UTILIZATION OF CANOLA SEED FRACTIONS IN RUMINANT FEEDS

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By

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ABSTRACT

Canola fibre-protein and can-sugar are the two by-products arising from a process for separating high quality protein fractions from canola meal. In the first trial chemical characteristics of fibre-protein and can-sugar were examined in comparison with commercial canola and soy meal. In the second trial in situ rumen degradability and kinetics of test feed was studied. Based on the findings of those two trials, available energy values were estimated based on NRC (2001) while protein contents potentially absorbable at small intestine were predicted using both NRC (2001) and DVE/OEB models. Subsequently a mixture of fibre-protein and can-sugar was used as an additive to dehydrated alfalfa pellet and two dairy cow trials were conducted to determine the palatability and examine effect on lactation performances of blended alfalfa pellet feeding in comparison with standard alfalfa pellet. Palatability difference was evaluated by "Paterson -two choice alternating access method" through a 7 day experimental period using 6 lactating Holstein cows. In the lactating performance trial, 6 cows were randomly assigned into two groups and two treatments were allocated over three experimental periods in a switchback design.

Can-sugar consisted of water soluble components (CP 15.6 %DM; SCP 96.2 %CP; NFC 99.9 %CHO) with non-protein nitrogen as the main CP fraction (NPN 96.2 %CP). Fibre-protein was a highly fibrous material (NDF: 55.6%; ADF: 46.3%; ADL: 24.1%) comparing to canola meal (NDF: 25.4%, ADF: 21.2%, ADL: 9.0%) due to presence of higher level of seed hulls in fibre-protein. Comparing to canola meal, fibre-protein contained 9% less CP and 1/4 of that consisted of undegradable ADIP. Rumen

degradability of can-sugar was assumed as immediate and total as it was water soluble. Most of the ruminally undgradable nutrient components present in canola meal appeared to be concentrated into fibre protein during the manufacturing process and as a result fibre-protein has shown a consistently lower effective degradability of DM, OM, CP NDF and ADF comparing to both canola and soy meal. Available energy content in can-sugar was marginally higher than that of canola meal while fibre-protein contained only 2/3 that of canola meal. The predicted absorbable protein content at small intestine was about 1/2 that of canola meal. These results indicate that fibre-protein can be considered as a secondary source of protein in ruminant feed and a mixture of fibre-protein and can-sugar would nutritionally complement each other to formulate into a cheaper ingredient in ruminant ration. In the palatability study, there was no significant difference ($P>0.05$) in intake preference or finish time between the blended and standard alfalfa pellets. The results from the lactation study showed that there was no significant difference ($P>0.05$) in milk yield, dairy efficiency or milk composition between the blended and standard alfalfa pellets. The results from the two studies indicated that fibre-protein and can-sugar fractions could be used as an additive to alfalfa dehydrated pellet at 15% inclusion rate without compromising its palatability or the performance of dairy cows.

For future studies it is proposed to conduct feeding trials with varying levels of inclusions to alfalfa pellet to know the nutritional effect of fibre-protein and can-sugar while ascertain optimum inclusion rate.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A or S	Intercept of the degradation curve at time zero and represents the fraction that immediately disappears from the nylon bag
ADF	Acid detergent fibre
ADIN	Acid detergent insoluble nitrogen
ADIP	Acid detergent insoluble protein
ADL	Acid detergent lignin
ADS	Acid detergent solution
AECP	Absorbable endogenous protein that passes into small intestine
AMCP	Absorbable true microbial protein synthesised in the rumen
AP	Absorbable protein
ARUP	Absorbable true feed protein that escape rumen degradation
B or D	Insoluble but potentially degradable fraction
CA	Carbohydrate fraction A as per CNCPS
CB	Carbohydrate fraction B as per CNCPS
CC	Carbohydrate fraction C as per CNCPS
CHO	Carbohydrate
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
D	Water insoluble but potentially degradable fraction during rumen in situ incubation
DCP	Digestible crude protein

DE	Digestible energy
DE_{1X}	DE at maintenance level
DE_{3X}	DE at production level when intake is equal to 3 times maintenance intake
DM	Dry matter
DMI	Dry matter intake
DOM	Digested organic matter
dRUP	Digestibility of RUP
DVE	Truly absorbable protein content in the small intestine as per DVE/OEB system
ECP	Correction for endogenous protein losses during digestion process
ED	Effective degradable fraction
EE	Ether extract
FC	Fibre carbohydrate
FCM	Fat corrected milk
FOM	Fermentable organic matter
GDP	Gross domestic product
Kd	Rate constant for in-situ rumen degradation of fraction B (or D) % /h
Kp	Rumen passage rate %/ h
L or T_0	Lag time in h
MCP_{FOM}	Microbial protein synthesis, potentially possible from energy extracted during anaerobic rumen fermentation
MCP_{RDP}	Microbial protein synthesis potentially possible from available rumen degradable protein

ME	Metabolizable energy
MJ	Mega joule
MP	Metabolizable protein
MPE	Milk production efficiency
MT	Metric ton
NDF	Neutral detergent fibre
NDFn	NDF adjusted for protein (NDFn = NDF – NDIP)
NDIP	Neutral detergent insoluble protein
NDS	Neutral detergent solution
NE	Net energy
NE_G	Energy retention or gain in beef cattle
NE_L	Net energy for lactation
NE_M	Net energy for maintenance in growing animals
NFC	Non-fibre carbohydrate
NPN	Non-protein nitrogen
OEB	Rumen degraded protein balance as per DVE/OEB system
P	Actual degradation after time “t”
PA	Protein fraction A as per CNCPS
PAF	Processing adjustment factor
PB	Protein fraction B as per CNCPS
PC	Protein fraction C as per CNCPS
R	Rest of the organic matter content other than crude protein and ether extract (R = OM – CP – EE)

RDP	Rumen degradable protein
RU	Rumen undegradable fraction
RUP	Rumen undegradable protein
SC	Structural carbohydrate
SCP	Soluble crude protein
t	Time in h
tdCP	Truly digestible crude protein
tdFA	Truly digestible fatty acid
TDN	Total digestible nutrients
TDN_{1X}	TDN at maintenance level
tdNDF	Truly digestible neutral detergent fibre
tdNFC	Truly digestible fraction in non-fibre carbohydrate
VFA	Volatile fatty acid

1. INTRODUCTION

Canola is an oil-seed crop developed from rapeseed (*Brassica napus* and *Brassica campestris / rapa*) by Canadian plant breeder's in 1970's. Unlike with traditional rape seed, canola contains low levels of "erucic acid" in the oil portion (<2% of total fatty acids in the oil) and low levels of anti-nutritional compounds called "glucosinolates" in the meal portion (<30 μmol of alkenyl glucosinolates per gram of oil-free dry matter of seed) (Bell 1993). Today canola oil has become one of the most popular all-purpose vegetable oils whilst the canola meal is widely used as a source of protein in livestock feeding. The current annual contribution of canola to the Canadian economy is estimated to be $11 billion (1.1% of GDP). Average canola seed production in Canada is over 7 million metric tonnes (MT) per year (Table 1.1) with a record production of 9.7 million MT reported during crop year of 2005-06. Out of total seed production, about 3 million MT of seed is crushed domestically and produces around 1.2 million MT of oil and 1.8 million MT of canola meal. Presently two thirds of canola meal produced in Canada is exported mainly to USA, European Union and Taiwan.

The protein component in the canola meal is rated as the highest nutritional quality protein of vegetable origin based on its amino acid composition and low anti-genicity. However due to it's comparatively high level of crude fibre (12%) and phytic acid (3.1 - 3.7%), it has a limited use in aquaculture, swine or poultry (Bell 1993; Higgs et al. 1995). Therefore it is traded as a low valued animal feed ingredient, usually at two thirds of the price of soybean meal (AgBiotech-INFOSOURCE 2004; MCN Bioproducts Inc. 2005; Canola Council of Canada 2007).

In December 2006, the Canadian federal government has announced a renewable fuel standard of 2% for diesel by year 2012. This amounts to 600 million litres of biodiesel per annum. As the main source of biodiesel in Canada would be from canola oil, there would be a substantial increase in supply of canola meal. The main market competitor for canola is soy bean, which has a highly diversified market due to inclusion in numerous food ingredients and products derived from non-oil portion of the soybean.

Table 1.1 Average production of canola seed, oil and meal during years 1996 to 2006 in Canada

	Average annual production from 1996-2006 (million MT)
Total seed production	7.1
Domestic crush	3.0
Export	3.7
Total oil production	1.2
Domestic utilization	0.4
Export	0.8
Total meal production	1.8
Domestic utilization	0.6
Export	1.2

Source: Canola Council of Canada (2007)

In contrast, canola market price is currently driven only by two products, i.e. oil and meal. As a result the crush margin of canola faces higher volatility in comparison to much more stable soybean (Figure 1.1).

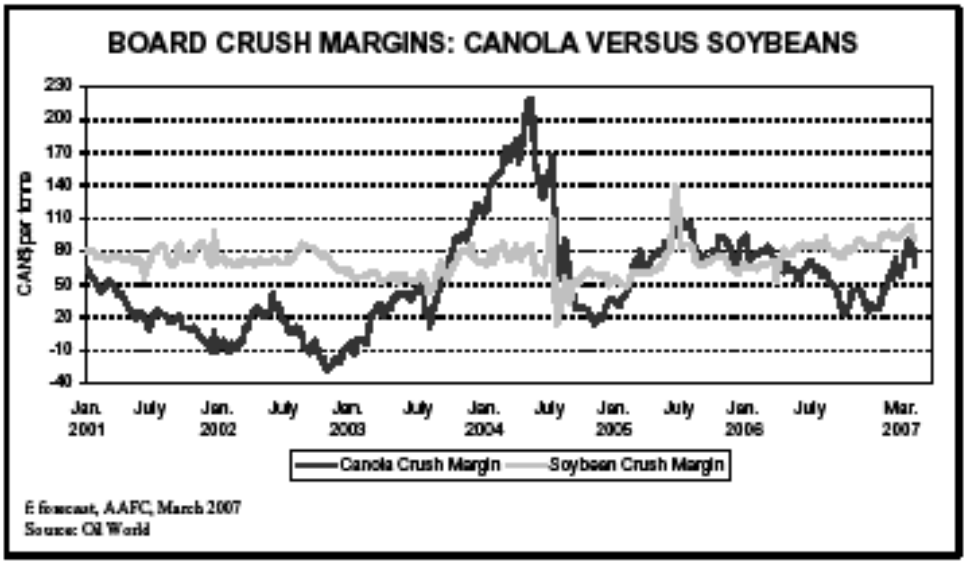

Figure 1.1 Board crush margins of canola and soybeans (Source: Agriculture and Agri-Food Canada, 2007)

Hence, in order to achieve a more stable market and to maximise the returns for canola, it is important to create a diversified market through value addition to canola. Considering the superior amino acid profile (Thiessen et al. 2004), higher protein efficiency ratio and low antigenicity of canola protein (Drew 2004), meal portion of canola seems to hold the potential for a significant value addition to canola. The development of technology similar to canola meal fractionation and protein extraction process developed by MCN Bioproducts Ltd, Saskatoon (SK), represents an opportunity in this direction.

In this fractionation process, two protein concentrates are extracted which would target lucrative aquaculture and mono-gastric markets, and be the main economic drivers of the process. Apart from the two protein concentrates, two other end products (i.e. "fibre-protein" fraction and "canola-sugar" fraction) result from the fractionation process. These two products amount to more than 50% of total fraction yield and need to be utilized for a commercially viable fractionation process. By the nature of the fractionation process, fibre-protein would contain most of the fibrous material while

can-sugar would contain other non-protein components and is expected to be more suitable for ruminant feeding.

The overall objective of the current study was to conduct a comprehensive evaluation of fibre-protein and can-sugar as ruminant feed ingredients. The literature review in this thesis is focused on some of the feed evaluation techniques currently used for ruminant feedstuffs and then on nutritional evaluation of different types of canola products in relation to cattle. The first hypothesis in the current research was that "fibre-protein" and "canola sugar" fraction (can-sugar) can be used as feed ingredients in ruminant rations and the second hypothesis was that "fibre-protein" and "can-sugar" fractions can be included in dairy rations without affecting their palatability and performances. The first hypothesis was tested by conducting chemical and ruminal degradation characteristic studies followed by predicting available nutrients (energy and protein) using advanced nutrition models. The second hypothesis was tested by adding fibre-protein and can-sugar to alfalfa at pelletizing and conducting a palatability and a lactation performance trial with blended pellet in comparison with standard alfalfa pellet.

2. REVIEW OF LITERATURE

2.1 Cornell Net Carbohydrate and Protein System for feed evaluation

The Cornell Net Carbohydrate and Protein System (CNCPS) was published first in 1992 and 1993 in four companion papers (Fox et al. 1992; Russell et al. 1992; Sniffen et al. 1992; O'Connor et al. 1993) and since then it has undergone improvements and refinements. The CNCPS is a mathematical model to evaluate cattle ration and animal performance based on principles of rumen fermentation, feed digestion, feed passage and physiological status of the animal (Fox et al. 2004). The CNCPS model uses information on animal, feeds, management, and environmental conditions as inputs to formulate rations and consist of several sub-models, either empirical or mechanistic, i.e. maintenance, growth, pregnancy, lactation, body reserves, feed intake and composition, rumen fermentation, intestinal digestion, metabolism, and nutrient excretion. The animal related sub-models use equations to predict the animal requirements as per different physiological states and environmental conditions. The CNCPS also predicts the total supply of metabolizable energy and protein to the animal by using degradation and passage rates of carbohydrate and protein in the feeds.

In the feed composition sub-model, the CNCPS has two levels of solutions depending on the availability of feed compositional information. At the level-1, empirical equations developed by Weiss et al. (1992), are used to compute Total Digestible Nutrients (TDN) and Metabolizable Protein (MP) if the feed ingredients are not completely described chemically. In level-2 approach, TDN and MP values are estimated using feed degradation rates (Kd values) and passage rates (Kp values) and, their relationship i.e. Kd/(Kd+Kp) and Kp/(Kd+Kp). In CNCPS, each nutrient component, (i.e. crude protein (CP), soluble crude protein (SCP), neutral detergent insoluble protein (NDIP), acid

detergent insoluble protein (ADIP), neutral detergent fibre (NDF), fat, lignin, and ash) is assigned with its own Kd value, which could be modified according to the feed processing (Russel et al. 1992; Fox et al. 2004). In level-2, the CNCPS identifies different fractions in both feed carbohydrate and protein pools that are having different ranges Kd values. The estimation of rumen degradation and escape amounts of carbohydrate and protein in a feedstuff therefore, depends on proportion of different fractions of carbohydrate and protein rather than the total carbohydrate and CP contents in the feed (unlike Level-1 prediction).

2.1.1 Carbohydrate fractions

In CNCPS, carbohydrates are broadly categorised by Fox et al. (2004) either as fibre carbohydrates (FC) or non-fibre carbohydrates (NFC) (Figure 2.1.) These two fractions were described by Sniffen et al. (1992) in his original paper as structural carbohydrate (SC) and non-structural carbohydrate (NSC). The FC is neutral detergent fibre (NDF), which is the feed component insoluble in neutral detergent solution (NDS) and consists of cellulose, hemicellulose and lignin. The NFC is calculated by difference as the dry matter minus NDFn (NDF adjusted for protein i.e. NDFn = NDF - NDIP), CP, ash, and fat.

i.e. NFC = DM – NDFn – CP – Ash – Fat

The carbohydrates are further categorised into four fractions CA, CB1, CB2 and CC. The CA fraction is non-fibre carbohydrates minus starch. It contains mostly sugars/polysaccharides that are water soluble and rapidly fermentable in the rumen. In addition, fraction CA may contain organic acids and short oligosaccharides. Particularly in forages and silages there can be a considerable amount of organic acid, which are not utilized by rumen microorganisms with efficiency similar to sugar digestion. Therefore, microbial growth from organic acid fraction (of CA) of silage is discounted by 50% in CNCPS model (Fox et al. 2004).

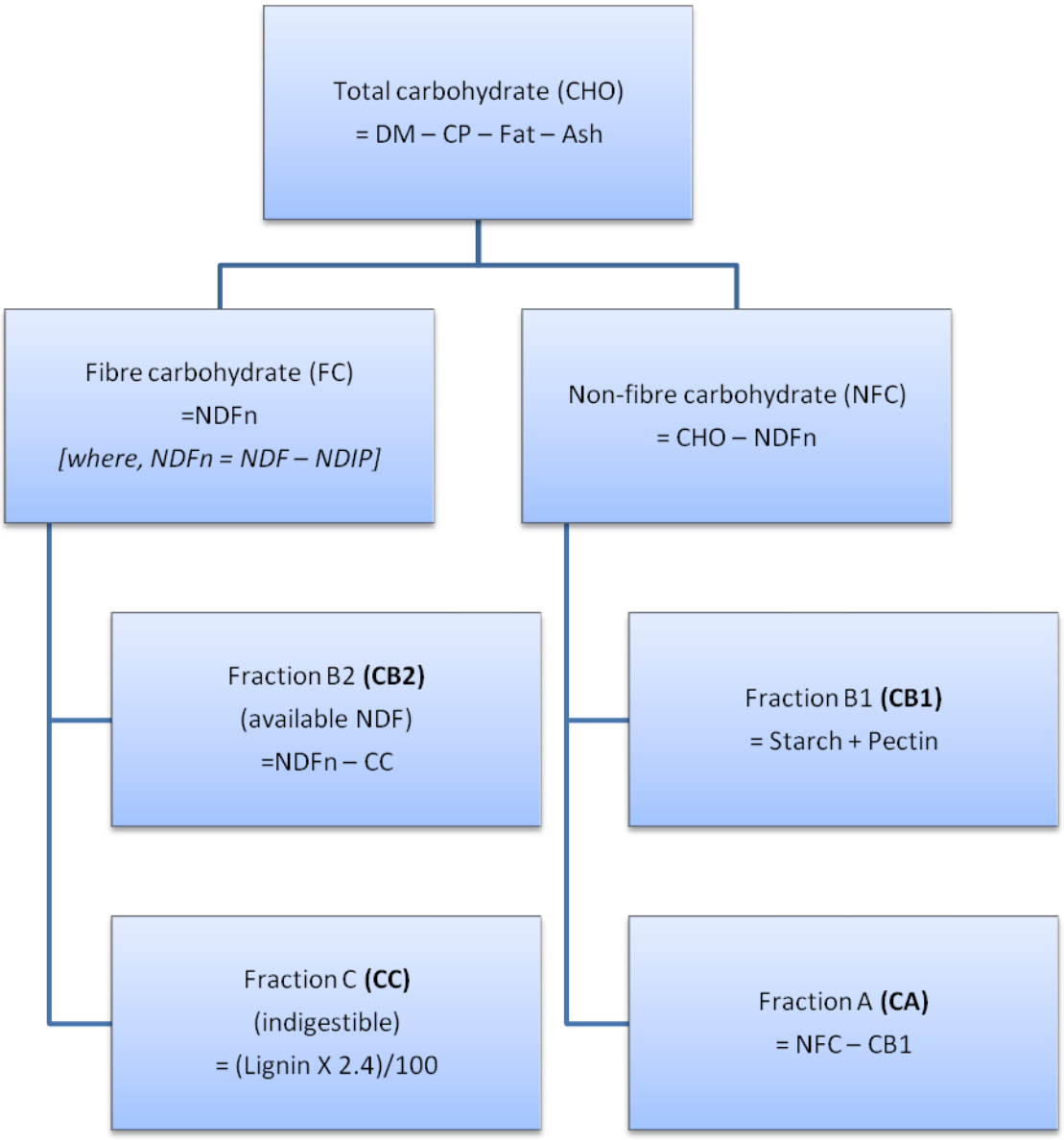

Figure 2.1. Carbohydrate fractions as per Cornell Net Carbohydrate and Protein System

Adapted from Fox et al. 2004

The tabulated degradation rate (Kd) values of fraction CA for large variety of feed ingredients shows a range of 200-350% /h while lowest Kd value of 10% /h for grass and alfalfa silage and, highest Kd value of 500% /h for beet and cane molasses were reported (Fox et al. 2000; Sniffen et al. 1992). It is assumed that almost all of this CA fraction is degraded in the rumen and the small amount that might escape the fermentation is 100% digestible in the intestine (Fox et al. 2004; Fox et al. 2000). The fraction CB1 consists of non-fibre carbohydrate minus sugar that contains mainly starch and pectin. It has a slower degradation rate than fraction CA with Kd values ranging from 10%/h for dry whole corn to 50%/h for steam flaked wheat. The carbohydrate fraction CB2 consists of available NDF that shows lower degradable rate than CB1. The degradation rate (Kd) of CB2 fraction range from 3%/h (for hay and straw) to 20%/h (molasses) depending on feed type, stage of maturity and processing. The fraction CC is undegradable fibre associated with lignin and estimated as Lignin X 2.4 (%DM).

Lanzas et al. (2007) noted that division of NFC fraction into two fractions (CA and CB1) is not precise since they do not accommodate variability in NFC digestibility caused by different processing treatments and role of NFC on rumen volatile fatty acids (VFA) production and pH. They proposed a more comprehensive division of dietary carbohydrates into eight fractions: VFA (CA1); lactic acid (CA2); other organic acid (CA3); sugars (CA4); starch (CB1); soluble fibre (CB2); available NDF (CB3); unavailable NDF (CC). Lanzas et al. (2007) claimed that new fractionation scheme would provide better description of silage with varying quality and dietary NFC content.

2.1.2 Protein fractions

The CNCPS protein fractions are described as a percentage of total crude protein with a similar design to carbohydrate fractions (Figure 2.2.). In the CNCPS, dietary protein is partitioned into three main fractions i.e. Fraction A (PA), true protein (PB) and unavailable protein (PC). The PB fraction is subdivided further into three fractions PB1, PB2 and PB3 that have different Kd values.

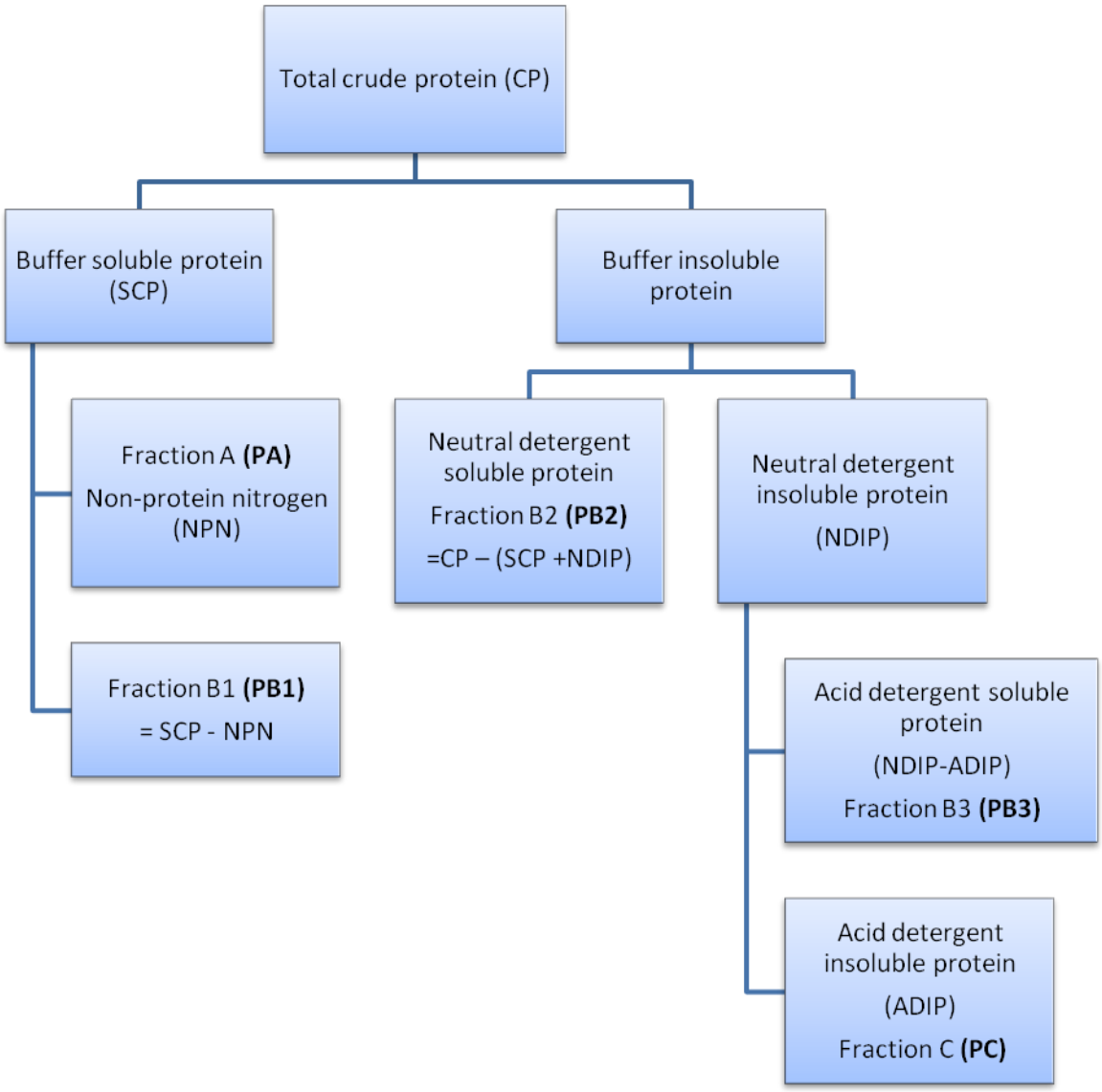

Figure 2.2. Protein fractions as per Cornell Net Carbohydrate and Protein System

Adapted from Fox et al. 2004

The protein fractionation in CNCPS is based on solubility in buffer and detergent solutions. The protein fraction A (PA) is assumed to consist of non-protein nitrogen (NPN), which enters immediately into ammonia pool in the rumen. It was reported, however that as much as two thirds of NPN can be peptides and amino acids in high quality alfalfa silage. Since both peptide and amino acids are utilised by NFC bacteria more efficiently than ammonia, two thirds of NPN in high quality alfalfa silage should be included in PB1 fraction (Fox et al. 2004). The protein B1 (PB1) contains rapidly and almost completely degradable true protein fraction in the rumen. Both PA and PB1 fractions are soluble in buffer solution and the PB1 is computed as the difference between buffer soluble protein (SCP) and NPN (i.e. PB1 = SCP – NPN). The Kd values of PB1 have a range from 135%/h (corn grain, grass hay) to 350%/h (wet barley) depending on feedstuff and processing.

