Characteristics and Utilization of Canola Seed Fractions in Ruminant Feeds A processing and value added research

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1. INTRODUCTION

Canola is the second most economically important crop grown in Canada. 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. About 98% of canola meal is exported by Canada to USA and due to its 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 comparing to soy meal.

Canola meal fractionation process, developed by MCN Bio Product intends to add value and to create new sources of demand to canola. In this fractionation process (figure 1), high quality protein is separated from white flake (un-toasted oil meal) suitable for specific livestock feed markets, particularly as a cost-wise attractive substitute for animal-based protein such as fishmeal in aquaculture. The by-product arising from the fractionation includes a canola sugar fraction (Can-Sugar) that contains soluble carbohydrates plus soluble minerals and a fibrous protein fraction (Fibre-Protein) containing hulls and some dockage. The protein concentrates are the main economic drivers of the process that would target for lucrative aquaculture and mono-gastric market. However, as the fibre protein and Can-sugar fractions consist of 55% of overall fractionation process, they should also be utilised to make the fractionation an economically viable process and the most likely market for these two fractions would be the ruminants.

Being newly developed products, there are no specific studies being done with “fibre-protein” and “can-sugar” on their nutritive value. The present study was conducted to examine the chemical characteristics of “Fibre-Protein” and “Can- Sugar” fractions and predict their potential nutrient supply to ruminants using two different models: the Dutch (DVE/OEB) system and NRC-2001 model. For comparison purpose, the evaluation of two fractions was done along with commercially available canola meal and soy meal.

2. MATERIALS AND METHODS

2.1. Samples and Sample Preparation for chemical analysis

Adequate amount of samples from three (3) batches of Canola meal and soy meal were obtained from commercial supplier “Saskatchewan Cooperative”. Three samples of fibre-protein drawn from three different batches were collected from the manufacturer of canola protein concentrates. Due to limitation in production, only one batch of can-sugar in dried powder form was available at the time of this study and therefore only one sample of can-sugar was tested along with other feeds.

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 0.5mm screen to obtain samples for starch analysis.

2.2 Chemical Analysis of Feed Samples

Samples were analysed according to the Association of Official Analytical Chemists (AOAC, 1990) for dry mater (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 (ADF method 973.18 followed by 72% H2SO4 treatment).

The Neutral Detergent Fibre (NDF) contents were determined without sodium sulfite according to procedure proposed by Van Soest et al 1991 while Acid Detergent Insoluble Crude Protein (ADICP) and Neutral detergent Insoluble Crude Protein (NDICP) 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) contents were obtained by precipitating 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. Then 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 carbohydrates (CHO) and non-fibrous carbohydrate (NFC) contents were calculated according to the following equations suggested by Sniffen et al., (1992) and NRC (2001).

Total CHO = 100 – CP – Ash – Ether Extract;
Non-fibrous CHO = 100 – (NDF-NDICP) - CP – EE - Ash

2.3. Energy Value Estimation

The gross energy values of feed samples were determined with the use of bomb calorimeter (Parr 1281, Parr Instruments Company, Moline, Illinois). The Total Digestible Nutrient at maintenance (TDN_{1X}), and energy values of DE_{3X}, ME_{3X}, NEL_{3X} related to dairy cattle were calculated using the summative approach proposed by Weiss et al., (1992) and recommended by NRC (2001). The energy values of ME, NEm, and NEg related to beef cattle were estimated from the NRC beef (1996).

2.4. Prediction of Microbial Protein Synthesis and potential protein supply to the small intestine

Both Dutch (DVE/OEB) Model (Tamminga et al, 1994; Yu et al, 2003B) and NRC (2001) model (NRC, 2001) were used in this study to predict the amount of true protein absorbable from the small intestine. In order to obtain the rumen degradation parameters those are needed for prediction of microbial synthesis protein and bypass fraction of feed protein, a nylon bag rumen incubation trial was carried with two Frisian cows fitted with wide cannulae (internal diameter: 10 cm)

2.5 Statistical Analysis

All the data of treatments were analysed statistically using mixed procedure of SAS software (SAS institute, inc. 1999). Each set of data were analysed as a complete randomised design with three batches as replicates. Means were separated using LSD procedure.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition

The fibre-protein has shown a significantly lower ash content (4.3%) and higher organic matter content (95.7%) while can-sugar had a higher ash content and lower organic matter comparing to both canola and soy meals (Table 1). This indicates that major portion of mineral matter in canola meal was concentrated in to can-sugar fraction during the extraction process. The ether extract contents were very low around 1% or less in all the ingredients except for canola meal. The ether extract 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 McKinon (1995). A 13% ether extract 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.

