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Ruminal Degradabilities and Intestinal Digestibilities of Canola Meals and the Production Response of Cows Fed Canola Meal With Varying Concentration of Starch Sources

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RUMINAL DEGRADABILITIES AND INTESTINAL DIGESTIBILITIES OF CANOLA MEALS AND THE PRODUCTION RESPONSE OF COWS FED CANOLA MEAL WITH VARYING CONCENTRATION OF STARCH SOURCES

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

A = Rapidly degradable fraction of CP

AA = Amino acids

ADF = Acid detergent fiber

ADICP = Acid detergent insoluble crude protein

ADIN = Acid detergent insoluble nitrogen

AH = Alfalfa haylage

Ala = Alanine

AOAC = Association of official analytical chemists

Arg = Arginine

Asp = Aspartic

B = Potentially degradable fraction of CP

BSC = Body condition score

BW = Body weight

C = Undegradable fraction of CP

CHO = Carbohydrate

CM = Canola meal

CNCPS = Cornell net carbohydrate and protein system

CO_2 = Carbon dioxide

CP = Crude protein

CS = Corn silage

CSM = Cotton seed meal

^oC = Celsius

C = Cubic

Cys = Cystine

DDGS = Distillers dry grain with solubles

DE = Digestible energy

DM = Dry matter

DMI = Dry matter intake

DNA = Deoxyribonucleic acid

EAA = Essential amino acids

ECM = Energy corrected milk

EE = Ether extract

ENU = Efficiency of nitrogen utilization

FA = Fatty acids

FCM = Fat corrected milk

fNDF = Forage neutral detergent fiber

Glu = Glutamine

Gly = Glysine

HB = Hull-less barley

HCl = Hydrochloric acid

His = Histidine

IADP = Intestinal absorbable digestible protein

IDP = Intestinal digestible protein

Ile = Isoleucine

Kd = Rate of degradation

Kp = Rate of passage

L = Linear

Leu = Leucine

Lys = Lysine

ME = Metabolisable energy

Met = Methionine

MMt = Million metric tons

MP = Metabolizable protein

MUN = Milk urea nitrogen

N = Nitrogen

NAN = Non ammonia nitrogen

NANMN = Non ammonia nitrogen microbial nitrogen

NCN = Non casein nitrogen

NDF = Neutral detergent fiber

NDICP = Neutral detergent insoluble crude protein

NEAA = Non-essential amino acids

NE_L = Net energy of lactation

NFC = Non-fiber carbohydrate

NH_3 = Ammonia

NPN = Non-protein nitrogen

NRC = National research council

NSC = Non-structural carbohydrate

OM = Organic matter

Phe = Phenylalanine

Pro = Proline

PUN = Plasma urea nitrogen

Q = Quadratic

RDP = Rumen degradable protein

RNA = Ribose nucleic acid

SBM = Soybean meal

SCC = Somatic cell count

Ser = Serine

SI = Small intestine

SNF = Solid non-fat

SP = Soluble protein

TDP = Total digestible protein

Thr = Threonine

TMR = Total mix ration

TP = Total protein

Trp = Tryptophan

TTD = Total tract digestibility

Tyr = Tyrosine

USDA = United States Department of Agriculture

Val = Valine

VFA = Volatile fatty acids

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ABSTRACT

RUMINAL DEGRADABILITIES AND INTESTINAL DIGESTIBILITIES OF CANOLA MEALS AND THE PRODUCTION RESPONSE OF COWS FED CANOLA MEAL WITH VARYING CONCENTRATION OF STARCH SOURCES

Nadeesha Jayasinghe

2014

Canola meal (CM) is a by-product in the manufacturing process of canola oil which can be performed with or without using solvent extraction. It is a protein supplement which has grown its importance in dairy cattle feeding competing with soybean meal (SBM). However, final quality of the feed depends on the oil extraction process and the production conditions used by the individual processing plant. Two studies were conducted to investigate: 1) the variability among the CM produced in different processing plants and 2) the best starch source or the most suitable proportion of corn and barley to be fed with CM, in order to optimize dairy cattle performance.

The first study consisted of an *in situ* and *in vitro* experiment where ruminal disappearance kinetics and intestinal digestibility parameters were estimated in seven CM samples obtained from different processing plants located in Canada. Canola meal 5, 6, 7, 9, 10, and 11 were obtained from processing plants using solvent extraction and CM12 was from a plant using mechanically extraction. Disappearance and digestibility parameters for CM were compared with SBM. Ruminal degradation and intestinal digestibility varied significantly among treatments. However, values obtained for degradability and digestibility parameters were in agreement with NRC with slight variations. Data from this study suggests that, variabilities in the chemical composition of

CM may be because of the production variabilities occurring during CM manufacturing process in different processing plants.

In the second experiment, CM was used as the primarily protein supplement with four different ratios of corn and barley 1) 100:0, 2) 67:33, 3) 33:67, and 4) 0:100. Sixteen multiparous Holstein cows were used in 4×4 Latin square design. Production parameters were not significantly different among treatments. Milk production was averaged for 41.2 kg/d and the efficiency of milk production averaged 1.53. There were no significant differences between the milk protein fractions among treatments. However, ruminal propionate and acetate to propionate ratio varied quadratically. Ruminal pH, NH_3-N, and plasma glucose concentration were similar to all treatments. Apparent total tract digestibilities of nutrients (except starch) were linearly decreased as the proportion of barley starch increased in the diet. However, total tract starch digestibility averaged 95.5% and was not affected by varying proportions of corn and barley in the diet. Results of this study concludes that there is no significant effect of starch source on animal performances when CM was used as the major protein source, and similar production responses of feeding corn can be obtained by feeding barley or by mixing corn and barley in different proportions.

Keywords: Canola meal, degradability, digestibility, dairy cattle, corn, barley

INTRODUCTION

Global canola production has grown rapidly over the past 40 years, rising from the sixth largest oil crop to the second largest (USDA Economic Research Service, 2012). In the United States, canola seed production has continuously increased during the past few years, and the increment in the production in 2013 was 60% compared to that of 2009 (USDA Foreign Agricultural Service, 2013). Canola production is concentrated mostly in Northern plains of United States where the drier and shorter growing seasons are less favorable for corn and soybean production (USDA Economic Research Service, 2012). Almost all the harvest is crushed into oil and meal within the country. The meal component remaining after the oil extraction, is a good source of protein for livestock feed (Mailer et al., 2008) and has become the second most used protein supplement in the animal feed industry (Huhtanen et al., 2011). Relative to the composition of milk protein, canola meal (CM) has an excellent balance of AA close to that of milk protein (Piepenbrink and Schingoethe, 1998). More importantly, CM possesses an excellent rumen degradable protein (RDP) profile that may stimulate microbial growth in the rumen (Piepenbrink and Schingoethe, 1998) and the production of microbial crude protein. However, variability occurs during the production process in oil plants causes changes in the nutritional properties of the meal, mainly result in a decrease in the concentration and digestibility of protein, individual AA (especially Lys) along with carbohydrates (González-Vega et al., 2011). Thus, an accurate estimation of the degradability and digestibility parameters of dry matter (DM), crude protein (CP) and AA of CM, which is being produced by different processing plants, is important to producers to formulate rations in order to meet the protein requirement of the animal

without overfeeding or underfeeding. In the first experiment, it was hypothesized that, processing conditions used by different processing plants have impacted on the final quality of the meal.Therefore, the objective of the first study was to evaluate the variability of the ruminal degradation and intestinal digestibility of DM, CP and AA of 7 CM samples obtained from different processing plants located in Canada and compare that with SBM using *in situ* and *in vitro* techniques.

Genetic improvements made in dairy cattle have doubled the milk production during the last decade. Moreover, it is quite challenging to meet their elevated nutrient requirement using current feeding systems (Overton et al., 1995). In order to meet animal's elevated nutrient requirement, research efforts have mainly focused on two feeding options namely: 1) providing feedstuffs that are not readily degraded in the rumen and pass to the small intestine (SI) to absorb, and 2) optimizing ruminal fermentation via synchronizing the supply of energy and ruminally available protein to make extensive use of the rumen inhabitant microflora (Overton et al., 1995). Feeding rumen undegradable feedstuffs are costly and do not ensure enhanced production all the time. Therefore, second feeding option may be a better way to meet the challenge in fulfilling animal's requirement and to obtain better production performance. Corn and barley are the widely used grain sources in dairy cattle diets that varies in the starch content and degradability in the rumen (Huntington et al., 1997; Herrera-Saldana et al., 1990). It is important to investigate an alternative starch source for corn, as it keeps increasing its price in animal feed industry. In Canada, barley is the primarily used starch source in dairy and beef cattle diets (Nikkhah, 2012). Whole grain and hull-less barley provide higher amounts of Lys, Met, and Cys than corn, which are considered as limiting

amino acids for the milk synthesis (Huntington et al., 1997; NRC, 2001). Thus, barley can be a better match with CM to be included in dairy rations rather than using corn. Most of the synchronization studies have used factorial designs in which the quantity of one source of non-structural carbohydrate was totally replaced with a similar quantity of another source of non- structural carbohydrate in the presence of different protein sources. Thus, it would be beneficial to determine different proportions of two sources of non-structural carbohydrate (corn and barley) in the same diet, in the presence of CM as the primary protein supplement on the effect of animal performance. Therefore, in the second experiment, it was hypothesized that, cows fed a combination of CM and, corn and barley will have a greater production through a better synchronization of ruminally available protein and energy. Hence the second experiment was conducted in order to determine the best starch source or the best proportion of corn and barley to be fed, and to evaluate the effect of starch source on lactating dairy cow performance in the presence of CM as the main protein source in the diet.

CHAPTER 1

LITERATURE REVIEW

1.1. Use of Canola Meal in Dairy Cattle Diets

Canola meal (CM) has become the most used protein supplement in animal feed industry after soybean meal. Feeding CM has been reported similar or equally satisfactory milk production and composition compared to that of soybean meal, in silage based diets. Being a low cost protein supplement and possessing similar production responses compared to soybean meal must have increased canola meal's usage as freely as soybean meal in the animal feed industry.

1.1.1. History of "Canola" Variety Development

All cruciferous seeds and plants contain glucosinolates, a large group of sulphur-containing secondary plant metabolites (Tripathi and Mishra, 2006) which breakdown in to products associated with deleterious effects (Fenwick & Curtis, 1980). These breakdown products are volatile and strongly pungent and exhibit potentially goitrogenic and hepatotoxic effects upon digestion (Fenwick, 1982). Compared to ruminants, deleterious effects of glucosinolates are greater in nonruminant animals (Tripathi and Mishra, 2006).

Oilseed rape species that are derived from the *Brassica* genus of the Cruciferae (Brassicaceae) family, also known as the mustard or cabbage family is believed to be first originated in the Mediterranean area and was grown in India over 4000 years ago (Environment Directorate, 2011). Thereafter, it became an important oilseed crop in temperate zone countries where most other oilseed crops do not grow vigorously. However, there is no clear evidence to prove the exact time-period when rapeseed oil

became edible or for its use as a fuel for lamp lighting or as an ingredient for soap and candles production (Bell et al., 1984).

Rapeseed was first introduced to Canada from Europe in the 1940's (Environment Directorate, 2011). Thereafter, both rapeseed and its meal (RSM) became important ingredients in animal feeds (DePeters and Bath, 1986). However, due to the fact that, consumption of greater amount of rapeseed with high levels of erucic acid could create potential impacts on health, stimulated Canadian plant breeders to develop genetically modified rapeseed with lesser concentrations of erucic acid. After several years of intensive backcrossing and selection, the first low-erucic acid rapeseed varieties, *Brassica napus* and *Brassica campestris* were released in late 1960's (Bell, 1993) and the name "canola" was adopted in late 1970's in North America in order to distinguish the low-erucic acid variety from other types of rapeseed varieties (Environment Directorate, 2011).

In 1990's, low-glucosinolate rapeseed variety *Brassica juncea* was developed through a cross between an Indian *Brassica juncea* line. Later, breeding programs were initiated to combine the low-glucosinolate characteristics with low-erucic acid trait and to increase the oil content. Thereafter, the term "canola" was registered and started to adopt by many countries to describe the air-dried, oil-free meal contains less than 2% erucic acid and the oil contains less than 30 μmol/g glucosinolates obtained from the species *B. napus*, *B. campestris* and *B. juncea* (Environment Directorate, 2011).

1.1.2. Growth and Distribution of Canola

Earlier varieties of rapeseed contained 110 to 150 μmoles of glucosinolates in the oil-free meal which caused deleterious effects on the livestock (Bell, 1993). With the

development of low-glucosinolate rapeseed varieties in 1970's, production of high-glucosinolate cultivars was eliminated in Canada up to a greater extent (Bell, 1993).

Soybean meal is the most widely-used protein supplement in animal feed industry. Due to the fact that drier and shorter growing seasons in the northern latitudes are less favorable for soybean growth, CM has become the common protein supplement in those areas over the soybean meal (SBM) (Huhtanen et al., 2011). In 2009, canola became the second-highest, commodity-producing oilseed globally with a volume of 60.62 million metric tonnes (MMt), and the third largest source of plant-based oil (after palm and soybean) producing a volume of 22.35 MMt of oil (Environment Directorate, 2011). According to the global oilseed-meal production statistics published by the Environment Directorate in 2011, the share of soybean for the global oilseed meal production was 67% and the contribution of canola (including rapeseed) was 13%.

Canola is produced extensively in Europe, Canada, Mexico, India, China, Japan, Australia, and to a more limited extent in the United States (Environment Directorate, 2011). With the introduction of the variety "canola", Canadian canola producers expanded the lands under cultivation by a greater extent than ever before (Harker et al., 2012). In connection to that, use of CM in feed industry also increased tremendously (Martineau et al., 2013).

In 2009, as a region, the European Union was the world's largest producer of low-erucic acid rapeseed or canola with a production of 21.4 MMt, followed by China at 13.5 MMt, Canada at 11.8 MMt, India at 7.2 MMt and Australia at 1.9 MMt. However, as a country, Canada was the largest exporter of canola seed and oil, accounting for 41.8% and 29.8%, respectively, of world exports (Environment Directorate, 2011).

Canola production in Canada has been steadily increasing and the production in 2012 was 13.3 million metric tonnes of canola seed per year (Canola Council of Canada, 2012). The Canola Council of Canada is targeting to reach the production volume of 15 million metric tonnes by 2015. In accordance with the Canola Council of Canada 2012 annual reports, 50% of production will be exported whereas the rest will be crushed within the country to extract oil for the food and biodiesel industries. According to the National Agricultural Statistical Service annual prospective planning report in 2013, in the United States, 1.37 million acres of lands were under canola cultivation and produced 1.11 million metric tons of canola seed which was an increment of 60% compared to that of 2009 (USDA Foreign Agricultural Service, 2013).

1.1.3. Processing of Canola Meal (CM)

1.1.3.1. Extraction of Oil

Canola or rapeseed is grown primarily for its high oil content (Fenwick, 1982). Canola seed is small and round, 1 to 2 mm in diameter and contains approximately 42 to 43% oil on a dry matter (DM) basis, which is approximately double that of soybean (Newkirk, 2009). In the early days, presence of glucosinolates and erucic acid in original rapeseeds led the majority of extracted oil to be used in the lubrication industry (Fenwick, 1982). However, with the success of plant breeders in removing undesirable components created a new avenue for the canola oil to be used in the human food industry as a premium edible vegetable oil enrich of oleic acid which exerts cardioprotective effects in humans (Ashes et al., 1992; Newkirk, 2009).

Oil extraction from canola seeds can be performed with or without using solvent extraction (Adams et al., 2006). Oil is being expelled from the seeds using cold pressing

or double pressing technique in the processing plant where solvent extraction is not used (Adams et al., 2006; Spragg and Mailer, 2007, Newkirk, 2009). Meal produced by the mechanical extraction contains a higher oil content compared to that of solvent extraction. As it is mentioned in the literature, oil content of the mechanically extracted CM is extremely variable, ranging from a minimum of 8 to a maximum of 30% of DM (Thacker and Petri, 2009). Moreover, similarly to all plant-based oils, higher degree of unsaturation makes the oil susceptible to rancidity. Therefore, the mechanically extracted CM reduces the possibility of longer storage and at the farm level discourages its use in animal feeding compared to solvent extracted meal (Guadagnin et al., 2013). Thus, majority of CM is been produced via solvent extraction as it removes more oil and produces a more storable product (Unger, 1990).

1.1.3.2. Production Steps in a Canola Meal Processing Plant

According to Mailer et al. (2008), "an animal feed which is rich in fiber content, decreases its digestibility and thus, reduces the value of the feed". Since, canola seed has a high concentration of lignin present in the seed hull (12 to 24% of the seed hull DM; Bell et al., 1984) makes it resistant in both ruminal and small intestinal degradation in cattle. Apart from lignin, cellulose and pentosans from cell walls can also be present in the seed hull (Mailer et al., 2008). Therefore, some form of processing is necessary for an effective utilization of canola seed within the digestive tract of the animal (Khorasani et al., 1992).

The main aim of processing CM should be to optimize the small intestinal supply of the most limiting AA for milk synthesis (i.e., Met, Lys, His, and Phe) by minimizing the load of ruminally soluble N (Schingoethe, 1996). Crushing was identified as the

primary method of processing used to improve utilization of nutrients within the seed (Aldrich et al., 1997). As the canola oil is characterized by a greater unsaturated fatty acid composition, inclusion of crushed seeds should be limited to less than 4% of the diet DM to avoid detrimental effects on ruminal fermentation, milk production, and milk composition (Newkirk, 2009). Therefore, canola seed is traditionally crushed and oil is separated from the meal by solvent extraction.

Once the canola seeds arrives the processing plant, it undergoes through a strict grading procedure following the grading standards established by the Canadian Grain Commission. Prior to processing, all the dockage materials are removed. In order to prevent shattering which occurs when the cold seed enters the flaking unit, the seed is preheated to approximately to 35°C in grain dryers. In order to rupture as many cell walls as possible without damaging the quality of the oil, the cleaned seed coat is physically ruptured by roller mills set for a narrow clearance. Having a thickness of 0.30 to 0.38 mm is an important parameter to be maintained in flakes which are being produced (Newkirk, 2009).

Flakes are then conditioned by passing them through a series of steam heated drum or stack type cookers. Cooking basically serves to thermally rupture oil cells and denature hydrolytic enzymes. Temperature of the cooker rapidly increases to 80 to 90°C at the beginning in order to inactivate the myrosinase enzyme present in canola. Myrosinase enzyme hydrolyzes the small amounts of glucosinolates in canola and produces undesirable breakdown products which affect both oil and meal quality. Thus, an inactivation of this enzyme is important to produce better quality oil and meal. In the next step, the cooked canola seed flakes are pressed in a low pressure continuous screw

press or in an expeller. Pressing units are made in a way that holding pressed cake inside, while allowing the oil fraction to flow between the bars. This action removes part of the oil without generating excessive pressure and heat. The objective of pressing is to remove usually 50 to 60% of the seed oil content while maximizing the output of the expellers and producing an acceptable press cake (Newkirk, 2009).

Since pressing cannot remove all the oil out of the seed, the press cake is subjected to solvent extraction to remove the remaining oil. Remaining cake coming out of pressing contains approximately 18 to 20% oil (Newkirk, 2009). Hexane solvent is specifically used for the vegetable oil industry with various mechanical designs of solvent extractors for moving the cake and the miscella (solvent plus oil) in opposite directions to generate a continuous counter current extraction. Basket and continuous loop type extractors are commonly used for canola. Regardless of the extractor type, the cake is deposited in the extractor which is then flooded with solvent. During the continuous counter current extraction, press cake is sprayed with miscella. Thus, oil contains a higher ratio of solvent in proportion. Gravity causes the solvent to percolate through the cake bed, diffusing into, and saturating the cake fragments. The hexane saturated meal contains less than 1% oil when it leaves the solvent extractor, after a fresh solvent wash (Newkirk, 2009).

In a desolventizer toaster, the solvent is removed by heating it on a series of steam heated plates. Meal is then stripped and dried at 95 to 115°C. It takes approximately 30 minutes for this whole process. After completing the drying process, meal averages 12 to 18 % moisture. This procedure is called prepress solvent extraction and the resultant meal usually contains less than 3% oil (Newkirk, 2009). The double-pressing process or

the mechanical extraction is similar to the prepress solvent extraction process, but solvent extraction, desolventization, drying, and cooling is not used. Instead, it uses a second press to squeeze out the additional oil contains in the pre-pressed seeds. In the cold-pressing process, pre-conditioning step is excluded and seeds are directly subjected to pressing by expellers where it maintains the temperature at 60 ºC throughout the process (Adams et al., 2006).

Stripped-out meal is next granulated to a uniform consistency using a hammer mill and is either pelleted or sent directly to storage as a mash (Newkirk, 2009). Temperature during the final step, desolventizing, and toasting of the meal can heavily affect the final meal quality (Newkirk, 2009). Heat applied during both expeller and solvent-extraction processes have detrimental effects upon meal quality. It negatively affects total lysine content and reactive lysine levels (Maison, 2013) in both expeller and solvent meals as a result of the occurrence of Maillard reactions due to the presence of heat and moisture applied to the meals containing amino acids (AA) and reducing sugars. These reactions result in decreased availability of AA (especially lysine) along with carbohydrates (González-Vega et al., 2011). However, provision of heat becomes important to reduce, glucosinolate content in expeller and solvent meals (Spragg and Mailer, 2007). Therefore, it can be postulated that there are differences in processing of CM which may result in variations in the nutritional composition of the meal among different processing plants.

Chemical treatment of canola with alkaline hydrogen peroxide was demonstrated as an alternative method to crushing with a reduced ruminal biohydrogenation of fatty

acids relative to crushed seed and improved postruminal disappearance of fatty acids relative to feeding whole seed (Aldrich et al., 1997).

1.1.4. Composition of Canola Meal

National Research Council (NRC, 2001) book values for the mechanically extracted CM are listed in Table 1.1. However, these values can be varied due to different reasons including climatic and geological variabilities (Bell, 1993) as well as due to different production technologies used in processing plants (Steingass et al, 2013).

1.1.4.1. Factors Affect the Composition of Canola Meal

Digestible energy (DE) or the metabolizable energy (ME) obtained from CM upon digestion varies with the animal species. Typical ME values used in feed formulation in MJ kg^{-1} dry matter, are 8.7, 9.2, 13.2 and 72.1, respectively, for broiler chicken, adult chicken, growing pigs and dairy cattle. Removal of glucosinolates by plant breeding has improved the ME values of the meal compared to that of original rapeseed meal (Bell, 1993). Hull represents about 16% of the seed weight and about 30% of meal weight. It is largely a fiber-rich fraction which remains in the meal after the oil extraction. Yellow-seeded *B. rapa* is characterized by a relatively low fiber content in comparison to brown-seeded *B. rapa*, mainly due to its thinner hull. Moreover, yellow-seeded strains have been shown to be significantly greater in oil and protein content and a lower lignin content (Stringam et al., 1974; Theander et al., 1977), which eventually improved its energy digestibility than the brown seed variety (Bell, 1993).