The PB2 fraction is soluble in neutral detergent solution and partly degraded in the rumen. The rumen degradability rate (Kd) of PB2 has a range from 3%/h to 16%/h depending on the ingredient type and processing. The ruminal degradation amount of PB2 depends on the relative rate of passage (Kp) to Kd. The PB3 fraction is insoluble in NDS but soluble in acid detergent solution (ADS) and considered to be associated with plant cell wall. Its degradability is very low (0.05% to 0.55 % /h depending on ingredient type) and therefore most of the PB3 escapes rumen degradation (Fox et al. 2000; Sniffen et al. 1992).

The PC fraction is acid detergent insoluble protein (ADIP). PC fraction contains proteins associated with lignin, tannins and heat damaged proteins such as Maillard products and assumed to be indigestible. In CNCPS, the intestinal digestibility of both PB1 and PB2 is assumed to be 100% while 80% digestibility is assigned for PB3 (Fox et al. 2004; Fox et al. 2000, O'connor et al. 1993).

2.2 Energy value estimation in feed ingredients

Measurement of gross energy in a feedstuff is a relatively simple procedure. However due to the complex nature of rumen degradability and the variability found in relation to feed digestibility and metabolism particularly in forages, the gross energy cannot be used practically in ration formulation. On the other hand, accurate determination of energy value of feedstuffs is important to ensure optimum production, growth, product quality and composition, animal health, reproduction and to minimise feed wastage. Although, chemical analysis is used to determine many constituents in feed ingredients, the available feed energy that defines nutritive characteristic of a feedstuff cannot be determined using routine analytical procedure (Weiss et al. 1993; Weiss 1998). The available energy in feedstuff is commonly expressed as total digestible nutrient (TDN), digestible energy (DE), metabolizable energy (ME) or net energy (NE). Digestibility trials are used to determine TDN and DE while metabolism trials are used to measure urinary energy and gaseous (methane) energy and thereby determine ME. In order to measure NE, "whole body calorie metric facilities" are needed. As these animal trials are expensive and need sophisticated equipment, the available energy values are usually predicted mathematically using equations based on chemical composition of feedstuffs with or without their digestibility values.

The energy value of a feedstuff is primarily determined by;

1. Fat content due to its high energy density,
2. Content of non-fibre carbohydrates (NFC) due to their high digestibility and,
3. Content and digestibility of fibrous carbohydrates due to their high level in ruminant rations

Robinson et al. (2004) noted that energy contribution of fat and NFC are generally similar among different feedstuffs thus use of universal predictive equations for energy

from fat and NFC content cause lesser inaccuracies. However, due to high qualitative differences in fibrous carbohydrates among feedstuff depending on many factors (cultivar type, season, harvesting time, region where it is grown) such a universal approach for fibre may not be accurate.

The mathematical models used for energy systems vary among different regions and countries, i.e. North America (NRC), Europe [ARC (UK), UFL (France), VEM (Netherlands & Belgium)], Australia (SCA) and the comparative studies conducted on these systems have given variable results indicating that differences exist in basic assumptions used among these models (Robinson et al. 2004; Vermorel and Coulon 1998; Yan et al. 2003).

2.2.1 NRC system for estimating feed energy

The NRC system is based on net energy for lactation (NE_L) since 1978 (NRC 1978). In dairy animals energy requirements for both maintenance and milk production are expressed in NE_L units, on the premise that metabolizable energy (ME) is used at similar efficiency for both maintenance (62%) and lactation (64%). In the latest NRC publication (NRC 2001), both DE and TDN values were estimated using a summative chemical approach.

2.2.1.1 NRC 2001 estimation of TDN in feedstuffs

NRC (2001) calculation of TDN at maintenance level (TDN_{1X}) was based on the summative chemical approach suggested by Weiss et al. (1992). It uses the concentration of NDF, lignin, CP, ash, ether extract, ADF and NDF along with their digestion coefficients to predict the theoretical "truly digestible" nutrient components.

a. Truly digestible fraction in non-fibre carbohydrate (tdNFC)

$$= 0.98\ (100 - (\text{NDF-NDIP}) - \text{CP} - \text{EE} - \text{Ash}) \times \text{PAF}$$

(PAF= Processing adjustment factor and EE = Ether extract)

Weiss et al. (1992) reported estimated true digestibility of 0.85 to 1.2 with an average of 0.98 for NFC. NRC (2001) recommends using 0.98 as digestibility of NFC in their equation. The physical processing steam and heat tends to increase the digestibility of starch. NRC (2001) reported a true digestibility of 0.98 and 0.90 at 1X maintenance and at 3X maintenance respectively. NRC (2001) calculated PAF by dividing in vivo starch digestibility of different feeds by 0.90 in their tabulated PAF values.

b. Truly digestible crude protein (tdCP)

Weiss et al. (1992) has reported that digestibility of forage protein highly correlated with ADIP content (as a % of CP) and digestibility coefficient of forage CP = exp [- 1.2 × (ADIP/CP)]. For concentrates, Weiss et al. (1992) suggested to use equation for digestibility coefficient of CP =1 – (0.4 × (ADIP/CP). Subsequently, NRC (2001) recommends the following equations to compute tdCP.

I. For concentrates (tdCPc) = {1 – [0.4 × (ADIP/CP)]} × CP

II. For forages (tdCPf) = CP x exp [- 1.2 × (ADIP/CP)]

c. Truly digestible fatty acid (tdFA)

The digestibility of fatty acids (FA) is assumed as 100%, thus tdFA = FA

If the fatty acid contents are not available, it is assumed that FA = Ether extract -1, thus

tdFA = EE – 1 (if EE < 1, then FA=0)

d. Truly digestible neutral detergent fibre (tdNDF)

I. Based on lignin content (L) as per sulphuric acid procedure

$= 0.75 \times (NDF – NDIP – Lignin) \times (1 – (L / (NDF – NDIP))^{0.667})$ or

II. Using 48 h in vitro or in situ estimate of NDF digestibility

e. Metabolic fecal TDN

NRC (2001) used metabolic fecal TDN value of 7 as reported by Weiss et al. (1992) to subtract from the sum of "truly digestible" nutrients since TDN is based on "apparent" digestibility.

Hence, the NRC (2001) summative equation for TDN of feeds at maintenance (TDN_{1X}) was given as;

$$TDN_{1X}\ (\%) = tdNFC + tdCP + (tdFA \times 2.25) + tdNDF - 7$$

The tdNFC, tdNDF, tdCP and tdFA were expressed as percent of dry matter. The above equations are valid only for feedstuffs of plant origin. NRC (2001) suggested different summative approaches to estimate TDN for animal protein meals and for fat supplements.

Animal protein meal

Some of the animal protein meals may contain significant amount of NDIP. However as these NDIP are not structural carbohydrates (cellulose, hemicellulose) or lignin, a different equation was suggested by NRC (2001) to estimate TDN_{1X}.

TDN equation for animal protein:

$$TDN_{1X}\ \% = (tdCP) + (FA \times 2.25) + 0.98\ (100 - CP - Ash - EE) - 7$$

Fat supplements

Two different TDN_{1X} equations were given by NRC (2001) for fat supplements based on whether the supplement contains glycerol or not. NRC (2001) assumes the ether extract in glycerol containing fat sources have 10% glycerol and 90% fatty acids and glycerol digestibility is 100% at 1X maintenance.

Accordingly TDN_{1X} equation for fat supplements containing glycerol is calculated as,

$$TDN_{1X}\ \% = (EE \times 0.1) + [(FA\text{-digestibility} \times (EE \times 0.9) \times 2.25]$$

For the fat supplements that does not contain glycerol,

$$TDN_{1X}\ \% = FA - \text{digestibility} \times EE \times 2.25$$

2.2.1.2 NRC 2001 estimation of Digestible Energy (DE) in feed stuffs

NRC (2001) has discarded its earlier (NRC 1989) method of direct computation of DE from TDN by multiplying TDN value with an average heat combustion value of 4.409 Mcal/kg. As different nutrients were reported to be having different heat combustion values (i.e. carbohydrate: 4.2 Mcal/kg, protein: 5.6 Mcal/kg, long chain fatty acids: 9.4 Mcal/kg, and glycerol: 4.3 Mcal/kg) apparent DE at maintenance was computed as,

DE_{1X} (Mcal/kg)

$$= (tdNFC/100 \times 4.2) + (tdNDF/100 \times 4.2) + (tdCP/100 \times 5.6) + (FA/100 \times 9.4) - 0.3$$

Where, tdNFC, tdNDF, tdCP and FA are given as % DM.

Metabolic fecal TDN value of 7 was multiplied by its assumed heat combustion value of 4.4 Mcal/kg (= 0.3 Mcal/kg) and subtracted from sum of truly digestible energy values to obtain the apparent DE value.

Similar to TDN_{1X} computations, different equations for DE_{1X} were suggested by NRC (2001) for animal protein meals and fat supplements as shown below.

Animal protein supplements

$$= (tdNFC/100 \times 4.2) + (tdCP/100 \times 5.6) + (FA\ /100 \times 9.4) - 0.3$$

Fat supplements with glycerol

$$= (EE/100 \times 0.1 \times 4.3) + [(FA\text{-digestibility} \times (EE/100 \times 0.9) \times 9.4]$$

Fat supplements without glycerol = FA-digestibility × (EE/100) × 9.4

2.2.1.3 Energy value discount

Since its 1978 publication NRC recognizes a reduction in digestible energy concentration in the diet as the DMI increases, an important factor for present day cows with intake more than 3x maintenance. In both 1978 and 1989, NRC has used a constant reduction of 4% in energy value per unit of maintenance energy output above maintenance, to obtain intake corrected DE (discounted DE), for diets having more than 60% TDN_{1X} (NRC 2001; Robinson 2007). In NRC (2001), a variable discount was proposed using both "diet TDN_{1X}" and intake level (over and above maintenance level intake) as factors.

> Discount = (TDN_{1X} – (0.18 x TDN_{1X} – 10.3)) x intake/ TDN_{1X}; (where, intake = incremental intake above maintenance)

There were, however, doubts being raised about the accuracy of NRC discounting method. Robinson (2007) observed that, the introduction of a new discounting method has resulted in an overall energy value reduction of 5% comparing to NRC 1978 and 1989 values at 1X maintenance level, which reduces further with the increase in intake level above maintenance. He has shown further that according to the NRC 2001 energy discounting method, a present day high producing cow with an energy output equivalent to 9X maintenance, then need to consume 78 kg DM per day or 12.6 % DM of body weight. Using published data between 1990 and 2005 in his study, Robinson (2007) has shown that the NRC assumption that energy concentration in a diet decreases as the energy output of the cow increases is fundamnatelly incorrect. He noted that while the increase in DM intake decreases net energy for lactation (NE_L) density in a diet, increase in NE_L output (xM) in fact increases energy density in diets and concluded that application of equations using both expected DM intake and energy output by a cow is necessary for accurate estimations.

2.2.1.4 NRC 2001 estimation of Metabolizable Energy (ME) in feed stuffs

The NRC uses "discounted DE (DE_P)" values to derive ME values. The NRC (2001) has modified its earlier equation (ME_P (Mcal/kg) = 1.01 × DE_P – 0.45) to accommodate diets

containing more than 3% EE since the earlier equation tends to underestimate ME of high fat diets ("$_p$" stands for the production level intake 3xM).

If the EE <3%: ME_P (Mcal/kg) = 1.01 × DE_P (Mcal/kg) – 0.45

If the EE >3%: ME_P (Mcal/kg) = 1.01 × DE_P (Mcal/kg) – 0.45 + 0.0046 × (EE-3)

For fat supplements: ME_P (Mcal/kg) = DE_P (Mcal/kg)

2.2.1.5 NRC 2001 estimation of Net Energy for lactation (NE_L) in feed stuffs

In the earlier NRC publications (1989 and before), TDN or DE was directly converted to NE_L for all feeds. This has amounted to similar or very close efficiency of converting DE to NE_L for all types of feed. The new equation for NE_L prediction is based on ME_P for feeds with less than 3% EE is as shown below.

NE_{Lp} (Mcal/kg) = [0.703 × ME_P (Mcal/kg)] – 0.19

Similar to ME_p calculation, modification to above equation was recommended for feeds with more than 3% EE on the basis that average efficiency of converting fat to NE_L is equal to 80%.

NE_{Lp} (Mcal/kg) = (0.703 × ME_P) – 0.19 + {[(0.097 × ME_P + 0.19)/97] × (EE-3)}

For fat supplements: NE_{Lp} (Mcal/kg) = 0.8 × ME_p (Mcal/kg)

2.2.1.6 NRC 1996/2000 estimation of feed Net Energy for maintenance and gain in beef cattle

The concept of a net energy system for maintenance (NE_M) and for energy retention or gain (NE_G) was introduced first in 1963 by California net energy system (Garrett and Johnson, 1983). The current NRC models for NE_M and NE_G were published in 1996 (NRC, 1996), where it is assumed that DM intake of growing animals is 3 times maintenance (3xM) and the conversion efficiency of DE to ME is 82% (ME = 0.82 x DE_{1X}. The ME value then converted to NE_M and NE_G using the following equations.

Net Energy for maintenance (NE_M Mcal/kg)

$$=1.37 \times ME - 0.138 \times ME^2 + 0.0105 \times ME^3 - 1.12$$

Net Energy for gain (NE_G Mcal/kg)

$$= 1.42 \times ME - 0.174 \times ME^2 + 0.0122 \times ME^3 - 1.65$$

For fat supplements, it is assumed that $ME_p = DE_p$ (100% efficient conversion from DE to ME) and conversion efficiency of ME to NE_M is 80% and ME to NE_G is 55%.

Accordingly for fat supplements,

$NE_M = ME_P \times 0.8$ and $NE_G = ME_P \times 0.55$

2.2.2 European systems for estimating feed energy

Some of the European systems (French system and UK -ADAS system) predict ME from gross energy (GE), which is either measured or calculated from feed composition and organic matter digestibility (Robinson et al. 2004; Vermorel and Coulon, 1998). In the UK-ADAS system it is assumed that in vitro rumen fluid in-vitro organic matter digestibility (ivOMD) (using rumen fluid) represents digestibility of organic matter and DE is converted to NE at an efficiency of 82%.

(1) ME (MJ/kgDM) = GE X ivOMD × 0.82, when GE is measured

(2) ME (MJ/kgDM) = 0.82 × (((2.4 × CP) + (3.9 × EE) + (1.8 × R)) × ivOMD), based on composition where R is the content of rest of the organic matter i.e. R=OM – CP − EE.

(Robinson et al. 2004)

The comparative studies on different energy systems have demonstrated that there were significant differences in predicted energy values and accuracy (Robinson et al. 2004; Vermorel and Coulon, 1998) and the choice of method would depend on the cost and complexity of procedure.

2.3 In situ nylon bag technique for estimating ruminal degradability and kinetics of feed nutrients

The first citation of the use of fibre bag technique to investigate ruminal digestion of feeds was far back as 1938 by Quin et al. who have used cylindrical natural silk bags in their study (Ørskov et al. 1980). Today, the in situ nylon bag technique is used widely to investigate ruminal degradation kinetics and fermentation characteristics of feeds. This technique is uncomplicated and allows rapid estimations for larger number of feed samples. However, there are several methodology related factors that influence the repeatability in nylon bag measurements.

Some of the main sources of variations in nylon bag measurements that has been reported are,

1. Pore size of bag material
2. Sample size to bag surface area ratio
3. Particle size of sample
4. Position in the rumen where bags are incubated
5. Timing of insertion and removal of bags
6. Diet composition fed to experimental animals
7. Feeding frequency of experimental animals
8. Type of animal used and between animal variations
9. Bag rinsing procedure
10. Incubation time mathematical model used to interpret data

(Ørskov et al. 1980; Vanzant et al. 1998)

2.3.1 Estimation of degradation kinetics and degradability

2.3.1.1 Mathematical models for estimating degradation kinetics

Both nonlinear and logarithmic-linear transformation models have been used to estimate degradation parameters in the nylon bag in situ studies. Non-linear models are used more extensively for NDF degradation studies while logarithmic-linear transformation models were used mostly in protein degradation trials (Stern et al. 1997). The most often used nonlinear model was first reported by Ørskov and McDonald (1979) for the estimation of protein degradability,

$$P = A + B\ (1 - e^{-Kd \times t})$$

where;

P = actual degradation after time "t"

A= intercept of the degradation curve at time zero and represents the fraction that immediately disappears from the nylon bag

B = insoluble but potentially degradable fraction in time

Kd = rate constant for the degradation of fraction "B"

The "A", "B" and "Kd" are constants and fitted by iterative least-squares procedure. The "A" and "B" expressed as %, represent the degradable fraction and 100 – (A +B) correspond to undegradable fraction (C).

This equation was later modified by inclusion of a "lag time (L)" to improve the accuracy when dealing with neutral detergent residues (Robinson et al. 1986) and low degradable feeds (Dhanoa, 1988) as shown below.

$$P = A + B\ (1 - e^{-kd \times [t-L]})$$

It has been reported that estimations of fractions "A" and "B" from model with the lag time were different from estimations from model without lag time (Robinson et al. 1986). However, the estimates of effectively degradable fractions were similar with both equations at a variety of passage rates (Denham et al. 1989).

2.3.1.2 Estimation of effective degradable and rumen undegradable fractions

The amount of effectively degraded fraction of a feed depends on potentially degradable fraction (B), passage rate (Kp) and degradation rate (Kd). As proposed by Ørskov and McDonald (1979) the effective degradability (ED) of a nutrient can be calculated as,

$$ED = A + B \left(\frac{Kd}{Kd + Kp} \right)$$

Using the same parameters, rumen undegradable fraction (RU) can be calculated as,

$$RU = C + B \left(\frac{Kp}{Kd + Kp} \right) \qquad \text{(Yu et al. 2003A; 2003B)}$$

The passage rate depends on the level of intake and type of feed and the choice of Kp value which would substantially affect the calculated values of ED and RU. In different protein evaluation systems, different values for Kp are assumed in the calculations. NRC (2001) assumes a passage rate of 5% while French-PDI system and Nordic system uses 6% and 8% /h, respectively. In the Dutch DVE-system two different Kp values are used for roughages (4 – 4.5% /h) and concentrates (6% /h) (Muia et al. 2001; NRC, 2001; Tamminga et al. 1994; Yu et al. 2003A, 2003B). When the dry matter intake (DMI) is known, NRC (2001) suggests using three different equations to calculate Kp value using DMI (as a % of body weight) as a variable for wet-forages, dry-forages and concentrates.

2.4 Prediction of protein supply to small intestine

In earlier days crude protein (CP) content was used in dairy ration formulation. This was later replaced by digestible crude protein (DCP) system (Dutch VEM-system) and absorbable protein (AP) or metabolizable protein (MP) system (North America-NRC, United Kingdom) (NRC 1989; Santos et al. 1998; Tamminga et al. 1994). The DCP was an inadequate system to predict the amount of true protein absorbed from the intestine as it does not specify the extent of ruminal protein degradation nor the amount of synthesised microbial protein (Tamminga et al. 1994). In the CP system used earlier, it was assumed that ruminal degradation of CP in all feedstuffs were equal and converted to MP with equal efficiency. The MP (or AP) system on the other hand has recognized difference among the feedstuff in relation to proportion of dietary proteins escaping rumen degradation where the MP was defined as true protein digested and absorbed in the intestine comprising of synthesised microbial protein from rumen degradable protein (RDP) and ruminally undegradable protein (RUP) (Van Soest, 1994). In the MP system a fixed intestinal digestibility of 80% was assumed for RUP (NRC 2001) and an increase in milk yield was expected when the RUP content in a diet was increased in relation to RDP. However, Santos et al. (1998) in their review study have observed that milk yield has failed to respond to higher RUP proportion in diets indicating inadequacy of RDP-RUP model. Some of the reasons attributed to this lack of response were decrease in microbial synthesis (due to lower RDP), poor amino acid profile in RUP and low digestibility of RUP (Santos et al. 1998). In addition RDP-RUP model has not considered the contribution of endogenous protein to MP (NRC 2001).

The Dutch DVE/OEB model (Tamminga et al. 1994) and NRC 2001 dairy model are two modern protein evaluation systems that have been developed and currently being used extensively in some European countries and North America to predict MP supply to small intestine.

2.4.1 DVE/OEB model

The DVE/OEB model was developed by Tamminga et al. (1994) using elements of French-PDI system and concept of Scandinavian protein balance in the rumen. In this model both feed protein value and animal protein requirement were expressed as the amount of truly absorbable protein (DVE value) in the small intestine (Yu et al. 2003A). The DVE value comprises of three components.

1. Absorbable true feed protein that escape rumen degradation (ARUP)
2. Absorbable true microbial protein synthesised in the rumen (AMCP)
3. Correction for endogenous protein losses during digestion process (ECP)

The DVE value of a feedstuff was expressed as;

DVE = ARUP + AMCP – ECP

2.4.1.1 Estimation of absorbable rumen undegraded protein (ARUP)

As shown earlier, rumen undegradable CP fraction (RUP %CP) in a feed was estimated from rumen degradation parameters derived from in situ (nylon bag) incubation as,

$$\text{RUP \%CP} = C + B \times \left(\frac{kp}{kd + kp} \right)$$

where,

C = undegradable fraction of CP (as a % of CP)

B = potentially degradable fraction of CP (as a % of CP) = 100 – C – soluble fraction %CP

Kp = passage rate (for roughages 4.5 %/h and concentrate 6% /h)

Kd = degradation rate constant for B fraction (% /h)

The amount of rumen undegradable protein (RUP %DM) was then calculated as,

$$\text{RUP \%DM} = 1.11 \times (\text{RUP \%CP}/100) \times \text{CP \%DM}$$

The factor 1.11 was adopted from French-PDI system, which represents the regression coefficient of in vivo data over in situ degradation data (Tamminga et al. 1994; Yu et al. 2003A). For the feedstuffs lacking in situ data, an assumed value of 35% was used for RUP %CP by Tamminga et al. (1994) in their study.

