MOLECULAR STRUCTURAL AND NUTRITIONAL EVALUATION OF FABA BEAN PLANTS AS HAY AND SILAGE FOR RUMINANTS: EFFECT OF TANNIN CONCENTRATION, CUTTING STAGE, AND FROST-DAMAGE

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By

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ABSTRACT

As new faba bean varieties are available in western Canada and the production is increasing, there is a possible use of this legume as a fodder. The overall objective of this research was to systematically evaluate whole plant faba bean as hay and silage for dairy cows. The effect of the tannin concentration (high vs. low) and the effect of cutting stages (flower, mid pod, and late pod) were determined.

In the first study (Chapter 3), the high tannin SSNS-1 and the low tannin Snowdrop variety were harvested at 77, 88, and 97 days from planting (flower, mid pod, and late pod stages, respectively). The results showed that dry matter (DM) yield and the Feed Milk Value (FMV) of whole plant faba bean hay (artificially air dried) was lower ($P< 0.05$) at flower stage than at late pod stage (7.68 vs. 12.74 t/ha; 1.35 vs. 1.57 kg milk/kg DM hay, respectively). This study indicates that late pod stage may be the alternative to harvest the whole plant faba bean as the yield and production performance are superior.

In the second study (Chapter 4) whole plant faba bean silage was evaluated. The results indicated that the whole plant faba bean silage had a similar protein concentration of 22 %DM in all the cutting stages. Additionally, the DVE and the FMV^{DVE} were lower ($P< 0.05$) at mid pod stage than at late pod stage (59 vs. 68 g/kg DM and 1.20 vs. 1.37 kg milk/kg DM silage, respectively). This study suggests that at late pod stage the predicted production performance is higher.

The third study (Chapter 5) determined the nutritive value of frost damaged whole plant faba bean hay. The results showed that the low tannin frost damaged hay had lower metabolizable protein (MP) (-4 g/kg DM) and lower FMV^{NRC} (-0.09 kg milk/kg DM Hay) than the high tannin

frost damaged hay. This study suggests that the nutritive value of frost damaged whole plant faba bean hay is lower than the normal whole plant faba bean hay.

The objectives of the third study (Chapter 6) were to carry out dairy production performance and metabolic trials with whole plant faba bean silage from Chapter 4. The inclusion of whole plant faba bean silage in high producing milking cows increased significantly ($P < 0.05$) the fat corrected milk (3.5% FCM), fat yield and efficiency (FCM/DMI) (56.39 vs. 51.98; 2.11 vs. 1.89 kg/cow/d; and 2.15 vs. 1.91, respectively). This study indicates that the inclusion of whole plant faba bean silage at late pod stage significantly improve the performance of dairy cows.

In the fourth study (Chapter 7) an intrinsic molecular structure analysis was performed on whole plant faba bean silage from Chapter 4. The results indicated that the total carbohydrates (TC) area was higher ($P < 0.05$) in low tannin silage at late pod stage than at mid pod stage (+3.45 AU) than the other silages. Amide II area was higher in the high tannin silage at late pod stage than the high tannin silage at mid pod stage (+2.50 AU). Principle component analysis (PCA) detected differences between whole plant faba bean silage, while carbohydrate and protein related structures can be used to predict nutrient utilization and availability with good estimation power ($R^2 > 0.74$).

In conclusion, whole plant faba bean should be harvested at late pod stage to obtain a higher yield, and superior predicted production performance as hay and silage. The inclusion of whole plant faba bean silage at late pod stage in high producing milking cows rations improved the performance. On the other hand, molecular structures of the whole plant faba bean silage were affected by the tannin concentration and by the cutting stage, also some of those structures are correlated to nutrient profiles and metabolic characteristics of the silage and can be used to predict them with good accuracy.

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TABLE OF CONTENTS

PERMISSION TO USE STATEMENT..i
ABSTRACT..ii
ACKNOWLEDGEMENTS..iv
TABLE OF CONTENTS...v
LIST OF TABLES..xii
LIST OF FIGURES...xvi
LIST OF ABBREVIATIONS...xviii
1. GENERAL INTRODUCTION..1
2. LITERATURE REVIEW...3
2.1 Origin and Distribution of faba bean...3
2.2 Botanical Description..4
2.3 Faba Production..4
2.4 Environmental Impact...5
2.5 Faba bean as Animal Feed...6
2.5.1 Nutritional Features..6
2.6 Nutritional Value for Monogastrics..7
2.6.1 Nutritional Value in Swine Diets..7
2.6.2 Nutritional Value in Poultry Diets..10
2.7 Faba Bean for Ruminants..11
2.7.1 Faba Bean Seeds...11
2.7.2 Faba bean Forage..13
2.8 Feed Evaluation Techniques and Methods..16
2.8.1 Application of Cornell Net Carbohydrate and Protein System V6.5 in Feed Evaluation...............16
2.8.2 Energy Evaluation in Feeds and Animal Diet Ingredients...17
2.8.3 Assessing Rumen Fermentation/Degradation Kinetics of Feed Nutrients Using the In Situ Technique....17
2.8.4 Hourly Effective Rumen Degradation Ratios/Potential N-to-Energy Synchronization....................19
2.8.5 Evaluation of Intestinal Digestibility of Feed Nutrients Using Three-Step In Vitro Techniques........19

2.8.6 Prediction of Truly Digestible Protein Supply to Small Intestine in Dairy Cattle ..20
2.8.7 Feed Milk Value Determination in Dairy Cattle 20
2.8.8 Fourier transform infrared spectroscopy-FTIR 21
2.9 Literature Review Summary/Conclusions, Overall Research Objectives and Hypothesis 26
2.9.1 Project Hypotheses 26
2.9.2 Project Objectives....26
3. COMPREHENSIVE PHYSIOCHEMICAL AND NUTRITIONAL EVALUATION OF FABA FORAGE VARIETIES FOR HAY: EFFECT OF VARIETY/TANNIN CONCENTRATION AND GROWTH STAGE ON DETAILED FEED AND FEEDING VALUES OF FABA FORAGE HAY GROWN AT THREE STAGES IN WESTERN CANADA WITH COOL CLIMATE CONDITIONS IN RUMINANT LIVESTOCK SYSTEMS. 29
3.1 Abstract....29
3.2 Introduction....30
3.3 Materials and Methods....32
3.3.1 Ingredients and Sample Preparation....32
3.3.2 Chemical Analysis of Whole Plant Faba Bean Hay 32
3.3.3 Energy Profile of Whole Plant Faba Bean Hay 33
3.3.4 Protein and Carbohydrate Subfractions....34
3.3.5 Rumen In Situ Incubation 35
3.3.5.1 Samples....35
3.3.5.2 Animals and Diets....35
3.3.5.3 Rumen Incubation Procedure 35
3.3.5.4 Chemical Analysis of In Situ Samples 36
3.3.5.5 Rumen Degradation Kinetics....36
3.3.6 Hourly Effective Rumen Degradation Ratios and Potential N-to-Energy Synchronization....38
3.3.7 Intestinal Digestion of Rumen Undegraded Protein....38
3.3.8 Nutrient Supply with the DVE/OEB System 39
3.3.9 Nutrient Supply to Dairy Cows with the NRC-2001 Model....39
3.3.10 Feed Milk Value....40
3.3.11 Statistical Analysis....40
3.4 **Results and Discussion**....41

3.4.1 *Yield* 41
3.4.2 *Chemical Profile* 43
3.4.3 *Energy Profile* 47
3.4.4 *Protein and Carbohydrate Subfractions* 47
3.4.5 *In Situ DM Degradation Kinetics* 51
3.4.6 *In Situ Crude Protein Degradation Kinetics* 53
3.4.7 *In Situ NDF Degradation Kinetics* 55
3.4.8 *In Situ Starch Degradation Kinetics* 57
3.4.9 *Hourly Effective Degradation Ratios between Available N and Available CHO* 59
3.4.10 *Intestinal Availability of Rumen Bypass Nutrients* 64
3.4.11 *Nutrient Supply with the DVE/OEB System* 68
3.4.12 *Nutrient Supply with the NRC-2001 Model* 69
3.4.13 *Feed Milk Value* 70
3.5 Conclusions 74
4. COMPREHENSIVE PHYSIOCHEMICAL AND NUTRITIONAL EVALUATION OF FABA FORAGE VARIETIES FOR SILAGE: EFFECT OF VARIETY/TANNIN CONCENTRATION AND GROWTH STAGE ON DETAILED FEED AND FEEDING VALUES OF FABA FORAGE SILAGE GROWN AT THREE STAGES IN WESTERN CANADA WITH COOL CLIMATE CONDITIONS IN RUMINANT LIVESTOCK SYSTEMS. 75
4.1 Abstract 75
4.2 Introduction 77
4.3 Materials and Methods 78
4.3.1 *Ingredients and Sample Preparation* 78
4.3.2 *Fermentation Profile of Silage* 78
4.3.3 *Chemical Analysis of Whole Plant Faba Bean Silages* 79
4.3.4 *Energy Profile of Whole Plant Faba Bean Silages* 79
4.3.5 *Protein and Carbohydrate Subfractions of Whole Plant Faba Bean Silages* 79
4.3.6 *Rumen In Situ Incubation of Whole Plant Faba Bean Silages* 79
4.3.6.1 *Samples* 79
4.3.6.2 *Animals and Diets* 80
4.3.6.3 *Rumen Incubation Procedure* 80
4.3.6.4 *Chemical Analysis of In Situ Silage Samples* 80
4.3.6.5 *Rumen Degradation Kinetics of Whole Plant Faba Bean Silages* 80

4.3.7 Hourly Effective Rumen Degradation Ratios and Potential N-to-Energy Synchronization.....81
4.3.8 Intestinal Digestion of Rumen Undegraded Protein in Whole Plant Faba Bean Silages.....81
4.3.9 Nutrient Supply to Dairy Cows with the DVE/OEB System.....81
4.3.10 Nutrient Supply to Dairy Cows with the NRC-2001 Model.....81
4.3.11 Feed Milk Value of Whole Plant Faba Bean Silage.....81
4.3.12 Statistical Analysis.....81
4.4 Results and Discussion.....82
4.4.1 Yield.....82
4.4.2 Fermentation Profile of Whole Plant Faba Bean Silage.....85
4.4.3 Chemical Profile of Whole Plant Faba Bean Silages.....88
4.4.4 Energy Profile of Whole Plant Faba Bean Silages.....92
4.4.5 Protein and Carbohydrate Subfractions in Whole Plant Faba Bean Silages.....94
4.4.6 In Situ DM Degradation Kinetics of Whole Plant Faba Bean Silages.....99
4.4.7 In Situ CP Degradation Kinetics.....102
4.4.8 In Situ NDF Degradation Kinetics of Whole Plant Faba Bean Silages.....105
4.4.9 In Situ Starch Degradation Kinetics of Whole Plant Faba Bean Silages.....107
4.4.10 Hourly Effective Degradation Ratios between Available N and Available CHO..110
4.4.11 Intestinal Availability of Rumen Bypass Nutrients of Whole Plant Faba Bean Silages.....114
4.4.12 Nutrient Supply to Dairy Cows with the DVE/OEB System.....119
4.4.13 Nutrient Supply to Dairy Cows with the NRC-2001 Model.....121
4.4.14 Feed Milk Value of Whole Plant Faba Bean Silages.....122
4.5 Conclusions.....126
5. COMPREHENSIVE PHYSIOCHEMICAL AND NUTRITIONAL EVALUATION OF FROST DAMAGED FABA FORAGE VARIETIES FOR HAY: EFFECT OF VARIETY/TANNIN CONCENTRATION ON DETAILED FEED AND FEEDING VALUES OF FROST DAMAGED FABA FORAGE HAY GROWN IN WESTERN CANADA WITH COOL CLIMATE CONDITIONS IN RUMINANT LIVESTOCK SYSTEMS.....127
5.1 Abstract.....127
5.2 Introduction.....128
5.3 Materials and Methods.....129
5.3.1 Ingredients and Sample Preparation.....129

5.3.2 Yield and Chemical Analysis 129
5.3.3 Energy Profile 130
5.3.4 Protein and Carbohydrate Subfractions 130
5.3.5 Rumen In Situ Incubation 130
5.3.5.1 Samples 130
5.3.5.2 Animals and Diets 130
5.3.5.3 Rumen Incubation Procedure 130
5.3.5.4 Chemical Analysis of In Situ Residual Samples 130
5.3.5.5 Rumen Degradation Kinetics 131
5.3.6 Hourly Effective Rumen Degradation Ratios and Potential N-to-Energy Synchronization 131
5.3.7 Intestinal Digestion of Rumen Undegraded Protein 131
5.3.8 Nutrient Supply with the DVE/OEB System 131
5.3.9 Nutrient Supply with the NRC-2001 Model 131
5.3.10 Feed Milk Value of Frost Damage Whole Plant Faba Bean Hay 132
5.3.11 Statistical Analysis 132
5.4 Results and Discussion 132
5.4.1 Yield and Chemical Profile of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration 133
5.4.2 Energy Profile of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration 135
5.4.3 Protein and Carbohydrate Subfractions of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration 136
5.4.4 In Situ DM Degradation Kinetics of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration 138
5.4.5 In Situ CP Degradation Kinetics of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration 139
5.4.6 In Situ NDF Degradation Kinetics of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration 140
5.4.7 In Situ Starch Degradation Kinetics of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration 141
5.4.8 Hourly Effective Degradation Ratios between Available N and Available CHO of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration.. 142

5.4.9 *Intestinal Availability of Rumen Bypass Nutrients of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration*....................................145

5.4.10 *Nutrient Supply with the DVE/OEB System from Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration*...147

5.4.11 *Nutrient Supply with the NRC-2001 Model from Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration*...147

5.4.12 *Feed Milk Value of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration*..148

5.5 Conclusions...150

6. DAIRY PERFORMANCE AND METABOLIC TRIALS WITH WHOLE PLANT FABA SILAGE: DEVELOPMENT EFFICIENT FEEDING STRATEGY OF WHOLE PLANT FABA SILAGE TO FIND MAXIMUM REPLACEMENT OF BARLEY AND CORN SILAGE IN HIGH PRODUCTION LACTATION DAIRY COWS TO BENEFIT PULSE GROWERS AND DAIRY PRODUCERS 151

6.1 Abstract...151

6.2 Introduction..152

6.3 Materials and Methods..153

6.3.1 *Silage Preparation*...154

6.3.2 *Animals, Diets, and Experimental Design*...154

6.3.3 *Milk Sampling*..155

6.3.4 *Feed Chewing Activity*...156

6.3.5 *Total Collection*...156

6.3.6 *Rumen Fermentation Characteristics*...157

6.3.7 *Chemical Analysis*..158

6.3.8 *Statistical Analysis*...159

6.4 Results and Discussion..159

6.4.1 *Feed Intake, Body Weight and Efficiency*..159

6.4.2 *Milk Yield and Composition*..163

6.4.3 *Digestibility of Principal Nutrients*..166

6.4.4 *N balance and N utilization*..168

6.4.5 *Energy Partitioning*...171

6.4.6 *Chewing Activity*..174

6.4.7 *Rumen Fermentation Characteristics*...176

6.5 Conclusions...179

7. ASSOCIATION OF MOLECULAR STRUCTURE SPECTRAL FEATURES WITH NUTRIENT UTILIZATION AND AVAILABILITY OF FABA FORAGE VARIETIES FOR SILAGE WITH EFFECT OF VARIETY/TANNIN CONCENTRATION AND GROWTH STAGE IN RUMINANT LIVESTOCK SYSTEM: AN APPROACH WITH ADVANCED MOLECULAR SPECTROSCOPY 180

7.1 Abstract 180

7.2 Introduction 182

7.3 Materials and Methods 183

7.3.1 ATR-FT/IR Sample Preparation and Spectra Collection 183

7.3.2 Univariate Molecular Spectral Analysis of Structure Profiles 183

7.3.3 Multivariate Molecular Spectral Analysis of Structure Profiles 184

7.3.4 Statistical Analysis 184

7.4 Results and Discussion 185

7.4.1 Univariate Molecular Spectral Analysis: Effect of Variety and Maturity Stage ... 185

7.4.2 Multivariate Molecular Spectral Analyses 190

7.4.3 Molecular Structure Spectral Profiles in Relation with Chemical and Energy Profiles 195

7.4.4 Molecular Structure Spectral Profiles in Relation with Rumen Degradation Kinetics of Primary Nutrients 205

7.4.5 Molecular Structure Spectral Profiles in Relation with In Vitro Digestion and Metabolic Characteristics 216

7.4.6 Multiple Regression Analysis with Prediction Model Variable Selection 229

7.5 Conclusions 233

8. GENERAL DISCUSSION, OVERALL CONCLUSION, AND IMPLICATIONS 234

9. LITERATURE CITED 242

10. APPENDIX 259

LIST OF TABLES

Table 2.1. Chemical composition of faba bean seeds (beans) and, for comparison, values of barley grain, soybean meal and pea seed. Adapted from O'Kiely et al., n.d. 7
Table 2.2. Growth performance of grower-finisher pigs fed diets based on either faba bean or soybean meal. Adapted from Zijlstra et al., 2008. 9
Table 2.3. Performance of broiler chicken (two studies) fed faba bean-containing diets made with low- or high-tannin varieties. Adapted from Crepon et al., 2010. 10
Table 2.4. Rumen CP degradation characteristics of lupin seed, faba bean and peas evaluated by different laboratories. Adapted from Yu et al., 2002. 12
Table 2.5. Rumen starch degradation characteristics of lupin seed, faba bean and peas evaluated by different laboratories. Adapted from Yu et al., 2002. 12
Table 2.6. Concentrate intake, milk yield, milk composition, blood protein and blood urea as influenced by the different diets fed to animals. Adapted from Volpelli et al., 2010. . 13
Table 2.7. Effect of harvest date on the yield of faba bean forage. Adapted from Fraser et al., 2001. 14
Table 2.8. Effect of harvest date on the chemical composition of faba beans. BC, buffering capacity all values g/kg DM, except DM content (g/kg FM) and BC (mequiv. 100 g $^{-1}$). Adapted from Fraser et al., 2001 14
Table 2.9. Chemical composition of whole-crop legume forages after 45 d of ensiling. Adapted from Mustafa and Seguin, 2003. 15
Table 2.10. Ruminal nutrient kinetic parameters and effective degradability of whole-crop legume silages. Adapted from Mustafa and Seguin, 2003 16
Table 3.1. Effect of variety (tannin concentration) and stage of cutting on yield of whole plant faba bean hay 42
Table 3.2. Effect of variety (tannin concentration) and stage of cutting on chemical composition of whole plant faba bean hay 44
Table 3.3. Effect of variety (tannin concentration) and stage of cutting on energy profile of whole plant faba bean hay 48
Table 3.4. Effect of variety (tannin concentration) and stage of cutting on CNCPS fractions of whole plant faba bean hay 50
Table 3.5. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics of dry matter of whole plant faba bean hay 52
Table 3.6. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics of crude protein of whole plant faba bean hay 54
Table 3.7. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics of fiber (NDF) of whole plant faba bean hay 56
Table 3.8. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics of starch of whole plant faba bean hay 58
Table 3.9. Effect of variety (tannin concentration) and stage of cutting on potentially available N to available CHO synchronization of whole plant faba bean hay 60
Table 3.10. Effect of variety (tannin concentration) stage of cutting on intestinal digestibility and total tract digestion of whole plant faba bean hay.. 65

Table 3.11. Effect of variety (tannin concentration) and stage of cutting on metabolic characteristics, true nutrient supply and feed milk value of whole plant faba bean hay72
Table 4.1. Effect of variety (tannin concentration) and stage of cutting on yield of whole plant faba bean silage..84
Table 4.2. Effect of variety (tannin concentration) and stage of cutting on fermentation profile of whole plant faba bean silage...86
Table 4.3. Effect of variety (tannin concentration) and stage of cutting on chemical composition of whole plant faba bean silage...89
Table 4.4. Effect of variety (tannin concentration) and stage of cutting on condensed tannin concentration of whole plant faba bean silage...90
Table 4.5. Effect of variety (tannin concentration) and stage of cutting on energy profile of whole plant faba bean silage...93
Table 4.6. Effect of variety (tannin concentration) and stage of cutting on CNCPS fractions of whole plant faba bean silage...96
Table 4.7. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics of dry matter of whole plant of faba bean silage......................................100
Table 4.8. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics of crude protein of whole plant faba bean silage......................................103
Table 4.9. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics of fibre (NDF) of whole plant faba bean silage..106
Table 4.10. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics of starch of whole plant faba bean silage...108
Table 4.11. Effect of variety (tannin concentration) and stage of cutting on potentially available N to available CHO synchronization of whole plant faba bean silage........................111
Table 4.12. Effect of variety (tannin concentration) and stage of cutting on intestinal digestibility and total tract digestion of whole plant faba bean silage.......................................116
Table 4.13. Effect of variety (tannin concentration) and stage of cutting on metabolic characteristics, true nutrient supply and feed milk value of whole plant faba bean silage 124
Table 5.1. Effect of variety (tannin concentration) on chemical composition of frost damaged whole plant faba bean hay...134
Table 5.2. Effect of variety (tannin concentration) on energy profile of frost damaged whole plant faba bean hay...136
Table 5.3. Effect of variety (tannin concentration) on CNCPS fractions of frost damaged whole plant faba bean hay...137
Table 5.4. Effect of variety (tannin concentration) on rumen degradation kinetics of dry matter of frost damaged whole plant faba bean hay..138
Table 5.5. Effect of variety (tannin concentration) on rumen degradation kinetics of crude protein of frost damaged whole plant faba bean hay..140
Table 5.6. Effect of variety (tannin concentration) on rumen degradation kinetics of fibre (NDF) of frost damaged whole plant faba bean hay..141
Table 5.7. Effect of variety (tannin concentration) on rumen degradation kinetics of starch of frost damaged whole plant faba bean hay...142

Table 5.8. Effect of variety (tannin concentration) on potentially available N to available CHO synchronization of frost damaged whole plant of faba bean hay 143
Table 5.9. Effect of variety (tannin concentration) on intestinal digestibility and total tract digestion of frost damaged whole plant faba bean hay 146
Table 5.10. Effect of variety (tannin concentration) on metabolic characteristics, true nutrient supply and feed milk value of frost damaged whole plant faba bean hay.................149
Table 6.1. Ingredients and nutrient composition of the four dairy dietary treatments...............156
Table 6.2. Intake, body weight and feed efficiency of Holstein cows fed TMRs with different levels of inclusion of whole plant faba bean silage 161
Table 6.3. Milk yield and composition of Holstein cows fed TMRs with different levels of inclusion of whole plant faba bean silage 164
Table 6.4. Digestibility of primary nutrients in dairy cows fed TMRs with different levels of inclusion of whole plant faba bean silage 167
Table 6.5. N balance and N utilization in dairy cows fed TMRs with different levels of inclusion of whole plant faba bean silage 170
Table 6.6. Energy partitioning in dairy cows fed TMRs with different levels of inclusion of whole plant faba bean silage 173
Table 6.7. Chewing activity in dairy cows fed TMRs with different levels of inclusion of whole plant faba bean silage 175
Table 6.8. Rumen fermentation characteristics of dairy cows fed TMRs with different levels of inclusion of whole plant faba bean silage 178
Table 7.1. Effect of variety (tannin concentration) and stage of cutting on molecular structure of whole plant of faba bean silage..187
Table 7.2. Correlation between carbohydrate structure spectral peaks and chemical and energy profiles of whole plant faba bean silage...196
Table 7.3. Correlation between protein structure spectral peaks and chemical and energy profiles of whole plant faba bean silage. 199
Table 7.4. Correlation between protein and carbohydrates structure spectral areas and chemical and energy profiles of whole plant faba bean silage...203
Table 7.5. Correlation between carbohydrate structure spectral peaks and rumen degradation kinetics of primary nutrients of whole plant faba bean silage.................................206
Table 7.6. Correlation between protein structure spectral peaks and rumen degradation kinetics of primary nutrients of whole plant faba bean silage. 210
Table 7.7. Correlation between protein and carbohydrates structure spectral areas and rumen degradation kinetics of primary nutrients of whole plant faba bean silage...............214
Table 7.8. Correlation between carbohydrate structure spectral peaks and *in vitro* digestibility characteristics, metabolic characteristics and predicted production performance of whole plant faba bean silage. 218
Table 7.9. Correlation between protein structure spectral peaks and *in vitro* digestibility characteristics, metabolic characteristics and predicted production performance of whole plant faba bean silage. 222

Table 7.10. Correlation between protein and carbohydrates structures spectral areas and *in vitro* digestibility characteristics, metabolic characteristics and predicted production performance of whole plant faba bean silage..226
Table 7.11. Multiple regression analysis to choose the most important protein and carbohydrates spectral characteristics for predicting chemical, energy and rumen degradation kinetics parameters of whole plant faba bean silage...231
Table 7.12. Multiple regression analysis to choose the most important protein and carbohydrates spectral characteristics for predicting intestinal digestion parameters and metabolic characteristic of whole plant faba bean silage...232
Table 10.1. Chemical composition of the ingredients used in the dairy production and metabolic trials 259

LIST OF FIGURES

Figure 1.1. Chemical structure of condensed tannin........1
Figure 1.2. Diagram of the entire whole plant faba bean project........2
Figure 2.1. Chemical structure of vicine and convicine........8
Figure 2.2. Functional groups of IR spectrum of primary amine, aniline (left) and Hexanoic acid (right).........22
Figure 2.3. Infrared spectrum of whole plant faba bean silage (ca. 1712-879 cm^{-1})........23
Figure 2.4. Schematic of a Michelson interferometer........24
Figure 3.1. Effect of tannin concentration on hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of whole plant faba bean hay. Optimum ratio = 32 EN/ECHO g/kg.........61
Figure 3.2. Effect of cutting stage on hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of whole plant faba bean hay. Optimum ratio = 32 EN/ECHO g/kg.........62
Figure 4.1. Effect of tannin concentration on hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of whole plant faba bean silage. Optimum ratio = 32 EN/ECHO g/kg.........112
Figure 4.2. Effect of stage of cutting on hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of whole plant faba bean silage. Optimum ratio = 32 EN/ECHO g/kg.........113
Figure 5.1. Effect of tannin concentration on hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of frost damaged whole plant faba bean hay. Optimum ratio = 32 EN/ECHO g/kg.........144
Figure 7.1. a) Effect of tannin concentration on principal components analysis (PCA) of whole plant faba bean silage using FTIR vibrational spectroscopy at complete spectra region (ca. 700 to ca. 4000 cm^{-1}); b) Effect of stage of cutting on principal components analysis (PCA) of whole plant faba bean silage using FTIR vibrational spectroscopy at complete spectra region (ca. 700 to ca. 4000 cm^{-1}); PCA: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2)........192
Figure 7.2. a) Effect of tannin concentration on principal components analysis (PCA) of whole plant faba bean silage using FTIR vibrational spectroscopy at amide region (ca. 1484 to ca. 1712 cm^{-1}); b) Effect of stage of cutting on principal components analysis (PCA) of whole plant faba bean silage using FTIR vibrational spectroscopy at amide region (ca. 1484 to ca. 1712 cm^{-1}); PCA: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).........193
Figure 7.3. a) Effect of tannin concentration on principal components analysis (PCA) of whole plant faba bean silage using FTIR vibrational spectroscopy at complete carbohydrate region (ca. 879 to ca. 1485 cm^{-1}); b) Effect of stage of cutting on principal components analysis (PCA) of whole plant faba bean silage using FTIR spectroscopy at complete carbohydrate region (ca. 879 to ca. 1485 cm^{-1}); PCA: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).........194
Figure 10.1. Whole plant faba bean at flower stage........260
Figure 10.2. Whole plant faba bean at mid pod stage........261

Figure 10.3. Whole plant faba bean at late pod stage 262
Figure 10.4. Whole plant faba bean silage at late pod stage used in the dairy production and metabolic trials 263

LIST OF ABBREVIATIONS

abs	Absorbance
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
ARUP	Truly absorbed rumen undegraded protein in the small intestine (NRC Dairy model)
ATR	Attenuated total reflection
AU	Absorbance units
BCP	Rumen bypass feed crude protein (DVE/OEB system)
BDM	Rumen bypass dry matter
BDNDF	Rumen bypass feed neutral detergent fiber
BST	Rumen bypass starch
BW	Body weight
BWG	Body weight gain
Ca	Calcium
CA1	Volatile fatty acids (Acetic + Propionic + Butyric + Isobutyric)
CA4	Sugar (rapidly degradable carbohydrate fraction)
CB1	Starch (intermediately degradable carbohydrate fraction)
CB2	Soluble fiber (intermediately degradable carbohydrate fraction)
CB3	Digestible fiber (available neutral detergent fiber or slowly degradable carbohydrate fraction)
CC	Indigestible fiber (unavailable neutral detergent fiber)

CEC	Cellulosic compounds
CHO	Carbohydrate
CLA	Hierarchical cluster analysis (or HCA)
CP	Crude protein
CT	Condensed tannin
D	Degradable fraction
dBDM	Intestinal digestibility of rumen bypass dry matter
dBNDF	Intestinal digestibility of rumen bypass fiber
dBST	Intestinal digestibility of rumen bypass starch
$DE_{p3\times}$	Digestible energy at a production level (3× maintenance)
dIDP	Intestinal digestibility of rumen bypass protein
DM	Dry matter
DMI	Dry matter intake
DPB	Degraded protein balance
DVBE	Truly absorbed bypass feed protein in the small intestine
DVE	Total truly digested protein in the small intestine (DVE/OEB system)
DVME	Truly absorbed rumen synthesized microbial protein in the small intestine
ECM	Energy corrected milk
ECP	Rumen endogenous protein
ED_CHO	Effectively degraded carbohydrate
ED_N	Effectively degraded nitrogen
EDCP	Effective degraded crude protein
EDDM	Effective degraded dry matter
EDNDF	Effective degraded neutral detergent fiber

EDST	Effective degraded starch
EE	Ether extracts (crude fat)
FCM	Fat corrected milk
FMV	Feed milk value
FTIR	Fourier-transform infrared spectroscopy
IADP	Intestinal digestible rumen bypass protein
IDBDM	Intestinal digestible rumen bypass dry matter
IDBNDF	Intestinal digestible rumen bypass neutral detergent fiber
IDST	Intestinal digestible rumen bypass starch
Kd	Degradation rate of degradable fraction
Kp	Passage rate
LIG	Ligneous compounds
MCP_{RDP}	Microbial protein synthesized in the rumen based on rumen degraded protein
MCP_{TDN}	Microbial protein synthesized in the rumen based on available energy (total digestible nutrients at a production level)
ME	Metabolizable energy
$ME_{p3\times}$	Metabolizable energy at a production level (3× maintenance)
MP	Metabolizable protein (NRC Dairy model)
MREE	Microbial protein synthesized in the rumen based on available energy
MREN	Microbial protein synthesized in the rumen based on rumen degraded feed crude protein
MUN	Milk urea nitrogen
N	Nitrogen
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein

NE_g	Net energy for gain
NE_L	Net energy of lactation
$NE_{Lp3\times}$	Net energy for lactation at a production level (3× maintenance)
NE_m	Net energy for maintenance
NFC	Non-fiber carbohydrate
NH_3	Ammonia
NPN	Non-protein nitrogen
NSTC	Non-structural carbohydrates
OEB	Degraded protein balance (DVE/OEB system)
OM	Organic matter
P	Phosphorus
PA1	Ammonia
PA2	Soluble true protein (rapidly degradable true protein)
PB1	Insoluble true protein (moderately degradable true protein)
PB2	Fiber bound protein (slowly degradable true protein)
PC	Indigestible protein
PCA	Principal Component Analysis
PDI	Pellet durability index
RDNDF	Rumen degradable fiber
RDP	Rumen degradable protein
RUDM	Rumen undegradable dry matter
RUNDF	Rumen undegradable neutral detergent fiber
RUP	Rumen undegradable protein
S	Soluble fraction (washable in NDF)

SCC	Somatic cell count
SCP	Soluble crude protein
SNF	Solids-non-fat
ST	Starch
STC	Structural carbohydrates
STC1	Structural carbohydrates peak 1
STC2	Structural carbohydrates peak 2
STC3	Structural carbohydrates peak 3
STC4	Structural carbohydrates peak 4
T0	Lag time
TC	Total carbohydrates
TC1	Carbohydrate peak 1
TC2	Carbohydrate peak 2
TC3	Carbohydrate peak 3
tdCP	Truly digestible crude protein
TDDM	Total digestible dry matter
tdFA	Truly digestible fatty acid
$TDN_{1\times}$	Total digestible nutrients at a maintenance level
TDNDF	Total digestible fiber
tdNDF	Truly digestible neutral detergent fiber
tdNFC	Truly digestible non-fiber carbohydrate
TDP	Total digestible crude protein
TDST	Total digestible starch
U	Rumen undegradable fraction

uNDF	Undigestible neutral detergent fiber
VFA	Volatile fatty acids.
WSC	Water soluble carbohydrates.
WG	Weight gain

1. GENERAL INTRODUCTION

In western provinces of Canada, the forage part of dairy rations usually consists mainly of barley silage and in less amounts, alfalfa, corn, and pea silages, while in Ontario and Quebec corn silage and alfalfa silage are the most common ones (McCartney' and Horton, 1997; Abeysekara, 2003). Besides that, the production of faba bean (*Vicia faba*) is increasing in Canada and most of this production is used for animal feeds, while a lower proportion is used for exports and food (Gilmour, 2016; Saskatchewan Pulse Growers, 2018). Faba bean is a legume with high nutritive content in its seeds, especially protein and starch (Heuzé et al., 2015). Faba bean seeds are used for human consumption and also have been used as animal feed, particularly monogastric livestock (Duc et al., 2010). The University of Saskatchewan has been working on new varieties. Now, there are two different types, the high tannin, and the low tannin ones (Fleurly and Barker, 2016). Tannins are phenolic plant secondary compounds distributed in legumes (Theodoridou, 2010), the condensed tannins are normally considered as antinutritional factors which negatively affect palatability and digestion in monogastrics.

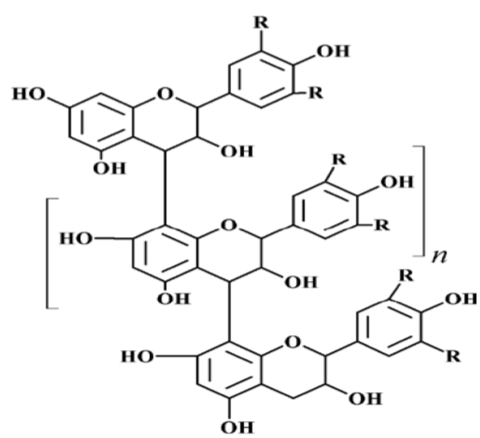


Figure 1.1. Chemical structure of condensed tannin. Adapted from Addisu, 2016.

However, condensed tannins have positive effects on ruminants when concentration is optimal (1 - 4 %DM) (Theodoridou, 2010; Mueller-Harvey, 2006; Matthäus and Angelini; 2005; Frutos et al., 2004). On the other hand, information on the use of the faba bean seeds in ruminants is available and showed that have positive effects when included in the rations (Volpelli et al., 2010; Crepon et al., 2010). However, the use of the whole plant for ruminants is rare. Previous studies have suggested that the inclusion of faba bean silage did not negatively affect the performance in dairy cows (Ingalls et al., 1978; Ingalls et al., 1974). The aim of this project was to systematically study the whole plant faba bean as hay and as silage and determine the effects of the tannin concentration and cutting stage on the nutritional and molecular characteristics. The diagram of the whole plant faba bean project discussed in this thesis is showed in Figure 1.2. In chapters 3 and 4, the effect of tannin concentration and cutting stage was determined. In chapter 5 the effect of tannin concentration was determined. The silage used in chapter 6 was determined based on the results of chapter 4. In chapter 7 the effect of tannin concentration and cutting stage was determined.

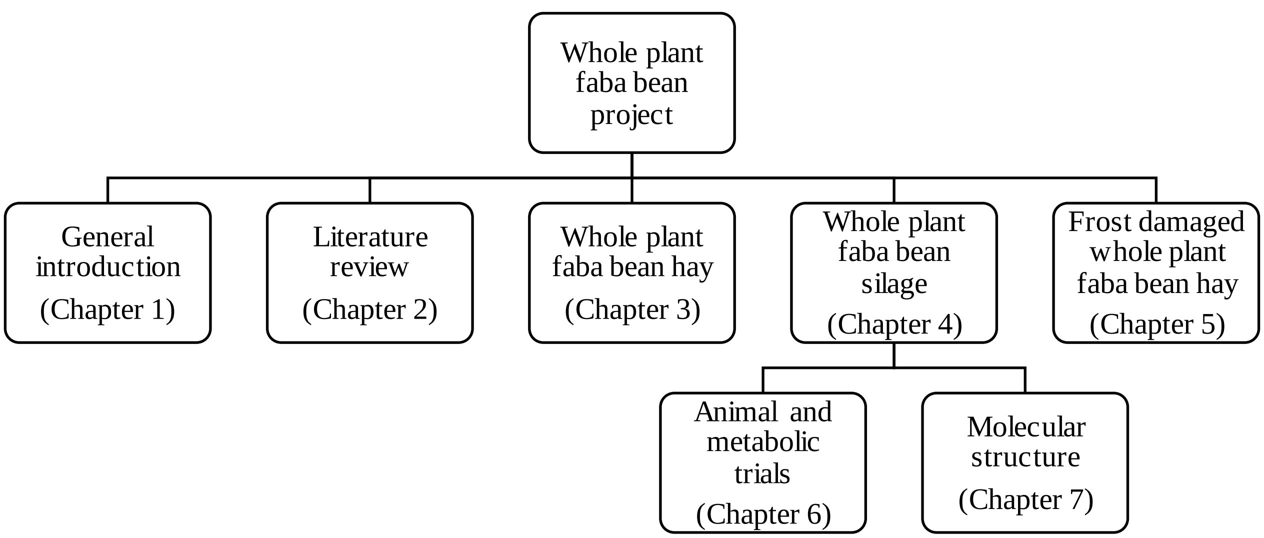


Figure 1.2. Diagram of the entire whole plant faba bean project.

2. LITERATURE REVIEW

2.1 Origin and Distribution of faba bean

Faba bean is a legume botanically known as *Vicia faba* L. originated in the southwestern Asia as principle center, and in the Mediterranean region (north Africa) as secondary center. It is widely cultivated around the world and commonly known as faba bean, broad bean, or horse bean (Tanno and Willcox, 2006; Muehlbauer and Tullu, 1997; Hanelt and Mettin, 1989; Harlan, 1969). Its name originates from one of the forms of the Greek verb “to eat” which highlights its use for food and feed by the ancient Greeks and Romans (Duc et al., 2010; Muratova, 1931). The earliest known archaeological Faba bean, one of the oldest domesticated food legumes, remains are from the Neolithic period (6800 to 6500 B.C.) from Israel (Vaughan and Geissler, 1997). In China, it was used for human consumption almost 5000 years ago, the Egyptians cultivated this crop 3000 years ago and later the Hebrews in biblical times (Singh and Bhatt, 2012; Mihailovic et al., 2005). Before 1000 B.C. Faba beans culture was very stablished in Britain and other European countries (Link et al., 2008). It is possible that faba bean was introduced by Europeans as a garden crop into India during the Sultanic period (1206 to 1555), during which its cultivation has been mentioned (Razia Akbar, 2000; Naqvi, 1984). The earliest forms of *Vicia faba* were small seeded, the large-seeded types were originated more recently 1000-1200 years ago in Iraq and then spread across north Africa to Europe and America (Link et al., 2008). The migration of faba beans towards South America, especially the Andean Region, probably occurred in the 15th century, helped by Spanish and Portuguese ship travelers (Duc et al., 2010). The wild progenitor of faba bean remain unknown (Kosterin, 2014) or extinct (Abbo et al., 2013). However, some wild species (*Vicia narbonensis* L. and *Vicia galilaea* Plitmann and Zohary) are taxonomically closely related to the cultivated faba bean (Singh, et al., 2013).

2.2 Botanical Description

The diversity of this specie was described by use of seed size. The small seeded group as *Vicia faba minor*, (100 seed weight <40 g or 800-1200 seeds = 454 g; >1.2 in long pods); the medium-large seeded group as *Vicia faba equine,* (100 seed weight 40-80 g or 500-800 seeds = 454 g; ½-3/4 in long pods), and the *Vicia faba major* with seeds than more a gram per seed (100 seed weight >80 g or 200-500 seeds = 454 g; >3/4 in long pods) (Landry, 2014; Link et al., 2008). Faba bean is an annual crop, sown in autumn (where temperatures remain above -5˚C, especially Mediterranean types) or in spring and, even though primarily grown for its edible seeds (beans), it can also be used as a whole-crop (O'Kiely et al., n.d). *Vicia faba* is an upright annual forage legume that can reach up to 1.5-2 m in height. It has a taproot and many fibrous lateral roots that explore up to 90 cm of soil layer (Muehlbauer and Tullu, 1997) flowers are large (up to 3-4 cm long) and white or white with black/dark purple spots. The fruit is a dehiscent cylindrical pod, up to 10 cm long and 1-2 cm in diameter. The pods are green when young and turn to dark brown or black at maturity (Heuzé et al., 2015; Hekneby et al., 2006). Faba beans require a cool, relatively humid climate and are grown at higher altitudes in tropics with temperatures from 7 to 30°C (Davies, 1985; Duke, 1981), however, the optimum temperature levels for production range from 18 to 27℃ (Slinkard et al., 1994; Duke, 1981). Additionally, temperatures above 27°C shorten the growing period and adversely affect pollination (Etemadi et al., 2015; Muehlbauer and Tullu, 1997).

2.3 Faba Production

Faba bean is a major food and feed legume because of the high nutritional value of its seeds, which are abundant in protein and starch. The 2014 production in Africa was 1,397,361; Asia 1,522,758; Americas 213,461; Europe 678,692 and Oceania 327,700 tonnes of faba bean (FAOSTAT, 2017). Currently faba beans are major crop in many countries including China, Ethiopia, and Egypt, and are widely grown for human food in the Mediterranean region and in parts of Latin America (Razia Akbar, 2000; Naqvi, 1984). In developed countries, faba bean provides an alternative to soybean meal for animal feed, this being particularly important in the more industrialized countries. The feed market for dry seeds has mostly been developed in Europe (0.7 Mt consumed by pigs and poultry); in addition, straw or silage is sometimes used to feed ruminant animals (Duc et al., 2010). In the United States, faba beans are not grown in large quantities and are used almost exclusively for livestock pasturage, hay, and silage (Singh and Bhatt, 2012; Oplinger, 1982). The production of pulses has been dramatically increasing in Canada since the 90's due to the rapid growth of international market opportunities, as well as the implementation of new technologies by the breeders and producers, particularly faba bean (Booker, 2016; Gilmour 2016). In 2013, were seeded 20 thousand ha of faba bean in Canada specially Alberta, Saskatchewan, and Manitoba, while in 2015 that number reached more than 60 thousand ha (Saskatchewan Pulse Growers, 2017). Canadian production of faba bean is destined to feed about 62%, for exports 28% and for food 10% (LMC, 2017). Breeding programs at the University of Saskatchewan (Saskatoon, Canada) started with the large-seeded tannin types (colored flowers) for the food market, and the second stream is a very early small-seeded, low-tannin type (white-flowered) for the cool, wet regions of Saskatchewan and Alberta for use in flexible farming systems that include livestock and protein extraction. The most common varieties grown in Saskatchewan in 2015 were the smaller seeded low-tannin varieties Snowbird and CDC Snowdrop (Fleurly and Barker, 2016)

2.4 Environmental Impact

The biological capacity that faba bean posses to fix nitrogen (N) was suggested as the major agronomic and economic advantage for including *Vicia faba* L. in a cereal crop rotation (Landry, 2014; Al Barri, 2012; Duc et al., 2010). Faba bean has a high degree of N fixation, in Australia was reported to contribute 270 kg/ha of N to the soil, resulting in increasing yields (+1-1.5 t/ha) and protein content (+0.7-1%) of the following wheat crop (Heuzé et al., 2015; Lang et al., 1993). These improvements were not only due to N fixation but also to positive effects of the faba bean crop for controlling certain crown rot and nematodes which causes diseases in wheat crop (Etemadi et al., 2015; Matthews and Marcellos, 2003). Faba bean crop is also used as green manure, to provide large quantities of N to spring-sown species such as maize. In Europe, faba bean can be sown in September with field pea in order to be mulched and ploughed down during spring (Heuzé et al., 2015; Clerc, 2013; Singh et al., 2013). Another important service that faba bean can provide is intercropping, which is a technique that mimics natural systems and aims to decrease weed disease, and crop competition by using components that have synergistic cultural requirements (Strydhorst et al., 2008). Additionally, benefits to the soil when replacing a cereal with a faba bean crop include increased microbial diversity, soil sanitation, soil structure, and less water use (Jensen et al., 2010). The adaptability of faba, similar to that of other pulses and oilseed mustards, to conservation and no-till is also quite promising feature (Landry, 2014).

2.5 Faba bean as Animal Feed

2.5.1 Nutritional Features

Vicia faba L. seeds which are abundant in protein and energy has a long history of several and valuable uses in feed and food (Al Barri, 2012; Crepon et al., 2010). Their low oil content means they do not need to be processed for oil extraction before being used for feed, such as soybeans. Faba bean seeds are high in protein (25-33% DM) and starch (40-48% DM) and are therefore a valuable source of protein and energy for livestock (Etemadi et al., 2015; Heuzé et al., 2015). The protein of faba beans contains similar or even higher proportions of lysine, when compared to protein from soybean meal or lupins (Degussa, 2006). Protein quality is mainly limited due to a low concentration of amino acids (sulfur-containing) methionine and cysteine (Link et al., 2008). The total fiber content of faba bean seed is widely variable depending on the seed variety (Vidal-Valverde et al., 1998). It has been reported to range from 5 to 26% (Gunawardena, 2009). Chemical composition of faba bean is presented in Table 2.1.

Table 2.1. Chemical composition of faba bean seeds (beans) and, for comparison, values of barley grain, soybean meal, and pea seed. Adapted from O'Kiely et al., n.d.

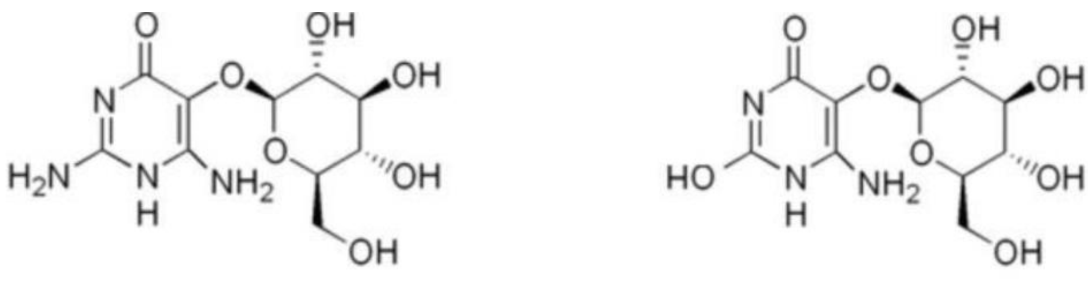
Constituent	Faba bean	Barley grain	Soybean meal	Pea seed
DM (%)	86.6	87.1	87.9	86.6
CP (%DM)	29.0	11.8	51.8	23.9
NDF (%DM)	15.9	21.7	13.7	14.2
ADF (%DM)	10.7	6.4	8.3	7.0
EE (%DM)	1.4	2.0	2.0	1.2
Starch (%DM)	44.7	59.7	5.0	51.3
Sugar (%DM)	3.6	2.0	9.4	4.9
Ash (%DM)	3.9	2.6	7.1	3.5
ME (Mcal/kg)	3.13	2.96	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

ME: metabolizable energy; DM: dry matter; EE: ether extracts (crude fat); CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber.

2.6 Nutritional Value for Monogastrics

2.6.1 Nutritional Value in Swine Diets

Faba beans are high in protein and energy and are very palatable to pigs (Blair, 2007), but the use of faba beans in pig diets may be limited due to the presence of antinutritional factors such as vicine, convicine, which are known to cause favism (disorder involving an allergic-like reaction to faba bean, caused by glucose-6-phosphate dehydrogenase deficiency).



Vicine Convicine

Figure 2.1. Chemical structure of vicine and convicine. Adapted from Rizzello et al., 2016.

Additionally, condensed tannins which may reduce feed intake and nutrient digestibility, especially for protein, and thereby reduce growth in swine (Gunawardena, 2009; Marquardt et al.,1977; Jansman, 1993). Among the antinutritional factors found in faba beans, tannins are the most detrimental to the pig (Garrido et al., 1991) and it can be concluded that tannins in faba beans reduce their nutritional value for pigs, both for energy and for protein (Crepon et al., 2010). In growing and fattening pigs, the recommended maximum inclusion rate is 20%, however rates up to 30% (low tannin variety) have been tested with success (Heuzé et al., 2015). Faba bean has been shown not to alter the growth performance of growing or finishing pigs up to 10% of a compound feed (Landry, 2014; Crepon et al., 2010). A previous study with growing pigs showed that animals fed diets containing high-tannin or low-tannin faba beans (30%) had similar N retention, growth performance, and feed efficiency (Flis et al., 1999). Later studies have been more decisive on the benefits of low-tannin cultivars. Low-tannin faba beans resulted in same voluntary feed intake and similar carcass quality when they were included at a 30% dietary level to replace soybean meal in growing pig diets (Zijlstra et al., 2004). Low-tannin faba beans could be included at higher concentration (35 vs 20%) than high-tannin seeds in fattening pig diets (Royer et al., 2010). The large potential of this new zero tannin faba beans (variety "Snowbird") mainly as a feed for swine is well documented (Singh et al., 2013). This zero-tannin faba bean Snowbird has an attractive nutrient profile and does not alter ADFI or ADG of grower-finisher pigs at inclusion rates up to 30%, therefore it is concluded (Table 2.2) that Snowbird faba bean is a worthwhile energy and protein feedstuff to consider in swine feed formulation (Zijlstra et al., 2008).

Table 2.2. Growth performance of grower-finisher pigs fed diets based on either faba bean or soybean meal. Adapted from Zijlstra et al., 2008.

	Gilt		Barrow			P value		
Variable[z]	Faba bean	Soybean meal	Faba bean	Soybean meal	SEM	Diet	Gender	Diet×Gender
ADG (kg/d)								
Grower	0.864	0.873	0.926	0.99	0.02	0.1	<0.01	0.21
Finisher 1	0.913	0.924	1.032	1.069	0.03	0.37	<0.01	0.62
Finisher 2	1.154	1.084	1.054	0.992	0.05	0.21	0.08	0.94
Total	0.977	0.961	1.004	1.017	0.02	0.93	0.04	0.42
ADFI (kg/d)								
Grower	1.959	1.964	2.16	2.167	0.07	0.92	<0.01	0.99
Finisher 1	2.719	2.593	3.223	3.208	0.08	0.42	<0.01	0.53
Finisher 2	2.978	2.916	3.383	3.261	0.07	0.25	<0.01	0.7
Total	2.552	2.491	2.922	2.878	0.07	0.45	<0.01	0.9
Feed efficiency								
Grower	0.442	0.445	0.429	0.457	0.007	0.04	0.96	0.11
Finisher 1	0.336	0.358	0.320	0.334	0.009	0.08	0.06	0.66
Finisher 2	0.386	0.373	0.311	0.305	0.013	0.47	<0.01	0.78
Total	0.388	0.392	0.354	0.365	0.006	0.25	<0.01	0.59
Final body weight (kg)								
Grower	62.23	62.06	65.21	67.14	0.81	0.29	<0.01	0.21
Finisher 1	87.81	87.95	94.09	97.09	1.2	0.21	<0.01	0.25
Finisher 2	102.8	102.04	107.8	109.99	1.28	0.58	<0.01	0.26

[z] Least-square means based on five pen observations per diet regime and gender combination. ADG: average daily gain; ADFI: average feed daily intake.

2.6.2 Nutritional Value in Poultry Diets

Faba beans are also a good source of energy for broilers (Nalle et al., 2011; Masey O'Neillet al., 2012). In faba beans, energy value is dependent on the content of vicine, convicine, tannins, and processing (Koivunen et al., 2016; Crépon et al., 2010). Vicine and convicine reduce egg production in laying hens (Heuzé et al., 2015; Fru-Nji et al., 2007). The lowering of vicine, convicine, and tannin contents in faba bean seeds has a significant and additive positive impact on apparent metabolizable energy (ME) values in broiler chickens (Table 2.3.); additionally, pelleting has a positive effect which can be explained by a better starch and protein digestibility (Crépon et al., 2010; Lacassagne et al., 1988). Therefore, the recommended inclusion of high vicine-convicine was 7% for a hen diet, while low vicine-convicine could be added up to 20% (Landry, 2014). On the other hand, tannins have a significant negative impact on protein digestibility (Grosjean et al., 2000), and also reduce energy and starch digestibilities (Vilariño et al., 2009). In broilers, it has been possible to include up to 25% high-tannin or low-tannin faba bean with no effect on growth performance (Métayer et al., 2003), and also was reported that low-tannin faba beans included at 20% in broiler diets resulted in higher live weight gain and feed intake than those obtained with high-tannin seeds (Brévault et al., 2003).

Table 2.3. Performance of broiler chicken (two studies) fed faba bean-containing diets made with low- or high-tannin varieties. Adapted from Crepon et al., 2010.

Performance	Control Soya	Diet with faba bean		P
		Low 250g/kg	High 250g/kg	
Live weight gain (g/d)	52.1	51.1	51.8	NS
Food intake 1−56 d (g)	5844	5635	5716	NS
Feed conversion ratio 1−56 d	2	1.97	1.98	NS
		Low 200g/kg	High 200g/kg	
Live weight gain (g/d)	31.4^{ab}	31.8^{b}	30.5^{a}	<0.01
Food intake 1−56 d (g)	7385^{b}	7341^{b}	7135^{a}	<0.01
Feed conversion ratio 1−56 d	2.87	2.82	2.85	NS

2.7 Faba Bean for Ruminants

2.7.1 Faba Bean Seeds

Faba beans seeds are highly digestible by ruminants (OM digestibility 91%) and they are considered to be an excellent source of both protein and energy for ruminants (Heuzé et al., 2015). The protein is extensively and rapidly degradable in the rumen (Table 2.4.) and protein not degraded in the rumen should be accessible later in the intestinal tract (Ramos-Morales et al., 2010; Yu et al., 2002). The very soluble nature of the protein in faba beans that makes them easily degraded in the rumen will provide a pulse of nitrogenous substrate for rumen microbes (O'Kiely et al., n.d). The energy value of faba beans is at least as good as cereal grains such as barley, additionally some secondary metabolites such as condensed tannins which in faba beans are located mainly in the hull can protect protein from degradation in the rumen and allowing to increase the bypass protein (Frutos et al., 2004). For optimum microbial protein synthesis, the ratio of rumen available proteins to rumen available carbohydrates is a critical determinant, it has been reported that about 32 g N/kg available carbohydrates should be appropriate and sufficient for achieving optimum microbial growth, however faba bean is about 55 g N/kg available carbohydrates, which indicates a potential N loss in the rumen under certain conditions (Tamminga et al., 2007; Yu, 1999).

The starch in faba beans is highly degradable in the rumen (Table 2.5), however, some heat treatments such as pressure toasting, dry roasting, and extrusion, except of pelleting, can reduce the rate and extent of rumen degradation of both protein and starch in legume seeds, thus resulting in a potential increase in the supply of amino acids and/or starch to the small intestines (Yu et al., 2002; Goelema et al., 1998).

Table 2.4. Rumen CP degradation characteristics of lupin seed, faba bean, and peas. Adapted from Yu et al., 2002.

	Rumen Degradation Characteristics						
	CP (%)	S (%)	D (%)	U (%)	Kd (%/h)	ED (%)	BCP (%)
Faba bean	31.7	49	50.7	0.2	21.4	89.7	11.3
Faba bean	31.2	64.2	34.0	1.8	7.4	82.7	17.3
Lupin	35	26.2	73.8	0	7.6	80	20
Lupin	38.7	31.8	67.4	0.8	10.2	74.1	25.9
Peas	26.8	39.3	60.7	0	8.6	87.5	22.5
Peas	25	62.1	37.9	0	4.8	79	21.0

CP: crude protein; Kd: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; S: soluble fraction in the *in situ* incubation; BCP: rumen bypassed crude protein in DVE/OEB system; ED: effectively degraded crude protein.

Table 2.5. Rumen starch degradation characteristics of lupin seed, faba bean and peas. Adapted from Yu et al., 2002.

	Rumen Degradation Characteristics					
	St (%)	S (%)	D (%)	Kd (%/h)	ED (%)	BSt (%)
Lupin	6.7	91	9	12.5	88	12
Faba bean	42.2	41	59	12.2	79.6	20.4
Faba bean	36	44	56	8	78	22
Faba bean	36.4	50	49.9	9.8	76.1	23.9
Horse bean	41	58.2	41.8	5	71	29
Peas	42.6	24	76	14.1	77.7	22.3
Peas	41.8	50	50	7.1	74.3	25.7
Peas	49.1	52.2	47.8	4.6	67.6	32.4

St: starch; Kd: the degradation rate of D fraction; S: soluble fraction; D: degradable fraction; BSt: rumen bypass or undegraded feed starch; ED: effective degraded starch.

Some studies conducted in ruminants have shown favorable effects; high concentration of raw faba beans were fed in sheep, without negative effects on the palatability and digestibility of the diets (Liponi et al., 2009). Replacement of soybean meal with ground faba bean for lactating cows up to 30% was shown to not result in a comparative loss in production, and in some cases, with the addition of rapeseed meal, could even eliminate the need for soybeans (Landry, 2014; Crepon et al., 2010). Other research (Table 2.6.) also was conducted and study the effect of partially replacing soybean meal with faba beans and it was suggested that milk yield and composition were not affected by the use of faba beans in the diet (Volpelli et al., 2010).

Table 2.6. Concentrate intake, milk yield, milk composition, blood protein and blood urea as influenced by the different diets fed to animals. Adapted from Volpelli et al., 2010.

Item	Diet		SEM	Significance
	Soybean	Faba		
Concentrate intake/cow (kg/d)	7.87	7.81	0.270	ns
Milk yield (kg/d)	22.21	22.38	0.568	ns
Milk composition				
Fat (%)	3.9	3.93	0.159	ns
Protein (%)	3.47	3.39	0.037	ns
Lactose (%)	4.95	4.94	0.025	ns
Casein (%)	2.72	2.67	0.030	ns
Urea (mg/dl)	34.58	32.93	0.649	*
Fat yield (kg/d)	0.86	0.88	0.046	ns
Protein yield (kg/d)	0.75	0.75	0.029	ns
Casein yield (kg/d)	0.59	0.59	0.022	ns
Blood total protein (g/L)	72.48	64.03	3.037	ns
Blood urea (mmol/L)	5.76	5.75	0.171	ns

*P< 0.10; ns: not significant

2.7.2 Faba bean Forage

In dairy cows, the voluntary intake of dairy cows fed on fresh faba bean forage (whole plant) was high, however when the forage was ensiled, voluntary intake decreased with maturity but remained high compared to other silages (Heuzé et al., 2015). Their use as a source of forage is limited, however limited data from previous studies demonstrated that whole plant faba bean silage is comparable to grass-legume silage (Ingalls et al., 1974). Regardless to the yield and composition of whole plant faba bean forage, a study compares the yield and chemical composition of whole-plant faba bean forage when harvested at different stages of growth. The yield and chemical composition of faba bean forages at different stages of growth are presented in Table 2.7 and Table 2.8, respectively. This study suggested that delaying the harvest of field beans until 14 weeks of growth (pod full growth stage) gave the highest DM and CP yields, and also a higher starch content compared with the earlier harvesting dates (Fraser et al., 2001).

Table 2.7. Effect of harvest date on the yield of faba bean forage. Adapted from Fraser et al., 2001.

	Harvest date after sowing	Plant height (cm)	FM yield (kg/ha)	DM content (g/kg FM)	DM yield (kg/ha)
Faba beans	10 weeks	115	30642	121	3698
	12 weeks	137	38332	135	5167
	14 weeks	146	50679	153	7760

FM: fresh matter; DM: dry matter.

Table 2.8. Effect of harvest date on the chemical composition of faba beans, buffering capacity (BC) all values in g/kg DM, except DM content (g/kg FM) and BC (mequiv. 100 g $^{-1}$). Adapted from Fraser et al., 2001.

	Harvest date after sowing	DM	CP	WSC	Starch	ADF	NDF	BC
Faba beans	10 weeks	171	213	88	45	287	375	392
	12 weeks	230	187	104	73	291	372	345
	14 weeks	210	180	97	64	298	376	342

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; WSC: water soluble carbohydrates.

An older study (Ingalls et al., 1974) suggested that faba bean silage was higher in protein (20.1 vs. 16.1 %) 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. Apparent digestibilities of faba bean silage ration DM, CP and gross energy tended to be higher than those of grass-legume silage diet. Additionally, cows fed faba bean silage produced as much milk of similar protein and total solids contents as when grass-legume silage was fed. The feeding value of faba bean silage for lactating dairy cows as suggested those results is comparable to that of good quality grass-legume silage. Given proper growing, harvesting and ensiling practices, whole plant faba bean may be a satisfactory alternative to other protein- and energy-abundant forages (McKnight and MacLeod, 1977). More recent study has an objective to determine the ensiling characteristics, chemical composition (Table 2.9) and ruminal nutrient degradability (Table 10) of faba bean silage (faba bean was harvested between the beginning seed to full seed stage). This study suggested that during faba bean ensiling, the SCP was increased rapidly between day 0 and day 4 post-ensiling with no further changes between day 8 and day 45 post-ensiling, the changes of NPN were similar to those observed with SCP, regardless to NDICP declined sharply between day 0 and day 2 post ensiling and a significant decline in NDICP continued between any two ensiling periods up to day 45. The true protein fraction was declined rapidly between day 0 and day 4, and a gradual reduction in true protein was observed for between day 4 and day 45 post-ensiling (Mustafa and Seguin, 2003a).

Table 2.9. Chemical composition of whole-crop legume forages after 45 d of ensiling. Adapted from Mustafa and Seguin, 2003a.

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

DM: dry matter; CP: crude protein; SCP: soluble crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NPN: non-protein nitrogen.

The rumen degradation kinetics (Table 2.10) indicated that the *in situ* soluble protein fraction was highest for pea silage, intermediate for soybean silage, and lowest for faba bean silage, additionally the effective ruminal degradability of CP for the three silages was high, and higher for soybean silage and pea silage than for faba bean silage (Mustafa and Seguin, 2003a).

Table 2.10. Ruminal nutrient kinetics and effective degradability of whole-crop legume silages. Adapted from Mustafa and Seguin, 2003a.

	Legume Silages			SEM
	Faba bean	Soybean	Pea	
DM				
Soluble fraction (% DM)	44.8^{b}	45.0^{b}	47.8a	0.47
Potentially degradable fraction (% DM)	36.8^{a}	37.3^{a}	31.8b	0.89
Degradation rate (%/h)	7.0^{b}	8.7^{a}	8.9a	0.24
Lag time (h)	0.1^{b}	1.1^{a}	1.2^{a}	0.32
Effective degradability (%)	66.2^{b}	68.5^{a}	69.1^{a}	0.25
CP				
Soluble fraction (% CP)	49.4^{c}	59.4^{b}	70.3a	0.7
Potentially degradable fraction (% CP)	41.5^{a}	32.0^{b}	22.0^{c}	0.76
Degradation rate (%/h)	13.2^{a}	12.6^{a}	7.7^{b}	0.86
Lag time (h)	0.4	0.2	0.6	0.26
Effective degradability (%)	79.4^{b}	82.2^{a}	83.6^{a}	0.53
NDF				
Soluble fraction (% NDF)	10.4	10.1	9.9	0.48
Potentially degradable fraction (% NDF)	47.2	47	53.3	1.81
Degradation rate (%/h)	5.1	5.1	5.5	0.37
Lag time (h)	0.6^{a}	0.1^{b}	0.7^{a}	0.13
Effective degradability (%)	34.2	35.5	34.5	0.57

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber.

2.8 Feed Evaluation Techniques and Methods

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

The CP and carbohydrate subtractions are partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS), which was introduced at the beginning of the 1990's and has several updates (Tylutki et al., 2008; Raffrenato, 2011; Higgs et al., 2015; Van Amburgh et al., 2015). The Cornell Net Carbohydrate and Protein System (CNCPS) is used to determine the protein and carbohydrate factions, specifically the CNCPS 6.5. The CP fractions are determined using the following formulas: PA1 = ammonia × (SP/100) × (CP/100) which indicates ammonia; PA2 = SP × CP/100 − PA1 which is soluble true protein; PB1 = CP − (PA1 − PA2 − PB2 − PC) refers to insoluble true protein; PB2 = (NDICP − ADICP) × CP / 100) which refers to fiber-bound protein and PC = ADICP × CP / 100 which is indigestible protein. The Kd values for CP fractions are: PA1, Kd is 200 %/h; PA2, Kd range is 10-40 %/h; PB1, Kd range is 3-20 %/h; PB2, Kd 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 Kd values for CHO fractions are: CA1, Kd value is 0 %/h; CA2, Kd value is 7 %/h; CA3, Kd is 5 %/h; CA4, Kd range is 40-60 %/h; CB1, Kd range is 20-40 %/h; CB2, Kd range is 20-40 %/h; CB3, Kd 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 of sample) which are incubated in the rumen of a cannulated cow for 288 hours. After complete incubation, the bags are washed then dry for 48 h at 55 °C (Huhtanen et al., 1994).

2.8.2 Energy Evaluation in Feeds and Animal Diet Ingredients

Truly digestible nutrients, total digestible nutrient (TDN), digestible energy (DE), metabolizable energy (ME), and net energy (NE_L) at 1× and 3× maintenance were determined using the NRC-2001 dairy (NRC, 2001), while the NRC-1996 beef (NRC, 1996) is used to determine net energy for maintenance (NE_m) and net energy for gain (NE_g).

2.8.3 Assessing Rumen Fermentation/Degradation Kinetics of Feed Nutrients Using the In Situ Technique

The first-order degradation kinetics model described by Ørskov and McDonald (1979) and modified by Tamminga et al., (1994) is used to determined degradation characteristics of dry matter (DM), crude protein (CP), starch (ST), and neutral detergent fiber (aNDF). Results were calculated using SAS 9.4 with nonlinear (NLIN) procedure and iterative least-squares regression (Gausse

Newton method): R(t) = U + D × $e^{-Kd\times(t-T0)}$, where R(t) = residue present at t h incubation (%); U = undegradable fraction (%); D = potentially degradable fraction (%); Kd = degradation rate (%/h); and T0 = lag time (h).

The extent of degradation or effective degradability (ED) of each individual nutrient (DM, CP, ST, and NDF) is predicted according to NRC (2001). The effective degradability expressed in percentage is obtained with the following equation: ED = D × Kd / (Kp + Kd) + S, where S (%) is the soluble fraction for DM, CP, and ST and washable fraction for NDF. The effective degradability of nutrients expressed in grams per kilogram of DM were obtained with the following formulas: EDST (g/kg DM) = ST (g/kg DM) × %EDST; EDCP (g/kg DM) = CP (g/kg DM) × %EDCP; and EDNDF (g/kg DM) = NDF (g/kg DM) × %EDNDF.

The rumen undegradable (RU) or bypass (B) values of nutrients on a percentage basis according to NRC dairy (2001) and Tamminga et al., (1994) included: %BDM, %BCP, %BST, and %BNDF are estimated with the following equation: D × Kp / (Kp + Kd) + U, where Kp is the estimated passage rate from the rumen which is assumed to be 6 %/h for DM, CP, and starch and 2.5 %/h for NDF (Tamminga et al., 1994). The equations used to obtain the rumen undegradable bypass ST (BST), NDF (BNDF), and CP (RUP) in grams per kilogram of DM (g/kg DM) according to NRC dairy 2001 were: BST (g/kg DM) = ST (g/kg DM) × %BST; BNDF (g/kg DM) = NDF (g/kg DM) × %BNDF; and RUP^{NRC} (g/kg DM) = CP (g/kg DM) × %RUP. In the present study, rumen bypass protein (BCP) according to the Dutch system is also calculated as follows: BCP^{DVE} (g/kg DM) = 1.11 × CP (g/kg DM) × %BCP, where 1.11 referred to the regression coefficient between *in situ* RUP and *in vivo* RUP (Tamminga et al., 1994; Yu et al., 2002). Data from *in situ* rumen degradation characteristics of crude protein are used to predict the true protein supply to dairy cattle by DVE/OEB system and NRC model. The best treatment would be used for future dairy production and metabolic trials.

2.8.4 Hourly Effective Rumen Degradation Ratios/Potential N-to-Energy Synchronization

The effective rumen degradation ratios of N and carbohydrates (CHO) are calculated using the formula described by Sinclair et al., (1993). Hourly ED ratio N/CHO t = 1000 × (HEDN t – HEDN t - 1) / [(HEDNDF t – HEDNDF t - 1) + (HEDST t – HEDST t - 1)], where N/CHO t = ratio of N to CHO at time t (g N/kg CHO); HEDN t = hourly effective degradability of N at time t (g/kg DM); HEDN t - 1 = hourly effective degradability of N 1 h before t (g/kg DM); HEDCHO t = hourly effective degradability of CHO at time t (g/kg DM) which includes: HEDNDF t = hourly effective degradability of neutral detergent fiber at time t (g/kg DM); HEDNDF t -1 = hourly effective degradability of neutral detergent fiber at 1 h before t (g/kg DM); HEDST t = hourly effective degradability of starch at time t (g/kg DM); HEDST t -1 = hourly effective degradability of starch at 1 h before t (g/kg DM).