Environmental factors during the growing season such as moisture, heat stress or frost damage highly effect on the chemical composition of CM mainly through the changes occur in seed size, proportion of hull to the embryo, seed coat color and

composition of the hull (Bell, 1993). There can also be a difference when comparing the oil extraction methods used, i.e.solvent vs. mechanical. Since solvent extraction removes much of total oil than mechanical extraction CM produced in those plants may have higher protein and a lower fat content (Newkirk, 2009).

It is important to follow the temperature guidelines in order to ensure the high quality of CM. During the early processing, it should be guaranteed that the temperature is not too low (Newkirk, 2009). If the temperature is below 80˚C, myrosinase enzyme will convert glucosinolates present in the seed in to a toxic compound. On the other hand, prolonged high temperature periods during processing will result in decreased protein quality of the meal (Newkirk, 2009). High temperature during processing can result in an undesirable level of browning decreasing both protein and carbohydrate availability to the animal (González-Vega et al., 2011). Usually the crude protein (CP) content, digestibility and apparent metabolizable energy are numerically lower in the final product compared that of wet CM. As investigated in the literature, CM is a uniform and high quality product until it enters the final drying phase (Newkirk, 2009).This emphasizes that the desolventizer toaster phase temperature is much more a critical factor when determining the final quality of CM.

1.1.4.2. Protein and Amino Acid

The amino acid composition of CM compares very well with that of soybean meal. Soybean meal has higher lysine content and CM is an excellent source of sulfur containing amino acids, namely methionine and cystine (Newkirk, 2009). Amino acid composition of CM is as listed in Table 1.2.

According to the Canola Council of Canada guidelines in 2009, the minimum CP guarantee for Canadian CM is 36% (8.5% moisture basis), although the actual protein content usually varies from 36 to 39%. Furthermore, canola crusher has some influence on the protein composition of the meal by adjusting the level of oil and carbohydrate (Newkirk, 2009).

Of all the protein sources listed in the Table 1.3, CM has the best AA balance which is indicated by the relatively high level of its first limiting AA. Interestingly, rumen degradable protein (RDP) fraction of CM necessarily stimulates the microbial growth in the rumen (Piepenbrink and Schingoethe, 1998) by efficient provision of AA and peptides which readily incorporate into microbial mass and leads for efficient milk synthesis (Bach et al., 2005).

The rumen undegradable protein (RUP) fraction in CM contains essential AA that closely match that of milk protein (Newkirk, 2009). As observed by Brito et al. (2007), intestinal supply of total AA, essential AA (EAA), branched chain AA and limiting AA (basically methionine, lysine, histidine, and threonine) are numerically higher or at least comparable when CM is used as a protein supplement than when diets are supplemented with SBM or cottonseed meal (CSM). Hence, the improved milk production observed in CM fed cows is attributed to the amino acid profile of the RUP fraction of CM is being complementary to microbial protein (Brito and Broderick, 2007).

Milk protein score is another index which can be used to measure the protein quality of the feed ingredients for dairy cattle. The milk protein score of common ingredients as calculated for a corn, corn silage and alfalfa based diet indicated that, CM

had the highest score out of all the plant protein sources which was closer to that of rumen microbial protein (Schingoethe, 1991).

1.1.5 Feeding CM on Dairy Cattle Performance

1.1.5.1. Palatability

Canola meal is classified as a highly palatable protein source for ruminant animals. The reason for palatability has not been studied in depth. It may be a factor related to the high sucrose content in CM (Newkirk, 2009). In a study which was conducted on eating rate and preference in dairy heifers using common protein sources, Spörndly and Åsberg (2006) observed that heifers consumed 221 g of CM in the first three minutes while those fed SBM only consumed 96 g when they fed a mash diet which clearly demonstrated that the highly palatable nature of CM. Ravichandiran et al. (2008) fed rapeseed meal with varying levels of residual glucosinolates to five-month-old dairy calves and examined the impact of residual glucosinolate content upon feeding. Calves receiving low residual glucosinolate rapeseed meal (<20 umol/g) consumed the same quantity of feed compared to the control diet without rapeseed meal (1.10 vs 1.08kg, respectively), while calves fed high glucosinolate rapeseed meal (>100 umol/g) only consumed 0.76 kg. Hence, when including canola meal in a ration, it is important to ensure the meal is derived from modern low glucosinolate varieties.

1.1.5.2. Lactation Response to Canola Meal

Sanchez and Claypool (1983) compared CM, cottonseed meal (CSM) and SBM as a protein source for cows during early lactation, and found that cows fed CM tended to have greater milk production while having no effect on milk components and feed intake. Piepenbrink and Schingoethe (1998) observed similar milk production when CM was fed

as the protein supplement than when CM was fed in combination with other high quality protein sources that included blood meal, corn gluten meal, and fish meal. In this study it was determined that ruminal protein degradability of CM was greater compared to the other protein sources which brought the idea of matching the protein degradability with the starch source. However, CM was capable of supplying an effective AA profile to the intestine which closely matched with milk protein out of the four supplements (Piepenbrink and Schingoethe, 1998).

Maesoomi et al. (2006) compared the lactation response of the diets where the CSM was substituted by CM. Daily DMI, feed efficiency, milk yield, milk fat % and yield, and 4% fat-corrected milk (FCM) were not affected upon substitution. However, milk protein % and solids non-fat (SNF) % were increased by dietary substitution of CM for CSM. Furthermore, a greater digestibility of DM and CP were reported upon replacement. These improvements were made when the daily intake of CM was 3.4 kg/d which allowed 10% more forage inclusion compared to cotton seed meal (Maesoomi et al., 2006).

Brito and Broderick (2007) compared urea with three true protein sources namely: SBM, CSM and CM. True protein supplements were superior to urea as CP sources, improving feed intake and yields of milk fat and protein. The CM fed group had the highest DMI among the true protein sources. Furthermore, the cows fed the CM had the numerically greatest milk yield (Brito and Broderick. 2007).

In accordance with Huhtanen et al. (2011), milk yield, energy-corrected milk (ECM) and milk protein yield were significantly greater in CM and heat treated CM fed groups compared to SBM. Interestingly, CM supplementation increased DMI more than

SBM. And the increment was 2.6 and 1.6 kg DM/d, respectively for CM and heat-treated CM. Greater responses in DMI with CM compared with SBM could be because of increased supply and more balanced supply of AA that improves the performance and as a result of increased energy demand. This suggests that improved performance with CM supplementation was at least partly derived from enhanced energy rather than protein intake (Newkirk, 2009). The abundance of essential AA and the extent to which it supplies AA may in part explain the consistent milk yield response found when CM is included in dairy cow rations.

In a meta–analysis of canola feeding studies, Martineau et al. (2013) reported the response of substituting various protein sources (SBM, corn gluten meal, cotton seed meal and distillers products) with CM. The database included 27 experiments with 88 experimental diets. Experiments that exceeded the dietary inclusion of CM of 17.2% of diet DM were not included in the database. Milk yield, FCM, ECM and milk protein yield responded positively ($P<0.001$) as a result to the substitution by CM. In addition, apparent N efficiency was also positive, mainly due to the positive response in milk protein yield (Martineau et al., 2013).

1.2. Protein Requirements of Lactating Dairy Cows

1.2.1. Protein Utilization by the Animal

Dairy cows consume protein to supply adequate amount of N for microbial growth in the rumen and to obtain AA for the maintenance and milk production (Clark et al., 1992). Dietary protein can be divided into RDP and RUP, where RDP is composed of non-protein and true protein N (Bach et al., 2005). True protein is degraded in the rumen into peptides and AA which eventually deaminate into ammonia-N, VFA, and CO_2

(Tamminga, 1979) or incorporate into microbial protein. Non-protein N is composed of N present in DNA, RNA, ammonia, and AA (Bach et al., 2005). Ammonia is the main N source in the rumen and in the presence of sufficient energy, ruminal microflora synthesize microbial protein (Russell et al., 1992). Therefore, N metabolism in the rumen can be divided into two distinct phases namely: protein degradation, which provides N sources for bacteria, and microbial protein synthesis (Bach, 2005).

1.2.2. Microbial Protein Synthesis in the Rumen

Microbial protein is the major protein supplement to the small intestine (SI) of ruminants, accounting for 50 to 80% of total absorbable protein (Storm and Ørskov, 1983). Along with microbial protein, dietary protein that escapes ruminal degradation and endogenous protein provide AA to the SI in order to fulfil metabolic demands of the animal (Clark, 1992). Peptides (mainly di- and tri- peptides) may contribute significantly to AA absorption from the intestines of ruminants (Remond et al., 2000) and may have nutritional benefits for the host animal (Webb and Matthews, 1998).

Feed protein supplements differ substantially in ruminal degradability and they supply different amounts of RDP and RUP with varying AA compositions (NRC, 2001). Because microbial protein is the major source of metabolizable protein to the lactating cow, the most effective RUP sources should have AA profiles that are complementary to microbial protein (Broderick, 1994). Degradation of OM and synthesis of microbial protein are depressed when N is deficient for the growth of ruminal bacteria (Smith, 1979). However, ruminal bacteria are efficient scavengers of ammonia and they can grow on relatively low concentrations of ammonia in ruminal fluid (Schaefer et al., 1980).

Satter and Slyter (1974) suggested that 2 to 5 mg of ammonia N/dL of ruminal fluid are adequate for maximizing passage of microbial N to the small intestine (SI).

A deficiency of any nutrient may decrease microbial protein synthesis in the rumen, passage of AA to the SI and eventually the milk production by the animal, but the two most important nutritional factors that are likely to be limited are energy and protein (Clark and Davis., 1980). In accordance with McCarthy et al. (1989), microbial N supplies about 35 to 66% of the non-amino N (NAN) that passes to the SI when large amounts of typical well balanced diets are consumed by dairy cows producing greater than 30 kg of milk daily. Therefore, protein supplements with low ruminal degradability increase the proportion of non-amino N microbial N (NANMN) and decrease the proportion of microbial N in NAN that passes to the SI. This emphasizes that the increment of RUP supply does not ensure improved milk protein production all the time (Santos et al., 1998). However, for high producing dairy cows, there should be sufficient protein in the diet to optimize microbial growth and fiber digestion in the rumen, and adequate amounts of essential AA to be available in the small intestine to provide for their increased metabolic and lactation demands (NRC, 2001; Cant et al., 2003).

1.2.3. Protein Degradability and Digestibility Fractionation of Feedstuff Using In situ and In vitro Techniques

1.2.3.1. Importance of Characterization of Feed Ruminal Degradability

During diet formulation, the attempt is to balance the protein in the diet for optimum ruminal degradation with the aim of maximizing microbial protein synthesis and to supply SI, the required dietary protein portion that escapes ruminal fermentation (Roe et al., 1991). Feed will vary with the protein degradation rate depending on its

proportions of non-protein nitrogen (NPN), true proteins and undegradable or unavailable protein. Therefore, each feed will have a characteristic protein degradation curve during ruminal fermentation based on its protein proportions. At present, there are numerous methods available for estimating ruminal protein degradation of feedstuffs. The *in situ* technique is one of the most practiced methods which involves the incubation of the feedstuffs in a rumen of a canulated animal at various time intervals to measure the ruminal CP degradability of that particular feed. However, this procedure is not practical for commercial laboratories (Roe et al., 1991) since it is time consuming and requires much labor (England et al., 1997). In vitro technique is another way of analyzing feed protein fractions within a laboratory environment inside an incubator. Therefore, it has been adopted by many of the researchers and commercial laboratories considering the easiness and less time required.

1.2.3.2. In Situ Technique

With regard to ruminal availability, feeds can be categorized into soluble, potentially degradable and undegradable portions which are commonly referred as A, B and C fractions (Nocek, 1988).The *in situ* procedure can be used to quantify these factions as well as the rate of ruminal degradation of the potentially degradable fraction. Furthermore, the number of time points during the digestion should be adequate in order to detect multiple rate components, lag time and the end point of the digestion. For concentrates and protein feed ingredients 48h incubation is sufficient, whereas for forage, 72h or 96h incubation is recommended (Nocek, 1988).

During an *in situ* study allows the intimate contact of the feed with the ruminal environment. Although it provides the actual rumen environment, the particular feed is

not subjected to mastication, rumination, and passage to complete the total rumen experience. Furthermore, many inherent factors of the *in situ* technique (e.g., bag pore size, sample size, sample particle size) can influence on the final result (Nocek, 1988). When the feedstuff is coarsely ground, it is associated with slower rates of digestion and greater variation, whereas finely ground feedstuffs are subjected to greater mechanical losses from the bags (Nocek, 1988). However, the variability is low for the materials which are smaller and more uniform compare to coarse ground. Grinding of feedstuff prior to incubation increases surface area per unit weight that is accessible for microbial attachment. Digestion rate reduces as sample size increases in relation to bag surface. When the bag is compacted, this restricts the microbial contact with feed particles, thus reducing the digestion rate. The diet that is given during the *in situ* procedure also has an impact upon increasing the digestion via removing the clogging materials associated with bag pores produced by bacteria. To obtain a reliable value, original samples should be corrected for bacterial contamination during every *in situ* study (Nocek, 1988).

1.2.3.3. In vitro Technique

In vitro techniques do not involve direct contact with animals. During an *in vitro* study, environment of the rumen or the SI is mimicked inside a laboratory using appropriate enzymes. Enzymatic digestion techniques are more suitable to measure relative differences between feedstuffs than providing absolute digestibility values. Prediction accuracy greatly depends on the relative complement of enzymes used in the incubation (Nocek, 1988).

In vitro studies can be performed in two ways: batch culture and continuous culture. In a batch culture or single or two-stage Tilly and Terry system, digestion is done

with rumen fluid for 48 h and followed by a second 48 h digestion using pepsin and weak acid to stimulate post-ruminal digestion. Protein degradation is supposed to be underestimated as ammonia is utilized for the microbial growth (Nocek, 1988). Continuous culture systems are much more suitable to alter ruminal environment compared to batch cultures. It incorporates a dual-effluent removal system for both liquids and solids. System has a rapid buffer input to maintain pH, which prevents a longer incubation time for digestion of particulate matter (Hoover, 1976). The major drawback associated with continuous culture systems is the difficulty in characterization of digestion rates and extents of individual feedstuffs without adding a marker for identification (Nocek, 1988).

1.2.4. Nitrogen Excretion Due to Overfeeding

Ruminant diets should supply sufficient RDP to support the growth of rumen microbes (Agricultural Research Council, 1980; NRC, 2001) whereas low RDP levels may compromise microbial growth, DM digestibility, and protein availability to the host. However, excess RDP that is not utilized for microbial growth is excreted in feces or deaminated into ammonia and excreted via urine and milk as urea resulting in inefficient N utilization by the animal (Broderick et al., 1992; Clark et al., 1992). Urinary urea contributes to environmental pollutants, such as atmospheric ammonia and nitrates in ground water (Tamminga, 1992). Under typical dairy cattle feeding conditions, manipulation of rumen protein degradation or the efficiency of N utilization (ENU) in the rumen is the most effective strategy to reduce N losses (Tamminga, 1996). Losses of N may be reduced by decreasing protein degradation in the rumen and (or) increasing N use by ruminal microorganisms (Reynal, 2003).

1.3. Carbohydrate Fermentation in the Rumen

1.3.1. Improving the Efficiency of N Use: Role of Ruminally Fermentable CHO

The fate of absorbed peptides and AA inside the microbial cell will depend on the availability of energy in the rumen (Tamminga, 1979). Furthermore, the availability of sufficient energy is vital for the N utilization within the animal. According to the calculated values reported by Tamminga (1992), in most of the dairy systems, the efficiency of utilization of dietary N for milk protein synthesis by dairy cows averages around 19 to 20%, a poor efficiency which is partially related to the N loss as ammonia occurs in the rumen (Tamminga, 1992). Since rumen microbes synthesis a large proportion of cell protein from ammonia-N (Hristov and Broderick, 1996), it will result an efficient transfer of ruminal ammonia-N into body and milk proteins by enhancing microbial protein synthesis in the rumen. Microbial protein yield can be increased by regulating primarily the energy intake and the CHO availability in the rumen (Hristov and Ropp, 2003).

Bacteria can use CHO and proteins as energy sources. Carbohydrates are the main energy source for bacteria because upon fermentation they yield more energy per unit of weight than protein (Stem et al., 1978). Among all CHO sources which are distinguished by the Cornell Net Carbohydrate and Protein System (CNCPS), cereal grains can be identified as the most common sources of readily available energy for livestock and account up to 60% of the total diet for high-yielding dairy cows (Herrera-Saldana et al., 1990). If energy is available AA will be transaminated or used directly for microbial protein synthesis using carbon skeletons of CHO in combination with ammonia. However, if energy is limiting, AA will be deaminated, and their carbon skeleton will be

fermented into VFA. Some ruminal bacteria are lacking mechanisms of AA transport from the cytoplasm to the extracellular environment and in such situations, excess AA should be absorbed and must be excreted from the cytoplasm as ammonia (Tamminga, 1979). Ruminal microbial protein synthesis depends on supply of adequate amounts and type of CHO as an energy source for the synthesis of peptide bonds. Readily fermentable CHO, such as starch or sugars are more effective than other CHO sources, such as cellulose in promoting microbial growth (Stern and Hoover, 1979).

A study completed by Cameron et al. (1991) demonstrated that infusions of increasing amounts of readily fermentable CHO decreased ammonia-N concentrations because of the improved N uptake by ruminal microbes. Hoover and Stokes (1991) suggested that, in pH controlled continuous culture fermenters maximized microbial growth is attained when the ratio between NFC to RDP is 2:1. Even though the CHO supplement in the diet is adequate, there can be a reduction of the passage of microbial protein and AA to the SI because of decrease in OM intake, reduced OM and fiber digestibilities in the rumen, energetic uncoupling of ruminal fermentation or a shortage of N constituents in the ruminal environment other than ammonia (Clark et al., 1992).

The ultimate goal of proper rumen nutrition is to maximize microbial growth and the amount of RDP that is captured into rumen microbial cells. Maximizing the capture of degradable N not only improves the supply of AA to the SI, but also decreases N losses. Carbohydrate greatly influences on the amount of ruminal ammonia-N incorporated into microbial protein (Firkins et al., 2007). In addition to the importance of the amounts of nutrient supply, synchronization between the rates of degradation of protein and CHO also becomes important. When rate of protein degradation exceeds the

rate of CHO fermentation, large quantities of N can be lost as ammonia, and vice versa when the rate of CHO fermentation exceeds protein degradation rate, microbial protein synthesis can be decreased (Nocek and Russell, 1988).

1.3.2. Readily Fermentable CHO Sources Available in the Feed Industry

Lactating dairy cows require comparatively large quantities of digestible energy for a high milk production (Yang et al., 1997) and for the maintenance and pregnancy. Carbohydrate provides more energy upon fermentation than proteins and fat.Therefore, dairy cow diets typically comprises of 70 to 80% of CHO (Nocek and Russell, 1988) and in United States, these diets contain 20 to 30% starch on DM basis (Ranathunga et al., 2010). In the provision of digestible energy, cereal grains are widely used to meet the energy demands for high milk production than forages (Yang et al., 1997).

Dairy rations formulated in western Canada and the United States use cereal grains such as barley, corn, wheat, or oats as the main readily fermentable carbohydrate sources due to their cost effectiveness which promotes them to be used as a source of digestible energy (Gozho and Mutsvangwa, 2008). Among grains used, corn is the heavily-used cereal grain in most of Canada, United States and southeastern Asia in order to provide starch in lactating dairy cow diets. In western North America and in Europe, barley is considered as the predominant grain used in dairy rations (Yang et al., 1997). Starch is the major nutrient that cereal grains provide to a dairy ration (Huntington, 1997). As mentioned in the review by Huntington (1997), cereal grains basically differ in their starch content, with wheat containing 77%, corn containing 72%, and barley and oats 57 to 58% starch on a DM basis.

The high starch content in corn has permitted its usage in high-producing dairy cow diets as the commonly used starch source, in order to meet the energy requirements and to promote microbial protein synthesis in the rumen (Ranathunga et al., 2010). Rates and extents of ruminal starch degradation are 92 to 94%, 80 to 90%, 55 to 70%, and 62% respectively for oats, wheat and barley, corn, and milo (sorghum) (Huntington, 1997; Herrera-Saldana et al., 1990). Therefore, grains can be ranked as oats, wheat, barley, corn, and sorghum according to the starch degradation rates in the rumen (Herrera-Saldana et al., 1990).

Rate and extent of fermentation of dietary carbohydrates in the rumen are important parameters that determine nutrient supply to the animal (Hall, 2004). Increased ruminally available energy content of diets for dairy cows will lead towards a greater microbial protein synthesis which will eventually enhance milk production through increased metabolizable nutrient supply (Gozho and Mutsvangwa, 2008).

Due to the surrounding fibrous hull and the pericarp, intact barley seed is highly resistant for digestion takes place in the rumen. Thus, barley possesses a low digestible energy compared with corn (Yang et al., 2000). Therefore, cows fed diets based on whole barley grains produce less milk in comparison with corn-based diets (Casper et al., 1989). Diets that are based on processed barley are rapidly fermented in the rumen would be a more economical source of readily fermentable NSC to increase utilization of NPN for rumen microbial protein synthesis (Casper et al., 1989).

Rapid fermentation occurs following ingestion of diets containing a large concentration of processed barley can increase the incidence of bloat, acidosis, laminitis, liver abscesses and feed intake problems related to digestive upsets (Yang et al., 1997).

Between 80 and 90% of wheat starch and barley starch is digested in the rumen; whereas for sorghum and corn, it ranges from 55 to 70% (Nocek and Tamminga, 1991).

In a study comparing oats, wheat, barley, corn, and sorghum as sources of starch in dairy cow diets, plasma urea N tended to be greater in cows fed the oats-based TMR compared with those fed the barley, corn, wheat, or sorghum (Gozho and Mutsvangwa, 2008). Cows fed oats-based TMR consumed 1.2 to 1.8 kg/d less starch compared to other treatments.

Since the total starch and ruminally degradable starch intake play a huge role in determining the efficiency of assimilation of ruminal ammonia-N for microbial growth, the lesser starch intake in oats diet fed to cows could potentially have limited the ability of ruminal microorganisms to utilize ruminal ammonia-N and thus, increased the rates of ureagenesis (Gozho and Mutsvangwa, 2008).

In the comparison of CP and starch degradation in corn and milo at 12 h, less than 80% of CP and less than 66% of starch had been degraded. These values conclude that, under normal rumen particulate outflow rates considerable amounts of CP and starch from these grains would escape rumen degradation and would be available for intestinal digestion (Herrera-Saldana et al., 1990).

Greater proportion of barley starch (80 to 90%) is digested in the rumen; whereas, the values for sorghum and corn ranged from 55 to 70% (Nocek and Tamminga, 1991). Therefore, animals fed barley-based diets may compensate for the apparent inefficiency of ruminal digestion of starch, through increased microbial protein synthesis (Herrera-Saldana et al., 1990) compared to corn.

Higher proportion of corn starch reached the small intestine compared to that of barley. Digestion of starch in the small intestine is found out to be more efficient than the digestion and the absorption of the resulting VFA in the rumen (Owens et al., 1986). Thus, corn fed diets are superior in efficient starch digestion compared to barley and wheat which possess a rapid starch fermentation in the rumen.