In Tamminga et al. (1994) study, it was assumed that undegradable CP after long term incubation (10-14 days) was indigestible in the small intestine and the digestibility of RUP (dRUP) was calculated from RUP %CP and the indigestible CP fraction (U %CP) estimated after long term incubation.

$$\text{dRUP \%} = \left(\frac{\text{RUP \%CP} - \text{U\%CP}}{\text{RUP \%CP}} \right) \times 100$$

The absorbable amount of rumen undegradable protein (ARUP) was then calculated as,

$$\text{ARUP \%DM} = \text{RUP \%DM} \times (\text{dRUP\%}/100)$$

2.4.1.2 Estimation microbial protein absorbable in the small intestine (AMCP)

Microbial protein synthesised in the rumen provides a major portion of amino acids to the small intestine of ruminants, which consist of ruminal bacteria, bacteria and protozoa. Similar to PDI-system, in the DVE/OEB model (Tamminga et al. 1994) microbial growth was estimated from fermentable organic matter (FOM) in the feed where FOM was calculated from digested organic matter (DOM %DM), corrected for crude fat, rumen undegraded CP, undegraded starch and end products of fermentation in ensiled feeds. The correction for fermentation end products was based on the assumption that rumen microorganisms can extract an equivalent of 50% of energy from major fermentation end products (lactic acid and alcohols) in ensiled feeds. Accordingly FOM was calculated as,

$$FOM = DOM – CFAT – RUP – USTA – (0.50 \times FP)$$

where,

FOM = fermented organic matter (%DM)

DOM = digested organic matter (% DM) estimated from long term in situ incubation

CFAT = crude fat (%DM)

RUP = RUP %CP × CP %DM

USTA = undegradable starch (%DM)

FP = estimated fermentation end products in ensiled feeds (% DM)

It was assumed that microbial crude protein (MCP) is synthesised in the rumen at the rate of 150 g per kg of FOM and MCP is calculated as,

$$MCP_{FOM} (\%DM) = 0.15 \times FOM \%DM$$

In the DVE/OEB model, amino acid content and digestibility of MCP was considered as 75% and 85% respectively, based on data from previous Dutch trials. Hence, content of ruminally synthesised absorbable microbial crude protein supplied to small intestine was calculated as,

$$AMCP \%DM = 0.75 \times 0.85 \times MCP_{FOM} \%DM = 0.6375 \times MCP_{FOM} \%DM$$

2.4.1.3 Estimation of endogenous protein losses in the digestion process (ECP)

The endogenous CP (ECP) lost in the digestive process consists of digestive enzymes, bile, peeled off epithelial cells and mucus. In the DVE/OEB model, apart from above losses, the amino acid losses during microbial re-synthesis were also included in the ECP component. The extent of ECP loss depends on characteristics of the feed and related directly to undigested DM excreted (Tamminga et al. 1994; Yu et al. 2003A, 2003B). The undigested DM (UDM) is the summation of undigested organic matter (UOM) and undigested inorganic matter represent by undigested ash (UASH).

Thus,

$$UDM\ \%DM = UOM\ \%DM + UASH\ \%DM$$

The UOM content is the difference between feed organic matter (OM) and digestible organic matter (DOM) both in dry matter basis, expressed as

$$UOM\ \%DM = OM\ \%DM - DOM\ \%DM$$

The digestibility of feed ash was assumed as 65% (Yu et al. 2003B) and therefore UASH was calculated as,

$$UASH\ \%DM = ASH\ \%DM \times 0.35$$

In the DVE/OEB model, it was assumed that there was loss of 50 g of metabolic crude protein per kg of UDM and the re-synthesis efficiency of metabolic protein was 67%. This amounts to 75 g of absorbable protein per kg of UDM to compensate for endogenous protein losses. Hence, loss of ECP was estimated as,

$$ECP\ \%DM = 7.5 \times UDM\ \%DM$$

2.4.1.4 Estimation of degradable protein balance

In addition to DVE value, the DVE/OEB model also predicts the rumen degraded protein balance (OEB value), which indicates the difference between microbial protein synthesis, potentially possible from available rumen degradable protein (MCP_{RDP}) and potentially possible from energy extracted during anaerobic rumen fermentation (MCP_{FOM}) (Yu et al., 2003A, 2003B, Tamminga et al., 1994).

$$OEB = MCP_{RDP} - MCP_{FOM}$$

The MCP_{RDP} was calculated using the equation,

$$MCP_{RDP}\ \%DM = CP\ \%\ DM \times [1 - (1.11 \times RUP\ \%CP/100)]$$

The MCP_{FOM} was calculated as,

MCP_{FOM} %DM = 0.15 × FOM %DM (assuming synthesis of 150 g MCP per kg of FOM)

A positive value of OEB, indicates potential loss of N from the rumen while negative value indicating an impaired microbial protein synthesis due to inadequate N in the rumen. Tamminga et al. (1994) recommended that care should be taken to prevent negative OEB value when formulating ration as shortage of N for rumen microorganism is too risky.

2.4.2 NRC 2001 Model

The NRC 2001 model, proposed for dairy cattle recognizes three components that contribute for MP reaching the small intestine (Yu et al. 2003A, 2003B).

1. Absorbable true feed protein that escapes ruminal degradation (ARUP)
2. Absorbable ruminally synthesised microbial protein (AMCP)
3. Absorbable endogenous protein that passes into small intestine (AECP)

The total metabolizable protein is then calculated as,

MP = ARUP + AMCP + AECP

2.4.2.1 Estimation of absorbable rumen undegradable protein (ARUP)

As stated earlier, RUP content reaching small intestine depends on rate of passage and ruminal degradability. The RUP digestibility values (in small intestine) estimated using mobile bag technique or three-step in vitro technique (Calsamiglia and Stern, 1995) from 54 studies were used in NRC 2001 to publish its tabulated mean digestibility values for a wide range of feeds. These digestibility values range from 50% (cotton hulls, almond hulls) to 93% (soybean meal) with digestibility of majority of the feedstuff lying between 75% and 85%. Because of lack of sufficient data at that time, both in NRC 1989 (Dairy) and NRC 1996 (Beef), the digestibility of RUP was assumed to be 80%. Yu et al. (2003A), in their comparison study of NRC 2001 model with DVE/OEB

system for forages, have used long term indigestible CP in nylon bags to estimate the digestibility of RUP similar to that of DVE/OEB system. However, considering the error term associated with mean value calculations and accuracy of assumptions being used in different methods, the digestibility estimations from different methods do not seems to vary significantly from each other for a majority of common feedstuffs.

Once the digestibility of RUP (dRUP) is decided ARUP was calculated as,

ARUP %DM = (dRUP% / 100) × RUP %DM

2.4.2.2 Estimation of absorbable ruminally synthesised microbial protein (AMCP)

In earlier version of NRC (NRC 1989), microbial crude protein that passes to small intestine was estimated from NE_L for dairy cows and from TDN intake for growing animals. In the NRC 1996 (Beef), MCP was predicted as 130 g per kg of TDN intake with downward adjustment for rations containing less than 40% forages. The NE_L based equation was developed from studies in which cows were fed with diets below 30 Mcal NE_L, equivalent to 3X maintenance and found to be over predicting MCP at higher levels of NE_L intakes (NRC, 2001). In the DVE/OEB system, MCP was predicted from FOM. However, NRC (2001) reported a wide range in efficiencies of microbial protein synthesis (from 12 to 54 g microbial N per kg of FOM). The efficiency of MCP synthesis is influenced to a great extent by availability of RDP relatively to availability of FOM. As discussed earlier, the relative availability of RDP to FOM was expressed as OEB value or degradable protein balance in the DVE/OEB system. The efficiency MCP synthesis from FOM is higher at negative degradable protein balance due to N recycling in the rumen where as at a positive balance, MCP synthesis become less efficient due to excess N in the rumen that is not utilized by microbes. The MCP synthesis efficiency at ideal level of zero protein balance was estimated at 186 g MCP per kg of FOM (30 g N per kg FOM) (NRC, 2001). Based on these observations NRC (2001) concluded that using a fixed linear function to predict MCP synthesis from FOM alone was less accurate, particularly at higher intake levels. The NRC (2001) estimation of MCP synthesis was therefore based on RDP balance where it was assumed that yield of MCP

was 130 g per kg of TDN (discounted for intake level) and the requirement of RDP is 1.18 x MCP yield.

Accordingly, if the RDP intake was more than 1.18 X MCP yield, MCP yield was calculated as,

MCP_{TDN} = 130 x TDN (discounted TDN value)

If the RDP intake was less than 1.18 X MCP yield, MCP yield was calculated as,

MCP_{RDP} = (1/1.18) x RDP = 0.85 x RDP

NRC 2001 assumed that MCP contains 80% true protein (and 20% nucleic acid) that is 80% digestible. Accordingly AMCP was calculated as,

AMCP = 0.8 x 0.8 x MCP = 0.64 x MCP

2.4.2.3 Estimation of absorbable endogenous protein (AECP)

Unlike in the DVE/OEB system where the ECP resulting during digestive process was considered as a loss to the total MP, in the NRC 2001 model ECP was considered as a source of protein contributing to the total MP supply to the small intestine (Yu et al. 2003A). NRC (2001) model assumed that 1.9 g of endogenous N was originated from a kg of dry matter intake (DMI). Accordingly, Yu et al. (2003A, 2003B) have calculated ECP as,

ECP (g/kg) = 6.25 x 1.9 x DMI (g/kg)

Based on the results of previous studies, NRC (2001) assumed that true protein content in ECP was 50% which was 80% digestible and therefore conversion of ECP to MP was assumed to be 40%. Accordingly absorbable ECP was estimated as,

AECP = 0.4 x ECP

2.4.3 Comparison of NRC 2001 model and DVE/OEB model

One of the main differences between two models was how the endogenous protein was treated in the model. The DVE/OEB model considered ECP as a loss in the overall protein system while NRC 2001 model treated ECP as contributory component in the total MP supply to small intestine.

The prediction of ruminal MCP synthesis in DVE/OEB system is solely based on FOM content. The underlining principle here is that microbes will convert 100% RDP into MCP provided there is no limit in available energy. The NRC 2001 in contrast identifies both RDP content and available energy as limiting factors for microbial synthesis whiles the efficiency of RDP conversion to MCP was assumed to be 85%. In the DVE/OEB model it was assumed that true protein in MCP was at 75% that has a digestibility of 85%, whereas in NRC model, both of these values were assumed to be 80%. Since multiplication of true protein content x digestibility gives almost same value (63.75 % vs. 64 %) for both models differences in these assumptions does not affect the predictions. The ARUP value predicted from DVE/OEB model is slightly higher than that of NRC 2001 model as it uses a regression coefficient of 1.11 as correction factor to convert in situ RUP values to in vivo values. However, a high correlation (R = 0.96 to 0.99) between predicted values for timothy and alfalfa from the two models were demonstrated by Yu et al. (2003A). They have observed higher predicted values for total MP with NRC 2001 model comparing to DVE/OEB model even though predicted AMCP values from DVE/OEB model were higher than NRC 2001 model for their forage samples. Since these predictions are based greatly on chemical composition of feedstuff, particularly due to variable proportions of different protein fractions with different degradability rates (Kd), NDF and lignin contents, the comparative overestimations or underestimations could vary with different types of feeds.

2.5 Canola products for cattle feeding

The suitability of canola products such as whole seed, press-cake, meal, hulls or screenings as an ingredient in ruminant rations has been studied by number of researchers in the past.

2.5.1 Canola seed

The chemical composition of canola seed as reported in some publications is shown in the Table 2.1. The fibre components in canola seed seems to vary considerably, due probably to contamination of seeds with plant materials during harvesting, use of different types of canola (i.e. *Brassica napus* or *Brassica campestris*) or differences in analytical procedures being used to determine NDF content. Canola seed contains 42-43% EE and around 20% CP and can be used as a protein and/or lipid source in ruminant rations (Beaulieu et al. 1990; Khorasani et al. 1992; Aldrich et al. 1997A, 1997B; Chichlowski et al. 2005; Leupp et al. 2006). Inclusion of canola seed containing high level of lipids, helps to increase the energy density in the ration, an important aspect particularly for today's high producing cows. In addition to that, canola oil fraction contains higher content of unsaturated fatty acids which are known to alter the fatty acid profile of ruminant products to contain more unsaturated C_{18} fatty acids (more than 60% of total fatty acids), a beneficial effect for health conscious consumers (Hussein et al. 1996; Aldrich et al. 1997A; Delbecchi et al. 2001; Chichlowski et al. 2005). Since canola seed has a highly lignified seed coat, which is resistant to both ruminal and small intestinal degradation, some form of processing is necessary for effective utilization of canola seed (Khorasani et al. 1992).

Table 2.1. Chemical composition of canola seed

Component	Reference		
	Mustafa et al. (2000)	NRC (2001)	Leupp et al. (2006)
Ether extract %DM	42.4	40.5	39.6
Crude Protein %DM	22.7	20.5	23.3
NPN %CP	13.7	-	-
SCP %CP	65.6	-	-
NDIP %CP	8.8	16.5*	-
ADIP %CP	5.7	6.3*	-
NDF %DM	16.6	17.8	31.3
ADF %DM	12.6	11.6	22.2
ADL %DM	4.8	2.7	
Ash %DM	4.3	4.6	4.1

*calculated from data given on % DM basis

Grinding, chemical treatment and heat treatment are some of the seed processing methods that were investigated in the past (Hussein et al. 1996; Aldrich et al. 1997A; Aldrich et al. 1997B). Since high unsaturated fatty acids could negatively affect ruminal fermentation and milk production; recommended maximum level of crushed seed in dairy ration was 4% of the ration DM (Kennelly, 1983). Treating canola seeds with alkaline hydrogen peroxide as an alternative to crushing was studied by some researchers (Hussein et al. 1995; Aldrich et al. 1997A, 1997B).

The DMI was not influenced due to inclusion of canola seed either crushed or chemically treated in steer diet (Hussein et al. 1995; Leupp et al. 2006) and lactating cow diets (Aldrich et al. 1997A; Chichlowski et al. 2005). Delbecchi et al. (2001) observed a slight (1 kg/day) but statistically significant drop in milk production when cows are fed with whole canola seed. In contrast, both Aldrich et al. (1997A) and Chichlowski et al. (2005) observed that there was no depression in milk production due to canola seed feeding either crushed or chemically treated (Aldrich et al. 1997A) or in ground form (Chichlowski et al. 2005).

As stated earlier, inclusion of canola seed to lactating cow diets evidently alters the milk composition particularly fatty acid composition favouring long chain unsaturated C_{18} fatty acid and isomers of conjugated lenoleic acid synthesis in milk (Aldrich et al. 1997A; Chichlowski et al. 2005). In addition, protein % in milk seems to be depressed when cows were fed with canola seed (Aldrich et al. 1997A; Delbecchi et al. 2001; Chichlowski et al. 2005), which was related to decline in ruminal propionate concentration (Delbecchi et al. 2001).

2.5.2 Canola press-cake

Canola press-cake is an intermediate product in the manufacturing process of canola oil after cooking and screw pressing stages and just before the solvent extraction of oil fraction (Figure 2.3). With partial removal of oil from seeds during screw pressing, oil concentration in resultant press-cake is turned out to be about 21% (Mustafa et al. 2000; Jones et al. 2001).

At the same time CP, ADF, NDF and ADL contents increases by 5-7% more than that of seeds. The CP content in canola press-cake reported to be 30% (Mustafa et al. 2000; Jones et al. 2001). With these high level of fat and protein, press-cake can be utilised as both protein and high energy sources similar to canola seed. The soluble protein content in press-cake was reported to be, lower than that of seed (56.7% vs. 65.6% as reported by Mustafa et al. 2000) that can be attributed to heat generated during screw pressing. The effective ruminal degradability of DM and CP in press-cake was observed to be lower than canola seed but higher than canola meal (Jones et al. 2001), which can attributed to the differences in heat received by the different material during the manufacturing process.

Jones et al. (2001) studied the effects of feeding heated and unheated canola press-cake to dairy cows, comparing to cows fed with similar level of tallow fat. Feeding press-cake in either form has increased the milk yield in multiparous cows but not in primiparous cows. On the other hand, primiparous cows have shown a higher milk production when heated press-cake or heated canola meal was included in the diets. Feeding press-cake has also increased the milk lactose % and lactose yield in both types of cows. This was attributed to higher availability of glucose and glucogenic precursors for lactose synthesis due to inhibition of short chain fatty acid formation in the presence of dietary long chain fatty acids. Similar effect on milk lactose % or yield however was not observed by Chichlowski et al. (2005) who have fed their cows with ground canola seeds (high in long chain fatty acids). Feeding press-cake has increased higher unsaturated C_{18} fatty acid concentration while reducing short chain fatty acid content in milk in both multiparous and primiperous cows (Jones et al. 2001) similar to the effect observed with canola seed feeding.

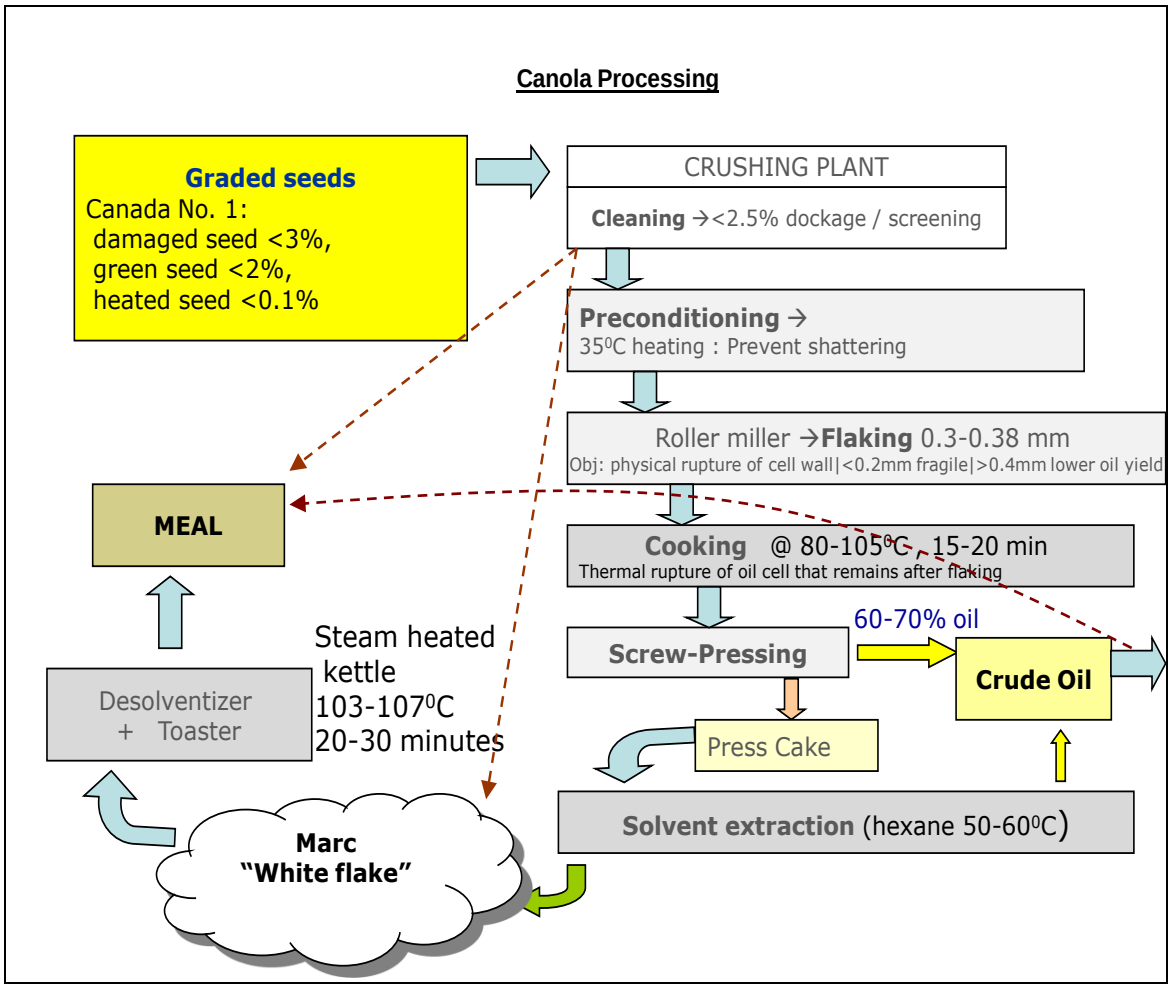

Figure 2.3. Flow chart of Canola oil and meal processing

(Adapted from Canola Council of Canada, 2007)

2.5.3 Canola meal

Canola meal is widely used in cattle diets and a popular ingredient in dairy rations due to its high protein quality. The trading rule setup by Canadian Oilseed Processors Association (COPA) in 1999 have stated that canola meal on as-fed basis should contain minimum of 34% CP and 2% EE while maximum content of moisture, glucosinolates and crude fibre at 12%, 30 μmol/g and 12%, respectively (Hickling 2001). The composition of canola meal however may vary with growing conditions as well as conditions during oil and meal extraction process. As shown in the Figure 2.3, canola meal is the by-product of oil extraction and undergoes several stages of processing from seed stage. The final quality of meal could be influenced by number of processing variables, particularly by the temperature utilized at seed cooking stage and, desolventizing and toasting stage. Rapid increase of temperature up to 80-90^{o}C at the seed cooking stage inactivates the myrosinase enzyme, which is responsible for hydrolyzing glucosinolates to undesirable products in oil and meal. Use of excessive heat during any stage however leads to formation of Maillard products that reduces the amino acid particularly lysine availability (Bell 1993; Hickling 2001). Level of addition of "gums" (phospholipid material removed during oil refining) to the meal would significantly affect the EE content and thus energy content while addition of dockage would increase the fibre content of meal.

The chemical composition of canola meal reported in different publications (Table 2.2), varies considerably indicating the possible crop related and manufacturing process related variations between the study materials. Crude protein is the major component in canola meal and two of its closest market competitors are soy meal and cotton seed meal in which the CP contents are reportedly higher than canola meal (Table 2.3). In canola meal the NPN content represented by fraction A is similar to other two meals. Comparison of NRC (2001) tabulated values of protein fractions B (degradable) and C (unavailable) indicate that canola meal is superior to cotton seed meal but lower than soy meal.

Table 2.2. Chemical composition of canola meal

Component	References			
	Mustafa et al. (1996)	Mustafa et al. (2000)[a]	NRC (2001)[b]	Maesoomi et al. (2006)
Ether extract %DM	4.7	3.2	5.4	2.4
Crude protein %DM	37.7	40.2	37.8	44.8
NPN %CP	24.3	15.5	23.2	13.9
SCP %CP	35.5	22.5	-	41.4[#]
NDIP %CP	10.5	19.2	16.7*	11.2[#]
ADIP %CP	4.6	5.2	6.3*	2.6
NDF %DM	26.7	32.1	29.8	27.5
ADF %DM	19.3	20.3	20.5	20.8
ADL %DM	6.3	8.2	9.5	-
Ash %DM	8.2	7.6	7.4	6.2

[a] solvent extracted meal
[b] mechanically extracted canola meal
*calculated from data given on % DM basis
[#]calculated from the data given

Table 2.3. Protein fractions (CNCPS) in canola meal in comparison to soy meal and cotton seed meal

	Canola meal	Soy meal	Cotton seed meal
Total CP %DM	37.8	49.9	44.9
Fraction A %CP	23.2	22.5	25.6
Fraction B %CP	70.4	76.8	55.5
Fraction C %CP	6.4	0.7	18.9

Adapted from NRC (2001)

The high fibre content in canola meal is due to presence of canola seed hull which amounts to about 30% of canola meal (Bell 1993). The addition of dockage to the meal portion at the tail end of processing (Figure 2.3) tends to increase the fibre content further. In the past, there were attempts to improve the nutritive value of canola meal fraction by reducing the fibre content through reducing hull content of meal before (front end de-hulling) or after (tail end de-hulling) oil extraction. However, due to certain problems associated with both types of de-hulling i.e. lack of product uniformity due to small and uneven seed varieties, loss of oil if it is front end de-hulling and poor separation of hull when de-hulling is done after oil extraction (Mustafa 1996; Ikebudu et al. 2000). Therefore up to now, front-end or tail-end de-hulling are not been considered as viable methods of separation by the industry.

Chemical composition of canola meal also varies due to heat treatment. McKinnon et al. (1995) has reported that both ADIP and NDIP content increased while SCP content reduced when the canola meal was heat treated. They have observed that increase in

NDIP and ADIP was temperature dependent. Particularly the ADIP content has increased by seven folds when the heat treatment temperature was 145^{o}C which they attributed to the significantly reduced intestinal CP disappearance from their mobile bags. On the other hand they noted that digestible RUP may be increased in canola meal by heat treatment at 125^{o}C. Mustafa et al. (2000) reported that soluble protein content in solvent extracted meal reduces by more than half after toasting, which they attributed to both high moisture and high heat prevailed at that processing stage.

The ruminal in situ degradability of crude protein in canola meal was reported to be comparatively higher than some protein supplements such as soybean meal (Kirkpatrick and Kennelly 1987) and corn gluten meal (Piepenbrink and Schingoethe 1998; Harstad and Prestløkken 2001), fish meal and blood meal (Piepenbrink and Schingoethe 1998). The amino acid (AA) profile of canola meal was reported as closest to AA profile of milk protein (Piepenbrink and Schingoethe 1998). In view of preserving canola meal AA for intestinal absorption, ruminal degradability of canola meal protein can however, be altered by treatment without affecting the post-ruminal digestibility as demonstrated by several researchers, i.e. acid treatment (Khorasani et al. 1989; 1993), lignosulfonate treatment (McAllister et al. 1993), moist-heat treatment (Moshtaghi Nia and Ingalls 1995) and moist-heat plus lignosulfonate treatment (Wright et al. 2005).