2.8.5 Evaluation of Intestinal Digestibility of Feed Nutrients Using Three-Step In Vitro Techniques

The *in vitro* intestinal digestion is determined following the three-step procedure described by Calsamiglia and Stern (1995) and later on modified by Gargallo et al., (2006). The procedure basically is as follows: dried ground (1 mm) residues from 12 or 16 h ruminal incubation were deposited into a centrifuge tube, after the addition of 10 ml of pepsin (Sigma P-7012) solution (in 0.1 N HCl with pH 1.9), the tubes were incubated for 1 h at 38 °C in a water bath, then 0.5 ml 1 N NaOH solution and 13.5 ml of pancreatin (Sigma P-7545) are included and vortexed. After, an incubation for 24 h at 38 °C in a water bath took place (tubes are vortexed every 8 hours), then 3 ml of tri-carboxylic acid (TCA) is added and tubes were vortexed and cooled to room temperature. Tubes are centrifuged for 15 min at 10000 g and then 5 ml of supernatant ai analyzed for N by the

Kjeldahl method. Intestinal digestion of protein is calculated as TCA-soluble N divided by the amount of N in the rumen 12 h or 16 h residue sample.

2.8.6 Prediction of Truly Digestible Protein Supply to Small Intestine in Dairy Cattle

2.8.6.1 Dutch DVE/OEB Systems

The protein digested in the intestine (DVE) and the degraded protein balance (OEB) are calculated using the following formulas: DVE (g/kg of DM) = DVME + DVBE − ENDP, where, DVME is the absorbable fraction of microbial crude protein, DVBE is the absorbable fraction of ruminally undegraded feed protein, and ENDP is a correction for endogenous protein losses in the digestion tract; OEB (g/kg of DM) = MREN – MREE, where MREN is the potential microbial protein synthesized in the rumen based on rumen degraded feed CP, and MREE the potential microbial protein synthesis based on energy extracted from anaerobic fermentation (Tamminga et al., 1994; Tamminga et al., 2007; Van Duinkerken et al., 2011).

2.8.6.2 National Research Council Dairy Model

Metabolizable protein (MP) and degraded protein balance (DPB) are calculated using the following formulas: MP (g/kg DM) = AMCP + ARUP + AECP, where AMCP was the absorbable microbial protein, ARUP is the truly absorbable rumen undegraded feed protein, and AECP was the truly absorbable endogenous protein in the small intestine; DPB (g/kg DM) = $RDP^{NRC} - 1.18 \times MCP_{TDN}$, where RDP^{NRC} is the rumen degraded protein, factor 1.18 = 1/0.85, and MCP_{TDN} was the microbial crude protein synthesis from energy (NRC, 2001)..

2.8.7 Feed Milk Value Determination in Dairy Cattle

Predicted production performance or feed milk value (FMV) is determined according the DVE/OEB system and the NRC model: FMV (kg milk/kg DM) = 0.67 × MP (g/kg DM) / 33, where MP was metabolizable protein (DVE value in DVE/OEB System), the assumed efficiency of metabolizable protein for lactation was 0.67, and 1 kg of milk contained 33 g protein (NRC, 2001).

2.8.8 Fourier transform infrared spectroscopy-FTIR

Spectroscopic methods with infrared light are sensitive to the composition and structure of biological components such as protein, carbohydrates, and lipids, among others. The uniqueness of the analysis relies on the intrinsic conformation of a sample in which the different molecular bonds absorb infrared (IR) light at different frequencies. This allows researchers to measure specific intensities at certain wavelengths for the determination of characteristic functional bands that help with the overall identification of the molecular conformation within a sample (Walker, 2007). A traditional IR spectrometer emits all IR frequencies simultaneously, including wavelengths from the near-IR (NIR), mid-IR (MIR), to far-IR (FIR).

Infrared spectroscopy studies the interaction of infrared light with matter. The most common regions for analyses include the NIR and MIR from which scientists obtain a plot of light intensity versus the light wavelength or wavenumber (Smith, 2011; Larkin, 2011). The resulting spectrum is useful to determine which molecules make up a sample and the concentration in which they are presented (Smith, 2011). The spectrum is plotted in absorbance units which measures the amount of light absorbed by the sample. Absorbance is linearly proportional to concentration, so absorbance units can be used to perform both quantitative and qualitative analyses of nutrients in feed (Smith, 2011).

Infrared spectroscopy is useful because the peak positions found in the spectrum correlate with the molecular structure of a sample (Smith, 2011). Various materials containing covalent bonds can absorb light at the infrared range, however, for the determination of the molecular functional groups, the molecule must be infrared active. This means that the molecule must have a dipole moment in order to absorb IR light (Khan et al., 2018). Essentially, the change in the molecular dipole moment caused by vibrations of the atoms is required in order for the energy to be transmitted from the IR light into the sample molecules (Larkin, 2011). Hence, a specific frequency of light is absorbed by a particular molecular bond which has its own natural vibrational frequency (Khan et al., 2018) which leads to a unique spectrum for each single material.

2.8.8.1 Band assignment and functional groups

Infrared light absorption produces changes in the molecules by stretching and bending vibrations of different molecular bonds. These alterations are of main importance to identify the functional groups of a molecule (Figure 2.2). Hence, functional groups could be observed at specific wavenumber ranges such as 3200 to 3550 cm^{-1} (O-H stretching), 2500 to 3000 cm^{-1} (Carboxylic O-H), 3300–3500 cm^{-1} (N-H stretch), 3500–3500 cm^{-1} (O=C-N-H stretch), 2950–2850 cm^{-1} (C-H stretch), 1620–1680 cm^{-1} (C=C stretch), 1780–1710 cm^{-1} (Carboxylic acid C=O), or 1690–1630 cm^{-1} (Amide C=O) (Khan et al., 2018). With this information is possible to analyze different materials in order to identify common functional groups on specific samples (Figure 2.3).

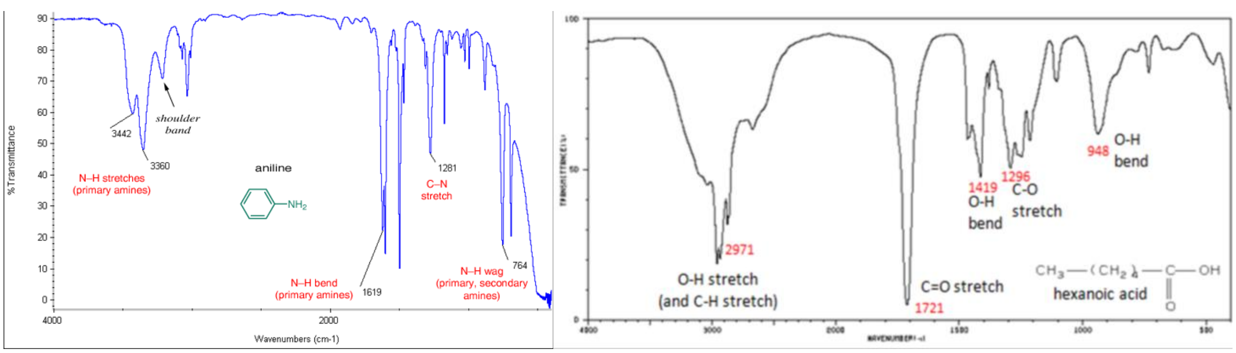


Figure 2.2. Functional groups of IR spectrum of primary amine, aniline (left) and Hexanoic acid (right). Adapted from University of Colorado (2007) and Kennepohl et al., (nd).

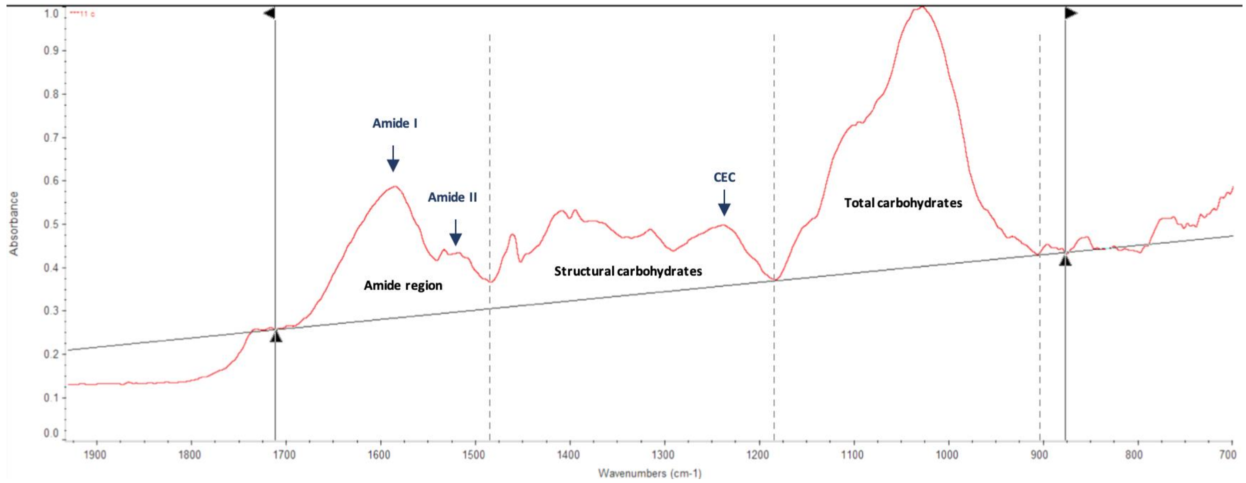


Figure 2.3. Infrared spectrum of whole plant faba bean silage (ca. 1712-879 cm^{-1})

2.8.8.2 Principals of FTIR spectroscopy

In FTIR spectroscopy IR light goes through the interferometer, and hits the sample giving each molecule a pulse of IR light and causing several atoms vibrations (Khan et al., 2018). Then the signals from the interaction of light with the sample reach a detector. The signal produced by different light absorptions create an interferogram that is later converted into a spectrum by a Fourier transform calculation (Khan et al., 2018). The Michelson interferometer is the one used in FTIR spectrometers (Figure 2.4.). It contains a beam splitter, a fixed mirror, and a moveable mirror. The beam splitter divides the incoming beam of light into two, it transmits half of the radiation to the fixed mirror while reflects the other half to the moving mirror. These two mirrors then reflect the radiation back to the beam splitter where they are recombined to next leave the interferometer to interact with the sample (Khan et al., 2018).

Alternative measurements for FTIR can be done using Attenuated Total Reflection (ATR) to avoid the disadvantages of other conventional transmission mode analysis (Khan et al., 2018). The equipment to perform ATR-FTIR contain horizontal crystals made up of zinc selenide, diamond, and germanium. In ATR-FTIR several materials from solids to liquids can be placed on the ATR crystal without any previous treatment or major sample preparation which makes this technique fast and simple (Khan et al., 2018).

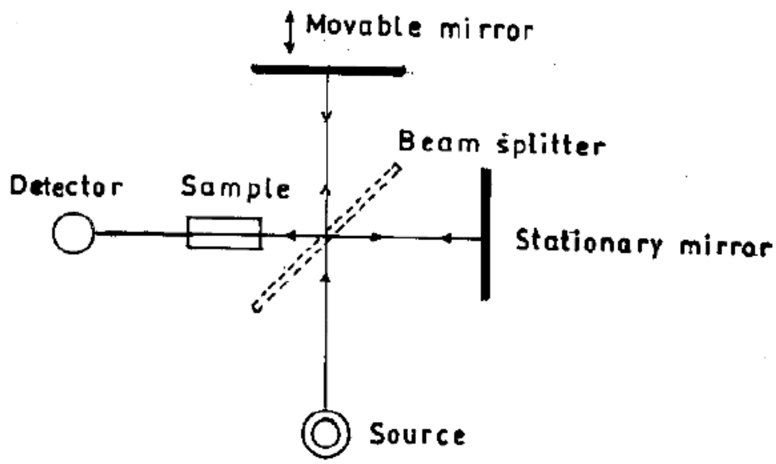


Figure 2.4. Schematic of a Michelson interferometer. Adapted from Jaggi and Vij (2007)

2.8.8.3 NIR vs. FTIR

Near-infrared spectroscopy (NIR) is a modern analytical technique in which materials are measured with a range of infrared light between 12500 and 4000 cm^{-1}. Bands that are observed in this region result from overtones, electronic transitions, and combination modes as NIR combines electronic and vibrational spectroscopy (Czarnecki et al., 2015). Analyses in the NIR present advantages such as high energy levels and the functionality of high sensitivity detectors in this region (Wilks, 2006). However, the overlapping of the absorption bands resulting from overtones from the fundamental bands in the MIR region, result in weaker band intensities compared to MIR, and hence, complicated calculations for quantitative analyses and demanding calibration procedures are needed in this technique (Czarnecki et al., 2015; Wilks, 2006). On the other hand, mid-infrared spectroscopy (MIR) is a vibrational spectroscopy technique used to study samples in the infrared range between 400 and 4000 cm^{-1}. Several organic functional groups have defined and characteristic absorptions in this region which helps in the identification and characterisation of the molecular structure. However, materials used for radiation transmission could be more expensive than the ones used for NIR (Wilks, 2006).

2.8.8.4 ATR-FTIR applications

Fourier transform infrared techniques have gain popularity along the years because they are sensitive, accurate, and fast compared to other spectrometers without FT. The broad analytical capacity of FTIR makes it a major tool to measure the absorption and emission of IR in most materials (Jaggi and Vij, 2007). FTIR technology ca be use for the analysis of several materials including organic molecules and nanomaterials which brings a broad application of this technique in different fields (Khan et al., 2018). As some examples, FTIR can be applied for biomedical imaging to study biological materials or to perform protein analyses by the determination of protein structure. Furthermore, a large variety of other elements can be studied using this technique including inorganic compounds, drugs, polymers, resins, among others (Khan et al., 2018). In fields of feed science, FTIR is used to detect molecular structural changes in several feeds relative to nutrient values and nutrient utilization (Ban, 2016).

2.9 Literature Review Summary/Conclusions, Overall Research Objectives and Hypothesis

Faba bean is widely known because of its high nutritive value as food and feed (Crepon et al., 2010; Heuzé et al., 2015). However, there is a lack of information about this legume when the whole plant is fed as forage to ruminants. There are several new varieties available in Canada but not systematically evaluation done; high tannin varieties which are used for human consumption and are mainly exported, and the low tannin varieties which are used for human and animal consumption. Additionally, the production of faba bean has been increased notably (Saskatchewan Pulse Growers, 2017).

2.9.1 Project Hypotheses

- Physiochemical, molecular structural and nutritional features of whole crop of faba bean plant as hay and silage for ruminants would be significantly impacted by variety/tannin concentration (low and high), growth stage /cutting time, and frost-damage.
- The alteration or changes of molecular structure features by variety/tannin concentration (low and high), growth stage /cutting time, frost-damage and forage processing would be highly associated with nutrient utilization and availability in cattle.

2.9.2 Project Objectives

Long-term:

- To systematically develop feeding strategy and maintain and increase business in competitive market for pulse producers and related industries for faba forage hay, faba silage, and faba bean as energy and protein concentrates (“try to extract high value from faba crop”).
- To help pulse producers and industry to maximize the utilization of whole plant faba bean.

- To build and develop alternative feeding strategies based on tannin content, variety, and processing impact to efficiently utilize faba hay and faba silage in sustainable livestock production systems for improving animal production and health.
- To increase basic knowledge of the nutritional relevance of faba forage hay and silage and apply this info to the production of high-quality feeding programs and aid faba variety/lines breeding programs.

Short-term:

- To carry out comprehensive physicochemical and nutritional evaluation of faba forage varieties for hay with a systematic approach: Effect of variety/tannin concentration and growth stage on detailed feed and feeding values of faba forage hay grown in western Canada (Saskatchewan) under cool climate conditions in ruminant livestock systems.
- To conduct comprehensive physicochemical and nutritional evaluation of faba forage varieties for silage with a systematic approach: Effect of variety/tannin concentration and growth stage on feed and feeding values of faba silage in ruminant livestock systems.
- To determine Feed Milk Value (FMV), absorbed microbial protein synthesis, metabolizable protein, degraded protein balance, and truly absorbed nutrient supply from faba bean harvested in western Canada (Saskatchewan) under cool climate growth conditions.
- To carry out dairy production performance and metabolic trials with faba silage: Development efficient feeding strategy of faba silage to find maximum replacement of barley silage or corn silage in high production lactation dairy cows to benefit of pulse growers and livestock producers.

- To reveal intrinsic molecular structure changes that affect nutrient utilization and availability in cattle by using cutting-edge molecular spectroscopic techniques with advanced molecular analysis techniques - chemometrics: Effect of varieties/tannin concentration and processing technology.
- To obtain all necessary feed nutritional characteristics for various advanced nutrient and diet formulation models: CNCPS, NRC, and DVE for diet formulation for cattle.
- To provide detailed faba data to assist Canadian companies and producer organizations to develop business overseas if applied and to provide detailed research data to faba bean breeders to assist develop new varieties that suitable grown in western Canada.

3. COMPREHENSIVE PHYSIOCHEMICAL AND NUTRITIONAL EVALUATION OF FABA FORAGE VARIETIES FOR HAY: EFFECT OF VARIETY/TANNIN CONCENTRATION AND GROWTH STAGE ON DETAILED FEED AND FEEDING VALUES OF FABA FORAGE HAY GROWN AT THREE STAGES IN WESTERN CANADA WITH COOL CLIMATE CONDITIONS IN RUMINANT LIVESTOCK SYSTEMS.

3.1 Abstract

Recently faba bean production in Canada is much higher than that in previous years but there is little information on its nutritive value for cattle. This study aimed to determine the effect of tannin concentration/variety (low tannin/Snowdrop variety; high tannin/SSNS-1 variety) and the effect of cutting stage (flower, mid pod, and late pod stages) on yield, chemical and energy profiles, CNCPS fractions, rumen degradation kinetics of principal nutrients, the hourly effective rumen degradation ratios, intestinal digestibility, metabolic characteristics, and Feed Milk Value (FMV) of whole plant faba bean hay (artificially air dried) grown in Western Canada. The results showed that dry matter (DM) yield of the whole plant faba bean was higher (P< 0.05) at late pod stage than flower stage (12.74 vs. 7.68 t/ha). Organic matter (OM), carbohydrates (CHO), and starch were lower (P< 0.05) at flower stage than at late pod stage (89.7, 65.6 and 0.7 vs. 91.8, 70.2 and 14.2 %DM, respectively). At late pod stage, whole plant faba bean hay had lower (P< 0.05) unavailable neutral detergent fiber (CC, 13.5 vs. 24.5 %CHO) and higher digestible fiber (CB3, 37.3 vs. 30.6 %CHO) than at flower stage. At late pod stage, a higher rumen undegradable protein (RUP) was shown than at flower stage (40 vs. 32 g/kg DM). The results showed that the total true protein supply (DVE) was higher (P< 0.05) at late pod stage than at flower stage (77 vs. 68 g/kg DM) and the degraded protein balance (OEB) was higher (P< 0.05) at flower and mid pod stages than at late pod stage (91 and 78 vs. 57 g/kg DM). The FMV value based on the DVE system was higher (P<

0.05) at late pod stage than at flower stage (1.57 vs. 1.35 kg milk/kg DM hay) and the FMV at mid pod stage (1.48 kg milk/kg DM hay) was similar to the previous values. The study concluded that both high and low tannin whole plant faba bean hay at late pod stage have attractive feed and feeding values and can be used as a potential ingredient in dairy rations. Further studies including animal and metabolic trials should be carried out to support this prediction.

Keywords: Faba bean, Tannin, Cutting stage, Dairy cattle.

3.2 Introduction

The production of pulses has been increasing in Canada since the 80's due to the rapid growth of international market opportunities, and the implementation of new technologies by the breeders and producers (Booker, 2016; Gilmour, 2016). In 2013, 20 thousand hectares of faba bean were seeded in Canada mainly in Alberta, Saskatchewan, and Manitoba. In 2015, the later production reached more than 60 thousand hectares. In the past years, Canadian faba bean exports have been as low as 10000 tonnes in 2013 and 2014 but rose above 25000 tonnes in 2018 (Saskatchewan Pulse Growers, 2018, 2017). Faba bean (*Vicia faba L.*) originated in the Mediterranean region (North Africa) or southwestern Asia, and it is a pulse crop capable of growing in cool and wet environments (Crepon et al., 2010; Tanno and Willcox, 2006; Muehlbauer and Tullu, 1997). There are two types of faba bean varieties which include the tannin and low tannin (zero or near zero tannin) varieties. Tannin beans are desired for human consumption as a food ingredient in both whole and fractionated forms. Low tannin beans can be used for both human and animal consumption (Saskatchewan Pulse Growers, 2017; Fleurly and Barker, 2016). Tannins are phenolic plant secondary compounds distributed in the plants, especially in legumes and brows (Theodoridou, 2010). Condensed tannins are considered as anti-nutritive compounds that affect palatability and digestion (protein and amino acid) in monogastrics but have possible beneficial effects on ruminants when concentration is optimal (Mueller-Harvey, 2006; Frutos et al., 2004; Hagerman and Butler, 1991). Ruminants are more tolerant to plants with high content of tannins and this is due to the microbial ecosystem responsible for fiber degradation in the rumen (Waghorn et al., 1998). Therefore, forages with low to moderate concentration of condensed tannins can contribute to higher retention of nitrogen in cattle (Cannas, 2015), because of the reduced rumen degradation of protein, more protein reaches the small intestine for digestion and absorption. On the other hand, Canada's forage resources include both native rangelands and cultivated crops. Forages are the foundation of diets for ruminant animals (Linn and Kuehn, 2017). Some dairy farmers use a mix of both silage and hay, while others might only feed silage or only feed hay (Alberta Milk, 2017). Hay purpose is to turn green, perishable forage into a product that can be easily transported and safely stored without danger of spoilage, while keeping losses of dry matter and nutrients to a minimum [The Food and Agriculture Organization (FAO), 2017]. In Canadian hay industry, pure timothy hay is the standard, brome-alfalfa is common for beef, and Orchard grass is common in British Columbia and Maritimes, but alfalfa hay and the mixture of alfalfa/timothy hay are also used (McCartney' and Horton, 1997). Timothy hay is mostly used as feed for cattle and horses and it is popular for its high palatability, relatively low protein, and high fibre content (Barr-Ag, 2013; Balasko and Nelson, 2003). On the other hand, alfalfa is well known as an excellent forage for high-producing cows (Jennings 2014). The quality of faba bean seed as food and feed has been examined and it is an excellent source of both protein and energy for ruminants (Heuzé et al., 2015; O'Kiely et al., n.d). However, the whole plant faba bean as fodder has received little attention (Sheaffer et al., 2001). Previously reported dry matter yields and crude protein concentration for faba bean forage have been 7.8 t/ha and 18 %, respectively (Fraser et al., 2001), thus faba bean appear to have similar potential as high yielding forages and high CP legumes (Strydhorst et al., 2008). For these reasons and due to the dramatically increased production in

Canada, we think that faba beans can become an additional option to produce high quality hay. However, its use as a forage source is very limited, and additionally there is not enough information on the chemical and energy profiles, CNCPS fractions as well as its rumen degradation kinetics and true nutrient supply to dairy cattle. The present study was conducted to determine the effects of the variety (tannin concentration) and cutting stage on whole plant faba bean hay in terms of chemical and energy profiles, CNCPS fractions, rumen degradation kinetics of primary nutrients and true nutrient supply to dairy cows.

3.3 Materials and Methods

3.3.1 Ingredients and Sample Preparation

Eighteen experimental plots (plot area = 36 square feet) were seeded (180 seeds per plot) by the Crop Development Centre (CDC) on May 3rd, 2017 in the research fields of the University of Saskatchewan. There was not any fertilization, neither apparent symptoms of disease, additionally the crop was not inoculated with rhizobium. Manually weed control is a common practice at the CDC fields, however in our faba bean plots was not required. Nine plots were randomly assigned to the low tannin faba bean variety Snowdrop, and nine plots were assigned to the high tannin faba bean variety SSNS-1. Snowdrop variety matures at 104 days, while SSNS-1 variety matures at 105 days. The whole plant faba bean from the plots was harvested at 77, 88, and 97 days old. Three plots of Snowdrop variety and 3 plots of SSNS-1 variety were harvested in each time period, collected in wool bags (2 or 3 bags per plot), and weighted (about 10 kg per bag). After harvesting (1 hour later), one bag per plot was artificially dried in an air drier room at 45 °C for 10 days at CDC field lab. Before drying, the bags were weighted.

3.3.2 Chemical Analysis of Whole Plant Faba Bean Hay

The samples were ground through a 1 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) and analyzed for sugars [Association of Official Analytical Chemists (AOAC) official method 974.06); CP (AOAC official method 984.13); EE (AOAC official method 920.39); Ash (AOAC official method 942.05) (AOAC 1990); NDICP, ADICP, and NPN were analyzed using the methods described in Licitra et al., (1996); SCP was estimated by incubating the sample with bicarbonate-phosphate buffer then filtering through Whatman filter paper (Roe et al., 1990); ADF, aNDF, and ADL were determined using the procedures of Van Soest et al., (1991); Cellulose and Hemicellulose were estimated according to the National Research Council-NRC (2001) as follows: Hemicellulose = NDF – ADF and Cellulose = ADF – ADL. Total, structural, and non-structural CHO were determined using NRC (2001) and Van Soest et al., (1991). Total carbohydrates (CHO) were estimated as: CHO = 100 - EE - CP - ash. Non-fiber carbohydrates were estimated as: NFC = 100 - (NDF - NDIP) - EE- CP – ash (NRC, 2001). Starch was analyzed using the Megazyme Total Starch Assay Kit (Wicklow, Ireland) and by the α-amylase/amyloglucosidase method (McCleary et al., 1999). Condensed tannins were analyzed with the HCl Butanol method published in Porter et al., (1986). Briefly, 20 to 30 mg of ground sample were weighed into a 16 x 125 mm test tubes (in quadruplicates: 4 tubes per sample), then 3 ml of HCl butanol reagent were added to all 4 tubes and covered with marbles. After one test tube (sample blank) of each sample was left at room temperature for 60 min, the rest 3 tubes were incubated in a water bath for 60 min. at 97° C. All tubes were vortexed every 10 min. After 60 min, the tubes were cooled at room temperature with cold water and then centrifuged for 10 min at 3000 rpm. The spectrophometer was turned on 20 min before the reading of the samples and then the wavelength was set at 550 nm. HCl butanol reagent (reagent blank) was used to zero the spectrophotometer. Absorbance (abs) of all solutions including sample blanks were read and results were expressed as absorbance at 550 nm per mg of sample. All samples were analyzed in duplicates and repeated if the error exceeded 5 %.

3.3.3 Energy Profile of Whole Plant Faba Bean Hay

Energy values, total digestible nutrients (TDN), digestible energy, metabolizable energy, and net energy are commonly used for estimation of available energy in feedstuffs. Truly digestible (td) crude protein (tdCP), non-fiber carbohydrates (tdNFC), neutral detergent fiber (tdNDF), fatty acids (tdFA; default calculation from EE), total digestible nutrients at 1× maintenance (TDN_{1x}), digestible energy at production level of intake (DE_{3x}), metabolizable energy at a production level of intake (ME_{3x}), and net energy at a production level of intake (NE_{L3x}) were determined using a summative approach from the NRC dairy (2001). Net energy for maintenance (NE_m) and net energy for gain (NE_g) were estimated using NRC beef (1996).

3.3.4 Protein and Carbohydrate Subfractions

The crude protein and carbohydrate subtractions were partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS 6.5) as follows: PA1 (ammonia) is calculated using the following formula: PA1 = ammonia × (SP/100) × (CP/100), and its degradation rate (Kd) is 200 %/h. PA2 (soluble true protein) is calculated as PA2 = SP × CP/100 − PA1 and its Kd range is 10-40 %/h. PB1 (insoluble true protein) is calculated with the following formula PB1 = CP − (PA1 − PA2 − PB2 − PC) and its Kd range is 3-20 %/h; PB2 fraction refers to fiber-bound protein and is equal to (NDICP − ADICP) × CP / 100) and its Kd range is 1-18 %/h. Last, the PC (indigestible protein) is calculated as PC = ADICP × CP / 100. The carbohydrate fractions are determined as CB2 (soluble fiber) which was calculated with the following formula: CB2 = NFC − CA1 − CA2− CA3 − CA4 − CB1 and its Kd range is 20-40 %/h. CA (volatile fatty acids) and is equal to CA1 = Acetic + Propionic + (Butyric + Isobutyric). CA2 (lactic acid) and its Kd value is 7 %/h. CA3 refers to other organic acids with Kd value 5 %/h. CA4 (water soluble carbohydrates) and its Kd range is 40-60 %/h. CB1 (starch) has a Kd range of 20-40 %/h. CB3 (digestible fiber) is calculated as CB3 = aNDFom – CC and its Kd range is 1-18 %/h, and CC (indigestible fiber) is calculated as CC =

(aNDFom × (Lignin × aNDFom) × 2.4)/100 or aNDFom × uNDFom (Higgs et al., 2015; Van Amburgh et al., 2015).

3.3.5 Rumen In Situ Incubation

3.3.5.1 Samples

Nine samples of whole plant faba bean hay of Snowdrop variety (3 of 77, 3 of 88, and 3 of 97 days old, respectively) and nine samples of whole plant faba bean hay of SSNS-1 variety (3 of 77, 3 of 88, and 3 of 97 days old, respectively) were ground through a 3 mm screen using the 8 inches Laboratory Mill (Christy & Norris LTD, Ipswich, England) in Department of Animal and Poultry Science, the University of Saskatchewan, Canada.

3.3.5.2 Animals and Diets

Four lactating Holstein cows on their third lactation and producing 32±5 kg of milk per day were used for the present study. Cows were fitted with a rumen cannula (Bar Diamond Inc, Parma, ID, USA) with an internal diameter of 8.8 cm and held at the Rayner Dairy Research and Teaching Facility (University of Saskatchewan, Saskatoon, Canada). The cows were kept in the regular tiestall/parlor and fed total mixed ration (TMR) based on dry matter basis 368 g/kg of barley silage, 173.2 g/kg alfalfa hay, 290 g/kg of lactating pellet, 151.5 g/kg of barley grain, and 17.3 g/kg of palmitic acid before and while the rumen incubation was conducted. The average daily feed intake was 30.36 kg of DM. The cows used for the present study were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 2009) 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.

3.3.5.3 Rumen Incubation Procedure

Rumen degradation characteristics were determined using the *in situ* method described by Yu et al., (2003). In brief: 1). About 7 g DM was weighed in a number-coded nylon bag (10 × 20 cm) with multi-bags for each treatment and each incubation time (2, 2, 2, 2, 3, 3, and 4 bags for incubation times 0, 2, 4, 8, 12, 24, and 48 h, respectively). The pore size of nylon bag was ca. 41 µm. These bags were tied about 2 cm below the top, allowing a ratio of sample size to bag surface area of 39 mg/cm^2. The weight of bag + string and bag + string + sample were recorded. 2). The rumen incubations were performed according to the "gradual addition/all out" schedule (the bags were inserted sequentially and retrieved at the same time). Samples were incubated in the rumen for 48, 24, 12, 8, 4, and 2 h. 3). After incubation, the bags were removed from the rumen and rinsed in a bucket of cold water to remove excess ruminal contents. Then, all the bags (including 0 h) were washed approximately 6 times by hand with cool water without detergent with 10 bags per round. 4). Washed residues were subsequently air-dried at 55°C for 48 h by placing all bags on stainless steel trays in a forced-air drying oven. All dried bags were exposed to lab room conditions (temperature and moisture) for at least 24 h, then weighed bag + string + residue. The *in situ* rumen incubation was performed for two experimental runs using the four fistulated cows.

3.3.5.4 Chemical Analysis of In Situ Samples

The samples of whole plant faba bean hay and their pooled residues of each incubation time point for each treatment were ground through a 1 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) and analyzed for CP using Leco protein/N analyzer, Model FP-528 (Leco Corp., St Joseph, MI, USA). Neutral detergent fiber (NDF) was analyzed using the procedures of Van Soest et al., (1991) combined with Ankom A200 filter bag technique (Ankom Technology, Fairport, NY, USA). Starch (ST) was analyzed using the Megazyme Total Starch Assay Kit (Wicklow, Ireland) and by the α-amylase/amyloglucosidase method (McCleary et al., 1997). All samples were analyzed in duplicates and repeated if the error was more than 5 %.

3.3.5.5 Rumen Degradation Kinetics

Degradation characteristics of DM, CP, NDF, and ST were determined using the first-order kinetics degradation model described by Ørskov and McDonald (1979) and modified by Tamminga et al., (1994). The results were calculated using the nonlinear (NLIN) procedure of SAS 9.4 and iterative least-squares regression (Gausse Newton method):

$$R(t) = U + D \times e^{-Kd \times (t - T0)},$$

where, R(t) = residue present at t h incubation (%); U = undegradable fraction (%); D = potentially degradable fraction (%); Kd = degradation rate (%/h); and T0 = lag time (h).

The bypass (B) or rumen undegradable (RU) values of nutrients on a percentage basis were calculated according to NRC Dairy (2001):

$$\%BDM; BCP \text{ or } BNDF = U + D \times Kp / (Kp + Kd)$$

$$\%BST = 0.1 \times S + D \times Kp / (Kp + Kd),$$

where, S = soluble fraction (%) or washable fraction (NDF); Kp = estimated passage rate from the rumen (%/h) and was assumed to be 6 %/h for DM, CP and ST, but 2.5 %/h for NDF (Tamminga et al., 1994). The factor 0.1 in the formula represented that 100 g/kg of soluble fraction (S) was escaped rumen fermentation.

The rumen undegradable or bypass DM, ST and NDF in g/kg DM were calculated as:

$$BDM\ (BST \text{ or } BNDF)\ (g/kg\ DM) = DM\ (ST \text{ or } NDF)\ (g/kg\ DM) \times \%\ BDM\ (BST \text{ or } BNDF).$$

The rumen undegradable protein (RUP) and rumen bypass protein (BCP) were calculated differently in the Dutch model (Tamminga et al., 1994) and NRC Dairy 2001 model (NRC 2001):

$$BCP^{DVE}\ (g/kg\ DM) = 1.11 \times CP\ (g/kg\ DM) \times RUP\ (\%),$$

$$RUP^{NRC}\ (g/kg\ DM) = CP\ (g/kg\ DM) \times RUP\ (\%),$$

where, 1.11 referred to the regression coefficient between *in situ* RUP and *in vivo* RUP (Tamminga et al., 1994).

The effective degradability (ED), or extent of degradation, of each nutrient was predicted according to NRC as:

$$\%EDDM\ (EDCP,\ EDNDF\ or\ EDST) = S + D \times Kd / (Kp + Kd)$$

$$EDDM\ (CP,\ NDF\ or\ ST) = DM\ (CP,\ NDF\ or\ ST)\ (g/kg\ DM) \times \%EDDM\ (EDCP,\ EDNDF\ or\ EDST)$$

3.3.6 Hourly Effective Rumen Degradation Ratios and Potential N-to-Energy Synchronization

The potential nitrogen to energy synchronization was determined by means of the effective rumen degradation ratios of N and energy/carbohydrate according to Sinclair et al., (1993) using the following equation: hourly ED ratio N/CHOt = 1000 × (HEDNt − HEDNt - 1) / [(HEDNDFt − HEDNDFt - 1) + (HEDSTt − HEDSTt - 1)], where N/CHOt (g N/kg DM) = ratio of N to CHO at time t; HEDNt, HEDNDFt, and HEDSTt (g/kg DM) = hourly effective degradability of nitrogen, neutral detergent fiber, and starch at time t, respectively; HEDNt − 1, HEDNDFt -1, and HEDSTt -1 (g/kg DM) = hourly effective degradability of N, NDF, and ST 1 h before t (Sinclair et al., 1993; Tamminga et al., 1990).

3.3.7 Intestinal Digestion of Rumen Undegraded Protein

A modified three-step *in vitro* procedure described by Calsamiglia and Stern (1995) and Gargallo et al., (2006) was used to determine intestinal digestibility. In summary, dried ground residues containing about 15 mg of N after 12 h ruminal preincubation were placed into a 50 ml centrifuge tube, in which 10 ml of pepsin (Sigma P-7012) solution (in 0.1 N HCl with pH 1.9) was added, then vortexed, and incubated for 1 h at 38 °C in a water bath. After incubation, 0.5 ml (1 N NaOH solution) and 13.5 ml of pancreatin (Sigma P-7545) were added, vortexed, and incubated at 38 °C for 24 h. Tubes were vortexed every 8 h intermittently. Then 3 ml of tri-carboxylic acid

(TCA) was added to stop enzymatic hydrolysis. Then tubes were vortexed and stayed at room temperature for 15 min, next they were centrifuged for 15 min at 10,000 g and the supernatant (5 ml) was analyzed for soluble N by the Kjeldahl method. Intestinal digestion of protein was calculated as TCA-soluble N divided by the amount of N in the rumen residue sample. The intestinal digestion data was used for the prediction of true protein supply to dairy cattle by DVE/OEB and NRC model.

3.3.8 Nutrient Supply with the DVE/OEB System

The Dutch evaluation system (DVE/OEB) predicts the true protein digested in the small intestine (DVE) and the rumen degradable protein balance (OEB). The DVE represents the protein value of a feed and it accounts for the digestible feed protein, microbial protein, and endogenous protein loss correction. Therefore, DVE is calculated as: DVE (g/kg of DM) = DVME + DVBE − ENDP, where DVME = absorbable fraction of microbial crude protein; DVBE = absorbable fraction of ruminally undegraded feed protein; ENDP = correction factor for endogenous protein lost during the digestion process. On the other hand, the OEB value is the difference between the potential microbial protein synthesis (MPS) on the basis of available rumen degradable feed protein (MREN) and that on the basis of available rumen degradable energy extracted from anaerobic fermentation (MREE). Thus, OEB (g/kg of DM) = MREN – MREE, where MREN = CP × [1 − (1.11 × RUP (% CP)/100)] and 1.11 factor represents the regression coefficient of *in vivo*, on *in situ* degradation data; MREE = FOM × 0.15 (FOM in g/kg) (Van Duinkerken et al., 2011; Tamminga et al., 2007, 1994).

3.3.9 Nutrient Supply to Dairy Cows with the NRC-2001 Model

Metabolizable Protein (MP) used in the NRC-2001 model is composed of three major protein sources, including the absorbable microbial protein (AMCP), the truly absorbable rumen undegraded feed protein (ARUP), and the truly absorbable endogenous protein in the small intestine (AECP); therefore, the total MP was calculated as follows: MP (g/kg DM) = AMCP + ARUP + AECP (NRC, 2001). Degraded protein balance (DPB) based on data from the NRC-2001 model is the difference between the potential microbial protein synthesis based on RDP and the potential microbial protein synthesis based on energy available for microbial fermentation in the rumen. Therefore, the DBP was calculated as follows: DPB (g/kg of DM) = $RDP^{NRC} - 1.18 \times MCP_{TDN}$.

3.3.10 Feed Milk Value

Protein metabolic characteristics from the DVE/OEB system and NRC dairy (2001) model were used to determine the Feed Milk Value (FMV). For FMV calculations, a factor of 0.67 was assumed as the efficiency of use of metabolizable protein for lactation (NRC, 2001) and 33 g protein /kg of milk was assumed to be present in the milk.

3.3.11 Statistical Analysis

Results from chemical profiles, energy values, protein and carbohydrate fractions, rumen degradation kinetics, hourly effective degradation ratios, intestinal digestibility of protein, predicted truly absorbed protein supply, and Feed Milk Values were analyzed using the Mixed model procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC, US). CRD and RCBD were used as experimental design with a 2 × 3 factorial treatment arrangement with the first factor related to tannin concentration (low and high) and the second factor including the cutting stage (flower, mid pod, and late pod stages). The model used for the analysis was as follows:

For chemical and nutrient profiles studies: $Y_{ijk} = \mu + F_i + H_j + (F_i \times H_j) + e_{ijk}$,

For in situ study and nutrient supply studies: $Y_{ijkl} = \mu + F_i + H_j + (F_i \times H_j) + R_k + e_{ijkl}$,

where Y_{ijk} or Y_{ijkl} was the observation of the dependent variable ijk or ijkl, μ was the population mean for the variable, F_i the effect of tannin concentration (i= 1,2); H_j the effect of cutting stage (j= 1,2,3), $F_i \times H_j$ the interaction between variables, R_k in situ experimental run, and e_{ijk} or e_{ijkl} the random error associated with observation ijk or ijkl. PROC NLIN-Gauss-Newton method of SAS was used to fit the rumen degradation data to the model. The difference among treatments was evaluated with a multiple comparison analysis using the Tukey method. The CRD and RCBD model assumption checking was carried out using Residual Analysis. The normality test was carried out using SAS Proc univaritate with Normal and Plot options. For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$.

3.4 Results and Discussion

3.4.1 Yield

The yield of fresh whole plant faba bean and the yield of whole plant faba bean hay are presented in Table 3.1. The stage of cutting on the yield of fresh matter (FM) of whole plant faba bean had no significant effect ($P > 0.05$). However, low tannin variety had higher ($P < 0.05$) FM yield (+17.26 t/ha) than the high tannin variety. Additionally, the stage of cutting had a significant ($P < 0.05$) effect on the dry matter (DM) yield of whole plant faba bean hay. The DM yield at late pod stage was higher than the DM yield at flower stage (+5.06 t/ha); however, the DM yield at mid pod stage was not significantly different from either the DM yield at flower or the DM yield at late pod stage. Kugler (2004) stated that as forage yield increases the forage quality decreases.

Compared with data of Bélanger et al., (2001) and Efetha el al. (2009), timothy DM yield is approximately 8 t/ha for the first cut which is similar to the DM yield of whole plant faba bean hay at flower stage, but lower than the DM yield at mid pod and mid pod stages. In the same study, timothy hay yielded 2 t/ha in the second cut which is lower when compared to the DM yield of whole plant faba bean hay at mid pod and mid pod stages. Alfalfa yield and forage quality are inversely related within a growth cycle (Orloff and Putnam 2007). The average yield of alfalfa ranged from 8.3 to 11.6 t/ha (Putnam et al., 2000) which is comparable with the flower and mid

Table 3.1. Effect of variety (tannin concentration) and stage of cutting on yield of whole plant faba bean hay fresh matter and dry matter.

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid Pod	Late Pod	SEM	Variety	Stage	V×S
Yield										
FM (t/ha)	48.64[b]	65.90[a]	7.350	52.37	60.75	58.68	8.006	0.02	0.55	0.36
DM (t/ha)	9.23	11.40	1.203	7.68[b]	10.52[ab]	12.74[a]	1.341	0.10	0.02	0.47
DM%	20.10[a]	17.07[b]	1.01	14.72[b]	17.83[b]	23.19[a]	1.129	0.01	<0.01	0.19
CP (t/ha)	1.95	2.39	0.195	1.72[b]	2.27[ab]	2.53[a]	0.221	0.06	0.03	0.33
aNDF (t/ha)	3.38	4.23	0.497	2.90	3.74	4.77	0.537	0.16	0.06	0.62
St (t/ha)	0.70	0.95	0.087	0.05[c]	0.68[b]	1.74[a]	0.106	0.06	<0.01	0.15

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; [a-c] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison: Tukey method. FM: fresh matter; DM: dry matter; CP: crude protein; aNDF: neutral detergent fiber; St: Starch; con: concentration.

pod stages of whole plant faba bean; however, those values are lower than the yield of whole plant faba bean hay at late pod stage. The yield of aNDF was similar (P> 0.05) among the three stages of cutting. The yield of CP was similar at mid pod and late pod stages, but these values were higher (P< 0.05) than at flower stage (+0.81, + 0.55 t/ha, respectively). The yield of starch was higher (P< 0.05) at late pod stage than mid pod stage (+ 1.06 t/ha) and flower stage (+ 1.69 t/ha). Additionally, Carter (1960) and LeClerc (1939) have suggested that higher DM and CP losses are more likely occur when forage is field dried than artificial dried, while most of the losses are in the form of leaves. Rain damage and losses by mowing are some factors which to account when field dried is used (Rotz, 1995).

3.4.2 Chemical Profile

The chemical profiles of whole plant faba bean hay are presented in Table 3.2. The tannin concentration had no significant (P> 0.05) effect on the contents of DM, Ash, OM, NDICP, ADICP, CHO, starch, sugar, and NFC. However, the stage of cutting affected significantly (P< 0.05) those components. The ash content was higher (P< 0.05) at the flower stage than that at the late pod stage (+2.1 %DM), but the ash content at the mid pod stage was similar to the values found at flower and late pod stages. The NDICP content was higher (P< 0.05) at the late pod stage than the flower stage (+0.4 %DM) and was also higher than the mid pod stage (+0.3 %DM). The NDICP values at flower stage and mid pod stage were similar. ADICP values were also similar at flower stage and mid pod stage and those values were lower (P< 0.05) than the value found at late pod stage (-0.3 and -0.4 %DM, respectively). The CHO content was higher (P< 0.05) at late pod stage than flower stage (+4.6 %DM). The CHO content at mid pod stage was similar to the ones found at flower and late pod stage. The starch content at flower stage was lower (P< 0.05) compared with the ones found at mid pod stage (-6.4 %DM) and late pod stage (-13.5 %DM). Also, at mid pod stage the starch content was lower than the one found at late pod stage (-7.1 %DM). At flower

Table 3.2. Effect of variety (tannin concentration) and stage of cutting on chemical composition of whole plant faba bean hay

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid Pod	Late Pod	SEM	Variety	Stage	V×S
Chemical composition										
DM (%)	93.1	93.3	0.10	93.9^{a}	93.0^{b}	92.8^{b}	0.11	0.11	<0.01	0.73
Ash (%DM)	8.7	9.6	0.37	10.3^{a}	8.9^{ab}	8.2^{b}	0.42	0.05	0.01	0.47
OM (%DM)	91.3	90.4	0.37	89.7^{b}	91.1^{ab}	91.8^{a}	0.42	0.05	0.01	0.47
EE (%DM)	1.4	1.4	0.07	1.5	1.4	1.3	0.09	0.55	0.11	0.58
FA (%DM)	0.4	0.4	0.07	0.5	0.4	0.3	0.08	0.51	0.11	0.55
Protein profile										
CP (%DM)	22.0	21.3	0.89	22.7	22.0	20.3	1.01	0.50	0.16	0.94
NDICP (%DM)	1.1	1.1	0.12	0.9^{b}	1.0^{b}	1.3^{a}	0.13	0.71	0.04	0.95
ADICP (% DM)	0.9	1.0	0.10	0.9^{b}	0.8^{b}	1.2^{a}	0.11	0.84	0.03	0.72
SCP (%DM)	13.6^{b}	15.2^{a}	0.66	15.5	14.5	13.3	0.74	0.04	0.07	0.70
NDICP (%CP)	5.1	5.0	0.68	4.1^{b}	4.5^{ab}	6.7^{a}	0.77	0.88	0.03	0.98
ADICP (% CP)	4.4	4.6	0.64	3.8^{b}	3.8^{b}	5.8^{a}	0.70	0.76	0.03	0.78
SCP (% CP)	61.9^{b}	71.2^{a}	1.32	68.6	66.0	65.1	1.62	<0.01	0.32	0.60
Carbohydrate profile										
CHO (%DM)	67.9	67.7	0.73	65.6^{b}	67.7^{ab}	70.2^{a}	0.87	0.84	0.01	0.62
Starch (%DM)	7.6	7.0	1.15	0.7^{c}	7.1^{b}	14.2^{a}	1.24	0.51	<0.01	0.85
Starch (% NFC)	21.8	21.2	3.26	2.4^{c}	20.9^{b}	41.1^{a}	3.41	0.76	<0.01	0.98
Sugar (%DM)	8.3	6.1	1.69	11.6^{a}	5.6^{ab}	4.5^{b}	1.99	0.31	0.04	0.26
Sugar (%NFC)	27.2	19.6	6.26	40.5	16.8	13.0	7.55	0.39	0.05	0.32
aNDF (%DM)	35.7	37.1	1.45	37.2	35.0	37.0	1.77	0.52	0.64	0.96
NDFn (%DM)	34.6	36.0	1.42	36.3	34.0	35.7	1.74	0.50	0.66	0.96
aNDFom (%DM)	35.3	36.6	1.45	36.8	34.6	36.5	1.78	0.53	0.66	0.95
NDF (%OM)	39.1	41.0	1.63	41.5	38.5	40.3	1.99	0.43	0.58	0.93
ADF (%DM)	28.8	30.1	1.74	30.8	29.1	28.5	1.99	0.54	0.60	0.57
ADL (%DM)	5.8	5.3	0.48	5.8	5.3	5.5	0.55	0.36	0.74	0.91
ADF (%NDF)	80.6	81.3	2.85	82.8	83.3	76.8	3.17	0.81	0.16	0.09
ADL (%NDF)	16.2	14.2	0.95	15.7	15.1	14.8	1.11	0.12	0.83	0.77

Table 3.2. ***Cont'd*** Effect of variety (tannin concentration) and stage of cutting on chemical composition of whole plant faba bean hay

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid Pod	Late Pod	SEM	Variety	Stage	V×S
Hemicellulose (%DM)	6.9	7.0	1.04	6.4	6.0	8.5	1.18	0.90	0.19	0.12
Cellulose (%DM)	23.0	24.8	1.30	25.0	23.8	22.9	1.50	0.27	0.54	0.49
NSC (%DM)	16.0	13.1	2.01	12.3	12.7	18.8	2.31	0.24	0.08	0.36
NFC (%DM)	33.3	31.7	0.96	29.3[b]	33.7[ab]	34.5[a]	1.17	0.26	0.02	0.56
NFC (%CHO)	49.1	46.8	1.74	44.9	49.7	49.2	2.13	0.36	0.24	0.82
Condensed tannins										
CT (abs nm / mg)	0.070a	0.048b	0.0027	0.063	0.061	0.053	0.0033	<0.0001	0.15	0.99
CT (% DM)	0.031a	0.012b	0.0023	0.024	0.023	0.017	0.0028	<0.0001	0.34	0.91
CT (g/kg DM)	0.310a	0.123b	0.0231	0.245	0.235	0.170	0.0282	<0.0001	0.15	0.99
CT (mg/kg DM)	310.49a	123.20b	23.101	245.28	235.62	169.62	28.293	<0.0001	0.16	1.00

H: high tannin variety; L: low tannin variety; 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: Tukey method; DM: dry matter; EE: ether extracts (crude fat); FA: EE - 1; CP: crude protein; OM: organic matter; SCP: soluble crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; aNDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: non-fiber carbohydrate; CHO: carbohydrate; NFC: non-fiber carbohydrate; NSC: non-structural carbohydrates; condensed tannins analysis: HCl Butanol method (Porter et al., 1986); CT: condensed tannins; abs: units of absorbance at 550 nm; con: concentration.

stage, the sugar content was higher ($P< 0.05$) than the value found at late pod stage (+7.1 %DM), and those values were not significantly different than that at mid pod stage. The NFC content was higher ($P< 0.05$) at the late pod stage than that at flower stage (+5.2 %DM); however, those values were similar to the NFC value found at mid pod stage. The tannin concentration had a significant effect on the SCP content, and the low tannin varieties showed a higher ($P< 0.05$) SCP content than the high tannin variety (+9.3 %DM).

Mature alfalfa hay is considered below average in quality which has 13 to 15 %CP, mid-bloom is considered average quality (16 to 17 %CP), and early-bloom and immature alfalfa are classified as high quality (18 % or higher) (Jennings, 2014). The CP of the high and low tannin whole plant faba bean hay at flower, mid pod, and late pod stages ranged from 20 to 22 %, therefore these values are comparable to the immature alfalfa. Jennings, (2014) observed that the NDF content of alfalfa ranged from 42 to 55 % at different maturity, those values are higher than the NDF content found in whole plant faba bean hay at flower, mid pod, and late pod stages. Timothy tends to be 2 % units lower in CP than other perennial grasses at similar maturity stages (Cherney and Chemey, 2002). Vinet et al., (1980) found that late cut timothy hay has about 5 and 42 % of CP and ADF content, respectively. At early cut stage, CP and ADF content in timothy hay is about 11 and 35 %, respectively. Therefore, the CP content of whole plant faba bean hay at flower, mid pod, and late pod stages was higher and ADF lower than timothy hay at both late and early cut stages. Condensed tannin concentration exceeding 50 g/kg of dry matter reduce growth and milk production; however, concentration from 10 to 40 g/kg of dry matter reduce the feed protein degradation in the rumen, therefore increasing the rumen bypass protein which could be absorbed in the small intestine (Addisu, 2016; Cannas, 2015). In our study, high tannin variety had higher concentration ($P< 0.05$) of condensed tannins than the low tannin varieties (+ 0.188 g/kg DM). However, these values may not have any positive or negative effect on animal performance.

3.4.3 Energy Profile

The energy profiles of whole plant faba bean hay are presented in Table 3.3. The level of truly digestible nutrients, total digestible nutrients, and energy values were not significantly ($P > 0.05$) affected by neither the variety nor the cutting stage except tdNFC which was affected by stage of cutting ($P < 0.05$). The total digestible nutrients (TDN) in alfalfa is reduced with maturity.

At early bloom it has 62 %TDN, mid bloom 60 %TDN, full bloom 57 %TDN, and 55 %TDN when mature (Jennings 2014). The TDN_{3x} whole plant faba bean hay at flower, mid pod, and late pod stages is comparable with the TDN value found with alfalfa hay at mid bloom stage. Timothy hay at early bloom has a TDN of 59 % and timothy hay at full bloom has a TDN of 57 %, which are comparable to the TDN_{3x} of whole plant faba bean hay at flower, mid pod, and late pod stages (Stanton, 2014).

3.4.4 Protein and Carbohydrate Subfractions

Crude protein and carbohydrate fractions were partitioned using the Cornell Net Carbohydrate and Protein System (CNCPS 6.5). Protein fractions include PA1, PA2, PB1, PB2, and PC; and carbohydrate fractions CA4, CB1, CB2, CB3, and CC. Each of them has different fermentation patterns and degradation rates. The values of protein and carbohydrate fractions of whole plant faba bean hay are presented in Table 3.4. The content of rapidly degradable true protein (PA2) and moderately degradable true protein (PB1) were significantly ($P < 0.05$) affected by the tannin concentration effect. The PA2 was lower and PB1 was higher in the high tannin variety than the low tannin variety. (-9.3 and +9.2 %CP, respectively, $P < 0.05$). Ammonia (PA1) and indigestible protein (PC) values were not significantly ($P > 0.05$) affected by the tannin concentration; however, the cutting stage effect affected significantly ($P < 0.05$) those values, PA1 was higher ($P < 0.05$) at the flower stage than the value found at late pod stage; however, those values were similar to the one found at mid pod stage.

Table 3.3. Effect of variety (tannin concentration) and stage of cutting on energy profile of whole plant faba bean hay

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid Pod	Late Pod	SEM	Variety	Stage	V×S
Truly digestible nutrient (%DM)										
tdNFC	32.6	31.1	0.94	28.7[b]	33.0[ab]	33.8[a]	1.15	0.26	0.02	0.56
tdCP	21.6	21.0	0.92	22.3	21.7	19.9	1.04	0.50	0.15	0.95
tdNDF	15.1	16.7	0.72	16.2	15.4	16.2	0.88	0.14	0.78	0.83
tdFA	0.4	0.4	0.07	0.5	0.4	0.3	0.08	0.51	0.11	0.55
Total digestible nutrient (%DM)										
TDN_{1x}	63.3	62.5	1.22	61.3	64.0	63.4	1.44	0.63	0.37	0.97
TDN_{3x}	58.1	57.4	1.12	56.3	58.7	58.3	1.33	0.63	0.37	0.97
Energy value (Mcal/kg)										
DE_{1x}	2.95	2.91	0.063	2.88	2.98	2.94	0.074	0.62	0.59	0.98
DE_{p3x}	2.71	2.67	0.059	2.65	2.74	2.70	0.069	0.59	0.60	0.99
ME_{p3x}	2.29	2.25	0.059	2.22	2.32	2.27	0.069	0.60	0.58	0.99
NE_{Lp3x}	1.42	1.39	0.041	1.37	1.44	1.41	0.048	0.55	0.59	0.98
ME	2.42	2.39	0.052	2.36	2.45	2.41	0.061	0.58	0.56	0.98
NE_m	1.54	1.51	0.046	1.48	1.56	1.53	0.054	0.59	0.57	0.99
NE_g	0.94	0.91	0.041	0.89	0.96	0.93	0.048	0.60	0.55	0.99

H: high tannin variety; L: low tannin variety; 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: Tukey method; tdCP: truly digestible crude protein; tdFA: truly digestible fatty acid; tdNDF: truly digestible neutral detergent fibre; tdNFC, truly digestible non-fibre carbohydrate. $TDN_{1\times}$: total digestible nutrient at one times maintenance. $DE_{3\times}$: digestible energy at production level of intake (3×); $ME_{3\times}$: metabolizable energy at production level of intake (3×); $NE_{L3\times}$: net energy for lactation at production level of intake (3×); NE_m: net energy for maintenance; NE_g: net energy for gain; con: concentration.

The PC content was higher ($P < 0.05$) at the late pod stage than the values found at flower and mid pod stage (+2.0 % and +2.0 %CP). Data found in the CNCPS feed library showed that alfalfa hay with 37 %NDF and 20 %CP has 31.9 %CP (PA2), 54.1 %CP (PB1), and 7.2 %CP (PB2) while timothy hay with 55 %NDF and 14 %CP has values of 31.6 %CP (PA2), 37.1 %CP (PB1), and 22.8 %CP (PB2). Compared to these CNCPS values, the PA2 fraction in both high and low tannin whole plant faba bean hay were higher while PB1 and PB2 fractions were lower in both varieties of whole plant faba bean hay. The tannin concentration did not affect significantly ($P > 0.05$) the carbohydrate fractions, but the cutting stage affected significantly ($P < 0.05$) all the carbohydrate fractions except the intermediately degradable carbohydrate fraction (CB2). The rapidly degradable carbohydrate fraction (CA4) was higher ($P < 0.05$) at the flower stage than the late pod stage (+11 %CHO), the CA4 value at mid pod stage was similar to the ones found at flower and late pod stages. The intermediately degradable carbohydrate fraction (CB1) was higher ($P < 0.05$) at late pod stage than the values found at flower (+19.2 %CHO) and mid pod stages (+9.8 %CHO). Additionally, the CB1 value at mid pod stage was higher ($P < 0.05$) than the value found at flower stage (+9.4 %CHO). The slowly degradable carbohydrate fraction (CB3) was lower ($P < 0.05$) at flower stage than the value found at late pod stage (-6.7 %CHO), but the CB3 fraction at mid pod stage was similar to the CB3 fractions at flower and late pod stage. The unavailable neutral detergent fiber (CC) was similar at the mid pod stage and late pod stage; however, those values were lower ($P < 0.05$) than the value found at the flower stage (-7.2 and -11 %CHO, respectively).

The carbohydrate fractions available in the CNCPS library for alfalfa hay (37 %NDF, 20 %CP) are CA4, 8.7 %CHO; CB1, 0.6 %CHO; CB2, 30.4 %CHO; CB3, 31.8 %CHO; CC, 24.2 %CHO and for timothy hay (55 %NDF; 14 %CP) are CA4, 13.5 %CHO; CB1, 2.9 %CHO; CB2, 11.6 %CHO; CB3, 57.1 %CHO; CC, 14.8 %CHO. In whole plant faba bean hay at flower stage, the CA4 value was higher than the ones found in alfalfa and timothy hay; however, at mid pod and

Table 3.4. Effect of variety (tannin concentration) and stage of cutting on CNCPS fractions of whole plant faba bean hay

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid Pod	Late Pod	SEM	Variety	Stage	V×S
Protein fractions										
PA1 (%CP)	0.1	0.1	0.01	0.1^{a}	0.1^{ab}	0.1^{b}	0.01	0.62	0.04	0.48
PA2 (%CP)	61.9^{b}	71.2^{a}	1.32	68.6	66.0	65.1	1.62	<0.01	0.32	0.60
PB1 (%CP)	32.9^{a}	23.7^{b}	1.21	27.2	29.5	28.2	1.48	<0.01	0.58	0.50
PB2 (%CP)	0.7	0.4	0.21	0.3	0.6	0.9	0.24	0.23	0.20	0.37
PC (%CP)	4.4	4.6	0.64	3.8^{b}	3.8^{b}	5.8^{a}	0.70	0.76	0.03	0.78
True Protein (%CP)	95.5	95.3	0.64	96.1^{a}	96.1^{a}	94.1^{b}	0.71	0.75	0.03	0.78
PA2 (%true protein)	64.9^{b}	74.6^{a}	1.25	71.4	68.7	69.1	1.54	<0.01	0.45	0.47
PB1 (%true protein)	34.4^{a}	24.9^{b}	1.28	28.3	30.6	30.0	1.56	<0.01	0.57	0.53
PB2 (%true protein)	0.8	0.5	0.22	0.3	0.6	0.9	0.25	0.23	0.18	0.36
Carbohydrate fractions										
CHO (%DM)	67.9	67.7	0.73	65.6^{b}	67.7^{ab}	70.2^{a}	0.87	0.84	0.01	0.62
CA4 (%CHO)	12.4	9.1	2.45	17.5^{a}	8.3^{ab}	6.5^{b}	2.86	0.29	0.03	0.23
CB1 (%CHO)	11.1	10.2	1.72	1.1^{c}	10.5^{b}	20.3^{a}	1.86	0.51	<0.01	0.89
CB2 (%CHO)	25.6	27.5	3.16	26.3	31.0	22.5	4.30	0.68	0.33	0.48
CB3 (%CHO)	32.3	35.0	1.27	30.6^{b}	33.0^{ab}	37.3^{a}	1.46	0.09	0.01	0.67
CC (%CHO)	18.6	18.2	1.52	24.5^{a}	17.3^{b}	13.5^{b}	1.86	0.86	<0.01	0.62

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a\text{-}b}$ Means with the different letters in the same row are significantly different ($P< 0.05$); Multi-treatment comparison: Tukey method; PA1: ammonia; PA2: soluble true protein (rapidly degradable true protein); PB1: insoluble true protein (moderately degradable true protein); PB2: fiber-bound protein (slowly degradable true protein); PC: indigestible protein; CHO: carbohydrate; CA4: water soluble carbohydrates (rapidly degradable carbohydrate fraction); CB1: starch (intermediately degradable carbohydrate fraction); CB2: soluble fiber (intermediately degradable carbohydrate fraction); CB3: digestible fiber (available neutral detergent fiber or slowly degradable carbohydrate fraction); CC: indigestible fiber (unavailable neutral detergent fiber); con: concentration.

late pod stages however, at mid pod and late pod stages CA4 value of the whole plant faba bean hay was lower than alfalfa hay and timothy hay. The CB1 value of alfalfa and timothy hay are comparable with the whole plant faba bean hay at flower stage, but at mid pod and late pod stages whole plant faba bean hay possess a higher CB1 value. The CB2 fraction of timothy hay is lower than the value found in alfalfa hay and the ones found in whole plant faba bean hay at flower, mid pod, and late pod stages. The CB3 fraction of alfalfa hay is comparable to the CB3 fraction of whole plant faba bean hay at flower and mid pod stages; however, it is lower than the CB3 fraction at late pod stage. The CB3 fraction of timothy hay is higher than the fraction found in alfalfa hay and whole plant faba bean hay at the three cutting stages. The CC fraction of the whole plant faba bean hay at flower stage is comparable with the CC fraction of alfalfa hay and those values are higher than the ones found in timothy hay and whole plant faba bean hay at mid pod and late pod stages.

3.4.5 In Situ DM Degradation Kinetics

The rate of degradation (Kd), rumen fractions (S, D, U), rumen undegradable dry matter (BDM), and effective degradability of DM (EDDM) of the whole plant faba bean hay are presented in Table 3.5. Detailed observation of the data revealed that all the characteristics were not significantly affected by the tannin concentration ($P > 0.05$). However, the cutting stage affected significantly ($P < 0.05$) all the characteristics except the undegradable fraction (U). The rate of degradation (Kd) was higher ($P < 0.05$) at flower stage than the value found at late pod stage (+4.12 %/h), and the Kd value at mid pod stage was similar to the ones found at flower and late pod stages. The soluble fraction (S) was lower ($P < 0.05$) at late pod stage than the values found at flower and mid pod stages (-8.1 and -3.7 %, respectively). In addition, the S fraction at mid pod stage was lower than the one found at flower stage (-4.4 %). However, the degradable fraction D showed an opposite outcome as it was higher at late pod stage than at flower and mid pod stages (49.8 vs. 39.6

Table 3.5. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics of dry matter (DM) of whole plant faba bean hay

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid Pod	Late Pod	SEM	Variety	Stage	V×S
Dry matter										
Kd (%/h)	10.63	11.26	0.585	12.80[a]	11.36[ab]	8.68[c]	0.716	0.46	0.01	0.63
Residue at 0 h (%)	65.8	64.8	0.73	61.1[c]	65.5[b]	69.2[a]	0.89	0.35	<0.01	0.59
S (%)	34.2	35.2	0.73	38.9[a]	34.5[b]	30.8[c]	0.89	0.35	<0.01	0.59
D (%)	44.9	45.0	0.98	39.6[c]	45.3[b]	49.8[a]	1.10	0.93	<0.01	0.88
U (%)	20.9	19.8	0.76	21.5	20.1	19.4	0.93	0.32	0.31	0.63
%BDM=%RUDM	37.4	36.0	0.80	34.3[b]	35.9[b]	39.9[a]	0.98	0.23	<0.01	0.53
%EDDM	62.6	64.0	0.80	65.7[a]	64.1[a]	60.1[b]	0.98	0.23	<0.01	0.53

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; [a-c] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison: Tukey method; Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the *in situ* incubation; D: degradable fraction; U: rumen undegradable fraction; BDM or RUDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter; con: concentration.

and 45.3 %, respectively). The rumen undegradable dry matter (RUDM) at flower and mid pod stages were similar, but those values were lower ($P < 0.05$) than the one found at late pod stage (343 and 359 vs. 399 g/kg DM, respectively). The effective degradable dry matter (EDDM) were similar at flower and mid pod stages and higher than the value found at late pod stage (657 and 641 vs. 601 g/kg DM, respectively).

Alfalfa has a higher soluble fraction (41 vs. 19 %), lower degradable fraction (37 vs. 51 %), lower undegradable fraction (20 vs. 29 %), higher Kd (10 vs. 3 %/h), and higher EDDM (756 vs. 649 g/kg DM) than timothy (Yu et al., 2004). The soluble fraction of alfalfa is comparable to the soluble fraction of dry matter of whole plant faba bean hay at flower stage, and those values and the correspondent values at mid pod and late pod stages were higher than the soluble fraction of dry matter of timothy. On the other hand, the degradable fraction of timothy hay is similar to the degradable fraction found at the late pod stage in whole plant faba bean hay while the degradable fraction at flower stage was comparable to the degradable fraction of alfalfa. The undegradable fraction is higher in timothy than the ones found at flower, mid pod, and late pod stages in whole plant faba bean hay. The Kd value of alfalfa is similar to the Kd value of the whole plant faba bean hay at three stages of cutting and those values were higher than the Kd values found in timothy. The EDDM is higher in alfalfa than timothy hay and whole plant faba bean hay, and the lower EDDM is found in the whole plant faba bean hay at late pod stage.

3.4.6 In Situ Crude Protein Degradation Kinetics

In this study, characteristics of rumen degradation kinetics of crude protein of whole plant faba bean hay are presented in Table 3.6. The tannin concentration affected significantly ($P < 0.05$) the rate of degradation of crude protein (Kd), showing that low tannin variety had higher Kd value than the high tannin variety (21.14 vs. 14.93 %/h). Also, the tannin concentration affected

Table 3.6. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics (crude protein) of whole plant faba bean hay

Item	Variety (V, Tannin con.)		SEM	Stage of Cutting (S)			SEM	P value		
	H	L	SEM	Flower	Mid Pod	Late Pod	SEM	Variety	Stage	V×S
Crude Protein										
CP (g/kg DM)	220	213	8.9	227	220	203	10.1	0.50	0.16	0.94
Kd (%/h)	14.93^{b}	21.14^{a}	1.699	23.45^{a}	18.73^{a}	11.92^{b}	1.958	0.01	<0.01	0.09
Residue (0 h, %)	43.5	41.7	1.68	36.6^{b}	44.7^{a}	46.5^{a}	2.04	0.46	0.01	0.97
S (%)	56.5	58.3	1.68	63.4^{a}	55.3^{b}	53.5^{b}	2.04	0.46	0.01	0.97
D (%)	37.0	34.7	1.48	28.6^{b}	38.5^{a}	40.4^{a}	1.81	0.31	<0.01	0.92
U (%)	6.5	7.0	0.71	8.0^{a}	6.2^{ab}	6.1^{b}	0.76	0.44	0.03	0.69
%BCP=%RUP	17.6	15.7	1.12	14.3^{b}	16.0^{b}	19.7^{a}	1.24	0.11	<0.01	0.82
BCP (g/kg DM, DVE)	42^{a}	37^{b}	2.3	36^{b}	39^{ab}	44^{a}	2.6	0.03	0.02	0.68
RUP (g/kg DM, NRC)	38^{a}	33^{b}	2.1	32^{b}	35^{ab}	40^{a}	2.3	0.03	0.02	0.68
%EDCP=%RDP	82.4	84.3	1.12	85.7^{a}	84.0^{a}	80.3^{b}	1.24	0.11	<0.01	0.82
EDCP=RDP (g/kg DM)	182	180	9.1	194	185	163	10.2	0.85	0.05	0.97

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a\text{-}b}$ Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison: Tukey method; Kd: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; S: soluble fraction in the *in situ* incubation; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded crude protein; con: concentration.

significantly both the bypass crude protein (BCP^{DVE}) and the rumen undegradable crude protein (RUP^{NRC}). The high tannin variety showed higher ($P< 0.05$) BCP and RUP than the low tannin variety (42 and 38 vs. 37 and 33 g/kg DM). The cutting stage affected significantly all the characteristics except the effectively degraded of crude protein (EDCP g/kg DM). The rate of degradation of crude protein (Kd) at flower and mid pod stages were similar (23.45 and 18.73 %/h), but those values were higher ($P< 0.05$) than the one found at late pod stage (+11.53 and +6.81 %/h, respectively). The soluble fraction of crude protein was similar at mid pod stage and late pod stage, but those values were lower ($P< 0.05$) than the one found at flower stage (55.3 and 53.5 vs. 63.4 %). The degradable fraction was lower at flower stage than the values found at mid pod and late pod stages, which were similar (-9.9 and -11.8 %, respectively). The undegradable fraction of crude protein (U) was higher ($P< 0.05$) at flower stage than at late pod stage (8.0 vs. 6.1 %), but the U fraction at mid pod stages was similar to the ones at flower and late pod stages. The BCP^{DVE} and RUP^{NRC} are higher ($P< 0.05$) at late pod stage than at flower stage (44 and 40 vs. 36 and 32 g/kg DM), the BCP^{DVE} and RUP^{NRC} values at mid pod stage were similar to the ones at flower and late pod stages.