1.3.3. Lactation Response of Feeding Corn and Barley

According to the study done by Casper et al. (1990) comparing corn and barley in the presence of SBM and urea, there was no significant difference on milk production for the cows fed barley or corn. Four percent fat corrected milk (FCM) and solid corrected milk (SCM) were lower ($P < 0.10$) for barley fed group. Milk fat percentage was also greater in cows fed corn. However, milk protein % was not affected by the grain source (Casper et al., 1990).

In an experiment conducted by Grings et al. (1992), corn was compared with three densities of barley (44, 49, or 53 lb/bu) on production in early lactation using 24 multiparous lactating Holstein cows. Diets containing 49 lb/bu barley were formulated to be isocaloric with corn diet. No effect ($P > 0.10$) of grain source or density on milk production and composition was found for the cows in early lactation (Grings et al., 1992).

Yang et al. (1997) fed a group of cows of diets containing corn, barley and hull-less barley (HB) and measured the variability in milk production. For the primiparous group, milk production and yields of 4% FCM and SCM were greater for corn treatment ($P < 0.05$). For the older cows fed corn had a greater milk production ($P < 0.05$) and numerically higher yields of 4% FCM and SCM than barley and HB. Milk production

and yields of 4% FCM and SCM were similar for barley and HB diets for both groups of cows. Milk composition was unaffected by diet except for lactose. For multiparous cows, corn diet fed group had the higher lactose content than barley and HB diets (Yang el al., 1996).

Overton et al. (1995) fed dairy cows with different proportions of corn and barley (corn was replaced linearly with 25% of barley) in the presence of SBM as the major protein supplement in diets. As the barley starch increased in the diets, milk production decreased quadratically. Moreover, 3.5% FCM and CP% decreased linearly as barley replaced corn in diets. CP yield and the fat % varied quadratically as barley increased in the diets.

Casper et al. (1999) conducted a study using corn and barley as starch sources with two protein supplements (SBM and extruded SBM). Production of milk, 4% FCM, SCM were greater for cows fed corn than fed barley. Higher starch content in corn might have affected on greater production. Milk composition was unaffected by grain type except for lactose. Cows fed corn had higher lactose compared to that of barley (Casper et al., 1999).

CONCLUSIONS

Canola meal is the most widely used protein supplement in dairy rations in the United States after soybean meal. Among all the plant-based protein sources, CM has the best balance of EAA which closely matches with rumen microbial AA content. Moreover, the RDP fraction necessarily stimulates ruminal microbial protein synthesis and its RUP fraction supplies AA to the animal which are complimentary to the microbial protein that is attributed to an increased milk production.

The availability of sufficient energy is vital for an efficient utilization of dietary N for milk synthesis via enhanced microbial protein synthesis. Among CHO and proteins as energy sources, ruminal microbes prefer CHO, as they yield more energy upon fermentation. Out of all CHO sources listed, cereal grains are the most commonly used readily available energy source for livestock. Corn and barley are the cereal grains used in dairy rations than any other cereals. So far, researchers have focused on replacing corn with other CHO sources. However, none of them were evaluated the effect of combining two different cereal grain sources in the presence CM as the main protein supplement on animal's performance. Therefore, the combination of corn and barley in different ratios can provide a better synchronization with CM in order to enhance microbial protein synthesis and eventually the milk production via reducing dietary N waste.

Table 1.1. Chemical composition of canola meal (Adapted from NRC, 2001)

Item	Value (% of DM)
CP	37.8
RUP (% of CP)	35.7
NDF	29.8
ADF	20.5
Lignin	9.5
Ash	7.4
EE	5.4

Table 1.2. Amino acid composition of canola meal (Adapted from Canola council of Canada, 2009)

Amino acid	Proportion as a % of CP on DM basis
Ala	4.36
Arg	5.78
Asp + Asparagine	7.25
Cys	2.39
Glu + Glutamin	18.1
Gly	4.92
His	3.11
Ile	4.33
Leu	7.06
Lys	5.56
Met	2.06
Phe	3.83
Pro	5.97
Ser	4.00
Thr	4.39
Try	1.33
Tyr	3.22
Val	5.47

Table 1.3. Feed ingredients and rumen microbial amino acid composition compared to milk protein (The first limiting amino acid in each ingredient is highlighted; adapted from NRC, 2001)

	Amino acid as a % of milk protein							
Item	Milk,% EAA	Rumen microbe	CM	SBM	Corn gluten meal	Cottonseed meal	Sunflower meal	DDGS
Arg	7.2	139	197	225	99	361	288	149
His	5.5	**73**	138	111	85	120	113	120
Ile	11.4	107	83	89	80	64	87	86
Leu	19.5	81	**82**	88	190	71	133	130
Lys	16.0	119	84	87	**23**	**61**	**50**	37
Met	5.5	84	95	**58**	95	67	102	87
Phe	10.0	104	103	116	141	125	110	**34**
Thr	8.9	121	113	98	84	85	98	102
Trp	3.0	90	115	93	40	93	97	77
Val	13.0	85	88	78	79	77	90	96

CHAPTER 2

RUMINAL DEGRADABILITY AND INTESTINAL DIGESTIBILITY OF PROTEIN AND AMINO ACIDS IN CANOLA MEAL

ABSTRACT

Differences in processing by different plants may result in canola meal (CM) with varying nutritional composition. The Dairy NRC (2001) estimated CM to be 35.7% rumen undegradable protein (RUP) with an intestinal digestibility of 75% when DMI was set at 4% of BW. Seven CM samples (CM5, CM6, CM7, CM9, CM10, CM11, and CM12) were obtained from different processing plants and 1 soybean meal (SBM) to evaluate the variability in ruminal degradability and intestinal digestibility of CP. Dacron bags containing 5 g of each feed were incubated in the rumen in replicates for 0, 2, 4, 8, 12, 16, 24 and 48 h using three ruminally cannulated lactating cows. The rate of passage was calculated at 6.6%/h. The A fraction (rapidly degradable CP) varied from 17.8% to 26.6%, respectively, for CM5 and CM10 ($P < 0.05$). The B fraction (slowly degradable CP) was least for CM12 (62.4%) and greatest for CM5 (79.9%), whereas the C fraction (undegradable CP) was least for SBM (0.6%) and greatest for CM12 (14.6%). The rate of degradation of B fraction, Kd (%/h) was least for CM12 (4.0%/h) and greatest for SBM (11.1%/h). The RUP (% of CP) was least for SBM (31.0%), whereas, greatest for CM12 (53.8%), while the IDP (measured by pepsin-pancreatin digestion) ranged from 71.6% for CM10 to 94.5% for SBM. The total digestible protein (TDP) was greatest for SBM (98.2%) and CM ranged from 85.1% to 90.8% for CM12 and CM10 ($P < 0.01$), respectively. The mean ruminal and intestinal digestibilities of CM are in agreement with NRC, however considerable variation exists among CM processing plants.

Keywords: Canola meal, degradability, digestibility, protein, amino acids.

2.1. INTRODUCTION

Canola meal is the primary source of protein in Western Canada and some parts of United States where Soy is a low-yielding crop (Mulrooney et al., 2009; Huhtanen et al., 2011). Amino acid profile of CM closely matches with that of milk and rumen microbes, and when fed, it is speculated to be efficiently converted for milk synthesis of dairy cows (NRC, 2001). Therefore, CM has become an important protein supplement in dairy cattle diets competing with SBM. Canola meal is a by-product in the manufacturing process of canola oil (Theodoridou and Yu, 2013), which can be performed with or without using solvent extraction (Adams et al., 2006). Double pressing and cold pressing techniques are involved in the processing which does not use a solvent for the oil extraction (Adams et al., 2006; Spragg and Mailer, 2007, Newkirk, 2009). Quality of the meal is affected by several factors namely: seed variety, growing conditions, geological variability such as soil types, and processing conditions (Bell, 1993). Final quality of the meal depends on the processing condition and the oil extraction technique adapted by the individual processing plant (Steingass et al., 2013). The purpose of using different heat processing techniques is to decrease the rumen fermentation and the degradation of nutrients, while enhancing the availability of protein or essential amino acids that reaches the small intestine for digestion and absorption (Schingoethe, 1996). Moreover, heating is one of the factors that affect the rumen degradability of protein from a particular feedstuff (Dakowski et al., 1995). However, undesirable high temperatures used in the oil extraction steps increase the incidences of Maillard reactions occurring between AA and reducing sugars, potentially decreasing the availability for intestinal digestion because of

the overprotection created (Dakowski et al., 1995; González-Vega et al., 2001). Therefore, the application of heat must be well-balanced between beneficial and destructive levels (Dakowski et al., 1995). In ruminants, feed proteins extensively degrade in the rumen. Hence, protein supply to the animal from a particular feed is highly dependent on the delivery of amino acids to the duodenum and their digestibility within the SI rather than the amount ingested with the diet (Dakowski et al., 1995). Therefore, it is crucial to know the actual amounts of protein and AA that are being supplied and absorbed by the cow from a particular feedstuff in order to formulate rations to avoid overfeeding and underfeeding. It is hypothesized that, processing conditions adapted by different processing plants have impacted on the final quality of the meal. Moreover, accurate information on the supply of protein and amino acids provided by CM is also insufficient for the producers. Therefore, the objective of this study was to compare ruminal degradation and intestinal digestion and absorption of protein and amino acids supplied by CM produced by different processing plants located in Canada.

2.2. MATERIAL AND METHODS

2.2.1. Animals and In situ Procedure

Experimental procedure was approved by the South Dakota State University (Brookings) Institutional Animal Care and Use Committee. Three ruminally cannulated lactating Holstein cows (197 ± 16 DIM, 757.7±25 kg BW) producing an average of 66.5 ± 9.2 lb/d of milk, were fed for ab libitum consumption of a diet containing 56% of forages and 44% concentrates (Table 2.1). Chemical composition of the feed that offered to the cows during the trial is presented in Table 2.2. Seven CM samples were obtained from seven different processing plants across Canada and all those samples were

manufactured from seeds harvested in a same growing season, so that climatic factor affecting on seed nutritional profile thought to be negligible. Commercially available SBM was used as the control along with CM to examine ruminal degradability and intestinal digestibility parameters for dry matter, crude protein and individual AA. Out of seven CM samples, CM5, CM6, CM7, CM9, CM10, and CM11were solvent extracted products, wherein CM12 was the only mechanically extracted product. The chemical compositions of CM and SBM used in this study are listed in Table 2.3. Feed samples were ground to pass through 2 mm screen. Five grams of ground feedstuff were weighed into replicated Dacron bags measuring 10 x 20 cm with a pore size of 50 µm (Ankom Technology, Macedon, NY) and heat sealed. Samples were weighed in duplicates for 0, 2, 4, and 8 h time points. Six bags were prepared for 12 h time point for each feedstuff to ensure enough residues for the intestinal digestibility analysis and for 16, 24 and 48 h time points, three bags were prepared for each feedstuff. Prior to incubation in the rumen, bags were soaked in warm water (39˚C) for 20 minutes. Bags were placed in a large mesh nylon bag with a weight and suspended below the particulate mat layer in the ventral sac of the rumen. Samples were incubated for 0, 2, 4, 8, 12, 16, 24 and 48 h. Bags for all the time points (except 0 h), were placed in the rumen in a reverse order so that they were all taken out at once. Duplicate blank bags corresponding to each time point were inserted into the rumen along with the treatment bags in order to correct for the microbial attachment and for the material that was being accumulated during the incubation period. Immediately after removal from the rumen, the mesh bags were cleaned with tap water to remove attached feed particles. Individual nylon bags were then rinsed in a domestic washing machine under appropriate settings with cold water. Washing cycle was repeated

until effluent water ran clear. Bags were dried for 48 h at 55˚C in a forced air Despatch oven (style V-23: Despatch Oven Co., Minneapolis, MN). Dry matter disappearance was calculated by the weight difference of the original sample and the residue of the post-ruminal incubation. Bags of 16 h time point were air dried to analyse the AA composition.

2.2.2. In vitro Procedure

Intestinal digestibility of RUP (IDP) and AA were determined using modified 3 step procedure described by Gargallo et al. (2006). One gram of dried sample residues from the 12 h time point was weighed into nylon bags (Ankom R510, pore size 50 µm, Ankom Technology, Macedon, NY) in triplicate and heat sealed. Thirty nylon bags filled with sample and 2 blank bags were placed in each incubation bottle of a Daisy II incubator (Ankom Technology, Macedon, NY). Two liters of prewarmed 0.1 N HCl solution (pH 1.9) containing 1 g/L of pepsin (P-7000, Sigma, St. Louis, MO) was added into incubation bottles. Bags were incubated with constant rotation at 39ºC for 1 h. After incubation, liquid was drained and bags were rinsed with tap water until the rinse water was clear. After rinsing bags were introduced back to the incubation bottles filled with 2 L of a prewarmed pancreatin solution (0.5 M KH_2PO_4 buffer of pH 7.75, containing 50 ppm of thymol and 3 g/L of pancreatin) and were incubated with constant rotation at 39°C for 24 h. After removal, bags were thoroughly washed with tap water and dried at 55°C for 48 h. After weighing, residues for each sample were pooled by cow and were used for further analysis.

2.2.3. Chemical Analysis

Individual feed ingredients (CM and SBM) and TMR samples were ground to pass through a 2 mm screen using Wiley mill (Model 3; Arthur H. Thomas., Philadelphia, PA) and then reground to 1 mm using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). Dried ground feed samples were sent to Dairyland laboratories (Arcadia, WI) for the chemical analysis. One gram of ground feed samples were dried for 3 h at 105ºC. Neutral detergent fiber (Van Soest et al., 1991) and acid detergent fiber (Robertson and Van Soest, 1981) were analysed using an ANKOM fiber analyzer (ANKOM Technology Corp., Fairport, NY). Lignin was analysed following the method described by Van Soest (1963). Ash, Ca, P, K, Mg, and S were analysed according to AOAC procedures (2006). Ether extract of the samples was determined using an Ankom^{XT10} extractor with petroleum ether as the solvent (method 920.39; AOAC, 2006). Starch content was determined using an amylase kit (Megazyme International Ireland Ltd., Wicklow, Ireland; AOAC, 2006; method 996-11).

Nitrogen concentration of original feeds, residues after ruminal incubation, and residues after pepsin/pancreatine digestion were analysed using by using Elementar rapid N Combustion analyzer (Rapid N-cube, Elemetar Americas Inc., Mt. Laurel, NJ) according to AOAC (2006, method 968.06). Soluble protein, neutral detergent insoluble CP (NDICP), and acid detergent insoluble CP (ADICP) were analysed according to the methods described by Licitra et al. (1996). Ultra performance liquid chromatography equipped with a pre-column derivatization technology was used to determine the AA composition of original feed stuff and residues (Waters ACQUITY UPLC system, Milford, MA) (AOAC, 2006; method 994.12).

2.2.4. Mathematical and Statistical analysis

Ruminal degradation constants of CP were analysed using nonlinear model (Ørskov and McDonald, 1979; SAS Institute, 2001) without using lag phase as it was negligible. The model used to determine the ruminal degradation of CP (%) at time t (Y) was described by the following equation.

$$Y = A + B\ [1 - e^{-Kd(t)}],$$

Where A = rapidly degradable CP fraction that disappears at 0h after the rinsing procedure; B = potentially degradable CP fraction; Kd =Rate of degradation of B fraction and t = Time of incubation (h). Ruminally undegradable fraction C was calculated by 100 − (A + B). Rumen degradable protein fraction of CP was calculated using the equation given below.

$$RDP = A + B\left[\frac{Kd}{Kd + Kp}\right]$$

Where Kp = Particulate passage rate (%/h) from the rumen as calculated by NRC (2001) for concentrates.

$$Kp = 2.904 + 1.375 \times X1 - 0.020 \times X2,$$

Where X1 = DMI, % of BW; X2 = Concentrate, % of diet DM. The passage rate in this study was estimated to be 6.626 ± 0.07%/h. Rumen undegradable crude protein fraction (RUP) of the samples was calculated by 100 − RDP%. Intestinally absorbable digestable protein (IADP) was determined as, RUP × IDP. Total tract digestibility of CP was calculated by the summation of RDP and IADP fractions. Ruminal degradability (%) for each AA was calculated as: (AA concentration of the original sample − AA concentration of the residue after 12 h ruminal incubation)/ AA concentration of the original sample × 100. Intestinally digestion (%) for each AA was calculated as: (AA concentration of the

residue after 12 h ruminal incubation − AA concentration of the residue after intestinal digestion)/AA concentration of the residue after 12 h ruminal incubation × 100. Contribution of RUP to intestinally absorbable AA (g/kg of CP) was calculated for each AA as: (100 − % rumen degradability at 16 h) × % intestinal disappearance *in situ* × AA concentration in the feed/10.

Fraction A, B, C, RDP, RUP, IDP, IADP and total tract digestibility (TDP), intestinal digestibility of individual AA, and intestinally absorbable AA were analysed using mix procedure of SAS (SAS Institute, 2001) for each feed. The model for all variables was Y = feedstuff with cow as a random variable. Mean comparisons for the data were performed using the Tukey test with $P < 0.05$ designated as significant.

2.3. RESULTS AND DISCUSSION

2.3.1. *Feed Composition*

Chemical composition of the original CM samples obtained from 7 different processing plants is presented in Table 2.3. Crude protein of CM, as a % of DM ranged from 38.09 to 42.95, respectively for CM12 and CM10. Crude protein percentage of SBM was 51.68, which was greater than all the CM samples. Dakowski et al. (1995) evaluated the effect of applying different temperatures and moisture levels during the manufacturing process of rapeseed meal on rumen degradation and intestinal digestibility of protein and AA. Crude protein content of the original CM samples in the present study (except CM12), is in agreement with the finding of Dakowski et al. (1995). Protein fraction that is recovered in ADICP can be used as an estimate of measuring unavailable protein of a particular feed (Dakowski et al., 1995). Plegge et al. (1985) observed threefold increment in ADIN fraction of the SBM that was roasted at a temperature of

145°C. However, the increment in ADIN was not significant when the temperatures of 115 and 130°C were used. Similarly, Dakowski et al. (1995) observed an increment in ADIN fraction of rapeseed meal samples, from 6.6% to 17.4%, respectively when the processing temperatures increased from 130°C to 150°C. Supporting to the findings of both studies, in the present study, mechanically extracted CM (CM12) had the greatest ADICP (% of CP) fraction of 9.46, among all 7 CM samples. Soluble protein of CM as a % of CP was also least in CM12 (14.52). Considering the previously mentioned facts, it can be suggested that, high temperature generated during the mechanical extraction process reduces a substantial amount of ruminally degradable CP from CM.

The NDICP (Table 2.3) was least in CM10 (10.37) and was greatest for CM9 (23.37) of the CM, whereas for SBM, it was 2.54% of CP. Values obtained for EE varied from 1.93 to 11.93% for CM, respectively for CM10 and CM12. Solvent-extracted CM samples (CM5, CM6, CM7, CM9, CM10 and CM11) had less residual oil concentrations in the meal than in mechanically extracted CM12 sample (1.9 to 3.5% vs. 12%). As the solvent extraction facilitates are more efficient at oil removal, residual oil concentration is expected to be less in the resulting meal (Spragg and Mailer, 2007; Newkirk, 2009). Moreover, Guadagnin et al. (2013) highlighted that, having a high residual oil content in the CM produced by expeller or the mechanical extraction process, decreases allowable storage time at the farm level because of increased possibility of rancidity and thus, this has discouraged its use in animal feeding compared to solvent-extracted CM. Obtained residual oil ranges for the CM samples are in agreement with findings by Maison et al. (2013), where it ranged from 1 to 2% vs. 8 to 13%, respectively for solvent and mechanically extracted meals. Homolka et al. (2007) compared solvent-extracted

rapeseed meal with expeller double-pressed rapeseed meal. Supporting to the values of the present study, EE value for expeller meal ranged from 10.9 to 17.9%, whereas solvent-extracted meal, was 3.9%. Moreover, having a low residual oil concentration in the meals resulted in greater amounts of CP in solvent extracted CM in the present study compared to mechanically-extracted meals. Crude protein of the final product can be further increased by removing seed hulls which include crude fiber, NDF and ADF in greater proportions (Bell, 1993). Neutral detergent fiber content varied considerably across CM samples from 23.9 to 30.2 as a % of DM. Steingass et al. (2013) compared 10 CM samples obtained from different processing plants located in Germany in their study. Variability in the NDF content was found among samples. Neutral detergent fiber value was correlated with NDIN fraction. Researchers speculated that the effects of varying heat intensity applied during the production process would have increased the linkages between N fraction and fiber in the feed sample and thus, being recovered in the NDF fraction (Steingass et al., 2013).

Amino acid composition of the original feed samples is presented in Table 2.4. The least value for AA concentration was reported in CM12 (the mechanically-extracted CM sample), except for Val and Trp. Moreover, Lys concentration in CM12 was 6 to 14 units less than the other CM samples, which indicates that, the ruminally availability of Lys may be affected by the production conditions used by the particular processing plant. This can be problematic as Lys is assumed to be one of the first limiting AA in milk production (NRC, 2001). In a study conducted by Dakowski et al. (1996), a decrease in total Lys and the ruminally available Lys contents was observed when CM were treated even with moderate heat treatments. Lysine supply as a proportion of EAA, ratio between

lysine to Met and total AA concentration in the original feed samples were also reported low in CM12. Homolka et al. (2007) used rapeseed meal samples manufactured via solvent extraction and expeller double press (mechanical extraction) process in their study, and observed similar low values for CP and AA content in the expeller processed meal. Low values reported for CP and AA content in the original CM12 sample reflects that, the processing conditions during the mechanical extraction might have influenced on the protein value of the feed to a greater extent compared to that with solvent extraction. Soybean meal has a greater AA content in the original feed when it compared with CM. However, concentrations of Met and Cys were less in SBM than CM, and support the fact that SBM is a poor source of Met.

Though it is postulated there are greater concentration losses of protein because of the heat application during desolventizing and toaster stages in the solvent extraction process, in the practical scenario, much higher heat damage occurs within the double press expeller or the mechanical extraction process than the solvent extraction due to the heat generated during the double pressing process (Spragg and Mailer, 2007).

2.3.2 *Rumen Degradation Kinetics of Crude protein and Amino Acids*

Rumen degradation values of CP fraction for CM and SBM samples are as presented in the Table 2.5. Rapidly degradable CP of CM (A fraction) varied from 17.8 to 26.6%, respectively for CM5 and CM10 ($P < 0.05$). The potentially degradable fraction for CM (B fraction) was least for CM12 (62.4%) and greatest for CM5 (79.9%) ($P < 0.05$). Soybean meal had the least undegradable fraction (C fraction) among all, whereas the range for C fraction for CM varied from 2.3% (CM5 and CM11) to14.6% (CM12). An experiment conducted by Steingass et al. (2013) found that the average

values for fraction A, B, and C for CM were 7.7%, 87.7%, and 9.3%, respectively. Compared to their findings, fraction A in the present study is much greater (7.7% vs. 17.8 to 26.6%) while fraction B is lower (87.7% vs. 62.4 to 79.9%). Previous authors calculated small particle loss by subtracting water soluble fraction from total washing loss at 0 h time point. Accounting for the small particle loss in their study must be the reason for obtaining values which are lesser than the present study. Homolka et al. (2007) compared the rumen degradation kinetics of CP fraction of expeller-processed rapeseed meal with solvent-extracted rapeseed meal using steers. Fraction A varied from 53.6 to 61.6% for expeller processed meals and was 14.7% for solvent-extracted meals. Values obtained for fraction A in the present study do not support their findings. Moreover, the potentially degradable fraction in Homolka et al. (2007) study varied from 32.2 to 40.1% for expeller processed meal and for solvent extracted meal, it was 78.6%. Even though the B fraction for the expeller meal was much greater in the present study (62.4% vs. 32.2 to 40.1%), observed value for solvent extracted meals is in agreement with the previous author's findings (66.3% to 79.9% vs. 78.6%). Variability in animals (cows vs. steers, goats, and sheep) used for these kinds of experiments exhibits differences in the values observed and makes it hard to compare different studies. However, findings of the present study closely match with the results obtained for ruminal degradation kinetics for CM by Boila and Ingalls (1992). The degradation parameters of their study were as follows: fraction A was 28.2% and fraction B was 68.4%. Undegradable fraction of CP for solvent-extracted CM samples in the present study varied from 1.5 to 7.4%, and for mechanically-extracted CM, it was 14.6%. The fact that mechanically-extracted products supply greater concentration of RUP supports the findings of a study done by Titgemeyer

and Shirley (1997), where they found higher estimates of RUP in expeller processed SBM produced by modifying heat inputs compared to that with solvent-extracted SBM.