Even though there was no significant difference between total carbohydrate content in fibre-protein and can-sugar fractions, they were significantly higher ($P<0.05$) than Canola meal by 16% and 18% respectively. In can-sugar, the total carbohydrate was almost totally (99.9%) represented by non-fibre carbohydrates. As the starch content in can-sugar was only 1.1% it indicates that carbohydrate fraction of can-sugar consists mainly of sugars. The non-fibre carbohydrate in fibre-protein was lowest (20.2 %DM) and significantly different from canola meal (29.3%) and soy meal (36.6%). In fibre-protein that amounts to only 32% of total carbohydrate whereas, non-fibre carbohydrate represents 63% and 89% of total carbohydrate in canola meal and soy meal. When non-fibre contents 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 manufacturing process of fibre-protein and can-sugar.

A significantly higher($P<0.05$) NDF and ADF contents 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. The contents of fibre components observed with canola meal in this study agree with those values reported by other publications (NRC, 2001; Mustafa et al, 1996). McKinnon et al (1995) has reported a similar ADF content (46.7%) but with a higher NDF content (65.8%) for untreated canola hulls which is likely with hulls from “front-end de-hulling” due to remains of seed embryo that are possibly associated with hemicellulose.

Very low acid detergent lignin (ADL) content (< 1%) was observed in can-sugar and soy meal. 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%) 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 Cornell Net Carbohydrate and Protein System (CNCPS), indigestible carbohydrate/fibre fraction is calculated by multiplying ADL by factor 2.4 (lignin x 2.4) (Sniffen et al, 1992). 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). In canola meal the calculated indigestible portion of NDF amounts to 21.6% against its NDF content of 25.4 %DM while in soy meal indigestible NDF portion is only 1.4% against its total NDF content of 8.8%.

Highest crude protein content of 48.4% was observed in soy meal followed by canola meal (39.6%), fibre-protein (30.9%) and can-sugar (15.6%) as shown in the table 2. McKinnon et al (1995) has reported a protein content of 15.4% in canola hulls, which is less than half of canola meal. The difference of 9% ($P<0.05$) in crude protein content observed in fibre-protein comparing to canola meal could therefore be attributed to presence of hulls in fibre-protein. On the other hand, fibre-protein seems to contain parts of seed other than hulls that has resulted a protein content of 30.9%.

There was no significant difference ($P>0.05$) in soluble crude protein (SCP) content of fibre-protein and canola meal which is about 25% of total crude protein where as SCP content in soy meal was only 14.4%. In contrast the crude protein component in can-sugar observed to be comprised mainly by soluble crude protein which in turn represented by 87% of non-protein nitrogen (NPN). In the process of manufacturing, most of the available true proteins in the aqueous extract were separated enzymatically as protein concentrates and can-sugar fraction would therefore contain nitrogenous substances mainly in the form of non-protein nitrogen. On dry matter basis the NPN content in fibre-protein (4.3%) is significantly less than ($P<0.05$) canola meal (7.1%). However there was no significant difference ($P>0.05$) between fibre-protein (13.9%CP) and canola meal (17.8%CP) when the share of NPN in crude protein was considered. This indicates NPN that consist of ammonia, peptides and amino acids (Sniffen et al, 1992) were equally distributed in the two fractions (figure 1: dewatered cake and aqueous extract) before "enzymatic fractioning" stage and the NPN in canola meal is mostly confined to can-sugar fraction after enzymatic separation.

As the fibre component in Can-sugar is almost zero, NDICP and ADICP contents were insignificant. The NDICP and ADICP contents in soy meal were significantly lower than ($P<0.05$) canola meal. The protein fraction associated with 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.

3.2 Digestible Nutrients and Energy content

As shown in the Table 3, there was no significant difference in gross energy between fibre-protein, canola meal and soy meal while can-sugar has shown significantly lower ($P<0.05$) gross energy content than other ingredients. The lower gross energy value in can-sugar could be attributed to its comparatively higher ash content (19.3%). However the total digestible nutrient (TDN) content in can-sugar was not significantly different ($P>0.05$) from that of canola meal or soy meal and significantly higher than ($P<0.05$) fibre-protein. This was owing to the presence of significantly higher percentage of non-fibre carbohydrate in can-sugar. The TDN value of soy meal was significantly higher than canola meal due to its comparatively higher digestible crude protein and non-fibre carbohydrate fractions.