Alfalfa has higher soluble fraction (44 vs. 23 %), lower undegradable fraction (9 vs. 35 %), higher Kd (13 vs. 8 %/h), and higher EDCP (797 vs. 505 g/kg DM) than timothy (Yu et al., 2004). Whole plant faba bean hay had higher soluble fraction, lower undegradable fraction, higher Kd, and similar EDCP than the alfalfa used in the study of Yu et al., (2004)

3.4.7 In Situ NDF Degradation Kinetics

The results of NDF rumen degradation characteristics and uNDF at 288h incubation are presented in Table 3.7. The tannin concentration did not affect significantly any of the rumen degradation characteristics of NDF; however, the cutting stage affected significantly ($P< 0.05$) the rate of degradation of NDF (Kd), which was higher ($P< 0.05$) at flower stage than the ones at mid

Table 3.7. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics (NDF) of whole plant faba bean hay

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid Pod	Late Pod	SEM	Variety	Stage	V×S
Fiber (NDF) Degradation										
NDF (g/kg DM)	357	371	14.5	372	370	350	17.7	0.52	0.64	0.96
Kd (%/h)	5.56	4.67	0.530	7.83^{a}	4.31^{b}	3.21^{b}	0.649	0.26	<0.01	0.84
Residue (0h, %)	82.5	79.5	2.72	82.8	82.9	77.3	3.33	0.46	0.42	0.36
S (%)	17.5	20.5	2.72	17.2	17.1	22.7	3.33	0.46	0.42	0.36
D (%)	45.0	49.8	4.84	42.5	47.9	51.8	5.47	0.36	0.36	0.57
U (%)	37.5	29.7	3.12	40.4^{a}	35.0^{ab}	25.5^{b}	3.81	0.10	0.05	0.78
%BNDF=%RUNDF	62.2	60.4	2.27	58.9	63.4	61.5	2.72	0.55	0.49	0.42
RUNDF (g/kg DM, NRC)	221	222	8.4	218	220	227	9.9	0.92	0.80	0.68
%EDNDF=%RDNDF	37.8	39.6	2.27	41.1	36.6	38.5	2.72	0.55	0.49	0.42
EDNDF=RDNDF (g/kg DM)	136	149	11.6	154	130	143	14.2	0.46	0.52	0.67
uNDF (288 h, CNCPS 6.5) (% DM)	12.59	12.26	1.069	16.17^{a}	11.67^{ab}	9.44^{b}	1.309	0.83	0.01	0.69

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a\text{-}b}$ Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method; Kd: the degradation rate of D fraction; S: washable fraction; D: degradable fraction; U: rumen undegradable fraction; BDNDF or RUNDF: rumen bypass or undegraded feed neutral detergent fiber; EDNDF or RDNDF: effective degraded neutral detergent fiber. uNDF: undigestible neutral detergent fiber; con: concentration.

pod and late pod stages, which on the other hand were similar (7.83 vs. 4.31 and 3.21 %/h, respectively). The undegradable fraction of NDF (U) was higher ($P < 0.05$) at flower stage than at late pod stage (+14.9 %), but the U fraction at mid pod stage was similar to the ones at flower and late pod stages. The undigestible neutral detergent fiber (uNDF288h) was lower ($P < 0.05$) at late pod stage than the one at flower stage (9.44 vs. 16.17 % DM). Furthermore, the uNDF288h at mid pod stage was similar to the ones at flower and late pod stages.

Alfalfa has similar degradable (70 %) and undegradable (29 %) fractions, higher Kd (6.45 vs. 3.51 %/h), and lower EDNDF (337 vs. 444 g/kg DM) than timothy (Yu et al., 2004). Whole plant faba bean hay had lower degradable fraction and comparable undegradable fraction at late pod stage with results published by Yu et al., (2004). The undegradable fraction of whole plant faba bean hay at flower and mid pod stages was higher than the one found by Yu et al., (2004) in alfalfa. The Kd value of alfalfa is similar to the Kd value of whole plant faba bean hay at flower stage, while the Kd value of timothy found by Yu et al. (2004) is comparable to the whole plant faba bean hay at mid pod and late pod stages. The EDNDF at three stages were lower than the ones found by Yu et al., (2004) in alfalfa and timothy. Effective degradability of NDF decreased with increasing stage of maturity for alfalfa and timothy, however in our study EDNDF was no affected by the stage of cutting. According to CNCPS feed library, the uNDF of alfalfa (37 %NDF, 20 %CP) is 15.83 %DM, and this value is comparable to the uNDF288h value of whole plant faba bean hay at flower stage, but higher than the value found at mid pod and late pod stages.

3.4.8 In Situ Starch Degradation Kinetics

The results of starch rumen degradation characteristics are presented in Table 3.8. The tannin concentration did not affect significantly any of the rumen degradation characteristics of starch. The cutting stage affected significantly all those characteristics. The rate of degradation of starch (Kd) was higher ($P < 0.05$) at mid pod stage than at flower stage (16.96 vs. 9.58 %/h). The value

Table 3.8. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics (Starch) of whole plant faba bean hay

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid Pod	Late Pod	SEM	Variety	Stage	V×S
Starch										
St (g/kg DM)	76	70	11.5	7c	71^{b}	142^{a}	12.3	0.51	<0.01	0.85
Kd (%/h)	12.50	15.46	1.478	9.58^{b}	16.96^{a}	15.40^{ab}	1.810	0.18	0.03	0.60
Residue (0 h, %)	51.5	63.3	6.11	78.2^{a}	45.8^{b}	48.2^{b}	7.09	0.13	0.01	0.46
S (%)	48.5	36.7	6.11	21.8^{b}	54.2^{a}	51.8^{a}	7.09	0.13	0.01	0.46
D (%)	48.3	59.8	5.42	68.8^{a}	45.2^{b}	48.1^{ab}	6.46	0.13	0.04	0.47
U (%)	3.2	3.5	1.121	9.4^{a}	0.6^{b}	0.1^{b}	1.28	0.83	<0.01	0.93
%BSt	20.2	22.4	2.54	37.0^{a}	12.7^{b}	14.2^{b}	3.11	0.55	<0.01	0.32
BSt (g/kg DM)	13	11	2.7	3^{b}	9^{b}	23^{a}	3.1	0.47	<0.01	0.64
%EDSt	79.8	77.6	2.54	63.0^{b}	87.3^{a}	85.8^{a}	3.11	0.55	<0.01	0.32
EDST (g/kg DM)	65	61	9.5	5^{c}	62^{b}	121^{a}	10.3	0.62	<0.01	0.71

H: high tannin variety; L: low tannin variety; 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: Tukey method; Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction; D: degradable fraction; U: rumen undegradable fraction; BSt: rumen bypass or undegraded feed starch; EDST: effective degraded starch; con: concentration.

at late pod stage was not significantly different from the ones at flower and mid pod stages. The soluble fraction of starch (S) was similar at mid and late pod stages and those values were higher (P< 0.05) than the one at flower stage (54.2 and 51.8 vs. 21.8 %). The degradable fraction (D) was lower at mid pod stage than at flower stage (-23.6%), and the D fraction at late pod stage was similar to the D fractions at flower and mid pod stages. The undegradable fraction (U) was similar at mid pod and late pod stages; however, those values were lower (P< 0.05) than the one at flower stage (-8.8 and -9.3 %, respectively). The bypass starch (BSt) was higher (P< 0.05) at late pod stage than the ones at flower and mid pod stages which were similar (23 vs. 3 and 9 g/kg DM). The effective degraded starch (EDST) was lower (P< 0.05) at flower stage than the mid pod stage (-57 g/kg DM) and EDST at mid pod stage was lower (P< 0.05) than the one found at late pod stage (-59 g/kg DM). The whole plant faba bean hay at late pod stage had higher yield of starch, therefore had a higher bypass starch and higher EDST than the correspondent values found at flower and mid pod stages. Legume starches have higher amylose content than cereal starches (Punia et al., 2019) which may affect the effective degradation of starch.

3.4.9 Hourly Effective Degradation Ratios between Available N and Available CHO

The effect of tannin concentration and stage of cutting of whole plant faba bean hay on the hourly effective degradation ratios between available nitrogen (N) and available carbohydrates (ED ratio of N/CHO) at different incubation times are shown in Table 3.9. The effect of tannin concentration on the hourly effective degradation of whole plant faba bean hay is shown in Figure 3.1 while the effect of stage of cutting is presented in Figure 3.2.

Detailed observation of the data revealed that tannin concentration had no significant effect (P> 0.05) on the ratio of N/CHO, neither on the overall ratio of ED_N/ED_CHO. The effect of tannin concentration on the ratio at individual hours was not significant from h0 to h18; however, high tannin whole plant faba bean hay had higher (P< 0.05) ratios of ED_N/ED_CHO at h20 (21 vs. 12

Table 3.9. Effect of variety (tannin concentration) and stage of cutting on potentially available N to available CHO synchronization of whole plant faba bean hay

Item	Variety (V, Tannin con.)		SEM	Stage of Cutting (S)			SEM	P value		
	H	L		Flower	Mid pod	Late Pod		Variety	Stage	V×S
Ratio of N/CHO (g/kg)	84	79	5.6	98^{a}	84^{ab}	64^{b}	6.8	0.55	0.01	0.93
Ratio of ED_N/ED_CHO (g/kg)	135	125	10.6	171^{a}	131^{ab}	87^{b}	13.0	0.54	<0.01	0.94
Ratio at individual h (g/kg)										
h0	243	220	32.3	373^{a}	208^{b}	114^{b}	39.6	0.62	<0.01	0.79
h1	150	170	15.8	172^{a}	202^{ab}	106^{b}	19.3	0.37	0.01	0.23
h2	137	146	13.8	141^{a}	180^{ab}	103^{b}	16.9	0.65	0.02	0.47
h3	124	126	12.5	116^{a}	160^{ab}	99^{b}	15.3	0.95	0.04	0.73
h4	113	109	11.5	96	141	96	14.1	0.78	0.07	0.89
h6	94	82	10.1	68	109	87	12.3	0.43	0.10	0.91
h8	77	62	8.8	49	83	77	10.8	0.25	0.09	0.80
h10	63	47	7.6	36	62	67	9.4	0.17	0.08	0.71
h12	51	36	6.4	27^{b}	47^{ab}	57^{a}	7.8	0.12	0.05	0.63
h14	41	27	5.3	20^{b}	34^{ab}	47^{a}	6.5	0.09	0.04	0.61
h16	33	20	4.3	16^{b}	25^{ab}	39^{a}	5.3	0.06	0.03	0.56
h18	26	15	3.6	12^{b}	19^{ab}	32^{a}	4.4	0.05	0.02	0.57
h20	21^{a}	12^{b}	2.9	10^{b}	14^{ab}	26^{a}	3.6	0.04	0.02	0.50
h22	17^{a}	9^{b}	2.4	8^{b}	10^{ab}	21^{a}	3.0	0.04	0.02	0.49
h24	14^{a}	7^{b}	2.0	6^{b}	8^{b}	17^{a}	2.4	0.02	0.02	0.43

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a\text{-}b}$ Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method; ED: effective degradability; N: nitrogen; CHO: carbohydrates; con: concentration.

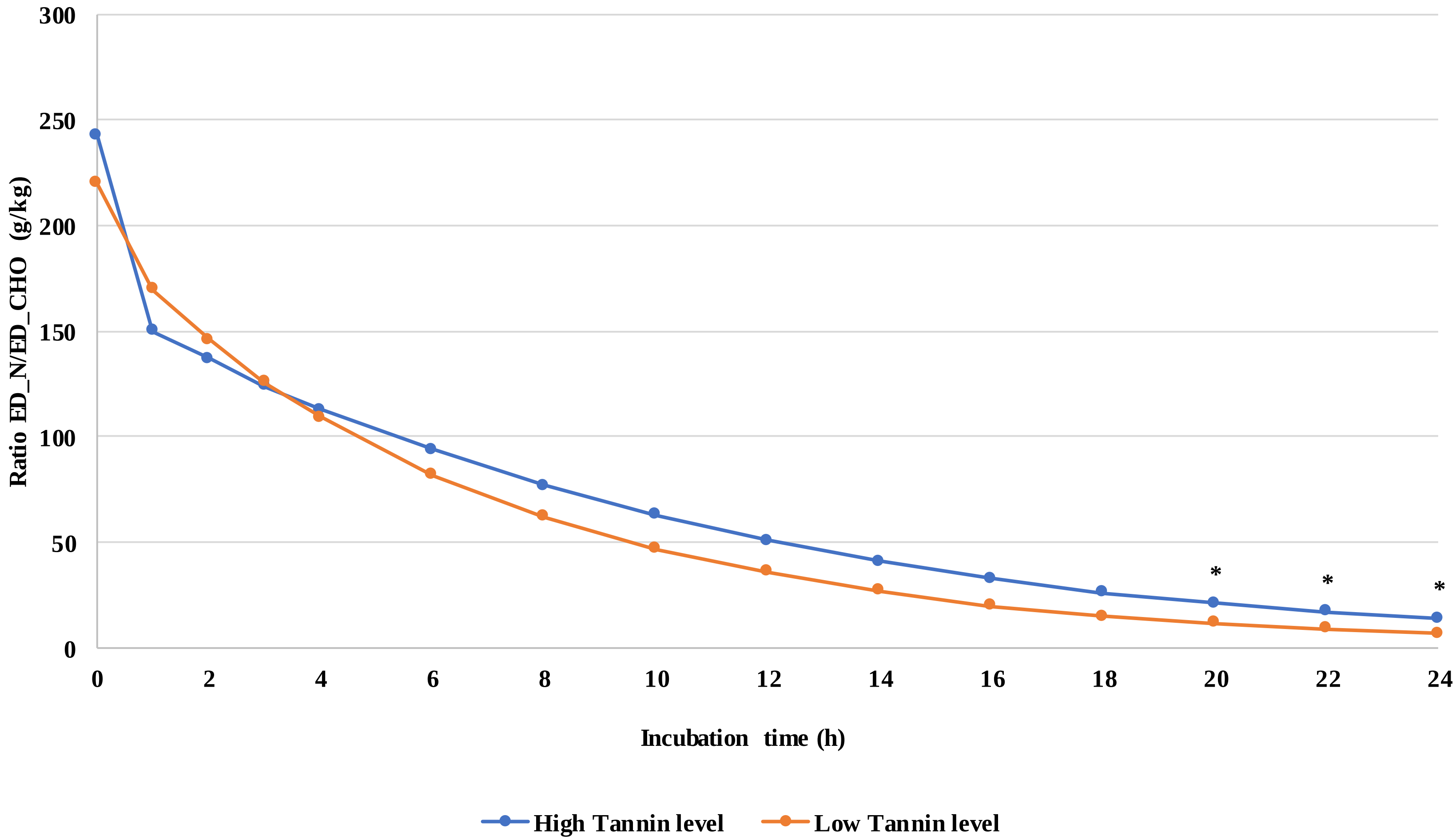


Figure 3.1. Effect of tannin concentration on hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of whole plant faba bean hay. Optimum ratio = 32 EN/ECHO g/kg. * Means are significantly different (P< 0.05).

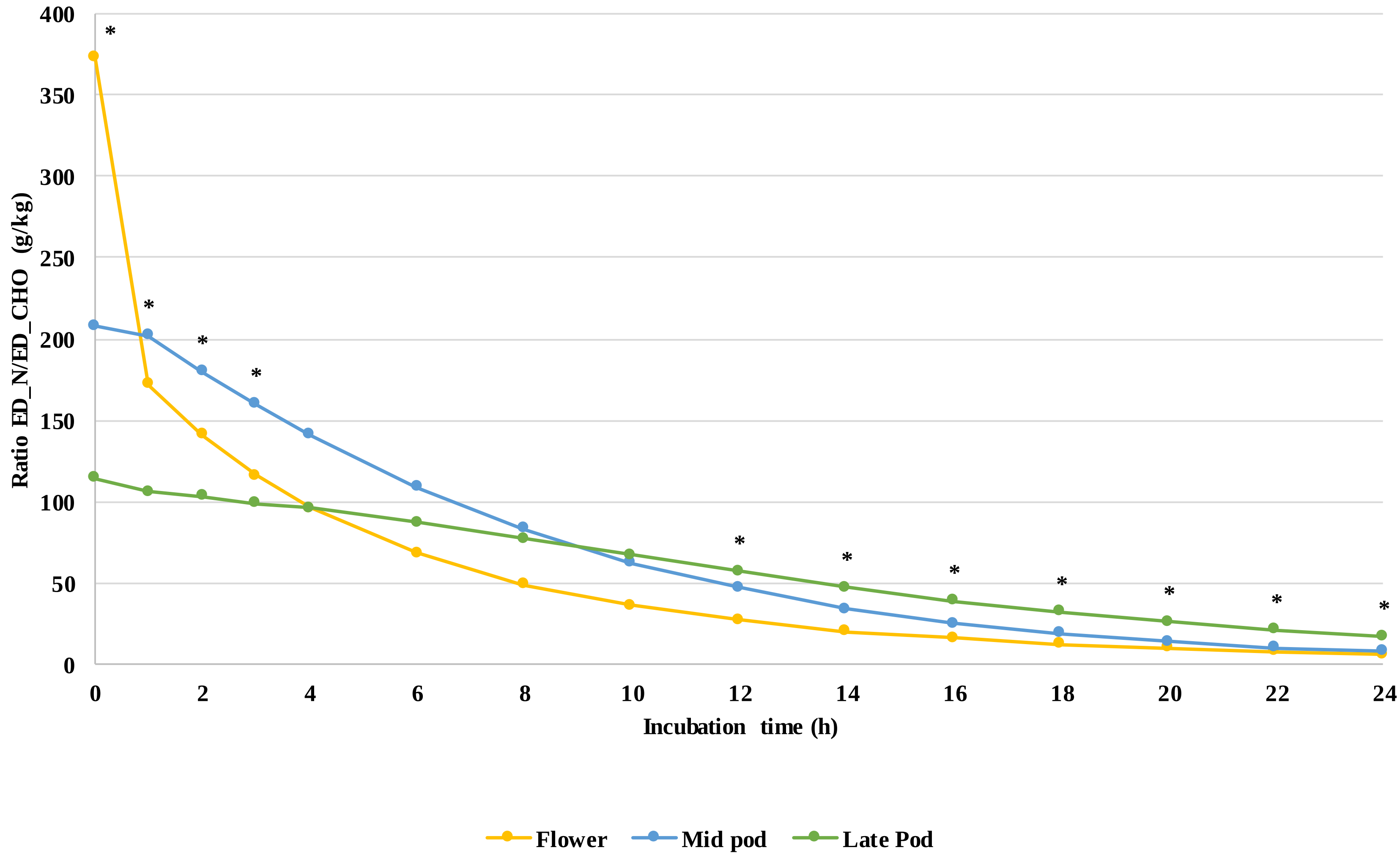


Figure 3.2. Effect of cutting stage on hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of whole plant faba bean hay. Optimum ratio = 32 EN/ECHO g/kg. * Means are significantly different ($P < 0.05$).

g/kg), h22 (17 vs. 9 g/kg), and h24 (14 vs. 7 g/kg) than the low tannin whole plant faba bean hay. On the other hand, the stage of cutting significantly affected the ratio of N/CHO and the overall ratio of ED_N/ED_CHO. At flower stage, those characteristics were higher (P< 0.05) than the late pod stage (98 vs. 64 g/kg and 171 vs. 87 g/kg, respectively); however, the ratio of N/CHO and the overall ratio of ED_N/ED_CHO at mid pod stage was not significantly different (P> 0.05) from the ratios found at flower and late pod stages. The individual ratios from h0 to h3 were higher (P< 0.05) at flower stage than late pod stage (373 vs. 114; 172 vs. 106; 141 vs. 103 and 116 vs. 99 g/kg, respectively) and from individual h4 to h8 the ratios were not significantly affected by the stage of cutting, but from h12 to h24 the ratios were higher (P< 0.05) at late pod stage than at flower stage. The hourly effective rumen degradation ratios (ED_N/ED_CHO) at the first degradation hours (h1 to h3) were higher at flower stage than the ratios found at late pod stage. The flower stage ratios reduced drastically (from 333 to 116 g/kg), while at late pod stage the reduction was gradual (from 114 to 99 g/kg). However, from h12 to h24 the ratios were higher at late pod stage than at flower stage. At flower stage they might be more possible nitrogen losses than at mid pod and late pod stages.

Rodríguez (2018), evaluated the high and low tannin faba bean seeds and found that the ratio of N/CHO and the overall ratio of ED_N/ED_CHO were not significantly different between high and low tannin faba bean seeds; however, at individual hours h6, h8, h10, h12, and h16 the high tannin seeds showed a higher ratio of ED_N/ED_CHO than low tannin seeds. On the other hand, in the present study the effective degradation ratios of high and low whole plant faba bean hay were similar at individual hours (h6, h8, h10, h12, and h16) and different at h20, h22 and h24. In published research an ideal ratio of 25 g of N/kg of OM or 32 g of N/kg of CHO for dairy cattle is denoted which yields a maximum microbial protein with a minimum N loss (Tamminga et al., 2007, 1994; Sinclair et al., 1993). Several studies indicate that higher ratios between effective degradability of N and energy compared to the ideal suggests a potential N loss from the rumen or insufficient energy for the rumen microorganisms. Conversely, a lower ratio than the ideal implies a N deficiency required for microbial growth (Nuez-Ortin and Yu, 2010).

3.4.10 Intestinal Availability of Rumen Bypass Nutrients

The effect of tannin concentration and stage of cutting on the intestinal digestible rumen bypass and total digestible DM, CP, NDF and ST of the whole plant faba bean hay are presented in Table 3.10. Detailed observation of the data revealed that the tannin concentration did not affect (P> 0.05) any of the characteristics of the intestinal digestibility of DM; however, the stage of cutting significantly affected some of those. At mid pod and late pod stages the intestinal digestibility of DM (%dBDM)

was higher (P< 0.05) than at flower stage (50.3 and 55.6 vs. 40.9 %), the intestinal digestible rumen bypass DM (IDBDM) was higher (P< 0.05) at late pod stage than at mid pod stage (222 vs. 180 g/kg DM), and at mid pod stage the value was higher (P< 0.05) than the one found at flower stage (180 vs. 140 g/kg DM). The total digestible DM (TDDM) was not significantly affected (P> 0.05) by the stage of cutting. The tannin concentration did not affect significantly the characteristics of intestinal digestibility of crude protein except for the intestinal absorbable feed protein (IADP) which was higher (P< 0.05) in the high tannin whole plant faba bean hay than the low tannin whole plant faba bean hay (21 vs. 19 g/kg DM).

The stage of cutting did not affect (P> 0.05) the intestinal digestibility of CP (%dIDP) neither the total digested protein (TDP), but the intestinal absorbable feed protein (IADP) was higher (P< 0.05) at late pod stage than at flower and mid pod stages (24 vs. 18 and 19 g/kg DM).

The low tannin whole plant faba bean hay showed a higher (P< 0.05) intestinal digestibility of NDF (%dBNDF) than the high tannin whole plant faba bean hay (42.3 vs. 37.8 %), also the intestinal digestible rumen bypass NDF (IDBNDF) was higher (P< 0.05) in the low tannin whole

Table 3.10. Effect of variety (tannin concentration) and stage of cutting on intestinal digestibility and total tract digestion of whole plant faba bean hay

Item	Variety (V, Tannin con.)		SEM	Stage of Cutting (S)			SEM	P value		
	H	L	SEM	Flower	Mid pod	Late Pod	SEM	Variety	Stage	V×S
Dry matter										
%dBDM	48.2	49.7	1.72	40.9^{b}	50.3^{a}	55.6^{a}	2.06	0.55	<0.01	0.86
%IDBDM	18.1	18.0	0.75	14.0^{c}	18.0^{b}	22.2^{a}	0.86	0.92	<0.01	0.92
IDBDM (g/kg DM)	181	180	7.5	140^{c}	180^{b}	222^{a}	8.6	0.92	<0.01	0.92
%TDDM	80.7	82.0	0.82	79.7	82.1	82.3	1.00	0.27	0.17	0.64
TDDM (g/kg DM)	807	820	8.2	797	821	823	10.0	0.27	0.16	0.65
Crude protein										
%dIDP	56.2	56.7	3.85	55.9	54.8	58.7	4.02	0.84	0.40	0.29
IADP (g/kg DM)	21^{a}	19^{b}	1.3	18^{b}	19^{b}	24^{a}	1.4	0.04	0.01	0.45
IADP (g/kg CP)	98	88	3.7	79^{b}	86^{b}	115^{a}	4.6	0.08	0.01	0.54
TDP (g/kg DM)	203	199	10.0	212	204	187	11.1	0.65	0.14	0.93
TDP (g/kg CP)	923	931	10.5	936	926	919	11.1	0.30	0.21	0.51
%IADP (%CP)	9.8	8.8	0.37	7.9^{b}	8.6^{b}	11.5^{a}	0.46	0.08	<0.01	0.54
%TDP (%CP)	92.3	93.1	1.05	93.6	92.6	91.9	1.11	0.30	0.21	0.51
Fiber (NDF)										
%dBNDF	37.8^{b}	42.3^{a}	2.18	34.0^{b}	42.8^{a}	43.3^{a}	2.38	0.04	<0.01	0.40
%IDBNDF	23.5	25.7	2.14	20.0^{b}	27.0^{a}	26.8^{a}	2.27	0.17	0.01	0.39
IDBNDF (g/kg DM)	84^{b}	94^{a}	6.7	75^{b}	94^{a}	98^{a}	7.0	0.03	<0.01	0.48
%TDNDF	61.3	65.4	1.51	61.1	63.6	65.3	1.85	0.08	0.32	0.43
TDNDF (g/kg DM)	220	243	11.7	228	224	241	14.4	0.19	0.68	0.77
Starch										
%dBST	87.6	88.8	2.84	73.0^{b}	94.3^{a}	97.2^{a}	3.39	0.76	<0.01	0.80
% IDBST	16.8	18.6	2.34	27.3^{a}	12.0^{b}	13.8^{b}	2.87	0.59	0.01	0.32
IDBST (g/kg DM)	12	10	2.7	2^{b}	9^{b}	23^{a}	3.1	0.47	<0.01	0.64
%TDST	96.6	96.2	1.09	90.3^{b}	99.3^{a}	99.6^{a}	1.23	0.75	<0.01	0.91
TDST (g/kg DM)	77	71	11.7	7^{c}	71^{b}	144^{a}	12.6	0.51	<0.01	0.86

Table 3.10. *Cont'd*. Footnote:

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method; dBDM: intestinal digestibility of rumen bypass dry matter; IDBDM: intestinal digested rumen bypass dry matter; TDDM: total digested dry matter dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digested crude protein; TDP: total digested crude protein; dBST: intestinal digestibility of rumen bypass starch; IDBST: intestinal digested rumen bypass starch; TDST: total digested starch; dBNDF: intestinal digestibility of rumen bypass neutral detergent fiber; IDBNDF: intestinal digested rumen bypass neutral detergent fiber; TDNDF: total digested neutral detergent fiber; con: concentration.

plant faba bean hay than that in the high tannin whole plant faba bean hay (94 vs. 84 g/kg DM). The tannin concentration did not affect (P> 0.05) the total digestible NDF (TDNDF). Stage of cutting significantly affected some of the characteristics of the intestinal digestibility of NDF, the intestinal digestibility of NDF (%dBNDF) was higher (P< 0.05) at mid pod and late pod stages than at flower stage (42.8 and 43.3 vs. 34.0 %). The intestinal digestible rumen bypass NDF (IDBNDF) was higher (P< 0.05) at mid pod and late pod stages than at flower stage (94 and 98 vs. 75 g/kg DM). The total digestible NDF (TDNDF) was not affected (P> 0.05) by the stage of cutting.

The characteristics of intestinal digestibility of starch were not significantly affected by tannin concentration (P> 0.05); however, the stage of cutting affected those characteristics. The intestinal digestibility of bypass starch (%dBST) was higher (P< 0.05) at mid pod and late pod stages than at flower stage (94.3 and 97.2 vs. 73 %). The intestinal digestible bypass starch (IDBST) was higher (P< 0.05) at late pod stage than at flower and mid pod stages (23 vs. 2 and 9 g/kg DM). The total digested starch (TDST) was higher (P< 0.05) at late pod stage than mid pod stage (144 vs 71 g/kg DM) and at mid pod stage the value was higher than at flower stage (71 vs. 7 g/kg DM). High tannin whole plant faba bean hay had higher IADP (+2 g/kg DM), lower dBNDF (-4.5 %), and lower IDBNDF (-10 g/kg DM) than low tannin whole plant faba bean hay. At late pod and mid pod stages, the dBDM was higher than at flower stage (+14.7 and +9.4 %). At late pod stage the IDBDM, the IADP, the IDBNDF, the IDBST, and TDST were higher than at flower stage (+82, +6, +23, +21, and +137 g/kg DM, respectively). IDBDM and TDST values increased with maturity.

When the faba bean seeds were evaluated not significant difference between high and low tannin faba bean seeds in terms of IADP (g/kg DM) were observed (Rodríguez, 2018); however, the values in Rodríguez (2018) were higher than the ones found in whole plant faba bean hay. In contrast with the findings of the present study, in Rodríguez (2018), the %dBNDF and IDBNDF were not significantly different between high and low tannin faba bean seeds, but those values were higher in both whole plant faba bean hay than the ones found in the seeds. In agreement with our results, all the characteristics of starch digestion were not significantly different except for the digestibility of starch (%dBST) and the total digested starch (%TDST) which were higher in the high tannin faba bean seeds (Rodríguez, 2018).

3.4.11 Nutrient Supply with the DVE/OEB System

Metabolic characteristics and true nutrient supply based on the DVE/OEB system are presented in Table 3.11. The truly digestible nutrient supply characteristics were not significantly affected by the tannin concentration ($P > 0.05$) except for the truly absorbed bypass protein in the small intestine (DVBE) which was higher ($P < 0.05$) in the high tannin whole plant faba bean hay than the low tannin whole plant faba bean hay (24 vs. 21 g/kg DM). The degraded protein balance (OEB) and the total true protein supply (DVE) were not affected by the tannin concentration ($P > 0.05$). The stage of cutting affected significantly the microbial protein synthesized in the rumen based on rumen degraded feed crude protein (MREN) which was higher ($P < 0.05$) at flower stage than at late pod stage (191 vs. 159 g/kg DM). The MREN at mid pod stage (181 g/kg DM) was similar at flower and mid pod stages ($P > 0.05$).The DVBE value was higher ($P < 0.05$) at late pod stage compared to the flower and mid pod stages which were similar (26 vs. 20 and 21 g/kg DM). The OEB was higher ($P < 0.05$) at flower stage than at late pod stage (91 vs. 57 g/kg) and the OEB at mid pod stage was not different from both values ($P > 0.05$). The DVE was lower ($P < 0.05$) at flower stage than at late pod stage (68 vs. 77 g/kg DM), while the DVE at mid pod stage was similar to the DVE values found at flower and late pod stages ($P > 0.05$). The truly absorbed bypass protein in the small intestine (DVBE) was higher in the high tannin whole plant faba bean hay (+3 g/kg DM) than that in the low tannin whole plant faba bean hay, and also was higher at late pod stage than at flower and mid pod stages (+6 and +5 g/kg DM). The microbial protein synthetized in the rumen based on rumen degraded feed crude protein (MREN) and the degraded protein balance (OEB) was higher at flower stage, but the total true protein supply (DVE) was lower than at late pod stage (+32, +34, -9 g/kg DM). The DVE values increased with whole plant faba bean maturity, while the OEB values decreased.

According to Rodríguez (2018), the truly digestible nutrient supply, the degraded protein balance (OEB), and the total true protein supply (DVE) were not significantly different between high and low tannin faba bean seeds. The DVE and the OEB values found in the seeds were higher than the ones found in the whole plant faba bean hay (Rodríguez, 2018; Yu et al., 2004). Yari et al., (2012) evaluated alfalfa hay at three stages of maturity and found DVE values of 57, 51, and 39 g/kg DM at early bud, late bud, and early flower, respectively. Those values were lower than the DVE values of whole plant faba bean hay at flower, mid pod, and late pod stages. According to the chemical composition and nutritional values of feedstuffs CVB (2016) the DVE values of red clover hay, poor quality grass hay, average quality grass hay, good quality grass hay and alfalfa hay are 71, 40, 58, 73 and 75 g/kg DM respectively. The OEB in alfalfa hay was higher at early bud stage (54 g/kg DM) (Yari et al., 2012), while in the whole plant faba bean hay was higher at flower and mid pod stages (91 and 78 g/kg DM). The OEB values of red clover hay, poor quality grass hay, average quality grass hay, good quality grass hay and alfalfa hay are 37, 3, 8, 31 and 28 g/kg DM respectively (CVB, 2016).

3.4.12 Nutrient Supply with the NRC-2001 Model

Metabolic characteristics and true nutrient supply based on the NRC-2001 model are shown in Table 3.11. The tannin concentration affected the truly absorbed rumen undegraded feed protein (ARUP) which was higher ($P < 0.05$) in the high tannin whole plant faba bean hay than the low tannin whole plant faba bean hay (21 vs. 19 g/kg DM). ARUP was significantly affected by the stage of cutting as well ($P < 0.05$), showing a lower value at flower and mid pod stages compared to late pod stage (18 and 19 vs. 24 g/kg DM). The degraded protein balance (DPB) and the metabolizable protein (MP) as same as the values predicted with the DVE/OEB system were not significantly affected by the tannin concentration (P> 0.05). The MP was not significantly affected (P> 0.05) by the stage of cutting based on this model (there was a tendency to P= 0.06); however, the DPB was higher at flower stage than at late pod stage (108 vs. 74 g/kg DM) and the DPB value at mid pod stage was similar to both previous values. The truly absorbed rumen undegraded feed protein (ARUP) was higher in the high tannin whole plant faba bean hay than the low tannin whole plant faba bean hay (+2 g/kg DM), also ARUP was higher at late pod stage than at flower and mid pod stages (+6 and +5 g/kg DM). A tendency was found in the effect of stage of cutting in the metabolizable protein (MP). The degraded protein balance (DPB) was higher at flower stage than at late pod stage (+34 g/kg DM), similar pattern was observed on the predicted OEB. The DPB value reduced with maturity of the whole plant faba bean. The metabolic characteristics and true nutrient supply, MP and DPB (based on TDN system: NRC dairy) were not significantly different in the study of Rodríguez (2018) between high and low tannin faba bean seeds; however, the MP value of the seeds (119 g/kg DM) was higher than the MP value of the hay (73 g/kg DM). Yari et al., (2012) found that the MP was 78, 72, and 66 g/kg DM at early bud, late bud, and early flower when they evaluated the alfalfa hay at three stages of maturity. In our study the MP at flower, mid pod, and late pod stages was 68, 72, and 76 g/kg DM, respectively.

The DPB of the alfalfa hay was lower at early flower (15 g/kg DM) (Yari et al., 2012), while the DPB in the whole plant faba bean hay was lower at mid pod and late pod stages (95 and 74 g/kg DM). A study conducted by Khalilvandi et al., (2010) in Sainfoin hay determined that the MP value was 58 g/kg DM, that value is lower that the MP value found in whole plant faba bean hay at the three cutting stages

3.4.13 Feed Milk Value

Feed Milk Values are presented in Table 3.11. The Feed Milk Value (FMV) based on the DVE system was not affected (P> 0.05) by the tannin concentration; however, we found that at late pod stage the FMV was higher (P< 0.05) than at flower stage (1.57 vs. 1.35 kg milk/kg DM hay) and the FMV at mid pod stage (1.48 kg milk/kg DM hay) was similar to those at late pod and flower stages. According to the NRC-2001 model (NRC, 2001) the tannin concentration and stage of cutting did not affect the FMV (P> 0.05), but a tendency of P= 0.06 was observed. Based on the DVE/OEB system, in agreement with the NRC model, the tannin concentration did not affect the FMV. The FMV with the DVE/OEB system was lower in high tannin whole plant faba bean hay and higher in low tannin whole plant faba bean hay compared with the corresponded values predicted with the NRC-2001 model. Based on the DVE/OEB system at late pod stage the FMV was higher than at flower stage (+0.2 kg milk/kg DM hay). At mid pod stage and at late pod stages the FMV predicted with the DVE/OEB system were higher than the corresponded values predicted with the NRC-2001 model, while the FMV at flower stage was lower.

According to Rodríguez (2018), the FMV of high and low tannin faba bean seeds based on the DVE/OEB system and NRC model were not significantly different. In this study those values were similar between high and low tannin whole plant faba bean hay, but the FMV of the faba bean seeds was higher than the FMV of the hay (+1 kg milk/kg DM feed).

Table 3.11. Effect of variety (tannin concentration) and stage of cutting on metabolic characteristics, true nutrient supply and feed milk value of whole plant faba bean hay

Item	Variety (V, Tannin con.)		SEM	Stage of Cutting (S)			SEM	P value		
	H	L		Flower	Mid pod	Late Pod		Variety	Stage	V×S
Truly digestible nutrient supply to dairy cows based on non-TDN system: DVE system										
BCP (g/kg DM)	42^{a}	37^{b}	2.3	36^{b}	39^{ab}	44^{a}	2.6	0.03	0.02	0.68
MREE (g/kg DM)	101	103	1.0	100	104	102	1.2	0.18	0.14	0.73
MREN (g/kg DM)	178	176	9.1	191^{a}	181^{ab}	159^{b}	10.2	0.90	0.05	0.98
DVME (g/kg DM)	64	66	0.6	64	66	65	0.7	0.18	0.14	0.73
DVBE (g/kg DM)	24^{a}	21^{b}	1.4	20^{b}	21^{b}	26^{a}	1.6	0.04	0.01	0.45
Degraded protein balance (OEB of silage) and Total true protein supply (DVE of Hay) to dairy cows										
DVE (g/kg DM)	73	72	2.1	68^{b}	73^{ab}	77^{a}	2.4	0.63	0.02	0.55
OEB (g/kg DM)	77	74	8.4	91^{a}	78^{ab}	57^{b}	9.4	0.72	0.02	0.96
FMV (kg milk/kg DM Hay)	1.48	1.46	0.043	1.37^{b}	1.48^{ab}	1.57^{a}	0.048	0.61	0.02	0.53
Truly digestible nutrient supply to dairy cows based on TDN system: NRC dairy										
RUP (g/kg DM)	38^{a}	33^{b}	2.1	32^{b}	35^{ab}	40^{a}	2.3	0.03	0.02	0.68
MCP$_{TDN}$ (g/kg DM)	76	75	1.5	73	76	76	1.7	0.63	0.37	0.97
MCP$_{RDP}$ (g/kg DM)	155	153	7.7	165	157	139	8.7	0.85	0.05	0.97
AMCP (g/kg DM)	48	48	0.9	47	49	48	1.1	0.63	0.37	0.97
ARUP (g/kg DM)	21^{a}	19^{b}	1.3	18^{b}	19^{b}	24^{a}	1.4	0.04	0.01	0.45
Degraded protein balance (DPB of Hay) and Total metabolizable protein supply (MP of Hay) to dairy cows										
MP (g/kg DM)	74	71	2.1	69	72	76	2.4	0.15	0.06	0.82
DPB (g/kg DM)	93	92	7.5	108^{a}	95^{ab}	74^{b}	8.5	0.93	0.01	0.95
FMV (kg milk/kg DM Hay)	1.51	1.44	0.042	1.41	1.46	1.55	0.048	0.14	0.06	0.81

H: high tannin variety; L: low tannin variety; 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: Tukey method; BCP: ruminally undegraded feed CP: calculated according the formula in DVE/OEB system; MREE: microbial protein synthesized in the rumen based on available energy; MREN: microbial protein synthesized in the rumen based on rumen degraded feed crude protein; DVME: truly absorbed rumen synthesized microbial protein in the small intestine; DVBE: truly absorbed bypass feed protein in the small intestine; DVE: truly absorbed protein in the small

Table 3.11. *Cont'd*. Footnote

intestine; OEB: is a balance between microbial protein synthesis from rumen degradable CP and that from the energy extracted during anaerobic fermentation in the rumen. RUP: ruminally undegraded feed CP: calculated according the formula in NRC-2001 dairy model; MCP_{TDN}, microbial protein synthesized in the rumen based on available energy (discounted TDN); $MCP_{RDP,}$: microbial protein synthesized in the rumen based on available protein; AMCP: truly absorbed rumen-synthesized microbial protein in the small intestine; ARUP: truly absorbed rumen-undegraded feed protein in the small intestine; MP: metabolizable protein; DPB: reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy-TDN available for microbial fermentation in the rumen; con: concentration.

3.5 Conclusions

The tannin concentration and the cutting stage affected some of the nutritive and metabolic characteristics of whole plant faba bean hay (artificially air dried). Based on these studies it was concluded that both low and high tannin whole plant faba bean hay have attractive nutrient profiles and can be used in future studies to determine important characteristics as palatability and performance in dairy cows. On the other hand, at late pod stage the yield of primary and bypass nutrients was higher. Additionally, these results reveal that whole plant faba bean hay at mid pod and late pod stages showed better predicted performance than whole plant faba bean hay at flower stage. Although, the results from the current study were from chemical, *in situ* and *in vitro* techniques, and modeling, further studies including animal and metabolic trials should be performed to confirm these results and develop an adequate feeding strategy.

4. COMPREHENSIVE PHYSIOCHEMICAL AND NUTRITIONAL EVALUATION OF FABA FORAGE VARIETIES FOR SILAGE: EFFECT OF VARIETY/TANNIN CONCENTRATION AND GROWTH STAGE ON DETAILED FEED AND FEEDING VALUES OF FABA FORAGE SILAGE GROWN AT THREE STAGES IN WESTERN CANADA WITH COOL CLIMATE CONDITIONS IN RUMINANT LIVESTOCK SYSTEMS.

4.1 Abstract

Faba bean is known as a high nutritive seed, however information about the use of the whole plant as silage for ruminants is scarce, therefore this study aimed to determine the effect of tannin concentration/variety (low tannin/Snowdrop variety; high tannin/SSNS-1 variety) and the effect of stage of cutting (mid pod stage at 88 days old; late pod stage at 97 days old) on yield, chemical and energy profiles, CNCPS fractions, on the rumen degradation kinetics of principal nutrients, the nitrogen to energy degradation ratios, *in vitro* intestinal digestibility, metabolic characteristics and predicted production performance of whole plant faba bean silage. A completely Randomized Design and RCBD with 2 × 3 factorial treatment arrangement were used as the experimental designs. Data was analyzed using the Mixed model procedure of SAS version 9.4. The results indicated that dry matter (DM) yield of whole plant faba bean silage was lower ($P < 0.05$) at flower stage than at late pod stage (7.34 vs. 12.20 t/ha). The pH, ammonia (NH_3), acetic acid and propionic acid were higher ($P < 0.05$) in whole plant faba bean silage at flower stage than at mid pod and late pod stages which were similar (5.39 vs. 4.35 and 4.51; 16.32 vs. 6.62 and 5.66 % of total N; 6.33 vs. 2.35 and 1.70 %DM; 1.44 vs. 0.04 and 0.06 %DM, respectively). Crude protein (CP) was similar in all different silage varieties and in all different cutting stages (22 %DM), however neutral detergent fiber (aNDF) at mid pod and late pod stage was similar, but lower ($P < 0.05$) than at flower stage (36.0 and 34.4 vs. 45.3 %DM). Starch content was higher ($P < 0.05$) at late pod stage than at mid pod and flower stages (17.2 vs. 9.4 and 1.3 %DM). Net energy of lactation (NEL_{3x}) was similar at mid pod and late pod stages but higher ($P< 0.05$) than at flower stage (1.45 and 1.46 vs. 1.13 Mcal/kg DM). At late pod stage a higher ($P< 0.05$) rumen undegraded crude protein based in the NRC-2001 Dairy model (RUP^{NRC}) and a higher ($P< 0.05$) bypass starch (BSt) were found at mid pod stage (33 vs. 25 and 32 vs. 18 g/kg DM respectively). The results indicated that the overall ratio of hourly effective degradation ratios between available N and available carbohydrates (ED_N/ED_CHO) was lower ($P< 0.05$) at late pod than at mid pod stage (-35 g/kg), but the intestinal absorbable feed protein (IADP) and the total digested starch (TDST) were higher ($P< 0.05$; 84 vs. 61 g/kg CP and 175 vs. 95 g/kg DM, respectively). The total true protein supply (DVE) and the Feed Milk Value based on the DVE system (FMV^{DVE}) were lower ($P< 0.05$) at mid pod stage than at late pod stage (59 vs. 68 g/kg DM and 1.20 vs. 1.37 kg milk/kg DM silage), while based on the NRC, the metabolizable protein (MP) tended to be lower (67 vs. 73 g/kg DM), and the Feed Milk Value (FMV^{NRC}) was lower ($P< 0.05$) at mid pod stage than at late pod stage (1.36 vs. 1.48 kg milk/kg DM hay). It was concluded that faba bean should be harvested at late pod stage in order to support high yield and high nutritional value in the silage, also at late pod stage the silage showed superior predicted production performance. Therefore, whole plant faba bean silage at late pod stage has the potential to be used as an ingredient in dairy cattle rations. Further animal and metabolic studies should be conducted to support these results.

Keywords: Whole plant faba bean; tannin concentration; cutting stage; silage; metabolizable protein.

4.2 Introduction

In North America dairy farms use barley, corn, or alfalfa as forage. Furthermore, in western Canada whole crop cereals are commonly used as a forage source for cattle (Kennelly, 1995). In western provinces dairy rations consist mainly of barley grain and barley silage, while in Ontario and Quebec corn grain, corn silage and alfalfa are more common (McCartney' and Horton, 1997). In Saskatchewan, barley silage is the most popular forage especially as silage (Abeysekara, 2003). Making silage, a widespread technique, is a process that takes place because of the fermentation of sugars present in the crop to organic acids under an anaerobic environment (Horrocks and Vallentine, 1999). Previous studies have shown that this chopped and fermented forage improves shelf-life, and additionally was demonstrated that increase palatability for dairy and beef cattle (Borreani et al., 2017; Bonnefield, 2016; Kaiser and Piltz, 2003). Nowadays there is a concern on mycotoxins because sometimes it is a problem for cereal grain and cereal silage in western Canada, additionally it seems that this issue is increasing (McKinnon, 2014; Heeg and Swamy, 2008). However, the production of legumes, especially Faba bean, has increased in the prairies, but its attention as a fodder is reduced. Faba bean, is an annual legume, which is predominantly used for grain production. The use of faba bean plant as forage is limited around the world, however data from a limited previous study indicated that whole plant faba bean silage is similar to grass-legume silage (Ingalls et al., 1974). Currently there are two types of faba bean grown in western Canada, eg. Saskatchewan, the tannin ones for food, and low tannin ones for food and feed (Saskatchewan Pulse Growers, 2017; Fleurly and Barker, 2016). Tannins are secondary compounds spread in plants, particularly legumes and brows (Theodoridou, 2010). Forages with low to moderate concentration of condensed tannins (1-4 %DM) contribute to higher performance (higher growth rates and higher milk yield), while concentration of tannins exceeding 5 %DM in the diet significantly reduce feed intake, negatively affecting growth and milk production (Addisu, 2016;

Cannas, 2015). The present study aimed to determine the effects of the variety (tannin concentration) and cutting stage on whole plant faba bean silage in terms of chemical profile, energy values, CNCPS fractions, rumen degradation kinetics of primary nutrients and predicted true nutrient supply to dairy cows. This study was carried out because of the lack of information on those aspects of the current faba bean varieties grown in western Canada and because of the availability of this crop which can become a supplemental feed source for dairy cattle.

4.3 Materials and Methods

4.3.1 Ingredients and Sample Preparation

On May 3rd, 2017, eighteen experimental plots of 36 square feet each were seeded with 180 seed per plot by the Crop Development Centre (CDC) at the University of Saskatchewan, Canada. Nine plots were randomly assigned to the low tannin faba bean variety Snowdrop, and nine plots were assigned to the high tannin faba bean variety SSNS-1. The whole plant faba bean from the plots was harvested at 77, 88 and 97 days old. In each of the three periods were harvested 3 plots of Snowdrop variety and 3 plots of SSNS-1 variety and collected in wool bags and weighted. After harvesting the samples were wilted for 2 days and chopped to 1-inch length, then 6 minisilos were filled (3 with Snowdrop variety and 3 with SSNS-1 variety) in each harvesting date with about 2.5 kg of chopped whole plant faba bean per minisilo. Fermentation process took place for 120 days until the minisilos were opened and further analysis were carried out.

4.3.2 Fermentation Profile of Silage

Whole plant faba bean silage samples were processed for analysis of pH, ammonia, and VFA as described by Zahiroddini et al., (2004) and Addah et al., (2012). Briefly the procedure comprehends: 15 gr of fresh silage samples were combined with 135 mL double distilled water and blended at 18,000 rpm for 30 s in a commercial blender (Oster® 12 speed blender, Sunbeam Corporation Ltd., Brampton, ON). The suspension was filtered through two layers of cheese cloth and the pH was measured immediately in duplicate using an Accumet Research AR 50 dual channel pH meter (Fisher Scientific, Waltham, MA). Two sets of 2 ml centrifuge tubes were prepared, 1 for VFA and 1 for ammonia, in which were deposit with a pipette 1 ml of clear liquid from previous step, all samples were kept in ice for further steps, 0.2 ml of phosphoric acid were added to the tubes for VFA analysis and 0.1 ml of CA (100%w/V) to the tubes for ammonia and then all tubes were vortexed and frozen at -20°C until analyzed. Concentration of VFA was quantified using a gas chromatograph (Model 5890, Hewlett-Packard Lab, Palo Alto, CA) and ammonia was determined with phenol and hypochlorite reagents following the procedure described by Broderick and Kang (1980).

4.3.3 Chemical Analysis of Whole Plant Faba Bean Silages

Chemical profile in whole plant faba bean silages was determined following official methods. Detailed information was included in Chapter 3 (3.3.2).

4.3.4 Energy Profile of Whole Plant Faba Bean Silages

Total digestible nutrients and energy values of whole plant faba bean silages were determined using the NRC-2001 dairy and the NRC-1996 beef. Detailed information was included in Chapter 3 (3.3.3).

4.3.5 Protein and Carbohydrate Subfractions of Whole Plant Faba Bean Silages

The Cornell Net Carbohydrate and Protein System (CNCPS 6.5) was used to determine the protein and carbohydrate factions in whole plant faba bean silages. Detailed information was included in Chapter 3 (3.3.4).

4.3.6 Rumen In Situ Incubation of Whole Plant Faba Bean Silages

4.3.6.1 Samples

Samples of whole plant faba bean silage of Snowdrop variety (6 samples in total: 3 of 88 and 3 of 97 days old, respectively) and samples of whole plant faba bean silage of SSNS-1 variety (6 samples in total: 3 of 88 and 3 of 97 days old, respectively) were ground through a 3 mm screen using the 8 inches Laboratory Mill (Christy & Norris LTD, Ipswich, England) in the Department of Animal and Poultry Science, University of Saskatchewan, Canada. Whole plant faba bean silages of 77 days old (at flower stage) were removed from this and further studies because of the poor quality shown in previous studies.

4.3.6.2 Animals and Diets

Four lactating Holstein cows on their third lactation were used in this study. They were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 2009). The animal study was approved (Animal Use Approval Protocol # 19910012) by Animal Research 125 Ethics Board (AREB) at the University of Saskatchewan, Canada. Detailed information was included in Chapter 3 (3.3.5.2).

4.3.6.3 Rumen Incubation Procedure

The *in situ* method described by Yu et al., (2003) was used to determine rumen degradation characteristics of primary nutrients. Detailed information was included in Chapter 3 (3.3.5.3).

4.3.6.4 Chemical Analysis of In Situ Silage Samples

The samples were analyzed for CP, Neutral detergent fiber (NDF), and Starch (ST) at Cumberland Valley Analytical Services (CVAS, Hagerstown, MD) using the official methods listed previously. Detailed information was included in Chapter 3 (3.3.5.4).

4.3.6.5 Rumen Degradation Kinetics of Whole Plant Faba Bean Silages

The first-order degradation kinetics model described by Ørskov and McDonald (1979) and modified by Tamminga et al., (1994) was used to determined degradation characteristics of dry matter (DM), crude protein (CP), starch (ST), and neutral detergent fiber (aNDF). Detailed information was included in Chapter 3 (3.3.5.5).

4.3.7 Hourly Effective Rumen Degradation Ratios and Potential N-to-Energy Synchronization

The effective rumen degradation ratios of N and carbohydrates (CHO) were calculated using the formula described in Sinclair et al., (1993). Detailed information was included in Chapter 3 (3.3.6).

4.3.8 Intestinal Digestion of Rumen Undegraded Protein in Whole Plant Faba Bean Silages

The *in vitro* intestinal digestion was determined following the three-step procedure described by Calsamiglia and Stern (1995) and later on modified by Gargallo et al., (2006). Detailed information was included in Chapter 3 (3.3.7).

4.3.9 Nutrient Supply to Dairy Cows with the DVE/OEB System

The protein digested in the intestine (DVE) and the degraded protein balance (OEB) were calculated. Detailed information was included in Chapter 3 (3.3.8).

4.3.10 Nutrient Supply to Dairy Cows with the NRC-2001 Model

Metabolizable protein (MP) and degraded protein balance (DPB) were calculated. Detailed information was included in Chapter 3 (3.3.9).

4.3.11 Feed Milk Value of Whole Plant Faba Bean Silage

Predicted production performance or Feed Milk Value (FMV) was determined according the DVE/OEB System and the NRC model. Detailed information was included in Chapter 3 (3.3.10).

4.3.12 Statistical Analysis

Results were analyzed using the Mixed model procedure of SAS version 9.4. (SAS Institute, Inc., Cary, NC, US). Completely Randomized Design and RCBD were used as experimental designs with a 2 × 3 factorial treatment arrangement. The first factor related to tannin concentration (low and high) and the second factor related to the cutting stage (flower, mid pod, and late pod stages). The models used were:

For chemical and nutrient profiles: CRD model: $Y_{ijk} = \mu + F_i + H_j + (F_i \times H_j) + e_{ijk}$, and

For In situ and modeling nutrient supply: RCBD model: $Y_{ijkl} = \mu + F_i + H_j + (F_i \times H_j) + R_k + e_{ijkl}$,

where, Y_{ijk} or Y_{ijkl} was the observation of the dependent variable ijk or ijkl, μ was the population mean for the variable, F_i the effect of tannin concentration (i= 1,2); H_j the effect of cutting stage (j= 1,2,3), $F_i \times H_j$ the interaction between variables, R_k in situ experimental runs (k= 1,2,3), and e_{ijk} or e_{ijkl} the random error associated with observation ijk or ijkl.

PROC NLIN-Gauss-Newton method of SAS was used to fit the rumen degradation data to the model. The difference among treatments was evaluated using the Tukey method. The CRD and RCBD model assumptions with NIID were checked using SAS Residual Analysis. The normality test was carried out using SAS Proc Univaritate with Normal and Plot options. 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 Yield

The stage of cutting on the yield of fresh matter (FM) of whole plant faba bean (Table 4.1) had no significant effect ($P > 0.05$) on fresh matter (FM) yield, however high tannin variety had lower ($P < 0.05$) FM yield (-17.26 t/ha) than the low tannin variety. Silage yield was affected by tannin concentration, low tannin variety silage showed higher ($P < 0.05$) yield of silage (+13.29 t of silage/ha) than high tannin variety silage, while the yield of DM of silage was affected by the stage of cutting, at late pod stage the yield was higher ($P < 0.05$) than at flower stage (+4.86 t of DM of silage/ha), the corresponding values of the silage at mid pod stage were similar than the values of the silage at flower and late pod stages. The DM content ranged from 17.0 % at flower stage, 24.4%

at mid pod stage to 28.5 % at late pod stage. The DM was higher (P< 0.05) in the high tannin silage (+1.9 %).

In a study (Berkenkamp and Meeres, 1986) in which faba bean was seeded with a 200 kg/ha rate and faba bean was harvested in different locations of Alberta Canada in 3 consecutive years, when the first lower pod turned black, it was found that the dry matter yield ranged from 3.47 to 12.68 t of DM/ha. That higher yield is similar than the one we obtained. Caballero (1989) harvested whole plant faba bean at 3 time points: L1: seed with less than 20% DM (undeveloped pods at the bottom nodes); L2: seed about 30-35% DM (green, fully developed pod and seeds), and L3: seed over 85% DM (brown pods and mature seeds) and found that the yield at L1 was 3.44 and 5.52 t of DM/ha in rainfed conditions and irrigation conditions, respectively, at L2 was 6.07 and 8.66 t of DM/ha in rainfed conditions and irrigation conditions, respectively, and at L3 was 4.28 and 6.84 t of DM/ha in rainfed conditions and irrigation conditions, respectively. L2 time point can be comparable to our late pod stage. It is important that the seed rate used in that study was about 115 kg/ha. In our study, faba bean was grown under rainfed conditions only, the seed rate approximately 190 kg/ha for Snowdrop variety and 160 kg/ha for SSNS-1 variety and we observed higher DM yield than Caballero's results. Fraser et al., (2001) grew faba bean, the seeding rate was 280 kg/ha and the harvesting dates after sowing were 10, 12 and 14 weeks. They found that the DM yield of faba bean was 3.69, 5.16 and 7.76 t of DM/ha, those values are lower than the ones we obtained at similar age at harvesting. Comparing with an important forage used in Western Canada, Nair (2017) suggested that barley is commonly harvested at mid-dough stage of maturity to optimize DM yield and nutrient quality. Preston et al., (2017b) found that the DM yield of common barley varieties (CDC Cowboy, CDC Copeland, and Xena) at mid-dough stage were 6.44, 5.38 and 5.55 t of DM/ha, respectively (at early-dough stage DM yield is even lower 3.90 t of DM/ha), those values were lower than the DM yield values that we found for whole plant faba bean silage.

Table 4.1. Effect of variety (tannin concentration) and stage of cutting on yield of whole plant faba bean silage.

Item	Variety (V, Tannin con.)		SEM	Stage of Cutting (S)			SEM	P value		
	H	L	SEM	Flower	Mid pod	Late Pod	SEM	Variety	Stage	V×S
Yield										
FM (t/ha)	48.64^{b}	65.90^{a}	7.350	52.37	60.75	58.68	8.006	0.02	0.55	0.36
Silage (t/ha)	37.47^{b}	50.76^{a}	5.977	43.70	46.05	42.60	6.379	0.01	0.82	0.29
DM (t/ha)	8.84	11.48	1.411	7.34^{b}	10.93^{ab}	12.20^{a}	1.533	0.05	0.02	0.42
DM (%)	24.24^{a}	22.34^{b}	0.586	16.98^{c}	24.43^{b}	28.45^{a}	0.718	0.04	<0.01	0.31
CP (t/ha)	1.98	2.52	0.303	1.66^{b}	2.43^{ab}	2.67^{a}	0.327	0.05	0.02	0.39
aNDF (t/ha)	3.37	4.47	0.572	3.46	4.06	4.24	0.623	0.05	0.43	0.30
St (t/ha)	0.92	1.13	0.165	0.09^{c}	0.95^{b}	2.03^{a}	0.188	0.29	<0.01	0.36

H: high tannin variety; L: low tannin variety; 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: Tukey method. FM: fresh matter; DM: dry matter; CP: crude protein; aNDF: neutral detergent fiber; St: Starch, con: concentration.

Additionally, Carter (1960) have suggested that higher DM losses occurred when forage was processed as hay than as silage, that statement was supported by Rotz (1995), however in our study the dry matter yield of whole crop faba bean hay and whole plant faba bean silage was similar. Also, Carter (1960) suggested that protein losses between barn dried hay and silage are small, which supported the finding in our study. In whole plant faba bean hay (Chapter 3) the starch yield was lower than in whole plant faba bean silage, suggesting a slightly higher nutrient loss when forage is processed as artificially dried hay.

4.4.2 Fermentation Profile of Whole Plant Faba Bean Silage

The fermentation characteristics of the whole plant faba bean silage are presented in Table 4.2. The variety did not affect ($P > 0.05$) all the characteristics, however the stage of cutting did affect them. At mid pod and late pod stages the pH was similar ($P > 0.05$), but those pH values were lower ($P < 0.05$) than the pH at flower stage (-1.04 and -0.88 respectively). The ammonia (NH_3) was higher ($P < 0.05$) at whole plant faba bean silage at flower stage than at mid pod and late pod stages which had similar NH_3 values (+9.73 and +10.69 % of Total N). Acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and isocaproic acid were higher ($P < 0.05$) at flower stage than at mid pod and late pod stages which had similar values (+3.98 and +4.63 %DM; +1.40 and +1.38 %DM; +0.91 and +0.90 %DM; +3.80 and +3.67 %DM; +0.42 and +0.42 %DM; +1.56 and +2.17 %DM, respectively).

A study by Vargas Bello (2007) demonstrated that soybean silage at full pod stage and alfalfa silage at early bloom stage had a pH of 5.29 and 4.89, respectively. In our study, the pH value a flower stage was similar to the pH value of soybean silage, and the pH value a mid pod and late pod stages were similar to the pH of alfalfa silage presented by Vargas Bello (2007). Mustafa and Seguin (2003a) suggested that soybean can be used for a well-fermented silage and in his study the pH was 4.50.

Table 4.2. Effect of variety (tannin concentration) and stage of cutting on fermentation profile of whole plant faba bean silage

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid pod	Late Pod	SEM	Variety	Stage	V×S
pH	4.73	4.77	0.121	5.39^{a}	4.35^{b}	4.51^{b}	0.138	0.78	<0.01	0.89
NH_3 (%DM)	0.38	0.44	0.050	0.71^{a}	0.29^{b}	0.24^{b}	0.062	0.40	<0.01	0.85
NH_3-N (% of Total N)	8.81	10.28	1.440	16.35^{a}	6.62^{b}	5.66^{b}	1.689	0.42	<0.01	0.91
Acetic Acid (%DM)	3.01	3.91	0.699	6.33^{a}	2.35^{b}	1.70^{b}	0.856	0.38	<0.01	0.93
Propionic Acid (%DM)	0.34	0.69	0.178	1.44^{a}	0.04^{b}	0.06^{b}	0.218	0.19	<0.01	0.28
Isobutyric Acid (%DM)	0.23	0.40	0.157	0.92^{a}	0.01^{b}	0.02^{b}	0.192	0.45	0.01	0.55
Butyric Acid (%DM)	1.20	1.43	0.454	3.80^{a}	0.00^{b}	0.13^{b}	0.556	0.72	<0.01	0.79
Isovaleric Acid (%DM)	0.11	0.17	0.102	0.42^{a}	0.00^{b}	0.00^{b}	0.117	0.61	0.02	0.76
Valeric Acid (%DM)	0.07	0.01	0.053	0.13	0.00	0.00	0.065	0.43	0.30	0.53
Isocaproic Acid (%DM)	3.81	3.96	0.122	5.13^{a}	3.57^{b}	2.96^{c}	0.149	0.40	<0.01	0.46
Caproic Acid (%DM)	0.17	0.10	0.136	0.41	0.00	0.00	0.166	0.70	0.17	0.86
Total VFA (%DM)	8.84	10.67	1.454	18.58^{a}	5.98^{b}	4.88^{b}	1.781	0.41	<0.01	0.84

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a-c}$ Means with the different letters in the same row are significantly different ($P < 0.05$). Multi-treatment comparison: Tukey method. NH_3; ammonia; con: concentration.

Additionally, they demonstrated that the pH of pea silage and faba silage was about 4.00. It is important to add that those legumes were harvested between the beginning seed to full seed stages (Soybean: 76 days old; Peas: 84 days old; Faba bean: 75 days old). Nair et al., (2016) found across six different varieties of barley silage (samples harvested at mid-dough stage) that the average pH of the silage samples was 4.05, indicating adequate ensiling. In our study, at late and pod stages, we found pH values within the ones suggested for legumes silage by Kung and Shaver (2001), however at flower stage the pH is higher than those suggested values. The NH_3 found in our silage was higher at flower stage, Whiter and L. Kung (2001) found that NH_3 concentration of alfalfa silage (harvested at the early bloom stage) was 0.29 % of DM for alfalfa silage with 30% of DM content and 0.12 %DM for alfalfa silage with 54% of DM content after 45 days of ensiling. High concentration of NH_3 reveal that protein was extensively degraded and indicates poor fermentation, studies suggested that if adequate silage fermentation took place NH_3 concentration should be <10 % of total N (Kaiser and Piltz, 2003; Pahlow et al., 2002). The high NH_3 in whole plant faba bean silage at flower stage may contribute to its higher pH. NH_3 of silage of common barley varieties in Canada (CDC Cowboy, CDC Copeland, and Xena) at mid-dough stage was about 0.18 %DM (Preston et al., 2017b) which is lower than the NH_3 values found in whole plant faba bean silage in our study. Fraser et al., (2001) found that acetic acid content was 1.61, 2.00 and 1.62 %DM, propionic acid was 0.04, 0.12 and 0.14 %DM in faba bean silage (harvested at 10, 12 and 14 weeks, respectively) after 90 days of ensiling. In our study, whole plant faba bean silage at flower stage showed higher values for all the volatile fatty acids. Silages with high concentration of acetic acid (>3 %DM) suggested that energy and DM recovery are probably less than ideal and possible reduced animal intake may be observed. Additionally high concentration of acetic acid (>4–6 %DM) are observed in silages with more than 70% of moisture, in which the predominant fermenters are enterobacteria, clostridia, or heterolactic acid bacteria (Kung et al., 2018; Silva de

Oliveira et al., 2016; Kung and Shaver, 2001). Preston et al., (2017b) found that the acetic acid, propionic acid, and butyric acid in barley silage harvested at mid-dough stage were 0.30, 0.02 and 0.49 %DM, respectively. In our study, the pH, NH_3, and VFA were higher at flower stage and may be related to the higher moisture content at this stage and therefore possible clostridial fermentation occurred.

4.4.3 Chemical Profile of Whole Plant Faba Bean Silages

The effect of tannin concentration and cutting stage on the chemical profiles of whole plant faba bean silage are presented in Table 4.3. The soluble crude protein (SCP), acid detergent lignin (ADL) and cellulose were affected by the tannin concentration. The ADL was higher ($P< 0.05$) in the high tannin silage (+1.7 %DM) while SCP and cellulose were lower ($P< 0.05$) than that in the low tannin silage. (11.4 vs 14.0 %DM and 27.7 vs 30.1 %DM). The stage of cutting affected significantly ($P< 0.05$) some characteristics. The organic matter (OM) was similar in the last two stages and those values were higher ($P< 0.05$) than the OM content found in flower stage (+3.2 and +3.3 %DM respectively). The CP content was similar and averaged 22 %DM (similar than in the hay from Chapter 3). The neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were higher ($P< 0.05$) at flower stage and SCP was lower ($P< 0.05$) than SCP at mid pod and late pod stages which were similar (4.1 vs. 0.9 and 1.2 %DM; 2.5 vs. 0,7 and 0.8 %DM; 9.9 vs. 15.1 and 13.2 %DM). Ether extract (EE) and fatty acids (FA) showed interactions ($P< 0.05$, variety ×cutting stage, V×S). Low tannin silage at mid pod stage showed higher EE and FA than the high tannin silage at late pod stage (2.7 vs. 1.2 and 1.7 vs. 0.2 %DM respectively).