However, average ruminal degradation kinetics for CM in the present study is in agreement with NRC (2001) recommendations with slight variations. Greatest ruminal degradation rate (Kd) was reported in SBM of 11.1 %/h. For CM, Kd varied from 9.7 to 4.0 %/h, respectively for CM10 and CM12. Soybean meal had the highest RDP among all feedstuffs. Rumen degradable protein in CM varied from 46.2 (CM12) to 67.7% (CM10). Small SP fraction in the original meal led to a greater concentration of B fraction of CP in CM12 sample. Consequently, CM12 sample had the greatest C fraction and the least RDP fraction among all CM. These results supports the conclusion made by Spragg and Mailer (2007) regarding the Australian oilseed processing conditions that there was significant heat damage occurring within the expeller screw press compared to solvent extraction. Effective degradability of CP observed for CM in the present study averaged 57.1% of CP, which agrees with the findings of Steingass et al. (2013). Kendall et al. (1991) who used steers to study CP degradation of rapeseed meal samples obtained from five processing plants demonstrated that RDP varied from 44.3% to 59.0 % at 5%/h rumen outflow rate and a large variation in ruminal degradation kinetics. Findings by Homolka et al. (2007) on RDP of CP of solvent extracted rapeseed meal support the present study, where RDP ranged from 53.4 to 67.7%. However, under a particulate passage rate of 6.0%/h, RDP obtained in their study was much higher for the mechanical-extracted CM sample compared to that with present study (84.4 to 88.7% vs. 46.2%). Variability in the values observed in previous studies compared to the present study is mainly due to the difference (dairy cow vs. beef steers), which has a huge impact on

ruminal passage rate. Ruminal CP degradation of the meals varied in the present study could be attributed to the variation in the seed quality and the processing technology used by individual processing plants. According to Bell (1993), harvesting season, climatic variations during crop growth and nutrient availability in the soil has huge impacts upon seed quality. Canola meal samples used in this experiment were from the same cropping season. Therefore, cropping season has no impact across the treatments. However, other geological variations might have affected on seed quality. Other than the seed quality, variability in production technology used in individual plants probably caused the biggest impact upon the nutritional composition variability as observed. Steingass et al. (2013) used 10 CM samples obtained from different processing plants located in Germany, and observed considerable variations in CM produced by different plants. Newkirk et al. (2003) observed precaecal AA digestibility in broiler chicken using 26 non-toasted (meal produced without passing through a desolvetizer/toaster) and 31 toasted meals obtained from different processing plants. Authors did not find much variability of lysine digestibility in non-toasted meals (87% to 92%) compared with toasted ones (66% to 86%) and deduced that, ruminal CP degradation is more affected by process technology than by quality of raw material. Apart from the seed quality and production process adopted by individual producers, discrepancies observed in ruminal degradation kinetics in different studies could be attributed to difference in the animal species used for the experiment (dairy cow vs. beef steer, goat, sheep and poultry), various outflow rates, different feed particle sizes, conditions of the animals used, time of the year when the experiment conducted and methods of calculation by different authors.

Amino acid content of the feeds after 12 h ruminal incubation is presented in Table 2.6. In all cases, the concentration of AA in feeds determined after ruminal incubation was lower than the original samples. Except Lys, contents of other individual AA in the post ruminal residue were greater in the CM12 sample among all CM. Ruminal degradation of individual AA varied considerably among and within feed samples (Table 2.7). Among CM samples, His, Arg and Trp were the most degraded, Lys and Met were intermediate, and Ile, Leu, Phe, Thr and Val were the least degraded in the rumen. Ruminal degradability values in CM12 sample reflects that, heat generated during mechanical extraction resulted a significant reduction in the digestibility of EAA. In SBM, Arg, His, Lys and Trp were the most degraded among EAA. A previous study by Mjoun et al. (2010) compared different SBM products and DDGS, and observed Arg, His, and Lys as the most degraded EAA within SBM products. In the residue after 12 h ruminal incubation, total EAA, total NEAA and total AA contents were higher in SBM compared to CM. In an in vitro experiment comparing SBM, rapeseed meal, blood meal and urea using Holstein steers, Lardy et al. (1993) showed that rapeseed meal supplementation had greater ($P < 0.01$) flows of Thr, Ser, Pro, Cys, Met, Arg, Ile, and Hyp than did SBM supplementation. Moreover, greater total AA flow was observed in rapeseed meal supplementation than SBM ($P < 0.05$) (Lardy et al., 1993).

Nutritive value of a dietary protein for ruminants highly depends on the amount and the AA composition of RDP which enters and digests in the SI. The flow of undegradable protein into the lower gut can be increased if ruminal degradation is reduced (Dakowski et al., 1995). Therefore, considering the AA composition of the post ruminal incubation residues and the ruminal degradability values, it can be claimed that

mechanically-extracted CM sample (CM12) supplies more nutrients than solvent-extracted products.

2.3.3. *Intestinal digestibility of Crude Protein and Amino Acids*

Intestinal digestibilities of CP are reported in Table 2.8. The RUP as a % of the CP for CM varied from 32.3 (CM10) to 53.8 (CM12). The RUP value for SBM was less (31.0%) than that of CM samples. Intestinal digestible protein fraction of RUP was greatest for SBM compared to that of CM. Among CM samples, IDP varied from 71.6% to 77.4%, respectively for CM10 and CM9. Dakowski et al. (1996) compared rapeseed meals treated at different temperature and moisture conditions. In their study, IDP values varied from 70.2% to 74.1% for rapeseed meals. Findings of previous authors support the present study with slight variability. However, observed discrepancies in the results may be mainly due to the way of analysing of intestinal digestibility; in-vitro technique vs. mobile bag technique, and the duration of incubation; 12 h vs. 16 h. Intestinal absorbable digestible protein fraction of RUP was least for CM10 (23.1%) and greatest for CM12 (39.4%). Among CM samples, CM12 had the least RDP value and the ruminal degradability of EAA. However, owing to the highest intestinal absorbable digestible protein fraction (IADP) in CM12 proved that protein fraction undegraded in CM12 in the rumen was utilized well in the SI. Moreover, CM12 or the mechanically extracted CM sample has claimed it as a promising way of shifting protein digestion from the rumen to the SI by means of proper processing.

Intestinal digestibility of EAA from RUP is listed in Table 2.9. Essential amino acids from SBM had the highest intestinal digestibility. Among CM, intestinal digestibility of total AA varied from 79.0% (CM10) to 93.7% (CM9). Least intestinal

digestibility observed in CM10 was owing to the least IDP% obtained. However, intestinal digestibility of EAA of CM12 (92.7%) did not significantly differ from CM9.

Estimated intestinal absorbable AA supply by RUP is presented in Table 2.10. Least supply of EAA by RUP was observed in CM10 (72.5 g/ Kg of CP), whereas, CM12 had the greatest supply (194.4) after SBM (295.3). Difference observed in absorbable AA supply among feedstuffs mainly accountable for the differences in rumen degradability and to a lesser extent to intestinal digestion (Mjoun et al., 2010). Rumen undegradable protein fraction, IDP%, IADP%, and intestinally digestibility of AA were least in CM10. However, rumen degradability of AA of CM10 was the greatest among CM. In the present study, intestinally digestibility played a vital role of estimating the supply of intestinally absorbable AA by RUP from the feedstuffs than the rumen degradability. The ratio of absorbable Lys to Met in the present study was greater for SBM (3.9) compared with mean value for that of CM (2.6). According to NRC (2001) recommendations, maximum milk protein production will be achieved when diets are formulated at concentrations of 7.2 and 2.4% as a percentage of MP for Lys and Met, respectively, which is corresponding to a Lys to Met ratio of 3. Among the feedstuffs tested, feeding SBM is expected to result in maximal milk protein synthesis compared to that of CM. However, mean value for the Lys to Met ratio of solvent-extracted CM was 2.7, whereas for mechanically-extracted product, it was 2.2. Considering the above fact, greater milk protein synthesis can be expected when fed solvent-extracted CM than mechanically-extracted CM. Therefore, during ration formulations, accurately estimating AA flow to the SI is vital, even though that is hard to achieve.

2.3.4. *Total Digestible Protein and Amino Acids*

As mentioned in Table 2.8, significantly greatest TDP was obtained for SBM at 98.2%. According to Mjoun et al. (2010), TDP for SBM was 99.0%. Observed TDP value for SBM in the present study is smaller than the findings of previous authors. However, difference between values is not huge. Among CM samples, TDP varied from 85.1 to 90.8 %, respectively for CM12 and CM10. The large C fraction in ruminal degradation kinetics caused CM12 to have greater RUP concentration compared that with solvent extracted products. However, the TDP fraction (which is the summation of RDP and IADP) is least in the mechanically extracted CM, mainly due to the small RDP fraction. Average intestinal digestible protein for CM was 74.6% and ranged from 71.6% to 77.4%. Values obtained in the present study are less than the values demonstrated in a similar study (average IDP: 74.56 vs. 79.8 and IDP range: 71.6 to 77.4 vs. 75.4 to 83.6) that used 10 CM samples from different processing plants performed by Steingass et al. (2013) in Germany. The difference may be because of the geological changes which could have affected on seed quality or the variability in the production technologies used in two different countries. Considering the IADP fraction, CM averaged 32.01, which is greater than that of SBM (29.2%). This indicates that CM may provide more protein available for absorption in the small intestine than SBM. Considering this fact, CM could be considered as a better source of RUP than SBM. However, TDP value speculates that, protein portion of SBM is more available to the animal than CM.

Total digestibility (TD) of EAA is presented in Table 2.11. Corresponding to the greatest TDP value, SBM had the greatest TD of EAA among all. Total digestibility of all the EAA in SBM was above 99%, which confirms it as an excellent protein source to be

fed. Except for Trp, CM12 had the least TD of EAA among 7 other CM samples. Even though it had the greatest intestinally digestibility of AA and estimated intestinally absorbable AA supply by the RUP fraction, TD decreased because of the least RDP fraction observed in the sample. Among CM, Arg, His, Trp, and Met were most digested, Leu, Lys and Phe intermediate, and Ile, Thr and Val were least digested. However, in SBM, no such differences were observed in TD of EAA. Overall, the extent of disappearance of individual EAA reflected the amount and the CP disappearance of the particular feed.

2.3.5. *Comparison of ruminal degradation kinetics and intestinal digestibility parameters of canola meal with National Research Council book values*

Mean values for ruminal degradation and intestinal digestibility kinetics for 7 CM samples were compared with NRC (2001) values in Table 2.12. Values for A fraction, B fraction and IDP are in agreement with NRC values. Mean value obtained in the present study for Kd was much smaller, whereas a higher RUP% compared to that of NRC. However, considering these variations, using a constant value for a particular feedstuff in ration balancing will lead either to overestimate or to underestimate the protein supply to the animal.

CONCLUSIONS

Some of mean ruminal and intestinal digestibilities of CM (A fraction, B fraction and IDP) are in agreement with NRC values. However, it can be concluded that, considerable variations exists among CM produced in Canada. The variability in degradation and digestibility parameters of protein and AA supply in CM may be because of variability in the production technology used in individual processing plants as the

other factors affecting on variability (i.e- harvest year, geological variability) are similar in all CM samples.

Thus, in ration formulation, these variabilities should be taken into account rather than using book values in order to supply nutrients to dairy cows to meet requirements.

Table 2.1. Ingredient composition of the total mixed ration fed to cows during the *in situ* experiment

Ingredient	% of DM
Corn silage	33.10
Alfalfa haylage	17.26
Alfalfa hay	5.67
High moisture corn	14.03
Whole cotton seed	5.99
Distillers dry grains with solubles	5.46
QLF Dairy sugar[1]	3.78
Soybean meal, 44% CP	3.59
Corn dry fine	3.22
Soyplus bypass SBM[2]	2.24
Wheat midds	1.46
Limestone	0.93
Sodium bicarbonate	0.83
Fat liquid[3]	0.65
Blood meal (pork)	0.62
Salt bulk	0.37
Urea 46%	0.20
Magnesium oxide 54%	0.20
VTM lac Avail4[4]	0.16
Yeast[5]	0.09
Omnigen[6]	0.09
Vit E 20,000 IU/lb	0.03
Rumensin[7]	0.01
Biotin 1% 4536mg/lb	0.01

[1]QLF Dairy sugar (Quality Liquid Feeds, Dodgeville. WI).
[2]SoyPlus (West Central Soy, Ralston, IA).
[3]Energizer 4-19W (Quality Liquid Feeds, Dodgeville. WI).
[4]VTM lac Avail4 (International Nutrition, Omaha, NE).
[5]Yeast gladiator (Hubbard Feeds, Mankato, MN).
[6]Omnigen (Prince Agri Products, Teaneck, NJ).
[7]Rumensin, 90g/lb (Elanco Animal Health, Greenfield, IN).

Table 2.2. Formulated chemical composition of the total mix ration fed to cows during the *in situ* experiment

Nutrient composition	% of DM[1]
Dry matter	47.96
CP	17.31
RDP[2]	10.22
RUP[2]	7.09
ADF	18.23
NDF	28.76
pefNDF	21.77
NFC[3]	42.53
Starch	25.75
Sugar	7.04
Total fat	4.97
NE_L[2] (Mcal/ lb)	0.81
Ca	1.02
P	0.38
Mg	0.39
K	1.33
S	0.21
Cl	0.48
Vit. A (KIU/lb)	2.83
Vit. D (KIU/lb)	0.71
Vit. E (KIU/lb)	15.84

[1]Units in % of DM unless noted.
[2]Calculated using NRC.
[3]NFC = 100- (% NDF+ % CP+ ether extract+ % ash).

Table 2.3. Analyzed chemical composition of canola meals and soybean meal

	Feedstuffs[1]							
Item[2]	CM5	CM6	CM7	CM9	CM10	CM11	CM12	SBM
DM, %	92.2	91.9	91.9	91.8	92.1	92.4	94.1	92.3
CP	41.5	42.0	40.1	41.1	43.0	41.0	38.1	51.7
SP,[3] % of CP	15.5	19.5	25.8	23.5	30.6	22.9	14.5	24.8
NDICP,[4] % of CP	19.4	19.2	10.5	23.4	10.4	13.4	20.9	2.5
ADICP,[5] % of CP	6.6	7.2	6.8	7.1	6.4	7.3	9.5	1.1
NDF	27.6	29.3	27.4	30.2	23.9	26.2	26.1	9.4
ADF	20.8	22.4	20.6	21.3	19.4	22.2	22.3	5.8
Ether extract	3.2	2.8	3.5	4.0	1.9	3.5	11.9	1.4
Starch	1.8	1.6	2.1	1.7	1.0	1.7	0.8	1.5
NFC[6]	10.6	10.2	11.4	9.9	11.3	10.1	9.9	17.4
Lignin (H_2SO_4)	8.9	10.0	8.6	9.8	7.4	9.6	10.4	0.1
Ash	7.5	8.5	7.5	7.3	7.5	7.5	7.8	6.8
Ca	0.8	1.0	0.8	0.8	0.8	0.1	0.7	0.4
P	1.2	1.2	1.3	1.2	1.3	1.2	1.2	0.8
Mg	0.6	0.6	0.7	0.6	0.7	0.7	0.6	0.3
K	1.3	1.4	1.4	1.3	1.5	1.3	1.3	2.5
S	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.5
Cl	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

[1]Feedstuff were solvent extracted canola meal = CM5, CM6, CM7, CM9, CM10 and CM11, mechanically extracted canola meal = CM12, SBM = Soybean meal.
[2]Units in % of DM unless noted.
[3]SP (% of CP) = soluble protein in borate buffer.
[4]NDICP (% of CP) = neutral detergent insoluble CP.
[5]ADICP (% of CP) = acid detergent insoluble CP.
[6]NFC = 100-(%NDF+ %CP+ ether extract+ % ash).

Table 2.4. Amino acid composition of canola meal and soybean meal products

	Feedstuffs[1]							
AA, g/kg of CP	CM5	CM6	CM7	CM9	CM10	CM11	CM12	SBM
Arg	48.8	49.8	43.5	46.3	51.4	48.1	42.8	87.4
His	20.7	21.6	18.9	20.3	22.1	21.1	18.1	31.1
Ile	31.9	33.2	28.8	31.3	34.2	32.2	28.8	55.6
Leu	58.8	59.5	52.3	56.5	60.1	57.0	50.5	93.7
Lys	43.7	44.2	40.8	40.6	49.1	44.8	34.8	76.6
Met	16.9	16.6	14.7	16.5	17.1	16.3	14.0	17.0
Phe	34.1	34.1	30.0	32.6	34.7	32.9	29.2	61.4
Thr	36.1	36.0	32.0	34.7	36.5	34.6	29.7	47.3
Val	42.6	44.8	39.6	42.9	47.1	44.2	39.7	61.4
Trp	9.8	10.1	8.7	10.2	10.8	10.9	9.1	18.0
Total EAA[2]	343.5	349.8	309.2	331.9	363.1	342.1	296.6	549.5
Ala	36.6	36.6	32.6	34.9	37.4	35.3	31.2	52.6
Asp	60.0	61.0	51.7	57.7	60.8	57.7	50.7	138.5
Cys	19.4	18.8	16.9	18.4	19.8	18.7	15.7	16.3
Glu	137.1	137.3	120.7	131.6	140.1	132.3	115.6	207.9
Gly	41.5	41.4	37.0	39.8	42.4	40.0	35.6	50.8
Pro	49.5	47.8	41.9	45.5	49.8	46.8	40.4	56.9
Ser	34.3	34.1	29.6	32.4	33.6	31.6	27.0	56.4
Tyr	23.4	23.3	20.9	21.8	23.4	22.4	19.4	42.6
Nonessential AA[3]	417	413	364	394	422	398	348	627
Lys, % of EAA	12.7	12.6	13.2	12.2	13.5	13.1	11.7	13.9
Met, % of EAA	4.9	4.7	4.8	5.0	4.7	4.8	4.7	3.1
Lys:Met	2.6	2.7	2.8	2.4	2.9	2.7	2.5	4.5
Total AA[4]	761	763	673	726	785	740	644	1176

[1]SBM= Soybean meal; CM= Canola meal.
[2]Sum of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val, Trp.
[3]Sum of Ala, Asp, Cys, Glu, Gly, Pro, Ser, Try.
[4]Total AA = Essential AA+ Nonessential AA.

Table 2.5. Rumen degradation kinetics crude protein for canola meal and soybean meal

	Feedstuffs[1]								
Item[2]	CM5	CM6	CM7	CM9	CM10	CM11	CM12	SBM	SEM
A, %	17.8^{b}	21.7^{bc}	26.4^{c}	24.8^{c}	26.6^{a}	25.1^{a}	23.1^{ab}	23.0^{c}	1.18
B, %	79.9^{a}	76.8^{ab}	66.3^{cd}	69.8^{bcd}	69.6^{bcd}	72.6^{abc}	62.4^{d}	76.5^{ab}	1.84
C, %	2.3^{b}	1.5^{b}	7.4^{b}	5.4^{b}	3.8^{b}	2.3^{b}	14.6^{a}	0.6^{b}	1.42
Kd, %/ h	5.6^{bc}	5.2^{c}	9.1^{ab}	4.6^{c}	9.7^{ab}	6.2^{bc}	4.0^{c}	11.1^{a}	1.32
RDP, % of CP	53.9^{c}	55.2^{c}	64.6^{ab}	53.4^{c}	67.7^{a}	59.2^{bc}	46.2^{d}	69.0^{a}	2.05

[a-d] Means in rows with different superscripts differ significantly ($P < 0.05$).

[1]SBM=Soybean meal; CM= Canola meal.

[2]A = rapidly degradable fraction of CP; B = potentially degradable fraction of CP; C = undegradable fraction of CP; Kd = rate of degradation of fraction B; RDP = A + B [Kd/(Kd + Kp); RUP = B [Kp/(Kd + Kp)] + C, where Kp is the rate of passage from the rumen calculated to be 6.6%/h (NRC, 2001).

Table 2.6. Amino acid composition of canola meal and soybean meal residues after 12 h of ruminal incubation

	Feedstuff[1]								
AA, g/kg of CP	CM5	CM6	CM7	CM9	CM10	CM11	CM12	SBM	SEM
Arg	24.1^{b}	24.1^{b}	13.2^{c}	25.6^{b}	10.7^{c}	19.6^{bc}	29.3^{b}	42.8^{a}	3.38
His	9.9^{bc}	10.1^{bc}	5.4^{de}	10.8^{bc}	4.4^{e}	8.2^{cd}	12.2^{b}	15.7^{a}	1.31
Ile	18.4^{b}	19.1^{b}	10.9^{c}	20.8^{b}	9.2^{c}	15.8^{bc}	21.5^{b}	32.2^{a}	2.53
Leu	32.1^{b}	32.8^{b}	18.3^{cd}	34.9^{b}	15.0^{d}	27.0^{bc}	37.1^{b}	53.8^{a}	4.28
Lys	22.5^{b}	22.8^{b}	13.8^{c}	24.2^{b}	12.1^{c}	20.0^{bc}	23.1^{b}	38.5^{a}	3.07
Met	8.5^{ab}	8.7^{ab}	4.9^{cd}	9.5^{ab}	4.1^{d}	7.3^{bc}	10.1^{a}	9.9^{ab}	0.96
Phe	19.1^{b}	19.8^{b}	11.1^{c}	20.7^{b}	9.2^{c}	16.0^{bc}	22.0^{b}	34.5^{a}	2.65
Thr	19.9^{b}	20.5^{ab}	12.0^{cd}	21.6^{ab}	9.7^{d}	17.2^{bc}	22.1^{ab}	26.3^{a}	2.30
Val	24.3^{b}	25.6^{b}	14.5^{cd}	27.4^{ab}	11.9^{d}	21.2^{bc}	28.5^{ab}	35.6^{a}	3.01
Trp	4.3^{bc}	4.6^{b}	2.6^{cd}	4.9^{b}	2.0^{d}	3.8^{bcd}	5.2^{b}	9.1^{a}	0.67
EAA[2]	183.0^{b}	206.6^{b}	110.9^{c}	189.3^{b}	88.3^{c}	156.1^{bc}	211.1^{b}	298.4^{a}	25.00
Ala	20.0^{b}	21.7^{b}	11.6^{cd}	22.1^{b}	9.6^{d}	17.1^{bc}	22.6^{b}	30.4^{a}	2.48
Asp	33.2^{bc}	34.7^{bc}	20.0^{cd}	36.3^{b}	16.0^{d}	28.2^{cd}	38.1^{b}	73.0^{a}	5.20
Cys	7.6^{ab}	7.9^{ab}	7.3^{cd}	8.3^{ab}	3.4^{d}	6.3^{bc}	9.0^{a}	8.7^{ab}	0.85
Glu	58.2^{bc}	59.5^{bc}	29.8^{de}	60.9^{bc}	22.8^{e}	45.6^{cd}	73.4^{b}	98.1^{a}	7.74
Gly	9.2^{b}	8.9^{b}	9.1^{b}	9.1^{b}	8.9^{b}	8.6^{b}	10.9^{a}	11.1^{a}	0.43
Pro	22.6^{bc}	23.5^{bc}	12.9^{de}	24.2^{abc}	10.5^{e}	19.0^{cd}	27.0^{ab}	31.2^{a}	2.72
Ser	16.5^{b}	16.7^{b}	9.6^{cd}	16.7^{b}	7.6^{d}	13.9^{bc}	17.6^{b}	27.5^{a}	2.02
Try	14.2^{b}	14.7^{b}	8.5^{c}	15.5^{b}	7.2^{c}	12.3^{bc}	15.5^{b}	25.2^{a}	1.91
NEAA[3]	199.4^{b}	179.6^{bc}	110.3^{cd}	224.4^{b}	91.4^{d}	66.6^{cd}	234.1^{b}	323.8^{a}	26.8
Total AA[4]	382.4^{b}	386.2^{b}	221.2^{cd}	413.8^{b}	179.7^{d}	322.6^{bc}	445.2^{b}	622.1^{a}	49.7

[a-e] Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]SBM= Soybean meal; CM= Canola meal.