Several experiments have been done in the past to study the effect of canola meal in dairy rations in comparison with other popular oil meals (Table 2.4). Brito and Broderick (2007) reported that dry matter intake of cows fed with canola meal was significantly higher than that of soybean meal. Sanchez and Claypool (1983) using 30 high producing Holstein cows over 10 weeks long experimental period demonstrated that solid non-fat (SNF) yield and milk yield was higher in cows fed with canola meal comparing to those fed with cottonseed meal and soybean meal even though there was no difference in SNF percentages. More recent comparison studies with lactating cows have however, shown that milk yield and milk component yield observed with canola meal diets were similar to that with soybean meal (Mustafa et al. 1997; Brito and Broderick 2007) and cottonseed meal (Maesoomi et al. 2006; Brito and Broderick 2007).

Brito and Broderick (2007) have observed that milk composition in cows fed with canola and soybean meal were similar while cows fed with cottonseed meal have shown a lower fat and protein content than with canola meal. Mustafa et al. (1997) has reported a slightly lower milk protein percentage in cows fed with canola meal but similar protein yield comparing to soybean meal. Comparing to cottonseed meal diets, a slightly higher milk protein and SNF content in cows fed with canola meal diets have been reported by Maesoomi et al. (2006).

In the past studies, canola meal has been used with beef cows (Patterson et al. 1999) weaned calves, yearlings and feedlot (Mckinnon et al. 1993). In their study on crude protein requirement of large frame cattle, McKinnon et al. (1993) have used canola meal as the only protein source in the test diets up to 19 % dietary CP without affecting the feedlot performances. Patterson et al. (1999) have reported there were no differences in body condition score, calf birth weights or reproductive performances in beef cows fed with canola meal comparing to sunflower meal or cull beans. Zinn (1993) studied the digestible energy (DE), ruminal and total tract digestion of canola meal and soybean meal in feedlot cattle fed with high energy diet. He has observed that canola meal has a lower DE value than soybean meal (3.52 vs. 3.61 Mcal/kg). He has also reported that ruminal escape protein in canola meal was greater than soybean meal contrary to higher in situ degradation values reported with canola meal in other studies as stated elsewhere.

Table 2.4. Effect of canola meal supplement on dry matter intake and milk yield of lactating cows in comparison with soy meal and cottonseed meal

Reference	Oil meal supplement		
	Canola meal	Soy meal	Cottonseed meal
	Dry matter intake (kg/d)		
Mustafa et al. (1997)	19.9	20.1	-
Maesoomi et al. (2006)	23.4	-	23.9
Brito and Broderick (2007)	24.9	24.2	24.7
	Milk yield (kg/d)		
Sanchez and Claypol (1983)	37.67	34.45	36.50
Mustafa et al. (1997)	33.4	33.6	-
Maesoomi et al. (2006)	28.0	-	27.0
Brito and Broderick (2007)	41.1	40.0	40.5

2.5.4 Canola screening

Canola screening is the byproduct originated at the seed cleaning stage. It may consist of a mixture of canola seeds, some cereal seeds, weed seeds, chaff and dust (Beames et al. 1986; Darroch et al. 1990). Canola screenings studied by Stanford et al. (2000) consisted of 60% canola seed (immature, cracked or whole seeds), 25% weed seeds and 14% dust/dirt. Beames et al. (1986) have reported that rapeseed screening contained 17 – 21% CP, 15 – 25% EE, 23 – 33% ADF, and 6 – 9% ash. This type of screens are termed as "fines" while the screenings containing less CP (10 – 16%) and less ether extract (7-16%) are identified as "coarse" screenings (Stanford et al. 2000). The chemical composition of canola screening tested by Stanford et al. (2000) consisted of 15% CP, 9% EE, 45% NDF, 31% ADF, and 14% Ash. There is very little information on nutritional values or utilization of canola screening with cattle. Wiesen et al. (1990) have studied effect of rapeseed screenings in dairy rations on milk production, milk composition and feed intake. They have offered "fine" screenings (21.7% CP and 19.4 % EE) in pellet form at three dietary inclusion levels (0%, 7% and 14%) and observed that inclusion of rapeseed screenings did not affect the DMI, milk yield, milk fat, milk protein and SNF content. However, they have observed a significant increase in unsaturated fatty acid content in milk from cows fed with screenings. The effects of feeding "coarse" canola screenings on voluntary feed intake and total tract nutrient digestibility of beef steers was studied by Pylot et al. (2000). They reported the intake of steers fed with screenings tended to be lower than that of control diet (alfalfa-brome hay/barley). The apparent total tract digestibility of DM, NDF, ADF and fat were lower but there was no difference in CP digestibility of canola screening comparing to the control diet. They have observed a lower DMI but higher digestibility of DM, CP and fat when canola screenings were processed (grinding and pelleting). There was however, no processing effect on fibre digestion.

Stanford et al. (2000), in their digestibility trial with lamb, observed decrease in apparent digestibility of DM, OM, ADF and NDF as well as N retention when canola screen content was increased more than 45% of the diet. They attributed this decrease to

impaired rumen microbial population and lower digestibility of N in canola screenings. In the production performance trial, Stanford et al. (2000) observed a linear and quadratic increase in DMI and decrease in feed conversion efficiency with increasing dietary canola screenings. They also observed the carcass weight and overall carcass fatness (as measured by grade rule) linearly decreased, which they attributed to reduced fibre digestibility and N retention with increasing dietary screening content. They also reported a decrease in saturated fatty acid and increase in polyunsaturated fatty acid in both subcutaneous and kidney fat when canola screening content increased in the diet.

2.5.5 Canola hull

As mentioned earlier, Bell (1993) reported that content of canola hull amounts to about 30% of canola meal and dehulling may be done to improve the nutritive value of the meal. There is very limited published data available on utilization of canola hulls. McKinnon et al. (1995) have studied nutritional value, voluntary intake of canola hull based diets and effect of ammoniation on in situ rumen degradability of hulls. They have reported that both dry matter intake and apparent nutrient digestibility decreased linearly as inclusion of hulls increased from 0% to 75% into alfalfa based diets of growing lambs. These researchers have used hulls from front-end de-hulling that contained high level of ether extract (13% DM), which they surmise as a possible reason for low dry matter intake and digestibility due to its interference with rumen microbial activity. According to their chemical analysis, canola hull contained 15% CP, 47% ADF, 66% NDF, and 0.9% ADIN on dry matter basis. This ADIN content would be equivalent to 36.5% of ADIP on CP basis. Similar values for ADIP and CP were reported in NRC 2001 under its feed composition table. The degradation rate (Kd) of true protein fraction (PB fraction) and digestibility of rumen undegradable protein (RUP) in canola hulls were reported to be 5.3% /h and 70% respectively (NRC, 2001). The ammoniation of hulls has reduced the soluble fraction and effective degradability of nutrients and failed to improve the degradability.

2.6 Canola protein fractionation: process and products

In view of the high quality of protein in canola meal, particularly in relation to essential amino acid requirement of fish, there were attempts to fractionate and concentrate protein component of meal as a substitute for fishmeal (Mwachireya et al. 1999; Thiessen et al. 2004). Recently a proprietary processing technology was developed by MCN Bioproducts Inc., to fractionate oil extracted canola meal portion (desolventized non-toasted flake, i.e. white flake) with the intention of catering for specific livestock feed markets, particularly as a cost-wise attractive substitute for animal-based protein such as fish meal, thereby enhancing the value addition to canola. In this process, canola meal portion (before toasting) is subjected to "aqueous de-hulling" followed by fractionation of the aqueous-extract to obtain high protein concentrates CanPro-SP (soluble protein) and CanPro-IP (insoluble protein) (Figure 2.4).

Since this is a relatively novel process, there is only limited published information available on the performances of different fractions. A study conducted on performance of rainbow trout fed with canola protein concentrates has shown promising results indicating future market demand for the new ingredient (Drew, 2004).

In this process, besides the two protein concentrates, two other end products materialize. They are "fibre-protein" fraction (CanPro-FP) that contains mainly hulls with some dockage and "can-sugar" fraction that contains water soluble components. These two fractions amount to respectively 35% and 18% of total output. Even though protein concentrates are the main economic drivers of the process that would target for lucrative aquaculture and mono-gastric market, fibre-protein and can-sugar that are the by-products of fractionation process, should also be utilised to make the fractionation a commercially viable process.

The preliminary analysis conducted by MCN Bioproducts has shown that fibre-protein contains high level of crude fibre (25.4%) while can-sugar contains hardly any fibre. The crude protein contents in fibre-protein and can-sugar were 31% and 17% respectively (Table 2.5). In can-sugar, glucosinolate content reported to be 7.3 μmol/g. Apart from

these data, there are no other published data available on "fibre-protein" or "can-sugar" fractions. There is an urgent need to evaluate chemical profile and nutritive values related to ruminants for both domestic and international markets.

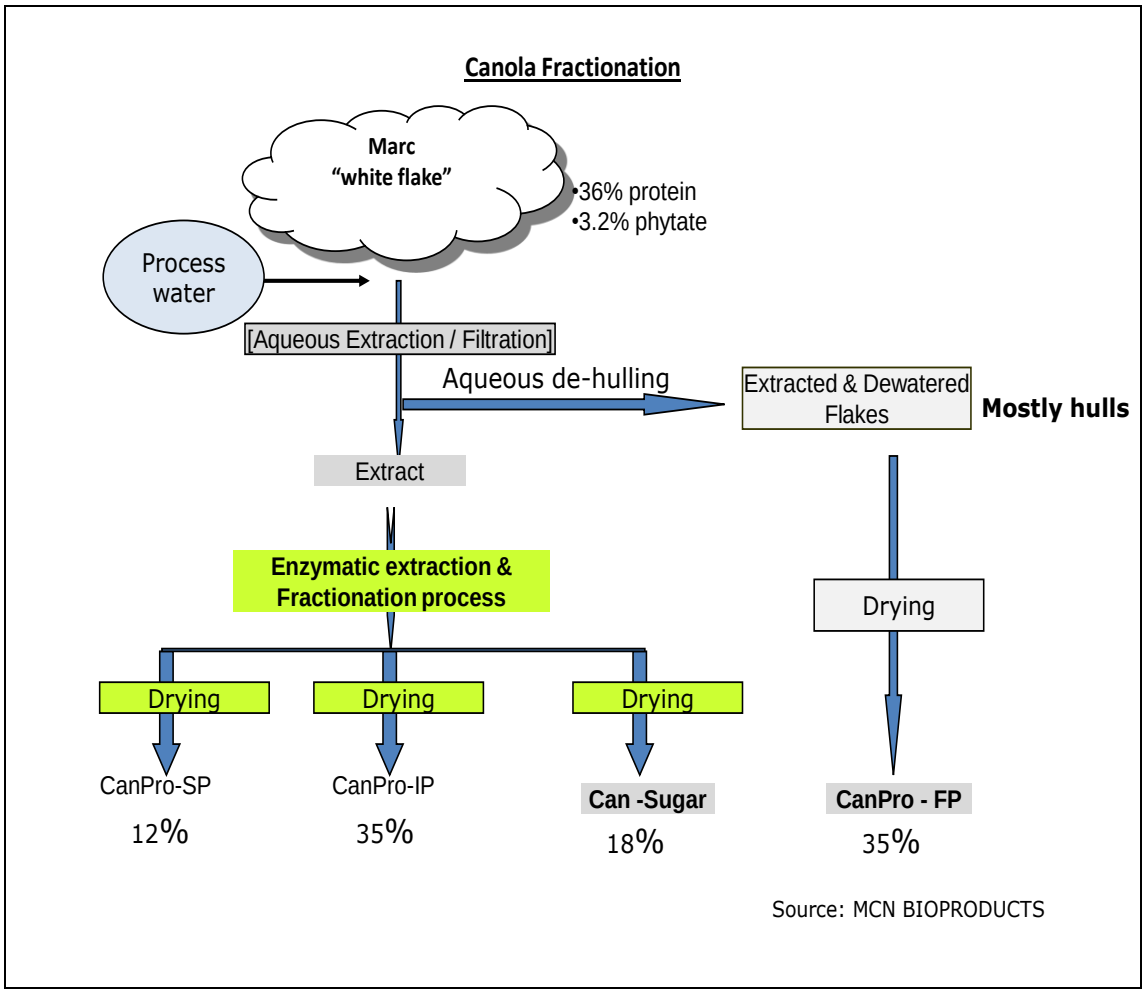

Figure 2.4. Canola fractionation process (Adapted from MCN Bioproducts Inc., 2005)

Table 2.5. Dry matter, crude protein, crude fibre, ether extract, ash, gross energy, phytic acid and glucosinolate contents in different canola fractions.

Component (as-is-basis)	Fibre-protein	Can-sugar	CanPro-SP	CanPro-IP
Dry matter %	94.3	93.5	95.2	97.4
Crude protein %	30.7	16.7	60.0	67.9
Crude fibre %	25.4	0.1	0.4	3.9
Ether extract %	0.5	0.1	0.2	0.3
Ash %	2.9	20.9	9.7	10.1
Gross energy (kcal / g)	4.18	3.52	4.5	4.8
Phytic acid %	0.9	0.0	0.0	0.0
Glucosinolates (μmol / g)	1.0	7.3	3.8	3.4

Source: MCN Bioproducts Inc. (2005)

3. CHEMICAL CHARACTERIZATION, IN SITU RUMEN DEGRADATION AND NUTRITIVE VALUES OF CANOLA FIBRE-PROTEIN AND CAN-SUGAR FRACTIONS IN COMPARISON WITH COMMERCIAL CANOLA MEAL AND SOY MEAL

3.1 Introduction

Recently there have been studies to develop processing technology to fractionate oil extracted canola meal portion with the intention of catering for specific livestock feed markets, particularly as a cost-effective alternative for animal-based protein such as fish meal (Mwachireya et al. 1999; Thiessen et al. 2004). In one such study, the meal portion of canola was fractionated to extract protein concentrates. In this process, in addition to the protein concentrates, two other end products occur, which are named as "fibre-protein" fraction and "can-sugar" fraction. The fibre-protein contains mainly hulls with some dockage present in the raw material (canola meal) while can-sugar, which is available in dried form, contains mainly the water soluble non protein components of canola meal. In order to obtain the maximum advantage from a commercially operated canola protein fractionation process, it is important to utilise both fibre-protein and can-sugar.

The CP content in fibre-protein and can-sugar reported to be 31% and 17%, respectively. However, in view of the presence of high fibre level (25% crude fibre) in fibre-protein and high ash content (21 %) in can-sugar, the value of these ingredients in monogastric diets would be limited and the most likely market would be as dietary ingredients for adult ruminants. Being newly developed products, there is very little nutritional information available on fibre-protein and can-sugar.

The objective of this study was to evaluate fibre-protein and can-sugar as feed ingredients in ruminant diets. This was achieved by examining the chemical and rumen degradation characteristics and determining the nutritive value of fibre-protein and can-sugar fractions as dietary components for dairy cattle in comparison with commercial canola meal and soy meal.

3.2 Materials and methods

3.2.1 Chemical characterization

3.2.1.1 Samples

Samples from three batches of canola meal and soy meal were obtained from a commercial supplier (Federated Cooperatives Limited, Saskatoon, Canada). Three samples of fibre-protein were collected from three different pre-commercial batches available at MCN Bioproducts Inc. (Saskatoon, SK, Canada). Due to limitation in production only one batch of can-sugar in dried powder form was available and therefore only one sample of can-sugar was tested along with other feeds.

3.2.1.2 Chemical analysis of samples

All the samples were ground through a 1mm screen using laboratory scale hammer mill (Retsch ZM-1, Brinkmann Instruments (Canada) Ltd., Ontario) prior to chemical analysis. Part of the samples was ground through the same mill using 0.5 mm screen to obtain samples for starch analysis.

Samples were analysed according to the Association of Official Analytical Chemists (AOAC, 1990) for dry mater (DM) (method 930.15), ether extract (EE) (method 920.39), crude protein (CP) (Kjeldahl method using Kjeltec 2400 auto analyser), ash (method 924.05), acid detergent fibre (ADF) (method 973.18 using ANKOM 200 fibre analyzer) and acid detergent lignin (ADL) (ADF method 973.18 followed by 72% H_2SO_4 treatment). The neutral detergent fibre (NDF) content was determined with heat stable α-amylase but without sodium sulfite according to procedure proposed by Van Soest et al. (1991). The acid detergent insoluble crude protein (ADIP) and neutral detergent insoluble crude protein (NDIP) were determined as per the procedure of

Licitra et al. (1996) using ANKOM 200 fibre analyser followed by Kjeldahl method using Kjeltec 2400 auto analyser. Non-protein nitrogen (NPN) content was determined by precipitating the true protein fraction with the use of tungstic acid and calculating the difference between total crude protein and crude protein content of precipitate (residual) according to Licitra et al. (1996). To obtain the soluble crude protein (SCP), samples were incubated in bicarbonate-phosphate buffer followed by filtering through Whatman No. 54 filter paper and residue analyzing for Kjeldahl crude protein. SCP was calculated as the difference between total crude protein and residual crude protein as proposed by Roe et al. (1990). The α-amylase amyloglucosidase method was used to determine the starch content of samples (Megazyme Total starch Assay kit, Megazyme, NSW, Australia). The total carbohydrate (CHO) and non-fibrous carbohydrate (NFC) contents were calculated according to the following equations as suggested by Sniffen et al., (1992) and NRC (2001).

Total CHO = 100 – CP – Ash – EE;

NFC = 100 – (NDF-NDIP) – CP – EE – Ash

3.2.1.3 Partitioning protein fractions

The CNCPS procedure proposed by Sniffen et al. (1992) was used to partition the total CP content into fraction A, B and C (i.e. PA, PB and PC). The PB fraction was partitioned further into three sub-fractions PB1, PB2 and PB3.

3.2.2 In situ rumen incubation

3.2.2.1 Samples

The same feed samples utilized for chemical analysis were used for in situ study, however without grinding. The can-sugar was found to be totally soluble in water. As such it was not incubated in the rumen assuming instantaneous degradation.

3.2.2.2 Animals and diets

Two non-lactating Frisian cows, weighing approximately 650 kg and ruminally fistulated with 10 cm cannulae (Bar Diamond Inc., Parma, ID, USA) were utilized. The cows were individually fed a balanced totally mixed ration (15 kg d^{-1} as fed) that contained 60% barley silage (approx 35% dry matter), 26% standard dairy concentrate (containing barley, wheat, oats, soy meal, canola meal, minerals and vitamins), 10% alfalfa hay and 4% alfalfa dehydrate. The animals were fed twice a day at 0800 and 1600 h, housed in individual pens (size 20' X 30') with free access to water and exercise ground. The animals were cared for according to the guidelines of the Canadian Council on Animal Care (1993).

3.2.2.3 Rumen incubation

The nylon bags were made out of "nytex" nylon material (Screen Tech Inc., CA) with a pore size of 40 µm. The bags with the dimensions of 10 cm x 20 cm were stitched with a nylon thread and needle holes were sealed with waterproof and non-degradable glue. The approximate sample weight was 7 g and the sample weight to bag surface area ratio was 35-39 mg per sq. cm. The maximum number of bags placed in the rumen at any time was 30. Weighted polyester mesh bags were used to hold the nylon bags in place within the rumen. Incubations were carried out for 72, 48, 24, 12, 8, 4, 2, 0 h on a "gradual addition-all out" schedule. All treatments (feed samples from 4 different feed types) were randomly allocated to two animals as per each incubation duration. The experiment was repeated over three periods/runs consecutively. On removal of bags from the rumen, they were immediately rinsed under a cold stream of water to prevent further microbial degradation and to remove excess ruminal contents.

All the bags including those representing 0 h were then hand-rinsed in a plastic tub filled with cold tap water until the rinse water was clear. The bags were laid flat on metal trays, once the excess water was drained out and the bags were dried in a forced air oven at 55ºC for 48h. End of the drying, residues were pooled together as per treatment, duration of incubation and period/run for analysis of DM, organic matter (OM), CP, NDF and ADF.

3.2.2.4 Rumen degradation characteristics

Rumen degradation parameters for DM, OM, CP, NDF and ADF were determined by using the first order rumen degradation kinetic equation proposed by Ørskov & McDonald (1979) modified with inclusion of lag time (Robinson et al. 1986; Dhanoa, 1988);

Equation: $R(t) = U + (100 - S - U) \times e^{-Kd\,(t-T0)}$;

Where, R(t), U, S, Kd and T0 represent residue% at t h of incubation, un-degradable fraction (%), water soluble fraction (%), degradation rate (%/h) and lag time respectively. The potentially degradable fraction (D) = 100 – S – U. The iterative non-linear regression procedure (PROC NLIN-Gauss-Newton method of SAS Institute Inc., 1999) was used to fit data to the model.

The effectively degraded fractions (ED) and ruminally un-degraded fractions (RU) of each nutrient were calculated using the following equations (Yu et al. 2003A; 2003B; NRC, 2001).

$ED = S + D \times \left(\frac{kd}{kd + kp}\right)$; $RU = U + D \times \left(\frac{kp}{kd + kp}\right)$; where, Kp is the outflow of digesta from rumen, which was assumed to be equal to 6% /h (Yu et al. 2003B, Tamminga et al. 1994).

3.2.2.5 Chemical analysis

In situ samples were prepared for chemical analysis by grinding to pass through a 1mm screen using a laboratory scale hammer mill (Retsch ZM-1, Brinkmann Instruments (Canada) Ltd., Ontario). Samples were analysed according to the Association of Official Analytical Chemists (AOAC, 1990) for DM (method 930.15), EE (method 920.39), CP (Kjeldahl method using Kjeltec 2400 auto analyser), ash (method 924.05), ADF (method 973.18 using ANKOM 200 fibre analyzer) and ADL (ADF method 973.18 followed by 72% H_2SO_4 treatment). The NDF content was determined without sodium sulfite according to procedure proposed by Van Soest et al. (1991).

3.2.3 Energy value estimation

The gross energy value of feed samples was determined with the use of a bomb calorimeter (Parr 1281, Parr Instruments Company, Moline, Illinois). The Total Digestible Nutrient values at maintenance (TDN_{1X}) were calculated using the summative approach proposed by Weiss et al. (1992) and recommended by NRC (2001). It uses the concentration of NDF, lignin, CP, ash, ether extract, ADF and NDF along with their theoretical digestion coefficients to predict the theoretical "truly digestible" nutrient components. The equations used are as follows.

TDN_{1X} (%) = tdNFC + tdCP + (tdFA × 2.25) + tdNDF – 7 where,

a. tdNFC (truly digestible fraction in non-fibre carbohydrate)

= 0.98 (100 − (NDF-NDICP) − CP − EE − Ash) × PAF

(PAF= Processing adjustment factor =1.00 for experimental feeds)

b. tdCP (truly digestible crude protein for concentrates)

= (1 – (0.4 × (ADICP/CP))) × CP

c. tdFA (truly digestible fatty acid)

= FA = EE – 1 (if EE < 1, then FA=0)

d. tdNDF (truly digestible neutral detergent fibre)

= 0.75 × (NDF – NDICP – Lignin) × (1 – (L/(NDF – NDICP))0.667)

e. Metabolic fecal TDN value of 7 was subtracted from the sum of digestible nutrients as TDN is based on apparent digestibility.

The digestible energy values at maintenance level (DE_{1X}) were derived using the NRC (2001) equation;

DE_{1X} (Mcal/kg)= (tdNFC/100) × 4.2 + (tdNDF/100) × 4.2 + (tdCP/100) × 5.6 + (FA/100) × 9.4 - 0.3

In the above equation, tdNFC, tdNDF, tdCP and tdFA were expressed as percent of DM.

The DE values at productive level of intake (DE_P: i.e. DE_{3X}) were determined by multiplying the DE_{1X} by a discount value calculated based on intake level (over and above maintenance level) as given below.

$$\text{Discount} = (TDN_{1X} - (0.18 \times TDN_{1X} - 10.3)) \times \text{intake} / TDN_{1X}$$

The metabolisable energy values at productive level (ME_P) were calculated from DE_P values and the equation used for conversion depended upon the percentage of EE in the test material as shown below.

If the EE <3% $\quad ME_P \text{ (Mcal/kg)} = 1.01 \times DE_P - 0.45$

If the EE >3% $\quad ME_P \text{ (Mcal/kg)} = 1.01 \times DE_P - 0.45 + 0.0046 \times (EE - 3)$

The net energy for lactation system (NE_L) at a given intake level was derived in turn from ME previously calculated for respective intake level, using equations given in NRC (2001). The equations were based on EE content of the ingredient.

