalfa. Can. J. Anim. Sci. **83**:279–290. doi:10.4141/A02-053.

Yu, P., Goelema, J.O., Leury, B.J., Tamminga, S., and Egan, A.R. 2002. An analysis of the nutritive value of heat processed legume seeds for animal production using the DVE/OEB model: A review. Anim. Feed Sci. Technol. **99**: 141–176. doi:10.1016/S0377-8401(02)00114-1.

Yu, P., Meier, J.A., Christensen, D.A., Rossnagel, B.G., and McKinnon, J.J. 2003. Using the NRC-2001 model and the DVE/OEB system to evaluate nutritive values of Harrington (malting-type) and Valier (feed-type) barley for ruminants. Anim. Feed Sci. Technol. **107**: 45–60. doi:10.1016/S0377-8401(03)00062-2.

Zhang, X., Yang, W., and Blasiak, W. 2011. Modeling study of woody biomass: interactions of cellulose, hemicellulose, and lignin. Energy Fuels **25**: 4786–4795. doi:10.1021/ef201097d.

Zijlstra, R.T., Lopetinsky, K., and Beltranena, E. 2008. The nutritional value of zero-tannin faba bean for grower-finisher pigs. Can. J. Anim. Sci. **88**: 293–302. NRC Research Press. doi:10.4141/CJAS07146.

ZoBell, D.R., Wiedmeier, R.D., Olson, K.C., and Treacher, R. 2000. The effect of an exogenous enzyme treatment on production and carcass characteristics of growing and finishing steers. Anim. Feed Sci. Technol. **87**: 279–285. doi:10.1016/S0377-8401(00)00202-9.

Zohary, D., and Hopf, M. 1973. Domestication of Pulses in the Old World: Legumes were companions of wheat and barley when agriculture began in the Near East. Science **182**: 887–894. doi:10.1126/science.182.4115.887.