[2]Sum of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val.

[3]Sum of Ala, Asp, Cys, Glu, Gly, Pro, Ser, Try.

[4]Total AA = Essential AA+ Nonessential AA.

Table 2.7. Ruminal degradability (%) of essential amino acids from soybean and canola meal residues after 12h ruminal incubation[1]

	Feedstuff[2]								
AA	CM5	CM6	CM7	CM9	CM10	CM11	CM12	SBM	SEM
Arg	50.7^{c}	51.5^{c}	69.6^{ab}	44.7^{cd}	79.2^{a}	59.3^{bc}	31.4^{d}	51.0^{c}	5.45
His	52.3^{c}	53.4^{c}	71.4^{ab}	46.7^{c}	80.0^{a}	61.2^{bc}	32.6^{d}	49.3^{c}	5.34
Ile	42.5^{cd}	42.6^{cd}	62.2^{ab}	33.7^{cd}	73.2^{a}	51.0^{bc}	25.4^{d}	42.2^{cd}	6.19
Leu	45.4^{c}	44.8^{c}	64.9^{ab}	38.3^{cd}	75.1^{a}	52.7^{bc}	26.5^{d}	42.5^{cd}	6.07
Lys	48.5^{cd}	48.4^{cd}	66.0^{ab}	40.3^{cd}	75.4^{a}	55.4^{bc}	33.6^{d}	49.8^{c}	5.58
Met	49.7^{c}	47.4^{c}	66.6^{ab}	42.4^{cd}	76.3^{a}	55.3^{bc}	27.9^{d}	41.9^{cd}	5.73
Phe	44.0^{c}	41.9^{c}	63.0^{ab}	36.6^{cd}	73.4^{a}	51.2^{bc}	24.8^{d}	43.8^{c}	6.10
Thr	44.8^{c}	42.9^{c}	62.5^{ab}	37.9^{cd}	73.3^{a}	50.1^{bc}	25.6^{d}	44.4^{c}	5.92
Val	43.0^{cd}	42.9^{cd}	63.4^{ab}	36.2^{cd}	74.6^{a}	52.1^{bc}	28.0^{d}	42.0^{cd}	5.94
Trp	55.8^{cd}	54.7^{cd}	70.3^{ab}	51.9^{cd}	81.9^{a}	64.6^{bc}	43.1^{d}	49.4^{d}	4.92
EAA[3]	46.7^{c}	40.9^{cd}	64.2^{ab}	43.0^{cd}	75.7^{a}	54.4^{bc}	28.8^{d}	45.7^{b}	6.13

$^{a–d}$Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Rumen disappearance (%) at 12 h of incubation using in-situ technique.

[2]SBM= Soybean meal; CM= Canola meal.

[3]Sum of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val.

Table 2.8. Rumen undegradable protein and intestinal digestibility parameters of canola meal and soybean meal

	Feedstuffs[1]								
Item	CM5	CM6	CM7	CM9	CM10	CM11	CM12	SBM	SEM
RUP, % of CP	46.1^{b}	44.8^{b}	35.4^{cd}	46.6^{b}	32.3^{d}	40.8^{bc}	53.8^{a}	31.0^{d}	2.05
IDP[2], %	76.8^{bc}	75.8^{bcd}	72.0^{de}	77.4^{b}	71.6^{e}	75.3^{bcde}	73.0^{cde}	94.5^{a}	2.50
IADP[3],% of CP	35.4^{ab}	34.0^{ab}	25.5^{cd}	36.0^{ab}	23.1^{d}	30.7^{bc}	39.4^{a}	29.2^{bcd}	2.08
TDP[4], %	89.3^{bc}	89.1^{c}	90.1^{bc}	89.4^{bc}	90.8^{b}	89.3^{bc}	85.1^{d}	98.2^{a}	0.73

[a-e] Means in rows with different superscripts differ significantly ($P < 0.05$).

[1]SBM= Soybean meal; CM=Canola meal.

[2]IDP = estimated intestinal protein digestibility (Gargallo et al., 2006).

[3]IADP = intestinally absorbable dietary protein = RUP × IDP.

[4]TDP =total digestible dietary protein = RDP + IADP.

Table 2.9. Intestinal digestibility (%) of essential amino acids from rumen undegradable protein of canola meal and soybean meal[1]

	Feedstuffs[2]								
AA	CM5	CM6	CM7	CM9	CM10	CM11	CM12	SBM	SEM
Arg	93.9^{ab}	94.4^{ab}	89.0^{bc}	94.9^{ab}	83.1^{c}	93.4^{ab}	93.4^{ab}	99.5^{a}	1.94
His	92.8^{ab}	93.3^{ab}	86.3^{bc}	94.0^{ab}	80.4^{c}	91.9^{ab}	92.8^{ab}	99.2^{a}	2.27
Ile	90.8^{b}	91.7^{ab}	85.6^{b}	91.7^{ab}	78.0^{c}	90.2^{b}	91.2^{ab}	98.9^{a}	2.31
Leu	92.3^{b}	93.0^{ab}	88.2^{b}	94.0^{ab}	81.0^{c}	91.8^{b}	92.0^{b}	98.8^{a}	1.81
Lys	91.5^{b}	91.9^{ab}	85.3^{bc}	92.7^{ab}	79.4^{c}	91.0^{b}	91.4^{b}	99.1^{a}	2.28
Met	93.4^{ab}	94.0^{ab}	89.6^{bc}	94.9^{ab}	84.3^{c}	93.4^{ab}	93.6^{ab}	99.0^{a}	1.79
Phe	91.7^{ab}	92.4^{ab}	86.4^{b}	93.4^{ab}	79.0^{c}	90.9^{b}	91.7^{ab}	98.9^{a}	2.15
Thr	91.4^{ab}	91.7^{ab}	85.3^{b}	92.8^{ab}	77.0^{c}	90.6^{b}	91.4^{ab}	98.8^{a}	2.35
Val	90.1^{b}	91.2^{ab}	84.3^{b}	92.1^{ab}	76.3^{c}	89.1^{b}	91.1^{ab}	98.7^{a}	2.45
Trp	93.5^{ab}	95.0^{ab}	89.6^{ab}	96.1^{ab}	87.5^{b}	90.6^{ab}	98.1^{a}	99.3^{a}	2.82
Total EAA[3]	91.9^{b}	93.3^{ab}	87.0^{b}	92.9^{ab}	79.6^{c}	91.2^{b}	92.1^{ab}	99.0^{a}	2.07
Total AA[4]	91.9^{ab}	92.7^{ab}	84.8^{cd}	93.7^{ab}	79.0^{d}	90.6^{bc}	92.7^{ab}	98.9^{a}	2.07

$^{a–c}$Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Intestinal disappearance (%) of 16-h rumen residues using in vitro technique.

[2]SBM= Soybean meal; CM= Canola meal.

[3]Sum of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val, Trp.

[4] Total AA = EAA + NEAA.

Table 2.10. Estimated intestinal absorbable[1] amino acids (g/ Kg of CP) supplied by rumen undegradable protein of soybean and canola meal

	Feedstuffs[2]								
AA	CM5	CM6	CM7	CM9	CM10	CM11	CM12	SBM	SEM
Arg	22.7^{ab}	22.8^{ab}	11.8^{bc}	24.3^{ab}	9.1^{c}	18.3^{ab}	27.4^{ab}	42.6^{a}	3.35
His	9.2^{b}	9.4^{b}	4.7^{cd}	10.2^{b}	3.7^{d}	7.5^{bc}	11.3^{b}	15.6^{a}	1.30
Ile	16.7^{b}	17.5^{b}	9.3^{c}	19.3^{b}	7.4^{c}	14.3^{bc}	19.6^{b}	31.8^{a}	2.49
Leu	29.7^{b}	30.5^{b}	16.2^{cd}	32.8^{b}	12.5^{d}	24.8^{bc}	34.2^{b}	53.1^{a}	4.18
Lys	20.7^{b}	20.9^{b}	11.8^{c}	22.5^{b}	9.9^{c}	18.2^{bc}	21.1^{b}	38.1^{a}	3.05
Met	8.0^{ab}	8.2^{ab}	4.4^{cd}	9.0^{ab}	3.5^{d}	6.8^{bc}	9.4^{ab}	9.8^{a}	0.95
Phe	17.6^{b}	18.3^{b}	9.6^{c}	19.3^{b}	7.5^{c}	14.6^{bc}	20.1^{b}	34.1^{a}	2.15
Thr	18.3^{b}	18.8^{b}	10.3^{cd}	20.0^{ab}	7.8^{d}	15.6^{bc}	20.2^{ab}	26.0^{a}	2.26
Val	21.9^{b}	23.4^{b}	12.2^{cd}	25.2^{b}	9.4^{d}	18.9^{bc}	26.0^{b}	35.1^{a}	2.96
Lys:Met	2.6^{cde}	2.6^{de}	2.7^{c}	2.5^{e}	2.8^{b}	2.7^{cd}	2.2^{f}	3.9^{a}	0.04
EAA[3]	168.9^{b}	192.6^{b}	96.7^{cd}	176.5^{b}	72.5^{d}	142.6^{bc}	194.4^{b}	295.3^{a}	24.71
NEAA[4]	184.3^{b}	165.6^{b}	90.9^{cd}	211.5^{b}	73.8^{d}	150.7^{bc}	218.5^{b}	319.9^{a}	33.02
Total AA[5]	353.2^{b}	358.2^{b}	187.5^{cd}	388.0^{b}	146.3^{d}	293.3^{bc}	412.9^{b}	615.2^{a}	49.54

[a-f] Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Absorbable AA supplied by RUP is defined as (100 − % rumen degradability at 12 h) × (% intestinal disappearance *in situ*) × AA concentrations in the feed/10.

[2]SBM= Soybean meal; CM= Canola meal.

[3]Sum of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val.

[4]Sum of Ala, Asp, Cys, Glu, Gly, Pro, Ser, Try.

[5]Total AA = Essential AA+ Nonessential AA.

Table 2.11. Total digestibility (%) of amino acids of canola meal and soybean meal[1]

	Feedstuffs[2]								
AA	CM5	CM6	CM7	CM9	CM10	CM11	CM12	SBM	SEM
Arg	97.2^{bc}	97.3^{b}	96.7^{c}	97.2^{b}	97.0^{bc}	97.4^{b}	95.5^{d}	99.7^{a}	0.28
His	96.7^{bc}	96.9^{b}	96.1^{c}	96.8^{b}	96.7^{bc}	97.0^{b}	95.2^{d}	99.6^{a}	0.34
Ile	94.9^{b}	95.2^{b}	94.6^{b}	95.4^{b}	94.8^{b}	95.3^{b}	93.5^{c}	99.3^{a}	0.56
Leu	96.0^{b}	96.1^{b}	95.9^{b}	96.4^{b}	95.8^{b}	96.2^{b}	94.1^{c}	99.3^{a}	0.47
Lys	95.8^{bc}	95.8^{bc}	95.0^{cd}	95.7^{bc}	95.6^{bc}	96.1^{b}	94.3^{d}	99.6^{a}	0.42
Met	96.8^{b}	96.9^{b}	96.5^{b}	97.1^{b}	96.8^{b}	97.1^{b}	95.4^{c}	99.4^{a}	0.34
Phe	95.5^{b}	95.6^{b}	95.0^{b}	95.9^{b}	95.1^{b}	95.6^{b}	93.8^{c}	99.4^{a}	0.48
Thr	95.4^{bc}	95.3^{bc}	94.4^{c}	95.6^{b}	94.6^{c}	95.4^{bc}	93.7^{d}	99.3^{a}	0.49
Val	94.5^{bc}	95.0^{b}	94.3^{bc}	95.0^{b}	94.7^{b}	94.9^{b}	93.6^{c}	99.2^{a}	0.54
Trp	97.6^{ab}	97.7^{ab}	96.9^{ab}	98.2^{ab}	97.8^{ab}	96.8^{b}	98.9^{ab}	99.7^{a}	0.80
EAA[3]	95.9^{b}	96.0^{b}	95.4^{b}	96.1^{b}	95.7^{b}	96.1^{b}	94.4^{c}	99.4^{a}	0.43
NEAA[4]	96.4^{b}	96.6^{b}	94.7^{c}	96.7^{b}	95.8^{bc}	96.0^{b}	95.5^{bc}	99.4^{a}	0.37

[a-d] Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Total digestibility (%) of AA is defined as (AA concentration in the feed-AA concentration of the residue of intestinal digestion) / (AA concentration in the feed).

[2]SBM= Soybean meal; CM= Canola meal.

[3]Sum of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val, Trp.

[4]Sum of Ala, Asp, Cys, Glu, Gly, Pro, Ser, Try.

Table 2.12.Comparison of the ruminal degradability and intestinal digestibility parameters obtained for canola meal with National Research Council book value

Item	Calculated mean value for CM	NRC (2001) book value
A fraction, % of CP	23.6	23.2
B fraction, % of CP	71.0	70.4
C fraction, % of CP	5.3	6.4
Kd, %/h	6.3	10.4
RUP (% of CP)	47.3	35
IDP, %	74.5	75
TDP, %	89.0	-

CHAPTER 3

USE OF CANOLA MEAL IN DAIRY COW DIETS WITH VARYING CONCENTRATION OF STARCH SOURCES

ABSTRACT

Synchronization of the degradability of non-structural carbohydrate and rumen degradable protein has been identified as an effective method of increasing intestinal AA flow through increased microbial protein synthesis and efficient ruminal fermentation, thereby increased performance of dairy cows. Canola meal can be a suitable protein source to be matched with either corn or barley when fed. Therefore, to determine the performance of lactating cows fed corn and barley starches at varying proportions in diets containing CM as the major source of supplemental protein, 12 multiparous and 4 primiparous Holstein cows were used in a Latin square design for 16 wk, including 10 d covariate period. The ratio of starch from ground corn and rolled barley within each treatment was 100:0, 67:33, 33:67, and 0:100. Varying proportions of corn and barley had no effect on dry matter intake (26.5 kg/d) or milk production (41.2 kg/d). Also milk components except lactose % and milk urea nitrogen (MUN) were not affected by treatments. Lactose percentage and MUN responded cubically to the variability in starch proportions. Treatments did not have significant affects upon the efficiency of milk production that averaged 1.53. Replacing corn with barley had no effect on any of the milk N fractions. Average total protein % and casein % of total protein were 3.2% and 79.7%, respectively. Propionate concentrations and acetate to propionate ratio responded quadratically. Propionate concentration was greater in the diets that had mixed starches,

whereas acetate to propionate ratio was greater in the diets that had sole corn or barley. Isovalerate concentration decreased linearly when corn was replaced with barley. However, ruminal NH_3-N concentration (11.5 mg/dL) and pH (6.7) were not affected by treatments. Varying proportions of corn and barley did not have significant effect on blood glucose concentration (73.8 mg/dL). Apparent total tract digestibilities of DM, OM, and NDF decreased linealy and, CP and ADF tended to decrease linearly when the proportion of barley starch increased in the diet. Total tract digestibility of starch did not affect by the treatments and averaged 95.5%. Production performance of lactating cows were not affected by feeding varying proportions of corn and barley when the diets were formulated with CM as the primarily protein supplement.

Keywords: Canola meal, lactating dairy cows, starch source, corn, barley.

3.1. INTRODUCTION

As the genetic capacity of dairy cows increases to produce more milk, formulation of diets to meet elevated nutrient requirements has become more challenging (Overton et al., 1995). Under normal feeding conditions, the amount of microbial protein that is being synthesized in the rumen is not enough to fulfill the protein requirement of the high producing cows (Leng and Nolan, 1985). Thus, supplementation of greater amounts of protein in the diet to pass to the small intestine (SI) to digest and absorb has been justified as a promising way of providing enough protein to the animal (Leng and Nolan, 1985; Overton et al., 1995). However, feeding greater amounts of RUP to dairy cows to increase the amount of total protein outflow to the SI has not always resulted in an increased milk production (Voss et al., 1988). The lack of responses to an elevated

RUP supplementation are mainly because of the decrease microbial protein synthesis because of the substitution of RDP, and the insufficient supply of limiting AA by the RUP source (Aldrich et al., 1993). Therefore, optimization of microbial growth through the synchronization of energy and RDP in order to enhance microbial protein synthesis may be a more logical strategy than formulating diets with excess amounts of RUP, as the end products of rumen fermentation supplies high quality protein and most of the animal's energy requirement (Sutton, 1985; NRC, 2001).

Canola meal is considered as an effective source of RDP because of its extensive degradation occurs in the rumen (Wright et al., 2004). Corn and barley are the most widely used non-structural CHO supplements in dairy diets (Yang et al., 1997; Casper et al., 1999; Gozho and Mutsvangwa, 2008). The objective of study was to investigate the performance of lactating cows fed corn and barley starches at varying proportions in diets, containing CM as the major source of supplemental protein. It is hypothesized that, cows fed a combination of CM and, corn and barley will have a greater production through efficient synchronization of energy and protein. In addition, combination of two starch sources differ in degradability in the rumen, can be a better match with the protein source, in order to maximize microbial growth and protein synthesis.

3.2. MATERIAL AND METHODS

3.2.1. Cows and Experimental Design

The experiment and all animal use were approved by the South Dakota State University Institutional Animal Care and Use Committee guidelines. Twelve multiparous and four primiparous lactating Holstein cows, averaging 94 ± 25 DIM and 692 ± 61 Kg

BW were selected for a lactation experiment to evaluate the effect of the starch source on the lactation performance when CM was fed as the main protein source. Cows were randomly assigned to one of four experimental diets in a replicated 4×4 Latin square experimental design and were blocked into squares based on parity and milk production. Cows were housed in a free stall barn and were fed diets as total mix rations (TMR) using a Calan Broadbent feeding system (American Calan, Inc., Northwood, NH). There was a 10 d period prior to the beginning of the trial for the cows to adapt to the Calan doors. The study lasted for 16 wk divided into 4 periods of 4 wk each. The first and second weeks of each period were used to adapt the cows to diets and third and fourth weeks were used for sampling and data collection.

3.2.2. Experimental diets

Four experimental diets were formulated by varying the ratio between corn and the barley in the diet. Ingredient composition and the predicted nutrient compositions of experimental diets are as presented in Table 3.1 and Table 3.2, respectively. Ratios between corn to barley were, 1) 100:0, 2) 67:33, 3) 33:67, and 4) 0:100. Across all the treatments, starch concentration, as a % of diet DM, was similar. In all four diets, CM was used as the major protein supplement. All diets contained 56% forage (65% corn silage and 35% alfalfa haylage) and 45% concentrate on a DM basis. Forages were premixed for all diets in a mixer wagon (Patz 640 vertical mixer, Patz Corporation, Pound, WI). After addition of premixed forage, the respective experimental concentrate mix was added to the Calan Data Ranger (American Calan, Inc., Northwood, NH). All concentrate mixes were prepared at the South Dakota State University feed mill. Diets

were fed for ad libitum consumption once daily approximately at 0900 h allowing for 5 to10% orts.

3.2.3. Sampling measurements

Amounts of feed offered and refusals by individual animals were recorded daily throughout the experimental period to obtain net intake. Samples of corn silage (CS), alfalfa haylage (AH), cotton seed and each TMR were collected two times in third and fourth week of each period and composited by period for nutrient analysis. Individual ingredients in the concentrate mix and samples of each concentrate mix were collected every time with the arrival of new feed. All feed samples were stored at -20˚C until analysis. Dry matter content of CS and AH were measured weekly by drying the samples in a Despatch oven (style V-23: Despatch Oven Co., Minneapolis, MN) for 48 h in order to insure proper inclusion rate in the TMR mix on weekly basis. A set of TMR samples were collected during 3rd and 4th wk for particle size measurements on as-fed basis using the Penn State Particle Size Separator (Kononoff et al., 2003).

Composited feed samples were dried at 55˚C for 48 h in a Despatch oven (style V-23: Despatch Oven Co., Minneapolis, MN) and were ground to pass through a 4 mm screen on a Wiley mill (Model 3; Arthur H. Thomas., Philadelphia, PA). Samples were ground further to 1 mm by regrinding the samples in an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). All feed samples were sent to DairyLand laboratory (Arcadia, WI) for analysis of ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE) and crude protein (CP). Absolute dry matter (DM) was determined by drying approximately 1 g of sample at 105˚C for 3 h, for correction to

100%. Ash content was determined by heating samples for 8 h at 450˚C in a muffle furnace (Thermolyne, Model F1230, Thermolyne corporation, Dubuque, Iowa, U.S.A) (Understander et al., 1993). Samples were analyzed for NDF and ADF sequentially via Ankom filter bag analysis system (Ankom Technology Corp., Macedon, NY). The method for NDF was based upon procedures described by Van Soest et al. (1991) using heat stable α-amalylase and sodium sulphite and the ADF method was based on the procedures explained by Robertson and Van Soest. (1981). Ether extract was determined using Anom ^{XT10} extractor with petroleum ether as the solvent (method 920.39; AOAC, 2006). Crude protein of the samples was determined by using Elementar rapid N-Cube nitrogen determination (Elemetar Americas Inc., Mt. Laurel, NJ), based on AOAC 968.06 procedure (2006).