The carbohydrate (CHO) content was similar at mid pod and late pod stage and those values were higher ($P< 0.05$) than the CHO value at flower stage (+3.4 and +4.7 %DM). The starch content

Table 4.3. Effect of variety (tannin concentration) and stage of cutting on Chemical composition of whole plant faba bean silage

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid pod	Late Pod	SEM	Variety	Stage	V×S
Chemical Composition										
DM (%)	94.1^{a}	93.2^{b}	0.26	92.6^{c}	93.5^{b}	94.8^{a}	0.29	<0.01	<0.01	0.92
Ash (%DM)	9.6	9.7	0.30	11.8^{a}	8.6^{b}	8.5^{b}	0.33	0.65	<0.01	<0.01
OM (%DM)	90.4	90.3	0.30	88.2^{b}	91.4^{a}	91.5^{a}	0.33	0.65	<0.01	<0.01
EE (%DM)	1.7	2.0	0.15	2.0	2.2	1.3	0.17	0.12	<0.01	0.03
FA (%DM)	0.7	1.0	0.15	1.0	1.2	0.3	0.17	0.13	<0.01	0.03
Protein profile										
CP (%DM)	22.7	22.1	0.77	22.7	22.4	22.1	0.84	0.38	0.76	0.91
NDICP (%DM)	2.2	2.0	0.15	4.1^{a}	0.9^{b}	1.2^{b}	0.19	0.32	<0.01	0.03
ADICP (% DM)	1.4	1.3	0.11	2.5^{a}	0.7^{b}	0.8^{b}	0.14	0.31	<0.01	0.15
SCP (%DM)	11.4^{b}	14.0^{a}	0.62	9.9^{b}	15.1^{a}	13.2^{a}	0.69	<0.01	<0.01	0.26
NDICP (%CP)	9.7	8.8	0.84	18.2^{a}	4.1^{b}	5.4^{b}	1.03	0.49	<0.01	0.08
ADICP (% CP)	6.4	5.8	0.71	11.3^{a}	3.3^{b}	3.7^{b}	0.84	0.49	<0.01	0.28
SCP (% CP)	50.4^{b}	63.6^{a}	1.90	43.4^{b}	67.7^{a}	59.8^{a}	2.33	<0.01	<0.01	0.19
Carbohydrate profile										
CHO (%DM)	66.0	66.2	1.01	63.4^{b}	66.8^{a}	68.1^{a}	1.10	0.82	0.01	0.16
Starch (%DM)	10.2	8.4	0.85	1.3^{c}	9.4^{b}	17.2^{a}	0.97	0.07	<0.01	0.35
Starch (% NFC)	29.7	26.6	2.17	5.9^{c}	29.0^{b}	49.5^{a}	2.35	0.12	<0.01	0.37
Sugar (%DM)	0.3	0.3	0.08	0.4	0.4	0.2	0.10	0.92	0.26	0.50
Sugar (%NFC)	1.1	1.3	0.33	1.6	1.4	0.6	0.41	0.77	0.20	0.56
aNDF (%DM)	37.6	39.6	1.53	45.3^{a}	36.0^{b}	34.4^{b}	1.78	0.28	<0.01	0.38
NDFn (%DM)	35.4	37.7	1.51	41.3^{a}	35.1^{b}	33.3^{b}	1.74	0.22	<0.01	0.56
aNDFom (%DM)	37.1	39.2	1.52	44.5^{a}	35.8^{b}	34.1^{b}	1.76	0.26	<0.01	0.38
NDF (%OM)	41.7	44.0	1.72	51.4^{a}	39.5^{b}	37.6^{b}	1.99	0.27	<0.01	0.52
ADF (%DM)	35.0	37.0	1.43	43.4^{a}	33.5^{b}	31.1^{b}	1.57	0.15	<0.01	0.34
ADL (%DM)	7.3	6.9	0.53	10.4^{a}	5.9^{b}	5.0b	0.60	0.49	<0.01	0.43
ADF (%NDF)	93.0	93.2	1.43	95.8	93.0	90.5	1.76	0.95	0.14	0.69
ADL (%NDF)	18.8^{a}	17.1^{b}	0.76	22.9^{a}	16.4^{b}	14.5^{b}	0.83	0.03	<0.01	0.64

Table 4.3. ***Cont'd***. Effect of variety (tannin concentration) and stage of cutting on Chemical composition of whole plant faba bean silage

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid pod	Late Pod	SEM	Variety	Stage	V×S
Hemicellulose (%DM)	2.6	2.7	0.61	2.0	2.5	3.4	0.75	0.94	0.43	0.59
Cellulose (%DM)	27.7^{b}	30.1^{a}	1.00	32.9^{a}	27.6^{b}	26.1^{b}	1.10	0.03	<0.01	0.46
NSC (%DM)	10.6	8.7	0.85	1.7^{c}	9.8^{b}	17.4^{a}	0.97	0.07	<0.01	0.32
NFC (%DM)	30.6	28.5	1.12	22.2^{b}	31.7^{a}	34.8^{a}	1.37	0.22	<0.01	0.53
NFC (%CHO)	46.2	42.9	1.76	35.0^{b}	47.4^{a}	51.2^{a}	2.13	0.19	<0.01	0.64

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a-c}$ Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method; FM: fresh matter; DM: dry matter; EE: ether extracts (crude fat); CP: crude protein; OM: organic matter; SCP: soluble crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; aNDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; CHO: carbohydrate; NFC: non-fiber carbohydrate; NSC: non-structural carbohydrates; con: concentration; con: concentration.

Table 4.4. Effect of variety (tannin concentration) and stage of cutting on condensed tannin concentration of whole plant faba bean silage

Variety (V)	High Tannin Variety			Low Tannin Variety				P value		
Stage of Cutting (S)	Flower	Mid Pod	Late Pod	Flower	Mid Pod	Late Pod	SEM	Variety	Stage	V×S
CT (abs nm / mg)	0.117^{ab}	0.133^{ab}	0.136^{a}	0.092^{bc}	0.063^{cd}	0.046^{d}	0.0089	<0.01	0.33	0.01
CT (% DM)	0.070^{a}	0.083^{a}	0.085^{a}	0.050^{ab}	0.025^{bc}	0.010^{c}	0.0081	<0.01	0.21	0.01
CT (g/kg DM)	0.702^{a}	0.831^{a}	0.845^{a}	0.505^{ab}	0.250^{bc}	0.103^{c}	0.0725	<0.01	0.22	0.01
CT (mg/kg DM)	702.45^{a}	831.62^{a}	845.45^{a}	505.17^{ab}	250.71^{bc}	103.20^{c}	72.555	<0.01	0.23	0.01

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a-d}$ Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method. DM: dry matter; abs: units of absorbance at 550 nm; CT: condensed tannins.

ranged from 1.3 %DM at flower stage to 17.2 %DM at late pod stage (slightly lower values were observed in whole plant faba bean hay in Chapter 3), while the ash-corrected neutral acid detergent fiber (aNDFom), acid detergent fiber (ADF) and ADL were higher (P< 0.05) at flower stage than at mid pod and late pod stages which were similar (44.5 vs. 35.8 and 34.1 %DM; 43.4 vs. 33.5 and 31.1 %DM; 10.4 vs. 5.9 and 5.0 %DM respectively). The non-structural carbohydrates (NSC) ranged from 1.7 %DM at flower stage to 17.4 %DM at late pod stage. An interaction between the tannin concentration and the cutting stage was observed on the condensed tannin concentration (Table 4.4) of the whole plant faba bean silage. It was higher (P< 0.05) in the high tannin whole plant faba bean silage at flower, mid pod, and late pod stages than the low tannin at late pod stage. The low tannin at flower stage was similar than the high tannin whole plant faba bean silage at flower, mid pod, and late pod stages.

The condensed tannins in the whole plant faba bean silage are low and therefore they will not have any (positive or negative) impact on the animals. The condensed tannins in the whole plant faba bean hay (Chapter 3) were also low, however processing method may alter the condensed tannin concentration as we found higher condensed tannins in the silage than in the hay. Ruffino-Moya et al., (2019) totally found the opposite; the condensed tannins of Sainfoin and Sulla were higher when the forage was processed as hay. Huang et al., (2016) found a reduction in condensed tannins when Sanfoin was processed as silage than as hay.

Fraser et al., (2001) found that DM of faba bean silage of 10, 12 and 14 weeks old were 16.5, 21.6 and 19.7 %. The CP was 22.5, 20.5 and 20.5, respectively. In our study DM content was higher at late pod stage (28.5 %) which also is higher than the DM of faba bean silage 14 weeks of Fraser study. The CP in our study was similar (about 22 %DM) in all stages and seems to be similar to the CP of faba bean silage of 10 weeks old according to Fraser. The low DM content in our silage at flower stage may contributed with the high acetic acid concentration showed above in previous study. The sugars found by Fraser et al., (2001) averaged 1.7 %DM in the three silages, while in our silages averaged 0.3 %DM, the NDF averaged 42.3 %DM while in our silages averaged 38.5 %DM, additionally the starch content in our silages were higher (1.4, 9.4 and 17.2 %DM) than the ones found in Fraser silages (1.2, 3.4 and 8.0 %DM). Comparing with soybean and alfalfa silages Vargas Bello (2007) found that the CP, NDF, and NSC contents were (18.0 and 24.0 %DM; 46.0 and 45.0 %DM; 20.1 and 20.2 %DM). The CP of soybean silage is lower than the CP of whole plant faba bean silage, but it is comparable with the CP content found by Vargas Bello in alfalfa silage harvested at the early bloom stage. The NDF content is similar than the one we found at flower stage and higher than the ones we found a mid pod and late pod stages. The NSC are higher than the values we found in our study. According to Asekova et al., (2014) soybean harvested for forage before leaf drop has high CP content about 22 %DM, which is comparable with the CP value found in whole plant faba bean silage. Nair (2017) obtained CP, SCP, NDF, and starch values of six different barley silages which averaged 11.3, 7.1, 44.3, and 20.6 %DM, respectively. The CP and SCP contents found in whole plant faba bean silage in all thee stages are higher than the corresponding values found in barley silage, however barley silage has more NDF than whole plant faba bean silage at mid and late pod stages, but comparable to the NDF content of faba bean silage at flower stage. The starch content at late pod stage is comparable to the starch content Nair found in barley silage, while at flower and mid pod stages starch content is lower than in barley silage.

4.4.4 Energy Profile of Whole Plant Faba Bean Silages

The truly digestible nutrients, total digestible nutrients, and the energy values of whole plant faba bean silage are shown in Table 4.5. The tannin concentration did not affect ($P > 0.05$) any of those characteristics except for the truly digestible NDF (tdNDF) which was higher ($P < 0.05$) in the low tannin silage than the high tannin silage (15.7 vs 13.8 %). The cutting stage did affect the

Table 4.5. Effect of variety (tannin concentration) and stage of cutting on energy profile of whole plant faba bean silage

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid pod	Late Pod	SEM	Variety	Stage	V×S
Truly digestible nutrient (%DM)										
tdNFC	30.0	28.0	1.10	21.7^{b}	31.0^{a}	34.1^{a}	1.35	0.22	<0.01	0.53
tdCP	22.1	21.6	0.80	21.7	22.1	21.8	0.87	0.45	0.89	0.84
tdNDF	13.8^{b}	15.7^{a}	0.54	13.9	15.2	15.2	0.65	0.03	0.27	0.81
tdFA	0.7	1.0	0.15	1.0	1.2	0.3	0.17	0.13	<0.01	0.03
Total digestible nutrient (%DM)										
TDN_{1x}	60.5	60.4	1.20	52.6^{b}	64.1^{a}	64.8^{a}	1.35	0.94	<0.01	0.84
TDN_{3x}	55.6	55.5	1.10	48.3^{b}	58.8^{a}	59.5^{a}	1.24	0.95	<0.01	0.84
Energy value (Mcal/kg)										
DE_{1x}	2.85	2.83	0.061	2.51^{b}	2.99^{a}	3.02^{a}	0.068	0.84	<0.01	0.85
DE_{p3x}	2.61	2.60	0.056	2.31^{b}	2.75^{a}	2.77^{a}	0.062	0.86	<0.01	0.86
ME_{p3x}	2.19	2.18	0.057	1.88^{b}	2.33^{a}	2.35^{a}	0.064	0.88	<0.01	0.89
NE_{Lp3x}	1.35	1.34	0.040	1.13^{b}	1.45^{a}	1.46^{a}	0.045	0.85	<0.01	0.87
ME	2.33	2.32	0.051	2.06^{b}	2.46^{a}	2.48^{a}	0.056	0.82	<0.01	0.85
NE_{m}	1.46	1.45	0.046	1.20^{b}	1.57^{a}	1.59^{a}	0.051	0.87	<0.01	0.86
NE_{g}	0.87	0.86	0.042	0.64^{b}	0.97^{a}	0.98^{a}	0.047	0.85	<0.01	0.89

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a\text{-}c}$ Means with the different letters in the same row are significantly different ($P < 0.05$). Multi-treatment comparison: Tukey method; tdCP: truly digestible crude protein; tdFA: truly digestible fatty acid; tdNDF: truly digestible neutral detergent fibre; tdNFC, truly digestible non-fibre carbohydrate. $TDN_{1\times}$: total digestible nutrient at one times maintenance. $DE_{3\times}$: digestible energy at production level of intake (3×); $ME_{3\times}$: metabolizable energy at production level of intake (3×); $NE_{L3\times}$: net energy for lactation at production level of intake (3×); NE_{m}: net energy for maintenance; NE_{g}: net energy for gain; con: concentration.

truly digestible non-fibre carbohydrate (tdNFC) which was lower (P< 0.05) at flower stage than at mid pod and late pod stages which were similar (-9.3 and -12.4 %DM). Truly digestible fatty acids (tdFA) showed interaction (P< 0.05, variety × cutting stage, V×S). High tannin silage at late pod stage showed lower tdFA than the low tannin silage at mid pod stage (0.2 vs. 1.7 %DM). Low tannin silage at mid pod stage showed similar tdFA content to high tannin silage at flower and mid pod stages. The total digestible nutrients (TDN), net energy of lactation (NEL), and net energy of gain (NE_g) were similar a late pod and mid pod stages and those values were higher than the ones found at flower stage (+10.5 and +11.2 %DM; +0.32 and +0.33 Mcal/kg; +0.33 and +0.34 Mcal/kg respectively).

The total digestible nutrients (TDN) in soybean silage at full pod stage is 49 %DM, while in alfalfa silage at early bloom stage is 59 %DM. The net energy of lactation of soybean silage is 1.19 Mcal/kg, while the NE_L of alfalfa silage is 1.42 Mcal/kg (Vargas Bello, 2007). Comparing with whole plant faba bean silage at late pod stage, soybean silage, and alfalfa silage have lower TDN and lower NE_L which probably is due to their lower concentration of starch. A study conducted by Broderick (1985) showed that alfalfa silage has a NE_L of 1.35 Mcal/kg, while the corn silage has a NE_L of 1.60 Mcal/kg, however the NRC (2001) values showed that corn silage immature, normal and mature have a NE_L of 1.36, 1.45 and 1.35 Mcal/kg, respectively. Those values are lower than the NE_L of whole plant faba bean silage at late pod stage. Refat (2018) obtained a NE_L of 1.30, 1.40 and 1.30 Mcal/kg for CDC Cowboy, CDC Copland an Xena barley silages harvested at the mid-dough stage of maturity, respectively. Whole plant faba bean silage at mid pod and late pod stages showed higher NE_L than those values found in barley silage. However, TDN and NEL were slightly lower than in the hay (Chapter 3) mainly because of the slightly reduction of tdNDF and tdNFC.

4.4.5 Protein and Carbohydrate Subfractions in Whole Plant Faba Bean Silages

The crude protein and carbohydrate fractions of whole plant faba bean silages were partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS 6.5) and are presented in Table 4.6. Ammonia (PA1), slowly degradable true protein (PB2) and indigestible protein (PC) were not affected by the tannin concentration, while rapidly degradable true protein (PA2) and moderately degradable true protein (PB1) were significantly (P< 0.05) affected by the tannin concentration. The PA2 was higher (P< 0.05) and PB1 was lower (P< 0.05) in the low tannin variety than the high tannin variety whole plant faba bean silage (+9.3 and -9.9 %CP, respectively). The cutting stage effect affected significantly (P< 0.05) all CP fractions except for PA2. The PA1 was similar a mid pod and late pod stages and those values were lower (P< 0.05) than the value found at flower stage (-9.7 and -10.6 %CP). The PB1 was higher (P< 0.05) at late pod stage than at flower stage (+26.9 %CP), the value found at mid pod stage was similar to the one found at late pod stage. The PB2 and PC was higher (P< 0.05) at flower stage than at mid pod and late pod stage which were similar in both cases (+6.1 and +5.3 %CP; +8.0 and +7.9 %CP respectively). The carbohydrate fractions were only affected significantly (P< 0.05) by the cutting stage except for the rapidly degradable carbohydrate fraction (CA4) and the slowly degradable carbohydrate fraction (CB3). Volatile fatty acids (CA1) were similar in the whole plant faba bean silage at mid pod and late pod stages and those values were lower (P< 0.05) than the one found at flower stage (-16.1 and -16.9 %CHO). The intermediately degradable carbohydrate fraction (CB1) was ranged from 2.1 %CHO at flower stage to 25.4 %CHO at late pod stage. The intermediately degradable carbohydrate fraction (CB2) was lower (P< 0.05) at late pod stage than at mid pod stage (-16.5 %CHO), while the CB2 fraction at late pod stage was similar to those two previous values. The unavailable neutral detergent fiber (CC) was higher (P< 0.05) at flower stage than the values found at the mid pod stage and late pod stage which were similar (+12.7 and +16.2 %CHO respectively).

Table 4.6. Effect of variety (tannin concentration) and stage of cutting on CNCPS fractions of whole plant faba bean silage

Item	Variety (V, Tannin con.)			Stage of Cutting (S)				P value		
	H	L	SEM	Flower	Mid pod	Late Pod	SEM	Variety	Stage	V×S
Protein fractions										
PA1 (%CP)	8.8	10.3	1.44	16.3^{a}	6.6^{b}	5.7^{b}	1.69	0.42	<0.01	0.91
PA2 (%CP)	61.9^{b}	71.2^{a}	1.32	68.6	66.0	65.1	1.62	<0.01	0.32	0.60
PB1 (%CP)	19.6^{a}	9.7^{b}	2.71	-3.1^{b}	23.2^{a}	23.8^{a}	3.32	0.02	0.01	0.58
PB2 (%CP)	3.2	3.0	0.61	6.9^{a}	0.8^{b}	1.6^{b}	0.75	0.82	<0.01	0.26
PC (%CP)	6.4	5.8	0.71	11.3^{a}	3.3^{b}	3.7^{b}	0.84	0.49	<0.01	0.28
True Protein (%CP)	84.7	83.9	2.09	72.3^{b}	90.1^{a}	90.6^{a}	1.14	0.76	<0.01	0.89
PA2 (%true protein)	75.2	85.7	3.47	96.2^{a}	73.4^{b}	71.9^{b}	4.25	0.05	<0.01	0.79
PB1 (%true protein)	20.4	10.4	3.98	-5.8^{b}	25.7^{a}	26.3^{a}	4.87	0.10	<0.01	0.66
PB2 (%true protein)	4.4	3.9	0.79	9.7^{a}	0.9^{b}	1.8^{b}	0.97	0.67	<0.01	0.24
Carbohydrate fractions										
CHO (%DM)	66.0	66.2	1.01	63.4^{b}	66.8^{a}	68.1^{a}	1.10	0.82	0.01	0.16
CA1 (%CHO)	7.3	10.1	1.72	19.7^{a}	3.6^{b}	2.8^{b}	2.11	0.28	<0.01	0.57
CA4 (%CHO)	0.5	0.5	0.13	0.6	0.6	0.3	0.16	0.99	0.29	0.51
CB1 (%CHO)	15.4	12.3	1.33	2.1^{c}	14.1^{b}	25.4^{a}	1.50	0.06	<0.01	0.34
CB2 (%CHO)	23.0	19.9	2.76	12.6^{b}	29.1^{a}	22.7^{ab}	3.37	0.44	0.01	0.77
CB3 (%CHO)	29.6	32.8	1.40	31.1	31.3	31.1	1.57	0.05	0.99	0.07
CC (%CHO)	24.2	24.4	1.85	33.9^{a}	21.2^{b}	17.7^{b}	2.08	0.91	<0.01	0.05

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a-c}$ Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison: Tukey method; PA1: ammonia; PA2: soluble true protein (rapidly degradable true protein); PB1: insoluble true protein (moderately degradable true protein); PB2: fiber-bound protein (slowly degradable true protein); PC: indigestible protein; CHO: carbohydrate; CA1: volatile fatty acids (Acetic + Propionic + Butyric + Isobutyric); CA4: water soluble carbohydrates (rapidly degradable carbohydrate fraction); CB1: starch (intermediately degradable carbohydrate fraction); CB2: soluble fiber (intermediately degradable carbohydrate fraction); CB3: digestible fiber (available neutral detergent fiber or slowly degradable carbohydrate fraction); CC: indigestible fiber (unavailable neutral detergent fiber); con: concentration.

The CNCPS library showed that the protein fractions of alfalfa silage (38 %NDF, 20 %CP) are PA1 8.0 %CP; PA2, 53.7 %CP; PB1, 24.1 %CP; PB2, 7.5 %CP; PC, 6.6 %CP, and barley silage (56 %NDF; 10 %CP) are PA1 6.1 %CP; PA2 56.7 %CP; PB1, 19.4 %CP; PB2, 10.3 %CP; PC, 6.2 %CP. The carbohydrate fractions available in the CNCPS library for alfalfa silage (38 %NDF, 20 %CP) are CA1 9.3 %CHO; CA4, 6.4 %CHO; CB1, 0.9 %CHO; CB2, 11.5 %CHO; CB3, 38.5 %CHO; CC, 19.9 %CHO and barley silage (56 %NDF; 10 %CP) are CA1 6.8 %CHO; CA4, 2.4 %CHO; CB1, 7.3 %CHO; CB2, 1.4 %CHO; CB3, 53.3 %CHO; CC, 19.3 %CHO. The PA1 of alfalfa silage is higher than the PA1 value found in whole plant faba bean silage at mid and late pod stages, while the PA1 value of barley silage is comparable to the values of whole plant faba bean silage at mid and late pod stages. The PA1 value at flower stage is higher (more than 2 times) than the PA1 at mid pod and late pod stages and than the PA1 values of alfalfa and barley silages. The PA2 of whole plant faba bean silage averaged 66.5 %CP which is higher than the PA2 values of alfalfa and barley silages. The PB1 of alfalfa and barley silages are comparable to the PB1 values of whole plant faba bean silage at mid and late pod stages, however they are higher than the PB1 value in whole plant faba bean silage at flower stage and probably it is due to the higher level of protein degradation in the fermentation process which resulted in a higher concentration of PA1. The PB2 fraction of alfalfa is comparable to the PB2 fraction of whole plant faba bean silage, PB2 of barley silage is higher than the PB2 value of whole plant faba bean silage at flower, mid pod, and late pod stages. The PC fraction of whole plant faba bean silage at mid pod and late pod stages are similar but lower than PC at flower stage or PC values of alfalfa and barley silages. Whole plant faba bean silage at flower stage broke down more protein during fermentation and additionally has higher concentration of indigestible protein which are negative factors to be considered as good quality silage. Alfalfa silage showed a low CB1 content compared with, barley silage and whole plant faba bean silage at mid pod and late pod stages. CB1 fraction of whole plant faba bean silage at flower stage is almost zero. Alfalfa and barley silages showed higher CB3 values than whole plant faba bean silage. The CC fraction of whole plant faba bean silage at flower stage is higher than the values of alfalfa, barley and faba silage at mid pod and pod stages, which indicates that can provide less energy from fiber. Refat (2018) found that the protein fractions for CDC Cowboy, CDC Copland, and Xena barley silages (harvested at the mid-dough stage of maturity) were PA1 13.9, 12.6 and 13.8 %CP; PA2 51.5, 51.8 and 45.7 %CP; PB1, 23.1, 24 and 28.1 %CP; PB2, 2.3, 1.6 and 0.6 %CP and PC, 9.3, 10.1 and 11.8 %CP, respectively, while the carbohydrate fraction were CB1 11.2, 16.9 and 19.5 %CHO; CB2 6.9, 10.2 and 13.1 %CHO; CB3 33.8, 32.5 and 37.3 %CHO and CC 30.1, 22.4 and 22.8 %CHO, respectively. The PA1 values of those three varieties of barley silage are higher than the PA1 values of whole plant faba bean silage at mid and late pod stages. However, all those previous PA1 values are lower than PA1 value of whole plant faba bean silage at flower stage. These suggested the protein breakdown during fermentation process was higher in whole plant faba bean silage at flower stage than the other silages. PA2 values of barley silages are lower than the PA2 values of whole plant faba bean silage in any cutting stage. PB1 values of whole plant faba bean silage at mid and late pod stages are comparable to the PB1 values of those barley silages, which are higher than PB1 value of whole plant faba bean silage at flower stage. PB2 value of whole plant faba bean silage at flower stage was higher than PB2 value of barley silages but have comparable PC fraction. Whole plant faba bean silage at mid and late pod stages seems that went under a better fermentation process than the one at flower stage. At flower stage the breakdown of protein is higher, that is why it has a higher PA1 fraction than the whole plant faba bean silages at mid and late pod stages. It is noticed that the PA2 fraction is higher than the PA2 fraction found in barley silage which can be beneficial for rumen microbial production if energy levels are adequate, otherwise possible N loss can be observed. Xena silage showed higher CB1 content, however that value is lower than the CB1 value found in whole plant faba bean silage at late pod stage. In those barley silages, the CB2 fraction is lower than the CB2 value found in whole plant faba bean silage at late pod stage, while the CB3 values are similar, but whole plant faba bean silage at late pod stage showed a lower CC fraction than in barley silage. Consequently, faba bean silage at late pod can provide more energy from the CHO fractions than barley silage at mid-dough stage of maturity. Additionally, whole plant faba bean silage has lower PB1, CB2, CB3 but higher PB2 and CB1 than whole plant faba bean silage hay.

4.4.6 In Situ DM Degradation Kinetics of Whole Plant Faba Bean Silages

The effect of tannin concentration and cutting stage on the degradation characteristics of DM of whole plant faba bean silage are presented in Table 4.7. Interactions ($P < 0.05$) between tannin concentration and cutting stage were observed in rate of degradation (Kd), rumen bypass or undegraded feed dry matter (RUDM), and effective degraded dry matter (EDDM) from the *in situ* DM degradation. The high tannin variety at late pod stage had a higher ($P < 0.05$) Kd than the low tannin at mid pod and late pod stages which were similar (+4.20 and +4.98 %/h respectively), the Kd value of high tannin variety at mid pod stage was similar to the one found in the high tannin variety at late pod stage.

The low tannin variety at late pod stage had a similar RUDM to the corresponding values of the high tannin variety at mid pod and late pod stages, however the low tannin variety at late pod stage had a higher ($P < 0.05$) RUDM than the low tannin silage at mid pod stage (+3.1 % or +32 g/kg DM), while the EDDM of the low tannin variety silage at mid pod stage was higher ($P < 0.05$) than the EDDM values found in the high tannin variety silage at mid pod stage and than the low tannin variety silage at late pod stage. (+3.0 % or +30 g/kg DM and +3.1 % or +31 g/kg DM, respectively).

The degradation rate, and the effective degradability of dry matter in faba bean, soybean and pea silages are (7.00, 8.70 and 8.90 %/h; 66.2, 68.5 and 69.1%, respectively) (Mustafa and Seguin, 2003a). Those Kd values are comparable to the Kd values of the low tannin variety at mid pod and

Table 4.7. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics (dry matter) of whole plant of faba bean silage

Variety (V)	High Tannin Variety		Low Tannin Variety			P Value		
Stage of Cutting (S)	Mid pod	Late Pod	Mid pod	Late Pod	SEM	Variety	Stage	V×S
Dry matter								
Kd (%/h)	9.45^{ab}	12.32^{a}	8.12^{b}	7.34^{b}	0.840	<0.01	0.13	0.02
Residue at 0 h (%)	69.6	74.5	62.9	70.3	1.67	<0.01	<0.01	0.14
S (%)	30.4	25.5	37.1	29.7	1.67	<0.01	<0.01	0.14
D (%)	44.6	49.8	41.0	50.6	2.25	0.43	<0.01	0.24
U (%)	25.0	24.7	22.0	19.8	1.25	0.02	0.33	0.45
%BDM=%RUDM	42.4^{a}	41.2^{ab}	39.4^{b}	42.5^{a}	1.49	0.35	0.30	0.04
RUDM (g/kg DM)	424^{a}	412^{ab}	394^{b}	425^{a}	14.9	0.35	0.30	0.04
%EDDM	57.6^{b}	58.8^{ab}	60.6^{a}	57.5^{b}	1.49	0.35	0.30	0.04
EDDM (g/kg DM)	576^{b}	588^{ab}	606^{a}	575^{b}	14.9	0.35	0.30	0.04

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a\text{-}c}$ Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method; Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the *in situ* incubation; D: degradable fraction; U: rumen undegradable fraction; BDM or RUDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter.

late pod stages, however the Kd values of the high tannin variety silage at mid pod and late pod stages are slightly higher than the ones found by Mustafa and Seguin. The EDDM in faba bean soybean and pea silages are higher than the values found in whole plant faba bean silage which averaged 58.6 %. Therefore, faba bean silage in our study has a potential ability to contribute with more bypass fraction to be digested and absorbed in the small intestine.

In the study of Mustafa and Seguin (2003a), faba bean was harvested at 75 days old, while in our study were harvested at 88 and 97 days old for mid pod and late pod stages, respectively, the higher EDDM can be due to the stage of maturity in which faba bean was harvested, the more mature the less degradable in the rumen. According to Mustafa, Christensen and McKinnon (2000), pea, alfalfa and barley silages have Kd values of 5.80, 8.00 and 3.00 %/h and EDDM values of 67.0, 66.4 and 53.9 % respectively. Additionally, whole plant faba bean silage had lower EDDM consequently higher RUDM than whole plant faba bean hay (Chapter 3).

Comparing with the Kd value of whole plant faba bean silage which averaged 9.30 %/h, we can say that it is comparable to the Kd value of alfalfa silage, but it is higher than the Kd value found in pea and barley silages. The EDDM in whole plant faba bean silage ranged from 57.5 to 60.6 % which are lower than the ones observed in pea and alfalfa silage. Based on these results, whole plant faba bean silage can provide higher bypass DM than pea silage harvested at full-pod stage and alfalfa silage harvested at early bloom. Vargas Bello (2007) found that the Kd value of soybean (harvested at full pod stage) and alfalfa (harvested at early bloom stage) silages are 6.00 and 8.00%/h, while had EDDM values of 53.3 and 63.9 %. The Kd value of whole plant faba bean silage in our study ranged from 7.34 to 12.32 %/h, those values are higher than the Kd value of soybean silage, while the Kd value of alfalfa is within that range. The EDDM in whole plant faba bean silage averaged 58.6 % which is higher than the EDDM of soybean silage and lower than the EDDM of alfalfa silage.

4.4.7 *In Situ CP Degradation Kinetics*

Characteristics of rumen degradation kinetics of crude protein of the whole plant faba bean silages are presented in Table 4.8. The rate of degradation of crude protein (Kd) and the undegradable fraction (U) were not significantly ($P > 0.05$) affected by either tannin concentration effect or cutting stage effect. The Kd showed interaction ($P < 0.05$, variety × cutting stage, V×S). The Kd of low tannin silage at mid pod stage is higher than the Kd value of high tannin silage at mid pod stage and the low tannin silage at late pod stage (19.14 vs. 13.85 and 13.39 %/h, respectively), the Kd of low tannin silage at mid pod stage is similar to the high tannin silage at late pod stage. The soluble fraction of crude protein (S) was lower ($P < 0.05$) in the high tannin silage than in the low tannin silage (-11.8 %), while the degradable fraction (D) was higher ($P < 0.05$) in the high tannin silage than in the low tannin silage (+11.7 %). Additionally, the tannin concentration affected significantly both the bypass crude protein BCP^{DVE} and the rumen undegradable crude protein RUP^{NRC}, the low tannin variety silage showed lower ($P < 0.05$) BCP and RUP than the high tannin variety silage (28 and 25 vs. 37 and 33 g/kg DM, respectively). The soluble fraction of crude protein (S) was higher ($P < 0.05$) at mid pod stage than that at late pod stage (+13.3 %), while the degradable fraction (D) was lower ($P < 0.05$) at mid pod stage than that at late pod stage (-13.1 %). At late pod stage the BCP^{DVE} and RUP^{NRC} were higher ($P < 0.05$) than that at mid pod stage (+9 and +8 g/kg DM, respectively), consequently the effectively degraded of crude protein (EDCP) was lower ($P < 0.05$) at late pod stage than at mid pod stage (188 vs. 199 g/kg DM).

According to Mustafa and Seguin (2003b), Berseem clover (*Trifolium alexandrinum L.*) silage (harvested at the vegetative stage) and alfalfa silage (harvested at the early bloom stage) showed a Kd of 6.8 and 9.4 %/h; a soluble fraction of 46.1 and 75.5 %; a degradable fraction of 45.5 and

Table 4.8. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics (crude protein) of whole plant faba bean silage

Item	Variety (V, Tannin con.)			Stage of Cutting (S)			P value		
	H	L	SEM	Mid pod	Late Pod	SEM	Variety	Stage	V×S
Crude Protein									
CP (g/kg DM)	227	219	5.4	224	221	5.4	0.11	0.53	0.68
Kd (%/h)	15.55	16.27	1.051	16.50	15.32	1.051	0.64	0.45	0.02
Residue (0 h, %)	37.9^{a}	26.1^{b}	1.58	25.3^{b}	38.6^{a}	1.58	<0.01	<0.01	0.14
S (%)	62.1^{b}	73.9^{a}	1.58	74.7^{a}	61.4^{b}	1.58	<0.01	<0.01	0.14
D (%)	32.1^{a}	20.4^{b}	1.70	19.7^{b}	32.8^{a}	1.70	<0.01	<0.01	0.23
U (%)	5.8	5.7	0.45	5.7	5.8	0.45	0.75	0.64	0.56
%BCP=%RUP	14.7^{a}	11.4^{b}	0.69	11.1^{b}	15.1^{a}	0.69	<0.01	<0.01	0.33
BCP (g/kg DM, DVE)	37^{a}	28^{b}	1.9	28^{b}	37^{a}	1.9	<0.01	<0.01	0.60
RUP (g/kg DM, NRC)	33^{a}	25^{b}	1.7	25^{b}	33^{a}	1.7	<0.01	<0.01	0.60
%EDCP=%RDP	85.3^{b}	88.6^{a}	0.69	88.9^{a}	84.9^{b}	0.69	<0.01	<0.01	0.33
EDCP=RDP (g/kg DM)	193	194	5.0	199^{a}	188^{b}	5.0	0.87	0.02	0.50

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a-e}$ Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison: Tukey method; Kd: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; S: soluble fraction in the *in situ* incubation; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded of crude protein; con: concentration.

17.5%; an effective degradability of CP of 72.3 and 86.8 %. Compared with whole plant faba bean silage Berseem clover and alfalfa silages have lower Kd value. The soluble fraction found in whole plant faba bean silage in our study is comparable to the soluble fraction of alfalfa silage but higher than the soluble fraction of Berseem clover. The degradable fraction found in faba silage at mid pod stage is comparable to the degradable fraction of alfalfa, and the degradable fraction found in whole plant faba silage at late pod stage is slightly lower than the degradable fraction of Berseem clover. The effective degradability of CP in whole plant faba bean silage is about 86 %, which is similar to the effective degradability of CP found in alfalfa silage and higher than the value found in Berseem clover silage. Other study focused on faba bean, soybean and pea silage in which the Kd values were 13.2, 12.6 and 7.7 %/h; the soluble fraction 49.4, 59.4 and 70.3 %; the degradable fraction 41.5, 32.0 and 22.0 %; effective degradability of CP 79.4, 82.2 and 83.6 %, respectively (Mustafa and Seguin, 2003a). Additionally, whole plant faba bean silage had higher S fraction consequently lower RUP than whole plant faba bean hay (Chapter 3). The Kd values of whole plant faba bean silage at mid pod and late pod stages are comparable to the Kd values of faba bean and soybean silage presented by Mustafa and Seguin (2003) but higher than the Kd value of pea silage. The soluble fraction of whole plant faba bean silage in our study is comparable to the soluble fraction of pea silage and higher than the Kd values of faba silage and soybean silage, while the degradable fraction of whole plant faba bean silage at late pod stage is comparable to the degradable fraction of soybean silage. The effective degradability of CP of faba bean, soybean and pea silage and whole plant faba bean silage at mid pod and late pod stages seems to be comparable. In other study Mustafa et al., (2002) found an effective degradability of CP of three varieties of pea silage (harvested at full-pod stage) was averaged 88 %. Refat (2018) found that the Kd value of three different varieties of barley silage averaged 6.1 %/h, while the soluble fraction, degradable fraction, rumen undegradable protein, and effective degradability of CP are 48.6 %, 18.8 %, 57.6 g/kg DM and 78.6 g/kg DM, respectively. The Kd and the soluble fraction are higher in whole plant faba bean silage than the values found in barley silage, the degradable fraction of barley silage is comparable to the degradable fraction of whole plant faba bean silage at mid pod stage but is lower than the value found at late pod stage. The rumen undegradable protein (g/kg DM) is higher in barley silage, while the effective degradability of CP (g/kg DM) is lower. It is important to consider that barley silage have about the half of protein than faba bean silage does.

4.4.8 In Situ NDF Degradation Kinetics of Whole Plant Faba Bean Silages

The effect of tannin concentration and stage of cutting on NDF rumen degradation characteristics and uNDF at 288h incubation are presented in Table 4.9. The tannin concentration did not significantly affect the rumen degradation characteristics of NDF except for degradable fraction (D) and undegradable fraction (U). Low tannin variety silage showed higher ($P < 0.05$) D fraction and lower ($P < 0.05$) S fraction than high tannin variety silages (51.8 vs. 39.5 and 34.2 vs. 48.6 %, respectively). The cutting stage did not affect significantly ($P > 0.05$) any of the rumen degradation characteristics of NDF, but it was found that the effective degraded neutral detergent fiber (EDNDF) was 31.0 and 30.5 %, and the undigestible neutral detergent fiber (uNDF) was 14.2 and 12.0 % DM for whole plant faba bean silage at mid pod and late pod stages, respectively.

Pea silage (harvested at full-pod stage), alfalfa silage (harvested at early bloom), and barley silage (harvested at mid dough stage) have Kd values of NDF of 3.60, 4.90 and 2.50 %/h; soluble fractions 0.6, 4.7 and 4.8 %; degradable fractions 51.5, 44.9 and 58.2 %; effective degradability 23.5, 27.0 and 21.9 %, respectively (Mustafa, Christensen and McKinnon 2000). The Kd value of whole plant faba bean silage at mid pod and late pod stages averaged 4.20 %/h which is comparable to the Kd value of pea and alfalfa silage but is higher than the Kd of barley silage. The soluble fractions of whole plant faba bean silage are almost three times higher than the ones found in alfalfa and barley silage, while the degradable fraction in whole plant faba bean silage is slightly lower

Table 4.9. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics (NDF) of whole plant faba bean silage

Item	Variety (V, Tannin con.)			Stage of Cutting (S)			P value		
	H	L	SEM	Mid pod	Late Pod	SEM	Variety	Stage	V×S
Fiber (NDF) Degradation									
NDF (g/kg DM)	333	372	13.9	360	344	13.9	0.09	0.44	0.68
Kd (%/h)	4.95	3.46	0.623	4.16	4.25	0.623	0.13	0.93	0.43
Residue (0h, %)	88.1	86.0	4.00	87.0	87.2	4.00	0.62	0.97	0.31
S (%)	11.9	14.0	4.00	13.0	12.8	4.00	0.62	0.97	0.31
D (%)	39.5^{b}	51.8^{a}	3.98	44.4	46.9	3.98	0.04	0.61	0.20
U (%)	48.6^{a}	34.2^{b}	2.36	42.6	40.3	2.36	<0.01	0.50	0.51
%BNDF=%RUNDF	71.0	67.5	2.34	69.0	69.5	2.34	0.30	0.87	0.35
RUNDF (g/kg DM, NRC)	236	251	11.4	247	240	11.4	0.36	0.70	0.34
%EDNDF=%RDNDF	29.0	32.5	2.34	31.0	30.5	2.34	0.30	0.87	0.35
EDNDF=RDNDF (g/kg DM)	98	120	10.7	113	104	10.7	0.12	0.48	0.55
uNDF (288 h, CNCPS 6.5) (% DM)	11.8	14.5	1.28	14.2	12.1	1.28	0.17	0.28	0.33

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a-e}$ Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method; Kd: the degradation rate of D fraction; S: washable fraction; D: degradable fraction; U: rumen undegradable fraction; BDNDF or RUNDF: rumen bypass or undegraded feed neutral detergent fiber; EDNDF or RDNDF: effective degraded neutral detergent fiber. uNDF: undigestible neutral detergent fiber; con: concentration.

than the degradable fractions of pea, alfalfa and barley silages, but the effective degradability of NDF is higher in whole plant faba bean silage (averaged 30.8 %). Based on these results we can imply that animals can obtain more energy from the NDF of faba bean silage than from the NDF of pea, alfalfa, and barley silages. A study suggested that the effective degradability of NDF of faba bean silage, soybean silage and pea silage are 34.2, 35.5 and 34.5 respectively (Mustafa and Seguin, 2003a). These values are slightly higher than the values obtained in our study and the reason probably is the stage of maturity in which faba bean, soybean and pea were harvested (between the beginning seed to full seed stages), the closes value in our study was found in whole plant faba bean silage at mid pod stage (31%). Pea silage (harvested a at full-pod stage) had an effective degradability of 23.5 % (Mustafa et al., 2000), while pea silage (harvested between the beginning seed to full seed stages) had an effective degradability of 34.5 %. Based on those results and the results from our study, the maturity plays an important role when the NDF is degraded in the rumen. Refat (2018) found that the averaged uNDF of three varieties of barley silage is 19 %DM, while in our study whole plant faba bean silage at mid pod stage is 14.2 %DM and at late pod stage is 12. 1 %DM. Again, whole plant faba bean silage has a higher portion of NDF which can be utilized (degraded) by the microorganisms in the rumen than barley silage. Additionally, whole plant faba bean silage had lower S fraction and lower EDNDF than whole plant faba bean hay (Chapter 3).

4.4.9 In Situ Starch Degradation Kinetics of Whole Plant Faba Bean Silages

The effect of tannin concentration and cutting stage on starch rumen degradation characteristics are presented in Table 4.10. The tannin concentration did not significantly affect any of the rumen degradation characteristics of starch, however the cutting stage did affect significantly ($P< 0.05$) some characteristics. The rate of degradation of starch (Kd) was higher ($P< 0.05$) at mid pod stage than at late pod stage (18.96 vs. 15.50 %/h). The soluble fraction of starch

Table 4.10. Effect of variety (tannin concentration) and stage of cutting on rumen degradation kinetics (Starch) of whole plant faba bean silage

Item	Variety (V, Tannin con.)		SEM	Stage of Cutting (S)		SEM	P value		
	H	L		Mid pod	Late Pod		Variety	Stage	V×S
Starch									
St (g/kg DM)	147	119	11.7	94[b]	172[a]	11.7	0.09	<0.01	0.65
Kd	18.22	16.24	2.003	18.96[a]	15.50[b]	2.003	0.07	0.01	0.10
Residue (0 h, %)	64.9	64.0	5.98	70.9	57.9	5.98	0.92	0.16	0.30
S (%)	35.1	36.1	5.98	29.1	42.1	5.98	0.92	0.16	0.30
D (%)	64.1	63.0	5.76	69.6	57.5	5.76	0.89	0.18	0.29
U (%)	0.8	1.0	0.40	1.3	0.4	0.40	0.72	0.15	0.75
%BSt	16.9	17.9	1.83	18.0	16.8	1.83	0.68	0.61	0.66
BSt (g/kg DM)	27	23	2.8	18[b]	32[a]	2.8	0.32	0.01	0.83
%EDSt	83.1	82.1	1.83	82.0	83.2	1.83	0.68	0.61	0.66
EDST (g/kg DM)	123	98	10.8	78[b]	143[a]	10.8	0.09	<0.01	0.57

H: high tannin variety; L: low tannin variety; 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: Tukey method; Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction; D: degradable fraction; U: rumen undegradable fraction; BSt: rumen bypass or undegraded feed starch; EDST: effective degraded starch; con: concentration.

(S), the degradable fraction (D) and the undegradable fraction (U) were similar (P>0.05) at mid pod and late pod stages, but the rumen bypass or undegraded feed starch (BSt) and the effective degraded starch (EDST) were higher (P< 0.05) at late pod stage (+14 and +65 g/kg DM) and that is due to its higher starch content.

In comparison with whole plant faba bean hay at mid pod and late pod stages, we found that the Kd of starch is comparable with the Kd of silages at same stage of maturity, but the soluble fraction is higher and the degradable fraction is lower in whole plant faba bean hay, than in whole plant faba bean silage (53.0 % vs. 35.0 % and 47.0 % vs. 63.0 %, respectively). The rumen bypass starch is higher in whole plant faba bean silage at mid pod and late pod stages (averaged 25 g/kg DM) than the rumen bypass starch in whole plant faba bean hay at mid pod and late pod stages (averaged 16 g/kg DM) (Guevara-Oquendo et al., unpublished, In Chapter 3). Therefore, whole plant faba bean silage can provide higher starch to be absorbed in the small intestine than whole plant faba bean hay. Mustafa et al., (2000) studied the rumen degradation kinetics of pea silage (harvested at full-pod stage) and barley silage (harvested at mid dough stage). They found that the Kd, soluble fraction, degradable fraction, and effective degradability of starch are: 8.6 and 40.6 %/h; 63.0 and 88.7 %, 37.8 and 9.8 %; 86.3 and 97.4 %, respectively. Comparing with whole plant faba bean silage at mid pod and late pod stages, the Kd value of pea silage is lower, while the Kd value of barley silage is higher. The soluble fraction of whole plant faba bean silage at mid pod stage is 29 % while is 42% in whole plant faba bean silage a late pod stage, those values are lower than the ones found in pea and barley silages. The degradable fraction is higher in whole plant faba bean silage than in pea and barley silages, but the degradability of starch of whole plant faba bean silage seems to be comparable to the one found in pea silage but slightly lower than the one found in barley silage. Therefore, whole plant faba bean silage has a potential higher contribution of rumen bypass starch than barley silage. Additionally, whole plant faba bean silage had higher BSt than whole plant faba bean hay (Chapter 3).

4.4.10 Hourly Effective Degradation Ratios between Available N and Available CHO

The effect of tannin concentration on the hourly effective degradation of whole plant faba bean silage is shown in Figure 4.1 and the effect of cutting stage on hourly effective degradation of whole plant faba bean silage is presented in Figure 4.2. The hourly effective degradation ratios between available N and available carbohydrates (ED ratio of N/CHO) at different incubation times are presented in Table 4.11. The overall ratio of N/CHO and the overall ratio of ED_N/ED_CHO were not significantly (P> 0.05) affected by the tannin concentration, however those values were significantly (P< 0.05) affected by the cutting stage showing higher (P< 0.05) values at mid pod than at late pod stage (+10 and +35 g/kg, respectively). At individual hours from h3 to h22, the ED_N/ED_CHO were higher (P< 0.05) in the high tannin whole plant faba bean silage than the low tannin whole plant faba bean silage (+22, +23, +22, +23, +21, +19, +17, +15, +12 and +10 g/kg, respectively).The cutting stage affected the individual ED_N/ED_CHO from h4 to h14 showing lower (P< 0.05) values at mid pod stage than at late pod stage (-19, -17, -15, -14, -13 and -11 g/kg, respectively).The ED_N/ED_CHO values in the high tannin whole plant faba bean silage and in the low tannin whole plant faba bean silage seemed to decrease linearly or gradually from h0 till reach a value below than the optimal at h16 (high tannin silage) and h10 (low tannin silage). High ED_N/ED_CHO values were observed at h0 and immediately after we can notice that the high tannin whole plant faba bean silage keeps higher numerical values after 24 hours. Also it is important to notice that low tannin whole plant faba bean silage has a numerically higher ED_N/ED_CHO at h0 which can be related to the higher content of soluble crude protein and numerically higher concentration of ammonia (NH_3) that immediately release N, therefore a faster effective degradation of N is seen than in the high tannin whole plant faba bean silage.

Table 4.11. Effect of variety (tannin concentration) and stage of cutting on potentially available N to available CHO synchronization of whole plant faba bean silage

Item	Variety (V, Tannin con.)			Stage of Cutting (S)			P value		
	H	L	SEM	Mid pod	Late Pod	SEM	Variety	Stage	V×S
Ratio of N/CHO (g/kg)	76	72	1.9	79[a]	69[b]	1.9	0.16	<0.01	0.29
Ratio of ED_N/ED_CHO (g/kg)	127	122	3.4	142[a]	107[b]	3.4	0.38	<0.01	0.58
Ratio at individual h (g/kg)									
h0	285	316	70.5	411	190	70.5	0.76	0.06	0.98
h1	82	62	7.8	61	82	7.8	0.10	0.09	0.52
h2	79	58	6.7	59	79	6.7	0.06	0.07	0.57
h3	76[a]	54[b]	5.8	55	75	5.8	0.03	0.05	0.69
h4	73[a]	50[b]	4.9	52[b]	71[a]	4.9	0.01	0.03	0.69
h6	66[a]	42[b]	3.5	45[b]	62[a]	3.5	<0.01	0.01	0.90
h8	57[a]	34[b]	2.6	38[b]	53[a]	2.6	<0.01	<0.01	0.73
h10	49[a]	28[b]	2.4	32[b]	46[a]	2.4	<0.01	<0.01	0.57
h12	41[a]	22[b]	2.6	25[b]	38[a]	2.6	<0.01	0.01	0.48
h14	34[a]	17[b]	2.9	20[b]	31[a]	2.9	<0.01	0.03	0.56
h16	28[a]	13[b]	3.1	16	25	3.1	0.01	0.07	0.66
h18	22[a]	10[b]	3.1	12	20	3.1	0.03	0.11	0.77
h20	18[a]	8[b]	3.1	10	16	3.1	0.05	0.17	0.77
h22	15	6	2.9	8	13	2.9	0.07	0.21	0.90
h24	12	5	2.7	6	10	2.7	0.11	0.26	0.93

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; [a-e] Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method; ED: effective degradability; CHO: carbohydrates; con: concentration.

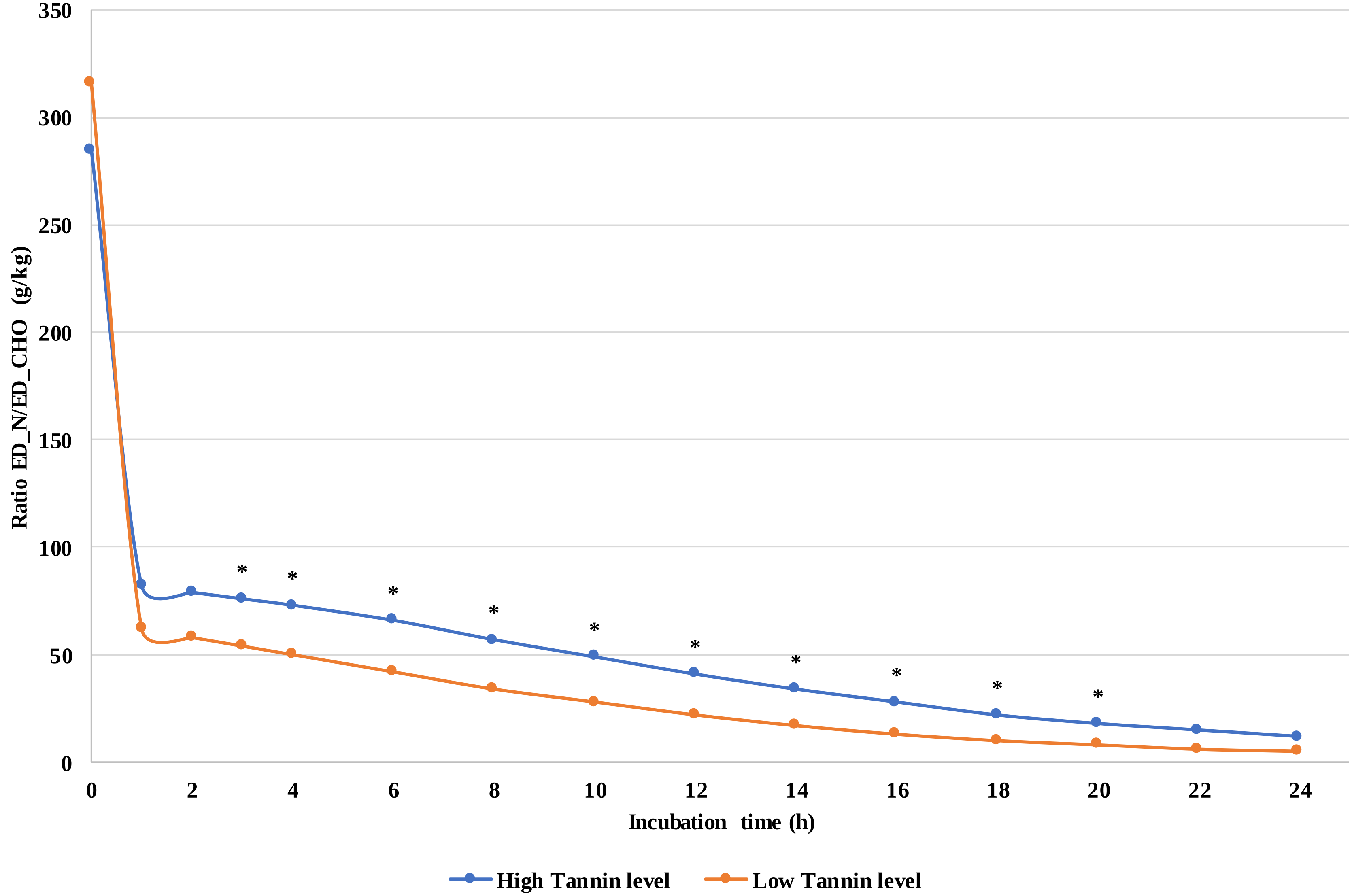


Figure 4.1. Effect of tannin concentration on hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of whole plant faba bean silage. Optimum ratio = 32 EN/ECHO g/kg. * Means are significantly different (P< 0.05).

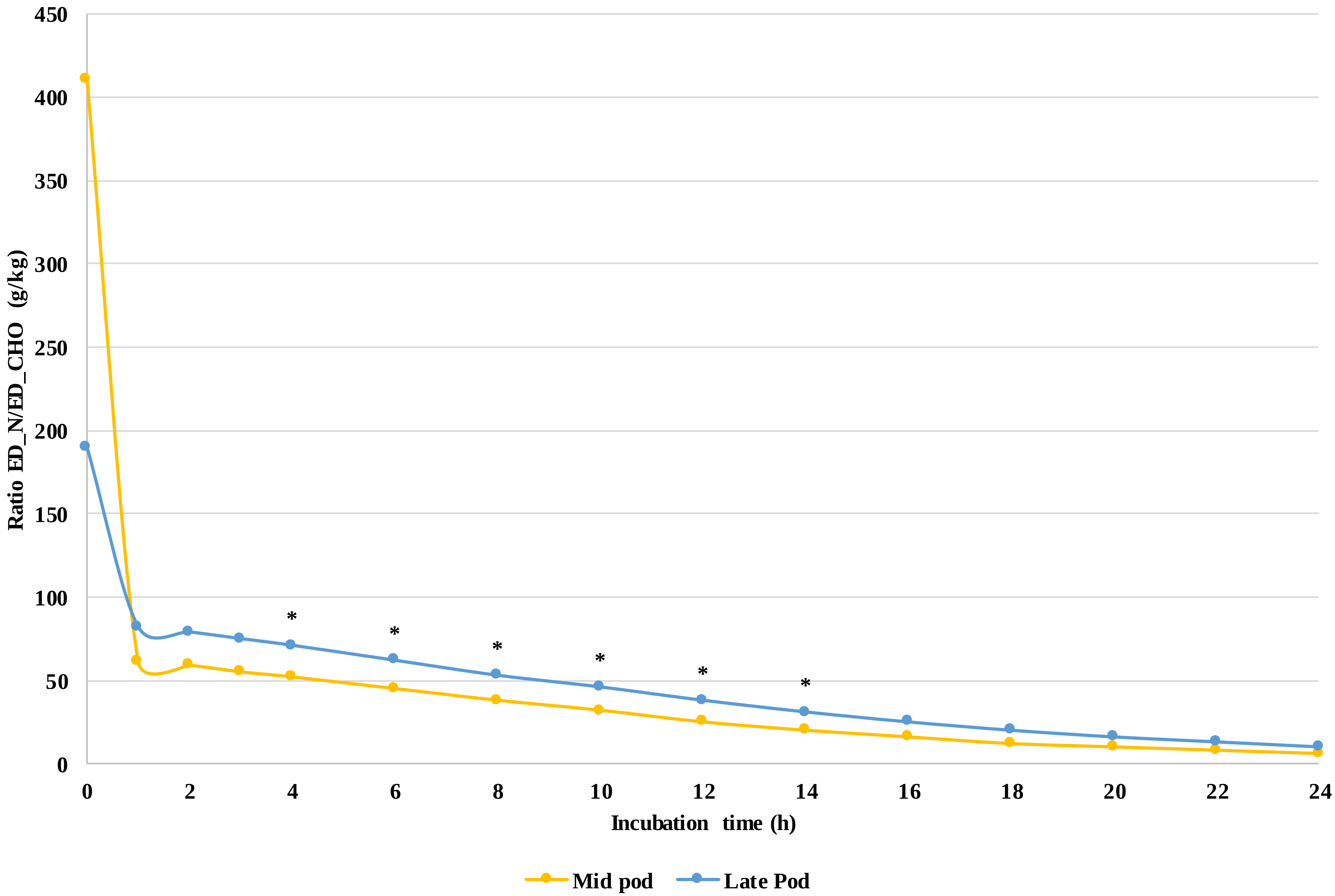


Figure 4.2. Effect of stage of cutting on hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of whole plant faba bean silage. Optimum ratio = 32 EN/ECHO g/kg. * Means are significantly different ($P < 0.05$).

Guevara-Oquendo et al., (unpublished, in Chapter 3) found that the tannin concentration did not affect the ED_N/ED_CHO at individual hours in whole plant faba bean hay. Therefore, processing the same forage differently can modify the effective degradation of N, probably because during silage fermentation protein breakdown and ammonia production take place.

On the other hand, whole plant faba bean silage at mid pod stage tended (P= 0.06) to have a higher ED_N/ED_CHO at h0 than whole plant faba bean silage at late pod stage which indicates that the N is released immediately so it can be related to the higher concentration of SCP and lower concentration of starch compared to whole plant faba bean silage at late pod stage, for that reason in the individual hours (except h0) the ED_N/ED_CHO is numerically higher always in whole plant faba bean silage at late pod stage. Although whole plant faba bean silage will not be used as a sole ingredient for dairy cattle, it is important to have in mind the ED_N/ED_CHO of individual ingredients that can integrate a TMR in order to have an ED_N/ED_CHO close to the ideal ratio (32 g of N/kg CHO) to maximize microbial synthesis and minimize N loss in dairy cattle. Higher ED_N/ED_CHO ratio than the ideal shows a probable nitrogen loss from the rumen or no enough energy supply to the microbes in the rumen, while a lower ratio than the ideal suggests no enough nitrogen supplied for rumen microorganisms to grow (Guevara-Oquendo et al., 2018; Tamminga et al., 2007, 1994; Sinclair et al., 1993). Therefore, whole plant faba bean silage at late pod stage showed a slower degradation of N which can be favorable because by itself can have close ratios of ED_N/ED_CHO to the ideal for a longer period of time that can contribute to a more stable microbial growth. Moreover, at individual hours from h0 to h6, the ED_N/ED_CHO were higher in the whole plant faba bean hay than whole plant faba bean silage, which may be associated to the slightly lower concentration of starch in the hay.

4.4.11 Intestinal Availability of Rumen Bypass Nutrients of Whole Plant Faba Bean Silages

The intestinal digestibility characteristics of DM, CP, NDF and Starch of the whole plant faba bean silage are shown in Table 4.12. The results showed that the intestinal digestibility of DM was not affected by the tannin concentration ($P > 0.05$), but the stage of cutting affected significantly ($P < 0.05$) some intestinal digestibility characteristics, the intestinal digestible rumen bypass DM (IDBDM) was lower ($P < 0.05$) at mid pod stage than at late pod stage (-3.4 % or -34 g/kg DM), the total digestible DM (TDDM) was not significantly affected ($P > 0.05$) by the stage of cutting and was 772 g/kg DM at mid pod stage and 798 at late pod stage.

The intestinal digestibility of rumen bypass protein (dIDP) showed interaction ($P < 0.05$, variety × cutting stage, V×S). The dIDP of low tannin whole plant faba bean silage at mid pod stage was higher ($P < 0.05$) than high tannin whole plant faba bean silage at mid pod stage (+8.1 %) and the dIDP values of high tannin and low tannin whole plant faba bean silages at late pod stage were similar to those two previous values. The tannin concentration did significantly affect some characteristics. The intestinal absorbable feed protein (IADP) was lower ($P < 0.05$) (-1.6 %CP or -3 g/kg DM or -16 g/kg CP) and the total digested protein (TDP) was higher ($P < 0.05$) (+1.8 %CP or +18 g/kg CP) in the low tannin whole plant faba bean silage than in the high tannin whole plant faba bean silage. Whole plant faba bean at late pod stage showed higher ($P < 0.05$) IADP and lower ($P < 0.05$) TDP than whole plant faba bean at mid pod stage (+23 g/kg CP and -16 g/kg CP respectively).

Some studies focused on different silages used the same *in vitro* study to determine the CP digestibility. Refat (2018) found that the dIDP values of corn silage and three different varieties of barley silages (Cowboy, Copeland and Xena) were 39.3, 33.8, 34.6 and 31.4 %, respectively; the IADP values were 20.0, 20.3, 20.8 and 16.4 g/kg DM, respectively, while the TDP values were 70.7, 71.8, 71.5 and 71.7 %CP, respectively. In our study, we found that whole plant faba bean

Table 4.12. Effect of variety (tannin concentration) and stage of cutting on intestinal digestibility and total tract digestion of whole plant faba bean silage

Item	Variety (V, Tannin con.)			Stage of Cutting (S)			P value		
	H	L	SEM	Mid pod	Late Pod	SEM	Variety	Stage	V×S
Dry matter									
%dBDM	46.4	49.7	2.28	44.5	51.6	2.28	0.35	0.07	0.36
%IDBDM	19.4	20.4	1.14	18.2^{b}	21.6^{a}	1.14	0.47	0.03	0.11
IDBDM (g/kg DM)	194	204	11.4	182^{b}	216^{a}	11.4	0.47	0.03	0.11
%TDDM	77.6	79.4	1.24	77.2	79.8	1.24	0.26	0.13	0.93
TDDM (g/kg DM)	776	794	12.4	772	798	12.4	0.26	0.13	0.93
Crude protein									
%dIDP	54.2	57.3	1.37	55.9	55.7	1.37	0.14	0.92	0.03
IADP (g/kg DM)	18^{a}	14^{b}	1.4	14^{b}	19^{a}	1.4	0.04	0.02	0.55
IADP (g/kg CP)	81^{a}	65^{b}	5.3	61^{b}	84^{a}	5.3	0.04	0.01	0.59
TDP (g/kg DM)	211	208	5.4	213	207	5.4	0.45	0.20	0.43
TDP (g/kg CP)	933	951	2.5	950	934	2.5	<0.01	<0.01	0.01
%IADP (%CP)	8.1^{a}	6.5^{b}	0.53	6.1^{b}	8.4^{a}	0.53	0.04	0.01	0.59
%TDP (%CP)	93.3	95.1	0.25	95.0	93.4	0.25	<0.01	<0.01	0.01
Fiber (NDF)									
%dBNDF	33.6	39.7	3.28	34.9	38.4	3.28	0.16	0.39	0.73
%IDBNDF	24.1	26.8	2.74	24.2	26.7	2.74	0.38	0.41	0.43
IDBNDF (g/kg DM)	79	100	8.8	85	93	8.8	0.10	0.49	0.38
%TDNDF	53.1	59.3	1.91	55.2	57.2	1.91	0.05	0.48	0.81
TDNDF (g/kg DM)	176^{b}	220^{a}	8.2	199	197	8.2	0.01	0.90	0.85
Starch									
%dBST	96.0	96.0	0.62	94.1^{b}	97.9^{a}	0.62	0.97	<0.01	0.92
% IDBST	16.2	17.2	1.75	16.9	16.5	1.75	0.66	0.83	0.64
IDBST (g/kg DM)	26	22	2.8	17^{b}	32^{a}	2.8	0.34	0.01	0.83
%TDST	99.3	99.3	0.16	98.9^{b}	99.7^{a}	0.16	0.97	0.01	0.98
TDST (g/kg DM)	149	121	11.9	95^{b}	175^{a}	11.9	0.09	<0.01	0.67

Table 4.12. ***Cont'd***.Footnote:

H: high tannin variety; L: low tannin variety; 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: Tukey method; dBDM: intestinal digestibility of rumen bypass dry matter; IDBDM: intestinal digested rumen bypass dry matter; TDDM: total digested dry matter dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IADP: intestinal digested crude protein; TDP: total digested crude protein; dBNDF: intestinal digestibility of rumen bypass neutral detergent fiber; IDBNDF: intestinal digested rumen bypass neutral detergent fiber; TDNDF: total digested neutral detergent fiber; dBST: intestinal digestibility of rumen bypass starch; IDBST: intestinal digested rumen bypass starch; TDST: total digested starch; con: concentration.

silage have higher dIDP values (averaged 55.7 %) than those observed for corn and barley silages, but the IADP values of corn and barley silages are comparable to the IADP value of whole plant faba bean silage at late pod stage (19.0 g/kg DM), but higher than the IADP value of whole plant faba bean silage at mid pod stage (14.0 g/kg DM). The TDP value of whole plant faba bean silage averaged 94.2 %CP which is higher than the TDP values of corn and barley silages. Therefore, more protein can be used by the animal when consume whole plant faba bean silage rather than when consume corn or barley silages. Tabacco et al., (2006) found that the intestinal CP digestibility of alfalfa silage (harvested at early bloom) was 39.8 %, which is comparable to the value found by Refat (2018) in barley silage, however, is lower than the value found in our study for whole plant faba bean silage at mid pod and late pod stages. Based on those results, we can consider that the CP of whole plant faba bean is highly digestible when compare to common silages used in western Canada, thus N losses in digestion may be lower when whole plant faba bean silage is fed, however Rodriguez (2018) determined the *in vitro* digestibility of high and low tannin faba bean seeds (3 low tannin types and 4 high tannin types) and found that the CP digestibility (dIDP) was averaged 83.1% for the low tannin seeds, while was 75.1 % the high tannin seeds; in our study the dIDP was similar between high and low tannin silages therefore we can notice that whole plant faba bean silage had almost 1.5 times lower CP digestibility when we compare with the faba bean seeds.

A study conducted by Di Marco et al., (2002) used the two-stage procedure described by Tilley and Terry (1963) to determine the DM and NDF digestibility of corn silage which were 61 and 32 %, respectively. Comparing to whole plant faba bean silage, corn silage used in that study had higher DM digestibility and slightly lower NDF digestibility compared to whole plant faba bean silage at mid and late pod stages, therefore energy from digestible NDF cannot be so different from that corn silage and whole plant faba bean silage at mid pod and late pod stages, however, it is important to clarify that the *in vitro* procedure used was different and the values may change when other *in vitro* study is carried out. Another study conducted by Miron et al., (2005) used the same procedure and obtained DM and NDF digestibility of sorghum silage (73.0% and 64.0% for the 1st cut early head and 70.7% and 62.2% for the 1st cut soft dough, respectively) which are higher than the DM and NDF digestibility of whole plant faba bean silage at mid pod and late pod stages. Again, those values are higher than the ones of whole plant faba bean silage at mid pod and late pod stages. The main source of energy for a high producing dairy cows is the starch; part of this starch which escape the rumen degradation is digested in the small intestine and absorbed as glucose (Ali et al., 2012), therefore whole plant faba bean silage at late pod stage can contribute with higher energy from starch for a high producing cow than whole plant faba bean silage at mid pod stage. Additionally, whole plant faba bean silage had slightly lower TDDM, TDNDF than whole plant faba bean hay (Chapter 3),but higher IDBST and similar dIDP.

4.4.12 Nutrient Supply to Dairy Cows with the DVE/OEB System

The DVE/OEB System characteristics are presented in Table 4.13. The tannin concentration significantly affected the ruminally undegraded feed CP (BCP) which was higher ($P < 0.05$) in the high tannin silage than in the low tannin silage (+9 g/kg DM). Also the truly absorbed bypass protein in the small intestine (DVBE) was higher ($P < 0.05$; +4 g/kg DM) but the microbial protein synthesized in the rumen based on available energy (MREE) and the truly absorbed rumen synthesized microbial protein in the small intestine (DVME) were lower ($P < 0.05$; -4 and -2 g/kg DM, respectively) than in the low tannin silage. The truly absorbed protein in the small intestine (DVE); the degraded protein balance (OEB), which reflects the difference between the potential microbial protein synthesis on the basis of available rumen degradable protein and that on the basis of available rumen degradable energy, were not significantly affected ($P > 0.05$) by the tannin concentration. The cutting stage significantly affected most of the DVE/OEB System characteristics. The BCP and DVBE were higher (P< 0.05), but the MREN was lower (P< 0.05) at late pod stage than at mid pod stage (+9, +6 and -12 g/kg DM respectively). The DVE was lower (P< 0.05), the OEB was higher (P< 0.05) at mid pod stage than at late pod stage (-9, + 14 g/kg DM respectively).

Boever et al., (1997) analyzed more than fifty different maize silages and found that the DVE value of this important feed for dairy cattle averaged 49 g/kg DM, while the OEB value averaged -23 g/kg DM. In our study the DVE of whole plant faba bean silage at mid pod and late pod stages (59 and 68 g/kg DM) are higher than the one observed for maize silage, therefore more truly absorbed protein in the small intestine can be obtained by animals fed whole plant faba bean silage, also the OEB value in whole plant faba bean silage is positive, while in maize silage is negative which can indicate a potential shortage of N supply which can negatively affect microbial growth. Visser and Hindle (1992) studied grass silage which consisted of approximately 95% of Lolium perenne and found that the DVE was 68 g/kg DM. Additionally, Boever et al., (2004) analyzed grass silage which included Holcus lanatus, Ranunculus acer, Phleum pratense, Poa trivialis and Agrostis tenuis. The grass was harvested from stem elongation to the beginning of flowering. It was found that the DVE of this silage was 68 g/kg DM and the OEB was 26 g/kg DM. Comparing with whole plant faba bean silage, those grass silages have similar DVE value to the one found in whole plant faba bean silage at late pod stage and higher than the one from whole plant faba bean silage at mid pod stage. The OEB value of the grass silage was positive and lower than the OEB value of whole plant faba bean silage. There is not a minimum requirement of a positive OEB value, however it is suggested to avoid negative OEB values which can implicate possible N deficiency in the rumen. Also the CVB (2016) reported that alfalfa silage (40%DM) has a DVE value of 43 g/kg DM which is lower than the one found in whole plant faba bean silage at mid and late pod stages; and a OEB value of 81 g/kg DM which is similar to the OEB value of whole plant faba bean silage at late pod stage but lower than the one found at mid pod stage, which suggests that alfalfa silage may have a lower predicted production performance than whole plant faba bean silage. Additionally, whole plant faba bean silage at late pod stage seems to contribute with higher truly absorbed protein in the small intestine than whole plant faba bean silage at mid pod stage, consequently the predicted production performance is higher for whole plant faba bean silage at late pod stage, also it is implied that whole plant faba bean silage at mid pod stage contributed with higher microbial protein synthesis from rumen degradable crude protein for that reason the higher degraded protein balance than in whole plant faba bean silage at late pod stage. Additionally, whole plant faba bean silage had slightly lower DVE value, but higher OEB value than whole plant faba bean hay (Chapter 3).