The DE, ME and NE values observed with canola meal and soy meal in this study were closer to those reported in nutrition composition tables of NRC 2001. Unlike with TDN, the digestible energy (DE), metabolizable energy (ME) and net energy (NE) values for can-sugar were significantly lower than those of soy meal. Comparison of energy values among feed ingredients has shown the same pattern for all the energy values (DE, ME, NE). There was no significant difference between canola meal and can-sugar in relation to DE, ME and NE values at any production level intake. The fibre-protein has shown the lowest energy values at all the intake levels while the soy meal showing the highest values.

3.4 Microbial Protein Synthesis and protein supply to the small intestine

3.4.1 Dutch (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 4. The results shows 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 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 ($P>0.05$). The lower level of FOM in turn has resulted a lower predicted values for microbial crude protein based on FOM (MCP_{FOM}) and absorbable microbial crude protein (AMCP) in fibre protein (48.2 g/kg DM and 30.7 g/kg DM respectively) comparing 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 rumen un-degradable protein (RUP) content in soy meal (234.8 g/kg DM) was significantly higher ($P<0.05$) than that of canola meal (163.8 g/kg) and fibre-protein (186.9 g/kg). Even though RUP content in fibre-protein was slightly higher than canola meal, the difference was not significant ($P>0.05$). However, the absorbable ruminally undegradable protein (ARUP) in fibre protein (62 g/kg DM) has shown a significantly lower value than that of canola meal (137.6 g/kg DM) and soy meal (234.8 g/kg). In soy meal, the ARUP content was equal to RUP content while in fibre-protein it was approximately 1/3 of its RUP content. This was due to complete degradation of protein in soy meal and only a partial degradation of protein in fibre-protein taken place during incubation. The loss of endogenous protein (that occur during digestive process due to extent of undigested dry matter in a feed), was significantly highest in fibre-protein (35 g/kg DM) followed by canola meal (12.8 g/kg DM) which in turn significantly higher than that of soy meal (4.3 g/kg).

Because of lower AMCP and ARUP along with higher ENDP, the DVE value of fibre-protein (57.8 g/kg DM) was predicted to be significantly the lowest. The DVE value of Canola meal (180.0 g/kg DM) was significantly lower than that of soy meal (291.4 g/kg DM). The results shows that all the feed ingredients were having positive OEB values that shows availability of feed protein exceeds the availability of energy (extracted during rumen fermentation) for microbial protein synthesis indicating a Nitrogen loss in the rumen.

3.4.2 NRC-2001 Model

As shown in the Table 4, predicted synthesis of microbial crude protein (MCP) and absorbable-MCP (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). There were no significant differences ($P>0.05$) in MCP and AMCP values between canola meal and soy meal.

The highest rumen un-degradable protein (RUP) was observed in soy meal (211.5 g/kg DM). Although there was no significant difference ($P>0.05$) in RUP between fibre-protein (168.4 g/kg DM) and canola meal (147.6 g/kg DM), the absorbable-RUP (ARUP) of fibre protein was significantly lower than canola meal (124.0) that amounted to less than 50% of ARUP in Canola meal. The lower digestibility of RUP observed in fibre-protein which contained a higher level of indigestible ADIP could be attributed for the low ARUP value.

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 nitrogen loss in the rumen.

3.4.3 Dutch 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 values derived from NRC-2001 for all the feed samples. However, in the present study AMCP and ARUP values predicted for fibre-protein and canola meal using DVE/EB were lower than those of the NRC-2001 values 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 Dutch model (DVE values), were 15% lower than predictions from NRC-2001 model (MP values). In the current study too, 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). While three feed samples are not adequate to do a comparison of two models, the inconsistent differences indicates major differences in some of the assumptions and concepts used in two models.

4. CONCLUSIONS

It was concluded that fibre-protein could be used as a secondary source of protein for ruminants while can-sugar can be used as a source of readily available energy. A mixture of fibre-protein and can-sugar, both of which are by-products of canola fractionation process could possibly be formulated as a cheaper ingredient in ruminant ration. As most of the observations in this study were based on chemical compositional analysis, a feeding trial is suggested to arrive at a more conclusive assessment of fibre-protein and can-sugar.