Cows were milked three times daily at 0700, 1500 and 2200 h in a double 8 parallel parlor and the milk production was recorded daily (Delaval- Alpro, Sweden). Two milk samples were collected at the end of week 3 and 4 of each period from each milking on two consecutive days. One set was sent to Heart of America DHIA laboratory (Manhattan, KS) to analyze fat, protein, lactose, milk solids, somatic cell and milk urea nitrogen (MUN) according to AOAC procedures (2006). Fat, protein and lactose were analyzed by mid infrared spectroscopy (Bentley 2000 Mid Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Milk urea nitrogen concentration was determined using chemical methods based on a modified Berthelot reaction (Chaney and Marbach, 1962; ChemSpec 150 Analyzer, Bentley Instruments). Somatic cell counts (SCC) were determined with a flow cytometer laser (Somacount 500, Bentley Instruments). Energy

corrected milk (ECM) was determined using the equation: [(0.327 x kg milk) + (12.95 x kg fat) + (7.2 x kg protein)] (Orth, 1992). The other milk sample was taken at each milking, compositied and analyzed for milk nitrogen fractions. Total milk protein (TP) (method 991.20; AOAC, 2006), non-protein nitrogen (NPN) (method 991.21; AOAC, 2006) and non-casein nitrogen (NCN) (method 998.05; AOAC, 2006) were analyzed. According to the methods described in AOAC (2006); true protein (method 991.23) and casein nitrogen (method 998.07) were calculated, respectively.

Cows were weighed on 3 d before the beginning of the feeding trial and on the last 3 d of each period. Body condition scores (BCS) were recorded on the same days of weighing, independently by three individuals using a scale made of 1 to 5, where 1 being emaciated and 5 being obese (Wildman et al., 1982).

Rumen fluid was collected via an esophageal tube fitted with a suction strainer at the end and a hand operated pump, on two consecutive days in wk 4 of each period at approximately 3 to 4 h post feeding. Rumen fluid collected at the beginning of each pump was discarded in order to minimize the saliva contamination. Ten milliliter aliquots of rumen fluid were mixed with 2 ml of 25% (wt/vol) meta-phosphoric acid to determine VFA concentrations. Another 10 ml aliquot of rumen fluid was mixed with 200 µL of 50% (v/v) sulfuric acid to determine rumen ammonia nitrogen (NH_3-N) concentration. Samples were frozen at -20˚ C until analysis. Rumen fluid samples were thawed and centrifuged at 30,000 × *g* for 20 min in a micro centrifuge (Model A-14, Jouan, Jouan Inc, P.O box 2716, Vinchester, VA, U.S.A). Ammonia- N concentration was measured following the assay described by Chaney and Marbach (1962).Volatile fatty acids

concentration were measured using an automated gas chromatograph (Model 6890, Hewlett-Packard, Palo Alto, CA) equipped with a 0.25 mm i.d x 15m column (Nukol, 17926 to 01C, Supelco, Inc., Bellefonte, PA) using 2-ethylbutyrate as an internal standard. Flow rate was 1.3 ml/ min of Helium. Column and detector temperature were maintained at 140˚C and 250˚C, respectively.

Blood was collected from the coccygeal artery and the subcutaneous abdominal vein, 3 to 4 h post feeding on two consecutive days of wk 4 of each period into 10 mL vacutainers containing Sodium Fluoride and Potassium Oxalate (Becton Dickinson and Co., Rutherfor, NJ). Samples were immediately placed on ice and transported to the laboratory where they were centrifuged (500 × g) at 10˚C for 20 minutes to separate the plasma. Obtained serum was stored at -20˚C until analysis. Serum samples were thawed and analyzed for glucose using glucose oxidase (Glucose kit, cat. no. G7521, Pointe Scientific, Canton, MI) according to the procedure described by Trinder (1969). Glucose concentration was determined using a Microplate spectrophotometer (Cary 50 Bio UV-visible spectrophotometer, Australia).

Fecal grab samples were collected in 6 h interval during the last 3 d of each period. Sampling time points were arranged in a manner that it represents the entire 24 h day in order to avoid diurnal variation. Similar volumes of feces on a wet basis from each cow for each time point were collected and composited. Refusals were also collected on the same days respective to fecal collection. Collected samples were stored at −20°C until analysis. Prior to analysis composites were thawed and samples were weighed in to duplicate pans for oven drying. Samples were oven dried at 55˚C in a forced air oven

(style V-23: Despatch Oven Co., Minneapolis, MN) until dried completely. After drying, the fecal samples were ground and analyzed for DM, NDF, ADF, CP, starch, and ash using the same procedures as previously described for feeds. Acid detergent insoluble ash (ADIA) content in TMR, refusals and feces was analysed as the internal marker in order to calculate the total tract digestibility of DM, organic matter (OM), NDF, ADF, CP, and starch following the method described by Merchen (1988).

3.2.4. Statistical Analysis

All data were analyzed using MIXED procedure of SAS (Version 9.3, Cary, NC, 2001). Means of DMI, milk yield, milk composition, body weight, BCS, rumen parameters, blood parameters and total tract digestibilities of nutrients were analyzed using the following model:

$$Y_{ijkl} = \mu + T_i + P_j + C_k\,(S_l) + S_l + (T_i \times S_l) + \varepsilon_{ijkl},$$

Where Y_{ijkl} is the variable of interest, μ is the overall mean, T_i is the effect of treatment i (i = 1 to 4), P_j is the effect of period j (j = 1 to 4), $C_k(S_l)$ is the effect of cow k (k = 1 to 4) nested in square l , S_1 is the effect of square l (l = 1 to 4), $T_i \times S_l$ is the interaction between treatment and square, and ε_{ijkl} is the residual error. Cow was considered as the experimental unit whereas cow nested with square was considered as the random variable. Polynomial contrasts were used to test the linear, quadratic, and cubic effects of varying the ratios between corn and barley on animal performance. Significance of main effects was declared at $P \leq 0.05$ and tendencies were discussed at $0.05 \leq P \leq 0.10$.

3.3. RESULTS AND DISCUSSION

3.3.1. Diet Evaluation

Nutrient composition of individual feed ingredients is listed in Table 3.2. Predicted nutrient compositions and the actual nutrient compositions of experimental diets are listed in Table 3.3 and Table 3.4, respectively. Crude protein values obtained from the experimental diets were slightly greater than the formulated diets. However, as the corn was replaced in the diet with barley by 1/3, a linear increment of CP was demonstrated from 100% corn diet to 100% barley diet. This is mainly due to the fact there was greater CP% in barley than in corn (12.9 vs. 9.06; Table 3.2). Starch content in actual diets is less than that of the formulated diets. It was mainly because of less starch content in the corn silage that was used for feeding compared to the corn silage which used for diet formulation (34.75% vs. 37.91%). Barley has less starch content than corn (52.5% vs. 72.1%; Table 3.2). Therefore, when barley replaced corn, it decreased the starch content in agreement with Overton et al. (1995) study where they replaced corn with barley linearly. As mentioned in the NRC (2001), Lys to Met ratio supplied by MP should be 3 to 1 in order to maximize milk and milk protein production. In all four diets, Lys to Met ratio was above 3. Moreover, MP balance (g/d) was positive in all the treatments.

The particle size distribution of dietary treatments is presented in Table 3.5. Proportions of particles retained in each sieve were similar in all four diets. The mean values of particle amounts retained in each pan in percentage were, 8.3, 37.0, 41.9, and 12.8, respectively from top to the bottom. Materials left in upper sieve and middle sieve

followed the recommendations for particle size of TMR suggested by Heinrichs and Kononoff (2003). However, there were more material left in the 4 mm sieve and less in the bottom pan than the recommendations.

3.3.2 Production Measures

Dry matter intake, body weight, milk yield, and composition are presented in Table 3.6. Varying the ratio between corn and barley in the diets did not significantly affect dry matter intake, body weight (BW), and BCS. However, BW change tended to decrease linearly ($P = 0.09$) when inclusion of barley increased from 0 to 100%. Milk yield, ECM yield, yields and percentages of individual milk constituents were similar for all treatments. No contrasts were observed for any of the parameters, except for lactose% MUN and somatic cell score (SCS). Cows receiving diets containing barley consumed amounts of DM equal to those diets containing corn. Hence, no significant effect of grain source was observed on DMI, in agreement with the findings of DePeters and Taylor (1985) and Grings et al. (1992). However, previous researchers (Casper and Schingoethe, 1989; McCarthy et al., 1989; Casper et al., 1999) found an increased DMI when the cows were fed corn over barley. Barley starch rapidly digests in the rumen than corn starch and thus, results in an elevated lactic acid concentration (Khorasani et al., 2001). Greater lactic acid concentration could potentially decrease ruminal pH which can ultimately create detrimental effects on inhabitant microflora, hence the digestion. Consequently, a decrease in DMI could occur in animals fed barley. Moreover, when corn was fed over barley, DMI was increased because of the fact that corn based diet contained about 5 percentage units more starch than barley (Clark et al., 1992). Dietary NDF for barley

containing diets in this present study was slightly greater than that for the diet containing corn (32.07 vs. 32.53). This was mainly because of the greater NDF content in barley grain than in corn (22.8 vs 6.8%). Mertens (1987), observed a decrease in DMI, when the NDF content in diet increased by several percentage units. Thus, slight increment observed in NDF in barley-based diet may have resulted in numerically least DMI of the cows fed 100% barley diet. Overton et al. (1995) conducted a study similar to the present study using SBM with varying starch sources. In their experiment, ratio of starch from ground shelled-corn to steam-rolled barley varied from, 1)100:0, 2) 75:25, 3) 50:50, 4) 25:75 and 5) 0:100). As the proportion of barley increased, previous authors observed a linear decrease in DMI and milk yield. However, results of the present study did not support the findings of Overton et al (1995).

Even though there was no significant effect of the starch source on milk production, it was numerically greatest for cows fed the 33:67 diet (41.55 kg). Clark et al. (1992) and Surber and Bowman (1998) reported 17% greater microbial N synthesis and greater meat production of steers fed barley than for those fed corn. The extent and the rate of starch degradation in barley must have matched up with the protein degradation in the rumen (Nikkhah, 2012) and, thus a better synchronization may have led to the increased production (Khorasani et al., 2001). Khorasani et al. (2001) speculated a greater microbial N synthesis when cows were fed a diet containing a combination of corn and barley than solely corn or barley. Nocek and Russell (1988) concluded that greater milk response from cows fed barley could be attributed to an increased propionic acid production in the rumen that stimulated more efficient milk production compared to corn.

In this study, feeding of diets containing combination of corn and barley increased the molar percentage of propionate in ruminal fluid compared with diets that contained solely corn or barley (Table 3.6). Propionate first converts to glucose in the liver which then converts to lactose, the key osmotic regulator in milk. Therefore, numerically greater milk yield observed in the diet that had the ratio of corn to barley of 33: 67, can be attributed to the greater propionate concentration reported.

Previous researchers (McCarthy et al., 1989; Casper et al., 1999) found a greater milk production for cows fed corn-based diet than those fed barley-based diet. As the reason for increased milk production observed when cows were fed a corn-based diet, McCarthy et al. (1989) suggested that the shift in the site of starch digestion of corn to the SI increased the glucose availability for lactose synthesis, which then eventually led for the increased production. Contrasting to the suggestions made by other authors, Nocek and Tamminga (1991) concluded that, net increase in glucose reaching the portal vein when the site of starch digestion shift to SI is negligible. However, according to Nocek and Tamminga (1991), feeding corn can derive glucose from the hydrolysis of starch that is reaching SI and may spare glucose derived from gluconeogenesis for gut metabolism, which may then increase the amount of glucose that is directed to milk synthesis. Others (DePeters and Taylor, 1985; Grings et al., 1992) observed no effect ($P>0.1$) of grain source on milk production.

The discrepancies observed in the production response between studies may be also related to the origin of the grain source. In a study conducted by Casper et al. (1990) using corn and barley, reported a decrease in 4% FCM for cows fed diets containing

barley. According to previous authors, non-structural carbohydrate solubility in Midwest originated barley was greater than corn.

When early lactation cows were fed corn and barley-based diets, Casper et al. (1990) observed a decrease milk fat from cows fed barley during early lactation. Gozho and Mutsvangwa (2008) compared the effect of four different grain sources, namely: corn, barley, wheat, and oats on lactation performance. Cows fed corn-based TMR resulted in greater fat content compared to that with barley or wheat-based TMR. Overton et al. (1995) identified a linear reduction in milk fat yield and a quadratic effect in the percentage of milk fat, as the proportion of corn replaced by barley, increased in the diet. Differences in milk fat content could be attributed to the differences in site and extent of digestion of grain source. However, the fact that there was no effect of grain source on milk fat yield or the fat percentage in the current study is in agreement with the findings of Grings et al. (1992) and Khorasani et al. (2001).

In the present study, dietary treatments did not effect milk protein % or milk protein yield, which is not surprising because milk protein content is hard to alter by manipulating of diets (Yang et al., 1997). Overton et al. (1995) found that when corn was replaced from barley linearly, milk protein % increased linearly as the proportion of barley starch increased in the diet. However, contrast to milk protein %, protein yield tended to decline quadratically, and was least in 0:100 barley diet. In the study done by Khorasani et al. (2001), milk protein % decreased in a quadratic manner, where the greatest milk protein% was observed in the diet had the ratio of 50:50, between corn and barley. In the present study, dietary treatments did not effect milk protein % or milk

protein yield, which is not surprising because milk protein content is hard to alter by manipulating of diets (Yang et al., 1997).

In this present study, lactose yield was similar in all four treatments. Moreover, milk lactose % exhibited a cubic effect ($P<0.05$) when corn was replaced with barley and was greatest in the diet where the ratio between corn and barley was 33 to 67. Greater milk lactose concentration observed in Casper et al. (1999) study for the cows fed corn than barley, was assumed to be related to a greater supply of glucose because of a greater NSC concentration in corn. According to the suggestions made by McCarthy et al. (1989), starch digestion shifts to the intestine when diets are based on corn, which could potentially increase the availability of glucose for lactose synthesis and, thus, an increased milk production of cows fed corn-based diets. Milk composition was not affected by feeding corn or barley as the cereal component in the diets in DePeters and Taylor (1985) study. According to the authors, lack of response in milk composition for feeding corn or barley was because cows consumed equal amounts of digestible energy since diets were formulated to be isocaloric.

Milk urea nitrogen concentration varied cubically when corn was totally replaced with barley. Milk urea nitrogen concentration was least (14.5 mg/dL) in cows fed the diet that had the ratio of corn to barley of 67: 33 and greatest (15.39 mg/dL) in cows fed diet with the ratio of corn to barley was 33 to 67. The diet that had the greatest MUN concentration had the least milk protein yield and the greatest ruminal NH_3-N concentration (Table 3.6). These effects suggest a potential to decrease nitrogen utilization efficiency in the animals fed 33 to 67 corn to barley diet. Milk urea nitrogen is

highly correlated with milk protein yield and ruminal NH_3 concentration (Sannes et al., 2002). Even though there was no significant reduction in milk production or milk protein yield and milk protein %, low MUN appear to be a useful indicator of inefficient use of dietary N in the diet that had the ratio between corn to barley of 33 to 67 (Sannes et al., 2002).

Somatic cell score (SCS) linearly increased from 1.03 to 1.23 with the proportion of barley increased in the diet. However, the reason for this variability in SCS is unexplainable.

Lack of response was observed for most of the production parameters of the animals fed diets varying ratios of corn to barley. Reasons for the lack of response observed can be explained as following. Fractions included in carbohydrate (starch, sugar, fructans, pectins, glucans, hemicelluloses and cellulose) have different degradation rates which can vary under different conditions. Degradation products of these compounds supply a single rumen carbohydrate pool where rumen microbes ferment them and fulfill their energy requirement while supplying VFA to the cow for milk synthesis. Cows eat multiple meals per day which includes multiple carbohydrate fractions that contribute to the rumen carbohydrate pool. However, after several meals, this pool approaches a steady-state. Thus, varying dietary starch content or the starch source do not change the energy availabile to the microbes or to the cow, despite differences in rumen degradation rates between carbohydrate fractions, as long as the total NFC and NDF levels are maintained (St-Pierre and Knapp, 2008). Furthermore, responses of lactating cows to different cereal grains depend on the factors such as,

dietary inclusion rate, processing of the cereal grains, composition of the basal ration and the level of intake (Khorasani et al., 2001). Therefore, when speculating the exact reason for the differences observed in different studies, direct comparisons are often not possible (Gozoa and Mutsvangwa, 2008).

3.3.3 Milk Protein Fractions

Results for the milk protein fractions are presented in Table 3.7.Varying the proportion of corn and barley in diets did not exhibit any significant effect on milk protein fractions. Total protein percentage in milk varied from 3.17 to 3.34. Milk non protein nitrogen which is approximately 5% of the total milk N comprises of end products of N metabolism, mainly urea (DePeters and Ferguson, 1992). In the present study, NPN varied from 0.22 to 0.27 as a % of total milk N. Slightly greater NPN observed in this study can be attributed to the greater MUN values observed. According to Bruhn and Franke (1977), total milk protein content was least in the summer and greatest in the winter, across four different dairy breeds. Moreover, diseases such as mastitis can cause variation in milk N fractions (DePeters and Cant, 1992).

Casein which is approximately 76 to 86 % of the total protein in milk is considered as the most important milk constituent in dairy manufacturing (DePeters and Cant, 1992). However, changing the proportion of corn to barley did not affect casein % in milk. Even though it is speculated that both amount and type of dietary protein influence the milk protein content and composition, magnitude of change is much smaller than that was observed for fat (Sutton, 1989).

3.3.4 Rumen measures and plasma metabolites

Corn and barley exhibit differences in starch content as well as the ruminal degradation rate (Herrera-Saldana et al., 1990; Huntington, 1997). Therefore, effects of dietary treatments in ruminal pH can be expected. However, in the present study, ruminal pH was not affected by dietary treatments (Table 3.8). Rumen fluid samples were collected via esophageal tubing which has the higher tendency of saliva contamination. It could have attributed to the lack of differences found in ruminal pH by varying starch sources (Gozho and Mustvangwa, 2008). Ruminal pH that varied from 6.65 to 6.8 demonstrate that, there was no acidosis conditions occurred by feeding any of the treatments.

Even though it is believed that inclusion of barley in a ration causes acidosis to cows, results of the present study demonstrates that, acidosis can be avoided if the diets are properly balanced. Overton et al. (1995) observed a linear decrease in ruminal pH as the corn was replaced by 25% of barley in the diet of lactating cows. McCarthy et al. (1989) observed a low ruminal pH in the cows fed barley. Similar to their findings, Surber and Bowman (1998) also observed a lesser ruminal pH in the rumen fluid, when beef steers were fed barley-based diet than for corn-based diet. Khorasani et al. (2001) expected to observe an increase lactic acid concentration in cows fed barley-based diet because of its rapid rate of starch digestion than corn, which can detrimentally effect on ruminal pH. However, they did not find any effect of grain source on ruminal lactic acid concentration or ruminal pH. In agreement with the finding of the present study, DePeters and Taylor (1985) and Casper et al. (1999) also did not observe any effect of grain source

on ruminal pH. Discrepancies observed in ruminal pH to the replacement of corn with barley could be attributed to various factors namely: forage source and inclusion level in the basal diet, grain variety, extent of processing of grain, inclusion level, method of sampling and the time of sampling relative to feeding.

In the present study, starch source did not affect ($P > 0.10$) ruminal NH_3-N concentration. However, ruminal NH_3-N concentration was greater than 5 mg/dL (varied from 11.08 to 11.67 mg/dL) for all the treatments, which is suggested for a maximum microbial protein synthesis in the rumen (Satter and Slyter, 1974). Greater NH_3-N concentration in the rumen fluid demonstrates lower N utilization efficiency of converting NH_3-N into microbial protein, which results in decreased milk protein synthesis (NRC, 2001).

Ruminal NH_3-N concentrations were less when cows were fed barley-based diets compared to those fed corn-based diets as observed by previous researchers (McCarthy et al., 1989; Casper et al., 1990; Casper et al., 1999). In a study done by Overton et al. (1995), linear reduction was observed in ruminal NH_3-N concentration when corn was replaced by 25% of barley. In contrast to their findings, Surber and Bowman (1998) observed a greater ruminal NH_3-N concentration for the steers fed barley-based diet than for corn fed diet. Moran (1986) compared the effect of the grain source (barley, wheat, and orts) on dairy cow's performance and did not observe dietary effect on ruminal NH_3-N concentration. Similarly, Khorasani et al. (1994) and Gozho and Mutsvangwa (2008) also found no treatment differences when feeding corn or barley on ruminal NH_3-N concentration, and supports the findings of the present study.

Concentration of total VFA in the rumen fluid was not affected by the dietary treatments (Table 3.8). However, 33 to 67 corn to barley diet had the numerically greatest total VFA concentration. Particular diet must have facilitated better growing conditions for the microbes, where they can produce greater VFA concentrations in the presence of CM as the main protein supplement. The fact that total VFA concentration in the rumen fluid was not affected by the dietary treatment is in agreement with the findings of previous researchers (DePeters and Taylor, 1985; Casper and Schingoethe, 1989; Casper et al., 1990; Nocek and Tamminga, 1991; Khorasani et al., 1994). McCarthy et al. (1989), Surber and Bowman (1998), and Khorasani et al. (2001) reported a decrease in ruminal total VFA concentration as a result of substituting corn for barley. In contrast, Casper et al. (1999) observed higher total VFA concentration for the cows fed corn than that for the animals fed barley. Linear decrease in total VFA content was observed in the study done by Overton et al. (1995), when corn was linearly replaced with barley.

In the present study, acetate concentration (Table 3.8) did not vary when corn was replaced from barley. Propionate concentration responded quadratically ($P < 0.05$) to the variation of starch, and was greater in the diets that had mix proportions of corn and barley. Greater propionate concentrations in the mixed diets demonstrate that, there was a better starch utilization by microbes in those diets than when fed a single starch source. However, variability in propionate did not reflect in milk lactose content or blood glucose.

According to previous studies, variability in the grain source did not have any effect on ruminal acetate concentration (DePeters and Taylor, 1985; Casper and

Schingoethe, 1989; Casper et al., 1990; Khorasani et al., 1994). Higher propionate concentration and a lower acetate concentration were observed by McCarthy et al. (1989) when the barley replaced corn in the diet. Similar to their findings, Casper et al. (1990) also reported a greater molar proportion of propionate when cows were fed barley than corn. Khorasani et al. (1994) reported a decrease propionate concentration when cows were fed barley than fed corn. In the study by Overton et al. (1995), replacement of 25% of corn linearly with barley resulted decrease acetate molar percentage and increase propionate molar percentage, which resulted decrease acetate to propionate ratio. Reflecting the variability observed in propionate concentrations, acetate to propionate ratio also exhibited a quadratic effect ($P < 0.05$), and diets had the mix proportions of corn and barley had the least ratio. Khorasani et al. (1994) did not observe any significant effect of starch source (corn vs. barley) on ruminal acetate to propionate ratio. In contrast, lower acetate to propionate ratio was reported by DePeters and Taylor (1985) in the dairy heifers fed barley than corn.