4.4.13 Nutrient Supply to Dairy Cows with the NRC-2001 Model

Metabolic Characteristics based on the NRC-2001 model are shown in Table 4.12. The tannin concentration affected significantly the ruminally undegraded feed CP (RUP) and the truly absorbed rumen-undegraded feed protein in the small intestine (ARUP) which were lower ($P< 0.05$) in the low tannin silage (-8 and -4 g/kg DM). Whole plant faba bean silage at late pod stage had higher ($P< 0.05$) RUP and ARUP but lower ($P< 0.05$) microbial protein synthesized in the rumen based on available protein (MCP_{RDP}) than whole plant faba bean at mid pod stage (+8, +5 and -9 g/kg DM respectively). The metabolizable protein (MP) tended ($P= 0.05$) to be higher (73 vs. 67 g/kg DM) and the degraded protein balance (DPB) was lower ($P< 0.05$; -12 g/kg DM) at late pod stage than at mid pod stage.

Some common ingredients used in western Canada for dairy cattle include corn and barley silages which MP values are 49.8 and 51.8 g/kg DM, and DPB values are -48.3 and -32.1 g/kg DM, respectively (Refat, 2018); barley grain (averaged of six different varieties) which has a MP value of 87.6 g/kg DM and a DPB value of -51.8 g/kg DM (Hart et al., 2011); canola meal has a

MP value of 153 g/kg DM and a DPB value of 140 g/kg DM, while carinata meal has a MP value of 136 g/kg DM and a DPB value of 259 g/kg DM (Xin and Yu, 2014; Ban, 2016) and oats (averaged of three different varieties) with a MP value of 95.6 g/kg DM and a DPB value of 58.3 g/kg DM (Tosta, 2019). Compared to barley and corn silages, whole plant faba bean silage at late pod stage has 0.4 times more metabolizable protein, while whole plant faba bean silage at mid pod stage has 0.3 times more metabolizable protein. Barley grain has about 20% more metabolizable protein than whole plant faba bean silage at late pod stage. Negative values observed in the degraded protein balance of corn silage, barley silage and barley grain may imply potentially impaired microbial protein synthesis because of N deficiency in the rumen for the microbes to grow. Canola and carinata meals have almost double more metabolizable from what whole plant faba bean silage at late pod stage has, but the degradable protein balance in canola and carinata meals are much higher than the degradable protein balance of whole plant faba bean silage which may implicate potentially more loss of N from the rumen. Additionally, oats have a higher concentration of metabolizable protein and lower degraded protein balance than whole plant faba bean silage. Whole plant faba bean silage compared with other silages used in North America seems to contribute with more metabolizable protein, however it provides less metabolizable protein than common grains and protein sources used for dairy cattle. Moreover, whole plant faba bean silage had slightly lower MP but higher DPB value than whole plant faba bean hay (Chapter 3).

4.4.14 Feed Milk Value of Whole Plant Faba Bean Silages

The Feed Milk Value based on the DVE System (FMV^{DVE}) and the Feed Milk Value based on the NRC model (FMV^{NRC}) are presented in Table 4.12. The feed milk value (FMV^{DVE}) were not significantly affected ($P > 0.05$) by the tannin concentration, however the FMV^{DVE} was lower (P<

0.05) at mid pod stage than at late pod stage (-0.17 kg milk/kg DM Silage). On the other hand, the FMV^{NRC} was higher ($P < 0.05$; +0.12 kg milk/kg DM Silage) at late pod stage than at mid pod stage.

The FMV^{NRC} of corn and barley silages are 1.01 and 1.05 kg milk/kg DM Silage respectively (Refat, 2018). Although the predicted production performance is relatively high in comparison with common barley and corn silages used in dairy rations, higher metabolizable protein and lower degraded protein balance observed al late pod stage contributed to have a higher predicted performance than at mid pod stage. Additionally, whole plant faba bean silage had lower FMV based on both models than whole plant faba bean hay (Chapter 3).

Table 4.13. Effect of variety (tannin concentration) and stage of cutting on metabolic characteristics, true nutrient supply, and feed milk value of whole plant faba bean silage

Item	Variety (V, Tannin con.)		SEM	Stage of Cutting (S)		SEM	P value		
	H	L		Mid pod	Late Pod		Variety	Stage	V×S
Truly digestible nutrient supply to dairy cows based on non-TDN system: DVE system									
BCP (g/kg DM)	37^{a}	28^{b}	1.9	28^{b}	37^{a}	1.9	<0.01	<0.01	0.60
MREE (g/kg DM)	95^{b}	99^{a}	1.7	96	98	1.7	0.05	0.36	0.17
MREN (g/kg DM)	189	191	5.0	196^{a}	184^{b}	5.0	0.69	0.02	0.48
DVME (g/kg DM)	61^{b}	63^{a}	1.1	61	63	1.1	0.05	0.36	0.17
DVBE (g/kg DM)	20^{a}	16^{b}	1.5	15^{b}	21^{a}	1.5	0.04	0.02	0.55
Degraded protein balance (OEB of silage) and Total true protein supply (DVE of silage) to dairy cows									
DVE (g/kg DM)	64	63	2.3	59^{b}	68^{a}	2.3	0.84	0.04	0.81
OEB (g/kg DM)	94	92	3.9	100^{a}	86^{b}	3.9	0.51	0.01	0.20
FMV^{DVE} (kg milk/kg DM Silage)	1.30	1.28	0.047	1.20^{b}	1.37^{a}	0.047	0.80	0.03	0.84
Truly digestible nutrient supply to dairy cows based on TDN system: NRC dairy									
RUP (g/kg DM)	33^{a}	25^{b}	1.7	25^{b}	33^{a}	1.7	<0.01	<0.01	0.60
MCP_{TDN} (g/kg DM)	77	77	1.1	76	77	1.1	0.82	0.59	0.54
MCP_{RDP} (g/kg DM)	164	165	4.3	169^{a}	160^{b}	4.3	0.87	0.02	0.50
AMCP (g/kg DM)	49	49	0.7	49	49	0.7	0.82	0.59	0.54
ARUP (g/kg DM)	18^{a}	14^{b}	1.4	14^{b}	19^{a}	1.4	0.04	0.02	0.55
Degraded protein balance (DPB of silage) and Total metabolizable protein supply (MP of silage) to dairy cows									
MP (g/kg DM)	72	68	1.7	67	73	1.7	0.10	0.05	0.88
DPB (g/kg DM)	102	103	4.1	109^{a}	97^{b}	4.1	0.75	0.01	0.26
FMV^{NRC} (kg milk/kg DM Silage)	1.46	1.38	0.035	1.36^{b}	1.48^{a}	0.035	0.11	0.05	0.89

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; $^{a\text{-}b}$ Means with the different letters in the same row are significantly different ($P<0.05$); Multi-treatment comparison: Tukey method; BCP: ruminally undegraded feed CP: calculated according the formula in DVE/OEB system; MREE: microbial protein synthesized in the rumen based on available energy; MREN: microbial

Table 4.13. *Cont'd*. Footnote

protein synthesized in the rumen based on rumen degraded feed crude protein; DVME: truly absorbed rumen synthesized microbial protein in the small intestine; DVBE: truly absorbed bypass feed protein in the small intestine; DVE: truly absorbed protein in the small intestine; OEB: is a balance between microbial protein synthesis from rumen degradable CP and that from the energy extracted during anaerobic fermentation in the rumen; RUP: ruminally undegraded feed CP: calculated according the formula in NRC-2001 dairy model; MCP_{TDN}, microbial protein synthesized in the rumen based on available energy (discounted TDN); MCP_{RDP}: microbial protein synthesized in the rumen based on available protein; AMCP: truly absorbed rumen-synthesized microbial protein in the small intestine; ARUP: truly absorbed rumen-undegraded feed protein in the small intestine; MP: metabolizable protein; DPB: reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy-TDN available for microbial fermentation in the rumen; FMV: feed milk value; The efficiency of use of metabolizable protein for lactation is 0.67 (source NRC, 2001), and protein composition in milk is assumed to be 33 g protein/1000 g milk; con: concentration.

4.5 Conclusions

Whole plant faba bean silage had higher concentration of condensed tannins than whole plant faba bean hay. Additionally, the tannin concentration affected the rate of degradation of dry matter in the whole plant faba bean silage but did not in the whole plant faba bean hay. Furthermore, the tannin concentration affected the rate of degradation of crude protein in the whole plant faba bean hay but did not in the whole plant faba bean silage. On the other hand, metabolizable protein and the predicted production performance were slightly higher in whole plant faba bean hay than in whole plant faba bean silage.

The tannin concentration and the cutting stage affected some of the nutritive and metabolic characteristics of whole plant faba bean silage. Whole plant faba bean silage at mid and late pod stages showed superior qualities than whole plant faba bean silage at flower stage. Additionally, either high or low tannin whole plant faba bean should be harvested at late pod stage in order to have more adequate N to energy ratios, more bypass protein and bypass starch to be digested in the small intestine and more metabolizable protein, in that event higher predicted production performance is expected. Therefore, whole plant faba bean silage can be considered as a high nutritive (high in protein, starch and energy) silage, besides it has an attractive digestibility characteristics and metabolic profile, thus it can be used as fodder for high producing dairy cows in western Canada. The results from the current study include and integrate results from well recognized chemical, *in situ* and *in vitro* procedures, but in order to have a complete information on whole plant faba bean silage feeding and metabolic trials should be carried out.

5. COMPREHENSIVE PHYSIOCHEMICAL AND NUTRITIONAL EVALUATION OF FROST DAMAGED FABA FORAGE VARIETIES FOR HAY: EFFECT OF VARIETY/TANNIN CONCENTRATION ON DETAILED FEED AND FEEDING VALUES OF FROST DAMAGED FABA FORAGE HAY GROWN IN WESTERN CANADA WITH COOL CLIMATE CONDITIONS IN RUMINANT LIVESTOCK SYSTEMS

5.1 Abstract

The objective of this study was to determine the effect of tannin concentration/variety (low tannin/Snowdrop variety; high tannin/SSNS-1 variety) on chemical and energy profiles, CNCPS fractions, rumen degradation kinetics of principal nutrients, nitrogen to energy degradation ratios, *in vitro* intestinal digestibility, metabolic characteristics, and predicted production performance of frost damaged whole plant faba bean hay harvested at 114 days of maturity. Complete Randomized Design and RCBD with one way treatment arrangement were used as experimental designs. Data was analyzed using the Mixed model procedure of SAS version 9.4. The results showed that high tannin frost damaged hay had higher ($P < 0.05$) organic matter (OM) and lower acid detergent insoluble crude protein (ADICP) than low tannin frost damaged hay (+2.5 %DM and -0.4 %DM). Starch and protein contents in this stage of maturity averaged (11.9 %DM and 16.8 %DM). Energy values were similar ($P > 0.10$), while high tannin hay had higher ($P < 0.05$) fiber-bound protein (PB2) and lower ($P < 0.05$) indigestible protein (PC, +2.3 and -3.1 %CP). Low tannin frost damaged hay had higher ($P < 0.05$) rumen undegraded crude protein (RUP, +2.8 %) and lower undegradable fraction of neural detergent fiber (U, -5.7 %) than high tannin frost damaged hay. High tannin frost damaged hay had higher ($P < 0.05$) intestinal digested rumen bypass dry matter (IDBDM) (+15 g/kg DM), higher ($P < 0.05$) intestinal digestibility of rumen bypass protein (dIDP) (+7 %), higher metabolizable protein (MP, +4 g/kg DM) and higher Feed Milk Value (FMV^{NRC}) (+0.09 kg milk/kg

DM Hay) than low tannin frost damaged hay. In conclusion, both high tannin and low tannin frost damaged hay have lower nutritive value at 114 days than non-frost damage hay at flower stage at 77 days, mid-pod stage at 88 days and late pod stage at 97 days. The high tannin frost damaged hay harvested at 114 days showed superior nutritive value than the low tannin frost damaged hay harvested at 114 days.

Keywords: Frost damage, whole plant faba bean hay, high tannin, low tannin, metabolizable protein, predicted performance.

5.2 Introduction

Faba bean was originated in the southwestern Asia as principle center, and in the Mediterranean region (north Africa) as secondary center (Tanno and Willcox, 2006). These are warmer areas and are not exposed to frost events most of the time. However, some cultivars that are grown in Europe, normally small seeded varieties are showed to be more resistant to freezing (Landry, 2014). These cultivars are known basically as winterhardy varieties. Frost tolerance is the more important parameter is the winter hardy varieties, however some varieties will be more tolerant than others and additionally will show low or high survival (Neugschwandtner et al., 2015; Herzog, 1987). Some studies have demonstrated that this frost tolerance is a heritability trait and also demonstrated that frost tolerance involved chemical and morphologic mechanisms (Link et al., 2010; Herzog, 1989). In Canada, especially Saskatchewan spring frosts are very common and depending on the severity of the frost the damages can vary too. Sometimes the damaged due to the frost can be visible and effects on the tissue can be observed, but most of the time can cause stress to the plant (Phelps, 2015; Meyer and Badaruddin, 2001). On the other hand, faba bean is one of the most resistant pulses to spring frosts in Canada, one of the reasons is that when germinate the plant leaves the cotyledon below the ground, hence protected from low temperatures. Nevertheless, sometimes when the material which is above the ground is affected, the faba bean can regrowth from the nodes that are below the ground (Phelps, 2016; Phelps, 2015; Meyer and Badaruddin, 2001; Brown and Blackburn, 1987). Data has indicated that frost damaged can reduce the productivity and also the quality of the seeds (Blaylock, 1995; Herzog, 1989). However, there is not information on the effect of frost damage on the nutritional profile of whole plant faba bean. Effort is needed to fully understand the effect of the frost damage (if occurred) on the chemical and nutrient profile of the high and low tannin varieties when the whole plant faba bean is processed as hay. For that reason, this study was conducted to test the frost damaged whole plant faba bean hay grown in western Canada in terms of chemical profile, energy profile, protein and carbohydrate fractions, rumen degradation kinetics, intestinal digestibility, nitrogen to energy synchronization, metabolic characteristics, and predicted production performance. The effect of variety (low or high tannin variety were also determined.

5.3 Materials and Methods

5.3.1 Ingredients and Sample Preparation

Four experimental plots (plot area = 36 square feet) were seeded (180 seeds per plot) by the Crop Development Centre (CDC, University of Saskatchewan, Saskatoon, Canada) on June 17th, 2017 in the crop research fields of the University of Saskatchewan. Two plots were randomly assigned to the low tannin faba bean variety Snowdrop, and two plots were assigned to the high tannin faba bean variety SSNS-1. On September 20th, 21st and 22nd the lowest temperature of the growth period was observed (0 °C). The frost damaged whole plant faba bean from the plots was harvested at 114 days old. After harvesting, the samples were dried in a drier room at 45 °C for 7 days.

5.3.2 Yield and Chemical Analysis

Chemical profile of frost damage whole plant faba bean hay was determined following AOAC official methods. Detailed information was included in Chapter 3 (3.3.2).

5.3.3 Energy Profile

Total digestible nutrients and energy values of frost damage whole plant faba bean hay were determined using the NRC-2001 dairy and the NRC-1996 beef. Detailed information was included in Chapter 3 (3.3.3).

5.3.4 Protein and Carbohydrate Subfractions

The Cornell Net Carbohydrate and Protein System (CNCPS 6.5) of frost damage whole plant faba bean hay was used to determine the protein and carbohydrate factions. Detailed information was included in Chapter 3 (3.3.4).

5.3.5 Rumen In Situ Incubation

5.3.5.1 Samples

The four frost damage whole plant faba bean hay samples of Snowdrop variety and SSNS-1 variety (114 days old) were ground through a 3 mm screen using the 8 inches Laboratory Mill (Christy & Norris LTD, Ipswich, England) in the Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada.

5.3.5.2 Animals and Diets

Four lactating Holstein cows on their third lactation were used in this study were and cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 2009). The experiments were approved (Animal Use Approval Protocol # 19910012) by Animal Research 125 Ethics Board (AREB) at the University of Saskatchewan, Canada.

5.3.5.3 Rumen Incubation Procedure

The *in situ* method described by Yu et al., (2003) was used to determine rumen degradation characteristics of primary nutrients. Detailed information was included in Chapter 3 (3.3.5.3).

5.3.5.4 Chemical Analysis of In Situ Residual Samples

The *In situ* samples were analyzed for CP, Neutral detergent fiber (NDF) and Starch (ST) at Cumberland Valley Analytical Services (CVAS, Hagerstown, MD) using the official methods listed previously. Detailed information was included in Chapter 3 (3.3.5.4).

5.3.5.5 Rumen Degradation Kinetics

The first-order degradation kinetics model described by Ørskov and McDonald (1979) and modified by Tamminga et al., (1994) was used to determined degradation characteristics of dry matter (DM), crude protein (CP), starch (ST), and neutral detergent fiber (aNDF) in frost damage whole plant faba bean hay. Detailed information was included in Chapter 3 (3.3.5.5).

5.3.6 Hourly Effective Rumen Degradation Ratios and Potential N-to-Energy Synchronization

The effective rumen degradation ratios of N and carbohydrates (CHO) of frost damage whole plant faba bean hay were estimated using the formula described in Sinclair et al., (1993). Detailed information was included in Chapter 3 (3.3.6).

5.3.7 Intestinal Digestion of Rumen Undegraded Protein

The *in vitro* intestinal digestion of frost damage whole plant faba bean hay was determined following the three-step procedure described by Calsamiglia and Stern (1995) and later on modified by Gargallo et al., (2006). Detailed information was included in Chapter 3 (3.3.7).

5.3.8 Nutrient Supply with the DVE/OEB System

The protein digested in the intestine (DVE) and the degraded protein balance (OEB) of frost damage whole plant faba bean hay were calculated. Detailed information was included in Chapter 3 (3.3.8).

5.3.9 Nutrient Supply with the NRC-2001 Model

Metabolizable protein (MP) and degraded protein balance (DPB) of frost damage whole plant faba bean hay were calculated. Detailed information was included in Chapter 3 (3.3.9).

5.3.10 Feed Milk Value of Frost Damage Whole Plant Faba Bean Hay

Predicted production performance or Feed Milk Value (FMV) of frost damage whole plant faba bean hay was determined according the DVE/OEB System and the NRC model. Detailed information was included in Chapter 3 (3.3.10).

5.3.11 Statistical Analysis

Results from chemical profiles, energy values, protein and carbohydrate fractions, rumen degradation kinetics, hourly effective degradation ratios, intestinal digestibility of protein, predicted truly absorbed protein supply, and Feed Milk Values in frost damage whole plant faba bean hay were analyzed using the Mixed model procedure of SAS version 9.4. (SAS Institute, Inc., Cary, NC, US). CRD and RCBD were used as experimental designs with one-way treatment arrangement (low and high varieties). The models used for the analysis were as follows:

For chemical and nutrient profile studies: CRD model: $Y_{ij} = \mu + F_i + e_{ij}$,

For *in situ*, *in vitro*, and modeling studies: RCBD model: $Y_{ijk} = \mu + F_i + R_j + e_{ijk}$,

where, Y_{ij} or Y_{ijk} was the observation of the dependent variable ij or ijk, μ was the population mean for the variable, F_i the effect of tannin concentration (i= 1,2); R_j the effect of *in situ* experimental runs (j= 1,2,3) and e_{ij} or e_{ijk} the random error associated with observation ij or ijk. The model assumptions of CRD and RCBD were tested using SAS Residual Analysis. The normality test was carried out using Proc Univariate with Normal and Plot options. PROC NLIN-Gauss-Newton method of SAS was used to fit the rumen degradation data to the model. The differences among treatments were evaluated with a multiple comparison analysis using the Tukey method. For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$.

5.4 Results and Discussion

5.4.1 *Yield and Chemical Profile of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration*

Effect of variety/tannin concentration on yield and chemical profiles of the frost damaged whole plant faba bean hay are presented in Table 5.1. There is a tendency for the DM, CP, and starch (St) to be higher in the high tannin frost damaged whole plant faba bean hay than in the low tannin frost damaged whole plant faba bean. The tannin concentration had no significant ($P > 0.05$) effect on most of the nutrients. However, DM, Ash and ADICP were higher ($P < 0.05$) in the low tannin frost damaged whole plant faba bean hay than that in the high tannin frost damaged whole plant faba bean hay (+0.7 %, +2.5 %DM and +3.1 %CP). Condensed tannins were higher in the high tannin frost damaged whole plant faba bean hay than in the low tannin frost damaged whole plant faba bean (+126.27 mg/kg DM).

Whole plant faba bean hay at flower, mid pod, and late pod stages showed higher CP content than the frost damaged whole plant faba bean hay, but they showed a lower ADICP and NDICP content. The frost damaged whole plant faba bean hay showed slightly higher CHO content, and similar starch content to the whole plant faba bean hay at late pod stage. Additionally, frost damaged whole plant faba bean hay showed higher aNDF and higher ADL than whole plant faba bean hay harvested at flower, mid pod, and late pod stages. Cromey et al., (2010) found that wheat grains were as much as 80% lighter in upper parts of affected spikes. Overall yield losses as a result of frost were between 13 and 33% in affected crops. Besides that, Yu and Racz (2009) found that frozen wheat had lower starch content, similar protein content and higher NDF content than normal wheat.

Table 5.1. Effect of variety (tannin concentration) on yield and chemical composition of frost damaged whole plant faba bean hay.

Item	Variety (V, Tannin con.)			P value
	H	L	SEM	
Yield				
FM (t/ha)	8.48	4.97	0.602	0.13
DM (t/ha)	5.74	3.80	0.336	0.06
DM (%)	67.74	77.22	3.879	0.08
CP (t/ha)	0.99	0.62	0.078	0.08
aNDF (t/ha)	2.57	1.79	0.202	0.11
St (t/ha)	0.77	0.40	0.085	0.09
Chemical Composition				
DM (%)	93.6[b]	94.3[a]	0.04	0.05
Ash (%DM)	7.1[b]	9.6[a]	0.05	0.01
OM (%DM)	92.9[a]	90.4[b]	0.05	0.01
EE (%DM)	0.7	0.9	0.15	0.17
Protein profile				
CP (%DM)	17.3	16.3	0.51	0.30
NDICP (%DM)	2.0	2.0	0.03	0.40
ADICP (% DM)	1.6[b]	2.0[a]	0.03	0.01
NDICP (%CP)	11.4	12.4	0.20	0.08
ADICP (% CP)	9.1[b]	12.2[a]	0.33	0.02
SCP (% CP)	77.6	82.3	2.38	0.30
Carbohydrate profile				
CHO (%DM)	74.9	73.3	0.55	0.17
Starch (%DM)	13.5	10.4	0.98	0.16
Starch (% NFC)	42.15	37.18	5.160	0.57
Sugar (%DM)	3.00	3.10	0.510	0.87
Sugar (%NFC)	9.39	10.94	1.500	0.32
aNDF (%DM)	44.69	47.16	1.051	0.24
NDFn (%DM)	42.71	45.14	1.025	0.24
aNDFom (%DM)	44.31	46.07	0.920	0.31
uNDF (%DM)	22.97	23.52	2.925	0.91
NDF (%OM)	48.12	52.16	1.050	0.13
ADF (%DM)	37.70	41.80	1.740	0.31
ADL (%DM)	9.10	8.00	0.680	0.45
ADF (%NDF)	84.44	88.56	5.413	0.64
ADL (%NDF)	20.42	17.00	1.870	0.33
Hemicellulose (%DM)	7.04	5.41	2.558	0.70
Cellulose (%DM)	28.60	33.70	1.070	0.16
NFC (%DM)	32.17	28.12	1.549	0.21
NFC (%CHO)	42.94	38.37	1.787	0.21
NSC (%DM)	16.50	13.50	1.030	0.27

Table 5.1. Cont'd. Effect of variety (tannin concentration) on yield and chemical composition of frost damaged whole plant faba bean hay.

Item	Variety (V, Tannin con.)		SEM	P value
	H	L		
CT (abs nm / mg)	0.058	0.043	0.0016	0.03
CT (% DM)	0.020	0.007	0.0013	0.03
CT (g/kg DM)	0.205a	0.078b	0.0129	0.03
CT (mg/kg DM)	204.54a	78.27b	12.970	0.03

H: high tannin variety; L: low tannin variety; 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: Tukey method; FM: fresh matter; DM: dry matter; CP: crude protein; aNDF: neutral detergent fiber; St: Starch; EE: ether extracts (crude fat); CP: crude protein; OM: organic matter; SCP: soluble crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; aNDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: non-fiber carbohydrate; CHO: carbohydrate; NFC: non-fiber carbohydrate; NSC: non-structural carbohydrates. abs: units of absorbance at 550 nm; CT: condensed tannins; con: concentration; con: concentration.

5.4.2 Energy Profile of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration

The effect of variety/tannin concentration on the energy profiles of frost damaged whole plant faba bean hay are presented in Table 5.2. The concentration of truly digestible nutrients, total digestible nutrients and energy values were not significantly ($P> 0.05$) affected by the variety. The total digestible nutrients (TDN_{3x}) averaged 51.7 %DM, while the net energy for lactation ($NE_{L3\times}$) averaged 1.15 Mcal/kg and the net energy of gain (NE_g) averaged 0.7 Mcal/kg.

The TDN_{3x}, the $NE_{L3\times}$, and the NE_g of frost damaged whole plant faba bean hay are lower than the correspondent values on whole plant faba bean hay at flower, mid pod, and late pod stages. Those results are supported by Yu and Racz (2009) which concluded that frozen wheat was lower in energy values (TDN_{3x}, the $NE_{L3\times}$, and the NE_g) than normal wheat. Additionally, Arnott and Richardson (2007) suggested that frost damaged cereal grains may have slightly lower energy and digestibility levels than unfrosted crops.

Table 5.2. Effect of variety/tannin concentration on energy profile of frost damaged whole plant faba bean hay

Item	Variety (V, Tannin con.) H	L	SEM	P value
Truly digestible nutrient (%DM)				
tdNFC	31.5	27.6	1.52	0.21
tdCP	16.7	15.5	0.51	0.25
tdNDF	16.3	19.1	1.30	0.27
Total digestible nutrient (%DM)				
TDN_{1x}	57.5	55.1	0.49	0.09
TDN_{3x}	52.8	50.6	0.46	0.09
Energy value (Mcal/kg)				
DE_{1x}	2.6	2.5	0.03	0.16
DE_{p3x}	2.4	2.3	0.03	0.15
ME_{p3x}	2.0	1.9	0.02	0.15
NE_{Lp3x}	1.2	1.1	0.02	0.18
ME	2.2	2.1	0.02	0.10
NE_m	1.3	1.2	0.02	0.14
NE_g	0.7	0.7	0.02	0.16

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; Multi-treatment comparison: Tukey method; tdCP: truly digestible crude protein; tdNDF: truly digestible neutral detergent fibre; tdNFC, truly digestible non-fibre carbohydrate. $TDN_{1\times}$: total digestible nutrient at one times maintenance. $DE_{3\times}$: digestible energy at production level of intake (3×); $ME_{3\times}$: metabolizable energy at production level of intake (3×); $NE_{L3\times}$: net energy for lactation at production level of intake (3×); NE_m: net energy for maintenance; NE_g: net energy for gain; con: concentration.

5.4.3 Protein and Carbohydrate Subfractions of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration

The effect of variety/tannin concentration on the values of protein and carbohydrate fractions of frost damaged whole plant faba bean hay are presented in Table 5.3. The content of slowly degradable true protein (PB2) and indigestible protein (PC) were affected by the tannin concentration in frost damaged whole plant faba bean hay. The low tannin frost damaged whole plant faba bean hay showed lower ($P< 0.05$) PB2 and higher ($P< 0.05$) PC than the high tannin frost damaged whole plant faba bean hay (-2.3 and +3.1 %CP respectively). The tannin concentration did not affect significantly (P> 0.05) any of the carbohydrate fractions in frost damaged whole plant faba bean hay.

The PB2 fraction of low tannin frost damaged whole plant faba bean hay is similar to the PB2 fraction of the whole plant faba bean hay at flower, mid pod, and late pod stages. However, the PB2 fraction of high tannin frost damage whole plant faba bean hay is higher than the PB2 fraction of whole plant faba bean hay at flower, mid pod, and late pod stages. The PC fraction increases with maturity and it is observed that the PC fraction of frost damaged whole plant faba bean hay is 3.5 times higher than the PC fraction at flower and mid pod stages and doubled than at late pod stage. The frost damaged whole plant faba bean hay shows lower CA4 than the whole plant faba bean hay at late pod stage. It is being noted that the CA4 fraction reduced with maturity. Additionally, it is found that frost damaged whole plant faba bean hay has higher CC fraction than whole plant faba bean hay at mid pod and late pod stages. Yu and Racz (2009) concluded that frozen wheat had higher PC fraction, lower CB1 and higher CC fractions than normal wheat.

Table 5.3. Effect of variety (tannin concentration) on CNCPS fractions of frost damaged whole plant faba bean hay

Item	Variety (V, Tannin con.)		SEM	P value
	H	L		
Protein fractions				
PA2 (%CP)	77.6	82.3	2.38	0.30
PB1 (%CP)	11.0	5.3	2.58	0.26
PB2 (%CP)	2.4[a]	0.1[b]	0.21	0.04
PC (%CP)	9.1[b]	12.2[a]	0.33	0.02
True Protein (%CP)	90.9[a]	87.7[b]	0.33	0.02
PA2 (%true protein)	85.3	93.8	2.93	0.18
PB1 (%true protein)	12.1	6.1	2.84	0.27
PB2 (%true protein)	2.6[a]	0.1[b]	0.22	0.03
Carbohydrate fractions				
CHO (%DM)	74.9	73.3	0.55	0.17
CA4 (%CHO)	4.0	4.2	0.70	0.78
CB1 (%CHO)	18.0	14.2	1.44	0.21
CB2 (%CHO)	21.0	19.9	3.09	0.84
CB3 (%CHO)	26.4	29.5	3.03	0.48
CC (%CHO)	30.7	32.1	4.11	0.83

Table 5.3. ***Cont'd***.Footnote:
H: high tannin variety; L: low tannin variety; 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: Tukey method; PA1: ammonia; PA2: soluble true protein (rapidly degradable true protein); PB1: insoluble true protein (moderately degradable true protein); PB2: fiber-bound protein (slowly degradable true protein); PC: indigestible protein; CHO: carbohydrate; CA4: water soluble carbohydrates (rapidly degradable carbohydrate fraction); CB1: starch (intermediately degradable carbohydrate fraction); CB2: soluble fiber (intermediately degradable carbohydrate fraction); CB3: digestible fiber (available neutral detergent fiber or slowly degradable carbohydrate fraction); CC: indigestible fiber (unavailable neutral detergent fiber); con: concentration.

5.4.4 In Situ DM Degradation Kinetics of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration

Rate of degradation (Kd), rumen fractions (S, D, U), rumen undegradable dry matter (BDM) and effective degradability of DM (EDDM) of frost damaged whole plant faba bean hay affected by variety/tannin concentration are presented in Table 5.4. All the characteristics were not significantly affected by the tannin concentration (P> 0.05). The effective degradable dry matter (EDDM) averaged 495.5 g/kg DM.

Table 5.4. Effect of variety (tannin concentration) on rumen degradation kinetics of dry matter of frost damaged whole plant faba bean hay

Item	Variety (V, Tannin con.)			P value
	H	L	SEM	
Dry matter				
Kd (%/h)	7.04	6.67	0.186	0.30
Residue at 0 h (%)	77.5	77.0	1.20	0.82
S (%)	22.6	23.0	1.20	0.82
D (%)	51.0	49.6	1.34	0.55
U (%)	26.6	27.4	1.60	0.37
%BDM=%RUDM	50.0	50.9	1.34	0.59
RUDM (g/kg DM)	500	509	13.4	0.59
%EDDM	50.0	49.1	1.34	0.59
EDDM (g/kg DM)	500	491	13.4	0.59

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method; Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction in the *in situ* incubation; D: degradable fraction; U: rumen undegradable fraction; BDM or RUDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter; con: concentration.

The Kd value of the frost damaged whole plant faba bean hay is lower than the Kd values of whole plant faba bean hay at flower and mid pod stages. The frost damaged whole plant faba bean hay shows higher RUDM and lower EDDM than the whole plant faba bean hay at flower, mid pod, and late pod stages. Yu and Racz (2009) found that frozen wheat had higher S fraction and lower D fraction of than normal wheat.

5.4.5 In Situ CP Degradation Kinetics of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration

The effect of variety/tannin concentration on rumen degradation characteristics of crude protein of frost damaged whole plant faba bean hay are presented in Table 5.5.The tannin concentration affected significantly ($P< 0.05$) the rumen undegradable crude protein (RUP), which was higher ($P< 0.05$) in the low tannin frost damaged whole plant faba bean hay than in the high tannin frost damaged whole plant faba bean hay (30.5 vs. 27.7 %). Also, the tannin concentration affected significantly ($P< 0.05$) the effective degraded crude protein (EDCP) which was lower in the low tannin frost damaged whole plant faba bean hay than in the high tannin frost damaged whole plant faba bean hay (-2.8 %). The other rumen degradation characteristics of crude protein were similar.

The Kd value is reducing with maturity, the Kd value of frost damaged whole plant faba bean hay is comparable with the Kd value of whole plant faba bean at late pod stage, but lower than the Kd value at flower and mid pod stages. However, the U fraction in the frost damaged whole plant faba bean hay is higher than the U fraction of the whole plant faba bean hay at flower mid pod, and late pod stages. The RUP^{NRC} of the frost damaged whole plant faba bean hay is slightly higher than the RUP^{NRC} of whole plant faba bean hay at late pod stage. Yu and Racz (2009) found that frozen wheat had higher S and U fractions and lower D fraction of than normal wheat.

Table 5.5. Effect of variety (tannin concentration) on rumen degradation kinetics (crude protein) of frost damaged whole plant faba bean hay

Item	Variety (V, Tannin con.)			P value
	H	L	SEM	
Crude Protein				
CP (g/kg DM)	173	163	5.1	0.30
Kd (%/h)	10.65	7.84	0.905	0.16
Residue (0 h, %)	57.2	54.6	3.58	0.65
S (%)	42.8	45.4	3.58	0.65
D (%)	46.2	42.4	4.23	0.59
U (%)	11.1	12.2	1.19	0.07
%BCP=%RUP	27.7^{b}	30.5^{a}	0.37	0.03
BCP (g/kg DM, DVE)	53	55	1.0	0.30
RUP (g/kg DM, NRC)	48	50	0.9	0.30
%EDCP=%RDP	72.3^{a}	69.5^{b}	0.37	0.03
EDCP=RDP (g/kg DM)	125	113	4.3	0.19

H: high tannin variety; L: low tannin variety; 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: Tukey method; Kd: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; S: soluble fraction in the *in situ* incubation; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded of crude protein; con: concentration.

5.4.6 In Situ NDF Degradation Kinetics of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration

The results of NDF rumen degradation characteristics and uNDF at 288h incubation of frost damaged whole plant faba bean hay affected by variety/tannin concentration are presented in Table 5.6. The tannin concentration did not affect significantly most of the rumen degradation characteristics of NDF, however the undegradable fraction of NDF (U) was higher (P< 0.05) in the low tannin frost damaged whole plant faba bean hay than in the high tannin frost damaged whole plant faba bean hay (+5.7 %), however the undigestible neutral detergent fiber (uNDF) was similar (P> 0.05; 23.24 %DM).

The Kd of NDF is reducing with maturity. The Kd of high tannin frost damaged whole plant faba bean hay is lower than the Kd of whole plant faba bean hay at flower and mid pod stages. The

U fraction after 72 h of incubation is higher in the frost damaged whole plant faba bean hay than the whole plant faba bean hay at late pod stage, but similar to the U fraction of whole plant faba bean hay at flower and mid pod stages. However, the uNDF after 288 h of incubation of frost damaged whole plant faba bean hay is higher than the uNDF of whole plant faba bean hay at flower, mid pod, and late pod stages.

Table 5.6. Effect of variety (tannin concentration) on rumen degradation kinetics (NDF) of frost damaged whole plant faba bean hay

Item	Variety (V, Tannin con.)			P value
	H	L	SEM	
Fiber (NDF) Degradation				
NDF (g/kg DM)	447	472	10.5	0.24
Kd (%/h)	2.66	4.21	0.682	0.25
Residue (0h, %)	92.5	90.5	1.75	0.34
S (%)	7.5	9.5	1.75	0.34
D (%)	54.6	46.9	1.57	0.09
U (%)	37.9[b]	43.6[a]	3.13	0.01
%BNDF=%RUNDF	75.7	71.5	1.79	0.34
RUNDF (g/kg DM, NRC)	338	337	5.7	0.84
%EDNDF=%RDNDF	24.3	28.5	1.79	0.34
EDNDF=RDNDF (g/kg DM)	109	135	10.0	0.21
uNDF (288 h, CNCPS 6.5) (% DM)	22.97	23.52	2.925	0.91

H: high tannin variety; L: low tannin variety; 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: Tukey method; Kd: the degradation rate of D fraction; S: washable fraction; D: degradable fraction; U: rumen undegradable fraction; BDNDF or RUNDF: rumen bypass or undegraded feed neutral detergent fiber; EDNDF or RDNDF: effective degraded neutral detergent fiber. uNDF: undigestible neutral detergent fiber; con: concentration.

5.4.7 In Situ Starch Degradation Kinetics of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration

The results of starch rumen degradation characteristics of frost damaged whole plant faba bean hay affected by variety/tannin concentration are presented in Table 5.7. The tannin concentration did not affect significantly ($P > 0.05$) any of the rumen degradation characteristics of starch. The effective degradable dry matter (EDDM) averaged 495.5 g/kg DM. The rumen bypass starch (BSt) was averaged 26 g/kg DM.

The Kd value of frost damaged whole plant faba bean hay is slightly lower than the Kd value of whole plant faba bean at late pod stage, however, the BSt (g/kg DM) is similar to the whole plant faba bean at late pod stage. Yu and Racz (2009) found that frozen wheat had lower bypass starch than normal wheat.

Table 5.7. Effect of variety (tannin concentration) on rumen degradation kinetics (Starch) of frost damaged whole plant faba bean hay

Item	Variety (V, Tannin con.)			P value
	H	L	SEM	
Starch				
St (g/kg DM)	135	104	9.8	0.16
Kd	12.93	8.93	1.442	0.14
Residue (0 h, %)	53.6	60.0	11.38	0.73
S (%)	46.4	40.0	11.38	0.73
D (%)	53.6	60.0	11.37	0.73
%BSt	17.1	23.7	2.77	0.23
BSt (g/kg DM)	25	27	1.7	0.52
%EDSt	82.9	76.3	2.77	0.23
EDST (g/kg DM)	112	80	10.8	0.17

H: high tannin variety; L: low tannin variety; 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: Tukey method; Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction; D: degradable fraction; U: rumen undegradable fraction; BSt: rumen bypass or undegraded feed starch; EDST: effective degraded starch; con: concentration.

5.4.8 Hourly Effective Degradation Ratios between Available N and Available CHO of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration

The effect of tannin concentration on the hourly effective degradation ratios between available N and available carbohydrates (ED ratio of N/CHO) at different incubation times of frost damaged whole plant faba bean hay is shown in Table 5.8 and Figure 5.1. Detailed observation of the data revealed that tannin concentration had a significant effect (P< 0.05) on the ratio of N/CHO which was higher (P< 0.05) in the high tannin frost damaged whole plant faba bean hay than that in the low tannin frost damaged whole plant faba bean hay (+ 3 g/kg). On the other hand, the tannin concentration did not significantly (P> 0.05) affect the overall ratio of ED_N/ED_CHO as well as the individual ratios of ED_N/ED_CHO at the different time points.

The ratio of N/CHO is lower in the frost damaged whole plant faba bean hay than that in the whole plant faba bean hay at late pod stage, also the overall ED ratio of N/CHO is lower in frost damaged whole plant faba bean hay than that in whole plant faba bean at flower, mid pod, and late pod stages because of the lower S fraction of CP. The ED ratio of N/CHO at h0 of frost damaged whole plant faba bean hay is comparable to the ratio of ED ratio of N/CHO at h0 of whole plant faba bean hay at late pod stage.

Table 5.8. Effect of variety (tannin concentration) on potentially available N to available CHO synchronization of frost damaged whole plant faba bean hay

Item	Variety (V, Tannin con.)			P value
	H	L	SEM	
Ratio of N/CHO (g/kg)	48[a]	45[b]	0.2	0.02
Ratio of ED_N/ED_CHO (g/kg)	74	69	3.8	0.46
Ratio at individual h (g/kg)				
h0	125	136	8.8	0.40
h1	86	57	13.3	0.26
h2	83	55	12.3	0.26
h3	79	53	11.4	0.25
h4	75	51	10.5	0.24
h6	67	46	8.8	0.24
h8	59	42	7.3	0.25
h10	51	38	5.9	0.26
h12	43	34	4.7	0.30
h14	36	30	3.7	0.37
h16	30	27	2.7	0.49
h18	25	24	2.0	0.73
h20	23	22	1.7	0.95
h22	17	18	0.9	0.06
h24	13	16	0.7	0.12

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; [a-e] Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method; ED: effective degradability; CHO: carbohydrates; con: concentration.

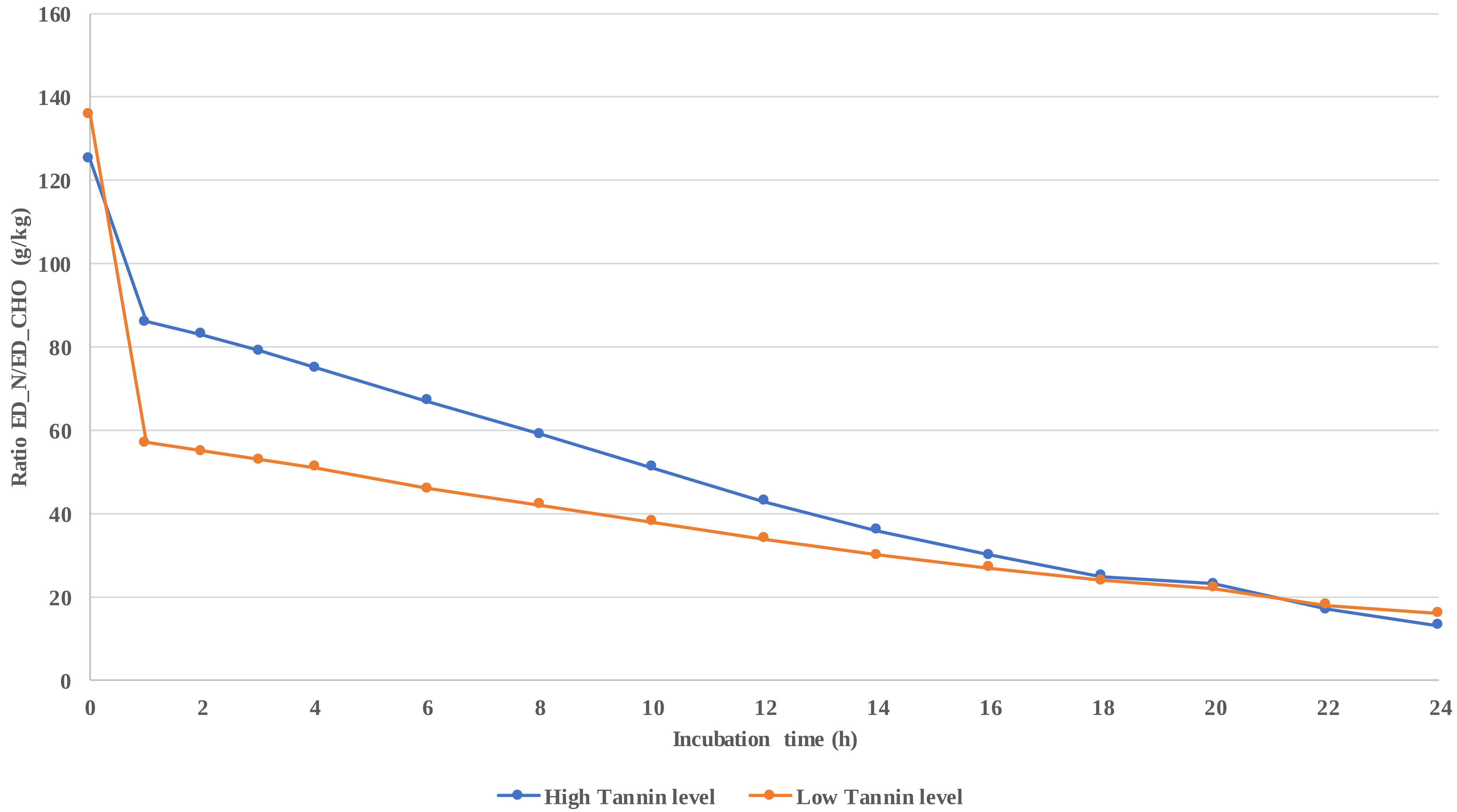


Figure 5.1. Effect of tannin concentration on hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of frost damaged whole plant faba bean hay. Optimum ratio = 32 EN/ECHO g/kg.

5.4.9 Intestinal Availability of Rumen Bypass Nutrients of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration

The effect of tannin concentration on the intestinal digestible rumen bypass and total digestible DM, CP, NDF and starch of the frost damaged whole plant faba bean hay are presented in Table 5.9. The data revealed that the tannin concentration did affect ($P< 0.05$) the intestinal digestible rumen bypass DM (IDBDM) which was higher ($P< 0.05$) in the high tannin frost damaged whole plant faba bean hay than that in the low tannin frost damaged whole plant faba bean hay (245 vs 230 g/kg DM). Additionally, the intestinal digestibility of CP (%dIDP) was higher ($P< 0.05$) in the high tannin frost damaged whole plant faba bean hay than that in the low tannin frost damaged whole plant faba bean hay (+ 7%). The other characteristics of intestinal digestibility of DM, CP, NDF and starch were similar between the high tannin and the low tannin frost damaged whole plant faba bean hay. The TDDM (%) of frost damaged whole plant faba bean hay is lower than the TDDM of whole plant faba bean hay at flower, mid pod, and late pod stages. The dIDP (%) and the TDP (%) of frost damaged whole plant faba bean hay are lower than the values in whole plant faba bean hay at flower, mid pod, and late pod stages. The TDNDF (%) of frost damaged whole plant faba bean hay is lower than the TDNDF (%) of whole plant faba bean hay at flower, mid pod, and late pod stages. However, the TDNDF (g/kg DM) is higher in the frost damaged whole plant faba bean hay than in the whole plant faba bean hay at flower, mid pod, and late pod stages, mainly because of the higher concentration of NDF in the frost damaged whole plant faba bean hay. The TDST (g/kg DM) of the frost damaged whole plant faba bean hay is comparable to the TDST (g/kg DM) of whole plant faba bean hay at late pod stage.

Table 5.9. Effect of variety (tannin concentration) on intestinal digestibility and total tract digestion of frost damaged whole plant faba bean hay

Item	Variety (V, Tannin con.)			P value
	H	L	SEM	
Dry matter				
%dBDM	49.0	45.3	3.18	0.18
%IDBDM	24.5[a]	23.0[b]	1.03	0.01
IDBDM (g/kg DM)	245[a]	230[b]	10.3	0.01
%TDDM	74.5	72.1	2.32	0.31
TDDM (g/kg DM)	745	721	23.1	0.31
Crude protein				
% dIDP	49.1[a]	42.1[b]	1.02	0.02
IADP (g/kg DM)	24	21	0.6	0.18
IADP (g/kg CP)	136	128	4.1	0.26
TDP (g/kg DM)	149	134	4.5	0.16
TDP (g/kg CP)	859	824	2.8	0.06
%IADP (%CP)	13.6	12.8	0.41	0.26
%TDP (%CP)	85.9	82.4	0.28	0.06
Fiber (NDF)				
%dBNDF	40.4	33.9	3.76	0.24
%IDBNDF	30.5	24.2	2.47	0.26
IDBNDF (g/kg DM)	136	114	10.7	0.20
%TDNDF	54.8	52.7	3.34	0.09
TDNDF (g/kg DM)	245	248	16.3	0.79
Starch				
%dBST	96.9	96.7	0.45	0.83
% IDBST	16.5	23.0	2.76	0.24
IDBST (g/kg DM)	24	26	1.8	0.55
%TDST	99.5	99.2	0.06	0.13
TDST (g/kg DM)	136	106	9.7	0.16

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; [a-f] Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method; dBDM: intestinal digestibility of rumen bypass dry matter; IDBDM: intestinal digested rumen bypass dry matter; TDDM: total digested dry matter dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digested crude protein; TDP: total digested crude protein; dBST: intestinal digestibility of rumen bypass starch; IDBST: intestinal digested rumen bypass starch; TDST: total digested starch; dBNDF: intestinal digestibility of rumen bypass neutral detergent fiber; IDBNDF: intestinal digested rumen bypass neutral detergent fiber; TDNDF: total digested neutral detergent fiber; con: concentration.

5.4.10 Nutrient Supply with the DVE/OEB System from Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration

The effect of variety/tannin concentration on metabolic characteristics and true nutrient supply based on the DVE/OEB System from the frost damaged whole plant faba bean hay are presented in Table 5.10. The truly digestible nutrient supply (DVE) tended (P= 0.09) to be affected by the tannin concentration (P> 0.05) with a higher value in high tannin frost damaged whole plant faba bean hay than low tannin forage damaged hay. However, the degraded protein balance (OEB) was not significantly affected by the tannin concentration (P> 0.05) with average of 25 g/kg DM.

The DVE value of the frost damaged whole plant faba bean hay is lower than the DVE value of whole plant faba bean hay at mid pod and late pod stages, but comparable to the DVE value of whole plant faba bean hay at flower stage. The OEB value of frost damaged whole plant faba bean hay is lower than the OEB value of whole plant faba bean hay at flower, mid pod, and late pod stages, mainly because of the lower EDCP found in frost damaged whole plant faba bean hay. Yu and Racz (2010) found that frozen wheat had lower DVE value than normal wheat.

5.4.11 Nutrient Supply with the NRC-2001 Model from Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration

The effect of variety/tannin concentration on metabolic characteristics and true nutrient supply to dairy cows based on the NRC-2001 model from the frost damaged whole plant faba bean hay are shown in Table 5.10. The tannin concentration did not affect most of these characteristics, however it significantly affected the metabolizable protein (MP) which was higher (P< 0.05) in the high tannin frost damaged whole plant faba bean hay than in the low tannin frost damaged whole plant faba bean hay (72 vs. 68 g/kg DM). Again, it did not affect the degraded protein balance (OEB) with average of 40 g/kg DM.

According to the NRC, the MP is slightly lower in the frost damaged whole plant faba bean hay than in the whole plant faba bean hay at flower, mid pod, and late pod stages. Also, the DPB value was lower because of the lower MCP_{RDP} in the frost damaged whole plant faba bean hay. Yu and Racz (2010) found that frozen wheat had lower MP value than normal wheat additionally, they found that both the frozen and normal wheat had negative DPB.

5.4.12 Feed Milk Value of Frost Damaged Whole Plant Faba Bean Hay: Effect of Varity/Tannin Concentration

The effect of variety/tannin concentration on Feed Milk Value (FMV) of the frost damaged whole plant faba bean hay based on the DVE system and the FMV based on the NRC 2001 model are presented in Table 5.10. The FMV^{DVE} and the FMV^{NRC} in the frost damaged whole plant faba bean hay were significantly affected ($P< 0.05$) by the tannin concentration. Those values were higher ($P< 0.05$) in the high tannin frost damaged whole plant faba bean hay than in the low tannin frost damaged whole plant faba bean hay (+0.15 and +0.09 kg milk/kg DM hay, respectively).

According to the DVE/OEB system, the FMV of the high tannin frost damaged whole plant faba bean hay is comparable to the FMV of whole plant faba bean hay at flower stage, but lower than at mid pod and late pod stages. According to the NRC model the predicted FMV values are higher than the FMV values predicted with the DVE/OEB system; however, the FMV^{NRC} of frost damaged whole plant faba bean hay is slightly lower than the FMV of the whole plant faba bean hay at late pod stage.

Table 5.10. Effect of variety (tannin concentration) on metabolic characteristics, true nutrient supply, and feed milk value of frost damaged whole plant faba bean hay

Item	Variety (V, Tannin con.)		SEM	P value
	H	L		
Truly digestible nutrient supply to dairy cows based on non-TDN system: DVE system				
BCP (g/kg DM)	53	55	1.0	0.30
MREE (g/kg DM)	92	86	2.8	0.17
MREN (g/kg DM)	120	108	4.2	0.18
DVME (g/kg DM)	59	55	1.8	0.17
DVBE (g/kg DM)	26	23	0.7	0.18
Degraded protein balance (OEB of hay) and Total true protein supply (DVE of hay) to dairy cows				
DVE (g/kg DM)	65	57	2.8	0.09
OEB (g/kg DM)	28	22	5.2	0.52
FMV (kg milk/kg DM Hay)	1.31[a]	1.16[b]	0.065	0.04
Truly digestible nutrient supply to dairy cows based on TDN system: NRC dairy				
RUP (g/kg DM)	48	50	0.9	0.30
MCP_{TDN} (g/kg DM)	69	66	0.6	0.09
MCP_{RDP} (g/kg DM)	106	96	3.6	0.19
AMCP (g/kg DM)	44	42	0.4	0.09
ARUP (g/kg DM)	24	21	0.6	0.18
Degraded protein balance (DPB of hay) and Total metabolizable protein supply (MP of hay) to dairy cows				
MP (g/kg DM)	72a	68b	0.5	0.03
DPB (g/kg DM)	44	36	3.9	0.27
FMV (kg milk/kg DM Hay)	1.46[a]	1.37[b]	0.010	0.02

H: high tannin variety; L: low tannin variety; SEM: standard error of mean; [a-h] Means with the different letters in the same row are significantly different (P< 0.05); Multi-treatment comparison: Tukey method; BCP: ruminally undegraded feed CP: calculated according the formula in DVE/OEB system; MREE: microbial protein synthesized in the rumen based on available energy; MREN: microbial protein synthesized in the rumen based on rumen degraded feed crude protein; DVME: truly absorbed rumen synthesized microbial protein in the small intestine; DVBE: truly absorbed bypass feed protein in the small intestine; DVE: truly absorbed protein in the small intestine; OEB: is a balance between microbial protein synthesis from rumen degradable CP and that from the energy extracted during anaerobic fermentation in the rumen. RUP: ruminally undegraded feed CP: calculated according the formula in NRC-2001 dairy model; MCP_{TDN}, microbial protein synthesized in the rumen based on available energy (discounted TDN); $MCP_{RDP,}$: microbial protein synthesized in the rumen based on available protein; AMCP: truly absorbed rumen-synthesized microbial protein in the small intestine; ARUP: truly absorbed rumen-undegraded feed protein in the small intestine; MP: metabolizable protein; DPB: reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy-TDN available for microbial fermentation in the rumen; con: concentration.

5.5 Conclusions

The condensed tannin concentration of frost damaged whole plant faba bean hay is comparable to the condensed tannin concentration of normal whole plant faba bean hay, but lower than whole plant faba bean silage. Besides, the protein content and the energy level are lower than the whole plant faba bean hay and silage, while the soluble crude protein is higher. Also, the rate of degradation of dry matter, crude protein, neutral detergent fiber and starch were lower than in whole plant faba bean hay and silage, but the bypass protein is higher in the frost damaged whole plant faba bean hay, while the metabolizable protein and the predicted production performance was almost similar than in the whole plant faba bean silage.

The tannin concentration had a significant effect on some of the characteristics of the chemical profile, protein fractions, and rumen degradation kinetics of primary nutrients in frost damaged whole plant faba bean hay. The tannin concentration in frost damaged whole plant faba bean hay greatly affected the truly digestible nutrient supply (DVE) and metabolizable protein (MP) but did not significantly affect the degraded protein balance (OEB or DPB value). The Feed Milk Value was significantly lower in the low tannin frost damaged whole plant faba bean hay. It was concluded that tannin concentration significantly affects nutrient supply in forage whole plant faba bean hay. However, frost damaged whole plant faba bean hay had slightly lower nutritive value and predicted production performance than normal whole plant faba bean hay at mid pod and late pod stages. Further studies including animal and metabolic trials should be performed to confirm these results and develop an adequate feeding strategy.

6. DAIRY PERFORMANCE AND METABOLIC TRIALS WITH WHOLE PLANT FABA SILAGE: DEVELOPMENT EFFICIENT FEEDING STRATEGY OF WHOLE PLANT FABA SILAGE TO FIND MAXIMUM REPLACEMENT OF BARLEY AND CORN SILAGE IN HIGH PRODUCTION LACTATION DAIRY COWS TO BENEFIT PULSE GROWERS AND DAIRY PRODUCERS

6.1 Abstract

The aim of this study was to determine the effect of partial (50% and 75%) and complete (100%) replacement of barley and corn silages with low tannin (Snowdrop variety) whole plant faba bean silage at late pod stage (97 days old) on high production dairy cows rations in terms of milk yield and component, feed intake and efficiency, digestibility of primary nutrients, rumen fermentation, and metabolic characteristics as well as feeding behaviour. A double 4 × 4 Latin square (8 early lactating cows: 4 cannulated and 4 non-cannulated) as an experimental design with four 25-d periods. The results showed that T100 (30.60 % whole plant faba bean silage) produced higher ($P < 0.05$) fat corrected milk (3.5% FCM) and higher ($P < 0.05$) energy corrected milk (ECM) than control diet T0 (18.37% corn silage + 12.23 % barley silage) (+4.35 and +3.48 kg/cow/d, respectively), but produced similar FCM and ECM to T50 (9.18% corn silage + 6.12 % barley silage + 15.30 % whole plant faba bean silage) and T75 (4.59% corn silage + 3.06 % barley silage + 22.95 % whole plant faba bean silage). Additionally, the fat yield was higher ($P < 0.05$) when whole plant faba bean silage was included (T50, T75, T100 vs. T0 control) in the diet (2.11 vs. 1.89 kg/cow/d). Efficiency (FCM/DMI) was higher ($P < 0.05$) when animals consumed T75 than T0 (2.21 vs. 1.91). The digestibility of starch was similar ($P > 0.10$) among T50, T75 and T100 but were lower ($P < 0.05$) than that in T0 (92.65 % vs. 96.13%). The inclusion of whole plant faba bean silage (T50, T75, T100 vs. T0 control) increased ($P < 0.05$) the total energy of the diets (1.91 vs. 1.65 Mcal/kg DMI) and significantly increased ($P < 0.05$) the percentage of energy for body weight

gain (BWG) and milk production (78.3 vs. 75.5 % of total energy). Rumen fermentation characterises (pH, ammonia, volatile fatty acids) were similar (P> 0.10) among all the treatments. In conclusion, the inclusion of whole plant faba bean silage at late pod stage improve fat corrected milk, energy corrected milk, milk fat yield, and efficiency without negatively affecting the intake of dry matter. Consequently, this study showed that whole plant faba bean silage is a highly nutritive alternative feed to improve the performance of dairy cows in western Canada.

Keywords: Cows, whole plant faba bean silage, milk yield, fat, efficiency, metabolic characteristics.

6.2 Introduction

Barley is well adapted to the different types of soils in temperate regions (Krishna, 2013) and normally is harvested at mid-dough stage in which the yield is about 8 tonnes of dry matter per hectare (Gill et al., 2013; Government of Alberta, 2020). In western Canada, different barley varieties are used as silage to fed beef and dairy cattle, but the concentration of protein in barley silage is relatively low when compared with some legume silages such as soybean and alfalfa (McKinnon, 2018; Vargas et al., 2008; Khorasani et al., 1993). Alfalfa silage is commonly used in Canada as well. Alfalfa silage is well recognized to have higher yield and quality than grass silages, but normally alfalfa silage has lower concentration of fiber and higher concentration of crude protein than grass silages (Bernardes et al., 2018; Ingalls et al., 1974). However, the disadvantages of soybean and alfalfa silages are sometimes the price and their almost null starch content. In western Canada, other legumes such as peas and faba bean are grown. These crops have a high crude protein and starch content which shows an interesting nutrient profile, beneficial when included in dairy rations as silage (Heeg, 2017; Mustafa and Seguin, 2003a; Hickling, 2003). For instance, pea silage has similar nutrient composition than alfalfa silage, but has higher concentration of starch, which makes it an adequate forage for dairy cattle (Fraser et al., 2001;

Mustafa et al., 2000). The digestibility of dry matter and fiber is similar to alfalfa silage but higher than barley silage, additionally it has been demonstrated that pea silage can replace alfalfa silage and barley silage without affecting milk production (Mustafa et al., 2000). On the other hand, the use of faba bean as forage is scarce about the world, however faba bean as silage was tested long time ago in Canada with promising results (Berkenkamp and Meeres, 1986; McKnight and MacLeod, 1977). Actual varieties available in Canada showed a dry matter yield about 12 tonnes per hectare (Guevara-Oquendo et al., 2019). Furthermore, the protein in faba bean silage is higher than in barley silage, while the starch content is comparable to barley silage (Nair et al., 2016; Preston et al., 2017a) and higher than in pea silage (Mustafa et al., 2000). Therefore, ensiled whole plant faba bean is a source of both protein and starch, which allows the reduction of both ingredients in the feed, as less amount of protein from alternative protein sources and less amount of fermentable carbohydrate from cereal grains, could be supplemented in the total mixed ration (TMR). Moreover, high producing milking cows in early lactation normally show low feed intake and high energy and protein demands (Buckley et al., 2003; NRC, 2001), consequently including high nutrient ingredients such as whole plant faba bean silage to meet their requirements can be an advantage. As faba bean production has increased and mycotoxin problem in cereals as well, it is possible to replace appropriately barley silage with faba bean silage if it is required (Guevara-Oquendo et al., 2019). For that reason, this project was conducted in order to develop the best faba bean silage feeding strategy in dairy cows which can provide similar or better production performance than the already established barley or barley/corn silage-based diets.

6.3 Materials and Methods

All cows involved in the present trial were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 2009) and were approved (Animal Use Approval

Protocol #19910012) by Animal Research 125 Ethics Board (AREB) at the University of Saskatchewan (Saskatoon, SK, Canada).

6.3.1 Silage Preparation

Faba bean cv. Snowbird (low tannin variety) was seeded on May 12th, 2018 and harvested on August 19th, 2018 at late pod stage (97 days old) in Melfort, SK, Canada (crop location NW 16 44 21 W2). The harvest time was chosen based on a previous study which compared whole plant faba bean silage at different stages of cutting and for different varieties (Guevara-Oquendo, unpublished; In Chapter 3). After harvesting, the fresh material was wilted to a targeted 45 %DM (August 20th and August 21st), and then chopped to 1-inch length (about 226 tonnes). Afterwards, a silage pile was constructed, packed, and covered with plastic on August 22nd. Fermentation process lasted for 150 days until the silage pile was opened. The chemical composition of all used ingredients is presented in Table 10.1 in the appendix.

6.3.2 Animals, Diets, and Experimental Design

Eight early lactating Holstein cows (3rd lactation) with average BW = 671 ± 57 kg and DIM = 68 ± 20 were kept in individual tie stalls (4 × 4 Latin square design (LSD): 4 cannulated cows and 4 non-cannulated cows) at the Rayner Dairy Research and Teaching Facility (RDRTF, Saskatoon, SK, Canada). Cows were allowed to adapt to the tie stalls 8 days prior the beginning of the first LSD experimental period.

Four different 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 early lactating cows at 60 days in milk and 25kg of assumed DMI.

The formulations included a control diet (T0) with 18.37% corn silage + 12.23% barley silage (on DM basis), no addition of whole plant faba bean silage; two diets with partial replacement of barley and corn silages (T50 and T75) with whole plant faba bean silage: T50 (9.18% corn silage + 6.12% barley silage + 15.30% whole plant faba bean silage) (on DM basis) and T75 (4.59% corn silage + 3.06% barley silage + 22.95% whole plant faba bean silage) (on DM basis); last, one diet with complete replacement of barley and corn silages (T100) which contained 30.60% of whole plant faba bean silage on DM basis. Cows were provided with ad libitum water and a total mixed ration (TMR) once a day at 0930 h. The offered feed was monitored constantly to keep refusals at 5 to 7% (on as fed basis). Every week, about 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.

Each experimental period consisted of 25 days with 4 days of diet change over, 14 days of diet adaptation, and 7 days of sample collection. Throughout the 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 (on as fed basis) (Table 6.1).

6.3.3 Milk Sampling

Cows were milked three times per day at 0700, 1500, and 2300 h at a milking parlor and yield was recorded from d 16 to d 25 at each milking time per each LSD experimental period. Milk samples were collected on d 19, d 20, and d 21 for three consecutive days, then 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 N, protein (%), lactose (%), and milk fat (%). Calculations for solids-non-fat, fat corrected milk, energy corrected milk, and yields of milk protein, lactose, and fat were performed following previous research of Refat et al., (2017) and Tosta et al., (2019).

Table 6.1. Ingredients and nutrient composition of the four dietary treatments for dairy production and metabolic trials

	Treatment % (T)			
Item	0	50	75	100
Ingredient, % of DM				
Corn Silage Jan 2019	18.37	9.18	4.59	0.00
Barley Silage Jan2019	12.23	6.12	3.06	0.00
Whole Plant Faba Bean Silage	0.00	15.30	22.95	30.60
Alfalfa Hay	10.41	10.41	10.41	10.41
Straw	4.09	4.09	4.09	4.09
Beet Pulp	7.81	7.81	7.81	7.81
RP10 Palmitic	1.52	1.52	1.52	1.52
Barley/Corn	27.70	31.05	32.17	32.91
Flaked Barley	19.77	22.16	22.96	23.48
Flaked Corn	7.93	8.89	9.21	9.43
Protein_2018	13.39	10.04	8.92	8.18
Canola Meal	9.58	7.18	6.38	5.85
Soybean Meal	3.81	2.86	2.54	2.33
Lactating Supplement	4.46	4.46	4.46	4.46
Water	0.01	0.01	0.01	0.01
Nutrient %DM				
CP	16.2	16.2	16.9	17.5
aNDF	33.9	32.8	31.6	30.1
Starch	22.8	24.0	23.7	24.5
Ash	7.36	8.05	7.55	7.49
Ca	0.78	0.81	0.83	0.86
P	0.44	0.42	0.41	0.40

T0=18.37% corn silage + 12.23 % barley silage; T50=9.18% corn silage + 6.12 % barley silage + 15.30 % whole plant faba bean silage; T75=4.59% corn silage + 3.06 % barley silage + 22.95 % whole plant faba bean silage; T100= 30.60 % whole plant faba bean silage.

6.3.4 Feed Chewing Activity

Cameras with a digital video recorder (QSEE, QC908 HD DVR, China) were set up at the tie stall area to monitor the cows chewing activity at d 19 to d 20 (24 h) from each experimental period. The recording started and finished at 0800 h (before feeding), and videos collected were analyzed for duration of eating and ruminating at 5 min intervals (Mugerwa et al., 1973).

6.3.5 Total Collection

All sample collections started in the morning before cows were fed (~ 0900 h). Refusals were removed from the bunks and weighed. Feed TMR and orts were collected daily from d 20 to d 25 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 then were pooled by cow and period, collected on paper bags, and dried in a forced-air oven at 55 °C for 48 h.

Urine and faeces samples were collected for 3 d every 6 h on d 22, d 23, and d 24 of each period. On d 21 urine catheters (Foley bladder catheters, 26 Fr, 75-mL ribbed balloon, lubricious- coated; C. R. Bard Inc., Covington, GA) were placed in all cows. 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. At each time point, urine containers were weighed, and urine volume was recorded to determine total urine excretion per cow, then about 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. Each collection day, 90 ml of urine samples were pooled by day, by cow, and by period based on the proportion of total urine collected on each time point and stored at −20°C for further analyses. Faeces were collected in large plastic containers and weight to determine total excretion per cow. About 600 g of faeces samples were taken into individual plastic bags per cow and stored at −20°C. At the end of the last experimental period, faeces samples were thawed at room temperature and about 400 g were pooled into Aluminium foil trays by cow, by day, and by period based the proportion of total faeces collected on each time point. Then, samples were dried in an air oven at 55 °C for 48 h (AOAC, 930.15) (AOAC, 2005).

6.3.6 Rumen Fermentation Characteristics

Rumen fluid samples were collected from the 4 cannulated cows on d 25 of each period at 0900 h every 3 h until 0900 h of the next day. Fluid and solid material were taken from the cranial ventral, rumen ventral, and caudal ventral sections of the rumen of each cow and filtered through

4 layers of cheesecloth into individual plastic buckets. The pH of the filtered solution was measured using a pH meter (Accumet AP110, Thermo Fisher Scientific, Waltham, MA) and results were recorded for future data analysis. Next, 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.