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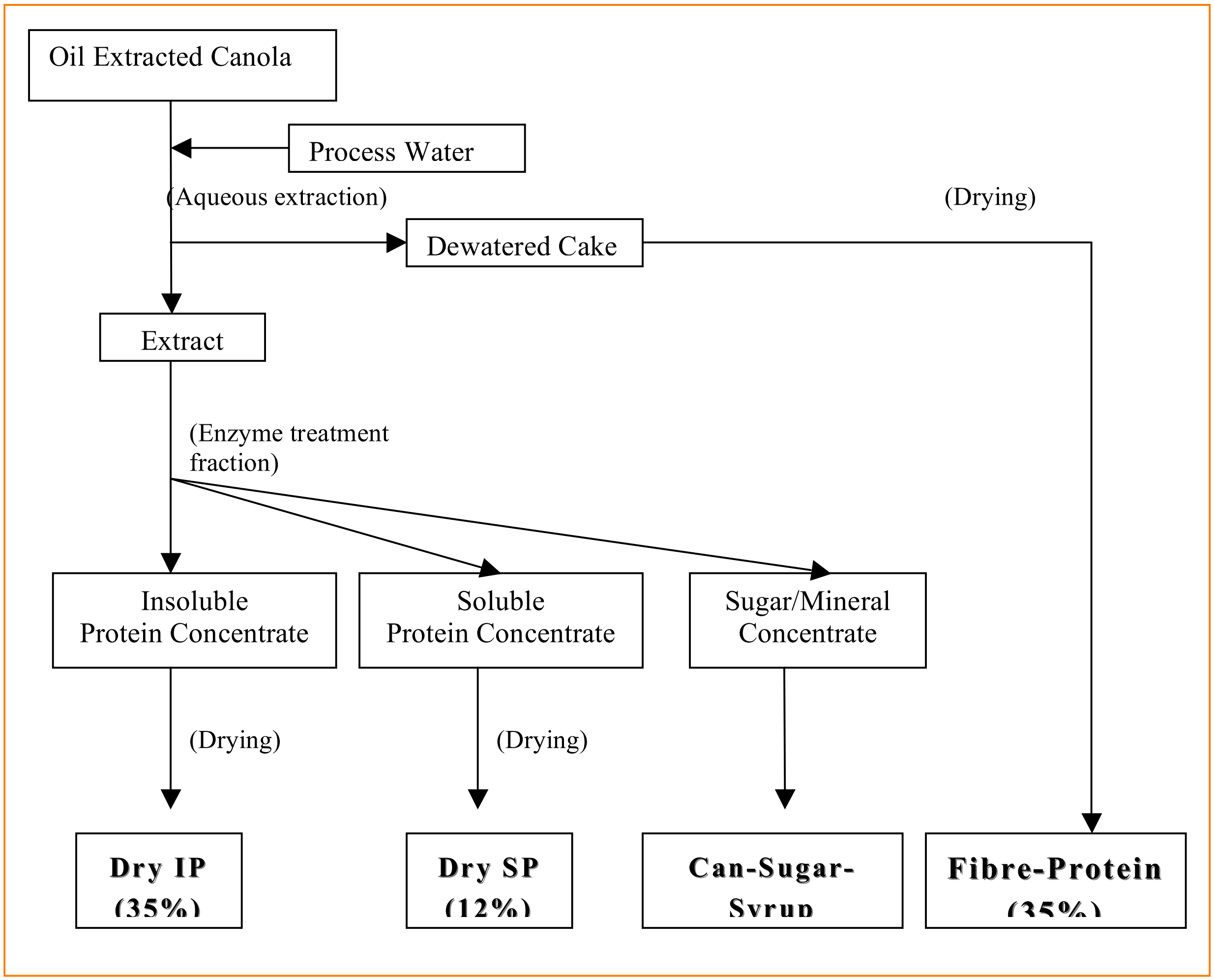
Figure 1. Canola Fraction Process



Source: www.mcnbioproducts.com

Table 1. Dry matter, Ash, organic matter, ether extract and carbohydrate composition of fibre protein and can-sugar compared with commercial Soy and Canola meal.

Component	Feed Ingredient				SEM
	Fibre-Protein	Can-sugar	Canola meal	Soy Meal	
DM %	91.8	87.6	90.2	92.1	1.68
Ash % DM	4.3^c	19.3^a	8.4^b	9.4^b	1.31
OM % DM	95.7^a	80.7^c	91.6^b	90.6^b	1.31
Ether extract % DM	1.5^b	0.3^b	5.3^a	1.1^b	0.38
Carbohydrates (CHO)					
Total CHO %DM*	63.2^a	64.9^a	46.7^b	41.1^c	0.60
Starch % DM	0.8	1.1	1.0	1.0	0.16
Non-Fibre CHO %DM	20.2^d	64.8^a	29.3^c	36.6^b	1.36
Non Fibre CHO %CHO	32.0^c	99.9^a	62.9^b	89.0^a	2.90
Neutral Detergent Fibre % DM	55.6^a	0.1^d	25.4^b	8.8^c	1.71
Acid Detergent Fibre % DM	46.3^a	0.1^d	21.2^b	6.1^c	1.62
Acid Detergent Lignin %DM	24.1^a	0.2^c	9.0^b	0.6^c	1.47
Hemicellulose[1] %DM	9.3^a	0.0^d	4.2^b	2.7^c	0.20
Cellulose[2] %DM	22.2^a	0.0^d	12.2^b	5.5^c	0.65