If the EE<3% $\quad NE_{Lp} \text{ (Mcal/kg)} = (0.703 \times ME_P) - 0.19$

If the EE>3% $\quad NE_{Lp} \text{ (Mcal/kg)} = (0.703 \times ME_P) - 0.19 + \{[(0.097 \times ME_P + 0.19)/97] \times (EE-3)\}$

The net energy values of feeds for maintenance and for gain in Mcal/kg were calculated using NRC (1996) equations for beef cattle as shown below.

Net Energy for maintenance (NE_M) $= 1.37 \times ME - 0.138 \times ME^2 + 0.0105 \times ME^3 - 1.12$

Net Energy for gain (NE_G) $= 1.42 \times ME - 0.174 \times ME^2 + 0.0122 \times ME^3 - 1.65$

The ME in the above equations were calculated as $ME = 0.82 \times DE_{1X}$

3.2.4 Prediction of microbial protein synthesis and potential protein supply to the small intestine

Both DVE/OEB model (Tamminga et al. 1994) and NRC (2001) model were used in this study to predict the amount of true protein absorbable from the small intestine. Both of these models have been developed based on principles used in existing protein evaluation systems such as French PDI (INRA 1978), ARC (1984), AAT-PVB (Madsen 1985), NRC (1985).

3.2.4.1 DVE/OEB Model

In the DVE/OEB system content of total truly digestible protein in the small intestine (DVE value) was derived from equation;

DVE = AMCP + ARUP – ENDP, where;

AMCP = absorbable microbial crude protein, ARUP = absorbable rumen un-degraded protein and, ENDP = Endogenous protein (lost in the digestive process).

3.2.4.1.1 Absorbable microbial crude protein (AMCP)

The fermentable organic matter (FOM) was calculated from apparently digested organic matter (DOM) as;

FOM = DOM – EE – RUP – RUST – FP, where,

DOM was assumed as the amount of organic matter (g/kg DM) disappeared after 72 h of in situ incubation while EE, RUP, RUST and FP were ether extract, rumen un-degraded protein, un-degraded starch and end products of fermentation for ensiled feeds respectively expressed as g/kg DM. FP was assumed to be zero for the test ingredients. In this system it is assumed that 150 g of microbial crude protein (MCP) is synthesised from one kg of FOM and accordingly MCP from FOM (MCP_{FOM}) was calculated as;

MCP_{FOM} (g/kg DM) = 0.15 × FOM (g/kg DM)

The amino acid N in MCP amounts to 75% of total N that has a digestibility of 85%.

Accordingly AMCP is calculated as;

AMCP (g/kg DM) = MCP_{FOM} (g/kg) × 0.75 × 0.85

3.2.4.1.2 Absorbable rumen undegraded protein (ARUP)

The fraction of rumen un-degraded protein of the total crude protein of feeds (RUP %CP) was determined as;

$$RUP\ (\%CP) = U + D \times \left(\frac{kp}{kd + kp} \right)$$ where,

Kp is the outflow of digesta from rumen assumed to be equal to 6%/h while U and D were rumen in situ degradation parameters for CP and expressed as % of CP.

The intestinal digestibility values of rumen un-degraded protein (dRUP) of feeds were calculated based on residue remained after 72 h of nylon bag incubation as;

$$dRUP\ \% = \left(\frac{RUP\ \%CP - residue\ \%CP\ at\ 72\ h\ incubation}{RUP\ \%CP} \right) \times 100$$

Then the amount of ARUP was calculated as;

ARUP = (1.11 × RUP %CP/100) × CP × dRUP% where ARUP and CP are in g/kg DM.

The Factor 1.11 in the equation was taken from French PDI-system as suggested by Tamminga et al. (1994).

3.2.4.1.3 Endogenous protein (ENDP) lost in digestive process

In the DVE/OEB model, ENDP that consists of digestive enzymes, bile, desquamated epithelial cells and mucus as well as amino acids lost in resynthesis of ENDP, is deemed as lost in the digestive process and its extent is considered to depend on undigested dry matter (UDM) excreted in the faeces (Yu et al. 2003A; Tamminga et al. 1994). In this system it is assumed that 75 g of absorbed protein per kg of undigested DM in faecal

excretion is required to compensate the ENDP loss and accordingly ENDP was calculated as;

ENDP = 75 × UDM where UDM and ENDP are in g / kg DM.

The values of UDM in different treatments were calculated as summation of undigested organic matter and undigested inorganic matter that were in the residues at the end of 72 hours of nylon bag incubation.

3.2.4.1.4 Degradable protein balance (OEB)

The degradable protein balance in DVE/OEB model indicates the difference between microbial protein synthesis potentially possible from ruminally degradable protein (MCP_{RDP}) of feed and that potentially possible from energy extracted during rumen fermentation (MCP_{FOM}) (Yu et al., 2003A; 2003B; Tamminga et al., 1994).

Accordingly the OEB values were calculated as;

$OEB = MCP_{RDP} - MCP_{FOM}$, where all values are in g/kg DM.

MCP_{RDP} was calculated as;

MCP_{RDP} (g/kg DM) = CP (g/kg DM) × [1 – (1.11 × RUP %CP/100)]

MCP_{FOM} was calculated as;

MCP_{FOM} (g/kg) = FOM (g/kg DM) × 0.15

3.2.4.2 NRC – 2001 Model

As per NRC 2001 model, the total metabolizable protein (MP) was calculated by totalling true protein fraction of ruminally synthesised microbial crude protein (AMCP), absorbable fraction of ruminally un-degradable feed CP (ARUP) and absorbable true protein content of endogenous protein (AECP) i.e. MP = AMCP + ARUP + AECP.

3.2.4.2.1 Absorbable ruminally undegradable feed CP (ARUP)

The RUP fraction of CP was calculated for each feed ingredient as;

RUP (%CP) = U + D $\times \left(\frac{kp}{kd + kp} \right)$; where U and D were expressed as % of CP and Kp value was assumed to be 6%/h for all the feeds.

The digestibility of RUP (dRUP) was estimated in this study as;

dRUP = [(RUP%CP − residue%CP at 72 h incubation)/RUP%] x 100

The ARUP was then calculated as;

ARUP (g/kg DM) = RUP (g/kg DM) × dRUP%; (where RUP (g/kg DM) = CP (g/kg DM) × RUP % CP)

3.2.4.2.2 Absorbable microbial crude protein (AMCP)

It is assumed in the NRC 2001, that MCP is synthesized at the rate of 130 g per kg of TDN (discounted) intake while the RDP requirement for MCP synthesis is 1.18 × MCP. Thus ruminally synthesized MCP was calculated as MCP (g/ kgDM) = 0.13 × TDN when RDP has exceeded 1.18 × "MCP based on TDN (MCP_{TDN})" (i.e RDP > 1.18 × 0.13 × TDN).

If the RDP was less than 1.18 × MCP_{TDN} then synthesized MCP was calculated as 0.85 × RDP. For all the calculations here, TDN (discounted) was taken as of 3 times maintenance intake (TDN_{3X}).

In the DVE/OEB model true protein content and digestibility were considered to be 75% and 85% respectively for MCP as stated before where as in NRC 2001 model, the true protein content in MCP is assumed to be 80% with a digestibility of 80%. Accordingly the AMCP was calculated as AMCP (g/kg DM) = 0.80 × 0.80 × MCP (g/kg DM)

3.2.4.2.3 Absorbable true protein content of endogenous protein (AECP)

The endogenous N that passes from reticulo-rumen in to small intestine is calculated in NRC 2001 based on dry matter intake as endogenous N (g/day) = 1.9 × DMI (kg/day). Based on this equation the endogenous crude protein (ECP) related to feed was calculated as ECP (g/kg DM) = 6.25 × 1.9 × Dry Matter (g/kg) (Yu et al. 2003A). Using the assumptions that true protein content in ECP is 50% that has a digestibility of 80% (NRC 2001), AECP values related to feeds were calculated as;

AECP (g/kg DM) = 0.5 × 0.8 × ECP (g/kg DM)

3.2.4.2.4 Degradable protein balance (DPB)

Analogous to DVE/OEB model, Yu et al. (2003B) have calculated degradable protein balance (DPB) using the data from NRC 2001 model where DPB indicates the difference between RDP value of a feed ingredient and RDP requirement for MCP synthesis based on discounted TDN value of the same feed ingredient.

Using the same formula DPB in present study was calculated as;

DPB (g/kg DM) = RDP – 1.18 × MCP_{TDN}

3.2.5 Statistical analysis

All the data were analysed statistically using "Mixed Procedure" of SAS software (SAS Institute, Inc. 1999). Each set of data were analysed as a completely randomised design with three batches as replicates. Means were separated using Fisher's protected LSD procedure and significance was declared at $P<0.05$. As there was only one sample of can-sugar was available, it was not included in the statistical analysis but shown separately for comparison in the relevant tables.

3.3 Results and discussion

3.3.1 Chemical composition and characteristics

The fibre-protein had a lower ($P<0.05$) ash content (4.3%) and higher organic matter content (95.7%) compared to both canola and soy meals (Table 3.1). The fibre- protein is the residue of the protein fractionation process and consists mainly of hulls, separated after aqueous filtration. The ash content in can-sugar was 19.3% indicating that a major portion of mineral matter in canola meal was concentrated in the can-sugar fraction during the extraction process. In addition, can-sugar may also contain minerals originated from chemicals used during enzymatic extraction of proteins. The EE contents were very low around 1% or less in all the ingredients except for canola meal (5.3%) which was closer to average EE content reported in NRC (2001). The EE content in fibre-protein (1.5%) that consists mainly of canola seed hulls is low in contrast to that was in canola hulls (13%) reported by McKinnon et al. (1995). An EE content of 13% is higher than even that of canola meal and this probably was due to presence of seed kernel in the hulls from "front-end de-hulling" used by these researchers.

The total CHO content in fibre-protein (63.2%) was closer to that of the can-sugar fraction (64.9%), but significantly higher ($P<0.05$) than canola meal and soybean meal by 16 and 22% respectively. In can-sugar, the total CHO was almost totally (99.9%) represented by NFC. As the starch content in can-sugar was only 1.1%, it indicates that the CHO fraction of can-sugar consists mainly of sugars. The NFC in fibre-protein was the lowest (20.2 %DM) and significantly different from canola meal (29.3%) and soy meal (36.6%). In fibre-protein this amounts to only 32% of total CHO whereas NFC represents 63% and 89% of total CHO in canola meal and soy meal, respectively. When the non-fibre content in fibre protein and can-sugar are compared with canola meal, it seems that structural carbohydrates in canola meal were isolated mostly into fibre-protein while non-structural carbohydrates consisting mainly of sugars were concentrated into can-sugar during the fractionation process.

The structural CHO content observed for canola meal in this study agrees with values reported in other publications (Maesoomi et al. 2006; NRC, 2001; Mustafa et al. 1996). A significantly higher ($P<0.05$) NDF and ADF content were observed in fibre-protein relative to canola meal (55.6% vs. 25.4% and 46.3% vs. 21.2%, respectively) which could be attributed to high level of hulls present in fibre-protein. McKinnon et al. (1995) has reported a similar ADF content (46.7%) but with a higher NDF content (65.8%) for untreated canola hulls obtained from front-end de-hulling.

Very low acid detergent lignin (ADL) content (< 1%) was observed in both can-sugar and soy meal samples. In comparison to canola meal, both cellulose and ADL contents were almost doubled in fibre-protein (12.2% vs. 22.2% and 9% vs. 24.1%, respectively) indicating that both cellulose and lignin are closely associated with the hulls of canola seed. Similar association between canola seed hull and cellulose/lignin was reported previously by Mustafa et al. (1996). In the CNCPS, indigestible carbohydrate/fibre fraction (fraction CC) is estimated by multiplying ADL% by factor 2.4 (lignin% X 2.4) (Sniffen et al. 1992; Fox et al. 2004). If the same principal is applied here, it would indicate that 24.1% of ADL present in fibre-protein would render its NDF (55.6 %DM) totally indigestible (where 2.4 X 24.1 = 57.84.> 55.6). This is comparable with a high indigestible NDF content in canola meal (estimated as 85%).

Table 3.1. Dry matter, ash, organic matter, ether extract and carbohydrate composition of fibre protein and can-sugar compared with commercial soy meal and canola meal.

	Feed Ingredient				
Component	Fibre-protein	Canola meal	Soy meal	SEM	Can-sugar
DM %	91.8	90.2	92.1	1.68	87.6
Ash % DM	4.3[b]	8.4[a]	9.4[a]	1.31	19.3
OM % DM	95.7[a]	91.6[b]	90.6[b]	1.31	80.7
Ether extract % DM	1.5[b]	5.3[a]	1.1[b]	0.38	0.3
Carbohydrates (CHO)					
Total CHO %DM	63.2[a]	46.7[b]	41.1[c]	0.6	64.9
Starch % DM	0.8	1	1	0.16	1.1
Non fibre CHO %DM	20.2[c]	29.3[b]	36.6[a]	1.36	64.8
Non fibre CHO %CHO	32.0[c]	62.9[b]	89.0[a]	2.9	99.9
Neutral detergent fibre % DM	55.6[a]	25.4[b]	8.8[c]	1.71	0.1
Acid detergent fibre % DM	46.3[a]	21.2[b]	6.1[c]	1.62	0.1
Acid detergent lignin %DM	24.1[a]	9.0[b]	0.6[c]	1.47	0.2
Hemicellulose[1] %DM	9.3[a]	4.2[b]	2.7[c]	0.2	0.0
Cellulose[2] %DM	22.2[a]	12.2[b]	5.5[c]	0.65	0.0

[a, b, c] Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM = Standard error of mean

[1] Hemicellulose = Neutral detergent fibre – Acid detergent fibre

[2] Cellulose = Acid detergent fibre – acid detergent lignin

The highest CP content was observed in soy meal (48.4%) followed by canola meal (39.6%), fibre-protein (30.9%) and can-sugar (15.6%) as shown in the Table 3.2. McKinnon et al. (1995) reported a protein content of 15.4% in canola hulls obtained from front-end de-hulling, which is only half of that of the value for fibre-protein and less than half of that for canola meal. The difference of 9% ($P<0.05$) in CP content observed in fibre-protein comparing to canola meal could therefore be attributed to concentration of hulls in fibre-protein. On the other hand, fibre-protein also contains parts of the seed other than hulls that has resulted in a protein content of 30.9%.

There is no difference ($P>0.05$) in SCP content of fibre-protein and canola meal which is about 25% of total CP where as SCP content in soy meal ($P<0.05$) was only 14.4%. In contrast the CP components in can-sugar were comprised mainly of SCP, which in turn represented up to 87% by NPN. In the manufacturing process (figure 2.4), most of the available true protein in the aqueous extract were separated as protein concentrates. The can-sugar fraction would therefore contain nitrogenous substances mainly in the form of NPN. On a dry matter basis, NPN content in fibre-protein (4.3%) is significantly less than ($P<0.05$) canola meal (7.1%). However, when the share of NPN in crude protein was considered, there was no significant difference ($P>0.05$) between fibre-protein (13.9 %CP) and canola meal (17.8 %CP). It seems NPN, consisting of ammonia, peptides and amino acids, was equally distributed in the two fractions (i.e. dewatered cake and aqueous extract) before the "fractionation" stage and the NPN in canola meal was mostly confined to the can-sugar fraction after enzymatic separation.

The protein fraction associated with the NDF and ADF in fibre-protein were significantly higher than ($P<0.05$) those of canola meal by 20% and 17% respectively. The presence of higher level of hull in fibre-protein is attributed to this difference. As the fibre component in can-sugar is almost zero, the NDIP and ADIP contents therein were insignificant. The significantly lowest ($P<0.05$) NDIP and ADIP contents were observed in soy meal (8.9 %CP and 1.4 %CP respectively).

Table 3.2. Crude protein and protein fractions in fibre-protein and can-sugar compared with commercial soy meal and canola meal.

	Feed Ingredient				
Component	Fibre-protein	Canola meal	Soy meal	SEM	Can-sugar
Crude protein %DM	30.9[c]	39.6[b]	48.4[a]	1.01	15.6
Soluble crude protein %DM	8.3[ab]	10.0[a]	7.0[b]	0.94	15.0
Soluble crude protein %CP	26.7[a]	25.2[a]	14.4[b]	2	96.2
Non-protein nitrogen[1] %DM	4.3[b]	7.1[a]	2.5[b]	0.93	13.0
Non-protein nitrogen[1] %CP	13.9[a]	17.8[a]	5.1[b]	1.98	83.5
Non-protein nitrogen[1] %SCP	52.0[bc]	70.6[ab]	33.6[c]	7.38	86.8
Neutral detergent insoluble protein %DM	12.6[a]	8.0[b]	4.3[c]	1.28	0.04
Neutral detergent insoluble protein %CP	40.9[a]	20.2[b]	8.9[c]	3.89	0.3
Acid detergent insoluble protein %DM	7.7[a]	3.2[b]	0.7[b]	0.93	0.0
Acid detergent insoluble protein %CP	24.9[a]	8.2[b]	1.4[b]	3.04	0.0
True protein %CP	61.2[c]	74.8[b]	94.5[a]	2.93	16.5

[a, b, c,] Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM = Standard error of mean

[1]Non-protein nitrogen is presented as crude protein (6.25 X N)

The crude protein associated with ADF is considered indigestible and the true protein (TP) fraction is considered as the portion of crude protein without NPN and ADIP. In the Cornell Net Carbohydrate and Protein System (CNCPS) this true protein fraction known as PB fraction, is subdivided further in to three fractions i.e. PB1, PB2 and PB3 (Sniffen et al. 1992). In this study (Table 3.3) it was observed that there is a significant difference ($P<0.05$) in TP content between different feed ingredients where highest level of TP (in total protein) was present in soy meal (94.5 %) followed by canola meal (74.8%), fibre-protein (61.2%) and can-sugar (16.5%). The TP values of canola meal and soy meal agree with those reported previously (NRC 2001; Mustafa et al. 1996).

The CP sub-fractions partitioned according to CNCPS are given in the Table 3.3. In can-sugar the largest fraction was PA that is instantly degradable and contains NPN. The indigestible protein fraction PC in fibre-protein amounted to approximately 25% of its total CP and it was significantly higher than ($P<0.05$) in other feed ingredients. On dry matter basis, there was no significant difference in the soluble true protein fraction PB1 between fibre-protein (4.0 %DM) and canola meal (2.9 %DM), which indicates equal proportion of PB1 was separated along with dewatered cake as with aqueous extract, during the aqueous extraction stage in the manufacturing process. This in turn has resulted a significantly higher ($P<0.05$) concentration of PB1 (on the basis of crude protein and true protein) in fibre-protein (12.8 %CP and 21.0 %TP) compared to canola meal (7.3 %CP and 9.7 %TP). Except for can-sugar in all the other ingredients, PB2 was the largest fraction of protein.

The PB2 fraction in soy meal was significantly higher than ($P<0.05$) in all other ingredients on both dry matter basis and crude protein basis. However there was no significant difference in concentration of PB2 in relation to true protein between canola meal (74.3 %) and soy meal (82.3 %) even though soy meal has shown a higher value.

Table 3.3. Protein fractions (associated with rumen degradation characteristics) of fibre-protein and can-sugar compared with commercial soy meal and canola meal.

	Feed Ingredients				
	Fibre-protein	Canola meal	Soy meal	SEM	Can-sugar
Total crude protein %DM	30.9[c]	39.6[b]	48.4[a]	1.01	15.6
Protein fraction (% CP)					
PA	13.9[a]	16.9[a]	4.1[b]	1.94	83.5
PB1	12.8[a]	7.3[b]	9.2[ab]	1.56	12.7
PB2	32.4[c]	55.5[b]	77.8[a]	3.22	3.5
PB3	15.9[a]	12.0[ab]	7.5[b]	1.85	0.3
PC	24.9[a]	8.2[b]	1.4[b]	3.04	0.0
True Protein (% CP)	61.2[c]	74.8[b]	94.5[a]	2.93	16.5
Protein fraction (% TP)					
PB1	21.0[a]	9.7[b]	9.8[b]	1.79	76.7
PB2	52.6[b]	74.3[a]	82.3[a]	2.98	21.6
PB3	26.4[a]	16.0[b]	7.9[b]	3.23	1.7
Protein fraction (% DM)					
PA	4.3[ab]	6.8[a]	2.0[b]	0.9	13.0
PB1	4.0	2.9	4.5	0.46	2.0
PB2	10.0[c]	22.0[b]	37.6[a]	0.94	0.6
PB3	4.9	4.8	3.6	0.79	0.04
PC	7.7[a]	3.2[b]	0.7[b]	0.93	0.0

[a, b, c] Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM = Standard error of mean

The slowly degradable PB3 fraction was observed to be higher in both fibre-protein and canola meal than that of soy meal. There was no significant difference in PB3 between canola meal (4.9 %DM) and fibre-protein (4.8 %DM). The degradation rates of PB1, PB2 and PB3 were reported to be 135% to 350%, 3% to 6% and 0.05% to 0.55% per h respectively (Fox et al. 2000; Sniffen et al. 1992). Based on these degradation rates and the distribution of three sub-fractions in each ingredient, soy meal can be expected to show a comparatively faster ruminal degradability compared to canola meal and fibre-protein.

3.3.2 In situ rumen degradation characteristics

3.3.2.1 In situ rumen kinetic parameters and degradability of DM

The Figure 3.1 illustrates the remaining in situ dry matter residue observed at different incubation periods for the tested feed ingredients. It shows that the fibre-protein has a lower zero hour soluble DM fraction and much higher ruminally undegradable DM portion comparing to soy meal and canola meal. There was no difference in degradation between canola meal and soy meal until the 8^{th} hour of incubation when more than 50% of DM from both feeds had escaped from the nylon bags. After the 8^{th} hour, the rate of degradation seems to decline in canola meal and showed a higher undegradable DM content compared to soy meal. As shown in the Table 3.4, the soluble fraction (S) of DM in fibre protein was 7 %, which amounts to only one third of that of canola meal (21.1%). There was no significant difference in the "S" fraction between canola and soy meal (23.0%). The potentially degradable fraction (D) of DM in soy meal (73%) was significantly higher than canola meal (64.1%) while a far greater difference in "D" was observed between fibre-protein (45.5%) and canola meal. The values of "S" and "D" fractions of canola meal and soy meal agree with those reported by Kirkpatrick and Kennelly (1987) while Mustafa (1996) has reported similar values for regular canola meal. Woods et al. (2003A) in their study have reported slightly higher "S" fraction (33%) and slightly higher "D" fraction (65%) for soy meal DM. Use of nylon bags with a higher pore size (50 µm) by Woods et al. (2003A) could be one of the reasons for

having a higher "S" fraction. However, the total degradable fraction ("S" + "D") of DM in soy meal reported by these researchers (97%) is in agreement with findings of the current study (96%).