6.3.7 Chemical Analysis

The dried TMR and orts 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 analyses of ash (AOAC, 942.05), CP (AOAC, 984.13) (AOAC, 2005), NDF (Van Soest et al., 1991), and starch (Hall, 2009).

Nitrogen balance and total tract digestibility were determined by the analysis of urine and faeces samples. Kjeldahl method (AOAC 984.13) was used to determine total N. 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. 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), CP (AOAC, 984.13), NDF (Van Soest et al., 1991), and starch (Hall, 2009) at Cumberland Valley Analytical Services (CVAS, Hagerstown, MD).

Rumen fluid was used for the analysis of rumen fermentation characteristics. Sample tubes were thawed overnight at 4°C and then analyzed for ammonia and VFA. Ammonia was determined based on the procedure from Broderick and Kang (1980), and VFA were determined using a gas chromatography-flame ionization detector (GC-FID) system the TRACE 1310 (Thermo Fisher Scientific, Waltham, MA, USA) at the Ministry of Agriculture Strategic Feed Research Chair Lab, University of Saskatchewan (Saskatoon, SK, Canada). Sample preparation for the later was performed according to an internal standard method as follows: 1) thawed samples were vortexed,

2) 1 ml of solution was pipetted 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) finally, the solution was diluted with 0.75 ml of double distilled water to reduce the concentration and viscosity of the rumen fluid.

6.3.8 Statistical Analysis

Results from the Latin Square Design (LSD) were analyzed using the Mixed model procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC, US). Milk production and composition, chewing activity, total tract digestibility, and metabolic studies were analyzed 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; Ti 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 final variance and covariance structure models were selected based on AIC and BIC values (Tempelman et al., 2001). The difference among treatments was evaluated using the Tukey method. Letter groupings in Proc Mixed was used SAS macro "pdmix800" (Saxton, 1998). Contrast statements were used to compare barley/corn silage and whole plant faba bean silage (SAS, 2003). Curvilinear responses (linear, quadratic, and cubic) to the increase level of whole plant faba bean silage was determined using orthogonal polynomial contrast with coefficients adjusted for unequal spacing using SAS version 9.4. For all statistical analyses, significance was declared at $P< 0.05$ and trends at $P\leq 0.10$.

6.4 Results and Discussion

6.4.1 Feed Intake, Body Weight and Efficiency

Feed intake, body weight and efficiency are presented in Table 6.2. The intake of dry matter (DMI), intake of organic matter, intake of ash and intake of starch were not significantly different (P> 0.05) among the four treatments. However, the intake of CP was higher (P< 0.05) in T100 (= 30.60 % whole plant faba bean silage) than T0 (=18.37% corn silage + 12.23 % barley silage) and T50 (=9.18% corn silage + 6.12 % barley silage + 15.30 % whole plant faba bean silage) (+0.44 and +0.58 kg/d, respectively), and the intake of CP in T75 (=4.59% corn silage + 3.06 % barley silage + 22.95 % whole plant faba bean silage) was similar to the other treatments. There was a quadratic response (P= 0.4) of CP intake to the increased inclusion level of whole plant faba bean silage in the TMR. The NDF intake was higher (P< 0.05) in T0 than T75 and T100 (+1.04 and +1.11 kg/d, respectively), the intake of NDF in T50 was similar (P> 0.05) to the other treatments. Inclusion of whole plant faba bean silage reduced significantly (P< 0.05) the NDF intake (-0.94 kg/d). There was a linear response (P< 0.01) of NDF intake to the increased inclusion level of whole plant faba bean silage in the TMR.

There was a tendency (P= 0.07) that the animals which consumed the diets which included whole plant faba bean silage had higher body weight, however the results showed that weight gain (WG) was higher (P< 0.05) in the treatments which include whole plant faba bean silage than control diet T0 with the barley/corn silage only (+0.44 vs -0.17 kg/d).

Efficiency FCM/DMI and efficiency ECM/DMI were higher (P< 0.05) in the treatments which included whole plant faba bean silage than the barley/corn silage treatment T0 (2.15 vs. 1.91 and 2.07 vs. 1.87, respectively). Both efficiency FCM/DMI and efficiency ECM/DMI had linear responses (P< 0.03) to the increased inclusion level of whole plant faba bean silage in the TMR.

Table 6.2. Intake, body weight, and feed efficiency of Holstein cows fed TMRs with different levels of inclusion of whole plant faba bean silage

	Treatment % (T)					Contrast, P value			
Item	0	50	75	100	SEM	Control vs. Treatments	Linear	Quadratic	Cubic
Intake (kg/d)									
DM	27.14	25.81	25.80	27.12	1.140	0.28	0.69	0.16	0.68
OM	25.19	23.71	23.86	25.08	1.083	0.22	0.63	0.13	0.81
Ash	1.95	2.10	1.94	2.04	0.076	0.32	0.50	0.63	0.05
CP	4.37^{b}	4.23^{b}	4.42^{ab}	4.81^{a}	0.174	0.39	0.01	0.04	0.90
NDF	9.13^{a}	8.45^{ab}	8.09^{b}	8.02^{b}	0.394	<0.01	<0.01	0.61	0.65
ST	6.26	6.21	6.13	6.75	0.357	0.73	0.24	0.27	0.34
Weight									
BW (kg)	679.95	690.63	688.85	690.94	9.737	0.07	0.06	0.55	0.54
WG (kg/d)	-0.17^{b}	0.43^{ab}	0.25^{ab}	0.66^{a}	0.209	0.02	0.03	0.77	0.27
Dairy efficiency									
Efficiency (FCM/DMI)	1.91^{b}	2.18^{a}	2.21^{a}	2.08^{ab}	0.090	0.01	0.03	0.06	0.65
Efficiency (ECM/DMI)	1.87^{b}	2.10^{a}	2.13^{a}	2.00^{ab}	0.082	0.01	0.03	0.05	0.58

SEM: standard error of mean; $^{a\text{-}b}$ Means with the different letters in the same row are significantly different ($P < 0.05$); T0=18.37% corn silage + 12.23 % barley silage; T50=9.18% corn silage + 6.12 % barley silage + 15.30 % whole plant faba bean silage; T75=4.59% corn silage + 3.06 % barley silage + 22.95 % whole plant faba bean silage; T100= 30.60 % whole plant faba bean silage; DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ST: Starch; OM: organic matter; BW: body weight; WG: weight gain; FCM (3.5%): fat corrected milk; ECM: energy corrected milk; Efficiency: FCM/DMI; Efficiency: ECM/DMI

Dairy cows fed barley silage diets had and averaged DMI of 25.6 kg/cow/d (Refat, 2018), however in our study the averaged DMI was 26.3 kg/cow/d when were fed dies which included whole plant faba bean silage, suggesting that the inclusion of whole plant faba bean silage did not negatively affected the DMI. On the other hand, in our study the inclusion of whole plant faba bean silage reduced significantly the NDF intake compared with the barley/corn silage diet, however that reduction did not impact in the DMI as in agreement with the results found in Bauchmein (1991) which suggested that the DMI was not affected when NDF concentration varied from 31, 34 to 37 % DM. However, Benchaar et al., (2014) replace barley silage (52% NDF) with corn silage (36% NDF) obtaining lower NDF content in the diet which had a higher inclusion of corn silage. Therefore, a higher DMI and similar NDF intake were observed when corn silage inclusion increased, however in our study as whole plant faba bean inclusion increased, CP intake quadratically increased, NDF intake linearly decreased and DMI remained similar.

It is normal for early lactating dairy cows to mobilize adipose tissue and lose weight because the energy provided by the diet is lower than the energy demand for milk production (Buckley et al., 2003; Bauman and Currie, 1980). The magnitude and duration of the negative energy balance in early lactation may negatively affect the reproductive performance of cows, particularly the probability of conception (Buckley et al., 2003; NRC, 2001; Domecq et al., 1997). The weigh gain of the animals fed diets with whole plant faba bean silage averaged 0.44 kg/d, while the barley/corn diet was -0.17 kg/d, suggesting that dairy cows in early lactation fed diets fed whole plant faba bean diets actually can sustain high production and in the mean time can gain weight which is one of the objectives in order to start cycling again.

The feed efficiency FCM/DMI and ECM/DMI were improved when whole plant faba bean silage was included in the diets. Refat (2018) suggested that the FCM/DMI and the ECM/DMI of a corn silage based diet was 1.61 and 1.57 respectively; while on average, supported by Eun et al., (2004), the FCM/DMI and the ECM/DMI of the barley silage based diets was 1.42 and 1.37 respectively. Those results suggested that corn silage diet has a higher feed efficiency than barley silage diet, however in Benchaar et al., (2014) occurred the opposite. In our study the barley/corn diet showed a FCM/DMI and an ECM/DMI of 1.91 and 1.87, but in average the FCM/DMI and the ECM/DMI of the whole plant faba bean silage diets are 2.15 and 2.07, respectively. Suggesting that there is a clear improvement in efficiency when whole plant faba bean silage is included in the diet (%).

6.4.2 Milk Yield and Composition

The milk yield and milk composition characteristics are presented in Table 6.3. The milk yield tended (P= 0.06) to be higher as the level of inclusion of whole plant faba bean silage increased. The yield of fat corrected milk (3.5 % FCM) was higher (P< 0.05) in T75 and T100 than in T0 (+4.97 and +4.36 kg/cow/d, respectively).

Also, the inclusion of whole plant faba bean silage significantly increased (P< 0.05) the 3.5 % FCM (+4.41 kg/cow/d). On the other hand, the yield of energy corrected milk (ECM) was higher (P< 0.05) in T75 and T100 than in T0 (+4.21 and +3.58 kg/cow/d, respectively), additionally the inclusion of whole plant faba bean silage increased significantly (P< 0.05) the ECM (+3.65 kg/cow/d). The yield of 3.5 % FCM and the yield of ECM in T50 were not significantly different (P> 0.05) from the correspondent values of T0, T75 and T100. In general, both FCM and ECM had significantly linear responses (P= 0.01) to the increased inclusion level of whole plant faba bean silage in the TMR.

The inclusion of whole plant faba bean silage tended to increase (P= 0.03) the fat content in the milk (4.11 vs. 3.86 %), while the inclusion of whole plant faba bean silage tended (P= 0.08) to decrease the crude protein content in the milk (2.96 vs. 3.04 %), but the inclusion of whole plant faba bean silage tended (P= 0.09) to increase the total solids in the milk (12.46 vs. 12.25 %).

Table 6.3. Milk yield and composition of Holstein cows fed TMRs with different levels of inclusion of whole plant faba bean silage

Item	Treatment % (T) 0	50	75	100	SEM	Contrast, P value: Control vs. Treatments	Linear	Quadratic	Cubic
Yield									
Milk (kg/cow/d)	49.06	50.64	51.81	51.15	2.996	0.09	0.06	0.59	0.51
3.5 % FCM (kg/cow/d)	51.98[b]	55.90[ab]	56.95[a]	56.34[a]	3.126	0.01	0.01	0.36	0.80
ECM (kg/cow/d)	50.77[b]	53.84[ab]	54.98[a]	54.35[a]	2.915	0.02	0.01	0.42	0.69
Milk composition									
Fat (%)	3.86	4.20	4.11	4.02	0.120	0.03	0.11	0.07	0.57
CP (%)	3.04	2.95	2.97	2.96	0.061	0.08	0.08	0.49	0.45
Lactose (%)	4.45	4.51	4.49	4.50	0.086	0.06	0.07	0.42	0.28
Total Solids (%)	12.25	12.56	12.47	12.35	0.126	0.09	0.32	0.09	0.71
SNF (%)	8.45	8.44	8.45	8.44	0.051	0.85	0.78	0.94	0.90
SCC (10^3 cells/ml)	352.7	325.6	481.0	474.7	366.45	0.61	0.32	0.78	0.46
MUN (mg/dl)	12.67[b]	14.59[ab]	15.27[ab]	17.34[a]	0.700	<0.01	<0.01	0.45	0.49
FPD	0.55	0.55	0.55	0.55	0.003	0.19	0.21	0.59	0.73
BHB	0.08	0.08	0.11	0.09	0.013	0.21	0.15	0.56	0.08
Milk component yield									
Fat (kg/cow/d)	1.89[b]	2.10[ab]	2.13[a]	2.11[a]	0.116	0.02	0.01	0.36	0.96
CP (kg/cow/d)	1.48	1.49	1.53	1.51	0.077	0.59	0.46	0.92	0.45
Lactose (kg/cow/d)	2.19	2.29	2.34	2.31	0.153	0.05	0.04	0.48	0.65
SNF (kg/cow/d)	4.15	4.27	4.37	4.31	0.253	0.17	0.11	0.67	0.54

SEM: standard error of mean; [a-b] Means with the different letters in the same row are significantly different (P< 0.05); T0=18.37% corn silage + 12.23 % barley silage; T50=9.18% corn silage + 6.12 % barley silage + 15.30 % whole plant faba bean silage; T75=4.59% corn silage + 3.06 % barley silage + 22.95 % whole plant faba bean silage; T100= 30.60 % whole plant faba bean silage; FCM (3.5%): fat corrected milk; ECM: energy corrected milk; CP: crude protein; SNF: solids-non-fat; SCC: somatic cells; MUN; milk urea nitrogen; FPD: freezing point depression; BHB: beta-hydroxybutyrate.

On the other hand, the milk urea nitrogen (MUN) increased as the level of inclusion of whole plant faba bean silage increased as well (+1.92, +2.6, and + 4.67 mg/dl, respectively). The yield of fat was higher (P< 0.05) in T75 and T100 than in T0 (+0.24 and +0.22 kg/cow/d), also the inclusion of whole plant faba bean silage increased (P< 0.05) the yield of fat (2.11 vs 1.89 kg/cow/d). The inclusion of whole plant faba bean silage tended (P= 0.05) to increase the lactose yield (2.31 vs. 2.19 kg/cow/d). The CP yield and the solids non-fat yield were similar (P> 0.05) among the treatments.

Refat (2018) suggested that milk yield, lactose yield, solids-not-fat yield, and ECM were higher when cows were fed corn silage diet than when were fed barley silage diets (40.14 vs. 35.32; 1.78 vs. 1.56; 3.37 vs. 2.92 and 40.21 vs. 36.2 kg/d, respectively). However, Refat (2018) found that the fat yield was similar (averaged 1.39 kg/d). Those results are supported by Benchaar et al., (2014) which found higher the milk yield, ECM and FCM in corn silage based diet than in barley silage based diet. In our study the milk yield, lactose yield and solids-not-fat yield were similar between the barley/corn diet and the whole plant faba bean diets (50.6, 2.28 and 4.28 kg/d), but the FCM, ECM and fat yield were higher when whole plant faba bean silage was included in the diets (56.39 vs. 51.98, 54.39 vs. 50.77 and 2.11 vs. 1.89 kg/d, respectively). Therefore, the milk yield and ECM are lower in barley silage diets compared with the milk yield of corn silage diets, barley/corn silage diet or whole plant faba bean silage diet. Also, higher fat yield is observed when whole plant faba bean silage is included in the diet. McKnight and Mcleod (1977) observed that when grass legume silage was replaced with faba bean silage, the milk yield was similar, this fact is supported by the study conducted by Ingalls et al., (1978), however milk fat was higher when animals were fed faba bean silage (22.6 kg/d and 4.09 vs. 3.77%). In our, study higher protein level, probably different amino acid profile (legumes are high in lysine) and different starch composition (legume starches have higher amylose than cereal starches) when faba bean was included may play an important role on the increment of the production. Milk urea nitrogen (MUN) is used as a management tool to monitor for protein feeding rates and nitrogen metabolism and is able to reflect the efficiency of utilization of dietary protein in lactating dairy cows (Bucholtz and Johnson, 2007; Kohn, 2007). According to Benchaar et al., (2014), the MUN level of barley silage diet was higher than the MUN level in corn silage diet, Hassanat et al., (2013) in his study showed similar MUN levels when replace alfalfa silage with corn silages, while Gozho and Mutsvangwa (2008) showed higher values of MUN in barley silage diets than those studies above. In our study the inclusion of whole plant faba bean silage increased significantly the MUN level (15.7 vs. 12.6 mg/dl).

6.4.3 Digestibility of Principal Nutrients

Digestibility of primary nutrients are presented in Table 6.4. Digestibility of dry matter (DM), organic matter (OM), and neutral detergent fiber (NDF) were similar (P> 0.05) among the treatments (67.86, 63.53 and 39.74 % respectively). The digestibility of ash was higher (P< 0.05) in T100 than in T0 (+11.72%), while the digestibility of ash in T50 and T75 were similar to the ones in T100 and T0. Also, the inclusion of whole plant faba bean silage increased significantly (P< 0.05) the digestibility of ash (41.26 vs. 31.22 %). On the other hand, the digestibility of crude protein were similar in T0 and T100 and were higher (P< 0.05) than in T50 (+4.84 and +4.29), while the digestibility of starch (ST) was similar (P> 0.10) among T50, T75 and T100 but were lower (P< 0.05) than in T0 (92.65 % vs. 96.13%).

The digestible DM, OM and ST were similar (P> 0.05) among the treatments (17.96, 15.66 and 5.83 kg/d respectively). The inclusion of whole plant faba bean silage increased significantly (P< 0.05) the digestible ash (0.85 vs. 0.62 kg/d) but reduced significantly (P< 0.05) the digestible NDF (3.27 vs. 3.88 kg/d). The digestible CP was higher in T100 than in T50 (+0.53 kg/d) while the digestible CP in T0 and T75 were similar (P> 0.05) to the corresponded values in T100 and

Table 6.4. Digestibility of primary nutrients in dairy cows fed TMRs with different levels of inclusion of whole plant faba bean silage

	Treatment % (T)					Contrast, P value			
Item	0	50	75	100	SEM	Control vs. Treatments	Linear	Quadratic	Cubic
Digestibility (%)									
DM	69.17	66.62	67.06	68.60	1.011	0.06	0.34	0.04	0.98
OM	64.80	62.65	62.60	64.07	1.496	0.12	0.31	0.15	0.76
Ash	31.22^{b}	40.43^{ab}	40.43^{ab}	42.94^{a}	3.393	<0.01	<0.01	0.48	0.50
CP	59.13^{a}	54.29^{b}	56.16^{ab}	58.58^{a}	1.689	0.04	0.51	0.01	0.51
NDF	41.66	40.01	39.76	37.56	2.863	0.28	0.13	0.80	0.70
ST	95.30^{a}	91.95^{b}	90.58^{b}	90.39^{b}	0.738	<0.01	<0.01	0.14	0.51
Digestible (kg/d)									
DM	18.81	17.21	17.27	18.58	0.841	0.12	0.46	0.07	0.71
OM	16.51	14.95	14.99	16.20	1.071	0.15	0.45	0.12	0.76
Ash	0.62^{b}	0.85^{ab}	0.79^{ab}	0.91^{a}	0.090	0.01	<0.01	0.62	0.17
CP	2.60^{ab}	2.32^{b}	2.49^{ab}	2.85^{a}	0.171	0.77	0.17	0.03	0.96
NDF	3.88^{a}	3.45^{ab}	3.25^{ab}	3.13^{b}	0.405	0.05	0.01	0.86	0.94
ST	5.98	5.71	5.55	6.11	0.348	0.50	0.97	0.20	0.32

SEM: standard error of mean; $^{a\text{-}b}$ Means with the different letters in the same row are significantly different ($P< 0.05$); T0=18.37% corn silage + 12.23 % barley silage; T50=9.18% corn silage + 6.12 % barley silage + 15.30 % whole plant faba bean silage; T75=4.59% corn silage + 3.06 % barley silage + 22.95 % whole plant faba bean silage; T100= 30.60 % whole plant faba bean silage; DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ST: Starch; OM: organic matter.

T50. Polynomial orthogonal contrast study showed that the digestible CP had a quadratic response (P< 0.03) to the increased inclusion level of whole plant faba bean silage in the TMR. In our study, there were no differences in terms of digestibility of OM and NDF when diary cows were fed the barley/corn diet and the whole plant faba bean diets. A study conducted by Eun et al., 2004 showed that the digestibility of DM, OM and NDF in a common barley silage diet is 59, 63 and 31.4 %, respectively. This result suggests that whole plant faba bean silage diets have higher digestibility of DM, similar digestibility of OM and higher digestibility of NDF, however nowadays Refat (2018) suggested that there are some barley varieties which showed improved NDF digestibility. Benchaar et al., (2014) demonstrated that the DM digestibility of a corn silage diet is higher than a barley silage diet, and according to our study the DM digestibility of whole plant faba bean diets is similar (68%) to the DM digestibility of the corn silage diet. Also, in that study, it was demonstrated that the NDF digestibility of the corn silage diet was about 46 %, while Yang et al., (2019) showed that the NDF digestibility of the corn silage diet used in this study was 51%. Those two numbers are higher than the NDF digestibility showed in the whole plant faba bean diets in our study. Additionally, in our study the CP and starch digestibilities were lower in the whole plant faba bean diets than the barley/corn diet. The slightly lower CP digestibility can be attributed to the lower level of inclusion of the protein from the concentrate in the whole plant faba bean diets. Eun et al., (2004) showed that the starch digestibility of a barley silage diet is about 93 %, while Benchaar et al., (2014) showed that the starch digestibility of a corn silage diet is about 98 %, that value is supported for by Hassanat et al., (2013). In our study, the whole plant faba bean diets averaged a starch digestibility of 90.1 %, suggesting that whole plant faba bean diets have lower starch digestibility than a barley or corn silage based diets, but when we observed the digestible starch compared with the barley/corn diet there are not differences (averaged 5.82 kg/d).

6.4.4 N balance and N utilization

Predicted and actual nitrogen balance and nitrogen utilization are presented in Table 6.5. Based on the prediction, nitrogen intake was higher (P< 0.05) in T100 than in T0 (+113.8 g/d), while nitrogen intake T50 and T75 were similar (P> 0.05) to correspondent T100 and T0 values. The milk nitrogen was similar (P> 0.05) among all the treatment (235.23 g/d). The inclusion of whole plant faba bean in the diet significantly increased (P< 0.05) the urinary N, fecal N, and manure N (282.4 vs. 222.3; 223.3 vs. 209.3 and 505.8 vs. 431.4 g/d, respectively).

Additionally, the nitrogen utilization efficiency was higher (P< 0.05) in T0 than in T100 (+4.2 g in milk/g intake), while nitrogen utilization efficiency in T50 and T75 were similar (P> 0.05) to correspondent T100 and T0 values.

On the other hand, the actual nitrogen balance and nitrogen utilization showed that the inclusion of whole plant faba bean in the diet did not increase (P> 0.05) the N intake (718.0 vs. 698.3 g/d), also N intake in T0 was similar to in T50 and were lower (P< 0.05) than in T100 (-72.2 and - 93.6 g/d). In general, the N intake had a quadratic response (P< 0.03) to the increased inclusion level of whole plant faba bean silage in the TMR.

The milk nitrogen was similar (P> 0.05) among all the treatment (235.23 g/d). The inclusion of whole plant faba bean in the diet significantly increased (P< 0.05) the urinary N, fecal N, and manure N (229.6 vs. 232.1; 264.7 vs. 234.2 and 494.4 vs. 425.9 g/d, respectively). The N utilization efficiency had a quadratic response (P= 0.03) to the increased inclusion level of whole plant faba bean silage in the TMR.

The predicted values overestimated the N intake in the diets which contained whole plant faba bean silage and underestimated the N intake in the control diet. Additionally, overestimated urinary N, fecal N, and manure N in all the treatments. On the other hand, the real nitrogen balance showed that there is no difference among the treatments in terms of nitrogen utilization efficiency. The inclusion of whole plant faba bean silage increases the N intake in the diets (+19.71 g/d) which is

Table 6.5. N balance and N utilization in dairy cows fed TMRs with different levels of inclusion of whole plant faba bean silage

	Treatment % (T)					Contrast, P value			
Item	0	50	75	100	SEM	Control vs. Treatments	Linear	Quadratic	Cubic
Predicted N utilization (g/d)									
N intake	660.67^{b}	718.17^{ab}	736.28^{ab}	774.47^{a}	23.383	<0.01	<0.01	0.90	0.64
Milk N	232.06	233.05	239.64	236.20	11.961	0.58	0.44	0.93	0.47
Urinary N	222.34^{b}	262.67^{ab}	273.30^{ab}	311.08^{a}	12.930	<0.01	<0.01	0.59	0.43
Fecal N	209.32^{b}	219.09^{ab}	222.17^{ab}	228.66^{a}	3.975	<0.01	<0.01	0.90	0.64
Manure N	431.36^{b}	481.88^{ab}	495.55^{ab}	539.84^{a}	16.590	<0.01	<0.01	0.66	0.45
N Utilization Efficiency (g in milk/g intake)	34.80^{a}	32.56^{ab}	32.38^{ab}	30.56^{b}	1.127	0.01	<0.01	0.87	0.37
Real N Utilization (g/d)									
N intake	698.27^{b}	676.90^{b}	706.25^{ab}	770.50^{a}	27.705	0.38	0.01	0.04	0.89
Milk N	232.06	233.05	239.64	236.20	11.961	0.58	0.44	0.93	0.47
Urinary N	191.82^{c}	202.59^{c}	228.57^{b}	257.68^{a}	7.359	<0.01	<0.01	0.03	0.62
Fecal N	234.18^{a}	263.47^{ab}	261.98^{ab}	268.74^{a}	14.273	<0.01	<0.01	0.42	0.45
Manure N	425.92^{c}	465.37^{bc}	491.06^{ab}	526.68^{a}	17.132	<0.01	<0.01	0.46	0.86
N Utilization Efficiency (g in milk/g intake)	32.75	35.05	34.13	30.66	1.165	0.68	0.28	0.02	0.73

SEM: standard error of mean; $^{a-b}$ Means with the different letters in the same row are significantly different ($P < 0.05$); T0=18.37% corn silage + 12.23 % barley silage; T50=9.18% corn silage + 6.12 % barley silage + 15.30 % whole plant faba bean silage; T75=4.59% corn silage + 3.06 % barley silage + 22.95 % whole plant faba bean silage; T100= 30.60 % whole plant faba bean silage. Urinary N (g/d) can be predicted as: 0.026 × BW(Kg) × MUN (mg/dl); N intake (g/d) can be predicted as: (urinary N (g/d) + milk N + 97) / 0.83. The endogenous losses are represented as 97 g/d and the fraction of feed N digested is assumed to be 0.83; Fecal N (g/d) can be predicted as: predicted N intake - predicted urinary N - milk N (Kohn, 2007).

related to the higher protein content in whole plant faba bean silage than in corn or barley silages, which increased the protein content of the whole plant faba bean silage diet.

Hassanat et al., (2013) showed a reduction of fecal and urinary N and an increment of milk N when alfalfa silage was replaced with corn silage, the decrease in urinary N losses as corn silage dietary proportion increased can be related to the decrease in ruminal concentration of ammonia. In the corn silage diet the protein source (soybean) increases, while in the alfalfa diet the protein source (soybean) decreases; and it's been showed that alfalfa protein has high solubility, higher than soybean (Wattiaux and Karg, 2004; Nagel and Broderick, 1992). That is comparable in our study, in which when the inclusion of whole plant faba bean silage increased, the inclusion of the protein source (soybean and canola) decreased. Although, we did not see any difference in the ammonia concentration in the rumen fluid between whole plant faba bean silage diets and barley/corn silage diet, the increment of MUN level may be related to the higher level of CP in the diet, therefore higher N intake, fecal and urinary N were observed compared to the barley/corn silage based diet. Cow manure can be a crop fertilizer; however, it can damage the environment as well, potential effects on air quality is a concern. The amount of NH_3 produced from cattle manure is correlated with the amount of urinary N in the manure, which is correlated with N intake (Weiss et al., 2009; Cole et al., 2005). A study conducted by Weiss and Wyatt (2006) suggested that cows fed the high crude protein diets (17 %DM with 10% RDP on DM basis) consumed more total and digestible N, excreted more N in feces and urine, and retained more N than cows fed the low crude protein diets (14 %DM with 10% RDP on DM basis). On the other hand, the N utilization efficiency is similar between whole plant faba bean silage diets and barley/corn silage diet and maybe because of the slightly numerical increment of protein in the milk when animals were fed whole plant faba bean silage.

6.4.5 Energy Partitioning

The energy values and energy partitioning are presented in Table 6.6. The inclusion level of whole plant faba bean silage in the diets increased significantly (P< 0.05) the total energy provided (50.22 vs. 44.24 Mcal/d), however the energy for maintenance and the energy for producing 1kg of milk were similar (P> 0.05) among all the treatments. On the other hand, the energy for body weight gain (BWG), the energy for total milk production of milk per day and the energy for body weight gain plus milk production were higher (P< 0.05) when whole plant faba bean silage was included in the diets (2.30 vs. -0.82, 37.10 vs. 34.56 and 39.45 vs. 33.60 Mcal/d, respectively). On the other hand, the energy partitioning showed that energy of maintenance as percentage of total energy is higher (P< 0.05) in T0 than in T100 (+2.76 %), additionally the inclusion of whole plant faba bean silage reduced significantly (P< 0.05) the percentage of energy that was used for maintenance (21.81 vs. 24.50 %), also the percentage of energy that was used for body weigh gain and the percentage of energy used for body weight gain plus milk production were higher when whole plant faba bean silage was included in the diets (4.32 vs. -2.35 and 78.20 vs. 77.50 %, respectively). Polynomial orthogonal contrast study showed that the NE_L had a quadratic response (P< 0.02) to the increased inclusion level of whole plant faba bean silage in the TMR.

Refat (2018) showed that the NE_L between the corns silage based diet and the barley silage diets was similar and averaged 1.42 Mcal/kg DMI, however in our study the whole plant faba bean silage diets showed a higher level of NE_L than the barley/corn silage diet (1.91 vs. 1.65 Mcal/kg DMI). Therefore, including whole plant faba bean silage increased the energy level in the diets. Additionally, it was showed that the energy used for milk production when cows were fed barley silage diet was 24.66 Mcal/d, but in our study the animals were fed whole plant faba bean silage diets showed higher energy used for milk production than the animals were fed the barley/corn silage diet (37.10 vs. 34.56 Mcal/d). Furthermore, the animals were fed whole plant faba bean silage diets showed positive energy for BWG. Some studies suggested that in high-yielding dairy

Table 6.6. Energy partitioning in dairy cows fed TMRs with different levels of inclusion of whole plant faba bean silage

	Treatment % (T)					Contrast, P value			
Item	0	50	75	100	SEM	Control vs. Treatments	Linear	Quadratic	Cubic
Calculated Energy (Mcal/d)									
Maintenance	10.65	10.77	10.75	10.78	0.114	0.07	0.06	0.55	0.56
BWG	-0.82^{b}	2.24^{ab}	1.28^{ab}	3.38^{a}	1.056	0.02	0.03	0.77	0.27
1kg milk	0.70	0.74	0.73	0.72	0.012	0.04	0.16	0.08	0.61
kg milk/day	34.56^{b}	37.03^{ab}	37.54^{a}	36.74^{a}	2.058	0.02	0.02	0.25	0.75
BW gain + milk	33.60^{b}	39.52^{ab}	39.38^{ab}	39.46^{a}	2.285	0.01	0.01	0.26	0.66
Total energy	44.24^{b}	50.29^{ab}	50.14^{ab}	50.24^{a}	2.352	0.01	0.01	0.27	0.66
NE_L/d (Mcal/kg DMI)	1.65^{b}	1.95^{a}	1.94^{a}	1.86^{ab}	0.063	<0.01	0.01	0.02	0.82
Net energy partitioning (%Total energy)									
Maintenance	24.50^{a}	21.74^{ab}	21.96^{ab}	21.74^{b}	1.090	0.01	0.01	0.28	0.52
BWG	-2.35^{b}	3.94^{ab}	2.51^{ab}	6.53^{a}	2.110	0.02	0.02	0.76	0.32
Milk	77.46	74.23	75.19	72.59	1.952	0.13	0.10	0.97	0.41
BW gain + milk	75.50^{b}	78.30^{ab}	78.04^{ab}	78.26^{a}	1.043	0.01	0.01	0.28	0.52

SEM: standard error of mean; $^{a\text{-}b}$ Means with the different letters in the same row are significantly different ($P< 0.05$); T0=18.37% corn silage + 12.23 % barley silage; T50=9.18% corn silage + 6.12 % barley silage + 15.30 % whole plant faba bean silage; T75=4.59% corn silage + 3.06 % barley silage + 22.95 % whole plant faba bean silage; T100= 30.60 % whole plant faba bean silage; BWG: body weight gain; Net energy required for maintenance was calculated as $BW^{0.75} \times 0.08$. Energy of BW change was assumed to be 5.114 Mcal kg^{-1} of gain or 4.924 Mcal kg^{-1} of loss. Milk energy was calculated as (0.0929 × milk fat concentration) + (0.0563 × milk true protein concentration) + (0.0395 ×146 milk lactose concentration) (NRC 2001). Estimated NE_L value was calculated by total net energy used for maintenance, BW gain + milk divided by DMI (Neal et al., 2014).

cows in early lactation, the negative energy balance is considered a physiological phenomenon (Van Straten et al., 2008), therefore assessing adaptation to this negative energy balance is key because a poor adaptation negatively affects health status, reproductive performance, and milk production (Poncheki, et al., 2015; Roche et al., 2007; Buckley et al., 2003). Therefore, animals fed whole plant faba bean silage diets may easily recover from the negative energy balance. On the other hand, dairy cows fed whole plant faba bean silage diet showed to have enough energy to partition higher percentage of that energy towards BWG and BWG plus milk production than dairy cows feed barley/corn silage diet. Bradford and Allen (2007) suggested that in order to meet requirements for maintenance, milk production, and reproduction, high-producing dairy cows require substantial amounts of energy. However, some data showed that increasing the energy in the diets can also limit dry matter intake (Allen et al., 2009), but also sometimes increasing crude protein is positive related to dry matter intake (Allen, 2000). Therefore, in our study including whole plant faba bean silage did not reduce the intake but increased substantially the energy level by increasing crude protein content and therefore partition more energy towards maintenance and milk production.

6.4.6 Chewing Activity

The chewing activity truly characteristics are shown in Table 6.7. The length of laying, standing and first meal were similar (P> 0.05) among all the treatments and averaged (808.3, 631.7 and 84.4 min/d, respectively). The length of eating and the length of eating one kilogram of dry matter were similar (P> 0.05) among all the treatments (362.0 min/d and 13.8 min/kg DM), however the length of eating one kilogram of NDF was longer (P< 0.05) when animals were fed T100 than T0 (+7.5 min/kg NDF), the length of eating one kilogram of NDF when animals were fed T50 and T75 was similar (P> 0.05) to that when eating T100 than T0. The period of time spent ruminating was similar (P> 0.05) among all the treatments (502.7 min/d, 19.1 min/kg DM and 60.1

Table 6.7. Chewing activity in dairy cows fed TMRs with different levels of inclusion of whole plant faba bean silage

	Treatment % (T)					Contrast, P value			
Item	0	50	75	100	SEM	Control vs. Treatments	Linear	Quadratic	Cubic
Laying (min/d)	789.3	807.8	837.3	798.7	48.28	0.61	0.67	0.66	0.55
Standing (min/d)	650.7	632.2	602.7	641.3	48.28	0.61	0.67	0.66	0.55
First Meal (min/d)	85.6	80.6	84.6	86.7	12.01	0.91	0.95	0.74	0.89
Eating									
Min/d	364.0	359.4	350.1	378.4	17.19	0.94	0.73	0.41	0.44
Min/kg DM	13.6	13.9	13.7	14.2	0.76	0.69	0.60	0.90	0.64
Min/kg NDF	40.8^{b}	42.6^{ab}	43.5^{ab}	48.3^{a}	2.59	0.12	0.02	0.41	0.57
Ruminating									
Min/d	550.9	485.4	490.9	483.8	27.17	0.04	0.06	0.43	0.65
Min/kg DM	20.9	19.0	18.7	17.9	1.08	0.07	0.05	0.81	0.77
Min/kg NDF	61.8	57.7	60.5	60.5	3.25	0.55	0.81	0.52	0.57
Total Chewing Activity									
Min/d	916.2	843.5	842.3	860.8	29.77	0.04	0.07	0.21	0.96
Min/kg DM	34.4	32.9	32.7	31.9	1.31	0.18	0.12	0.94	0.82
Min/kg NDF	102.0	100.7	105.0	108.1	4.12	0.54	0.18	0.43	0.68

SEM: standard error of mean; $^{a\text{-}b}$ Means with the different letters in the same row are significantly different ($P< 0.05$); T0=18.37% corn silage + 12.23 % barley silage; T50=9.18% corn silage + 6.12 % barley silage + 15.30 % whole plant faba bean silage; T75=4.59% corn silage + 3.06 % barley silage + 22.95 % whole plant faba bean silage; T100= 30.60 % whole plant faba bean silage; DM: dry matter; NDF: neutral detergent fiber.

min/kg NDF). However, the inclusion of whole plant faba bean silage in the diets reduced significantly (P= 0.04) the time spent ruminating (-64.2 min/d). The total chewing activity was similar (P> 0.05) among all the treatments (865.7 min/d, 32.9 min/kg DM and 103.9 min/kg NDF), but the inclusion of whole plant faba bean silage in the diets reduced significantly (P= 0.04) the time of total chewing activity (-67.3 min/d).

Refat (2018) determined that the time spent eating, ruminating, or total chewing activity were not affected when cows were fed different barley silage varieties. However, in that study and supported by Yang and Beauchemin (2006), the time spending eating was shorter, and the time spending ruminating was longer compared with the correspondent values in our study (311 vs. 362 min/d and 578 vs. 486 min/d, respectively). This can be attributed to the higher concentration of NDF found in the barley silage based TMR (34.5 %DM), while in our study the NDF was about 31.5 %DM. In agreement with our study, Beauchemin (1991) found that the total chewing time decreased as fiber content of the diet decreased. In that study it was found that animals fed a 31% NDF diet and a 34% NDF diet had a total chewing activity of 767 and 796 min/d, respectively. However, in our study, animals fed whole plant faba bean diets (31% NDF) had a total chewing activity of 848 min/d.

6.4.7 Rumen Fermentation Characteristics

The rumen fermentation characteristics of dairy cows fed TMRs with different levels of inclusion of whole plant faba bean silage are presented in Table 6.8. The pH and ammonia were similar (P> 0.05) among all the treatments (6.34 and 10.79 mg/dl, respectively). On the other hand, ever single volatile fatty acid analysed (acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, caproic acid) and the total VFA were similar (P> 0.05) among all the treatments (50.87, 22.89, 0.65, 11.41, 1.30, 1.38, 0.45 and 88.94 mmol/l, respectively), consequently the ratio acetic: propionic was similar (P> 0.05) with an average ratio of 2.33.

A study conducted by Gozho and Mutsvangwa (2008) suggested that the mean pH value for different barley silage based diets averaged 6.18, while the ammonia averaged 15.4 mg/dl. Hassanat et al., (2013) found a reduction in the mean pH and the ammonia concentration when replaced alfalfa silage with corn silage (6.31 vs. 6.07 and 10.44 vs. 6.79 mmol/l). In our study, the pH and the ammonia were similar in the barley/corn silage diet to that in the whole plant faba bean silage diets and averaged (6.34 and 6.35 mmol/l, respectively), suggesting that the pH in the whole plant faba bean silage diets is similar to that in barley silage diets and the ammonia is slightly lower than the ammonia found in barley and corn silage diets. The pH was in the normal range and negative effects on microbial protein synthesis and fermentation are unlikely to occur (Castillo-Lopez et al., 2014). Benchaar et al., (2014) demonstrated that acetic acid was reduced when barley silage was replaced with corn silage (from 64.5 to 61.4 mmol/l), propionic acid was increased (from 20.9 to 23.6 mmol/l), while the butyric acid remained similar and averaged 11.5 mmol/l. Comparing with the results in our study, the whole plant faba bean diets showed a lower acetic acid (averaged 51.20 mmol/l) than barley and corn diets, however the propionic acid and the butyric acid are similar to the ones found in the corn silage diet in the previous study. McKnight and Mcleod (1977) observed that when grass legume silage was replaced with faba bean silage, the VFA proportions were similar, this fact is supported by the study conducted by Ingalls et al., (1978) which also indicated that ruminal fluid ammonia-N concentration was higher for the cows receiving the grass-legume silage than for those fed faba bean silage.

Table 6.8. Rumen fermentation characteristics of dairy cows fed TMRs with different levels of inclusion of whole plant faba bean silage

	Treatment % (T)					Contrast, P value			
Item	0	50	75	100	SEM	Control vs. Treatments	Linear	Quadratic	Cubic
pH	6.38	6.34	6.42	6.25	0.120	0.54	0.25	0.44	0.11
NH_3 (mmol/l)	5.66	6.23	6.33	7.18	0.581	0.24	0.18	0.61	0.72
NH_3 (mg/dl)	9.61	10.60	10.76	12.20	0.988	0.24	0.18	0.61	0.72
VFA (mmol/l)									
Acetic Acid	49.86	51.79	49.90	51.93	2.521	0.46	0.43	0.96	0.24
Propionic Acid	23.04	23.46	20.94	24.13	2.460	0.92	0.96	0.65	0.19
Isobutyric Acid	0.62	0.64	0.65	0.68	0.051	0.46	0.30	0.85	0.92
Butyric Acid	10.76	11.50	11.92	11.47	0.638	0.24	0.25	0.55	0.63
Isovaleric Acid	1.28	1.21	1.37	1.33	0.152	0.88	0.64	0.80	0.46
Valeric Acid	1.46	1.37	1.34	1.36	0.096	0.20	0.18	0.60	0.83
Caproic Acid	0.47	0.45	0.43	0.44	0.080	0.62	0.53	0.93	0.75
Total VFA	87.57	90.28	86.52	91.40	4.536	0.63	0.53	0.85	0.25
Ratio									
Acetic: Propionic	2.25	2.33	2.51	2.23	0.285	0.44	0.65	0.33	0.13

SEM: standard error of mean; [a-b] Means with the different letters in the same row are significantly different ($P< 0.05$); T0=18.37% corn silage + 12.23 % barley silage; T50=9.18% corn silage + 6.12 % barley silage + 15.30 % whole plant faba bean silage; T75=4.59% corn silage + 3.06 % barley silage + 22.95 % whole plant faba bean silage; T100= 30.60 % whole plant faba bean silage, NH_3: ammonia.

6.5 Conclusions

The whole plant faba bean silage at late pod stage in the TMR for high producing dairy cows positively affected the performance and efficiency, without altering the ruminal fermentation parameter or negatively affecting the intake. The levels of inclusion of whole plant faba bean silage in the rations have demonstrated to be effective, hence it can be used as an alternative feeding strategy besides the barley and corn silage based diets. Therefore, based on the prediction from Chapter 4 and the confirmation of this study, whole plant faba bean silage at late pod stage is a high-quality feed and can be used as a high value ingredient for dairy cows in western Canada.

7. ASSOCIATION OF MOLECULAR STRUCTURE SPECTRAL FEATURES WITH NUTRIENT UTILIZATION AND AVAILABILITY OF FABA FORAGE VARIETIES FOR SILAGE WITH EFFECT OF VARIETY/TANNIN CONCENTRATION AND GROWTH STAGE IN RUMINANT LIVESTOCK SYSTEM: AN APPROACH WITH ADVANCED MOLECULAR SPECTROSCOPY

7.1 Abstract

The objectives of this study were to determine the effect of tannin concentration/variety (low tannin/Snowdrop variety; high tannin/SSNS-1 variety) and the effect of cutting stage (mid pod stage: 88 days old, and late pod stage: 97 days old) on molecular structure spectral profile of whole plant faba bean silage and determine its relationship with chemical profiles, energy profiles, rumen degradation characteristics, *in vitro* intestinal digestibility, metabolic characteristics, and predicted production performance. The total carbohydrates (TC) spectral peak area was higher (P< 0.05 in low tannin silage at late pod stage than at mid pod stage (+3.45 AU). The structural carbohydrates (STC) area was higher (P< 0.05) in low tannin silage at mid pod stage (+4.11 AU) than the high tannin silage at late pod stage, while the amide I area was lower (-1.40 AU) than the low tannin silage at late pod stage. Amide II area was higher in the high tannin silage at late pod stage than the high tannin silage at mid pod stage (+2.50 AU). The PCA spectral analyses used all the carbohydrate region (ca. 879-1485 cm^{-1}), which include NSTC, TC, and STC regions. A clear difference between whole plant faba bean silage at mid and late pod stages (Figure 5b) was observed and PC1 accounted for 83% of total variation. Structural carbohydrate peak number four (STC4) spectral height was strongly positively correlated with starch content (r= 0.94, P≤ 0.01), while α-helix and β-sheet were medium positively correlated with crude protein content (r= 0.62, P= 0.03 and r= 0.65, P= 0.02 respectively) and were strongly positively correlated with total digestible nutrients (TDN) and energy values (r= 0.76, P≤ 0.01). Also, amide I spectral area was

strongly positively correlated with total digestible nutrients (TDN) and energy values ($r= 0.85$, $P\leq 0.01$). Structural carbohydrate spectral peak number one (STC1) height was strongly negatively correlated with rumen undegradable protein (RUP; $r= - 0.82$, $P\leq 0.01$) and rumen bypass starch (BSt; $r= - 0.84$, $P\leq 0.01$), while amide I peak height and STC area were strongly negatively correlated with rumen undegradable protein (RUP; $r= - 0.83$, $P\leq 0.01$ and $r= - 0.90$, $P\leq 0.01$), but α-helix to β-sheet spectral peak height ratio was strongly negatively correlated with rumen undegradable protein (RUP; $r= 0.73$, $P= 0.01$). Structural carbohydrates peak number one (STC1) spectral height was strongly negatively correlated with intestinal digested crude protein (IADP; $r= -0.90$, $P\leq 0.01$) and metabolizable protein (MP; $r= -0.92$, $P\leq 0.01$), while α-helix peak height, β-sheet peak height, and amide I area were strongly positively correlated with MP ($r= 0.86$, $P\leq 0.01$; $r= 0.86$, $P\leq 0.01$ and $r= 0.71$, $P= 0.01$). Cellulosic compound (CEC) spectral area was strongly negatively correlated with Feed Milk Value based on the DVE-OEB system (FMV^{DVE}) and Feed Milk Value based on the NRC model (FMV^{NRC}) ($r= -0.95$, $P\leq 0.01$ and $r= -0.82$, $P\leq 0.01$ respectively). The results indicated that STC4, amide I, and α-helix peak heights were related with starch content and can be used to predict it with good estimation power ($R^2 > 0.96$), but crude protein, total digestible nutrients and net energy of lactation were predicted with no good estimation power ($R^2 < 0.67$). On the other hand, STC, CEC, and amide spectral areas were related with important rumen kinetic, digestibility, and metabolic characteristics and can be used to predict them with good estimation power ($R^2 > 0.74$). In conclusion, protein and carbohydrates molecular structures were affected by the tannin concentration and the stage of cutting. Additionally, both molecular structures can be related independently and synergistically with nutrient, energy, degradation, digestion, and metabolic characteristics. Furthermore, protein and carbohydrates molecular structures evaluated with advanced vibration molecular spectroscopy can work together to predict accurately some of those characteristics in nutrient utilization and availability in ruminant livestock systems.

Keywords: Protein, carbohydrates, molecular structure, spectral peaks and areas, functional group ratios, relation, vibrational spectroscopy with chemometrics.

7.2 Introduction

Study of the molecular structure features of feedstuffs is a key factor to understand the nutritive quality and value of any feed ingredient, its nutrient utilization, and consequently the influence on animal performance (growth, milk, and meat production) (Xin and Yu, 2013a, 2013b; Yu, 2005a; Yu et al., 2004). The determination of inherent characteristics of main feed nutrients offered to ruminants, such as starch, protein, and fiber, might give researchers an insight of the degradation behaviour and fate of these nutrients after feeding. For instance, the internal protein makeup and conformation within an ingredient could impact the extent of enzymatic activity along the gastrointestinal tract as well as the digestibility and absorption of protein metabolites in the small intestine (Yu and Prates, 2017), and these effects have been related to the percentage of molecular protein secondary structures, α-helix and β-sheet (Theodoridou and Yu, 2013; Yu, 2004).

For this reason, in the past few years, the interest on the molecular structure features of feed ingredients and the possible association with nutrient and metabolic characteristics in ruminants has been increasing, leading to a new open area of research not completely understood yet. Vibrational Fourier Transform Infrared (FTIR) spectroscopy has become a useful tool to gather information of different molecular characteristics in a variety of samples as it is a non-destructive and fast analytical technique for feed analyses (Chen et al., 2018). This technique is based on the atom's vibrations when an infrared radiation is directed into a sample, enabling the measurement of different energy absorptions within a molecule and with a spectrum unique to every sample

(Stuart, 2004; Rodriguez, 2018). A useful region from all the infrared spectra has been the mid-infrared section between ca. 4,000 cm^{-1} and 200 cm^{-1} as it gives valuable information about different functional groups among organic molecules (Yang and Yu, 2016). The objectives of this study were: (1) To determine the effect of tannin concentration/variety and cutting stage on molecular structure spectral profile of whole plant faba bean silage; (2) To determine the relationship between molecular spectral profiles and chemical profiles, energy profiles, rumen degradation characteristics, *in vitro* intestinal digestibility, metabolic characteristics, and predicted production performance in ruminant livestock systems.

7.3 Materials and Methods

7.3.1 ATR-FT/IR Sample Preparation and Spectra Collection

Low tannin type of Snowdrop variety and high tannin type of SSNS-1 variety of whole plant faba bean silages grown in different research plots of the Crop Development Center (CDC, University of Saskatchewan, Saskatoon, Canada) and cut at different maturity stages (mid pod stage: 88 days old, and late pod stage: 97 days old) were from previous studies (Chapter 4).

Dried samples (n=6 for Snowdrop variety, 3 at mid pod stage and 3 at late pod stage and n=6 for SSNS-1 variety, 3 at mid pod stage and 3 at late pod stage) of whole plant faba bean silage were ground trough a 0.1 mm screen using the Retsch ZM 200 grinder (Retsch Inc, Haan, Germany). The JASCO FT/IR 4200 vibration molecular spectroscopy with ATR (JASCO Corporation, Tokyo, Japan) within the mid-IR range (ca. 700-4000 cm^{-1}) was used to scan each sample at the molecular spectroscopy laboratory of the Animal and Poultry Science Department of the University of Saskatchewan (Saskatoon, SK, Canada). Each sample was scanned five times.

7.3.2 Univariate Molecular Spectral Analysis of Structure Profiles

The spectral data were analyzed by using OMNIC 7.3 (Spectra Tech., Madison, WI, USA), the spectral characteristics associated with protein molecular structure detected included: amide I and II height located at ca. 1589 and ca. 1535 cm^{-1} respectively, amide area from ca. 1484 to ca. 1712 cm^{-1}, amide I area from ca. 1542 to ca. 1712 cm^{-1}, amide II area from ca. 1484 to ca. 1542 cm^{-1} and their ratios; α-helix and β-sheet peak height located at ca. 1648 and ca. 1635 cm^{-1} respectively and their ratios. The spectral characteristics associated with carbohydrate molecular structure detected were total carbohydrates (TC) which area is located from ca. 906 to ca. 1184 cm^{-1} and includes three peaks (TC1, TC2 and TC3) located at ca. 1027 cm^{-1}, ca. 1075 cm^{-1} and ca. 1149 cm^{-1} respectively. Structural carbohydrates (STC) area located from ca. 1184 to ca. 1485 cm^{-1}, which includes four peaks (STC1, STC2, STC3 and STC4) located at ca. 1237 cm^{-1}, ca. 1316 cm^{-1}, ca. 1395 cm^{-1} and ca. 1461 cm^{-1}. Non-structural carbohydrates (NSTC) area located from ca. 879 to ca. 906 cm^{-1} with its main peak located at 896 cm^{-1}, cellulosic compounds (CEC) area located from ca. 1184 to ca. 1290 cm^{-1} with its main peak located at ca. 1237 cm^{-1} and ligneous compounds (LIG) peak located at ca.1515 cm^{-1}. Spectral bands of functional groups were detected based on the literature (Refat, 2018; Rodriguez, 2018). Protein secondary structures were determined using the second derivative function and the Fourier self-deconvolution (FSD) function of OMNIC 7.3 Software.

7.3.3 Multivariate Molecular Spectral Analysis of Structure Profiles

The molecular spectral multivariate principal component analysis (PCA) was carried out using the Unscrambler 10.3 software (Camo Analytics, Oslo, Norway) in order to compare the whole plant faba bean silages in this study and elucidate if there were protein and carbohydrate spectral differences between the low and high tannin whole plant faba bean silage and between the cutting stages by utilising the entire spectral information obtained (Yu, 2005b).

7.3.4 Statistical Analysis

Results were analyzed using the Mixed model procedure of SAS version 9.4. (SAS Institute, Inc., Cary, NC, US). The experimental design was a CRD (2 × 2 factorial) with the first factor tannin concentration (low and high) and the second factor stage of cutting (mid pod and late pod stages). The model used was:

$Y_{ijk} = \mu + F_i + H_j + (F_i \times H_j) + e_{ijk}$,

Where, Y_{ijk} was the observation of the dependent variable ijk, μ was the population mean for the variable, F_i the effect of tannin concentration (i= 1,2); H_j the effect of the stage of cutting (j= 1,2), $F_i \times H_j$ the interaction between variables, and e_{ijk} the random error associated with observation ijk. Multiple comparison analysis was determined using the Tukey method. The relationships between protein and carbohydrate structures with the chemical profiles, energy profiles, rumen degradation characteristics, *in vitro* digestion characteristics, metabolic characteristics, and predicted production performance of whole plant faba bean silage were analyzed using the PROC CORR of SAS version 9.4. (SAS Institute, Inc., Cary, NC, US) using the Spearman correlation method. Multiple regression analysis of protein and carbohydrates molecular structures with the *in vitro*, metabolic characteristics and predicted production performance of whole plant faba bean silage were analyzed using the PROC REG of SAS version 9.4 using the following model: $Y = a + b1 \times x1 + b2 \times x2 + ... + bn \times xn$. The model used a “STEPWISE” option with variable selection criteria: “SLENTRY = 0.05, SLSTAY = 0.05” and just the variables which were significant at $P< 0.05$ were left. For all statistical analyses, significance was declared at $P< 0.05$ and trends at $P \leq 0.10$.

7.4 Results and Discussion

7.4.1 Univariate Molecular Spectral Analysis: Effect of Variety and Maturity Stage

Protein and carbohydrate molecular features of whole plant faba bean silage affected by variety and cutting stage are presented in Table 7.1. Interactions ($P< 0.05$, variety × cutting stage, V×S) were observed in most of the characteristics in this study. The spectral intensity of peak number one of total carbohydrate region (TC1) and peak number three of total carbohydrate region (TC3) had higher ($P< 0.05$) absorbance units (AU) in low tannin whole plant faba bean silage at late pod

stage than that in the low tannin whole plant faba bean silage at mid pod stage (+0.031 and +0.018 AU respectively). High tannin whole plant faba bean silage at mid pod stage had similar TC1 and TC3 to low tannin whole plant faba bean silage at late pod stage. The non-structural carbohydrates (NSTC) spectral peak intensity had higher ($P < 0.05$) AU in low tannin whole plant faba bean silage at mid pod stage (0.021 AU) than the other three silages. The spectral peak number two of structural carbohydrates (STC2) had higher ($P < 0.05$) AU in low tannin whole plant faba bean silage at mid pod stage than low tannin whole plant faba bean silage at late pod stage and high tannin whole plant faba bean silage at mid pod stage which were similar to high tannin whole plant faba bean silage at late pod stage (+0.009, +0.013, and +0.027 AU respectively). The spectral peak number three of structural carbohydrates (STC3) had higher ($P < 0.05$) AU in low tannin silage at mid pod and late pod stages than high tannin silages at mid pod and late pod stages (0.157 and 0.161 vs. 0.140 and 0.137 AU), while ligneous compound (LIG) peak intensity had higher ($P < 0.05$) AU in high tannin silage at mid pod stage than low tannin silage at late pod stage (+0.014 AU).
Amide II peak had higher ($P < 0.05$) AU in high tannin silage at late pod stage than high tannin silage at mid pod stage and low tannin silages at mid pod and late pod stages (+0.057, +0.051 and +0.015 AU), while α-helix and β-sheet peaks had similar ($P > 0.05$) AU among high tannin silages at mid pod and late pod stages and low tannin silage at late pod stage, but had higher ($P < 0.05$) AU than low tannin silage at mid pod stage.

Total carbohydrate (TC) spectral peak areas were similar ($P > 0.05$) among high tannin silages at mid pod and late pod stages and low tannin silage at late pod stage, but higher ($P < 0.05$) than low tannin silage at mid pod stage (72.73, 71.45, 74.37 and 70.92 AU, respectively), while the non-structural carbohydrates (NSTC) peak area intensity was higher ($P < 0.05$) in low tannin silage at mid pod stage and high tannin silages at mid pod and late pod stages than low tannin silage at late pod stage (+0.09, +0.05 and +0.04 AU respectively). The structural carbohydrates (STC) peak area

Table 7.1. Effect of variety (tannin concentration) and stage of cutting on molecular structure spectral profile of whole plant faba bean silage

Variety (V)		High Tannin Variety		Low Tannin Variety			P value		
Stage of Cutting (S)		Mid pod	Late Pod	Mid pod	Late Pod	SEM	Variety (V)	Stage (S)	V×S
Peak Height and Center (cm^{-1})									
TC1	ca. 1027 cm^{-1}	0.573^{ab}	0.570^{b}	0.568^{b}	0.599^{a}	0.0075	0.11	0.05	0.02
TC2	ca. 1075 cm^{-1}	0.393	0.384	0.379	0.394	0.0062	0.72	0.45	0.01
TC3	ca. 1149 cm^{-1}	0.137^{a}	0.129^{b}	0.119^{c}	0.137^{a}	0.0035	0.02	0.03	<0.01
NSTC	ca. 896 cm^{-1}	0.013^{b}	0.012^{b}	0.021^{a}	0.011^{b}	0.0012	0.02	<0.01	<0.01
STC1	ca. 1237 cm^{-1}	0.112	0.100	0.117	0.108	0.0034	<0.01	<0.01	0.31
STC2	ca. 1316 cm^{-1}	0.098^{b}	0.084^{c}	0.111^{a}	0.102^{b}	0.0019	<0.01	<0.01	0.03
STC3	ca. 1395 cm^{-1}	0.140^{b}	0.137^{b}	0.157^{a}	0.161^{a}	0.0034	<0.01	0.76	0.04
STC4	ca. 1461 cm^{-1}	0.109	0.118	0.109	0.117	0.0013	0.94	<0.01	0.50
CEC	ca. 1237 cm^{-1}	0.084	0.076	0.086	0.078	0.0025	0.17	<0.01	0.98
LIG	ca. 1515 cm^{-1}	0.020^{a}	0.008^{bc}	0.012^{b}	0.006^{c}	0.0017	<0.01	<0.01	0.02
Amide I	ca. 1589 cm^{-1}	0.243	0.194	0.260	0.222	0.0065	<0.01	<0.01	0.10
Amide II	ca. 1535 cm^{-1}	0.100^{c}	0.157^{a}	0.106^{c}	0.142^{b}	0.0058	0.18	<0.01	<0.01
α-Helix	ca. 1648 cm^{-1}	0.166^{a}	0.172^{a}	0.110^{b}	0.155^{a}	0.0069	<0.01	<0.01	<0.01
β-Sheet	ca. 1635 cm^{-1}	0.202^{a}	0.207^{a}	0.158^{b}	0.193^{a}	0.0064	<0.01	<0.01	<0.01
Areas (cm^{-1})									
TC	ca. 906 to ca. 1184 cm^{-1}	72.73^{ab}	71.45^{ab}	70.92^{b}	74.37^{a}	1.045	0.52	0.21	0.01
NSTC	ca. 879 to ca. 906 cm^{-1}	0.28^{ab}	0.29^{ab}	0.33^{a}	0.24^{b}	0.017	0.98	0.02	<0.01
STC	ca. 1184 to ca. 1485 cm^{-1}	26.51^{b}	23.54^{c}	27.65^{a}	26.40^{b}	0.437	<0.01	<0.01	<0.01
CEC	ca. 1184 to ca. 1290 cm^{-1}	4.76	4.11	4.75	4.15	0.144	0.86	<0.01	0.79
Amide	ca. 1484 to ca. 1712 cm^{-1}	26.09	28.18	25.65	27.97	0.710	0.41	<0.01	0.77
Amide I	ca. 1542 to ca. 1712 cm^{-1}	22.98^{ab}	22.57^{ab}	22.12^{b}	23.52^{a}	0.490	0.91	0.16	0.01
Amide II	ca. 1484 to ca. 1542 cm^{-1}	3.10^{d}	5.60^{a}	3.52^{c}	4.44^{b}	0.247	<0.01	<0.01	<0.01
Ratios									
Height α-Helix: β-Sheet		0.818^{a}	0.832^{a}	0.699^{b}	0.802^{a}	0.0171	<0.01	<0.01	<0.01
Height Amide I: Amide II		2.553^{a}	1.248^{c}	2.453^{a}	1.576^{b}	0.1454	0.14	<0.01	0.01
Area Amide I: Amide II		7.671^{a}	4.046^{d}	6.279^{b}	5.321^{c}	0.3199	<0.01	<0.01	<0.01

Table 7.1. ***Cont'd***. Footnote:

SEM: standard error of mean; [a-d] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison: Tukey method; the peak area and the peak height presented in each functional group measurements were expressed in IR absorbance units (AU); total carbohydrates (TC) area located from ca. 906 to ca. 1184 cm^{-1} including three peaks (TC1, TC2 and TC3) located at ca. 1027 cm^{-1}, ca. 1075 cm^{-1} and ca. 1149 cm^{-1} respectively; non-structural carbohydrates (NSTC) area located from ca. 879 to ca. 906 cm^{-1} including a main peak located at 896 cm^{-1}; structural carbohydrates (STC) area located from ca. 1184 to ca. 1485 cm^{-1} including four peaks (STC1, STC2, STC3 and STC4) located at ca. 1237 cm^{-1}, ca. 1316 cm^{-1}, ca. 1395 cm^{-1} and ca. 1461 cm^{-1}; cellulosic compounds (CEC) area located from ca. 1184 to ca. 1290 cm^{-1} including a main peak located at 1237 cm^{-1}; ligneous compounds (LIG) peak located at 1515 cm^{-1}; amide I and amide II peaks located at ca. 1645 cm^{-1} and ca. 1535 cm^{-1} respectively; α-helix and β-sheet peaks located at ca. 1648 cm^{-1} and ca. 1635 cm^{-1} respectively; amide area located from ca. 1484 to ca. 1712 cm^{-1}; amide I area located from ca. 1542 to ca. 1712 cm^{-1}; amide II area located from ca. 1484 to ca. 1542 cm^{-1}; n=6 for Snowdrop variety, 3 at mid pod stage and 3 at late pod stage; n=6 for SSNS-1 variety, 3 at mid pod stage and 3 at late pod stage.

intensity was lower ($P< 0.05$) in high tannin silage at late pod stage than low tannin silage at mid pod stage (-4.11 AU). Amide II area intensity was higher ($P< 0.05$) in high tannin silage at late pod stage than low tannin silage at late pod stage, high tannin silage at mid pod and low tannin silage at mid pod stage (+1.16, +2.5 and +2.08 AU, respectively). The α-helix to β-sheet peak height ratio was lower ($P< 0.05$) in low tannin silage at mid pod stage than low tannin silage at late pod stage and high tannin silages at mid pod and late pod stages (-0.103, -0.119 and -0.133), while the amide I to amide II peak height ratio was higher ($P< 0.05$) in high and low tannin silages at mid pod stage than in high and low tannin silages at late pod stage (2.553 and 2.453 vs. 1.248 and 1.576). The amide I to amide II area ratio was higher ($P< 0.05$) in high tannin silage at mid pod stage than high tannin silage at late pod stage and low tannin silages at mid pod and at late pod stages (+3.625, +1.392 and +2.350).

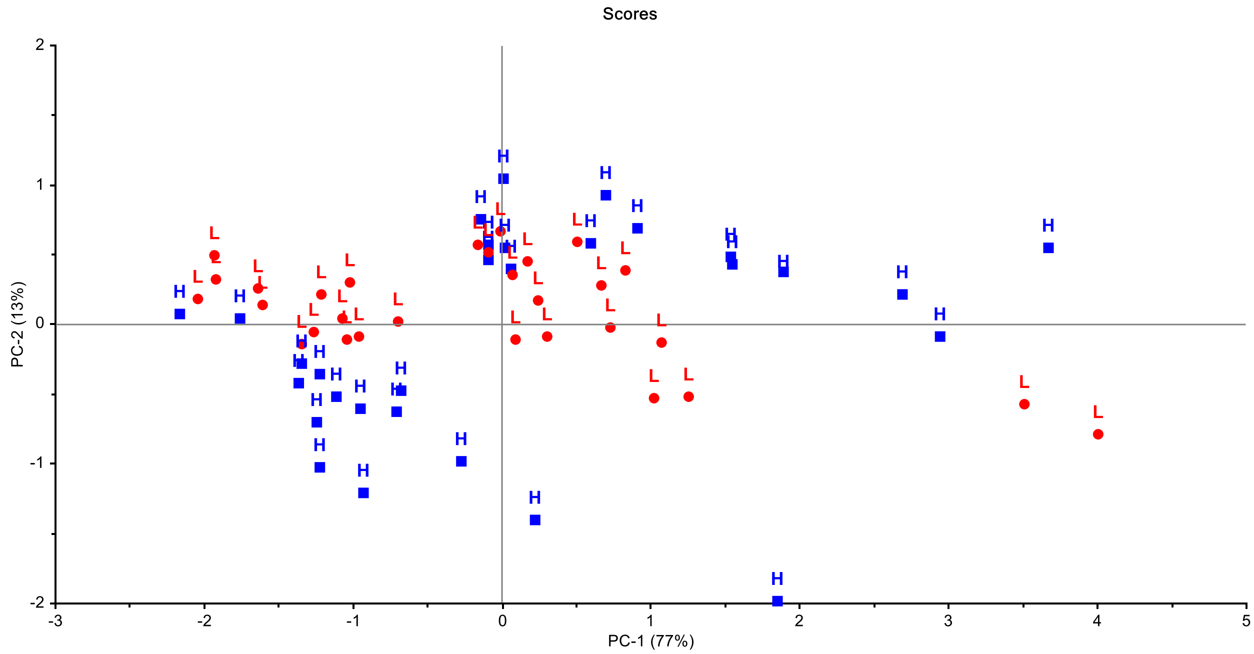
Rahman et al., (2019a) studied high and low tannin varieties of faba bean seed (FB9–4 and Snowdrop) and found that the STC peak heights located at ca. 1394 and ca. 1238 cm^{-1} had higher absorbance units for the high tannin seeds than low tannin seeds. Rodriguez (2018) studied five high tannin and three low tannin seed varieties and found that STC peak height located at ca. 1390 tended to have higher absorbance in the high tannin seed varieties than low tannin varieties, however in our study those two peaks had higher absorbance in the low tannin silage. Additionally, Rahman et al., (2019a) found that TC peaks and TC area were also higher in the high tannin seed than low tannin seed, while Rodriguez (2018) found the same, however in our study just TC3 was higher in the high tannin silage, while the other two TC peaks and TC area were similar between high and low tannin silages. Therefore, whole plant faba bean silage has different structural carbohydrates and total carbohydrates molecular features than the seeds. Rahman et al., (2019b) did not found significant difference between high and low tannin faba bean seed in terms of the amide I and amide II heights and amide I and II areas, Rodriguez (2018) found that high tannin seeds had higher amide I and amide II peak heights and areas; however in our study the total amide areas are similar in all four silages which correspond to the similar CP content (22% DM), amide I area is similar between the high tannin silage and the low tannin silage, while amide II area is higher in the high tannin silage. Additionally, amide area and amide II area changed as whole plant faba bean mature, we noticed an increment of amide area and amide II area in whole plant faba bean silage at late pod stage which can be related to more protein that comes from the seeds which are more developed as the crop reach maturity. The ratios of amide I to amide II may indicate changes in crude protein nutritive value of different feed ingredients (Khan et al., 2014). High tannin whole plant faba bean silage at mid pod stage had a higher amide I to amide II area ratio than the other three silages, but Rahman et al., (2019b) and Rodriguez (2018) agreed and have a higher amide I to amide II area ratio in the low tannin seeds. Therefore, other plant parts and silage fermentation process may affect this ratio; consequently, may affect the crude protein nutritive value. On the other hand, the protein secondary structure may affect the protein rumen degradation and the digestibility of rumen bypass protein because of the changes in solubility and because the difficulty for microbes to access and use their enzymes (Yu et al., 2010; Nuen-Ortiz and Yu, 2010). High tannin silage had higher absorbance in α-helix and β-sheet peaks and higher α-helix and β-sheet peaks ratio; the same pattern is observed in whole plant faba bean silage at late pod stage, consequently degradation and digestion behaviours of whole plant faba bean silage may be different for high and low tannin silage and also may be different for mid and late pod cutting stages.

7.4.2 Multivariate Molecular Spectral Analyses

Plots of principle component analysis (PCA) using the all spectra collected (ca. 700-4000 cm^{-1}) are shown in Figure 7.1. PCA was unable to detect a clear difference between high and low tannin whole plant faba bean silage and we can see some overlapping (Figure 7.1a). However, with PCA, we were able to detect a clear difference between whole plant faba bean silage at mid and late pod stages (Figure 7.1b), in which principal component one (PC1) accounted for more than 75% of its total variation. On the other hand, PCA using the amide region (ca. 1484 to 1712 cm^{-1}) detected a non-clear difference between high and low tannin whole plant faba bean silage because some overlapping was observed (Figure 7.2a), additionally the PCA in this region could detect a difference between whole plant faba bean silage at mid and late pod stages in which PC1 accounted for more than 70% of its total variation (Figure 7.2b). In this case the PC1 was the amide I peak height. Also, a PCA using all the carbohydrate region (ca. 879 to1485 cm^{-1}, which include NSTC, TC and STC regions) are shown in Figure 7.3. PCA in this region did not detect a clear difference between high and low tannin whole plant faba bean silage, however some overlapping was observed (Figure 7.3a), while a clear difference between whole plant faba bean silage at mid and late pod stages (Figure 7.3b) was observed and PC1 accounted for 83% of total variation.