Butyrate, isobutyrate and valerate concentrations did not differ by feeding varying proportions of corn and barley. Howeber, isovalerate concentration decreased linearly as barley starch increased in the diet. Previous researchers (Casper and Schingoethe, 1989; Casper et al., 1990) observed a reduction in butyrate concentration when barley replaced corn in diets. DePeters and Taylor. (1985) and McCarthy et al. (1989) found no difference in varying starch source on butyrate concentration supports the findings of present study. McCarthy et al. (1989) did not observe any difference in isovalerate and valerate concentrations by feeding differing starch sources. Replacement of corn with

25% of barley in Overton et al. (1995) study decreased butyrate and isovalerate in a quadratic manner, wherein molar percentage of valerate increased linearly as the proportion of barley starch increased. However, variabilities observed among different studies can be attributed to factors such as: variabilies in sampling techniques, composition of the basal ration, particle size of the grain sources, dietary intake and analytical procedures adapted (Moran, 1986; Khorasani et al., 2001).

The fact that plasma concentrations of glucose did not respond to varying proportions of corn and barley is in agreement with finding by Khorasani et al. (1994) and Gozho and Mutsvangwa (2008). Amount of glucose that is absorbed in the small intestine in dairy cows is limited due to the extensive ruminal fermentation of dietary starch. Therefore, plasma glucose synthesis during hepatic gluconeogenesis mainly uses ruminally derived propionate as the principle precursor (Huntington, 1997). Even though the ruminal propionate concentrations exhibited a quadratic effect in the present study, it did not reflect in plasma glucose concentration. In contrast to the present study findings, Grings et al. (1992) observed greater blood glucose concentration for the cows that fed barley compared to corn. According to their explanation, higher rate of starch degradation in the rumen expressed by barley must have resulted in greater glucose concentration.

3.3.5 Apparent total tract digestibility of nutrients

Apparent total tract nutrient digestibility results are shown in Table 3.9. Apparent total tract digestibilities of DM, organic matter (OM), and NDF decreased linearly and, CP and ADF tended to decrease linearly when the proportion of barley increased in the diet. However, starch digestibility averaged 95.5% was not affected by varying the

proportions of starch sources. Even though it was observed that the nutrient digestibility decreased as barley increased in the diets, production responses did not vary among treatments. May be this can be explained by the BW change of the animals where it tended to decrease as barley increased in the diets. Overton et al. (1995) observed no difference in total tract digestibility of DM and OM by varying proportions of corn and barley. However, they observed a decrease in the quantity of DM and OM digested when the proportion of barley starch increased in the diet. In agreement with the findings of Overton et al. (1995), DePeters and Taylor (1985) and McCarthy et al. (1989) also demonstrated little effect of replacing corn with barley on the apparent total tract digestibility of DM and OM. Amounts of undigested starch reaching hindgut of cows fed corn are greater than barley because of the slower rate of starch digestion from corn. Because of this reason, it is thought that feeding corn promotes greater bacterial protein synthesis in the hindgut. However, having no mechanism for enzymatic digestion in the hindgut, results in bacterial protein voided in to the feces (Orskov et al., 1970). Therefore, it is expected that there will be less total tract digestibility of CP in corn based diets compared to barley based diets. However, in contrast to this theory, in the present total tract digestibility of CP was tended (P=0.07) to linearly decrease as the inclusion of barley increased.

When corn was replaced with barley digestibilities of NDF and ADF in the present study linearly decreased. Similar to our findings Overton et al. (1995) observed a decrease in the digestibility of NDF from 51.6 to 46.5% and ADF from 44.0 to 32.6% when barley replaced corn. DePeters and Taylor (1985) observed a reduction in the total

tract digestibility of ADF when barley was fed over corn. However, McCarthy et al. (1989) did not observe such difference.

Digestibility of starch in the total tract was almost similar among treatments and averaged 95.5%. In dairy cows, starch digestion mainly occurs in the rumen (Huntington, 1997) and, barley expresses a greater starch digestion in the rumen than corn (Huntington, 1997; Herrera-Saldana et al., 1990). Therefore, it can be expected greater total tract starch digestion when barley was fed. McCarthy et al. (1989) and Overton et al. (1995) observed a decrease in starch digestibility when corn was replaced with barley. However, in contrast to that Robinson et al. (1995) observed a reduction in starch digestibility when barley was fed over corn. Discrepancies observed among different studies may be because of the differences in dietary inclusion amounts of corn and barley and the physical form of the diets that fed, such as pelleted, whole grain, and ground.

CONCLUSIONS

There is no significant effect of starch source in the diet on DMI, milk production, milk composition, ruminal measures (except propionate concentration and acetate to propionate ratio) and plasma glucose concentration of high producing dairy cows when fed with CM. However, apparent total tract digestibility of nutrients (except starch) linearly decreased when the proportion of barley starch increased in the diet. It can be suggested that, corn as a successful replacement for barley without creating detrimental effects on the cow performance when the rations are properly balanced. It is understood from the present study that, combination of starch sources of varying degradation rates have the potential to support greater production similar to an individual starch source in the presence of CM as the major protein supplement in the diet.

Table 3.1. Ingredients composition of experimental diets

	Diet[1]			
Ingredients, % of DM	100:0	67:33	33:67	0:100
Alfalfa haylage	19.6	19.6	19.6	19.6
Corn silage	36.3	36.3	36.3	36.3
Cottonseed	5.24	5.24	5.24	5.24
Corn grain, finely ground	11.2	7.45	3.72	0
Barley grain, rolled	0	4.95	9.89	14.8
Canola meal	12.2	12.1	11.9	11.7
Soybean hulls	5.94	4.89	3.84	2.79
Beet pulp, dried	2.80	2.80	2.80	2.80
Expellers soybean meal[2]	3.49	3.49	3.49	3.49
Energy Booster 100[3]	1.40	1.40	1.40	1.40
Rumen protected methionine [4]	0.12	0.12	0.12	0.12
Calcium carbonate	0.44	0.44	0.44	0.44
Salt	0.40	0.40	0.40	0.40
Sodium bicarbonate	0.56	0.56	0.56	0.56
Magnesium oxide	0.18	0.18	0.18	0.18
Mineral and vitamin premix[5]	0.17	0.17	0.17	0.17
Vitamin E	0.04	0.04	0.04	0.04
Rumensin[6]	0.01	0.01	0.01	0.01

[1]Treatments were 100C:0B with ratio between corn to barley was 100:0, 67C:33B with ratio between corn to barley 67:33, 33C:67B with ratio between corn to barley was 33:67, 0C:100B with ratio between corn to barley was 0:100.
[2]SoyPlus (West Central Soy, Ralston, IA).
[3]Energy Booster 100 (Milk Specialties, Dundee, IL).
[4]MetiPEARL™ (Kemin Industries, Inc., Des Moines, IA).
[5] Contained: 10% Mg; 2.6% Zn; 1.7 mg/kg Mn; 4640 mg/kg Fe; 4712 mg/kg Cu; 396 mg/kg I; 119 mg/kg Co; 140 mg/kg Se; 2640000 IU/ kg vitamin A; 528000 IU/kg vitamin D3; and 10560 IU/kg vitamin E (Land Ó Lakes Purina feed LLC, IA).
[6]Rumensin 90g/ lb (Elanco Animal Health, Greenfield, IN).

Table 3.2. Nutrient composition of individual ingredients

Item	Corn[1]	Barley[2]	Canola meal	Beet pulp	Soy Hulls	Soy plus	Cotton seed	Corn silage	Alfalfa haylage
DM[3]	97.4	97.6	97.4	97.8	98.0	98.0	97.3	38.9	41.4
CP[3]	9.06	12.9	43.1	8.55	13.4	46.2	22.3	7.73	23.5
Sol.P[4]	22.0	34.3	22.1	8.01	34.3	10.5	33.8	46.8	66.4
ADICP[4]	8.17	4.52	7.82	9.71	9.06	3.21	7.69	0.70	1.35
NDICP[4]	9.72	15.5	25.6	66.3	29.1	29.1	8.36	1.32	2.54
ADF[3]	1.97	7.24	21.6	23.8	47.1	10.3	31.9	23.1	35.5
NDF[3]	6.80	22.8	27.1	38.1	64.2	17.2	43.3	39.7	37.9
Lignin[3]	0.30	2.53	9.37	1.63	2.07	1.29	9.98	3.22	10.5
Starch[3]	72.1	52.5	1.59	1.08	2.38	1.48	0.17	34.8	0.66
Sugar[3]	4.57	7.21	10.7	17.9	5.68	13.5	5.67	1.57	1.95
EE[3]	3.67	2.11	3.26	0.63	2.34	7.27	17.0	3.39	3.62
Ash[3]	1.16	2.80	8.11	6.90	5.09	6.30	4.58	4.51	10.6
Ca[3]	0.04	0.09	0.21	0.85	0.52	0.38	0.15	0.28	1.85
P[3]	0.26	0.48	1.19	0.08	0.16	0.65	0.75	0.24	0.29
Mg[3]	0.12	0.10	0.66	0.32	0.22	0.33	0.42	0.28	0.44
K[3]	0.34	0.50	1.37	0.45	1.40	2.02	1.17	0.69	2.34
S[3]	0.12	0.16	0.89	0.31	0.17	0.39	0.26	0.14	0.25
Na[3]	0.03	0.01	0.03	0.09	0.05	0.04	0.02	0.01	0.05
Cl[3]	0.10	0.12	0.07	0.06	0.07	0.04	0.09	0.29	0.82

[1]Ground corn.
[2]Rolled barley.
[3]Values are in % of DM.
[4]Values are in % of CP.

Table 3.3. Predicted nutrient composition of experiment diets[1]

Nutrient composition	Diet[2]			
	100:0	67:33	33:67	0:100
Dry Matter, %	51.2	51.2	51.2	51.2
CP, % of DM	17.3	17.3	17.4	17.5
RDP, % of DM	10.0	10.1	10.2	10.3
RUP, % of DM	7.23	7.22	7.22	7.23
SP,% of DM	36.9	36.8	36.7	36.6
Lys, g/% of MP	6.59	6.60	6.61	6.61
Met, g/% of MP	2.21	2.21	2.20	2.20
Lys: Met as MP	2.98	2.99	3.00	3.01
MP balance, g/d	-133	-144	-95.0	-76.0
Starch (% of DM)	22.9	22.9	22.9	22.9
NDF, % of DM	33.4	33.4	33.4	33.4
fNDF, % of BW	0.76	0.76	0.76	0.76
ADF, % of DM	22.6	22.3	22.0	21.7
NFC, % of DM[3]	36.5	36.4	36.4	36.3
EE, % of DM	5.67	5.61	5.55	5.49
Ash, % of DM	7.12	7.14	7.16	7.19
Ca, % of DM	0.82	0.82	0.81	0.81
P, % of DM	0.42	0.43	0.44	0.45
K, % of DM	1.40	1.39	1.38	1.38
Mg, % of DM	0.37	0.36	0.35	0.35
S,% of DM	0.22	0.22	0.22	0.22
NE_L, Mcal/kg	1.57	1.57	1.57	1.57
ME balance, Mcal	-2.30	-2.60	-2.80	-3.10

[1]Estimated from AMTS software (Agriculture Modelling and Training Systems, Cortland, NY)

[2]Diets were 100C:0B with ratio between corn to barley was 100:0, 67C:33B with ratio between corn to barley 67:33, 33C:67B with ratio between corn to barley was 33:67, 0C:100B with ratio between corn to barley was 0:100.

[3]NFC=100 - (% NDF + % CP + % ether extract + % ash).

Table 3.4. Actual chemical composition of experimental diets

	Diet[1]			
Nutrient composition	100:0	67:33	33:67	0:100
DM%	51.4	50.9	51.2	50.6
CP, % of DM	17.6	17.6	17.7	17.8
RDP, % of DM[2]	9.83	9.93	10.0	10.1
RUP, % of DM[2]	7.73	7.71	7.70	7.69
SP, % of DM	6.61	6.69	6.77	6.85
ADICP, % of DM	7.60	7.42	7.24	7.17
NDICP, % of DM	10.0	10.1	10.1	10.1
Lys,% of MP[2]	6.64	6.65	6.65	6.65
Met, % of MP[2]	2.19	2.19	2.19	2.18
Lys: Met as MP[2]	3.03	3.04	3.05	3.05
MP balance, g/d[2]	127	135	143	150
Starch, % of DM	21.2	21.1	21.0	20.9
NDF, % of DM	32.1	32.2	32.4	32.5
fNDF, % of DM	22.2	22.2	21.3	21.7
ADF, % of DM	23.7	23.5	23.2	23.0
NFC, % of DM[3]	37.3	37.1	36.9	36.8
EE, % of DM	5.47	5.43	5.37	5.30
Ash, % of DM	7.58	7.59	7.62	7.65
Ca, % of DM	0.78	0.78	0.77	0.77
P, % of DM	0.39	0.40	0.41	0.42
K, % of DM	1.14	1.14	1.13	1.13
Mg, % of DM	0.43	0.43	0.43	0.42
S, % of DM	0.27	0.27	0.27	0.27
NE_L, Mcal/kg[2]	1.54	1.53	1.52	1.51
ME balance, Mcal[2]	1.18	0.70	0.25	-0.25

[1]Diets were 100C:0B with ratio between corn to barley was 100:0, 67C:33B with ratio between corn to barley 67:33, 33C:67B with ratio between corn to barley was 33:67, 0C:100B with ratio between corn to barley was 0:100.

[2]Estimated from AMTS software (Agriculture Modelling and Training Systems, Cortland, NY).

[3] NFC=100 - (% NDF + % CP + % ether extract + % ash).

Table 3.5. Particle distribution using penn state particle separator[1]

	Diet[2]					*P*-values[3]		
Item (% as fed basis)	100:0	67:33	33:67	0:100	SEM	L	Q	C
>19mm	7.14	8.32	7.81	9.90	0.96	0.10	0.65	0.34
8-19 mm	36.8	36.4	38.0	36.8	1.37	0.83	0.76	0.45
1.18-8 mm	41.9	42.7	41.4	41.5	1.32	0.67	0.70	0.67
<1.18 mm	14.1	12.5	12.5	11.9	1.12	0.22	0.66	0.68

[1]Particle size distribution of diets was measured using Penn State Particle Separator (PSPS; Kononoff and Heinrichs, 2003).

[2]Diets were 100C:0B with ratio between corn to barley was 100:0, 67C:33B with ratio between corn to barley 67:33, 33C:67B with ratio between corn to barley was 33:67, 0C:100B with ratio between corn to barley was 0:100.

[3]Contrasts: L = linear; Q = quadratic; and C = Cubic.

Table 3.6. Dry matter intake, Body weight, Body condition score, milk yield, milk composition, and feed efficiency of cows fed diets with varying ratios of corn and barley, in the presence of canola meal as the protein source

	Diet[1]					*P*-values[2]		
Item	100:0	67:33	33:67	0:100	SEM	L	Q	C
DMI (kg/d)	27.1	26.3	26.8	25.9	0.78	0.15	0.88	0.20
BW (kg)	656	656	659	651	12.5	0.64	0.46	0.57
BW change (kg/d)	0.38	0.57	0.20	0.02	1.91	0.09	0.33	0.38
BCS	3.08	3.07	3.10	3.11	0.38	0.44	0.61	0.61
BCS change	0.01	0	0.01	0	0.10	0.34	0.78	0.41
Production (kg/d)								
Milk	41.4	40.6	41.6	41.1	1.35	0.99	0.77	0.30
ECM[2]	40.7	40.2	40.5	40.8	1.13	0.88	0.57	0.82
Milk composition								
Fat (%)	3.46	3.56	3.45	3.58	0.13	0.50	0.84	0.18
Fat (kg/d)	1.42	1.42	1.41	1.44	0.05	0.77	0.68	0.65
Protein (%)	2.94	3.01	2.92	2.93	0.05	0.53	0.84	0.18
Protein (kg/d)	1.21	1.21	1.20	1.20	0.05	0.53	0.44	0.16
Lactose (%)	4.86	4.83	4.90	4.88	0.03	0.21	0.98	0.04
Lactose (kg/d)	2.02	1.98	2.04	2.01	0.04	0.66	0.98	0.82
SNF[3] (%)	8.63	8.71	8.71	8.70	0.04	0.22	0.24	0.74
SNF (kg/d)	3.52	3.52	3.64	3.61	0.12	0.27	0.92	0.49
Total solids (%)	12.1	12.3	12.2	12.3	0.15	0.25	0.73	0.14
Total solids (kg/d)	5.00	4.99	5.03	5.07	0.14	0.55	0.75	0.89
MUN (mg/dL)	14.8	14.5	15.4	15.1	0.29	0.09	0.96	<0.01
Feed efficiency[4]	1.49	1.54	1.52	1.55	0.04	0.36	0.75	0.59
SCS[5]	1.03	1.20	1.16	1.29	0.10	0.04	0.81	0.26

[1]Diets were 100C:0B with ratio between corn to barley was 100:0, 67C:33B with ratio between corn to barley 67:33, 33C:67B with ratio between corn to barley was 33:67, 0C:100B with ratio between corn to barley was 0:100.

[2]Contrasts: L = linear; Q = quadratic; and C = Cubic.

[3]ECM = [0.327 × milk (kg)] + [12.95 × fat (kg)] + [7.20 × protein (kg)]. Adapted from Orth (1992).

[4]SNF = Total solids - Fat.

[5]Feed efficiency = Energy corrected milk yield (kg)/ DMI (kg).

[6]SCS = log (SCC).

Table 3.7. Protein fractions of milk protein of cows fed diets with varying ratios of corn and barley, in the presence of canola meal as the protein source

	Diet[1]					*P*-values[2]		
Item	100:0	67:33	33:67	0:100	SEM	L	Q	C
Total CP, %	3.21	3.17	3.19	3.34	0.08	0.22	0.23	0.86
NPN[3], %	0.27	0.26	0.22	0.27	0.01	0.78	0.20	0.35
TP[4], %	2.94	2.91	2.95	3.07	0.08	0.23	0.35	0.99
Casein, %	2.54	2.51	2.59	2.67	0.10	0.28	0.52	0.84
Whey[5], %	0.41	0.40	0.37	0.40	0.05	0.66	0.68	0.64
TP, % of total CP	91.6	91.8	92.3	91.9	0.43	0.44	0.53	0.44
Casein, % of total CP	79.2	79.0	80.7	80.0	1.59	0.50	0.86	0.50

[1]Diets were 100C:0B with ratio between corn to barley was 100:0, 67C:33B with ratio between corn to barley 67:33, 33C:67B with ratio between corn to barley was 33:67, 0C:100B with ratio between corn to barley was 0:100.

[2]Contrasts: L = linear; Q = quadratic; and C = Cubic.

[3]NPN=Non-protein nitrogen.

[4]True protein = CP-NPN.

[5]Whey protein = NPN-NCN.

Table 3.8. Ruminal volatile fatty acid concentration and plasma metabolites of cows fed diets with varying ratios of corn and barley, in the presence of canola meal as the protein source

	Diet[1]					*P*-values[2]		
Item	100:0	67:33	33:67	0:100	SEM	L	Q	C
pH	6.80	6.65	6.78	6.68	0.09	0.54	0.79	0.13
NH_3-N, mg/dL	11.7	11.1	11.7	11.4	1.40	0.99	0.89	0.69
Total VFA, m*M*	61.2	60.3	63.0	60.0	7.47	0.98	0.87	0.74
VFA, mmol/100mol								
Acetate	66.8	66.2	65.2	66.3	0.83	0.43	0.21	0.40
Propionate	18.4	20.1	21.1	19.5	0.76	0.09	<0.01	0.45
Butyrate	9.97	9.53	9.60	9.81	0.41	0.80	0.37	0.80
Isobutyrate	3.10	2.41	2.33	2.67	0.45	0.35	0.14	0.89
Valerate	1.20	1.00	1.07	1.02	0.15	0.35	0.53	0.46
Isovalerate	0.88	0.70	0.67	0.61	0.13	0.05	0.53	0.61
A:P ratio[3]	3.78	3.34	3.17	3.59	0.15	0.13	<0.01	0.52
Serum Glucose, mg/dL	74.4	74.4	73.1	73.3	1.93	0.53	0.94	0.68

[1]Diets were 100C:0B with ratio between corn to barley was 100:0, 67C:33B with ratio between corn to barley 67:33, 33C:67B with ratio between corn to barley was 33:67, 0C:100B with ratio between corn to barley was 0:100.

[2]Contrasts: L = linear; Q = quadratic; and C = Cubic.

[3]Acetate to propionate ratio.

Table 3.9. Total tract digestibilities of the nutrients of cows fed diets with varying ratios of corn and barley, in the presence of canola meal as the primary protein source

	Diet[1]					*P*-values[2]		
Digestibility	100:0	67:33	33:67	0:100	SEM	L	Q	C
DM	71.0	68.9	66.1	65.5	2.06	0.02	0.66	0.71
OM	72.3	70.3	67.8	67.1	1.70	0.01	0.66	0.73
CP	71.5	69.7	68.3	67.6	1.74	0.07	0.72	0.96
NDF	51.6	47.0	45.4	42.7	2.90	0.02	0.71	0.72
ADF	54.2	50.9	48.5	47.3	2.78	0.05	0.68	0.97
Starch	95.8	95.5	94.9	95.6	0.65	0.66	0.40	0.47

[1]Diets were 100C:0B with ratio between corn to barley was 100:0, 67C:33B with ratio between corn to barley 67:33, 33C:67B with ratio between corn to barley was 33:67, 0C:100B with ratio between corn to barley was 0:100.

[2]Contrasts: L = linear; Q = quadratic; and C = Cubic.

GENERAL CONCLUSION

The first study evaluated the variability of CM that were produced in different processing plants located in Canada using an *in situ* experiment which was then connected to an *in vitro* analysis. Secondly, an animal experiment was conducted to evaluate the production response of feeding of CM as the primary protein source along with varying proportions of corn and barley to lactating dairy cows.

Proving the first hypothesis, results from the *in situ* and *in vitro* experiments suggest that, there was variability in the chemical composition of CM produced in different processing plants. Observed variability in different CM may be because of the variability in the processing technology adopted by different processing plants. Hence, ruminal degradability and intestinal digestibility parameters varied. Values obtained for A, B and C fractions and IDP were in agreement with that of NRC with slight variation. However, Kd and RUP showed much variation compared to that with NRC. Thus, emphasized the importance of taking this variability in to account rather than using constant book values in ration formulation in order to supply adequate nutrition to the dairy cows.

Results from the animal experiment indicate that production responses, mainly milk yields and milk composition did not differ by feeding corn or barley or different proportions of corn and barley along with CM as the major protein supplement. Replacing corn with barley did not have detrimental effects on the animal. Moreover, having no significant difference among treatments on milk production claimed that, by mixing corn and barley in appropriate ratios can obtain a production similar to feeding sole corn or barley. Disproving our second hypothesis, results demonstrated that, grain

source does not have an effect upon lacating cow performances when fed with CM. Because there is no production difference among treatments, whether to feed corn or barley will be determined by the prevailing economic situation.

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LITERATURE CITED

Adams, J., C. Cassarino, J. Lindstrom, L. Eslin, S. M. Lux, and F. H. Holcomb. 2006. Canola oil fuel cell demonstration: volume III-Technical, commercialization, and application issues associated with harvested biomass. Available at: http://dodfuelcell.cecer.army.mil/library_items/CanolaVol_III.pdf (Accessed Feb. 7, 2014).

Agriculture Research Council. 1980. The Nutrient Requirements of Ruminant Livestock. CAB International, Wallingford, UK.