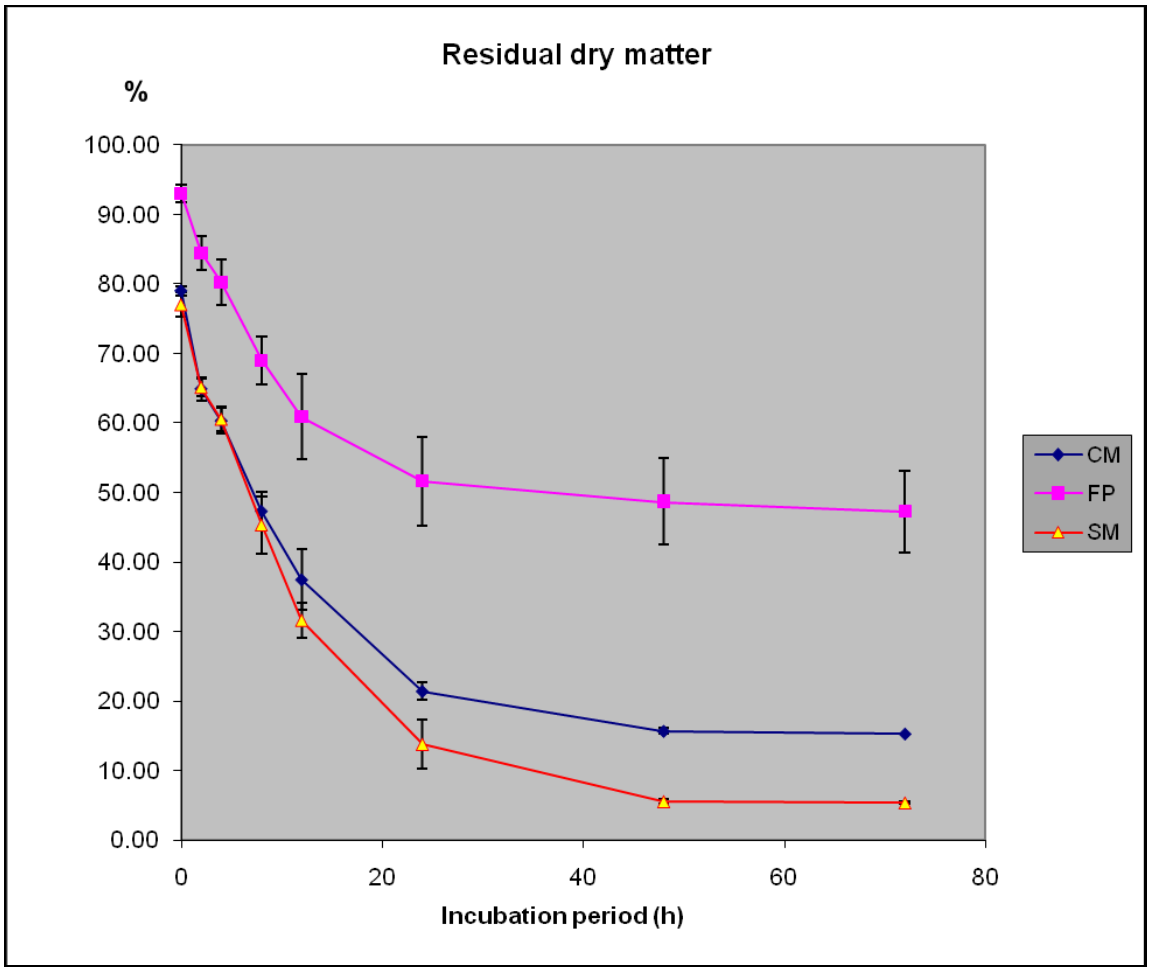

Figure 3.1. In situ ruminal disappearances of dry matter (DM) in fibre-protein in comparison with commercial canola meal and soy meal

Table 3.4. In situ rumen degradation kinetics of dry matter (DM) of fibre-protein in comparison with commercial canola meal and soy meal

Parameter	Feed Ingredient			SEM
	Fibre-protein	Canola meal	Soy meal	
S (% DM)	7.0[b]	21.1[a]	23.0[a]	0.71
D (% DM)	45.5[c]	64.1[b]	73.0[a]	2.20
U (% DM)	47.5[a]	14.8[b]	4.0[c]	2.56
Kd (% per hour)	9.9[a]	9.0[ab]	7.9[b]	0.49
T0 (hour)	0.20	0.01	0.25	0.086
EDDM (% DM)	35.3[b]	59.4[a]	64.5[a]	1.98
RUDM (% DM)	64.7[a]	40.6[b]	35.5[b]	1.98

[a, b, c] Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM = Standard error of mean

Almost half of the DM in fibre-protein seems to be ruminally un-degradable (47.5%) where as only 14.8% and 4% of DM was found to be un-degradable in canola meal and soy meal, respectively. The differences in parameters among the test ingredients can be attributed partly to level of the presence of seed hull, which were reflected by the lignin content. Canola meal with a lignin content higher than soy meal (9% vs. 0.6%), contains about 30% hulls (Bell and Shires 1982; Bell 1993) whilst fibre-protein by nature of its manufacturing process, tends to contain much higher level of hull which is indicated by its higher lignin content (24.1%). The degradability rate (Kd) of DM in fibre-protein (9.9 % /h) was closer to that of canola meal (9.0% /h). This is within the range of Kd for DM reported by Mustafa (1996), varying from 8.2% to 11.2% /h for canola meal obtained from different sources.

Even though the Kd value of soy meal (7.9% /h) was lower than canola meal (9.0% /h) the difference was not statistically significant ($P>0.05$). However, the difference in Kd value between soy meal and fibre-protein (9.9% /h) was found to be significant ($P<0.05$). Kirkpatrick and Kenelly (1987) has reported similar Kd values for canola meal and soy meal while slightly higher values of 15.1 and 11.6 % /h were reported by Khorasani et al. (1993) and Mustafa (1996) respectively.

At a ruminal passage rate (Kp) of 6% /h, 64.5% of DM in soy meal was degraded effectively in the rumen and not significantly different from EDDM of canola meal (59.4%). These values are comparable with EDDM values reported by Kirkpatrick and Kenely (1987) for canola meal (63 – 64%) and soy meal (67 – 69%) at 5% Kp. The EDDM value obtained for canola meal in this study was closer to that of rapeseed meal (61.2% at 6% Kp) as reported by Woods et al. (2003A) but slightly lower than those (66.5% to 69.6% at 5% Kp) reported by Mustafa (1996). In fibre-protein, only 35.3% of DM was ruminally degradable and significantly lower ($P<0.05$) than both canola meal and soy meal. Fibre-protein for the most part consists of canola hulls. McKinnon et al. (1995), has reported EDDM values of 31% and 40% respectively for defatted and

untreated canola hulls (from front-end dehulling) at a Kp value of 5% /h. These values are closer to EDDM content in fibre-protein, which has been calculated at 6% Kp.

3.3.2.2 In situ rumen kinetic parameters and degradability of OM

Rumen kinetic parameters for OM were similar to that of DM (Figure 3.2 and Table 3.5). The water-soluble "S" fraction of OM in fibre-protein (6%) was significantly lower ($P<0.05$) than that of canola meal (20.5%) and soy meal (19%) with no significant difference ($P>0.05$) between canola and soy meal. The highest potentially degradable fraction (D) was observed ($P<0.05$) in soy meal (80.5%) followed by canola meal (64.2%) and fibre-protein (46.5%). Only 0.5% of undegradable fraction of OM was observed in soy meal comparing to 15.3% that of in canola meal. The undegradable OM fraction in fibre-protein (47.5%) was approximately three times that of undegradable fraction in canola meal.

There was no significant difference ($P>0.05$) in degradability rate (Kd) of "D" fraction between canola meal (9.1%/h) and fibre-protein (9.8%/h). However, the Kd for soy meal (7.5%/h) was significantly lower than Kd of both canola meal and fibre-protein. This is in contrast to results reported by Woods et al. (2003A) who has reported a higher degradation rate for soy meal (12%/h) than rapeseed meal (9%/h). Figure 3.2 indicates that fibre-protein seemed to reach its maximum degradation much earlier (about 24 hours of incubation) than both canola meal and soy meal in which OM continued to decline up to 48 hours.

A significantly longer lag time (T0) was observed with OM degradation in soy meal (0.26 h) comparing to canola meal (0.0 h). However, the differences in lag time between fibre-protein (0.8 h) and soy meal (0.26) as well as between fibre-protein and canola meal were not significant ($P>0.05$). Out of the total OM, only 34.8% was degradable effectively in fibre-protein, which was significantly lower ($P<0.05$) than canola meal (59.1 %OM) and soy meal (63.7 %OM). The difference between soy meal and canola meal, however, was not significant ($P>0.05$). The EDOM content in fibre-protein comprises one third of dry matter (33.3 %DM) which was significantly lower than canola meal (54.8 %DM) and soy meal (56.1 %DM).

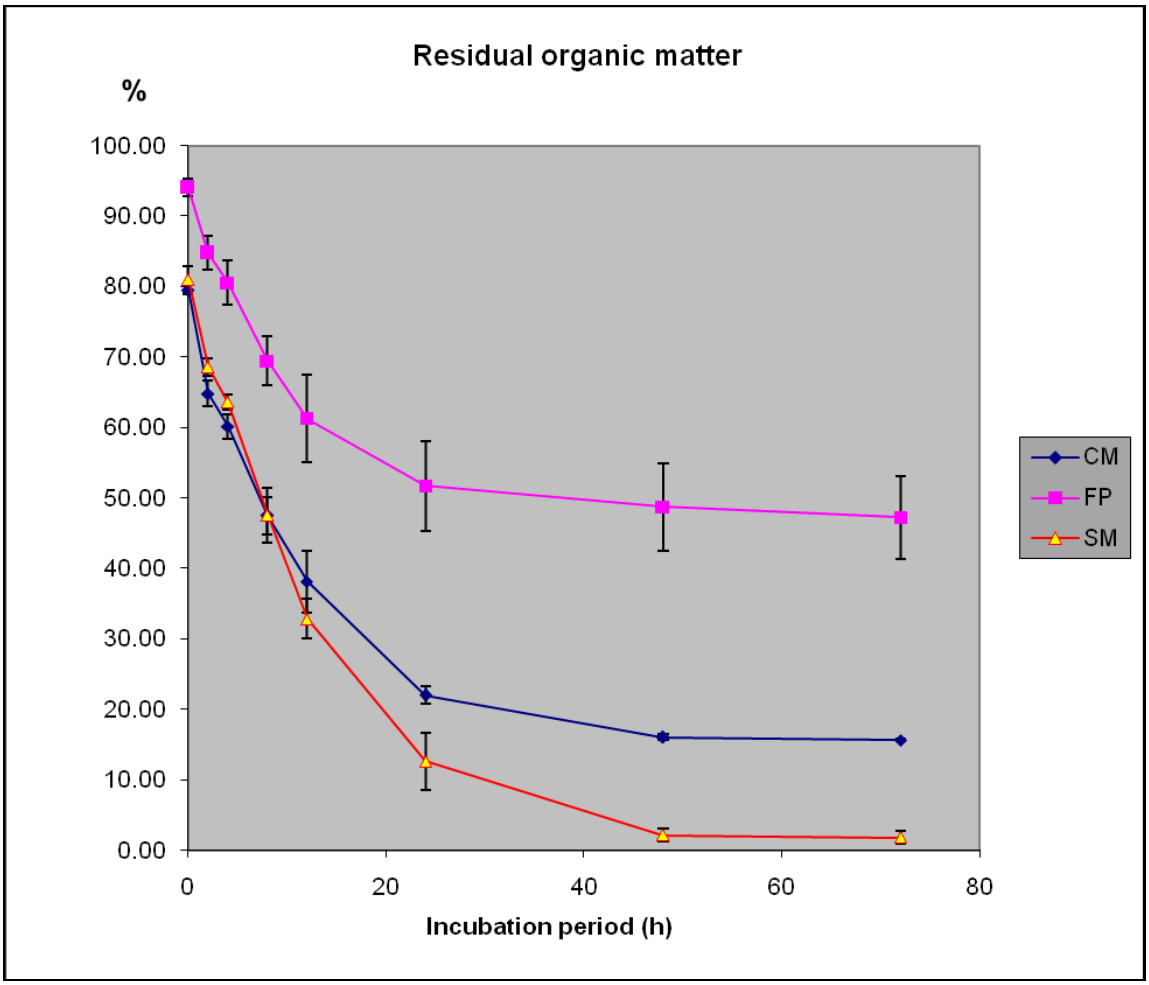

Figure 3.2. In situ ruminal disappearances of organic matter (OM) in fibre-protein in comparison with commercial canola meal and soy meal

Table 3.5. In situ rumen degradation kinetics of organic matter (OM) of fibre-protein in comparison with commercial canola meal and soy meal

Component	Feed Ingredient			SEM
	Fibre-protein	Canola meal	Soy meal	
S (% OM)	6.0[b]	20.5[a]	19.0[a]	0.71
D (% OM)	46.5[c]	64.2[b]	80.5[a]	2.22
U (% OM)	47.5[a]	15.3[b]	0.5[c]	2.58
Kd (% per hour)	9.8[a]	9.1[a]	7.5[b]	0.50
T0 (hour)	0.08[ab]	0.0[b]	0.26[a]	0.068
EDOM (% OM)	34.8[b]	59.1[a]	63.7[a]	1.99
EDOM (% DM)	33.3[b]	54.8[a]	56.1[a]	1.88
RUOM (% OM)	65.2[a]	40.9[b]	36.3[b]	1.99
RUOM (% DM)	62.4[a]	37.9[b]	32.0[b]	1.93

[a, b, c] Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM = Standard error of mean

Approximately 25% more ruminally un-degradable organic matter (RUOM) was present in fibre-protein on both OM and DM basis, comparing to canola meal. The difference in RUOM between canola meal and soy meal was not significant. Presence of lignin at a comparatively higher level in fibre-protein (24.1 %DM) can be attributed to the higher RUOM content found in fibre-protein (62.4 %DM).

3.3.2.3 In situ rumen kinetic parameters and degradability of CP

Figure 3.3 illustrates the amount of crude protein (as a % of initial CP content) remaining in the nylon bag at the end of each incubation period. In all three feed ingredients, most of the degradable crude protein was degraded before 24 hours of incubation. The soluble fraction (S) is represented by disappearance of CP at 0 h. Figure 3.3 shows that 0 h disappearance was highest in canola meal followed by fibre-protein and then by soy meal. This is evident from Table 3.6, where the lowest S fraction was observed in soy meal (0.7%) while canola meal has the highest (11.9%). The S fraction in fibre-protein (6.2%) was about 50% that of canola meal. As shown in the Table 3.6, the degradation rate of potentially degradable protein (Kd) was significantly highest in fibre-protein (15%/h) followed by canola meal (10.9 %/h) and soy meal (7.2%/h). These degradability rates of canola meal and soy meal closely agree with those reported in NRC (2001).

The Kd value represents the overall degradation rate of "B-fraction" of the crude protein (PB) and would depend on make-up of "B-fraction" since the different sub-fractions of PB (i.e. PB1, PB2, PB3) are having different rates of degradation. Hence, the differences in make-up of PB-fraction in the different ingredients could be attributed partly to differences in Kd values for protein observed in this study. There was no lag time (T0) for in situ degradation of protein in fibre-protein or canola meal while a short lag time (0.39 h) was observed in soy meal. The U (undegradable fraction) in fibre-protein (38.9%) was higher ($P<0.05$) than that of canola meal (7%) and soy meal (0%). Similar U values for soy meal were observed by Kirkpatrick and Kennelly (1987) and for canola meal by Kirkpatrick and Kennelly (1987), Khorasani et al. (1993) and Mustafa (1996).

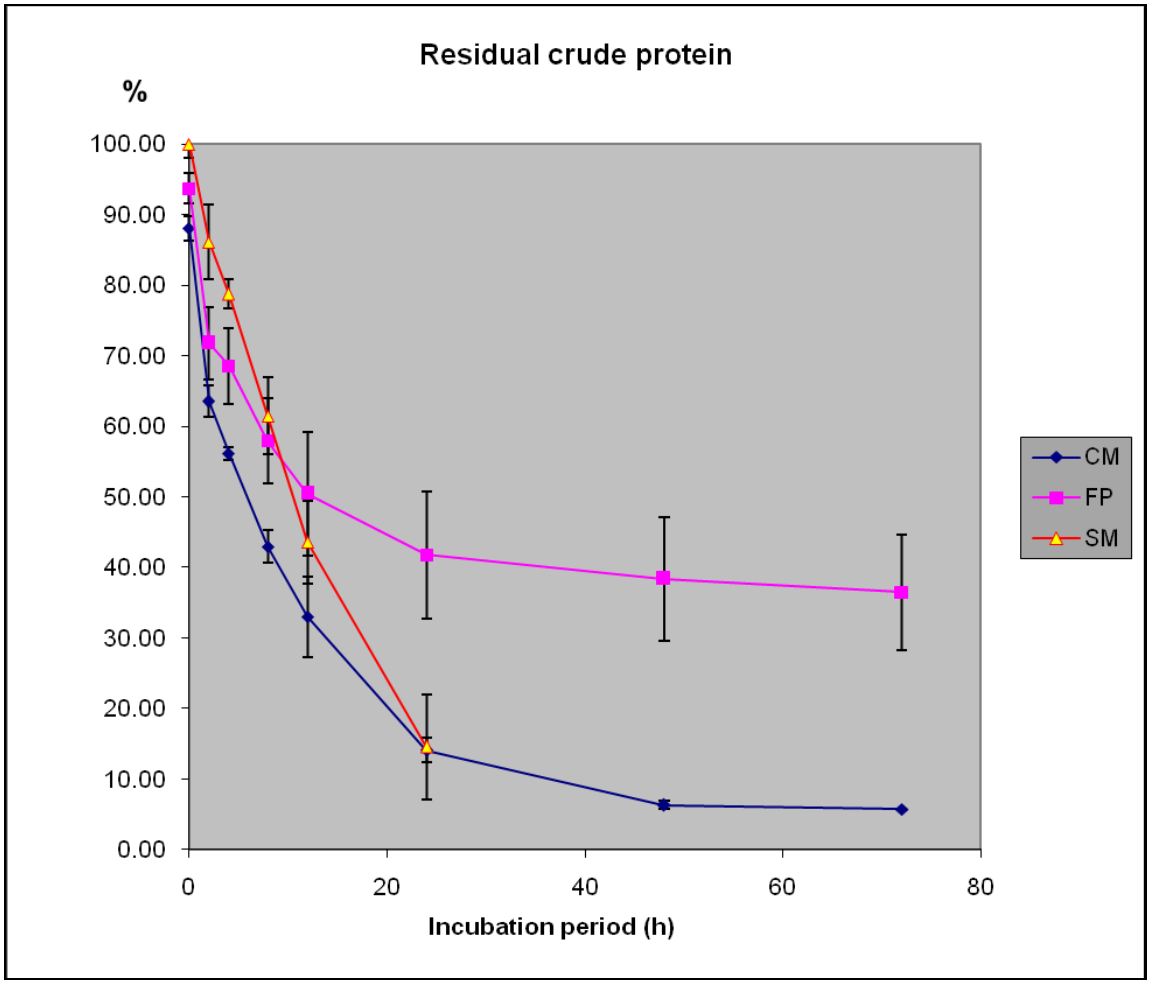

Figure 3.3. In situ ruminal disappearances of crude protein (CP) in fibre-protein in comparison with commercial canola meal and soy meal

Table 3.6. In situ rumen degradation kinetics of crude protein (CP) of fibre-protein in comparison with commercial canola meal and soy meal

Component	Feed Ingredient			SEM
	Fibre-protein	Canola meal	Soy meal	
S (% CP)	6.2[b]	11.9[a]	0.7[c]	1.03
D (% CP)	54. 8[c]	81.1[b]	99.3[a]	2.99
U (% CP)	38.9[a]	7.0[b]	0.0[b]	3.53
Kd (% per hour)	15.0[a]	10.9[b]	7.2[c]	0.71
T0 (hour)	0.0[b]	0.0[b]	0.39[a]	0.047
EDCP (% CP)	45.5[b]	64.2[a]	54.7[a]	3.04
EDCP (% DM)	14.1[b]	26.5[a]	25.6[a]	1.03
RUCP (% CP)	54.5[a]	35.8[b]	45.3[b]	3.04
RUCP (% DM)	16.8[b]	14.8[b]	21.2[a]	0.95

[a, b, c] Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM = Standard error of mean

It was reported in the past that ADIP content has a negative correlation with crude protein degradation (NRC 2001; McKinnon et al. 1995; Van Soest 1994). Similarly, the differences in U values observed between the tested ingredients in the current study were reflected by their indigestible ADIP contents (PC sub-fractions) where ADIP content in fibre-protein, canola meal and soy meal were respectively 24.9%, 8.2% and 1.4% as shown in Table 3.2. In canola hulls, ADIP component in the crude protein fraction was reported to be as high as 35% (McKinnon et al. 1995; NRC 2001). As the fibre-protein is rich in hull, its comparatively higher undegradable protein fraction is possibly due to its higher ADIP content contributed by hulls.

At a passage rate of 6%, the lowest ($P<0.05$) EDCP was observed in fibre-protein amounting to 45.5% of total crude protein compared to canola meal (64.2%) and soy meal (54.7%). Even though the EDCP % in soy meal was lower than canola meal, the difference was not significant ($P>0.05$). A similar observation was reported by Kirkpatrick and Kennelly (1987) where EDCP content of canola meal and soy meal were 63.2% and 60.2% respectively, at 5%/h Kp. The EDCP for canola meal in the current study is comparable with those reported by Mustafa (1996) for canola meal obtained from different sources (from 66.7% to 68.2% at 5% /h Kp). However, Khorasani et al. (1993; 1989) reported more than 70% EDCP in canola meal. The observed EDCP value for fibre-protein is closer to the EDCP value reported by McKinnon et al. (1995) for canola hulls (49.2 % at 5% passage rate) indicating a closer resemblance in crude protein between canola hulls and fibre-protein. On DM basis, ruminally degradable crude protein content in fibre-protein (14.1 % DM) was significantly far lower than that of canola meal (26.5 %DM) where as difference between canola meal and soy meal (25.6%) was not significant. Out of total CP, 54.5% of CP in fibre-protein was ruminally un-degradable, which was significantly higher than that of canola meal (35.8%) and soy meal (45.3%). However, on a dry matter basis, RUCP content in soy meal (21.2 %DM) was significantly higher than ($P<0.05$) that in both fibre-protein (16.8 %DM) and canola meal (14.8 %DM), where the difference between canola meal and fibre-protein was not significant ($P>0.05$). RUCP is the rumen bypass protein that is available for post-ruminal digestion. Even though bypass protein

content in soy meal was higher, major portion of that would be available to the animal post ruminally as CP in soy meal almost totally digestible. On the other hand, with a higher undegrable CP fraction (38.9 %CP), ruminal bypass protein in fibre-protein would be mostly unavailable to the host animal. Canola meal with only 7% undegradable CP fraction, would have a comparatively higher amount of available protein post ruminally despite its RUCP content being closer to that of fibre-protein. This was further substantiated by the content of undegradable protein C fraction (PC) in each feed ingredients as seen in the Table 3.3. The results also have shown a remarkable closeness between chemically derived PC fraction and in situ U fraction for both canola meal (8.2% vs. 7%) and soy meal (1.4% vs. 0%) even though the PC fraction in fibre protein (24.9%) was substantially lower than the U fraction (38.9%).

3.3.2.4 In situ rumen kinetic parameters and degradability of NDF

Figure 3.4 illustrates the residual NDF remaining at each incubation period. The curves of residual NDF for canola meal and fibre-protein seems to be closer for NDF unlike with curves for OM and CP where canola meal was observed to be closer to soy meal rather than to fibre-protein. This is to be expected since NDF portion in both canola meal and fibre-protein was mainly represented by canola hulls.

The rumen degradation kinetics of NDF for the different feed ingredients are given in the Table 3.7. The amount of soluble NDF fractions were very little (less than 1%) in all three ingredients and the difference was not significant ($P>0.05$). However, a significant difference ($P>0.05$) was observed among ingredients in potentially degradable fraction where soy meal (97.7%) was two times that of canola meal (48.3%) and three times that of fibre-protein (32.2%). Only a small fraction of undegradable NDF was observed in soy meal (1.9%) where as 51.1% NDF in canola meal was found to be undegradable.