Principal component analysis, a statistical data reduction method (Yu, 2005b), is a valuable tool for evaluating variations and identifying differences in molecular structures between feedstuffs (Allison et al., 2009; Ami et al., 2013). Principle component analysis was unable to detect clear differences between high and low tannin whole plant faba bean silages in terms of all spectra, amide region and carbohydrate region which may implicate high similarity between these two silages (supported by the results of chapter 4), but clear differences were observed between whole plant faba bean silage at mid pod stage and whole plant faba bean silage at late pod stage which suggests that at a molecular level whole plant faba bean silage changes with maturity, thus at late pod stage lower STC area was observed which can be related to the lower NSC from observed in Chapter 4, but the lower amide I peak height change could not be related to any protein fractions as those did no change as shown in Chapter 4. To our knowledge, no publications could be found

a)



b)

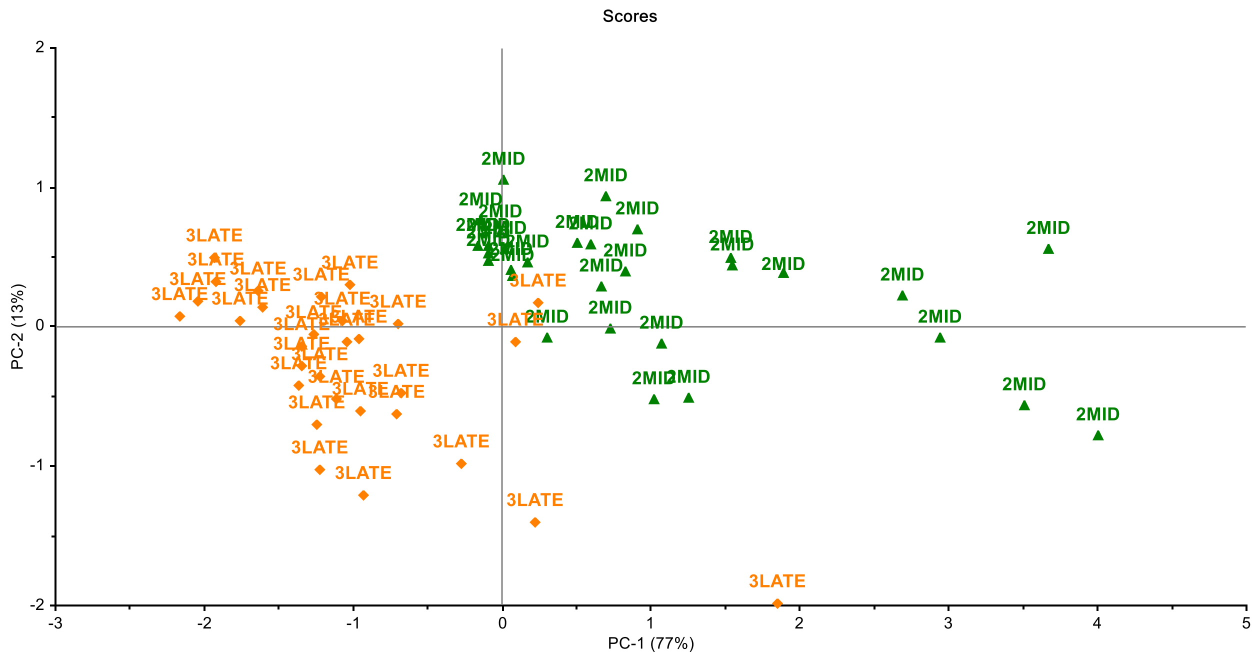
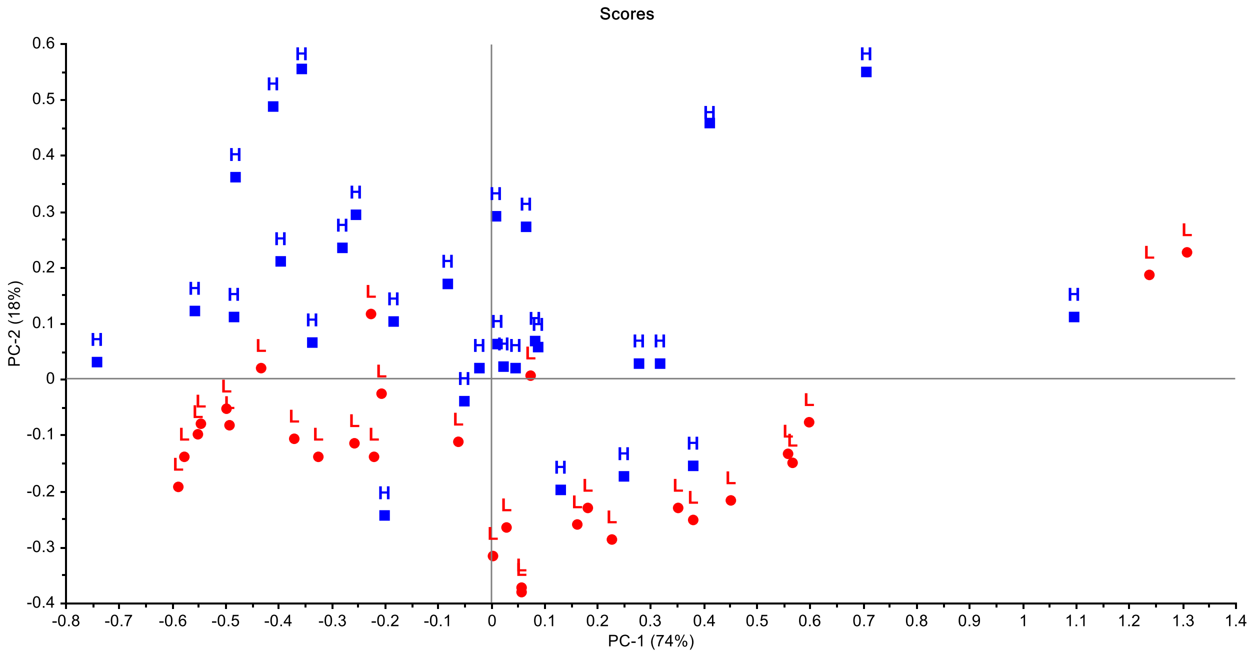


Figure 7.1. a) Effect of variety (tannin concentration) on principal components analysis (PCA) of whole plant faba bean silage using FTIR vibrational at complete spectra region (ca. 700 to ca. 4000 cm^{-1}); b) Effect of stage of cutting on principal components analysis (PCA) of whole plant faba bean silage using FTIR vibrational at complete spectra region (ca. 700 to ca. 4000 cm^{-1}); PCA: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

a)



b)

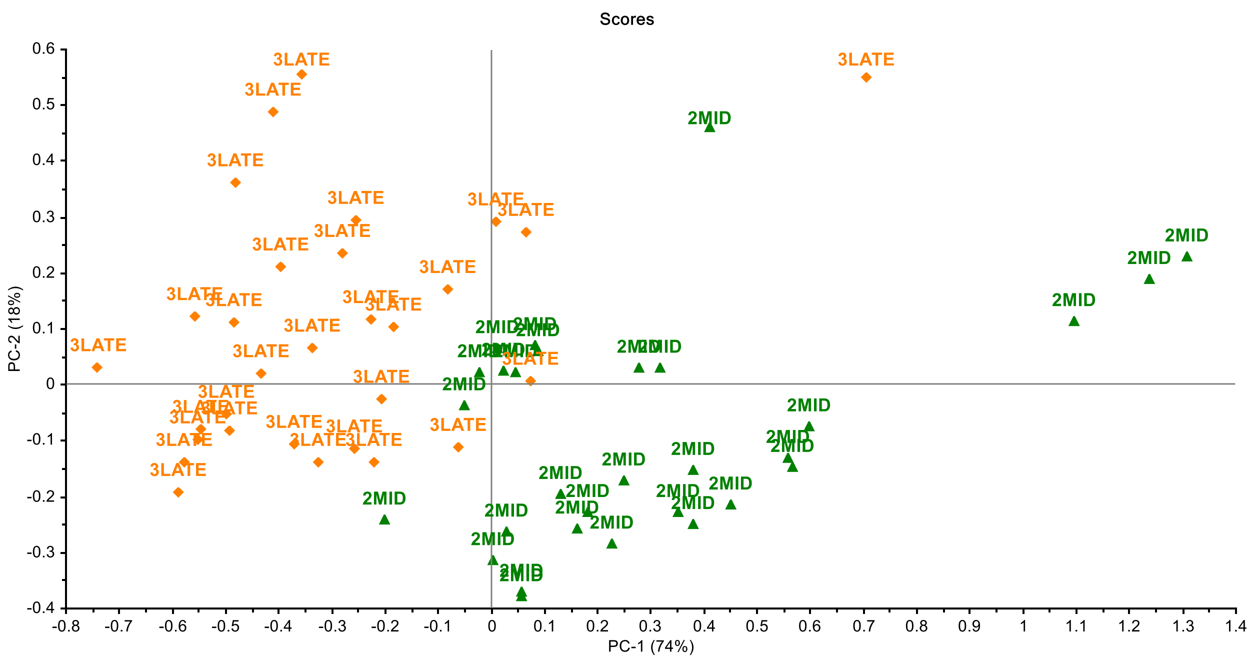
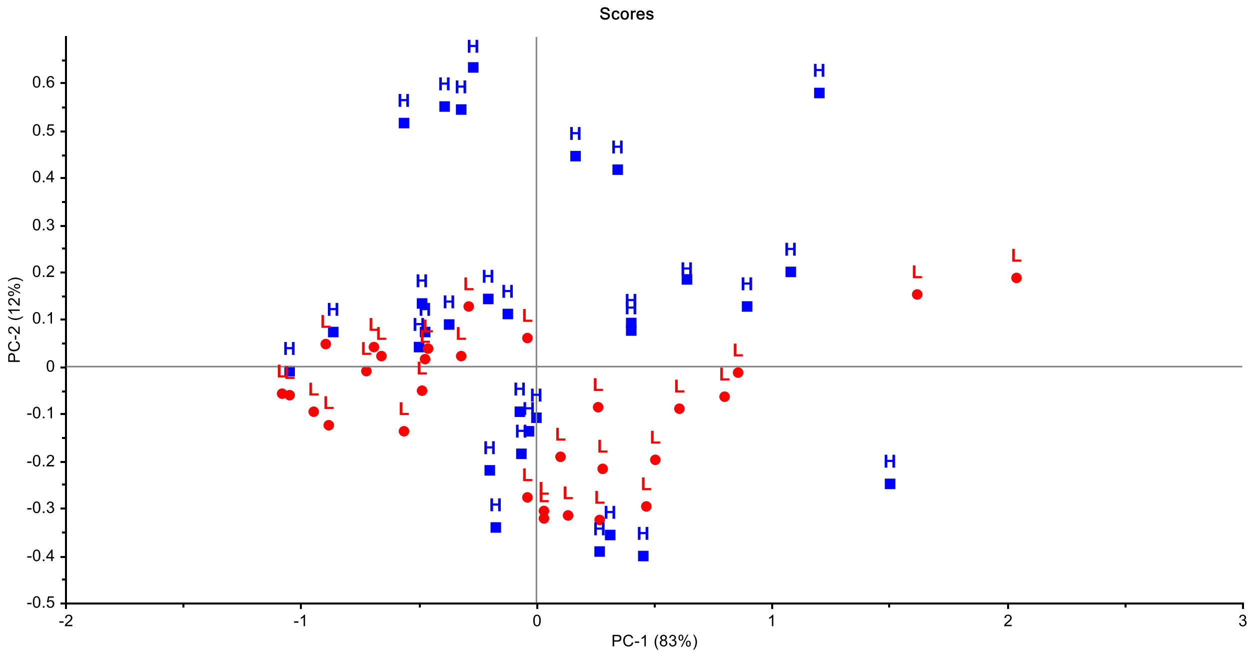


Figure 7.2. a) Effect of variety (tannin concentration) on principal components analysis (PCA) of whole plant faba bean silage using FTIR vibrational at amide region (ca. 1484 to ca. 1712 cm^{-1}); b) Effect of stage of cutting on principal components analysis (PCA) of whole plant faba bean silage using FTIR vibrational at amide region (ca. 1484 to ca. 1712 cm^{-1}); PCA: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

a)



b)

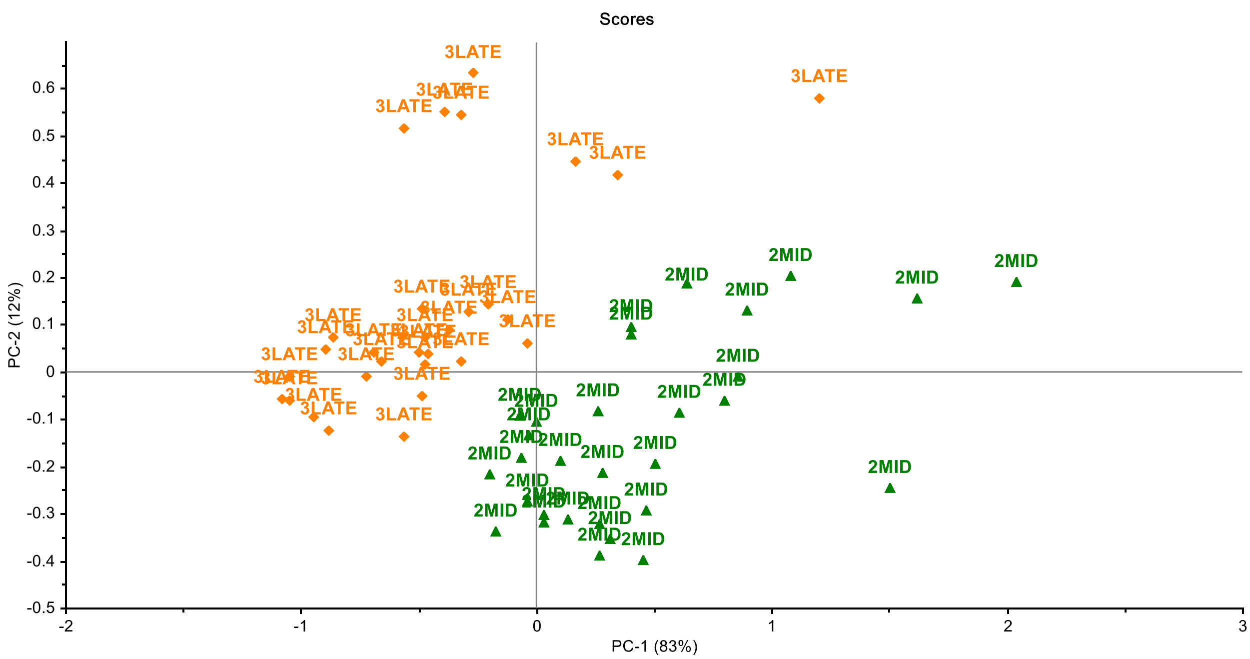


Figure 7.3. a) Effect of variety (tannin concentration) on principal components analysis (PCA) of whole plant faba bean silage using FTIR vibrational at complete carbohydrate region (ca. 879 to ca. 1485 cm^{-1}); b) Effect of stage of cutting on principal components analysis (PCA) of whole plant faba bean silage using FTIR at complete carbohydrate region (ca. 879 to ca. 1485 cm^{-1}); PCA: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

on the internal protein and carbohydrate structure of whole plant faba bean silage, therefore no comparison can be made.

7.4.3 Molecular Structure Spectral Profiles in Relation with Chemical and Energy Profiles

Results of correlation analysis between carbohydrate molecular structure spectral peaks with chemical and energy profiles of whole plant faba bean silage are shown in Table 7.2. The peak of NSTC had a significant correlation ($P< 0.05$) with ADL ($r= 0.69$) and hemicellulose ($r= -0.66$) and showed a tendency for correlation ($P< 0.10$) with total CHO ($r= -0.54$), starch ($r= -0.57$), sugars ($r= 0.58$), and NSC ($r= -0.57$). The four spectral peaks related to STC also showed associations with some chemical characteristics. Hence, a strongly significant correlation ($P< 0.01$) was observed between STC1 and SCP ($r= 0.80$), starch ($r= -0.84$), and NSC ($r= -0.84$); a significant correlation ($P< 0.05$) was observed with DM ($r= -0.70$), ADF ($r= 0.60$), ADL ($r= 0.61$), cellulose ($r= 0.60$), and NFC ($r= -0.68$); STC2 was strongly correlated ($P< 0.01$) with ADF ($r= 0.79$), cellulose ($r= 0.76$) , and SCP ($r= 0.84$) and showed significant correlations ($P< 0.05$) with DM ($r= -0.69$), aNDF ($r= 0.65$), NDFn ($r= 0.62$), ADL ($r= 0.64$), NFC ($r= -0.61$), starch ($r= -0.71$), and NSC ($r= -0.71$). The STC3 presented a significant correlation ($P< 0.05$) with SCP ($r= 0.66$) while the last peak of structural CHO (STC4) had a strongly significant correlation ($P< 0.01$) with ADL ($r= -0.82$) and significant correlations ($P< 0.05$) with DM ($r= 0.61$), NFC ($r= 0.67$), and SCP ($r= -0.62$). The peak of CEC was significantly correlated ($P< 0.05$) with NFC ($r= -0.70$), SCP ($r= 0.63$), starch ($r= -0.73$), and NSC ($r= -0.73$). The peak of ligneous compound was significantly ($P< 0.05$) correlated with ADICP ($r= -0.59$), starch ($r= -0.63$), and NSC ($r= -0.63$). In regard to the energy profiles, STC1 and STC2 showed a significant correlation ($P< 0.05$) with almost all total digestible nutrients (tdNFC, tdNDF, and tdFA), and with NE_m ($r= -0.70$, -0.69, respectively). A tendency ($P< 0.10$) was observed between STC1 and the majority of other energy values. The STC4 had a significant correlation ($P< 0.05$) with tdNFC ($r= 0.67$), tdFA ($r= -0.75$), and NE_m ($r= 0.61$).

Table 7.2. Correlation between carbohydrate structure spectral peaks and chemical and energy profiles of whole plant faba bean silage

Item	Peaks													
	NSTC		STC1		STC2		STC3		STC4		CEC		LIG	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Chemical Profile														
DM (%)	-0.47	0.13	-0.70	0.01	-0.69	0.01	-0.29	0.35	0.61	0.04	-0.51	0.09	-0.34	0.28
ASH (%DM)	0.36	0.25	0.19	0.56	-0.07	0.83	-0.15	0.63	-0.06	0.86	0.28	0.38	-0.10	0.76
OM (%DM)	-0.36	0.25	-0.19	0.56	0.07	0.83	0.15	0.63	0.06	0.86	-0.28	0.38	0.10	0.76
EE (%DM)	0.73	0.01	0.62	0.03	0.67	0.02	0.24	0.46	-0.75	0.01	0.43	0.17	0.63	0.03
CP (%DM)	0.06	0.85	-0.30	0.35	-0.39	0.21	-0.50	0.10	0.05	0.88	-0.17	0.59	0.50	0.10
CHO (%DM)	-0.54	0.07	-0.26	0.42	-0.11	0.73	0.15	0.65	0.25	0.43	-0.29	0.36	-0.41	0.18
aNDF (%DM)	0.15	0.64	0.56	0.06	0.65	0.02	0.49	0.11	-0.46	0.14	0.49	0.11	-0.12	0.71
NDFn (%DM)	0.19	0.56	0.54	0.07	0.62	0.03	0.44	0.15	-0.47	0.13	0.47	0.12	-0.13	0.68
ADF (%DM)	0.45	0.14	0.60	0.04	0.79	<0.01	0.44	0.15	-0.53	0.08	0.46	0.13	0.01	0.97
ADL (%DM)	0.69	0.01	0.61	0.04	0.64	0.02	0.16	0.62	-0.82	<0.01	0.48	0.12	0.53	0.08
Hemi (%DM)	-0.66	0.02	0.06	0.85	-0.17	0.59	0.20	0.53	0.01	0.97	0.27	0.39	-0.26	0.42
Cell (%DM)	0.35	0.27	0.60	0.04	0.76	<0.01	0.45	0.14	-0.46	0.14	0.46	0.14	-0.03	0.93
NFC (%DM)	-0.40	0.19	-0.68	0.02	-0.61	0.04	-0.27	0.40	0.67	0.02	-0.70	0.01	-0.18	0.57
NDICP (%DM)	-0.24	0.45	-0.28	0.37	-0.19	0.56	0.13	0.68	0.07	0.84	-0.25	0.44	-0.13	0.68
ADICP (%DM)	-0.27	0.40	-0.16	0.61	0.22	0.48	0.52	0.08	0.22	0.48	-0.13	0.68	-0.59	0.04
SCP (%DM)	0.36	0.25	0.80	<0.01	0.84	<0.01	0.66	0.02	-0.62	0.03	0.63	0.03	0.44	0.15
Starch (%DM)	-0.57	0.05	-0.84	<0.01	-0.71	0.01	-0.18	0.57	0.94	<0.01	-0.73	0.01	-0.63	0.03
Sugar (%DM)	0.58	0.05	0.37	0.24	0.22	0.49	-0.02	0.95	-0.43	0.16	0.24	0.45	0.55	0.06
NSC (%DM)	-0.57	0.05	-0.84	<0.01	-0.71	0.01	-0.18	0.57	0.94	<0.01	-0.73	0.01	-0.63	0.03
Energy Profile														
tdNFC (%DM)	-0.40	0.19	-0.68	0.02	-0.61	0.04	-0.27	0.40	0.67	0.02	-0.70	0.01	-0.18	0.57
tdCP (%DM)	0.07	0.84	-0.28	0.38	-0.38	0.23	-0.48	0.12	0.04	0.90	-0.17	0.59	0.52	0.08
tdNDF (%DM)	0.13	0.69	0.63	0.03	0.73	0.01	0.55	0.06	-0.42	0.17	0.56	0.06	-0.11	0.73
tdFA (%DM)	0.73	0.01	0.62	0.03	0.67	0.02	0.24	0.46	-0.75	0.01	0.43	0.17	0.63	0.03
TDN_{3x} (%DM)	-0.17	0.60	-0.55	0.06	-0.35	0.27	-0.15	0.63	0.52	0.08	-0.62	0.03	0.04	0.90
DE_{p3x} (Mcal/kg)	-0.20	0.52	-0.52	0.08	-0.42	0.17	-0.22	0.48	0.44	0.16	-0.56	0.06	0.16	0.63
ME_{p3x} (Mcal/kg)	-0.20	0.52	-0.52	0.08	-0.42	0.17	-0.22	0.48	0.44	0.16	-0.56	0.06	0.16	0.63

Table 7.2. *Cont'd*. Correlation between carbohydrate structure spectral peaks and chemical and energy profiles of whole plant faba bean silage

Item	Peaks													
	NSTC		STC1		STC2		STC3		STC4		CEC		LIG	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
NE_{Lp3x} (Mcal/kg)	-0.20	0.52	-0.52	0.08	-0.42	0.17	-0.22	0.48	0.44	0.16	-0.56	0.06	0.16	0.63
ME (Mcal/kg)	-0.20	0.52	-0.52	0.08	-0.42	0.17	-0.22	0.48	0.44	0.16	-0.56	0.06	0.16	0.63
NE_m (Mcal/kg)	-0.47	0.13	-0.70	0.01	-0.69	0.01	-0.29	0.35	0.61	0.04	-0.51	0.09	-0.34	0.28
NE_g (Mcal/kg)	0.36	0.25	0.19	0.56	-0.07	0.83	-0.15	0.63	-0.06	0.86	0.28	0.38	-0.10	0.76

Non-structural carbohydrates (NSTC) area located from ca 879 to ca 906 cm^{-1} including a main peak located at 896 cm^{-1}, structural carbohydrates (STC) area located from ca 1184 to ca 1485 cm^{-1} including four peaks (STC1, STC2, STC3 and STC4) located at ca 1237 cm^{-1}, ca 1316 cm^{-1}, ca 1395 cm^{-1} and ca 1461 cm^{-1} cellulosic compounds (CEC) area located from ca 1184 to ca 1290 cm^{-1} including a main peak located at 1237 cm^{-1} and ligneous compounds (LIG) peak located at 1515 cm^{-1}. +, positive correlation; -, negative correlation; r: correlation coefficient using spearman method; values of 'r' between 0 and ± 0.33: weak correlation, between ± 0.33 and ± 0.66: medium correlation, and between ± 0.66 and ± 1: strong correlation; $P < 0.10$, tendency; $P < 0.05$, significant; $P < 0.01$, strongly significant.

The peak of CEC had a significant correlation ($P < 0.05$) with tdNFC ($r = -0.70$) and TDN at 1× and 3× ($r = -0.62$ for both characteristics), while a tendency ($P < 0.10$) was observed with other energy values. Last, the peak intensity of ligneous compound had a significant correlation ($P < 0.05$) with tdFA ($r = 0.63$).

In this study, the peak of NSTC was positively correlated with ADL while the peaks of STC1, STC2 were positively correlated with ADL, ADF, cellulose, and SCP. On the other hand, the same peaks of STC were negatively correlated with NFC, starch, NSC, and NE_m while CEC area was negatively correlated with NFC, starch, NSC, and tdNFC. Positive correlations indicate a linear relationship between the chemical and energy characteristics with the spectral intensities related to CHO. On the contrary, negative correlations will show an opposite relationship between nutrient and energy profiles compared to molecular structure features. These results might indicate that correlated molecular characteristics are sensitive to different chemical and metabolic profiles. Rodriguez (2018) found that STC1, STC2 and STC3 were not correlated with the starch content, neither with the NE_L of faba bean seeds; while Prates et al., (2018) found a positive correlation of peak 2 (ca. 1370 cm^{-1}) with the NFC content. However, in our study the peak located at that point did not have correlation with NFC content.

Table 7.3 shows the results of the correlation between protein molecular structure spectral intensity peaks and chemical and energy profiles of whole plant faba bean silages. Amide I had a strongly significant correlation ($P < 0.01$) with SCP ($r = 0.80$) while both amide I and II spectral peaks showed a strongly significant correlation ($P < 0.01$) with ADL ($r = 0.81$, -0.83, respectively), starch ($r = -0.85$, 0.92, respectively), and NSC ($r = -0.85$, 0.92, respectively). Spectral intensity peaks of α-helix and β-sheet were strongly correlated ($P < 0.01$) with aNDF ($r = -0.83$, -0.84, respectively), NDFn ($r = -0.85$, -0.87, respectively), ADF ($r = -0.80$, -0.83, respectively), cellulose ($r = -0.76$, -0.80, respectively), and NFC ($r = 0.82$, 0.87, respectively) while these same spectral peaks showed a

Table 7.3. Correlation between protein structure spectral peaks and chemical and energy profiles of whole plant faba bean silage

Item	Peaks								Ratios			
	Amide I		Amide II		α-helix		β-sheet		Amide I: Amide II		α-Helix: β-Sheet	
	r	P	r	P	r	P	r	P	r	P	r	P
Chemical Profile												
DM (%)	-0.66	0.02	0.52	0.08	0.69	0.01	0.58	0.05	-0.53	0.08	0.64	0.03
ASH (%DM)	-0.01	0.98	0.16	0.61	-0.37	0.24	-0.41	0.19	-0.28	0.38	-0.48	0.11
OM (%DM)	0.01	0.98	-0.16	0.61	0.37	0.24	0.41	0.19	0.28	0.38	0.48	0.11
EE (%DM)	0.90	<0.01	-0.78	<0.01	-0.66	0.02	-0.63	0.03	0.80	<0.01	-0.70	0.01
CP (%DM)	-0.18	0.59	0.05	0.89	0.62	0.03	0.65	0.02	-0.08	0.79	0.43	0.17
CHO (%DM)	-0.27	0.40	0.12	0.70	0.19	0.56	0.19	0.56	-0.02	0.95	0.35	0.26
aNDF (%DM)	0.44	0.15	-0.42	0.18	-0.83	<0.01	-0.84	<0.01	0.41	0.19	-0.63	0.03
NDFn (%DM)	0.43	0.16	-0.42	0.18	-0.85	<0.01	-0.87	<0.01	0.40	0.20	-0.65	0.02
ADF (%DM)	0.65	0.02	-0.58	0.05	-0.80	<0.01	-0.83	<0.01	0.60	0.04	-0.58	0.05
ADL (%DM)	0.81	<0.01	-0.83	<0.01	-0.69	0.01	-0.69	0.01	0.85	<0.01	-0.67	0.02
Hemi (%DM)	-0.38	0.23	0.26	0.42	-0.15	0.63	-0.12	0.71	-0.31	0.32	-0.18	0.58
Cell (%DM)	0.61	0.04	-0.55	0.06	-0.76	<0.01	-0.80	<0.01	0.57	0.05	-0.51	0.09
NFC (%DM)	-0.54	0.07	0.56	0.06	0.82	<0.01	0.87	<0.01	-0.49	0.11	0.71	0.01
NDICP (%DM)	-0.24	0.44	0.26	0.42	0.07	0.84	0.18	0.58	-0.15	0.65	-0.09	0.79
ADICP (%DM)	-0.16	0.62	0.21	0.52	-0.19	0.55	-0.28	0.37	-0.17	0.59	-0.01	0.97
SCP (%DM)	0.80	<0.01	-0.57	0.05	-0.74	0.01	-0.65	0.02	0.61	0.04	-0.74	0.01
Starch (%DM)	-0.85	<0.01	0.92	<0.01	0.70	0.01	0.70	0.01	-0.90	<0.01	0.65	0.02
Sugar (%DM)	0.56	0.06	-0.41	0.18	-0.33	0.30	-0.24	0.45	0.47	0.12	-0.52	0.08
NSC (%DM)	-0.85	<0.01	0.92	<0.01	0.70	0.01	0.70	0.01	-0.90	<0.01	0.65	0.02
Energy Profile												
tdNFC (%DM)	-0.54	0.07	0.56	0.06	0.82	<0.01	0.87	<0.01	-0.49	0.11	0.71	0.01
tdCP (%DM)	-0.15	0.65	0.04	0.91	0.62	0.03	0.66	0.02	-0.06	0.85	0.41	0.18
tdNDF (%DM)	0.48	0.12	-0.42	0.17	-0.81	<0.01	-0.84	<0.01	0.40	0.20	-0.57	0.05
tdFA (%DM)	0.90	<0.01	-0.78	<0.01	-0.66	0.02	-0.63	0.03	0.80	<0.01	-0.70	0.01
TDN_{3x} (%DM)	-0.28	0.38	0.35	0.26	0.76	<0.01	0.79	<0.01	-0.28	0.38	0.66	0.02
DE_{p3x} (Mcal/kg)	-0.29	0.35	0.33	0.30	0.76	<0.01	0.82	<0.01	-0.27	0.40	0.61	0.04
ME_{p3x} (Mcal/kg)	-0.29	0.35	0.33	0.30	0.76	<0.01	0.82	<0.01	-0.27	0.40	0.61	0.04
NE_{Lp3x} (Mcal/kg)	-0.29	0.35	0.33	0.30	0.76	<0.01	0.82	<0.01	-0.27	0.40	0.61	0.04

Table 7.3. ***Cont'd***. Correlation between protein structure peaks and chemical and energy profiles of whole plant faba bean silage.

Item	Peaks								Ratios			
	Amide I		Amide II		α-helix		β-sheet		Amide I: Amide II		α-Helix: β-Sheet	
	r	P	r	P	r	P	r	P	r	*P*	r	*P*
ME (Mcal/kg)	-0.29	0.35	0.33	0.30	0.76	<0.01	0.82	<0.01	-0.27	0.40	0.61	0.04
NE_m (Mcal/kg)	-0.29	0.35	0.33	0.30	0.76	<0.01	0.82	<0.01	-0.27	0.40	0.61	0.04
NE_g (Mcal/kg)	-0.29	0.35	0.33	0.30	0.76	<0.01	0.82	<0.01	-0.27	0.40	0.61	0.04

Amide I and II peaks located at ca 1589 and ca 1535 cm^{-1} respectively, α-helix and β-sheet peaks located at ca 1648 and ca 1635 cm^{-1} respectively. +, positive correlation; -, negative correlation; r: correlation coefficient using spearman method; values of 'r' between 0 and ± 0.33: weak correlation, between ± 0.33 and ± 0.66: medium correlation, and between ± 0.66 and ± 1: strong correlation; P< 0.10, tendency; P< 0.05, significant; P< 0.01, strongly significant.

significant correlation (P< 0.05) with CP (r= 0.62, 0.65, respectively), ADL (r= -0.69 for both peaks), SCP (r= -0.74, -0.65, respectively), starch (r= 0.70 for both peaks), and NSC (r= 0.70 for both peaks). In terms of energy profiles, amide I and II spectral peaks were strongly correlated (P< 0.01) with tdFA (r= 0.90, -0.78, respectively). Spectral peaks of α-helix and β-sheet were strongly correlated (P< 0.01) with tdNFC (r= 0.82, 0.87, respectively) and tdNDF (r= -0.81, -0.84, respectively) and significantly correlated (P< 0.05) with tdCP (r= 0.62, 0.66, respectively) and tdFA (r= -0.66, -0.63, respectively). These same spectral peaks had a strong correlation (P< 0.01) with TDN_{1x}, TDN_{3x}, DE_{1x}, DE_{p3x}, ME_{p3x}, NE_{Lp3x}, ME, NE_m, and NE_g. The spectral peak ratios of amide I to amide II and α-helix to β-sheet were also correlated with some chemical and energy characteristics. Amide I to amide II peak ratio was strongly correlated (P<0.01) with ADL (r= 0.85), starch (r= -0.90), NSC (r= -0.90), and tdFA (r= 0.80) while it showed a significant correlation (P< 0.05) with ADF (r= 0.60) and SCP (r= 0.61). On the other hand, α-helix to β-sheet peak ratio had a significant correlation (P< 0.05) with ADL (r= -0.67), SCP (r= -0.74), starch (r= 0.65), and almost the majority of energy profiles.

In the current research, negative associations were found between amide II, α-helix, β-sheet peaks, and α-helix to β-sheet ratio with SCP and sugars while positive associations were presented between these spectral peaks and starch. Protein secondary structure β-sheet has been related with a lower protein solubility in feeds (Yu, P. 2005a), which indicates a lower rumen degradation of protein, hence higher bypass protein may be digested in the small intestine. On the contrary, amide I and the ratio of amide I to amide II showed a positive association with SCP and negative association with starch, the current results partially agree with Xin et al., (2013) and Yan et al., (2014). Only protein secondary structure spectral peaks and ratios showed positive correlations with CP and most energy values, nearly similar to data published by Rahman et al., (2019) on a research performed on faba bean seeds. Further research is still needed to fully understand associations between molecular values and nutrient characteristics. Different results among studies might suggest that different feeds have their unique specific inherent characteristics which could impact their nutrient uptake and metabolic behaviours in singular manners.

The correlation of protein and carbohydrate molecular structure spectral areas with chemical and energy profiles of whole plant faba bean silage is presented in Table 7.4. The CEC spectral area was strongly correlated (P< 0.01) with starch (r= -0.80) and significantly correlated (P< 0.05) with NFC (r= -0.71) and NSC (r= -0.80). The STC area showed strongly significant correlations (P< 0.01) with SCP (r= 0.87), starch (r= -0.80), and NSC (r= -0.80) while it showed a significant correlation (P< 0.05) with aNDF (r= 0.59), ADF (r= 0.74), ADL (r= 0.71), cellulose (r= 0.69), and NFC (r= -0.62). Amide area was strongly correlated (P< 0.01) with aNDF (r= -0.76), NDFn (r=-0.79), ADF (r= -0.79), cellulose (r= -0.78), NFC (r= 0.78), starch (r= 0.85), and NSC (r= 0.85) while it showed a significant correlation (P< 0.05) with ADL (r= -0.85). Moreover, amide I area had a significant correlation with aNDF (r= -0.59), NDFn (r= -0.78), ADF (r= -0.67), ADL (r= -0.59), cellulose (r= -0.66), and NFC (r= 0.78). Amide II area was strongly correlated (P< 0.001) with starch (r= 0.90) and NSC (r= 0.90), and strongly correlated (P< 0.01) with ADL (r= -0.86). The spectral intensity ratio of amide I to amide II was strongly correlated (P< 0.01) with starch (r= -0.83) and NSC (r= -0.83) and showed a significant correlation (P< 0.05) with ADL (r= 0.74).

In relation to the energy profiles, CEC spectral area was significantly correlated (P< 0.05) with tdNFC (r= -0.71), TDN_{1x} and TDN_{3x} (r= -0.65 for both characteristics) and showed a correlation tendency (P<0.10) with the other energy values (DE_{1x}, DE_{p3x}, ME_{p3x}, NE_{Lp3x}, ME, NE_m, and NE_g). The STC spectral area had a significant correlation (P< 0.05) with tdNFC (r= -0.62), tdNDF (r= 0.64), and tdFA (r= 0.71). Amide area had a strong correlation (P< 0.01) with tdNFC (r= 0.78) and significant correlations (P< 0.05) with all other energy values, except for tdCP.

Table 7.4. Correlation between protein and carbohydrate structure spectral areas and chemical and energy profiles of whole plant faba bean silage

Item	Areas												Ratio	
	NSTC		CEC		STC		Amide		Amide I		Amide II		Area Amide I: Amide II	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Chemical Profile														
DM (%)	-0.28	0.38	-0.51	0.09	-0.73	0.01	0.52	0.08	0.30	0.34	0.57	0.05	-0.55	0.07
ASH (%DM)	0.46	0.13	0.31	0.32	-0.06	0.86	-0.06	0.86	-0.41	0.18	0.31	0.33	-0.39	0.21
OM (%DM)	-0.46	0.13	-0.31	0.32	0.06	0.86	0.06	0.86	0.41	0.18	-0.31	0.33	0.39	0.21
EE (%DM)	0.35	0.27	0.49	0.11	0.71	0.01	-0.69	0.01	-0.37	0.24	-0.81	<0.01	0.73	0.01
CP (%DM)	0.20	0.53	-0.18	0.59	-0.28	0.38	0.47	0.12	0.49	0.10	0.07	0.82	0.06	0.85
CHO (%DM)	-0.55	0.07	-0.29	0.35	-0.15	0.65	-0.04	0.90	0.03	0.93	0.00	1.00	0.00	1.00
aNDF (%DM)	0.14	0.66	0.47	0.12	0.59	0.04	-0.76	<0.01	-0.72	0.01	-0.43	0.16	0.25	0.43
NDFn (%DM)	0.15	0.63	0.48	0.12	0.55	0.06	-0.79	<0.01	-0.78	<0.01	-0.43	0.17	0.24	0.46
ADF (%DM)	0.39	0.22	0.46	0.13	0.74	0.01	-0.79	<0.01	-0.67	0.02	-0.61	0.04	0.46	0.13
ADL (%DM)	0.45	0.14	0.55	0.06	0.71	0.01	-0.85	<0.01	-0.59	0.04	-0.86	<0.01	0.74	0.01
Hemi (%DM)	-0.39	0.21	0.24	0.46	-0.14	0.66	0.01	0.98	-0.19	0.56	0.29	0.37	-0.34	0.28
Cell (%DM)	0.27	0.39	0.43	0.16	0.69	0.01	-0.78	<0.01	-0.66	0.02	-0.58	0.05	0.43	0.17
NFC (%DM)	-0.49	0.11	-0.71	0.01	-0.62	0.03	0.78	<0.01	0.78	<0.01	0.50	0.10	-0.33	0.30
NDICP (%DM)	-0.24	0.46	-0.26	0.42	-0.08	0.81	0.16	0.62	0.11	0.73	0.11	0.73	-0.13	0.70
ADICP (%DM)	-0.10	0.75	-0.20	0.54	0.06	0.85	-0.06	0.85	-0.14	0.66	0.20	0.54	-0.28	0.38
SCP (%DM)	0.13	0.68	0.58	0.05	0.87	<0.01	-0.45	0.14	-0.15	0.65	-0.60	0.04	0.55	0.06
Starch (%DM)	-0.38	0.22	-0.80	<0.01	-0.80	<0.01	0.85	<0.01	0.51	0.09	0.90	<0.01	-0.83	<0.01
Sugar (%DM)	0.24	0.46	0.26	0.42	0.36	0.24	-0.33	0.30	-0.21	0.50	-0.47	0.12	0.36	0.25
NSC (%DM)	-0.38	0.22	-0.80	<0.01	-0.80	<0.01	0.85	<0.01	0.51	0.09	0.90	<0.01	-0.83	<0.01
Energy Profile														
tdNFC (%DM)	-0.49	0.11	-0.71	0.01	-0.62	0.03	0.78	<0.01	0.78	<0.01	0.50	0.10	-0.33	0.30
tdCP (%DM)	0.17	0.59	-0.17	0.59	-0.25	0.43	0.49	0.11	0.54	0.07	0.06	0.86	0.09	0.78
tdNDF (%DM)	0.15	0.65	0.51	0.09	0.64	0.03	-0.70	0.01	-0.64	0.03	-0.41	0.18	0.25	0.43
tdFA (%DM)	0.35	0.27	0.49	0.11	0.71	0.01	-0.69	0.01	-0.37	0.24	-0.81	<0.01	0.73	0.01
TDN_{3x} (%DM)	-0.28	0.38	-0.65	0.02	-0.38	0.22	0.73	0.01	0.86	<0.01	0.29	0.35	-0.11	0.73
DE_{p3x} (Mcal/kg)	-0.32	0.31	-0.58	0.05	-0.41	0.18	0.71	0.01	0.85	<0.01	0.27	0.40	-0.08	0.80

Table 7.4. ***Cont'd***. Correlation between protein and carbohydrate spectral structure areas and chemical and energy profiles of whole plant faba bean silage.

Item	Areas												Ratio	
	NSTC		CEC		STC		Amide		Amide I		Amide II		Area Amide I: Amide II	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
ME_{p3x} (Mcal/kg)	-0.32	0.31	-0.58	0.05	-0.41	0.18	0.71	0.01	0.85	<0.01	0.27	0.40	-0.08	0.80
NE_{Lp3x} (Mcal/kg)	-0.32	0.31	-0.58	0.05	-0.41	0.18	0.71	0.01	0.85	<0.01	0.27	0.40	-0.08	0.80
ME (Mcal/kg)	-0.32	0.31	-0.58	0.05	-0.41	0.18	0.71	0.01	0.85	<0.01	0.27	0.40	-0.08	0.80
NE_m (Mcal/kg)	-0.32	0.31	-0.58	0.05	-0.41	0.18	0.71	0.01	0.85	<0.01	0.27	0.40	-0.08	0.80
NE_g (Mcal/kg)	-0.32	0.31	-0.58	0.05	-0.41	0.18	0.71	0.01	0.85	<0.01	0.27	0.40	-0.08	0.80

Non-structural carbohydrates (NSTC) area located from ca 879 to ca 906 cm^{-1}, structural carbohydrates (STC) area located from ca 1184 to ca 1485 cm^{-1}, cellulosic compounds (CEC) area located from ca 1184 to ca 1290 cm^{-1}, amide area located from ca 1484 to ca 1712 cm^{-1}, amide I area located from ca 1547 to ca 1712 cm^{-1}, amide II area located from ca 1484 to ca 1547 cm^{-1}. +, positive correlation; -, negative correlation; r: correlation coefficient using spearman method; values of 'r' between 0 and ± 0.33: weak correlation, between ± 0.33 and ± 0.66: medium correlation, and between ± 0.66 and ± 1: strong correlation; P< 0.10, tendency; P< 0.05, significant; P< 0.01, strongly significant; P< 0.001, very strongly significant.

On the other hand, amide I spectral area intensity showed a strongly significant correlation ($P < 0.01$) with almost all energy values, except for tdCP and tdFA and had a significant correlation ($P < 0.05$) with tdNFC (r= 0.78) and tdNDF (r= -0.64). Amide II spectral area intensity was strongly correlated ($P < 0.01$) with tdFA (r= -0.81) while the spectral area intensity ratio of amide I to amide II was significantly correlated ($P < 0.05$) with tdFA (r= 0.73).

In this study no correlation between NSTC spectral area intensity and chemical and energy values was found. However, CEC and STC areas of spectral intensity were negatively correlated with NFC, starch, and TDN_{1x}, suggesting that at higher content of NFC and starch the spectral peaks of CEC and STC will be lower. As the cellulosic compound area increases there is a reduction in the starch content, and that could be the reason for the negative correlation of cellulosic compound area with the energy values. Moreover, STC area showed a positive correlation with almost all fiber profiles, and these data agrees with Yu (2012) but disagrees with the results from Xin et al., (2013) where no significant correlation was found between the area of STC and CHO chemical profiles (NDF, ADF, ADL, CHO, and cellulose). Amide areas of spectral intensity were negatively correlated with fiber compounds, SCP, and sugar while they were positively correlated with almost all energy values. These results were similar to the ones found by Rahman et al., (2019) but differ from data presented by Yang et al., (2014).

7.4.4 Molecular Structure Spectral Profiles in Relation with Rumen Degradation Kinetics of Primary Nutrients

The correlation between carbohydrate structure spectral peaks and rumen degradation kinetics of primary nutrients of whole plant faba bean silage is presented in Table 7.5. The spectral peak intensity of NSTC was significantly correlated ($P < 0.05$) with the Kd of CP (r= 0.59) and showed a tendency ($P < 0.10$) with other degradation characteristics such as %RUP (r= -0.51), %EDCP (r=

Table 7.5. Correlation between carbohydrate structure spectral peaks and rumen degradation kinetics of primary nutrients of whole plant faba bean silage

Item	Peaks													
	NSTC		STC1		STC2		STC3		STC4		CEC		LIG	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
DM														
Kd (%/h)	-0.01	0.97	-0.30	0.35	-0.66	0.02	-0.62	0.03	0.23	0.47	-0.11	0.73	0.22	0.49
S (%)	0.44	0.15	0.87	<0.01	0.83	<0.01	0.66	0.02	-0.61	0.04	0.67	0.02	0.36	0.26
D (%)	-0.48	0.11	-0.87	<0.01	-0.54	0.07	-0.18	0.57	0.77	<0.01	-0.82	<0.01	-0.46	0.13
U (%)	0.04	0.90	-0.01	0.97	-0.48	0.11	-0.77	<0.01	-0.17	0.60	0.20	0.53	0.13	0.69
%BDM=%RUDM	-0.19	0.55	-0.40	0.20	-0.23	0.47	-0.35	0.27	0.05	0.89	-0.36	0.25	-0.28	0.37
RUDM (g/kg DM)	-0.19	0.55	-0.40	0.20	-0.23	0.47	-0.35	0.27	0.05	0.89	-0.36	0.25	-0.28	0.37
%EDDM	0.19	0.55	0.40	0.20	0.23	0.47	0.35	0.27	-0.05	0.89	0.36	0.25	0.28	0.37
EDDM (g/kg DM)	0.19	0.55	0.40	0.20	0.23	0.47	0.35	0.27	-0.05	0.89	0.36	0.25	0.28	0.37
CP														
Kd (%/h)	0.59	0.04	0.33	0.29	0.15	0.63	-0.14	0.66	-0.33	0.29	0.38	0.22	0.08	0.81
S (%)	0.36	0.25	0.80	<0.01	0.84	<0.01	0.66	0.02	-0.62	0.03	0.63	0.03	0.44	0.15
D (%)	-0.41	0.18	-0.83	<0.01	-0.84	<0.01	-0.62	0.03	0.68	0.02	-0.64	0.03	-0.45	0.14
U (%)	0.06	0.86	0.18	0.57	0.06	0.86	-0.03	0.91	-0.21	0.52	0.22	0.48	-0.36	0.25
%BCP=%RUP	-0.51	0.09	-0.76	<0.01	-0.75	0.01	-0.49	0.11	0.63	0.03	-0.63	0.03	-0.65	0.02
BCP (g/kg DM, DVE)	-0.39	0.21	-0.82	<0.01	-0.86	<0.01	-0.69	0.01	0.60	0.04	-0.63	0.03	-0.45	0.14
RUP (g/kg DM, NRC)	-0.39	0.21	-0.82	<0.01	-0.86	<0.01	-0.69	0.01	0.60	0.04	-0.63	0.03	-0.45	0.14
%EDCP=%RDP	0.51	0.09	0.76	<0.01	0.75	0.01	0.49	0.11	-0.63	0.03	0.63	0.03	0.65	0.02
EDCP=RDP (g/kg DM)	0.39	0.22	0.20	0.53	0.26	0.42	0.03	0.91	-0.41	0.19	0.18	0.58	0.72	0.01
NDF														
Kd (%/h)	-0.03	0.93	0.03	0.93	-0.34	0.28	-0.57	0.05	-0.12	0.70	0.23	0.48	0.45	0.15
S (%)	0.43	0.16	0.42	0.17	0.36	0.25	0.09	0.78	-0.07	0.83	0.28	0.37	-0.16	0.63
D (%)	-0.16	0.62	-0.20	0.53	0.24	0.46	0.46	0.13	0.11	0.73	-0.33	0.29	-0.11	0.73
U (%)	-0.10	0.75	-0.04	0.90	-0.52	0.08	-0.67	0.02	-0.15	0.65	0.23	0.48	0.38	0.23
%BNDF=%RUNDF	-0.44	0.15	-0.38	0.22	-0.48	0.11	-0.24	0.44	-0.01	0.98	-0.17	0.61	0.12	0.71
RUNDF (g/kg DM, NRC)	-0.04	0.91	0.37	0.23	0.37	0.24	0.31	0.32	-0.61	0.04	0.43	0.17	0.16	0.63
%EDNDF=%RDNDF	0.44	0.15	0.38	0.22	0.48	0.11	0.24	0.44	0.01	0.98	0.17	0.61	-0.12	0.71
EDNDF (g/kg DM)	0.43	0.16	0.62	0.03	0.77	<0.01	0.52	0.08	-0.28	0.37	0.41	0.19	-0.05	0.88
St														
Kd (%/h)	0.17	0.59	0.21	0.52	-0.13	0.70	-0.30	0.34	-0.37	0.24	0.29	0.36	0.64	0.03
S (%)	-0.29	0.35	-0.14	0.66	0.07	0.83	0.35	0.27	0.36	0.24	-0.11	0.74	-0.20	0.54

Table 7.5. ***Cont'd***. Correlation between carbohydrate structure peaks and rumen degradation kinetics of primary nutrients of whole plant faba bean silage.

Item	Peaks													
	NSTC		STC1		STC2		STC3		STC4		CEC		LIG	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
D (%)	0.32	0.31	0.15	0.65	-0.09	0.78	-0.38	0.23	-0.36	0.25	0.14	0.67	0.23	0.46
U (%)	0.49	0.10	0.47	0.12	0.54	0.07	0.08	0.81	-0.43	0.16	0.40	0.20	0.21	0.50
%BSt	0.39	0.22	0.08	0.81	0.28	0.38	0.08	0.81	-0.11	0.74	-0.11	0.74	-0.24	0.45
BSt (g/kg DM)	-0.52	0.09	-0.84	<0.01	-0.61	0.04	-0.14	0.66	0.84	<0.01	-0.79	<0.01	-0.73	0.01
%EDSt	-0.39	0.22	-0.08	0.81	-0.28	0.38	-0.08	0.81	0.11	0.74	0.11	0.74	0.24	0.45
EDST (g/kg DM)	-0.59	0.05	-0.82	<0.01	-0.70	0.01	-0.17	0.59	0.95	<0.0001	-0.70	0.01	-0.63	0.03

Non-structural carbohydrates (NSTC) area located from ca 879 to ca 906 cm^{-1} including a main peak located at 896 cm^{-1}, structural carbohydrates (STC) area located from ca 1184 to ca 1485 cm^{-1} including four peaks (STC1, STC2, STC3 and STC4) located at ca 1237 cm^{-1}, ca 1316 cm^{-1}, ca 1395 cm^{-1} and ca 1461 cm^{-1} cellulosic compounds (CEC) area located from ca 1184 to ca 1290 cm^{-1} including a main peak located at 1237 cm^{-1} and ligneous compounds (LIG) peak located at 1515 cm^{-1}. +, positive correlation; -, negative correlation; r: correlation coefficient using spearman method; values of 'r' between 0 and ± 0.33: weak correlation, between ± 0.33 and ± 0.66: medium correlation, and between ± 0.66 and ± 1: strong correlation; P< 0.10, tendency; P< 0.05, significant; P< 0.01, strongly significant.

0.51), BSt g/kg DM (r= -0.52), and EDST g/kg DM (r= -0.59). The four spectral peaks related to STC also showed correlations with rumen degradation characteristics. Therefore, STC1 had a strong correlation (P< 0.01) with the S and D fractions of DM (r= 0.87, -0.87, respectively) and CP (r= 0.80, -0.83, respectively), and also with %RUP (r= -0.76), BCP and RUP g/kg DM (r= -0.82 for both characteristics), %EDCP (r= 0.76), BSt g/kg DM (r= -0.84), and EDST g/kg DM (r= -0.82). Moreover, STC1 had a significant correlation (P< 0.05) with EDNDF g/kg DM (r= 0.62). The spectral peak intensity of STC2 showed significant correlations (P< 0.05) with the Kd value of DM (r= -0.66), the S fraction of DM (r= 0.83) and CP (r= 0.84), the D fraction of CP (r= -0.84), %RUP (r= -0.75), BCP and RUP g/kg DM (r= -0.86 for both characteristics), %EDCP (r= 0.75), EDNDF g/kg DM (r= 0.77), BSt g/kg DM (r= -0.61), and EDST g/kg DM (r= -0.70). On the other hand, STC3 had a significant correlation (P< 0.05) with the Kd value (r= -0.62) and the S, and U fractions of DM (r= 0.66, -0.77, respectively), the S and D fractions of CP (r= 0.66, -0.62, respectively), BCP and RUP g/kg DM (r= -0.69 for both characteristics), and the U fraction of NDF (r= -0.67). Last, the spectral peak of STC4 had a strong correlation (P< 0.01) with EDST g/kg DM (r= 0.95) and was significantly correlated (P< 0.05) with S and D fractions of DM (r= -0.61, 0.77, respectively), S and D fractions of CP (r= -0.62, 0.68, respectively), %RUP (r= 0.63), BCP and RUP g/kg DM (r= 0.60 for both characteristics), %EDCP (r= -0.63), RUNDF g/kg DM (r= -0.61), and BSt g/kg DM (r= 0.84). The spectral peak intensity of CEC showed a strong correlation (P< 0.01) with the D fraction of DM (r= -0.82) and BSt g/kg DM (r= -0.79) while it had a significant correlation (P< 0.05) with the S fraction of DM (r= 0.67) and CP (r= 0.63), the D fraction of CP (r= -0.64), %RUP (r= -0.63), BCP and RUP g/kg DM (r= - 0.63 for both characteristics), %EDCP (r= 0.63), and EDST g/kg DM (r= -0.70). The spectral peak intensity of ligneous compound was significantly correlated (P< 0.05) with %RUP (r= -0.65), %EDCP (r= 0.65), EDCP g/kg DM (r= 0.72), Kd of starch (r= 0.64), BSt g/kg DM (r= -0.73), and EDST g/kg DM (r= -0.63).

The present data did not fully agree with Xin et al., (2013) and Yan et al., (2014) where they did not find any significant correlations between STC area and rumen degradation characteristics but found a strong correlation between amide area and EDCP, BNDF, and EDNDF, respectively. Moreover, Zhang and Yu (2012) did not find association between nutritional characteristics and protein related molecular structures suggesting that their results might be related to possible intrinsic interactions produced by the combination of different types of feed.

Table 7.6 shows the correlation between protein structure spectral peak intensity and rumen degradation kinetics of primary nutrients of whole plant faba bean silage. Amide I peak showed a strong correlation with the S fraction of DM (r= 0.82) and CP (r= 0.80), D fraction of CP (r= -0.85), %RUP (r= -0.83), BCP and RUP g/kg DM (r= - 0.83 for both), %EDCP (r= -0.78), BSt g/kg DM (r= -0.73), and EDST g/kg DM (r= -0.84). On the other hand, a significant correlation was observed between amide I peak with the D fraction of DM (r= -0.69) and EDNDF g/kg DM (r= 0.63). However, the peak of amide II had a very strong correlation ($P < 0.001$) with EDST g/kg DM (r= 0.91), showed a strong correlation ($P < 0.01$) with BSt g/kg DM (r= 0.81) and a significant correlation ($P < 0.05$) with the D fraction of DM (r= 0.63) and CP (r= 0.62), and %RUP (r= 0.60).A tendency for correlation ($P < 0.10$) was observed between amide II peak and the S fraction of DM (r= -0.51) and CP (r= -0.57) as well as with BCP and RUP g/kg DM (r= 0.56 for both). The peak of α-helix was strongly correlated ($P < 0.01$) with the S and D fractions of DM (r= -0.83, 0.77, respectively) and the D fraction of CP (r= 0.79) while it showed a significant correlation ($P < 0.05$) with the S fraction of CP (r= -0.74), BCP and RUP g/kg DM (r= 0.71 for both), RUNDF g/kg DM (r= -0.60), EDNDF g/kg DM (r= -0.69), BSt g/kg DM (r= 0.59), and EDST g/kg DM (r= 0.70). A tendency ($P < 0.10$) was observed with the Kd value of CP (r= -0.50), %RUP (r= 0.55), and %EDCP (r= -0.55). On the other hand, the peak of β-sheet was strongly

Table 7.6. Correlation between protein structure spectral peaks and rumen degradation kinetics of primary nutrients of whole plant faba bean silage.

Item	Peaks								Ratios			
	Amide I		Amide II		α-Helix		β-Sheet		Amide I: Amide II		α-Helix: β-Sheet	
	r	P	r	P	r	P	r	P	r	P	r	P
DM												
Kd (%/h)	-0.38	0.23	0.32	0.31	0.52	0.08	0.52	0.08	-0.40	0.20	0.27	0.40
S (%)	0.82	<0.01	-0.51	0.09	-0.83	<0.01	-0.74	0.01	0.57	0.05	-0.86	<0.01
D (%)	-0.69	0.01	0.63	0.03	0.77	<0.01	0.75	<0.01	-0.59	0.04	0.80	<0.01
U (%)	-0.27	0.39	-0.06	0.86	0.15	0.64	0.11	0.73	-0.06	0.85	0.12	0.71
%BDM=%RUDM	-0.28	0.38	-0.08	0.80	0.17	0.59	0.12	0.72	0.08	0.80	0.39	0.22
RUDM (g/kg DM)	-0.28	0.38	-0.08	0.80	0.17	0.59	0.12	0.72	0.08	0.80	0.39	0.22
%EDDM	0.28	0.38	0.08	0.80	-0.17	0.59	-0.12	0.72	-0.08	0.80	-0.39	0.22
EDDM (g/kg DM)	0.28	0.38	0.08	0.80	-0.17	0.59	-0.12	0.72	-0.08	0.80	-0.39	0.22
CP												
Kd (%/h)	0.21	0.51	-0.11	0.73	-0.50	0.10	-0.55	0.06	-0.01	0.98	-0.57	0.05
S (%)	0.80	<0.01	-0.57	0.05	-0.74	0.01	-0.65	0.02	0.61	0.04	-0.74	0.01
D (%)	-0.85	<0.01	0.62	0.03	0.79	<0.01	0.70	0.01	-0.67	0.02	0.80	<0.01
U (%)	-0.01	0.98	-0.09	0.77	-0.49	0.11	-0.56	0.06	0.05	0.88	-0.36	0.25
%BCP=%RUP	-0.83	<0.01	0.60	0.04	0.55	0.06	0.49	0.11	-0.63	0.03	0.63	0.03
BCP (g/kg DM, DVE)	-0.83	<0.01	0.56	0.06	0.71	0.01	0.62	0.03	-0.62	0.03	0.73	0.01
RUP (g/kg DM, NRC)	-0.83	<0.01	0.56	0.06	0.71	0.01	0.62	0.03	-0.62	0.03	0.73	0.01
%EDCP=%RDP	0.83	<0.01	-0.60	0.04	-0.55	0.06	-0.49	0.11	0.63	0.03	-0.63	0.03
EDCP=RDP (g/kg DM)	0.41	0.19	-0.38	0.22	0.07	0.82	0.11	0.74	0.38	0.23	-0.09	0.78
NDF												
Kd (%/h)	-0.19	0.56	-0.05	0.89	0.49	0.11	0.50	0.10	-0.03	0.93	0.38	0.22
S (%)	0.35	0.27	-0.15	0.64	-0.42	0.17	-0.45	0.14	0.17	0.60	-0.29	0.37
D (%)	0.09	0.78	-0.01	0.97	-0.06	0.85	-0.05	0.87	0.07	0.83	0.01	0.98
U (%)	-0.31	0.33	-0.01	0.97	0.47	0.12	0.44	0.15	-0.09	0.78	0.33	0.30
%BNDF=%RUNDF	-0.41	0.19	0.09	0.77	0.37	0.24	0.33	0.30	-0.17	0.60	0.25	0.44
RUNDF (g/kg DM, NRC)	0.28	0.38	-0.43	0.16	-0.60	0.04	-0.58	0.05	0.40	0.20	-0.56	0.06
%EDNDF=%RDNDF	0.41	0.19	-0.09	0.77	-0.37	0.24	-0.33	0.30	0.17	0.60	-0.25	0.44
EDNDF (g/kg DM)	0.63	0.03	-0.37	0.23	-0.69	0.01	-0.67	0.02	0.44	0.15	-0.51	0.09
St												
Kd (%/h)	0.10	0.76	-0.22	0.50	0.14	0.67	0.17	0.60	0.14	0.66	-0.07	0.82

Table 7.6. ***Cont'd***. Correlation between protein structure spectral peaks and rumen degradation kinetics of primary nutrients of whole plant faba bean silage.

Item	Peaks								Ratios			
	Amide I		Amide II		α-Helix		β-Sheet		Amide I: Amide II		α-Helix: β-Sheet	
	r	P	r	P	r	P	r	P	r	P	r	P
S (%)	-0.06	0.86	0.16	0.62	0.22	0.48	0.15	0.65	-0.12	0.71	0.30	0.35
D (%)	0.05	0.88	-0.15	0.65	-0.18	0.57	-0.12	0.72	0.10	0.76	-0.28	0.38
U (%)	0.46	0.13	-0.43	0.17	-0.50	0.10	-0.47	0.13	0.41	0.19	-0.39	0.21
%BSt	0.21	0.51	-0.08	0.81	-0.39	0.21	-0.33	0.29	0.15	0.63	-0.29	0.36
BSt (g/kg DM)	-0.78	<0.01	0.81	<0.01	0.59	0.04	0.58	0.05	-0.76	<0.01	0.61	0.04
%EDSt	-0.21	0.51	0.08	0.81	0.39	0.21	0.33	0.29	-0.15	0.63	0.29	0.36
EDST (g/kg DM)	-0.84	<0.01	0.91	<0.01	0.70	0.01	0.69	0.01	-0.90	<0.01	0.65	0.02

Amide I and II peaks located at ca 1589 and ca 1535 cm^{-1} respectively, α-helix and β-sheet peaks located at ca 1648 and ca 1635 cm^{-1} respectively. +, positive correlation; -, negative correlation; r: correlation coefficient using spearman method; values of 'r' between 0 and ± 0.33: weak correlation, between ± 0.33 and ± 0.66: medium correlation, and between ± 0.66 and ± 1: strong correlation; P< 0.10, tendency; P< 0.05, significant; P< 0.01, strongly significant.

correlated (P< 0.01) with the D fraction of DM (r= 0.75) and significantly correlated (P< 0.05) with the S fraction of DM (r= -0.74), S and D fractions of CP (r= -0.65, 0.70, respectively), BCP and RUP g/kg DM (r= 0.62 for both), EDNDF g/kg DM (r= -0.67), and EDST g/kg DM (r= 0.69). In regard to the protein molecular structure spectral intensity ratios, amide I to amide II had a strong correlation (P< 0.001) with EDST g/kg DM (r= -0.90) and showed a significant correlation (P< 0.05) with the D fraction of DM (r= -0.59), S and D fractions of CP (r= 0.61, -0.67, respectively), %RUP (r= -0.63), BCP and RUP g/kg DM (r= -0.62 for both), %EDCP (r= 0.63), and BSt g/kg DM (r= -0.76). The spectral peak intensity ratio of α-helix:β-sheet was strongly correlated (P< 0.01) with the S and D fractions of DM (r= -0.86, 0.80, respectively), S and D fractions of CP (r= -0.74, 0.80, respectively), %RUP (r= 0.63), BCP and RUP g/kg DM (r= 0.73 for both), %EDCP (r= -0.63), BSt g/kg DM (r= 0.61), and EDST g/kg DM (r= 0.65). A tendency (P<0.10) between this last ratio was observed with the Kd value of CP (r= -0.57), RUNDF g/kg DM (r= -0.56), and EDNDF g/kg DM (r= -0.51).

Data published by Yang et al., (2014) partially agrees with the current results, as several associations were different from the current results, but they found positive correlation between β-sheet to α-helix ratio peaks with RUP suggesting that higher ratio of protein secondary structures lead to a lower degradability of feed protein in the rumen. However, Yan et al., (2014) found consistent negative and positive associations between primary and secondary structure features and RUP and EDCP, respectively. Additionally, the results from this research agree with data published by Deng et al., (2020) in which they found significantly positive and negative correlations between rumen degradation characteristics of faba bean residues and protein related molecular structure characteristics. Theodoridou and Yu (2013) did not find any correlation between amide I, amide II or their ratio with the RUP in canola meal, but Yu and Nuez-Ortin (2010) found a negative correlation between the ratio of amide I to amide II with the undegradable fraction of crude protein in distiller grains. However, Yu and Nuez-Ortin (2010) found a positive correlation between the ratio of α-helix to β-sheet and RUP, and probably might be explained by the negative correlation observed between the ratio of α-helix to β-sheet with the D fraction of crude protein. However, in our study the ratio of α-helix to β-sheet had a positive correlation with the D fraction of crude protein.

The correlation between protein and carbohydrates molecular structure areas and rumen degradation kinetics of primary nutrients of whole plant faba bean silage is presented in Table 7.7. The spectral area intensity of NSTC showed a significant correlation ($P< 0.05$) with the Kd value of CP ($r= 0.67$). On the other hand, CEC spectral area had a strongly significant correlation ($P< 0.01$) with the D fraction of DM ($r= -0.86$), BSt g/kg DM ($r= -0.81$), and EDST g/kg DM ($r= -0.78$) while it showed a significant correlation ($P< 0.05$) with the S fraction of DM ($r= 0.64$) and D fraction of CP ($r= -0.61$). The spectral area of CEC also showed a tendency for correlation ($P< 0.10$) with %RUP ($r= -0.57$), BCP and RUP g/kg DM ($r= -0.57$ for both), and %EDCP ($r= 0.57$). The STC area had a strong correlation ($P< 0.01$) with the S fraction of DM ($r= 0.88$), S and D fractions of CP ($r= 0.87, -0.88$, respectively), %RUP ($r= -0.82$), %EDCP ($r= 0.82$), and EDST g/kg DM ($r= -0.80$); moreover, STC spectral area intensity showed a significant correlation ($P< 0.05$) with the D fraction of DM ($r= -0.65$), EDNDF g/kg DM ($r= 0.73$), and BSt g/kg DM ($r= -0.71$). In terms of protein related molecular structures, amide spectral area had a strongly significant correlation ($P< 0.01$) with EDST g/kg DM ($r= 0.84$) and significant correlations ($P< 0.05$) with the D fraction of DM ($r= 0.69$), RUNDF g/kg DM ($r= -0.62$), and BSt g/kg DM ($r= 0.64$). Amide I area had a significant correlation ($P< 0.05$) with the U fraction of CP ($r= -0.75$) and presented a tendency ($P< 0.10$) with the D fraction of DM ($r= 0.57$), the Kd value of CP ($r= -0.50$), RUNDF g/kg DM($r= -0.52$), and EDST g/kg DM ($r= 0.50$) while on the other hand, amide II area showed a strongly

Table 7.7. Correlation between protein and carbohydrates structure spectral areas and rumen degradation kinetics of primary nutrients of whole plant faba bean silage

Item	Areas												Ratio	
	NSTC		CEC		STC		Amide		Amide I		Amide II		Area Amide I: Amide II	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
DM														
Kd (%/h)	0.13	0.68	-0.08	0.80	-0.53	0.08	0.50	0.10	0.25	0.43	0.41	0.18	-0.34	0.29
S (%)	0.16	0.61	0.64	0.02	0.88	<0.01	-0.47	0.12	-0.21	0.50	-0.54	0.07	0.47	0.12
D (%)	-0.35	0.27	-0.86	<0.01	-0.65	0.02	0.69	0.01	0.57	0.05	0.56	0.06	-0.45	0.14
U (%)	0.33	0.30	0.29	0.35	-0.32	0.31	-0.17	0.60	-0.45	0.14	0.03	0.91	-0.06	0.86
%BDM=%RUDM	-0.09	0.78	-0.27	0.39	-0.27	0.39	-0.33	0.30	-0.37	0.24	-0.16	0.62	0.13	0.68
RUDM (g/kg DM)	-0.09	0.78	-0.27	0.39	-0.27	0.39	-0.33	0.30	-0.37	0.24	-0.16	0.62	0.13	0.68
%EDDM	0.09	0.78	0.27	0.39	0.27	0.39	0.33	0.30	0.37	0.24	0.16	0.62	-0.13	0.68
EDDM (g/kg DM)	0.09	0.78	0.27	0.39	0.27	0.39	0.33	0.30	0.37	0.24	0.16	0.62	-0.13	0.68
CP														
Kd (%/h)	0.67	0.02	0.43	0.16	0.17	0.59	-0.28	0.38	-0.50	0.10	0.03	0.91	-0.13	0.68
S (%)	0.13	0.68	0.58	0.05	0.87	<0.01	-0.45	0.14	-0.15	0.65	-0.60	0.04	0.55	0.06
D (%)	-0.13	0.68	-0.61	0.04	-0.88	<0.01	0.55	0.06	0.24	0.46	0.67	0.02	-0.61	0.04
U (%)	0.13	0.70	0.32	0.31	0.05	0.88	-0.55	0.06	-0.75	0.01	-0.06	0.86	-0.06	0.85
%BCP=%RUP	-0.31	0.33	-0.57	0.05	-0.82	<0.01	0.32	0.31	0.00	1.00	0.59	0.04	-0.57	0.05
BCP (g/kg DM, DVE)	-0.15	0.65	-0.57	0.05	-0.90	<0.01	0.41	0.19	0.08	0.81	0.59	0.04	-0.56	0.06
RUP (g/kg DM, NRC)	-0.15	0.65	-0.57	0.05	-0.90	<0.01	0.41	0.19	0.08	0.81	0.59	0.04	-0.56	0.06
%EDCP=%RDP	0.31	0.33	0.57	0.05	0.82	<0.01	-0.32	0.31	0.00	1.00	-0.59	0.04	0.57	0.05
EDCP=RDP (g/kg DM)	0.32	0.31	0.17	0.60	0.34	0.28	0.09	0.78	0.39	0.21	-0.36	0.26	0.45	0.14
NDF														
Kd (%/h)	0.33	0.30	0.22	0.48	-0.14	0.66	0.31	0.33	0.27	0.40	0.04	0.90	0.09	0.78
S (%)	0.35	0.27	0.25	0.43	0.34	0.28	-0.32	0.31	-0.40	0.20	-0.13	0.68	0.03	0.93
D (%)	-0.41	0.19	-0.36	0.26	0.06	0.86	-0.02	0.95	0.20	0.53	-0.10	0.76	0.08	0.80
U (%)	0.26	0.42	0.29	0.37	-0.31	0.33	0.22	0.48	0.10	0.75	0.12	0.71	-0.01	0.97
%BNDF=%RUNDF	-0.31	0.33	-0.08	0.81	-0.45	0.14	0.15	0.63	0.15	0.65	0.15	0.63	-0.09	0.78
RUNDF (g/kg DM, NRC)	0.00	1.00	0.48	0.11	0.41	0.19	-0.62	0.03	-0.52	0.08	-0.45	0.14	0.36	0.25
%EDNDF=%RDNDF	0.31	0.33	0.08	0.81	0.45	0.14	-0.15	0.63	-0.15	0.65	-0.15	0.63	0.09	0.78
EDNDF (g/kg DM)	0.29	0.35	0.33	0.30	0.73	0.01	-0.50	0.10	-0.40	0.20	-0.43	0.17	0.30	0.34
St														
Kd (%/h)	0.17	0.59	0.29	0.35	0.01	0.97	0.08	0.81	0.20	0.54	-0.12	0.71	0.13	0.70

Table 7.7. *Cont'd*. Correlation between protein and carbohydrates structure spectral areas and rumen degradation kinetics of primary nutrients of whole plant faba bean silage.