Aldrich, J. M., L. D. Muller, G. A. Varga, and L. C. Oriel, Jr. 1993. Non-structural carbohydrate and protein effects on rumen fermentation, nutrient flow, and performance of dairy cows. J. Dairy Sci. 76:1091-1105.

Aldrich, C.G., N. R. Merchen., J. K. Drackley., S. S. Gonzalez., G .C. Fahey, and L. L. Berger. 1997. The effects of chemical treatment of whole canola seed on lipid and protein digestion by steers. J. Anim. Sci. 75:502-511.

AOAC. 2006. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Ashes, J. R., P. St. Vincent Welch, S. K. Gulati, T. W. Scott, and G. H. Brown. 1992. Manipulation of the fatty acid composition of milk by feeding protected canola seeds. J. Dairy Sci. 75:1090-1096.

Bach, A. S. Calsamiglia, and M. D. Stern. 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88 :(E. Suppl.):E9-E21.

Bell, J. M., Keith, M. O., Blake, J. A. and McGregor, D. I. 1984. Nutritional evaluation of ammoniated mustard meal for use in swine feeds. Can. J. Anim. Sci. 64:1023-1033.

Bell, J. M. 1993. Factors affecting the nutritional value of canola meal: A review. Can. J. Anim. Sci. 73:679-697.

Berardinelli, J. G., J. Weng, P. J. Burfening, and R. Adair. 2001. Effect of excess degradable intake protein on early embryonic development, ovarian steroids, and blood urea nitrogen on days 2, 3, 4, and 5 of the estrous cycle in mature ewes. J. Anim. Sci.79:193-199.

Boila, J.R., and R.J. Ingalls.1992. *In situ* rumen digestion and escape of dry matter, nitrogen and amino acids in canola meal. Can. J. Anim. Sci.72: 891-901.

Brito, A. F. and G. A. Broderick. 2007. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. J. Dairy Sci. 90:1816-1827.

Broderick, G. A., and N. R. Merchen. 1992. Markers for quantifying microbial protein synthesis in the rumen. J. Dairy Sci. 75:2618-2632.

Broderick, G. A. 1994. Quantifying forage protein quality. Pages 200-228 in Forage Quality, Evaluation, and Utilization. G. C. Fahey, Jr., M. D. Collins, D. R. Mertens, and L. E. Moser, ed. American Society of Agronomy., Madison, WI.

Bruhn, J. C., and A. A. Franke. 1977. Monthly variations in gross composition of California herd milks. J. Dairy Sci. 60:696-700.

Cameron, M. R., T. H. Klusmeyer, G. L. Lynch, J. H. Clark, and D. R. Nelson. 1991. Effects of urea and starch on rumen fermentation, nutrient passage to the duodenum, and performance of cows. J. Dairy Sci. 74:1321-1336.

Canola Council of Canada, 2009. Canola Council of Canada, http://www.canolacouncil.org/uploads/feedguide/Canola Guide ENGLISH 2009 small.pdf (accessed Jan.02.14).

Cant, J. L., R. Berthiaume, H. Lapierre, P. H. Luimes, B. W. McBride, and D. Packeao. 2003. Responses of the bovine mammary glands to absorptive supply of single amino acids. Can. J. Anim. Sci. 83:341-355.

Casper, D. P., and D. J. Schingoethe. 1989. Lactational responses of early lactation dairy cows to diets varying in ruminal solubilities of carbohydrate and crude protein. J. Dairy Sci. 72:928-941.

Casper, D. P., D. J. Schingoethe, and W. A. Eisenbeisz. 1990. Response of early lactation dairy cows fed diets varying in source of non-structural carbohydrate and crude protein. J. Dairy Sci.73:1039-1050.

Casper, D.P., A. M. Harouna., M. J. Brouk, and D.J. Schingoethe. 1999. Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. J. Dairy Sci.82:1779-1790.

Chaney, A. L, and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.

Clark, J. H., and C. L. Davis. 1980. Some aspects of feeding high producing dairy cows. J. Dairy Sci. 63:873-888.

Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Symposium: nitrogen metabolism and amino acid nutrition in dairy cattle microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci.75:2304-2323.

Dakowski, P., M.R.Weisbjerg, and T. Hvelplund. 1996. The effect of temperature during processing of rape seed meal on amino acid degradation in the rumen and digestion in the intestine. Anim Feed Sci Technol. 58: 213-226.

DePeters, E. J., and S. J. Taylor. 1985. Effects of feeding corn or barley on composition of milk and diet digestibility. J. Dairy Sci. 68:2027-2032.

DePeters, E. J. and D. L. Bath. 1986. Canola meal versus cottonseed meal as the protein supplement in dairy diets. J. Dairy Sci. 69:148-154.

DePeters, E. J., and J. D. Ferguson. 1992. Non-protein nitrogen and protein distribution in the milk of cows. J. Dairy Sci. 75:3192-3209.

DePeters, E. J., and J. P. Cant. 1992. Nutritional factors influencing the nitrogen composition of bovine milk: A review. J. Dairy Sci.75:2043-2070.

England, M. L., G. A. Broderick., R. D. Shaver, and D. K. Combs. 1997. Comparison of *In Situ* and *In Vitro* techniques for measuring ruminal degradation of animal by-product proteins. J. Dairy Sci. 80:2925-2931.

Environment Directorate, Organisation for Economic Co-operation and Development, 2011. Revised consensus document on compositional considerations for new varieties of low erucic acid rapeseed (canola) key food and feed nutrients, anti-nutrients and toxicants. Paris.

Fenwick, G.R., R.F. Curtis, 1980. Rapeseed meal and its use in poultry diets. Anim. Feed Sci. Technol. 5: 255-298

Fenwick, G. R. 1982. The assessment of a new protein source-Rapeseed. Proc. Nutri. Soc. 41:277-288.

Firkins, J. L., Z. Yu, and M. Morrison. 2007. Ruminal nitrogen metabolism: perspectives for integration of microbiology and nutrition for dairy. J. Dairy Sci 90(E. Suppl.): E1-E16.

Gargallo, S., S. Calsamiglia, and A. Ferret. 2006. Technical note: A modified three-step in vitro procedure to determine intestinal digestion of proteins. J. Anim. Sci. 84:2163-2167.

González-Vega, J. C., B. G. Kim, J. K. Htoo, A. Lemme and H. H. Stein. 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. J. Anim. Sci.89:3617-3625.

Gozho, G. N, and T. Mutsvangwa. 2008. Influence of carbohydrate source on ruminal fermentation characteristics, performance, and microbial protein synthesisin dairy cows. J. Dairy Sci. 91:2726-2735.

Grings, E. E., R. E. Roffler, and D. P. Deitelhofpt. 1992. Evaluation of corn and barley as energy sources for cows in early lactation fed alfalfa-based diets. J. Dairy Sci.75:193-200.

Guadagnin, M., Cattani, M. and L. Bailoni. 2013. Effect of pressing and combination of three storage temperatures and times on chemical composition and fatty acid profile of canola expellers. Ital. J. Anim. Sci. 12:e43:265-269.

Harker, K. N., J. T. O'Donovan, T. K. Turkington, R. E. Blackshaw, N. Z. Lupwayi, E. G. Smith, H. Klein-Gebbinck, L. M. Dosdall, L. M. Hall, C. J. Willenborg, H. R. Kutcher, S. S. Malhi, C. L. Vera, Y. Gan, G. P. Lafond, W. E. May, C. A. Grant, and D. L. McLaren. 2012. High-yield no-till canola production on the Canadian prairies. Can. J. Plant Sci. 92:221-233.

Herrera-Saldana, R. E., J. T Huber and M. H. Poore, 1990. Dry matter, crude protein, and starch degradability of five cereal grains. J. Dairy Sci. 73:2386-2393.

Homolka, P., J. Harazim, and J. Trinacty. 2007. Nitrogen degradability and intestinal digestibility of rumen undegraded protein in rapeseed, rapeseed meal and extracted rapeseed meal. Czech J. Anim. Sci.52: 378-386.

Hoover, W. H., B. A. Crooker, and C. J. Sniffen.1976. The effects of differential solid liquid removal rates on protozoa numbers in continuous culture of rumen contents. J. Anim. Sci. 39:528-534.

Hoover, W. H., and S. R. Stokes. 1991. Balancing carbohydrates and protein for optimum rumen microbial yield. J. Dairy Sci. 74:3630-3644.

Hristov, A. N., and G. A. Broderick. 1996. Synthesis of microbial protein in ruminally cannulated cows fed alfalfa silage, alfalfa hay, or corn silage. J. Dairy Sci. 79:1627-1637.

Hristov, A.N., and Ropp, J.K. 2003. Effect of dietary carbohydrate composition on utilization of ruminal ammonia nitrogen for milk protein synthesis in dairy cows. J. Dairy Sci. 86: 2416-2427.

Huhtanen, P., M. Hetta and C. Swensson. 2011. Evaluation of canola meal as a protein supplement for dairy cows: A review and a meta-analysis. Can. J. Anim. Sci. 91: 529-543.

Huntington, G. B. 1997. Starch utilization by ruminants: From basics to the bunk. J. Anim. Sci. 75:852-867.

Kendall, E. M., J. R. Ingalls, and R.J. Boila. 1991. Variability in the rumen degradability and postruminal digestion of the dry matter, nitrogen and amino acids of canola meal. Can. J. Anim. Sci. 71: 739-754.

Kennelly, J. J., B. Robinson, and G. R. Khorasani. 1999. Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in early-lactation Holstein cows. J. Dairy Sci. 82:2486-2496.

Khorasani, G. R., G. de Boer, P. H. Robinson, and J. J. Kennelly. 1992. Effect of canola fat on ruminal and total tract digestion, plasma hormones, and metabolites in lactating dairy cows. J. Dairy Sci.75:492-501.

KhorasanI, G. R., G. De Boer, B. Robinson, and J. J. Kennelly.1994. Influence of Dietary Protein and Starch on Production and Metabolic Responses of Dairy Cows. J. Dairy Sci. 77:813-824.

Khorasani, G. R., E. K. Okine, and J. J. Kennelly. 2001. Effects of substituting barley grain with corn on ruminal fermentation characteristics, milk yield, and milk composition of Holstein cows. J. Dairy Sci. 84:2760-2769.

Lardy, G. P., G. E. Catlett, M. S. Kerley, and J. A. Paterson. 1993. Determination of the ruminal escape value and duodenal amino acid flow of rapeseed meal. J. Anim. Sci.71:3096-3104.

Leng, R. A., and J. V. Nolan. 1984. Nitrogen metabolism in the rumen. J. Dairy Sci. 67:1072-1089.

Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed. Sci. Technol. 57: 347-358.

Mailer, R. J., A McFadden, J. Ayton and B. Redden. 2008. Anti-nutritional components, fiber, sinapine and glucosinolate content, in Australian canola (Brassica napus L.) meal. J. Am. Soc. 85:937-944.

Maison, T. 2013. Evaluation of the nutritional value of canola meal, 00-rapeseed meal, and 00-rapeseed expellers fed to pigs. Dissertation for Doctor of Philosophy. University of Illinois, Urbana.

McAllister, T. A., K. J. Cheng, K. A. Beauchemin, D. C. R. Bailey, M. D. Pickard, and R. P. Gilbert. 1993. Use of lignosulfonate to decrease the rumen degradability of canola meal protein. Can. J. Anim. Sci.73:211-215.

McCarthy, R. D., Jr., T. H. Klusmeyer, J. L. Vicini, J. H. Clark, and D. R. Nelson. 1989. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. 72:2002-2016.

Martineau , R., D. R. Ouellet, and H. Lapierre. 2013. Feeding canola meal to dairy cows: A meta-analysis on lactation response. J. Dairy Sci. 96:1701-1714.

Maesoomi, S. M., G. R. Ghorbani, M. Alikhani, and A. Nikkhah. 2006. Short Communication: Canola meal as a substitute for cottonseed meal in diet of mid lactation holsteins. J. Dairy Sci. 89:1673-1677.

Merchen, N. R. 1988. Digestion, absorption and excretion in ruminants. Pages 172-201 in The Ruminant Animal Digestive Physiology and Nutrition. D. C. Church, ed. Waveland Press, Long Grove, IL.

Mertens, D. R. 1987. Predicting intake and digestibility using mathematical models of rumen function. J. Anim. Sci. 64:1548-1558.

Mjoun, K., K. F. Kalscheur, A. R. Hippen, and D. J. Schingoethe. 2010. Ruminal degradability and intestinal digestibility of protein and amino acids in soybean and corn distillers grains products. J. Dairy Sci. 93:4144-4154.

Moran, J. B. 1986. Cereal grains in complete diets for dairy cows: A comparison of rolled barley, wheat and oats and of three methods of processing oats. Anim. Prod. 43:27-36.

Mulrooney, C. N., D. J. Schingoethe, K. F. Kalscheur, and A. R. Hippen. 2009. Canola meal replacing distillers grains with solubles for lactating dairy cows. J. Dairy Sci. 92:5669-5676.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th Revised edition. National Academy Press, Washington, DC.

Newkirk, R.W, H. L. Classen., T.A. Scott, and M.J. Edney. 2003. The digestibility and content of amino acids in toasted and non-toasted canola meals. Can. J. Anim. Sci.83: 131-139.

Newkirk, R. 2009. Canola Meal: Feed Industry Guide. Pages 1-48. 4th ed. Canadian International Grains Institute, Winnipeg, MB, Canada.

Nikkhah, A. 2012. Barley grain for ruminants: A global treasure or tragedy. J. Anim. Sci. Biotech. 3:22-31.

Nocek, J. E., and J. B. Russell. 1988. Protein and energy as an integrated system: Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71:2070-2107.

Nocek, J. E., and S. Tamminga. 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74:3598-3629.

Normand, R., St-Pierre, and J.R. Knapp. 2008. Economics of replacing corn with other energy sources in ruminant diets. Pages 1-12. Renaissance Bucknell Nutrition Conference. The Ohio State University, Ohio, Columbus.

Orskov, E. R., C. Fraser, V. C. Mason, and S. O. Mann. 1970. Influence of starch digestion in the large intestine of sheep on caecal fermentation, caecal microflora and faecal nitrogen excretion. Brit. J. Nutr. 24:671-682.

Orskov, E. R., and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agr. Sci. 92:499-503.

Orth, R. 1992. Sample day and lactation report. DHIA 200 Fact sheet A-2. Mid-States DRPC, Ames, IA.

Overton, T.R., M.R. Cameron, J.P. Elliott and J.H. Clark. 1995. Ruminal fermentation and passage of nutrients to the deodinum of lactating cows fed mixtures of corn and barley. J. Dairy Sci. 78: 1981-1998.

Owens, F. N., R. A. Zinn, and Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. J. Anim. Sci. 63:1634-1648.

Piepenbrink, M. S., and D. J. Schingoethe. 1998. Ruminal degradation, amino acid composition, and estimated intestinal digestibilities of four protein supplements. J. Dairy Sci. 81:454-461.

Piepenbrink, M. S., D. J. Schingoethe, M. J. Brouk, and G. A. Stegeman. 1998. Systems to evaluate the protein quality of diets fed to lactating cows. J. Dairy Sci. 81:1046-1061.

Plegge, S. D., L.L. Berger, and G. C. Fahey, Jr. 1985. Effect of roasting temperature on the proportion of soybean meal nitrogen escaping degradation in the rumen. J.Anim. Sci. 61:1211-1218.

Ranathunga, S. D., K. F. Kalscheur., A. R. Hippen, and D. J. Schingoethe. 2010. Replacement of starch from corn with nonforage fiber from distillers grains and soyhulls in diets of lactating dairy cows. J. Dairy Sci. 93:1086-1097.

Ravichandiran, S., K. Sharma, N. Dutta, A.K. Pattanaik, J.S. Chauhan, A. Agnihotri and A. Kumar. 2008. Performance of crossbred calves on supplements containing soybean meal or rapeseedmustard cake with varing glucosinolate levels. Indian J. Anim. Sci. 78:85-90.

Remond, D., L. Bernard, and C. Poncet. 2000. Free and peptide amino acid net flux across the rumen and the mesenteric- and portal drained viscera of sheep. J. Anim. Sci. 78:1960-1972.

Reynal, S. M., and G. A. Broderick. 2003. Effects of feeding dairy cows protein supplements of varying ruminal degradability. J. Dairy Sci. 86:835-843.

Robertson, J.B., and P.J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Pages 123-158 in The Analysis of Dietary Fiber in Foods. W. P.T. James and O. Theander, ed. Marcel Dekker Inc., New York, NY.

Robinson, P.H., G.R. Khorasani, and J.J. Kennelly. 1995. Evaluation of linseed meal for dairy cows: Interaction with grain source on forestomach and whole-tract digestion. Can. J. Anim. Sci. 76: 209-214.

Roe, M. B., L. E. Chase, and C. J. Sniffen1.1991. Comparison of *in vitro* techniques to the *in situ* technique for estimation of ruminal degradation of protein. J. Dairy Sci. 74:1632-1640.

Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim. Sci. 70:3551-3561.

Sanchez, J. M., and D. W. Claypool. 1983. Canola meal as a protein supplement in dairy rations. J. Dairy Sci. 66:80-85.

Sannes, R. A., M. A. Messman, and D. B. Vagnoni. 2002. Form of rumen-degradable carbohydrate and nitrogen on microbial protein synthesis and protein efficiency of dairy cows. J. Dairy Sci.85:900-908.

Santos, F.A.P., J.E.P. Santos, C. B. Theurer, and J. T. Huber. 1998. Nutrition, feeding, and calves effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. J. Dairy Sci. 81:3182-3213.

SAS Institute. 2001. SAS User's Guide. Statistics. Version 9.01. SAS Institute Inc., Cary, NC.

Satter, L. D., and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Brit. J. Nutr.32:199-208.

Schaefer, D. M., C. L. Davis, and M. P. Bryant. 1980. Ammonia saturation constants for predominant species of rumen bacteria. J. Dairy. Sci. 63:1248-1263.

Schingoethe, D.J. 1991. Protein quality, amino acid supplementation in dairy cattle explored. Feedstuffs. p. 11.

Schingoethe, D.J. 1996. Balancing the amino acid needs of the dairy cow. Anim. Feed Sci. Technol. 60:153-160.

Smith, R. H. 1979. Synthesis of microbial nitrogen compounds in the rumen and their subsequent digestion. J. Anim. Sci. 49:1604-1614.

Sporndly, E. and T. Asberg. 2006. Eating rate and preference of different concentrate components for cattle. J. Dairy Sci. 89:2188-2199.

Spragg, J. C., and R. J. Mailer. 2007. Canola meal value chain quality improvement: A final report prepared for AOF and CRC. Project code: 1B-103-0506. Available at: http://www.porkcrc.com.au/Final_Report_1B-103.pdf Accessed Jan. 9, 2014.

Steingass, H., G. Kneer., G. Wischer, and M. Rodehutscord. 2013. Variation of *in situ* rumen degradation of crude protein and amino acids and in vitro digestibility of undegraded feed protein in rapeseed meals. Animal. 7: 1119-1127.

Stern, M. D., W. H. Hoover, C. J. Sniffen, B. A. Crooker, and P. H. Knowlton. 1978. Effects of non-structural carbohydrate, urea and soluble protein levels on microbial protein synthesis in continuous culture of rumen contents. J. Anim. Sci.47:944-956.

Stringam, G. D., D.I. McGregor and S.H. Pawlowski. 1974. Chemical and morphological characteristics associated with seed coat color in rapeseed. In Proceedings of the 4th International Rapeseed Congress, Giessen, Germany. 99-108.

Storm, E., and E. R. Orskov. 1983. The nutritive value of rumen micro-organisms in ruminants. 1. Large scale isolation and chemical composition of rumen micro-organisms. Brit. J. Nutr. 50:463-470.

Surber, L. M. M., and J. G. P. Bowman. 1998. Monensin effects on digestion of corn or barley high-concentrate diets. J. Anim. Sci. 76:1945-1954.

Sutton, J. D. 1985. Digestional absorbtion of energy substrates in the lactating cow. J. Dairy Sci. 68:3376-3393.

Sutton. J. D. 1989. Altering milk composition by feeding. J. Dairy Sci. 72:2801-2814.

Tamminga, S. 1979. Protein degradation in the fore stomachs of ruminants. J. Anim. Sci. 49:1615-1630.

Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75:345-357.

Tamminga, S. 1996. A review on environmental impacts of nutritional strategies in ruminants. J. Anim. Sci. 74:3112-3124.

Thacker, P.A., Petri, D., 2009. The effects of canola or mustard biodiesel press cake on nutrient digestibility and performance of broiler chickens. Asian Australas. J Anim. Sci. 22: 1531-1539.

Theander, O., P. Aman., G.E. Miksche and S. Yasunda.1977. Carbohydrates, polyphenols and lignin in seed hulls of different colors from turnip rapeseed. J. Agric. Food Chem. 25:270-273.

Theodoridou, K., and Y. Peiqiang. 2012. Effect of processing conditions on the nutritive value of canola meal and presscake. Comparison of the yellow and brown-seeded canola meal with the brown-seeded canola press-cake. J. Sci. Food Agr. 93:1986-1995.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 6: 24-27.

Titgemeyer, E.C., J.E. Shirley. 1997. Effect of processed grain sorghum and expeller soybean meal on performance of lactating cows. J. Dairy Sci. 80: 714-721.

Undersander, D., D. R. Mertens, and N. Thiex. 1993. Forage Analysis Procedures. National Forage Testing Association, Omaha, NE.

Unger, E.H. 1990. Commercial pressing of canola and rapeseed: crushing and oil extraction. Canola and Rapeseed: Production, Chemistry, Nutrition and processing technology. F. Shahidi (ed.) New York, N.Y.: Van Nostrand Reinhold. 1990. Ch. 14: 235-249.

USDA Economic Research Service. 2012. Canola growth. http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx#.U12Ye_ldW04. Accessed Jan. 18, 2014.

USDA Foreign Agricultural Service, Crop production summary. 2013. Crop yield and production in United States. http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1490. Accessed Jan. 20. 2014.

Van Soest, P.J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Voss, V. L., D. Stehr, L. D. Satter, and G. A. Broderick.1988. Feeding lactating dairy cows proteins resistant to ruminal degradation. J. Dairy Sci. 71:2428-2439.

Webb, K. E., and J. C. Matthews. 1998. Peptide absorption and its significance in ruminant protein metabolism. Pages 1–10 in Peptides in Mammalian Protein Metabolism. G. K.Grimble and F. R. C. Backwell, ed. Portland Press Ltd., London, UK.

Wildman, E.E., G. M. Jones., P. E. Wagner., R. L. Boman., H .F. Troutt Jr, and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to standard production characteristics. J. Dairy Sci. 65: 495-501.

Wright, C. F., M. A. G. von Keyserlingk, M. L. Swift, L. J. Fisher,J. A. Shelford, and N. E. Dinn. 2005. Heat- and lignosulfonate-treated canola meal as a source of ruminal undegradable protein for lactating dairy cows. J. Dairy Sci. 88:238-243.

Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2000. Effects of barley grain processing on extent of digestion and milk production of lactating cows. J. Dairy Sci. 83:554-568.

Yang, W. Z., K. A. Beauchemin, B. I. Farr, and L. M. Rode. 1997. Comparison of barley, hull-less barley, and corn in the concentrate of dairy cows. J. Dairy Sci.80:2885-2895.