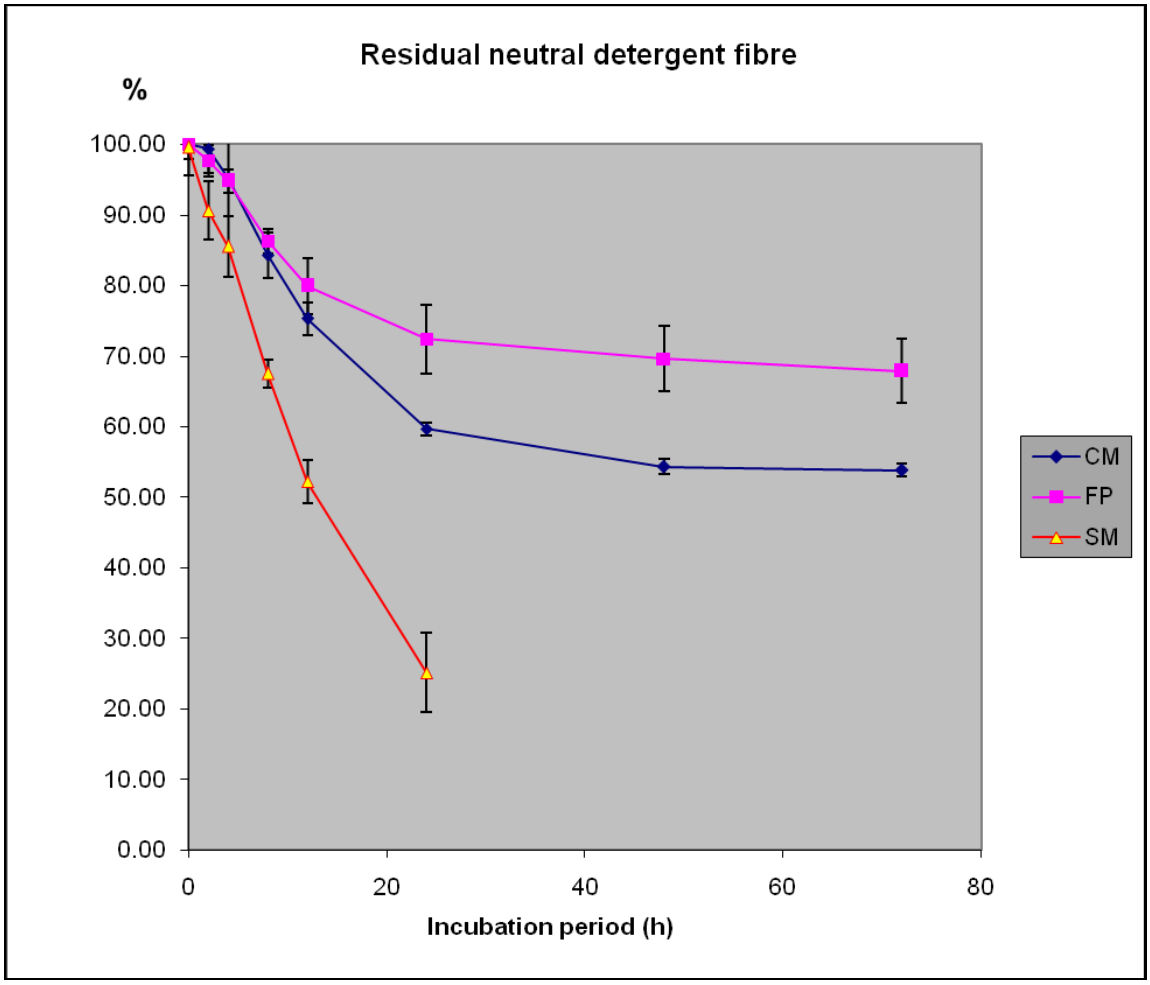

Figure 3.4. In situ ruminal disappearances of neutral detergent fibre (NDF) in fibre-protein in comparison with commercial canola meal and soy meal

Table 3.7. In situ rumen degradation kinetics of neutral detergent fibre (NDF) of fibre-protein in comparison with commercial canola meal and soy meal

Component	Feed Ingredient			SEM
	Fibre-protein	Canola meal	Soy meal	
S (% NDF)	0.5	0.7	0.5	0.58
D (% NDF)	32.2[c]	48.3[b]	97.7[a]	1.78
U (% NDF)	67.3[a]	51.0[b]	1.9[c]	1.94
Kd (% per hour)	9.0[a]	7.7[ab]	5.9[b]	0.73
T0 (hour)	1.12	1.52	0.86	0.290
EDNDF (% NDF)	19.7[c]	27.8[b]	49.0[a]	1.49
EDNDF (% DM)	10.9[a]	6.5[b]	4.5[c]	0.47
RUNDF (% NDF)	80.3[a]	72.2[b]	51.0[c]	1.49
RUNDF (% DM)	44.7[a]	16.9[b]	4.6[c]	1.71

[a, b, c] Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM = Standard error of mean

In fibre-protein, 67.3% of NDF (67.3%) was found to be undegradable. The degradability of NDF depends on proportion of its main components i.e. cellulose, hemicellulose and lignin. Lignin is known to be highly indigestible as well as render cellulose and hemicellulose that are associated with it, indigestible (Van Soest 1994). The lignin content in fibre-protein, canola meal and soy meal was 24.1, 9 and 0.6% respectively (Table 3.1). In CNCPS, undegradable fibre associated with lignin is calculated as 2.4 X Lignin%. Accordingly, the difference in U fractions in NDF observed among three ingredients under in situ conditions can be explained partly by lignin content. The Kd of NDF in fibre-protein (9%/h) was significantly higher than in soy meal (5.9%/h). Even though Kd of NDF in canola meal (7.7%/h) was lower than in fibre-protein and higher than in soy meal, the differences were not significant ($P>0.05$). A lag time of about 1 hour or more to commence NDF degradation was observed in all three ingredients; i.e. soy meal 0.86 h, fibre-protein 1.12 h and canola meal 1.52 h where time differences among ingredients were not significant ($P>0.05$). At passage rate of 6% /h, soy meal had the highest ($p<0.05$) effective NDF degradability at 49 %NDF, followed by canola meal (27.8 %NDF) and fibre-protein (19.7 %NDF). On a DM basis, the highest ($P<0.05$) effective degradable NDF was found in fibre-protein (10.9 %DM) in comparison to canola meal (6.5 %DM) and soy meal (4.5 %DM). This was due to the higher content of NDF in the fibre-protein.

Limited research has been carried out on degradation of fibre components in concentrate feeds. Mustafa (1996) working on canola meal reported slightly lower degradation rate (5.1%/h) and a longer lag time (2.8 h) for NDF in regular canola meal. He also reported a higher EDNDF value (46%) for canola meal at 5%/h Kp, which was possibly due to difference in chemical composition since his canola meal sample was with a comparatively lower lignin and higher hemicellulose content. The effective degradable NDF content in fibre-protein closely matches values reported by McKinnon et al. (1995) for canola hulls. They reported untreated canola hulls contained potentially degradable NDF content of 30.1% and EDNDF value of 19.4%. This indicates hulls as the major source of NDF in fibre-protein.

3.3.2.5 In situ rumen kinetic parameters and degradability of ADF

The in situ disappearances of ADF in test ingredients showed a pattern similar to NDF disappearance (Figure 3.5). The S fractions in all three ingredients were insignificant (Table 3.8). The ADF in soy meal was completely degradable which can be expected since its lignin content was very low (0.6 %DM). On the other hand, potentially degradable ADF fractions were significantly lower ($P<0.05$) in both canola meal (41.5%) and fibre-protein (23.6%) which are comparatively high in lignin content due to the presence of hulls. The D fraction in the hulls enriched fibre-protein was significantly lower than that of canola meal indicating the existence of comparatively higher percentage of hulls. The Kd values of both canola meal and fibre-protein were similar and significantly different from Kd value of soy meal. The lag time of ADF in all three ingredients were longer than their respective lag time of NDF indicating possible differences between hemicellulose and cellulose degradability in the rumen. The effectively degradable ADF in fibre-protein was only 14.7% compared to canola meal (25.1%) and soy meal (46.9%). The EDADF content in canola meal observed in this study is comparable with those reported by Mustafa (1996).

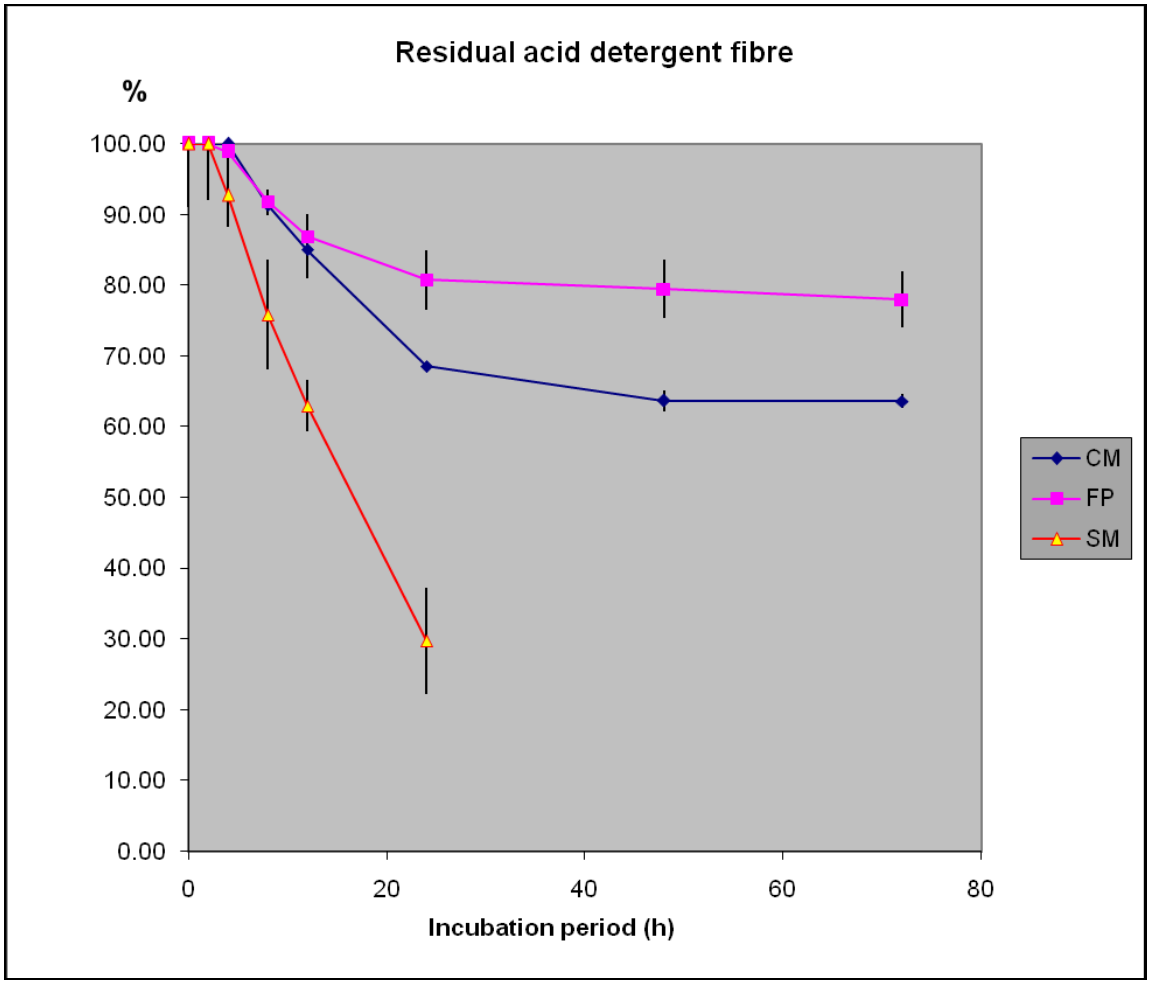

Figure 3.5. In situ ruminal disappearances of acid detergent fibre (ADF) in fibre-protein in comparison with commercial canola meal and soy meal

Table 3.8. In situ rumen degradation kinetics of acid detergent fibre (ADF) of fibre-protein in comparison with commercial canola meal and soy meal

Component	Feed Ingredient			SEM
	Fibre-protein	Canola meal	Soy meal	
S (% ADF)	0.04	0.48	0.33	0.216
D (% ADF)	23.6[c]	41.5[b]	99.7[a]	1.87
U (% ADF)	76.3[a]	58.1[b]	0.0[c]	1.76
Kd (% per hour)	9.9[a]	9.1[a]	5.3[b]	0.80
T0 (hour)	1.8	2.9	2.1	0.65
EDADF (% ADF)	14.7[c]	25.1[b]	46.9[a]	1.37
EDADF (% DM)	6.7[a]	4.8[b]	2.9[c]	0.31
RUADF (% ADF)	85.3[a]	74.9[b]	53.1[c]	1.37
RUADF (% DM)	39.6[a]	14.2[b]	3.3[c]	1.49

[a, b, c] Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM = Standard error of mean

3.3.3 Digestible nutrients and energy content

As shown in the Table 3.9, there was no significant difference (P>0.05) in gross energy between fibre-protein (4.36 Mcal/kg), canola meal (4.31 Mcal/kg) and soy meal (4.15 Mcal/kg). Can-sugar had lower gross energy content (3.06 Mcal/kg) than other ingredients, which could be attributed to its comparatively higher ash content (19.3%) and lower content of protein and EE. The lowest (P<0.05) TDN content was observed in fibre-protein (46.5%DM). The TDN value of soy meal (79.8%DM) was significantly higher than canola meal (71.9%DM) due to its comparatively higher digestible crude protein (48.1%DM vs. 38.3%DM) and non-fibre carbohydrate (35.9%DM vs. 28.7%DM) fractions. The TDN content in can-sugar (72.1%DM) was closer to that of canola meal. This was owing to the presence of high percentage of non-fibre carbohydrate in can-sugar (63.5%DM). The DE, ME and NE values estimated for canola meal and soy meal in this study were similar to those reported in nutrition composition tables of NRC 2001. Comparison of energy values among the feed ingredients showed the same pattern for all the energy values (DE, ME, NE) where fibre-protein had the lowest (P<0.05) energy values at all the intake levels while soy meal had the highest (P<0.05) values. The energy values of can-sugar were closer to those of canola meal.

3.3.4 Microbial protein synthesis and protein supply to the small intestine

3.3.4.1 DVE/OEB model

Predicted values of potential protein supply to dairy cattle from fibre-protein, canola and soy meal as per DVE/OEB model are given in Table 3.10. The results show that fibre protein was significantly lower (P<0.05) in fermented organic matter content (321.4 g/kg DM) than that of both canola meal (576.8 g/kg DM) and soy meal (637.9 g/kg DM). Interference of lignin, which was present at a higher level in fibre-protein, could be attributed to this low microbial degradability in fibre-protein. The difference in FOM between canola meal and soy meal was not significant (P>0.05).

Table 3.9. Truly digestible nutrients (td), total digestible nutrients (TDN), gross energy (GE) and predicted energy values at maintenance (1X) and production intake levels (3X) of fibre-protein and can-sugar compared with commercial canola meal and soy meal.

	Feed Ingredients				
	Fibre-protein	Canola meal	Soy meal	SEM	Can-sugar
Truly digestible nutrients (NRC 2001)					
tdNDF %DM	4.6[a]	2.3[b]	2.2[b]	0.79	0.03
tdNFC %DM	19.8[c]	28.7[b]	35.9[a]	1.34	63.5
tdCP %DM	27.8[c]	38.3[b]	48.1[a]	1.11	15.5
tdFA %DM	0.5[b]	4.3[a]	0.3[b]	0.33	0.0
Total digestible nutrients (NRC 2001)					
TDN_{1X} %DM	46.5[c]	71.9[b]	79.8[a]	2.25	72.1
Gross energy (Bomb calorie-meter)					
GE (Mcal/kg)	4.36	4.31	4.15	0.079	3.06
Predicted digestible energy value at maintenance level intake (1X)-NRC2001					
DE_{1X} (Mcal/kg DM)	2.33[c]	3.55[b]	4.02[a]	0.11	3.24
Predicted energy value at production intake level 3X for dairy cattle (NRC 2001)					
DE_{3X} (Mcal/kg DM)	2.16[c]	3.29[b]	3.72[a]	0.102	3.00
ME_{3X} (Mcal/kg DM)	1.73[c]	2.88[b]	3.31[a]	0.103	2.58
NE_{3X} (Mcal/kg DM)	1.03[c]	1.85[b]	2.14[a]	0.074	1.62
Predicted energy value for beef cattle (NRC 1996)					
ME (Mcal/kg DM)	1.91[c]	2.91[b]	3.30[a]	0.09	2.66
NE_m (Mcal/kg DM)	1.07[c]	1.96[b]	2.27[a]	0.077	1.74
NE_g (Mcal/kg DM)	0.52[c]	1.31[b]	1.58[a]	0.068	1.12

[a, b, c,] Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM = Standard error of mean

Table 3.10 Predicted values of potential protein supply to small intestine in dairy cattle from fibre-protein in comparison with commercial canola meal and soy meal using the Dutch model and NRC 2001 dairy model.

	Feed Ingredient			SEM
	Fibre-protein	Canola meal	Soy meal	
The DVE/OEB Model				
1. Absorbable microbial protein synthesis in the rumen (AMCP)				
FOM (g/kg DM)	321.4[b]	576.8[a]	637.9[a]	32.40
MCP_{FOM} (g/kg DM)	48.2[b]	86.5[a]	95.7[a]	4.86
AMCP (g/kg DM)	30.7[b]	55.16[a]	61.0[a]	3.10
2. Endogenous protein in the small intestine (ENDP)				
ENDP (g/kg DM)	35.0[a]	12.8[b]	4.3[c]	1.76
3. Truly absorbable rumen un-degraded protein in small intestine (ARUP)				
RUP (g/kg DM)	186.9[b]	163.8[b]	234.8[a]	10.51
ARUP (g/kg DM)	62.0[c]	137.6[b]	234.8[a]	4.95
Total truly digested protein in small intestine (DVE value)				
DVE(g/kg DM) = AMCP + ARUP – ENDP	57.8[c]	180.0[b]	291.4[a]	8.11
Degraded protein balance (OEB value)				
OEB (g/kg DM)	73.8[c]	162.1[b]	136.5[a]	6.87
The NRC 2001 Model				
1. Absorbable microbial protein synthesis in the rumen (AMCP)				
MCP (g/kg DM)	55.9[b]	91.8[a]	93.1[a]	1.24
AMCP (g/kg DM)	35.8[b]	58.7[a]	59.6[a]	0.80
2. Absorbable endogenous true protein in the small intestine (AECP)				
ECP (g/kg DM)	10.9	10.9	11.0	-
AECP (g/kg DM)	4.4	4.4	4.4	-
3. Absorbable rumen un-degraded true protein in the small intestine (ARUP)				
RUP (g/kg DM)	168.4[b]	147.6[b]	211.5[a]	9.47
ARUP (g/kg DM)	55.9[c]	124.0[b]	211.5[a]	4.46
Total metabolizable protein (MP)				
MP (g/kg DM) = AMCP + AECP + ARUP	96.1[c]	187.1[b]	275.5[a]	4.77
Degraded protein balance (DPB)				
DPB (g/kg DM)	74.5[b]	156.5[a]	145.6[a]	8.98

[a, b, c, d,] Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test. SEM = Standard error of mean

The lower level of FOM in turn has resulted in a significantly lower ($P<0.05$) predicted values of MCP_{FOM} and AMCP in fibre protein (48.2 g/kg DM and 30.7 g/kg DM, respectively) compared to canola meal (86.5 g/kg DM and 55.16 g/kg DM, respectively) and soy meal (95.7 g/kg DM and 61.0 g/kg DM, respectively). The MCP_{FOM} synthesis and its availability were similar between the canola meal and soy meal.

The RUP content in soy meal (234.8 g/kg DM) was significantly higher ($P<0.05$) than that of both canola meal (163.8 g/kg) and fibre-protein (186.9 g/kg). This was due to comparatively lower Kd value and higher D fraction of CP in soy meal. Even though RUP content in fibre-protein was slightly higher than canola meal, the difference was not significant ($P>0.05$). Despite that, ARUP content in fibre protein (62 g/kg DM) was significantly lower than that of canola meal (137.6 g/kg DM) as well as soy meal (234.8 g/kg). In soy meal, the ARUP content was equal to RUP content whereas in fibre-protein it was approximately 1/3 of its RUP content. The ARUP content depends on RUP digestibility (dRUP) which was estimated based on the CP residue remained after long time ruminal incubation of 72 h. As discussed earlier (section 3.3.2.3), CP degradation is negatively affected by ADIP content. It was also noted before that fibre-protein was having the highest ADIP content followed by canola meal while ADIP content in soy meal was less than 1% (Table 3.2). As such, a lower ARUP content in fibre-protein as well as a totally degradable RUP content in soy meal can be expected.

The loss of endogenous protein, that occurs during digestive process due to extent of undigested dry matter in a feed, was highest ($P<0.05$) in fibre-protein (35.0 g/kg DM) followed by canola meal (12.8 g/kg DM) which in turn was higher than that of soy meal (4.3 g/kg).

As a result of lower AMCP and ARUP along with higher ENDP, the DVE value of fibre-protein (57.8 g/kg DM) was predicted to be the lowest ($P<0.05$). The DVE value of canola meal (180.0 g/kg DM) was lower ($P<0.05$) than that of soy meal (291.4 g/kg DM). The results show that all the feed ingredients exhibited positive OEB values that shows availability of feed protein exceeds the availability of energy (extracted during

rumen fermentation) for microbial protein synthesis indicating a potential N loss in the rumen (Tamminga et al. 1994).

3.3.4.2 NRC 2001 model

As shown in the Table 3.10, predicted synthesis MCP and AMCP contents from fibre-protein (55.9 and 35.8 g/kg DM, respectively) were significantly lower ($P<0.05$) than that of canola meal (91.8 and 58.7 g/kg DM, respectively) and soy meal (93.1 and 59.6 g/kg DM, respectively). Similar to DVE/OEB model, there were no significant differences ($P>0.05$) in MCP and AMCP values between canola meal and soy meal.

The highest RUP content was observed in soy meal (211.5 g/kg DM). Although there was no significant difference ($P>0.05$) in RUP content between fibre-protein (168.4 g/kg DM) and canola meal (147.6 g/kg DM), the ARUP content in fibre-protein (55.9 g/kg DM) was significantly lower than that of canola meal (124.0). As discussed before, ARUP depends on digestibility of RUP and as result significantly the highest ARUP was observed in soy meal (211.5 g/kg DM) and lowest in fibre-protein. As the ECP was calculated in NRC 2001 based on feed dry matter, there was no difference in ECP or AECP between test feeds.

Owing to the differences in both AMCP and ARUP values, the total metabolizable protein (MP) contents in three feed ingredients were significantly different from each other. The MP content in fibre-protein (96.1 g/kg DM) found to be the lowest and amounted to approximately 50% MP in canola meal (187.1 g/kg DM) while the MP content in soy meal was observed to be 275.5 g/kg DM. The predicted protein balances (DPB) of all the three feed ingredients were found to be positive indicating a N loss in the rumen.

3.3.4.3 The DVE/OEB model vs. NRC 2001 model

A comparison between the two models was done previously by Yu et al. (2003A) where reasons behind differences in predicted values between models were discussed. In that study AMCP and ARUP values derived from DVE/OEB model, were consistently higher than those derived from NRC 2001 for all the feed samples (alfalfa and timothy

forages). However in the present study, AMCP and ARUP values predicted for fibre-protein and canola meal using DVE/OEB model were lower than those of the NRC model while opposite was true for soy meal. Yu et al. (2003A) observed that the amounts of total absorbable protein supply to small intestine predicted using DVE/OEB model (DVE values), were 15% lower than predictions from NRC 2001 model (MP values). In the current study also, the DVE values were found to be lower than MP values. However the differences between DVE and MP values were considerable in fibre-protein (57.8 vs. 96.1 g/kg DM) comparing to canola meal (180 vs. 187.1 g/kg DM) and soy meal (291.4 vs. 275.5 g/kg DM). In DVE/OEB model ENDP is considered as a loss. Thus, comparatively higher ENDP value estimated from DVE/OEB model for fibre-protein is the main reason for this larger difference observed between DVE and MP for fibre-protein. While three feed samples are not adequate enough to do a comparison of two models the inconsistent differences indicates major differences in some of the assumptions and concepts used in the two models.

3.4 Conclusions

The fibre-protein, which is the residual portion after aqueous extraction of the soluble fraction from canola meal, retained a sizeable amount of crude protein and can be classified as a source of protein. It is also a significant source of fibre. However, as demonstrated by both chemical and in situ analysis, most of the fibre was non-degradable. Unlike protein sources such as canola meal and soy meal, fibre-protein is higher in non-degradable protein leading to a lower metabolizable protein content.

Can-sugar, which is the remaining fraction after separation of protein from aqueous extract contained almost totally water soluble components i.e. carbohydrates, crude protein and minerals. Its protein as well as carbohydrate fractions were water soluble and therefore would be utilized rapidly by rumen microorganisms. Can-sugar was not analyzed for individual minerals in the current study. However, considering its comparatively high ash content, can-sugar may also supplement part of the mineral needs of animal.

It is concluded that fibre-protein can be used as a secondary source of protein while can-sugar can be used as a readily available energy source. As such a mixture of these two ingredients, both of which are by-products from canola meal protein extraction process, would complement each other and may be used as a cost effective ingredient in ruminant rations.

4. EFFECT OF CANOLA FIBRE-PROTEIN AND SUGAR FRACTIONS USED AS ADDITIVES IN DEHYDRATED ALFALFA PELLETS ON PALATABILITY AND LACTATION PERFORMANCE OF DAIRY COWS

4.1 Introduction

Canola is the second most economically important crop grown in Canada. About 98% of canola meal is exported by Canada to USA and due to high level of fibre (12% crude fibre) and phytic acid (3.1%), canola meal has limited use in aquaculture, swine and poultry feeding, thereby fetching a lower price compared to soy meal. The production of canola is expected to increase substantially within the next 10 years, particularly to meet the envisaged bio-diesel demand of 600 million litres per annum by year 2012. In order to maximise return from canola, it is necessary to add value to canola meal. Recently there were attempts to separate high quality protein from canola meal. Canola "fibre-protein" and "can-sugar" are the two by-products arising from one such method of canola meal fractionation. In the study of chemical and in situ rumen degradation characteristics and subsequent nutritive evaluation, it was observed that fibre-protein and can-sugar fractions can be used, respectively, as secondary protein source and readily available energy source (Chapter 3).