Item	Areas												Ratio	
	NSTC		CEC		STC		Amide		Amide I		Amide II		Area Amide I: Amide II	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
St														
Kd (%/h)	0.17	0.59	0.29	0.35	0.01	0.97	0.08	0.81	0.20	0.54	-0.12	0.71	0.13	0.70
S (%)	-0.20	0.53	-0.17	0.59	-0.03	0.93	0.30	0.34	0.33	0.30	0.17	0.59	-0.10	0.76
D (%)	0.27	0.40	0.20	0.54	0.02	0.95	-0.24	0.44	-0.29	0.37	-0.14	0.66	0.07	0.83
U (%)	0.57	0.05	0.35	0.27	0.56	0.06	-0.48	0.12	-0.46	0.13	-0.46	0.13	0.36	0.25
%BSt	0.22	0.48	-0.08	0.81	0.27	0.40	-0.33	0.30	-0.34	0.28	-0.20	0.54	0.14	0.66
BSt (g/kg DM)	-0.41	0.19	-0.81	<0.01	-0.71	0.01	0.64	0.02	0.38	0.22	0.74	0.01	-0.67	0.02
%EDSt	-0.22	0.48	0.08	0.81	-0.27	0.40	0.33	0.30	0.34	0.28	0.20	0.54	-0.14	0.66
EDST (g/kg DM)	-0.40	0.20	-0.78	<0.01	-0.80	<0.01	0.84	<0.01	0.50	0.10	0.89	<0.01	-0.82	<0.01

Non-structural carbohydrates (NSTC) area located from ca 879 to ca 906 cm^{-1}, structural carbohydrates (STC) area located from ca 1184 to ca 1485 cm^{-1}, cellulosic compounds (CEC) area located from ca 1184 to ca 1290 cm^{-1}, amide area located from ca 1484 to ca 1712 cm^{-1}, amide I area located from ca 1547 to ca 1712 cm^{-1}, amide II area located from ca 1484 to ca 1547 cm^{-1}. +, positive correlation; -, negative correlation; r: correlation coefficient using spearman method; values of 'r' between 0 and ± 0.33: weak correlation, between ± 0.33 and ± 0.66: medium correlation, and between ± 0.66 and ± 1: strong correlation; P< 0.10, tendency; P< 0.05, significant; P< 0.01, strongly significant.

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significant correlation (P< 0.01) with EDST g/kg DM (r= 0.89), and had a significant correlation (P< 0.05) with the S and D fractions of CP (r= -0.60, 0.67, respectively), %RUP (r= 0.59), BCP and RUP g/kg DM (r= 0.50 for both), %EDCP (r= -0.59), and BSt g/kg DM (r= 0.74). Finally, the spectral area intensity ratio of amide I to amide II was strongly correlated (P< 0.01) with EDST g/kg DM (r= -0.82), and significantly correlated (P< 0.05) with the D fraction of CP (r= -0.61) and BSt g/kg DM (r= -0.67). Also, it showed a tendency for correlation (P< 0.10) with the S fraction of CP (r= 0.55), %RUP (r= -0.57), BCP and RUP g/kg DM (r= -0.56 for both), and %EDCP (r= -0.57).

The present data did not fully agree with Xin et al., (2013) and Yan et al., (2014) where they did not find significant correlations between STC area and rumen degradation characteristics but found a strong correlation between amide area and EDCP, BNDF, and EDNDF, respectively. Variations between previous studies and the current research suggest that changes during the fermentation process while ensiling forages or processing feeds could affect the inherent molecular characteristics of a plant (Chen et al., 2014). Prates et al., (2018) found a positive correlation between the structural carbohydrates area and the rumen degradable starch, however in our study the structural carbohydrates area had negative correlation with the effective degraded starch. This is mainly explained by the negative correlation of cellulosic compound area and the total starch content. Furthermore, the correlation values obtained in the current research suggests that molecular spectral analyses could be used to relate molecular structure characteristics with different metabolic and different degradation characteristics in whole plant faba bean silages

7.4.5 Molecular Structure Spectral Profiles in Relation with In Vitro Digestion and Metabolic Characteristics

Results of correlation analysis between carbohydrate structure spectral peaks intensity and *in vitro* digestibility characteristics, metabolic characteristics, and predicted production performance of whole plant faba bean silages are shown in Table 7.8. The spectral intensity of NSTC peak had a significant correlation ($P < 0.05$) with %TDST (r= -0.67). The spectral peak intensity of STC1 had a strong correlation ($P < 0.01$) with IADP g/kg DM (r= -0.90), DVBE g/kg DM (r= -0.90), ARUP g/kg DM (r= -0.90), MP g/kg DM (r= -0.92), and FMV^{NRC} kg milk/kg DM silage (r= -0.92). It also showed a strong correlation ($P < 0.01$) with IDBDM g/kg DM (r= -0.75), IADP g/kg CP (r= -0.80), IDBST g/kg DM (r= -0.84), TDST g/kg DM (r= -0.84), BCP g/kg DM (r= -0.82), DVE g/kg DM (r= -0.83), FMV^{DVE} (kg milk/kg DM Silage (r= -0.83), and RUP g/kg DM (r= -0.82). Moreover, the spectral peak intensity of STC1 had a significant correlation ($P < 0.05$) with TDP k/kg CP (r= 0.63), %dBST (r= -0.75), and %TDST (r= -0.72). The spectral intensity of STC2 peak was strongly correlated ($P < 0.01$) with IADP g/kg DM (r= -0.84), TDP g/kg CP (r= 0.83), BCP g/kg DM (r= -0.86), DVBE g/kg DM (r= -0.84), RUP g/kg DM (r= -0.86), ARUP g/kg DM (r= -0.84), MP g/kg DM (r= -0.77), and FMV^{NRC} kg milk/kg DM silage (r= -0.77). Significant correlations ($P < 0.05$) were also observed between STC2 peak intensity and IADP g/kg CP (r= -0.72), TDNDF g/kg DM (r= 0.66), IDBST g/kg DM (r= -0.61), and TDST g/kg DM (r= -0.71). On the other hand, STC3 peak intensity had a significant correlation ($P < 0.05$) with TDP g/kg CP (r= 0.68), TDNDF g/kg DM (r= 0.59), BCP g/kg DM (r= -0.69), and RUP g/kg DM (r= -0.69). Last, the peak intensity of STC4 showed a strong correlation ($P < 0.01$) with TDST g/kg DM (r= 0.94), DVE g/kg DM (r= 0.89), and FMV^{DVE} kg milk/kg DM silage (r= 0.89). Furthermore, this last peak intensity had a strong correlation ($P < 0.01$) with %dBDM (r= 0.78), IADP g/kg CP (r= 0.76), %dBST (r= 0.86), and IDBST g/kg DM (r= 0.84). Significant correlations ($P < 0.05$) were also observed with IDBDM, TDDM, IADP, BCP, DVBE, RUP, ARUP, MP, and FMV^{NRC}.

Table 7.8. Correlation between carbohydrate structure spectral peaks and *in vitro* digestibility characteristics , metabolic characteristics, and predicted production performance of whole plant faba bean silage

Item	Peaks													
	NSTC		STC1		STC2		STC3		STC4		CEC		LIG	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
%dBDM	-0.53	0.08	-0.58	0.05	-0.22	0.50	0.27	0.40	0.78	<0.01	-0.70	0.01	-0.58	0.05
%IDBDM	-0.48	0.11	-0.75	<0.01	-0.34	0.28	0.03	0.93	0.67	0.02	-0.83	<0.01	-0.56	0.06
IDBDM (g/kg DM)	-0.48	0.11	-0.75	<0.01	-0.34	0.28	0.03	0.93	0.67	0.02	-0.83	<0.01	-0.56	0.06
%TDDM	-0.29	0.36	-0.50	0.10	-0.16	0.62	0.22	0.48	0.73	0.01	-0.67	0.02	-0.37	0.24
TDDM (g/kg DM)	-0.29	0.36	-0.50	0.10	-0.16	0.62	0.22	0.48	0.73	0.01	-0.67	0.02	-0.37	0.24
%dIDP	0.34	0.29	-0.12	0.72	0.23	0.47	0.33	0.30	0.26	0.42	-0.33	0.30	-0.25	0.44
IADP (g/kg DM)	-0.37	0.23	-0.90	<0.01	-0.84	<0.01	-0.57	0.05	0.71	0.01	-0.76	<0.01	-0.39	0.21
IADP (g/kg CP)	-0.56	0.06	-0.80	<0.01	-0.72	0.01	-0.34	0.29	0.76	<0.01	-0.68	0.02	-0.72	0.01
TDP (g/kg DM)	0.22	0.50	-0.22	0.49	-0.17	0.60	-0.33	0.30	-0.05	0.87	-0.19	0.55	0.53	0.08
TDP (g/kg CP)	0.44	0.15	0.63	0.03	0.83	<0.01	0.68	0.02	-0.45	0.14	0.41	0.18	0.45	0.14
%IADP (%CP)	-0.56	0.06	-0.80	<0.01	-0.72	0.01	-0.34	0.29	0.76	<0.01	-0.68	0.02	-0.72	0.01
%TDP (%CP)	0.44	0.15	0.63	0.03	0.83	<0.01	0.68	0.02	-0.45	0.14	0.41	0.18	0.45	0.14
%dBNDF	-0.28	0.38	-0.44	0.15	-0.10	0.75	0.28	0.38	0.49	0.11	-0.60	0.04	-0.28	0.38
%IDBNDF	-0.36	0.25	-0.42	0.18	-0.21	0.51	0.14	0.66	0.32	0.31	-0.44	0.16	-0.04	0.90
IDBNDF (g/kg DM)	-0.17	0.59	-0.22	0.48	0.08	0.80	0.36	0.25	0.14	0.66	-0.34	0.28	-0.12	0.71
%TDNDF	-0.05	0.87	-0.29	0.35	0.19	0.56	0.39	0.21	0.56	0.06	-0.55	0.06	-0.47	0.12
TDNDF (g/kg DM)	0.18	0.57	0.33	0.29	0.66	0.02	0.59	0.04	-0.10	0.75	0.12	0.70	-0.21	0.52
%dBST	-0.54	0.07	-0.75	0.01	-0.48	0.11	0.07	0.83	0.86	<0.01	-0.71	0.01	-0.78	<0.01
% IDBST	0.33	0.29	0.02	0.94	0.24	0.44	0.09	0.78	-0.05	0.87	-0.16	0.62	-0.26	0.41
IDBST (g/kg DM)	-0.52	0.09	-0.84	<0.01	-0.61	0.04	-0.14	0.66	0.84	<0.01	-0.79	<0.01	-0.73	0.01
%TDST	-0.67	0.02	-0.72	0.01	-0.55	0.06	0.05	0.88	0.90	<0.01	-0.62	0.03	-0.65	0.02
TDST (g/kg DM)	-0.57	0.05	-0.84	<0.01	-0.71	0.01	-0.18	0.57	0.94	<0.01	-0.73	0.01	-0.63	0.03
BCP (g/kg DM)	-0.39	0.21	-0.82	<0.01	-0.86	<0.01	-0.69	0.01	0.60	0.04	-0.63	0.03	-0.45	0.14
MREE (g/kg DM)	-0.27	0.41	-0.04	0.91	0.25	0.43	0.56	0.06	0.27	0.39	-0.16	0.62	-0.10	0.76
MREN (g/kg DM)	0.39	0.22	0.20	0.53	0.26	0.42	0.03	0.91	-0.41	0.19	0.18	0.58	0.72	0.01
DVME (g/kg DM)	-0.27	0.41	-0.04	0.91	0.25	0.43	0.56	0.06	0.27	0.39	-0.16	0.62	-0.10	0.76
DVBE (g/kg DM)	-0.37	0.23	-0.90	<0.01	-0.84	<0.01	-0.57	0.05	0.71	0.01	-0.76	<0.01	-0.39	0.21

Table 7.8. *Cont'd*. Correlation between carbohydrate structure spectral peaks and *in vitro* digestibility characteristics, metabolic characteristics, and predicted production performance of whole plant faba bean silage.

Item	Peaks													
	NSTC		STC1		STC2		STC3		STC4		CEC		LIG	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
DVE (g/kg DM)	-0.40	0.19	-0.83	<0.01	-0.48	0.11	-0.06	0.86	0.89	<0.0001	-0.90	<0.0001	-0.53	0.08
OEB (g/kg DM)	0.48	0.12	0.22	0.49	0.20	0.54	-0.10	0.76	-0.52	0.09	0.27	0.40	0.75	<0.01
FMV^{DVE} (kg milk/kg DM Silage)	-0.40	0.19	-0.83	<0.01	-0.48	0.11	-0.06	0.86	0.89	<0.0001	-0.90	<0.0001	-0.53	0.08
RUP (g/kg DM)	-0.39	0.21	-0.82	<0.01	-0.86	<0.01	-0.69	0.01	0.60	0.04	-0.63	0.03	-0.45	0.14
MCP_{TDN} (g/kg DM)	-0.17	0.60	-0.55	0.06	-0.35	0.27	-0.15	0.63	0.52	0.08	-0.62	0.03	0.04	0.90
MCP_{RDP} (g/kg DM)	0.39	0.22	0.20	0.53	0.26	0.42	0.03	0.91	-0.41	0.19	0.18	0.58	0.72	0.01
AMCP (g/kg DM)	-0.17	0.60	-0.55	0.06	-0.35	0.27	-0.15	0.63	0.52	0.08	-0.62	0.03	0.04	0.90
ARUP (g/kg DM)	-0.37	0.23	-0.90	<0.0001	-0.84	<0.01	-0.57	0.05	0.71	0.01	-0.76	<0.01	-0.39	0.21
MP (g/kg DM)	-0.37	0.23	-0.92	<0.0001	-0.77	<0.01	-0.43	0.17	0.71	0.01	-0.84	<0.01	-0.29	0.36
DPB (g/kg DM)	0.47	0.12	0.25	0.44	0.25	0.43	-0.06	0.85	-0.51	0.09	0.26	0.42	0.74	0.01
FMV^{NRC} (kg milk/kg DM Silage)	-0.37	0.23	-0.92	<0.0001	-0.77	<0.01	-0.43	0.17	0.71	0.01	-0.84	<0.01	-0.29	0.36

Non-structural carbohydrates (NSTC) area located from ca 879 to ca 906 cm^{-1} including a main peak located at 896 cm^{-1}, structural carbohydrates (STC) area located from ca 1184 to ca 1485 cm^{-1} including four peaks (STC1, STC2, STC3 and STC4) located at ca 1237 cm^{-1}, ca 1316 cm^{-1}, ca 1395 cm^{-1} and ca 1461 cm^{-1} cellulosic compounds (CEC) area located from ca 1184 to ca 1290 cm^{-1} including a main peak located at 1237 cm^{-1} and ligneous compounds (LIG) peak located at 1515 cm^{-1}. +, positive correlation; -, negative correlation; r: correlation coefficient using spearman method; values of 'r' between 0 and ± 0.33: weak correlation, between ± 0.33 and ± 0.66: medium correlation, and between ± 0.66 and ± 1: strong correlation; $P < 0.10$, tendency; $P < 0.05$, significant; $P < 0.01$, strongly significant.

The peak intensity of CEC had a strong correlation ($P < 0.01$) with DVE g/kg DM ($r = -0.90$) and FMV^{DVE} kg milk/kg DM silage ($r = -0.90$), and it showed a strong correlation ($P < 0.01$) with IDBDM g/kg DM ($r = -0.83$), IADP g/kg DM ($r = -0.76$), IDBST g/kg DM ($r = -0.79$), DVBE g/kg DM ($r = -0.76$), ARUP g/kg DM ($r = -0.76$), MP g/kg DM ($r = -0.84$), and FMV^{NRC} kg milk/kg DM silage ($r = -0.84$), moreover, significant correlations ($P < 0.05$) were also found with %dBST, TDDM, IADP, %dBNDF, %dBST, TDST, BCP, MCP^{TDN}, and AMCP. The ligneous compound peak intensity had a strong correlation ($P < 0.01$) with %dBST ($r = -0.78$) and OEB g/kg DM ($r = 0.75$) while it showed significant correlations ($P < 0.05$) with IADP g/kg CP ($r = -0.72$), IDBST g/kg DM ($r = -0.73$), TDST g/kg DM ($r = -0.63$), MREN g/kg DM ($r = 0.72$), MCP_{RDP} g/kg DM ($r = 0.72$), and DPB g/kg DM ($r = 0.74$).

Most carbohydrates peaks except for structural carbohydrate peak number four had negative correlation with some important characteristics of protein and starch digestion as well with metabolic nutrients from the DVE/OEB system and NRC model, which indicate that when structural carbohydrate peak number four height increases important characteristics such as intestinal digested crude protein, total digested starch, truly absorbed protein in the small intestine, metabolizable protein and feed milk value increases as well. Strong positive correlation between structural carbohydrate peak number one with the truly absorbed protein in the small intestine of carinata meal was found (Ban, 2016), however in our study that correlation was strong but negative, additionally in our study a negative correlation was found between cellulosic compound peak height and the truly absorbed protein in the small intestine, but Ban (2016) did not found that correlation significantly. Moreover, Rodriguez (2018) found no correlation between carbohydrate molecular structure and the truly absorbed protein in the small intestine and feed milk value based on the DVE/OEB system, which was not in agreement with our results.

Correlation between protein structure peaks and *in vitro* digestibility characteristics, metabolic characteristics, and predicted production performance of whole plant faba bean silages are presented in Table 7.9. Amide I peak intensity had a strong correlation (P< 0.01) with IADP g/kg DM and g/kg CP (r= -0.79, -0.85, respectively), TDP g/kg CP (r= 0.80), IDBST g/kg DM (r= -0.78), TDST g/kg DM (r= -0.85), BCP g/kg DM (r= -0.83), DVBE g/kg DM (r= -0.79), RUP g/kg DM (r= -0.83), and ARUP g/kg DM (r= -0.79). It also showed a significant correlation (P< 0.05) with %dBST (r= -0.73), MP g/kg DM (r= -0.70), and FMV^{NRC} kg milk/kg DM silage (r= -0.70). On the other hand, amide II peak intensity had a strong correlation (P< 0.01) with TDST g/kg DM (r= 0.92), strong correlations (P< 0.01) with %dBST (r= 0.88) and IDBST g/kg DM (r= 0.81) while it showed significant correlations (P< 0.05) with %dBDM, IADP, DVBE, DVE, FMV^{DVE}, ARUP, MP, and FMV^{NRC}. Furthermore, the secondary structures intensity of α-helix and β-sheet peaks showed a strong correlation (P< 0.01) with IADP g/kg DM (r= 0.82, 0.77, respectively), DVBE g/kg DM (r= 0.82, 0.77, respectively), MCP_{TDN} g/kg DM (r= 0.76, 079, respectively), AMCP g/kg DM (r= 0.76, 0.79, respectively), ARUP g/kg DM (r= 0.82, 0.77, respectively), MP g/kg DM (r= 0.86 for both peaks), and FMV^{NRC} kg milk/kg DM silage (r= 0.86 for both peaks). A significant correlation (P< 0.05) was also found between α-helix and β-sheet peaks with TDNDF g/kg DM (r= -0.63, -0.61, respectively), TDST g/kg DM (r= 0.70 for both peaks), BCP g/kg DM (r= 0.71, 0.62, respectively), DVE g/kg DM (r= 0.66, 0.69, respectively), FMV^{DVE} kg milk/kg DM silage (r= 0.66, 0.69, respectively), and RUP g/kg DM (r= 0.71, 0.62, respectively). The amide I to amide II peak intensity ratio had a strong correlation (P< 0.01) with TDST g/kg DM (r= -0.90) and strong correlations (P< 0.01) with %dBST (r= -0.82), IDBST g/kg DM (r= -0.76), and %TDST (r= -0.83). Moreover, this ratio was significantly correlated (P< 0.05) with IADP g/kg CP (r= -0.73), BCP (g/kg DM) (r= -0.62), DVBE g/kg DM (r= -0.62), DVE g/kg DM (r= -0.62), FMV^{DVE} kg milk/kg DM Silage (r= -0.62), RUP g/kg DM (r= -0.62), and ARUP g/kg DM (r= -0.62).

Table 7.9. Correlation between protein structure spectral peaks and *in vitro* digestibility characteristics, metabolic characteristics, and predicted production performance of whole plant faba bean silage

Item	Peaks								Ratios			
	Amide I		Amide II		α-helix		β-sheet		Amide I: Amide II		α-Helix: β-Sheet	
	r	P	r	P	r	P	r	P	r	P	r	P
%dBDM	-0.43	0.16	0.61	0.04	0.40	0.20	0.45	0.14	-0.51	0.09	0.47	0.12
%IDBDM	-0.52	0.08	0.52	0.08	0.41	0.18	0.45	0.14	-0.45	0.14	0.50	0.09
IDBDM (g/kg DM)	-0.52	0.08	0.52	0.08	0.41	0.18	0.45	0.14	-0.45	0.14	0.50	0.09
%TDDM	-0.26	0.42	0.53	0.08	0.41	0.19	0.48	0.12	-0.41	0.18	0.41	0.19
TDDM (g/kg DM)	-0.26	0.42	0.53	0.08	0.41	0.19	0.48	0.12	-0.41	0.18	0.41	0.19
%dIDP	0.16	0.62	0.31	0.32	-0.30	0.35	-0.16	0.62	-0.22	0.50	-0.39	0.21
IADP (g/kg DM)	-0.79	<0.01	0.63	0.03	0.82	<0.01	0.77	<0.01	-0.62	0.03	0.77	<0.01
IADP (g/kg CP)	-0.85	<0.01	0.74	0.01	0.58	0.05	0.54	0.07	-0.73	0.01	0.63	0.03
TDP (g/kg DM)	0.01	0.97	-0.06	0.85	0.48	0.11	0.54	0.07	0.07	0.83	0.30	0.34
TDP (g/kg CP)	0.80	<0.01	-0.48	0.12	-0.52	0.08	-0.44	0.15	0.55	0.06	-0.55	0.06
%IADP (%CP)	-0.85	<0.01	0.74	0.01	0.58	0.05	0.54	0.07	-0.73	0.01	0.63	0.03
%TDP (%CP)	0.80	<0.01	-0.48	0.12	-0.52	0.08	-0.44	0.15	0.55	0.06	-0.55	0.06
%dBNDF	-0.16	0.62	0.34	0.29	0.19	0.55	0.25	0.44	-0.24	0.44	0.18	0.59
%IDBNDF	-0.22	0.50	0.21	0.50	0.33	0.29	0.34	0.28	-0.18	0.57	0.27	0.39
IDBNDF (g/kg DM)	0.03	0.91	0.07	0.84	-0.12	0.70	-0.08	0.80	-0.02	0.95	-0.14	0.67
%TDNDF	0.01	0.97	0.30	0.35	0.02	0.96	0.06	0.85	-0.18	0.57	0.16	0.62
TDNDF (g/kg DM)	0.45	0.14	-0.23	0.46	-0.63	0.03	-0.61	0.04	0.29	0.35	-0.43	0.16
%dBST	-0.73	0.01	0.88	<0.01	0.46	0.14	0.48	0.12	-0.82	<0.01	0.45	0.14
% IDBST	0.16	0.62	-0.02	0.94	-0.33	0.29	-0.27	0.40	0.11	0.73	-0.25	0.44
IDBST (g/kg DM)	-0.78	<0.01	0.81	<0.01	0.59	0.04	0.58	0.05	-0.76	<0.01	0.61	0.04
%TDST	-0.77	<0.01	0.88	<0.01	0.62	0.03	0.63	0.03	-0.83	<0.01	0.58	0.05
TDST (g/kg DM)	-0.85	<0.01	0.92	<0.01	0.70	0.01	0.70	0.01	-0.90	<0.01	0.65	0.02
BCP (g/kg DM)	-0.83	<0.01	0.56	0.06	0.71	0.01	0.62	0.03	-0.62	0.03	0.73	0.01
MREE (g/kg DM)	0.07	0.83	0.12	0.70	0.09	0.79	0.09	0.78	-0.06	0.85	0.14	0.67
MREN (g/kg DM)	0.41	0.19	-0.38	0.22	0.07	0.82	0.11	0.74	0.38	0.23	-0.09	0.78
DVME (g/kg DM)	0.07	0.83	0.12	0.70	0.09	0.79	0.09	0.78	-0.06	0.85	0.14	0.67

Table 7.9. ***Cont'd***. Correlation between protein structure spectral peaks and *in vitro* digestibility characteristics, metabolic characteristics, and predicted production performance of whole plant faba bean silage

Item	Peaks								Ratios			
	Amide I		Amide II		α-helix		β-sheet		Amide I: Amide II		α-Helix: β-Sheet	
	r	P	r	P	r	P	r	P	r	P	r	P
DVBE (g/kg DM)	-0.79	<0.01	0.63	0.03	0.82	<0.01	0.77	<0.01	-0.62	0.03	0.77	<0.01
DVE (g/kg DM)	-0.57	0.05	0.71	0.01	0.66	0.02	0.69	0.01	-0.62	0.03	0.65	0.02
OEB (g/kg DM)	0.41	0.19	-0.44	0.15	-0.02	0.94	-0.02	0.96	0.39	0.21	-0.21	0.50
FMV^{DVE} (kg milk/kg DM Silage)	-0.57	0.05	0.71	0.01	0.66	0.02	0.69	0.01	-0.62	0.03	0.65	0.02
RUP (g/kg DM)	-0.83	<0.01	0.56	0.06	0.71	0.01	0.62	0.03	-0.62	0.03	0.73	0.01
MCP_{TDN} (g/kg DM)	-0.28	0.38	0.35	0.26	0.76	<0.01	0.79	<0.01	-0.28	0.38	0.66	0.02
MCP_{RDP} (g/kg DM)	0.41	0.19	-0.38	0.22	0.07	0.82	0.11	0.74	0.38	0.23	-0.09	0.78
AMCP (g/kg DM)	-0.28	0.38	0.35	0.26	0.76	<0.01	0.79	<0.01	-0.28	0.38	0.66	0.02
ARUP (g/kg DM)	-0.79	<0.01	0.63	0.03	0.82	<0.01	0.77	<0.01	-0.62	0.03	0.77	<0.01
MP (g/kg DM)	-0.70	0.01	0.62	0.03	0.86	<0.01	0.86	<0.01	-0.57	0.05	0.75	<0.01
DPB (g/kg DM)	0.43	0.17	-0.47	0.13	-0.03	0.93	-0.02	0.94	0.42	0.17	-0.18	0.59
FMV^{NRC} (kg milk/kg DM Silage)	-0.70	0.01	0.62	0.03	0.86	<0.01	0.86	<0.01	-0.57	0.05	0.75	<0.01

Amide I and II peaks located at ca 1589 and ca 1535 cm^{-1} respectively, α-helix and β-sheet peaks located at ca 1648 and ca 1635 cm^{-1} respectively. +, positive correlation; -, negative correlation; r: correlation coefficient using spearman method; values of 'r' between 0 and ± 0.33: weak correlation, between ± 0.33 and ± 0.66: medium correlation, and between ± 0.66 and ± 1: strong correlation; P< 0.10, tendency; P< 0.05, significant; P< 0.01, strongly significant.

A tendency ($P< 0.10$) was observed with MP g/kg DM (r= -0.57) and FMV^{NRC} kg milk/kg DM Silage (r= -0.57). Finally, the α-helix to β-sheet peak intensity ratio had a strong correlation ($P< 0.01$) with IADP g/kg DM (r= 0.77), DVBE g/kg DM (r= 0.77), ARUP g/kg DM (r= 0.77), MP g/kg DM (r= 0.75), and FMV^{NRC} kg milk/kg DM Silage (r= 0.75). Also, it showed a significant correlation ($P< 0.05$) with IADP g/kg CP (r= 0.63), IDBST g/kg DM (r= 0.61), TDST g/kg DM (r= 0.65), BCP g/kg DM (r= 0.73), DVE g/kg DM (r= 0.65), FMV^{DVE} kg milk/kg DM silage (r= 0.65), RUP g/kg DM (r= 0.73), MCP_{TDN} g/kg DM (r= 0.66), and AMCP g/kg DM (r= 0.66).

Protein molecular structure spectral peak heights except for amide I peak height had positive correlation with important characteristics of *in vitro* digestibility and metabolic characteristics as well as predicted production performance, which indicates that if amide I peak height increases those characteristics will decrease and vice versa. On the other hand, amide I to amide II peak height ratio had negative correlation with some *in vitro* digestibility and metabolic characteristics. Totally the opposite is seen when the α-helix to β-sheet peak heights ratio. Therefore, if the α-helix to β-sheet peak heights ratio increases important characteristics of *in vitro* digestibility, metabolic characteristics, and predicted production performance increase as well. Theodoridou and Yu (2013) studied canola meal and found that there were no significant correlations between any characteristics of the DVE/OEB system and the protein structures amide I, amide II, and their ratio, however in our study some negative and positive correlations were observed, additionally Theodoridou and Yu (3013) found that truly absorbed rumen-synthesized microbial protein in the small intestine based on the NRC model was correlated with amide I and amide II peak heights, but in our study we did not found any correlation. Moreover, the amide I to amide II peaks height ratio was correlated to the metabolizable protein and the degraded protein balance of canola meal based on the NRC, however in our study those correlations were not observed. Additionally, Huang (2015) found that metabolizable protein and degraded protein balance based on the NRC model were not significantly correlated with protein spectral profiles, but in our study some correlations were found more specifically with metabolizable protein. On the other hand, β-sheet peak height was correlated with the metabolizable protein and the degraded protein balance of canola meal based on the NRC model (Theodoridou and Yu, 2013), partially in agreement our study found that correlation just in the metabolizable protein. Additionally, Yari et al., (2013) indicated, in agreement with our results, that amide I and amide II peak heights ratio was negatively correlated with the truly absorbed bypass feed protein in the small intestine and the truly absorbed protein in the small intestine of alfalfa hay. In contrast, the α-helix and β-sheet peak heights ratio was positively correlated with the truly absorbed bypass feed protein in the small intestine and the truly absorbed protein in the small intestine, which also was in agreement with our study and the alfalfa results of Lei (2019).

Correlation between protein and carbohydrates structure spectral areas and *in vitro* digestibility characteristics, metabolic characteristics, and predicted production performance of whole plant faba bean silages are presented in Table 7.10. The spectral intensity of NSTC peak had a significant correlation ($P < 0.05$) with %dBDM ($r = -0.68$), IDBDM g/kg DM ($r = -0.59$), and %dBNDF ($r = -0.62$).CEC area had a strong correlation ($P < 0.01$) with DVE g/kg DM ($r = -0.95$) and FMV^{DVE} kg milk/kg DM silage ($r = -0.95$), a strong correlation ($P < 0.01$) with %dBDM ($r = -0.79$), IDBDM g/kg DM ($r = -0.85$), TDDM g/kg DM ($r = -0.78$), %dBST ($r = -0.76$), IDBST g/kg DM ($r = -0.81$), TDST g/kg DM ($r = -0.80$), MP g/kg DM ($r = -0.82$), and FMV^{NRC} kg milk/kg DM silage ($r = -0.82$), and some significant correlations ($P < 0.05$) were observed as well with IADP, %BNDF, %TDNDF, TDST, DVBE, MCP_{TDN}, AMCP, and ARUP. The spectral intensity of STC area was strongly correlated ($P < 0.01$) with BCP g/kg DM ($r = -0.90$) and RUP g/kg DM ($r = -0.90$), while it showed a strong correlation ($P < 0.01$) with IADP g/kg DM ($r = -0.86$), TDP g/kg CP (0.83), and TDST g/kg DM ($r = -0.80$).

Table 7.10. Correlation between protein and carbohydrates structure spectral areas and *in* vitro digestibility characteristics, metabolic characteristics, and predicted production performance of whole plant faba bean silage

Item	Areas												Ratio	
	NSTC		CEC		STC		Amide		Amide I		Amide II		Area Amide I: Amide II	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
%dBDM	-0.68	0.02	-0.79	<0.01	-0.41	0.19	0.55	0.07	0.53	0.08	0.48	0.11	-0.43	0.16
%IDBDM	-0.59	0.04	-0.85	<0.01	-0.52	0.08	0.36	0.26	0.30	0.34	0.37	0.24	-0.34	0.28
IDBDM (g/kg DM)	-0.59	0.04	-0.85	<0.01	-0.52	0.08	0.36	0.26	0.30	0.34	0.37	0.24	-0.34	0.28
%TDDM	-0.55	0.07	-0.78	<0.01	-0.32	0.31	0.59	0.04	0.62	0.03	0.40	0.20	-0.35	0.27
TDDM (g/kg DM)	-0.55	0.07	-0.78	<0.01	-0.32	0.31	0.59	0.04	0.62	0.03	0.40	0.20	-0.35	0.27
%dIDP	0.08	0.81	-0.36	0.26	0.15	0.63	0.21	0.51	0.10	0.75	0.16	0.62	-0.13	0.70
IADP (g/kg DM)	-0.20	0.54	-0.73	0.01	-0.86	<0.01	0.59	0.04	0.31	0.32	0.61	0.04	-0.55	0.07
IADP (g/kg CP)	-0.37	0.24	-0.66	0.02	-0.80	<0.01	0.49	0.11	0.16	0.62	0.71	0.01	-0.66	0.02
TDP (g/kg DM)	0.26	0.42	-0.19	0.56	-0.06	0.85	0.39	0.21	0.52	0.08	-0.08	0.80	0.24	0.46
TDP (g/kg CP)	0.16	0.62	0.34	0.29	0.83	<0.01	-0.21	0.51	0.17	0.60	-0.52	0.08	0.53	0.08
%IADP (%CP)	-0.37	0.24	-0.66	0.02	-0.80	<0.01	0.49	0.11	0.16	0.62	0.71	0.01	-0.66	0.02
%TDP (%CP)	0.16	0.62	0.34	0.29	0.83	<0.01	-0.21	0.51	0.17	0.60	-0.52	0.08	0.53	0.08
%dBNDF	-0.62	0.03	-0.65	0.02	-0.27	0.39	0.29	0.37	0.36	0.25	0.21	0.51	-0.23	0.47
%IDBNDF	-0.53	0.08	-0.47	0.12	-0.32	0.31	0.29	0.35	0.40	0.20	0.17	0.60	-0.17	0.60
IDBNDF (g/kg DM)	-0.48	0.11	-0.34	0.28	-0.07	0.83	-0.05	0.88	0.06	0.86	-0.03	0.93	-0.03	0.91
%TDNDF	-0.35	0.27	-0.64	0.02	-0.03	0.91	0.19	0.56	0.22	0.48	0.15	0.65	-0.17	0.59
TDNDF (g/kg DM)	-0.02	0.95	0.04	0.90	0.51	0.09	-0.48	0.12	-0.39	0.21	-0.34	0.29	0.18	0.57
%dBST	-0.43	0.17	-0.76	<0.01	-0.60	0.04	0.71	0.01	0.43	0.16	0.80	<0.01	-0.72	0.01
% IDBST	0.17	0.59	-0.14	0.66	0.23	0.47	-0.27	0.40	-0.28	0.38	-0.15	0.63	0.10	0.76
IDBST (g/kg DM)	-0.41	0.19	-0.81	<0.01	-0.71	0.01	0.64	0.02	0.38	0.22	0.74	0.01	-0.67	0.02
%TDST	-0.47	0.12	-0.71	0.01	-0.64	0.03	0.85	<0.01	0.58	0.05	0.84	<0.01	-0.74	0.01
TDST (g/kg DM)	-0.38	0.22	-0.80	<0.01	-0.80	<0.01	0.85	<0.01	0.51	0.09	0.90	<0.01	-0.83	<0.01
BCP (g/kg DM)	-0.15	0.65	-0.57	0.05	-0.90	<0.01	0.41	0.19	0.08	0.81	0.59	0.04	-0.56	0.06
MREE (g/kg DM)	-0.44	0.15	-0.27	0.39	0.09	0.78	0.28	0.38	0.51	0.09	0.10	0.76	-0.08	0.81
MREN (g/kg DM)	0.32	0.31	0.17	0.60	0.34	0.28	0.09	0.78	0.39	0.21	-0.36	0.26	0.45	0.14
DVME (g/kg DM)	-0.44	0.15	-0.27	0.39	0.09	0.78	0.28	0.38	0.51	0.09	0.10	0.76	-0.08	0.81

Table 7.10. ***Cont'd***. Correlation between protein and carbohydrates structure spectral areas and *in vitro* digestibility characteristics, metabolic characteristics, and predicted production performance of whole plant faba bean silage

Item	Areas												Ratio	
	NSTC		CEC		STC		Amide		Amide I		Amide II		Area Amide I: Amide II	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
DVBE (g/kg DM)	-0.20	0.54	-0.73	0.01	-0.86	<0.01	0.59	0.04	0.31	0.32	0.61	0.04	-0.55	0.07
DVE (g/kg DM)	-0.49	0.11	-0.95	<0.01	-0.62	0.03	0.71	0.01	0.59	0.04	0.59	0.04	-0.50	0.10
OEB (g/kg DM)	0.48	0.11	0.31	0.33	0.31	0.33	-0.04	0.90	0.15	0.63	-0.38	0.23	0.45	0.14
FMV^{DVE} (kg milk/kg DM Silage)	-0.49	0.11	-0.95	<0.01	-0.62	0.03	0.71	0.01	0.59	0.04	0.59	0.04	-0.50	0.10
RUP (g/kg DM)	-0.15	0.65	-0.57	0.05	-0.90	<0.01	0.41	0.19	0.08	0.81	0.59	0.04	-0.56	0.06
MCP_{TDN} (g/kg DM)	-0.28	0.38	-0.65	0.02	-0.38	0.22	0.73	0.01	0.86	<0.01	0.29	0.35	-0.11	0.73
MCP_{RDP} (g/kg DM)	0.32	0.31	0.17	0.60	0.34	0.28	0.09	0.78	0.39	0.21	-0.36	0.26	0.45	0.14
AMCP (g/kg DM)	-0.28	0.38	-0.65	0.02	-0.38	0.22	0.73	0.01	0.86	<0.01	0.29	0.35	-0.11	0.73
ARUP (g/kg DM)	-0.20	0.54	-0.73	0.01	-0.86	<0.01	0.59	0.04	0.31	0.32	0.61	0.04	-0.55	0.07
MP (g/kg DM)	-0.32	0.31	-0.82	<0.01	-0.78	<0.01	0.71	0.01	0.55	0.06	0.55	0.06	-0.43	0.16
DPB (g/kg DM)	0.43	0.16	0.27	0.40	0.33	0.30	-0.08	0.81	0.17	0.60	-0.41	0.19	0.44	0.15
FMV^{NRC} (kg milk/kg DM Silage)	-0.32	0.31	-0.82	<0.01	-0.78	<0.01	0.71	0.01	0.55	0.06	0.55	0.06	-0.43	0.16

Non-structural carbohydrates (NSTC) area located from ca 879 to ca 906 cm^{-1}, structural carbohydrates (STC) area located from ca 1184 to ca 1485 cm^{-1}, cellulosic compounds (CEC) area located from ca 1184 to ca 1290 cm^{-1}, amide area located from ca 1484 to ca 1712 cm^{-1}, amide I area located from ca 1547 to ca 1712 cm^{-1}, amide II area located from ca 1484 to ca 1547 cm^{-1}. +, positive correlation; -, negative correlation; r: correlation coefficient using spearman method; values of 'r' between 0 and ± 0.33: weak correlation, between ± 0.33 and ± 0.66: medium correlation, and between ± 0.66 and ± 1: strong correlation; P< 0.10, tendency; P< 0.05, significant; P< 0.01, strongly significant.

Also, significant correlations ($P < 0.05$) were observed with %dBST ($r = -0.60$), IDBST g/kg DM ($r = -0.71$), DVBE (g/kg DM) ($r = -0.86$), DVE g/kg DM ($r = -0.62$), FMV^{DVE} kg milk/kg DM silage ($r = -0.62$), ARUP g/kg DM ($r = -0.86$), MP g/kg DM ($r = -0.78$), and FMV^{NRC} kg milk/kg DM silage ($r = -0.78$). In relation with the protein molecular structure, the area of amide spectral intensity was strongly correlated ($P < 0.01$) with TDST g/kg DM ($r = 0.85$) and significantly correlated ($P < 0.05$) with several characteristics including TDDM, IADP, %dBST, IDBST, DVBE, DVE, FMV^{DVE}, MCP_{TDN}, AMPC, ARUP, MP, and FMV^{NRC}. Furthermore, amide I area had a strong correlation ($P < 0.01$) with MCP_{TDN} g/kg DM ($r = 0.86$) and AMCP g/kg DM ($r = 0.86$), and showed significant correlations ($P < 0.05$) with TDDM g/kg DM ($r = 0.62$), DVE g/kg DM ($r = 0.59$), and FMV^{DVE} kg milk/kg DM silage ($r = 0.59$). On the other hand, amide II area had a strong correlation ($P < 0.01$) with TDST g/kg DM ($r = 0.90$), and showed a strong correlation ($P < 0.01$) with %dBST ($r = 0.80$) and %TDST ($r = 0.84$) while it had significant correlations ($P < 0.05$) with IADP g/kg CP ($r = 0.71$), IDBST g/kg DM ($r = 0.74$), BCP g/kg DM ($r = 0.59$), DVBE g/kg DM ($r = 0.61$), DVE g/kg DM ($r = 0.59$), and FMV^{DVE} kg milk/kg DM silage ($r = 0.59$), RUP g/kg DM ($r = 0.59$), and ARUP g/kg DM ($r = 0.61$). Finally, the ratio of amide I to amide II had a strong correlation ($P < 0.01$) with TDST g/kg DM ($r = -0.83$) and significant correlations ($P < 0.05$) with IADP g/kg CP ($r = 0.66$), %dBST ($r = -0.72$), IDBST g/kg DM ($r = -0.67$), and %TDST ($r = -0.74$).

Non-structural carbohydrates, cellulosic compounds and structural carbohydrates areas and amide I to amide II area ratio were negatively correlated with most characteristics of *in vitro* digestibility, metabolic characteristics and feed milk value (independent of the evaluation system used) except for total digested starch, indicating that if those areas and ratio increase, those characteristics will increase too, but amide, amide I and amide II areas had positive correlation with hose characteristics. Ban (2016) found positive correlation between amide I and amide II areas with bypass crude protein of carinata seeds based on DVE/OEB system, but in our study bypass crude protein of whole plant faba bean silages was correlated with amide II area. Also, Ban (2016) found positive correlation strong between amide I and amide II areas with the truly absorbed protein in the small intestine of carinata meal, in agreement our study found a correlation but in our case they were weak, additionally in disagreement to the results in our study the truly absorbed protein in the small intestine of carinata meal was positive correlated with structural carbohydrates area, which was negative in our case. Lei (2019) found that truly absorbed protein in the small intestine of alfalfa was negatively correlated with amide I area, but in our study amide, amide I and amide II areas were positively correlated to truly absorbed protein in the small intestine. Rodriguez (2018) found, in agreement with our results, negative correlation between the cellulosic area and the metabolizable protein based on the NRC model. Prates et al., (2018) found a negative correlation between the structural carbohydrates area with the digestibility of starch

7.4.6 Multiple Regression Analysis with Prediction Model Variable Selection

In order to select the best spectral characteristics to predict important nutrient characteristics, the model selection technique was carried out. Multiple regression analysis was used to select the best protein and carbohydrates spectral characteristics to predict chemical, energy and rumen degradation kinetics characteristics (Table 7.11), *in vitro* digestibility, metabolic characteristics, and production performance (Table 7.12). The results indicated that structural carbohydrates and amide areas were highly associated with *in vitro* digestibility and metabolic characteristics. The equations showed that RUP (g/kg DM) can be predicted by α-Helix:β-Sheet Peak Height Ratio and STC Area (R^2 = 0.94), while BSt (g/kg DM) can be predicted by STC1 Peak Height, LIG Peak Height, Amide I Peak Height, α-Helix:β-Sheet Peak Height Ratio and CEC Area (R^2 = 0.98); IADP (g/kg DM) can be predicted by the STC area (R^2 = 0.84); DVBE (g/kg DM) and ARUP (g/kg DM) (R^2 = 0.84) as well. Additionally, α-helix peak height could predict with good accuracy (R^2 = 0.97) the TDST (g/kg DM); α-helix: β-sheet height ratio together with STC area could predict with good accuracy ($R^2 = 0.94$) the BCP (g/kg DM) and RUP (g/kg DM), while CEC area and amide area were the best characteristics to predict ($R^2 = 0.91$) DVE (g/kg DM) and FMV^{DVE} (kg milk/kg DM silage), and MP (g/kg DM) and FMV^{NRC} (kg milk/kg DM Silage) were predicted with reasonably good accuracy ($R^2 = 0.94$) by STC1 peak height.

Huang (2015) studied canola meal and found that metabolizable protein and degraded protein balance based on the NRC model were not predicted by any of the protein spectral profiles, that was in agreement with our findings, however Liu et al., (2012) and Peng et al., (2014) used protein spectral profiles to predict protein supply of feeds, but in our study amide area with CEC area could predict the truly absorbed protein in the small intestine based on the DVE/OEB system. Rodriguez (2018) found that the coefficients of determination in this multiple regression analysis between carbohydrates structures and metabolic characteristics of faba bean seeds were very low to be consider as precise predictors. However, Lei (2019) found that DVE/OEB metabolic characteristics of alfalfa can be predicted with protein and carbohydrates molecular structures. Moreover, Ban (2016) found that the truly absorbed protein in the small intestine based on the DVE/OEB system could be predicted using the cellulosic compound peak height, while in our study could be predicted by the cellulosic compound area together with amide area, which implicates that protein metabolic characteristics can be predicted not only by protein molecular structures, but also by carbohydrates molecular structures or both together.

Table 7.11. Multiple regression analysis to choose the most important protein and carbohydrates spectral characteristics for predicting chemical, energy and rumen degradation kinetics characteristics of whole plant faba bean silage.

Predicted Variable (Y)	variable selection (P< 0.05)	Predicted Equation Y = a + b1 × x1 + b2 × x2 + … + bn × xn	R^2	RSD	P value
CP (%DM)	NSTC Peak Height and β-Sheet Peak Height	CP (% DM) = 12.24 + 161.76 NSTC Peak Height + 40.67 β-Sheet Peak Height	0.67	0.64	0.01
Starch (%DM)	STC4 Peak Height Amide I Peak Height and α-Helix Peak Height	Starch (%DM) = -24.93 + 397.47 STC4 Peak Height -64.26 Amide I Peak Height + 52.58 α-Helix Peak Height	0.96	1.00	<0.01
TDN$_{3x}$ (%DM)	Amide I Area	TDN$_{3x}$ (%DM) = 32.17 + 1.18 Amide I Area	0.66	1.09	<0.01
NE$_{Lp3x}$ (Mcal/kg)	Amide I Area	NE$_{Lp3x}$ (Mcal/kg) = 0.57 + 0.04 Amide I Area	0.64	0.04	<0.01
BCP (g/kg DM)	α-Helix: β-Sheet Peak Height Ratio and STC Area	BCP (g/kg DM) = 65.04 + 50.06 α-Helix: β-Sheet Height Ratio - 2.77 STC Area	0.93	2.10	<0.01
RUP (g/kg DM)	α-Helix: β-Sheet Peak Height Ratio and STC Area	RUP (g/kg DM) = 58.59 + 45.10 α-Helix: β-Sheet Height Ratio - 2.49 STC Area	0.94	1.89	<0.01
BSt (g/kg DM)	STC1 Peak Height LIG Peak Height Amide I Peak Height α-Helix: β-Sheet Peak Height Ratio CEC Area	BSt (g/kg DM)= 5.46 – 1479.40 STC1 Peak Height - 1327.07 LIG Peak Height + 307.98 Amide I Peak Height + 106.20 α-Helix: β-Sheet Peak Height Ratio + 9.35 CEC Area	0.98	1.56	<0.01

TDN$_{3\times}$: total digestible nutrient at three times maintenance; NE$_{L3\times}$: net energy for lactation at production level of intake (3×); BCP: ruminally undegraded feed CP: calculated according the formula in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; BSt: rumen bypass or undegraded feed starch; RSD: residual standard deviation; R^2: coefficient of determination. All variables left in the final model were significant at the 0.05 alpha level.

Table 7.12. Multiple regression analysis to choose the most important protein and carbohydrates spectral characteristics for predicting intestinal digestion characteristics and metabolic characteristic of whole plant faba bean silage.

Predicted Variable (Y)	variable selection (P< 0.05)	Predicted Equation $Y = a + b1 \times x1 + b2 \times x2 + \ldots + bn \times xn$	R^2	RSD	P value
IADP (g/kg DM)	STC Area	IADP (g/kg DM) = 75.08 - 2.26 STC area	0.84	1.77	<0.01
TDST (%)	Amide I: Amide II Peak Height Ratio	TDST (%) = 100.64 - 0.69 Amide I: Amide II Height Ratio	0.74	0.27	<0.01
TDST (g/kg DM)	STC4 Peak Height, Amide I Peak Height and α-Helix Peak Height	TDST (g/kg DM) = -255.08 + 4070.24 STC4 Peak Height - 659.58 Amide I Peak Height + 528.55 α-Helix Peak Height	0.97	10.38	<0.01
DVBE (g/kg DM)	STC Area	DVBE (g/kg DM) = 83.34 - 2.51 STC Area	0.84	1.96	<0.01
DVE (g/kg DM)	CEC Area and Amide Area	DVE (g/kg DM) = 70.54 - 9.11 CEC Area + 1.24 Amide Area	0.91	2.18	<0.01
FMV^{DVE} (kg milk/kg DM Silage)	CEC Area and Amide Area	FMV^{DVE} (kg milk/kg DM Silage) = 1.43 - 0.18 CEC Area + 0.025 Amide Area	0.91	0.04	<0.01
ARUP (g/kg DM)	STC Area	ARUP (g/kg DM) = 75.08 - 2.26 STC Area	0.84	1.77	<0.01
MP (g/kg DM)	STC1 Peak Height	MP (g/kg DM) = 123.25 - 489.28 STC1 Peak Height	0.77	2.59	<0.01
FMV^{NRC} (kg milk/kg DM Silage)	STC1 Peak Height	FMV^{NRC} (kg milk/kg DM Silage) = 2.50 - 9.95 STC1 Peak Height	0.77	0.05	<0.01

IADP: intestinal digested crude protein; TDST: total digested starch; DVBE: truly absorbed bypass feed protein in the small intestine; DVE: truly absorbed protein in the small intestine; ARUP: truly absorbed rumen-undegraded feed protein in the small intestine; MP: metabolizable protein; FMV: feed milk value; RSD: residual standard deviation; R^2: coefficient of determination. All variables left in the final model were significant at the 0.05 alpha level.

7.5 Conclusions

The variety and cutting stage had a significant impact on the inherent molecular structure of whole plant faba bean silage. On the other hand, based on the multivariate analysis at complete spectral region, at amide region and at complete carbohydrate region, it was observed a clear difference between the mid pod and late pod stages. Additionally, protein and carbohydrate molecular structures were highly related with nutrient profiles, intestinal and metabolic characteristics from Chapter 4. Besides protein and carbohydrate molecular structure spectral features can be used to accurately predict some of these nutritional profiles and characteristics in ruminant livestock systems.

8. GENERAL DISCUSSION, OVERALL CONCLUSION, AND IMPLICATIONS

Faba bean is able to grow in wet and cool environments such as Canada. The production has dramatically increased, but there is not enough information on the use of the whole plant of this legume for ruminants. This project systematically investigated the effects of variety/tannin concentration, maturity stage (flower stage vs. mid pod stage vs. late pod stag), and frost damage on the nutritional values of whole plant faba bean as hay and silage for ruminants. The detailed chemical profiles, energy values, protein and carbohydrate fractions, rumen degradation kinetics, hourly effective degradation ratios of nitrogen to carbohydrates, intestinal nutrient digestion, metabolic characteristics, predicted production performance, molecular structure, interactive relationship between inherent molecular structure and nutrient utilization, and the effect when included in dairy rations were determined.

The third section (Chapter 3) showed that the tannin concentration and cutting stage of whole plant faba bean hay (artificially air dried) had a significant effect on yield, chemical profiles, CNCPS protein and carbohydrate fractions, as well as in the rumen degradation kinetics of primary nutrients, except for the energy profiles. The yield of fresh matter and dry matter of whole plant faba bean hay was higher than the average yield of alfalfa ranged found by Putnam et al., (2000). Furthermore, at late pod stage, the yield of organic matter and starch were higher than at flower and mid pod stages. Additionally, the high tannin whole plant faba bean hay showed higher bypass protein than the low tannin whole plan faba bean hay. At late pod stage, rumen bypass protein and the bypass dry matter were higher than that at the other cutting stages of flower and mid pod stages. The available nitrogen to available carbohydrates synchronization was affected by the tannin concentration and stage of cutting. Based on this study, with advancing maturity of whole plant faba bean, the ruminal nitrogen to energy synchronization of the hay was improved and closer to the optimum ratio across the curve (h16, h18 and h 20) at late pod stage. The ideal ratio (32 g of

N/kg CHO) to maximize microbial synthesis and minimize N loss in dairy cattle (Tamminga et al., 2007). Intestinal digestible characteristics were affected by the tannin concentration and stage of cutting. IDBDM and TDST values were increased with maturity. Various nutrient supply characteristics predicted with both DVE/OEB and NRC-2001 were affected by the tannin concentration and stage of cutting. However, the degraded protein balance and the total true protein supply were affected only by the stage of cutting, even when was difference between the high and low tannin hay in terms or bypass protein. Whole plant faba bean hay at late pod stage had higher estimated total true protein digested in the intestine (DVE) and lower degraded protein balance (OEB and DPB) than the whole plant faba bean hay at flower stage.

Based on the fourth section (Chapter 4), the results showed that the silage yield was affected basically by the stage of cutting and the higher silage dry matter yield and silage starch yield were observed when whole plant faba bean silage was harvested at late pod stage. Additionally, was suggested that whole plant faba bean silage at flower stage did not ferment properly and produced more ammonia (>10 % of total N) which may be result of extensive protein breakdown (Kaiser and Piltz, 2003), while at mid pod and late pod stages the silage achieved an adequate fermentation. There is not much difference in terms of chemical profiles between the low and high tannin whole plant faba bean silage. The high tannin whole plant faba bean silage showed higher acid detergent lignin and lower concentration of soluble crude protein than the low tannin whole plant faba bean silage. The CP remained similar among all the cutting stages (22 %DM), however at late pod stage a higher concentration of starch was found (17 %DM), therefore a higher energy value was obtained. Fraser et al., (2001) found that the CP in faba bean silage was about 21 %DM, while Nair (2017) found a starch content of 20 %DM in barley silage. Therefore, starch content in faba bean silage is slightly lower. Whole plant faba bean silage at mid pod stage showed similar energy values to late pod stage. At flower stage, the silage had higher concentration of indigestible fiber and WSC than at the mid pod and late pod stages. The whole plant faba bean silage at late pod stage showed higher rumen bypass dry matter, bypass crude protein and higher bypass starch than that at mid pod stage, the same trend was observed in the whole plant faba bean hay in Chapter 3. The hourly effective degradation ratios of nitrogen and carbohydrates were affected by the cutting stage, showing a smoother curve at late pod stage. The intestinal nutrient digestion characteristics showed that the low tannin whole plant faba bean silage had higher total digested protein than the high tannin variety, besides that at late pod stage also was noted higher intestinal digested dry matter, total digested protein and higher intestinal digested starch. The main source of energy for a high producing dairy cows is the starch; part of this starch which escape the rumen degradation is digested in the small intestine and absorbed as glucose (Ali et al., 2012), therefore whole plant faba bean silage at late pod stage can contribute with higher energy from starch for a high producing cow than whole plant faba bean silage at mid pod stage. In terms of total true protein supply and predicted production performance, high and low tannin whole plant faba bean silages were not significantly different. Refat, (2018) found that the metabolizable protein of corn and barley silages are values are 49.8 and 51.8 g/kg DM, respectively. However, those values are lower than the average metabolizable protein found in whole plant faba bean silage (70.0 g/kg DM). However, the cutting stage significantly affected nutrient supply with late pod stage showing higher performance than flower and mid pod stages.

The fifth section (Chapter 5) of studies suggested that tannin the tannin concentration affected some of the characteristics analyzed in the frost damaged whole plant faba bean hay. The frost damaged high tannin whole plant faba bean hay had higher organic matter, lower ADICP and lower indigestible protein fraction than the low tannin frost damaged whole plant faba bean hay, however they are similar in energy levels (1.15 Mcal/kg). The protein, starch and neutral detergent fiber of whole crop faba bean hay (Chapter 3) are 22 %DM, 14 %DM and 35 %DM respectively, which are slightly higher than the values found in frost damaged whole plant faba bean hay 16 %DM, 12 %DM, except for NDF which was higher in the frost damaged whole plant faba bean hay (45 vs. 35 %DM, respectively). Partially in agreement with our study, Yu and Racz (2009) found that frost damaged wheat had lower starch content, similar protein content and higher NDF content than normal wheat. Although the high tannin frost damaged whole plant faba bean hay showed lower bypass protein as a percentage, lower undegradable fraction of NDF, higher digestibility of crude protein, the metabolic characteristics based on the DVE system and the NRC model were similar. The predicted production performance was higher in the high tannin frost damaged whole plant faba bean hay than that in the low tannin frost damaged whole plant faba bean hay (1.31 vs. 1.16 kg of milk/kg of DM hay). These results also showed that important nutrients such as crude protein and starch, energy values and the predicted production performance (-0.23 kg of milk/kg of DM hay) of frost damaged whole plant faba bean hay were significantly lower than the correspondent values of normal whole plant faba bean hay (Chapter 3) at mid pod or late pod stages.

The sixth section (Chapter 6) of studies include the animal performance and metabolic trials using low tannin whole plant faba bean silage at late pod stage (decided based on the results of Chapter 4). The results demonstrated that the inclusion of low tannin whole plant faba bean silage at late pod stage tended to increase the milk yield and increased significantly the fat corrected milk (56 kg/cow/d vs. 52 kg/cow/d) and energy corrected milk (54 kg/cow/d vs. 50 kg/cow/d). Those results are related to the higher predicted production performance of whole plant faba bean silage than the predicted production performance of corn and barley silages in found in Chapter 4. Besides that, the inclusion of whole plant faba bean silage increased significantly the fat yield (2.1 vs. 1.9 kg/cow/d) when compared with the yield provided by the animals fed conventional barley/corn silage-based diet. Refat (2018) found that the average of fat yield when high milking cows were fed barley silage based diet was 1.39 kg/cow/d. Therefore, higher fat yield may be achieved with whole plant faba bean silage. On the other hand, the results showed that feed efficiency was also improved when animals consumed diets with whole plant faba bean silage, although the intake was not affected by the inclusion of whole plant faba bean silage. Additionally, it has been showed that the digestibility of ash was higher in the diets which included whole plant faba bean silage. The predicted nitrogen utilization overestimated the nitrogen intake and the nitrogen output, however on the real nitrogen utilization, it is observed that whole plant faba bean silage diets had similar nitrogen efficiency than the control barley/corn silage diet. Besides that, the energy level of the diet was increased when whole plant faba bean silage was included in the diet (1.92 vs. 1.65 Mcal/kg DMI), furthermore the addition of whole plant faba bean silage improved the energy partitioning and provided more energy to support milk production and body weight gain (78 vs 75 % of total energy). Studies suggested that in high-yielding dairy cows in early lactation, the negative energy balance is considered a physiological phenomenon (Van Straten et al., 2008; Roche et al., 2007), therefore assessing adaptation to this negative energy balance is key because an poor adaptation negatively affects health status, reproductive performance, and milk production (Poncheki et al., 2015). Thus, inclusion of whole plant faba bean silage has a positive effect on recovery from a negative energy balance. The energy level was increased by increased protein concentration and slightly reduction of neutral detergent fiber content. Besides that, the inclusion of whole plant faba bean silage did not affect the eating, ruminating, and total chewing activity in general, also did not negatively affected any rumen fermentation characteristics.

The seventh section (Chapter 7) studied the whole plant faba bean silage from Chapter 4 at a molecular level. The molecular spectral analysis with vibrational spectroscopy-ATR-Ft/IR coupled with chemometric showed some interactions between the variety and the cutting stage in spectral characteristics of functional groups. STC3 peak intensity was affected by the variety and not by the stage. This peak intensity was higher in the low tannin variety. The amide I peak intensity was similar among all the silages; however, the amide II peak intensity was higher in the high tannin variety at late pod stage. The STC area intensity was higher in the low tannin variety at mid pod stage than the other silages, also the amide II area intensity was higher in high tannin variety at late pod stage, but the total amide area was similar among all the treatments and probably is related to the similar protein concentration found in them. The ratios of amide I to amide II may indicate changes in crude protein nutritive value of different feed ingredients (Khan et al., 2014). Whole plant faba bean silage at mid pod stage had a higher amide I to amide II area ratio than whole plant faba bean silage at late pod stage, however based on the chemical profile and CNCPS from chapter 4 there are not any changes in the crude protein fractions. Based on the multivariate molecular spectral analysis, by using the whole spectra region, the amide region, and total carbohydrate region, it was possible to clearly differentiate between the whole plant faba bean silage at mid pod stage and at late pod stage, however it was not possible to differentiate the high tannin from the low tannin varieties. The results from the multivariate analysis using all spectra showed a difference between whole plant faba bean silage at mid and late pod stages. The principal component accounted for 75% of the total variation. Additionally, using the amide region, the PCA detected a difference between whole plant faba bean silage at mid and late pod stages. The principal component is this case is the amide I peak height which accounted for 70% of the total variation. Therefore, as we saw in Chapter 4, the crude protein content was similar between the two cutting stages, but we can see based on the multivariate analysis, supported by the univariate analysis, that the protein related molecular structure (Amide I) changes with maturity. Rodriguez (2018), found that the principal component is the Amide I and Amide II when the amide spectral region was analyzed from low and high tannin faba bean seeds; also it was found in that study that the soluble crude protein was lower in the high tannin varieties than in the low tannin varieties. Therefore, in that study molecular changes on the protein related structures may affect the proportion on the protein fractions. Using the total carbohydrate region, the PCA detected a difference between whole plant faba bean silage at mid and late pod stages. The principal component is the STC area which accounted for 83% of the total variation. Therefore, at mid pod stage there is a higher STC area than at late pod stage, which could be related to the lower content of NSC at mid pod stage as shown in Chapter 4. On the other hand, it was found that the protein and carbohydrate molecular structures (spectral peaks and areas intensities) are correlated to some important nutrient profiles, energy profile, rumen degradations characteristics, intestinal digestion characteristics and metabolic characteristics and predicted production performance. Additionally, it was found that STC1 peak high, STC area, amide I area and others can work alone or together to accurately predict important nutrients, energy profile, metabolic characteristics and even the predicted production performance as in agreement with the study conducted by Lei (2019) and Rodriguez (2018) which indicate that protein and carbohydrate molecular structures can used together to make an accurate prediction.

In conclusion, whole plant faba bean at late pod stage can be considered as a high yielding forage, however faba varieties with higher yields in drought areas must be developed. Besides, physiochemical, molecular structural and nutritional features of whole crop of faba bean plant as hay and silage were significantly impacted by tannin concentration and cutting stage. Whole plant faba bean hay showed interesting nutrient characteristics to be considered as a forage source in future animal trials; whole plant faba bean hay showed slightly higher predicted production performance than whole plant faba bean silage and frost damaged plant whole plant faba bean hay. Furthermore, whole plant faba bean silage at late pod stage showed higher predicted production performance than at mid pod stage, which was supported by the results in the animal trial. These results indicated that its inclusion improve the performance in high milking cows in western Canada. Therefore, these levels of inclusion of whole plant faba bean silage can be used as alternative feeding strategy. The changes of molecular structure features by tannin concentration and cutting stage would be highly associated with nutrient utilization and availability in cattle. Besides that, a clear difference between whole plant faba bean silage at mid pod and late pod stages was observed at the molecular level. Both, the protein and carbohydrate structures can be related with nutrient profiles, intestinal and metabolic characteristics and therefore the spectral profile of silage could be used as a fast tool to rapidly evaluate its nutritive quality and accurately predict some characteristics of true nutrient supply and animal performance in ruminant systems.

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10. APPENDIX

Table 10.1. Chemical composition of the ingredients used in the dairy production and metabolic trials

Item (%DM, except DM)	Corn Silage	Barley Silage	Faba bean Silage	Alfalfa Hay	Straw	Corn Grain	Barley Grain	Canola Meal	Soybean Meal	Beet Pulp
DM (%)	39.1	44.4	45.1	84.9	86.5	86.0	86.3	90.2	90.0	88.9
CP	9.6	12.8	22.5	12.2	4.3	8.4	12.9	41.5	51.5	10.3
SCP	4.8	8.4	12.9	5.2	1.9	1.0	1.9	12.4	10.3	2.4
NDICP	0.7	1.3	0.9	1.3	1.2	1.1	1.4	9.1	1.0	4.3
ADICP	0.5	0.8	0.7	1.1	0.9	0.6	0.6	2.5	0.9	1.4
CHO	83.5	78.0	69.4	77.4	87.4	86.5	81.5	45.7	39.0	82.1
aNDFom	48.5	39.8	35.5	49.4	77.7	9.0	20.1	27.7	10.0	38.8
ADF	28.2	22.1	27.9	36.9	51.4	3.7	9.2	20.4	6.8	22.4
ADL	3.0	4.5	4.5	7.7	8.5	1.2	3.0	7.3	0.8	1.9
Starch	21.3	20.1	20.0	1.5	1.0	75.7	55.4	2.6	1.9	0.3
Sugar (WSC)	1.1	3.8	0.9	7.1	1.4	1.6	1.9	10.3	10.9	13.9
NFC	35.0	38.2	33.8	28.0	9.7	77.5	61.4	18.0	29.0	43.3
NSC	22.4	23.9	20.9	8.6	2.4	77.1	57.3	12.9	12.8	14.2
Ash	4.8	5.5	6.6	7.5	6.0	1.2	3.1	8.0	6.7	6.9
Ca	0.2	0.3	0.6	0.9	0.2	0.1	0.1	0.8	0.3	0.7
P	0.3	0.3	0.3	0.2	0.1	0.3	0.4	1.2	0.7	0.1

DM: dry matter; CP: crude protein; SCP: soluble crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; aNDFom: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: non-fiber carbohydrate; CHO: carbohydrate; NFC: non-fiber carbohydrate; NSC: non-structural carbohydrates; WSC: water soluble carbohydrates; Ca: calcium; P: phosphorus.

Figure 10.1. Whole plant faba bean at flower stage

Figure 10.2. Whole plant faba bean at mid pod stage

Figure 10.3. Whole plant faba bean at late pod stage

Figure 10.4. Whole plant faba bean silage at late pod stage used in the dairy production and metabolic trials