Palatability is a major issue when it comes to feeding an unusual ingredient or a man-made ingredient. Palatability of a feedstuff is influenced by its oropharyngeal stimulants such as taste, odour and texture (Kaitho et al. 1997). Fibre-protein is enriched with canola hulls and other fibrous material found in canola meal and does not possess a detectable odour. Can-sugar is available as a highly hygroscopic powder consisting of water soluble fractions (i.e. sugars, nitrogenous compounds and minerals). In view of the possible low palatability of fibre-protein if fed alone due to its physical characteristics (Figure 4.1), it was decided to incorporate a combination of fibre-protein and can-sugar into alfalfa dehydrate pellets particularly since the combined chemical composition of fibre-protein and can-sugar are closer to that of dehydrated alfalfa.

Figure 4.1 Fibre-protein and can-sugar

In order to evaluate the potential of utilizing fibre-protein and can-sugar mixture as an additive to alfalfa pellet used in dairy cattle rations, two studies were conducted. The objective of study 1 was to determine effect of fibre-protein and can-sugar fractions used as additives in dehydrated alfalfa pellets on palatability of dairy cows. The objective of study 2 was to find the effect of fibre-protein and can-sugar blended alfalfa pellet on lactation performance, dry matter intake and apparent dry matter digestibility of dairy cows, compared with standard alfalfa pellet.

4.2 Materials and methods

4.2.1 Test feeds and feeding

The blended pellets were prepared at LED Pelleting in Zenon Park, Saskatchewan, Canada by mixing 85% standard dehydrated alfalfa with 15% of fibre-protein + can-sugar mixture. This mixing ratio was chosen as it was the optimum level at which the original color of alfalfa pellet could be maintained. The fibre-protein/can-sugar mixture contained 1/3 of can-sugar and 2/3 of fibre-protein. For comparison, standard alfalfa dehydrated pellets were prepared using the same source of alfalfa. Animals were fed ad-libitum, twice a day at 0800 and 1600 h throughout the experimental period of both studies. All the animals were housed in tie stalls and had access to free choice water. The animals were cared for according to the guidelines of the Canadian Council on Animal Care (1993).

4.2.2 Palatability study

Palatability difference between two test feeds was evaluated by "Two choice alternating access method" (Paterson, 1996) using six multiparous Holstein cows (body weight 737 ± 46 kg; days in milk 127 ± 36; milk yield 42 ± 5 kg) . During an adaptation period of 8 days, the two test feeds were given as top dressing to TMR on alternative days, starting from 0.5 kg on the first two days and gradually increasing to 2 kg by 7th/8th day. Following the preliminary period, palatability was measured for 7 days. Two test feeds were offered to animals one at a time in blue color tubs and exchanged the tubs at 5 minutes intervals, which continued for a maximum period of 30 minutes in the morning

(0800 h) and afternoon (1600 h) just before feeding the basal diet. Basal diet along with test feeds was balanced to meet the nutrient requirements as per NRC 2001 recommendations for lactating dairy cows (Table 4.1).

At a time, 0.5 kg of each test feed was placed in front of each animal in tubs thereby altogether 2 kg of both test feed taken together was offered per day per animal. The type of feed that was offered first was also alternated between morning and afternoon as well as between consecutive days to eliminate possible bias in a pattern of offering. At the end of 30 minutes, the remaining test feed in the tubs was measured. Eating time was recorded for each animal if an animal stop eating or finished the feed in a tub. The morning and afternoon intakes were summed up to find the daily intake of test feed by each cow and preference percentage was calculated as;

$$\text{Preference \%} = \frac{\text{Intake Pellet A}}{\text{Intake Pellet A} + \text{Intake Pellet B}} \times 100$$

4.2.3 Lactation performances study of dairy cows

Six multiparous Holstein cows (body weight 760 ± 55 kg; days in milk 155 ± 36) were used in this trial. The experimental design was a switchback/crossover that included two animal groups and three experimental periods. Animals were randomly assigned into the two groups. Each experimental period was 21 days long and consisted of 6 days adaptation period followed by 15 days measurement period.

Feed was offered twice a day at 0800 and 1600 h. Test feed pellets were mixed manually to the basal diet at the rate of 1 kg (dehydrated-pellet) per 21 kg (basal diet) (as fed basis). The ingredient and nutrient composition of the total rations (TMR), balanced to meet the nutrient requirements of lactating dairy cows as per NRC 2001 recommendations, are given in the Table 4.2.

Table 4.1 Ingredient and nutrient composition of base diet used in palatability study of lactating cows

Ingredient	g/kg DM
Alfalfa hay	159.4
Barley silage	320.1
Wheat distillers dried grain	20.7
Barley grain	287.2
Wheat grain	18.6
Oat grain	26.0
Canola meal	54.4
Soybean meal	59.6
Corn gluten meal	11.9
Canola oil	3.3
Molasses	11.1
Limestone	0.4
Dynamate™[1]	1.2
Sodium bicarbonate	3.1
Mineral-Vitamin Premix[2]	15.6
Cobalt-Iodized salt	3.1
Golden Flakes[3]	3.7
Niacin-Magnesium Mix[4]	0.4
Nutrient composition, % DM	
DM	54.4
CP	17.9
Ether Extract	3.0
NDF	35.5
ADF	21.9

[1] Pitman Moore, Inc., Mundelein, IL (potassium: 180 g/kg, sulphur: 220 g/kg, 110 g/kg magnesium, iron: 1,000 mg/kg). [2] Formulated to provide 45 mg manganese, 63 mg zinc, 17 mg copper, 0.5 mg selenium, 11,000 I.U. vitamin A, 1,800 I.U. vitamin D_3 and 30 I.U. vitamin E per kg of dairy concentrate. The mix also contributes 0.14% magnesium, 0.48% calcium, 0.26% phosphorus, 0.23% sodium and 0.38% chloride to the total dairy concentrate. Prepared by Federated Cooperatives Ltd., Saskatoon, Saskatchewan, Canada. [3] Dried fat supplement (Malaysian palm oil) distributed by Prairie Micro-Tech Inc., Regina, Saskatchewan, Canada. [4] Formulated to provide one gram of niacin and 0.3 grams of magnesium per kg of fresh cow concentrate.

Table 4.2 Ingredient and nutrient composition of total mixed rations used in the lactation performance study of dairy cows

	Standard Dehy. Ration	Blended Dehy. Ration
Ingredient composition, g/kg DM		
Alfalfa hay	148.1	148.0
Barley silage	297.4	297.3
Standard alfalfa dehydrated pellet	*71.0*	
Blended alfalfa dehydrated pellet		*71.1*
Wheat distillers' dried grain	19.2	19.2
Barley grain	266.8	266.8
Wheat grain	17.3	17.3
Oat grain	24.2	24.2
Canola meal	50.6	50.6
Soybean meal	55.3	55.3
Corn gluten meal	11.1	11.1
Canola oil	3.1	3.1
Molasses	10.3	10.3
Limestone	0.4	0.4
Dynamate™[1]	1.1	1.1
Sodium bicarbonate	2.9	2.9
Mineral-Vitamin Premix[2]	14.5	14.5
Cobalt-Iodized salt	2.9	2.9
Golden Flakes[3]	3.4	3.4
Niacin-Magnesium Mix[4]	0.4	0.4
Nutrient composition, % DM		
DM	56.1	56.1
CP	17.7	17.9
Ether Extract	3.0	3.1
aNDF	35.7	35.6
ADF	22.2	22.2
NE_L, Mcal/kg[5]	1.59	1.59

[1]Pitman Moore, Inc., Mundelein, IL (potassium: 180 g/kg, sulphur: 220 g/kg, 110 g/kg magnesium, iron: 1,000 mg/kg). [2]Formulated to provide 45 mg manganese, 63 mg zinc, 17 mg copper, 0.5 mg selenium, 11 000 I.U. vitamin A, 1800 I.U. vitamin D_3 and 30 I.U. vitamin E per kg of dairy concentrate. The mix also contributes 0.14% magnesium, 0.48% calcium, 0.26% phosphorus, 0.23% sodium and 0.38% chloride to the total dairy concentrate. Prepared by Federated Cooperatives Ltd., Saskatoon. [3]Dried fat supplement (Malaysian Palm Oil) distributed by Prairie Micro-Tech Inc., Regina, Saskatchewan, Canada. [4]Formulated to provide one gram of niacin and 0.3 grams of magnesium per kg of fresh cow concentrate. [5] Calculated based on equations from NRC (2001) at 3x production level.

The daily intake of each animal was recorded during the 15 days measurement period and closely monitored to prevent both under feeding as well as selective eating. Feed samples were collected on every other day to obtain cumulative samples of basal diet and two test feeds during the last 10 days of each experimental period. Feed samples were dried in a forced air oven set at 55^{o}C for 48 hours to obtain the DM content and calculate dry matter intake (DMI).

Grabbed fecal samples were drawn from each animal at 1930 h during the last three days of each experimental period and dried in a forced air oven set at 55^{o}C for 72 hours. Equal amounts of dried fecal samples were pooled together to obtain 3-days-cumulative samples for each animal during each period. Milking was done twice a day at 0600 and 1600 h. Individual milk yields were recorded during the last 10 days of each experimental period. Two milk samples were drawn at the end of milking from each animal for 3 consecutive days on the last Monday, Tuesday and Wednesday of each test period. One set of milk samples was frozen (at -20^{o}C) immediately after milking and the other set was refrigerated after adding a preservative tablet (Brotab "10" containing 7.83 mg 2-Bromo-2-Nitropropane-1,3 Diol and 0.35 mg Pimaricin, D&F Control Systems Inc., Dublin, CA, USA). Morning and afternoon milk samples on each day were pooled together, in quantities proportionate to morning and afternoon milk yields of each animal. The milk samples with the preservative were tested for milk fat, milk protein and lactose while frozen samples were analysed for milk urea (MU). Milk sample analysis was done at Saskatchewan Agriculture Provincial Dairy Laboratory, 4840 Wascana Parkway, Regina, Saskatchewan, Canada.

4.2.4 Chemical analysis

Feed and fecal samples were ground through a 1mm screen using laboratory scale hammer mill (Retsch ZM-1, Brinkmann Instruments (Canada) Ltd., Ontario) prior to chemical analysis. Feed samples were analysed for dry mater (DM), ether extract (EE), crude protein (CP), ash, acid detergent fibre (ADF), acid detergent lignin (ADL), neutral detergent fibre (NDF), acid detergent insoluble crude protein (ADIP), neutral detergent insoluble crude protein (NDIP), non-protein nitrogen (NPN) soluble crude protein (SCP)

and gross energy (GE) as described in the Chapter 3, section 3.2.1.2. Both feed and fecal samples were analysed for acid insoluble ash (AIA) using 2% HCl acid as per procedure published by Van Keulen and Young (1977) and concentration values of AIA in feed and feces were used to calculate the apparent DM digestibility.

4.2.5 Statistical analysis

The test feed intake and finish time data from palatability study was analysed using paired T-test procedure of SAS software (SAS Institute Inc., 1999). SAS procedure for T-test was used to analyze preference % data with H_0: μ=50%.

Mixed Procedure of SAS was used to analyse all the data from the lactation performance study. The period effect was assumed as fixed effect and means were separated using LSD procedure and significance was declared at $P<0.05$.

4.3 Results and discussion

The two types of pellets were remarkably similar looking and only a close examination shows the blended pellet having slight dark brown stains probably due to dark color of fibre-protein (Figure 4.2).

Table 4.3 shows the chemical composition of fibre-protein, can-sugar, and two types of alfalfa pellets being tested. It should be noted that fibre-protein and can-sugar data from the previous study (discussed in the Chapter 3), were included in this table for comparison purposes and they do not represent the fibre-protein and can-sugar blended with alfalfa pellet in the current study.

Blending of alfalfa with fibre-protein and can-sugar mixture (2:1 mixture) at 15% increased the total CP content of pellets by 2.4% (from 16.2% to 18.6%). The increase in CP was observed in all the CP components (i.e. SCP, NPN, NDIP and ADIP). However, the highest increase was observed in NPN component (1.5%) which can be attributed to comparatively higher NPN content in can-sugar.

Figure 4.2 Alfalfa dehydrated pellet blended with fibre-protein and can-sugar in comparison with standard alfalfa dehydrated pellet

Table 4.3 Chemical composition of fibre-protein, can-sugar, standard alfalfa dehydrated pellets and dehydrated alfalfa blended with 15% of fibre-protein + can-sugar mixture at 2:1 ratio

Component (% DM)	Feed Ingredient			
	Fibre-protein	Can-sugar	Standard alfalfa	Blended alfalfa
DM (%)	91.8	87.6	97.2	97.2
Ash	4.3	19.3	8.0	8.3
Organic matter	95.7	80.7	92.0	91.7
CP	30.9	15.6	16.2	18.6
EE	1.5	0.3	2.8	3.4
NDF	55.6	0.1	37.5	37.3
ADF	46.3	0.1	25.4	25.8
ADL	24.1	0.2	5.9	6.9
NDICP	12.6	0.0	5.2	6.0
ADICP	7.7	0.0	2.2	2.9
NPN[1]	4.3	13.0	3.2	4.7
Hemicellulose[2]	9.3	0.0	12.1	11.5
Cellulose[3]	22.2	0.0	19.5	18.9
G. Energy Mcal/kg	4.36	3.06	4.24	4.29

[1]Non-protein nitrogen is presented as crude protein (6.25 × N)
[2] Hemicellulose = Neutral detergent fibre – Acid detergent fibre
[3] Cellulose = Acid detergent fibre – acid detergent lignin

Even though fibre-protein has a very high fibre content (55.6% NDF), the blended pellet had a fibre content (37.3%) closer to the standard pellet (37.5%) which was due to inclusion of almost fibre free can-sugar at blending. On the other hand, lignin content increased by 1% in blended pellets due to lignin enriched fibre-protein.

Although can-sugar has high ash content, the increase in ash content of blended pellet was not noteworthy. Despite both fibre-protein and can-sugar were observed to be low in EE, slightly higher EE content was observed in blended pellet (3.4% vs. 2.8%). This indicates a possible difference between canola meals used to prepare fibre-protein for blending and fibre-protein used in the previous study since EE content in canola meal would affect the EE content in resultant fibre-protein.

Even though there was a difference in chemical composition, none of the parameters used to evaluate palatability showed any significant difference between two types of pellet (Table 4.4). The intake of blended pellet (969 g) was similar to that of standard alfalfa pellet (966 g). The average time taken to finish eating blended pellet (6.8 m) was marginally higher than that of the standard pellet (6.4 m) but the difference was not significant ($P>0.05$). The animal preference for both types of pellet was similar (50.1% vs. 49.9%).

The nutritional composition of the two TMRs were similar as shown in the Table 4.2. The DMI of the TMR by animals fed with standard alfalfa pellet (27.9 kg/day) was slightly higher than those fed with blended pellet (27.6) but the difference was not significant (Table 4.5). The DMI as a percentage of body weight was similar for both treatments. There was no significant difference observed in apparent DM digestibility between the blended and standard alfalfa pellet diets (64.5% and 63.5%, respectively).

There was no treatment effect ($P>0.05$) on milk, milk fat or milk protein yield (Table 4.6) which can be expected as the nutrient composition of the two diets was similar. The same milk production efficiency was observed with both blended alfalfa diet and standard alfalfa diet (1.39 milk kg per kg DMI).

Table 4.4 Palatability of blended alfalfa dehydrate pellet and standard alfalfa dehydrate pellet fed to dairy cows as indicated by mean intake, eating time and preference percentage

	Pellet type		SEM	P value
	Blended	Standard		
Intake : as-fed (g)	969	966	3.5	0.51
Eating time (minutes)	6.8	6.4	0.18	0.11
Preference (%)	50.1	49.9	0.10	0.53

SEM = Standard error of means

Table 4.5 Dry matter intake and apparent dry matter digestibility of dairy cows fed with standard alfalfa dehydrated pellet and alfalfa dehydrated pellet blended with canola fibre-protein and can-sugar

	Pellet type		SEM	P value
	Blended	Standard		
Dry matter intake kg d^{-1}	27.6	27.9	0.31	0.09
Dry matter intake % body weight	3.6	3.6	0.08	0.37
Apparent DM digestibility %	64.5	63.5	1.14	0.37

SEM = Standard error of means

Table 4.6 Milk yield, milk production efficiency and milk composition of dairy cows fed with pure alfalfa dehydrated pellet and alfalfa dehydrated pellet blended with canola fibre-protein and can-sugar

	Pellet type		SEM	P value
	Blended	Standard		
Yield (kg d^{-1})				
Milk yield	38.5	38.7	2.33	0.84
3.5% Fat corrected milk yield	37.4	38.0	2.68	0.60
Milk fat yield	1.28	1.31	0.114	0.50
Milk protein yield	1.18	1.18	0.068	0.86
Milk production efficiency (MPE)				
MPE (milk kg per kg DMI)	1.39	1.39	0.094	0.85
MPE (3.5% FCM kg per kg DMI)	1.04	1.02	0.034	0.35
Milk components				
Milk fat %	3.33	3.37	0.187	0.44
Milk protein %	3.05	3.04	0.071	0.77
Lactose %	4.43	4.40	0.137	0.06
Milk urea (m Mol L^{-1})	7.33	7.09	0.236	0.15

[a, b,] Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM = Standard error of mean

The milk production efficiency on the basis of fat corrected milk yield (at 3.5% FCM) was 1.04 kg/kg DMI with the blended alfalfa diet which was very close to that of the standard alfalfa diet (1.02 kg/kg DMI). There were no significant differences between the blended and standard alfalfa treatments in relation to fat (3.33% vs. 3.37%), protein (3.05% vs. 3.04%) or lactose (4.43% vs. 4.40%) content in milk.

The urea N content in milk (MUN) is an indicator of efficiency of N utilization in a lactating cow (Broderick and Clayton, 1997). Jonker et al. (1998) suggested 10 – 16 mg/dl of MUN as a desirable target. In the current study, MU content in milk of cows fed with blended alfalfa has shown a slightly higher value (7.33 m Mol/l) comparing to standard alfalfa (7.09 m Mol/l), but the difference was not statistically significant. These values are equivalent to 20.5 mg/dl and 19.9 mg/dl MUN, respectively, which while above the Jonker et al. (1998) target, are within the range reported in high producing Holstein cows fed with higher CP containing diets (Broderick and Clayton, 1997; Jonker et al. 1998).

4.4 Conclusions

The results of this study show that 2:1 of fibre-protein to can-sugar mixture can be added at 15% to standard alfalfa pellet without affecting the palatability or lactation performances of dairy cows, when the alfalfa pellets are included at the standard inclusion rate of 5% (as fed basis) of a TMR. The CP content of the pure alfalfa pellet in the current study was only 16% which was increased to 18% with the addition of fibre-protein and can-sugar mixture without compromising the composition or the lactation performances. This may be considered as an advantage since the optimal CP content in alfalfa according to Canadian Hay Association should be about 18%, particularly which are intended for export. However, the commercial viability of such inclusion would depend on the price of fibre-protein and can-sugar as well as the acceptance by the end users.

5. GENERAL DISCUSSION AND CONCLUSIONS

Fibre-protein and can-sugar are the by-products arising from a process of enzymatic protein separation in canola meal. While protein concentrates are intended to be utilized in aquaculture and monogastric feeding, utilization of fibre-protein and can-sugar would enhance the value addition to canola. The main objective of this thesis was to evaluate fibre-protein and can-sugar as potential feed ingredients in ruminant diets. This was accomplished first by investigating the chemical characteristics, in situ rumen degradation characteristics and determining the nutritive values of two ingredients in comparison with commercial canola meal and soy meal. Subsequently, potential of utilizing fibre-protein and can-sugar was examined by blending them with dehydrated alfalfa pellet and studying its palatability and lactation performance of cows fed alfalfa pellet contain a blend of fibre-protein and can sugar.

In the study of chemical characteristics, it was observed that during the aqueous extraction followed by protein separation; almost all the structural carbohydrates and fibre associated proteins present in canola meal were isolated in fibre protein while non-structural carbohydrates, NPN and mineral matter were isolated with can-sugar. Aqueous extraction is a dehulling process and canola hulls and dockage are separated into fibre-protein. Fibre-protein, however, contained a significant amount of crude protein although about 25% of crude protein comprise of undegradable ADIP. Similar to canola meal and soy meal, major CP portion in fibre-protein was intermediately degradable PB2 fraction. Due to high lignin content, potentially degradable fibre was found to be low in fibre-protein.

In situ rumen degradation kinetics was studied in relation to DM, OM, CP, NDF and ADF. The can-sugar was totally water soluble and assumed to be totally degradable at zero hour incubation. The fibre-protein had lower effective ruminal degradability in all the nutrients comparing to both canola and soy meal. During the protein fractionation,

35% of DM in canola meal is recovered with fibre-protein. Yet effective degradable DM content in fibre-protein was reduced by 24% compared to canola meal. EDCP in fibre-protein was 45.5% which is only 9% less than EDCP content in soy meal (54.7%). However over a long term incubation, CP in soy meal was totally degradable where as fibre-protein had a substantial portion of ruminally undegradable CP (39%) indicating lower post-ruminal availability. This has substantiated the findings of chemical characteristic study where 25% of CP consisted of undegradable ADIP. According to literature, degradability of fibre (NDF and ADF) is inversely related to lignin content in an ingredient. As mentioned earlier, during the manufacturing process lignin enriched canola hulls are concentrated into fibre-protein. Hence, lowered overall degradability of NDF and ADF in fibre-protein comparing to canola meal can be expected. There was no difference in gross energy content among tested ingredients. However, predicted available energy content (DE, ME and NE) in fibre-protein was only 50% that of canola meal. This was as a result of its lower digestible NFC, CP and fat content. Can-sugar on the other hand had an available energy content closer to canola meal which is mainly due to its high digestible NFC content.

The potential protein supply to small intestine of dairy cattle predicted by both DVE/OEB and NRC 2001 models for fibre-protein was approximately half that of canola meal, caused by higher undegradable and undigestible protein content in fibre-protein contributed likely by canola hulls. Fibre-protein similar to soy and canola meal had a positive degraded protein balance. As stated earlier, can-sugar has shown a comparatively superior content of available energy content but low in true protein. Since both can-sugar and fibre-protein are by-products of canola protein fractionation process, a mixture of can-sugar and fibre-protein would complement each other not only nutrition wise but also with regard to economically viable manufacturing operation.

Fibre-protein has a rough texture while can-sugar is a highly hygroscopic powdery material and both without any detectable odour which are influential factors for palatability. The output ratio of fibre-protein and can-sugar is roughly 2:1 in canola protein fractionation process. These two ingredients were mixed using the same ratio and added at the rate of 15% to dehydrated alfalfa at the pelletizing stage to obtain

blended alfalfa pellet without visible effect on colour of alfalfa pellet. As a result of blending, CP content of the pellet increased by 2.4%. The comparative study revealed that there was no difference in palatability between blended and standard alfalfa pellet fed to lactating dairy cows which have shown similar preferences to both types of pellet. Dehydrated alfalfa is a common ingredient included in the ration for high producing dairy cows. In the feeding trial conducted subsequent to the palatability study, alfalfa pellet was included at a rate of approximately 7% (on DM basis) into the ration and it was observed that blending has no effect on lactation performance in comparison to standard alfalfa pellet. Since the overall nutrient composition of total mixed ration (TMR) with blended pellet was very close to that of standard pellet, similar lactation performances were observed under two treatments.

The main focus of current thesis was to investigate the feasibility of utilizing fibre-protein and can-sugar as ruminant feed ingredients and find a possible marketing avenue domestically and internationally. This study has demonstrated those ingredients can be successfully utilized as an additive to alfalfa pellet. The present study was confined to single blending level at 15% to dehydrated alfalfa. There is possibility that optimum level of inclusion to alfalfa could be different from 15%. Likewise, fibre-protein and can-sugar could possibly be either included in the TMR directly or blended with standard concentrate pellets. The can-sugar has a significant amount of mineral matter and therefore a detailed mineral analysis would be advantageous. The DM contribution by the two ingredients to the total TMR was only about 1% which might have been too low to make a pronounced effect on lactation performance. Therefore, in order to obtain a better understanding of nutritional effects, it will be necessary to conduct feeding trials with higher levels of inclusion into TMR. Another area of interest would be the presence and possible nutritional role of tannin in hull loaded fibre-protein since canola hulls was reported to be containing up to 1574 mg condensed tannin per 100 g of hulls depending on the cultivar (Naczk et al. 1994). Condensed tannin was reported to have inhibitory effect on both cellulolytic and proteolytic bacteria in the rumen (McSweeney et al. 2001) and therefore inclusion level of fibre-protein into TMR may affect the overall ruminal degradability and post-ruminal protein availability.

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