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

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

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

Table 2. Crude protein and protein fractions in fibre protein and can-sugar compared with commercial Soy and Canola meal.

Component	Feed Ingredient				SEM
	Fibre-Protein	Can-sugar	Canola meal	Soy Meal	
Crude Protein %DM	30.9^{c}	15.6^{d}	39.6^{b}	48.4^{a}	1.01
Soluble crude protein %DM	8.3^{bc}	15.0^{a}	10.0^{b}	7.0^{c}	0.94
Soluble crude protein %CP	26.7^{b}	96.2^{a}	25.2^{b}	14.4^{c}	2.00
Non-protein nitrogen[1] %DM	4.3^{c}	13.0^{a}	7.1^{b}	2.5^{c}	0.93
Non-protein nitrogen[1] %CP	13.9^{b}	83.5^{a}	17.8^{b}	5.1^{c}	1.98
Non-protein nitrogen[1] %SCP	52.0^{bc}	86.8^{a}	70.6^{ab}	33.6^{c}	7.38
ND insoluble protein %DM	12.6^{a}	0.04^{c}	8.0^{b}	4.3^{c}	1.28
ND insoluble protein %CP	40.9^{a}	0.3^{c}	20.2^{b}	8.9^{c}	3.89
AD insoluble protein %DM	7.7^{a}	0.0^{b}	3.2^{b}	0.7^{b}	0.93
AD insoluble protein %CP	24.9^{a}	0.0^{b}	8.2^{b}	1.4^{b}	3.04
True Protein %CP	61.2^{c}	16.5^{d}	74.8^{b}	94.5^{a}	2.93

$^{a, b, c, d,}$ Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM Standard error of mean

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

Table 3. Truly digestible nutrients (td), Total digestible nutrients (TDN), Gross energy (GE) and predicted energy values at maintenance (1X) and production intake levels (3X, 4X) of fibre-protein and can-sugar compared with commercial canola meal and soy meal.

	Feed Ingredients				SEM
	Fibre-Protein	Can-sugar	Canola meal	Soy Meal	
Truly Digestible nutrients (NRC 2001)					
tdNDF %DM	4.6^a	0.03^b	2.3^b	2.2^b	0.79
tdNFC %DM	19.8^d	63.5^a	28.7^c	35.9^b	1.34
tdCP %DM	27.8^c	15.5^d	38.3^b	48.1^a	1.11
tdFA %DM	0.5^b	0.0^b	4.3^a	0.3^b	0.33
Total Digestible Nutrients (NRC 2001)					
TDN_{1X}	46.5^c	72.1^{ab}	71.9^b	79.8^a	2.25
Gross Energy (Bomb calorie-meter)					
GE (Mcal/kg)	4.36^a	3.06^b	4.31^a	4.15^a	0.079
Predicted Digestible Energy value at Maintenance level intake (1X)-NRC2001					
DE_{1X} (Mcal/kg DM)	2.33^c	3.24^b	3.55^b	4.02^a	0.110
Predicted energy value at production intake level 3X for dairy cattle (NRC 2001)					
DE_{3X} (Mcal/kg DM)	2.16^c	3.00^b	3.29^b	3.72^a	0.102
ME_{3X} (Mcal/kg DM)	1.73^c	2.58^b	2.88^b	3.31^a	0.103
NE_{3X} (Mcal/kg DM)	1.03^c	1.62^b	1.85^b	2.14^a	0.074
Predicted energy value at production intake level 4X for dairy cattle (NRC 2001)					
DE_{4X} (Mcal/kg DM)	2.08^c	2.88^b	3.16^b	3.57^a	0.097
ME_{4X} (Mcal/kg DM)	1.65^c	2.46^b	2.75^b	3.16^a	0.099
NE_{4X} (Mcal/kg DM)	0.97^c	1.54^b	1.75^b	2.03^a	0.071
Predicted energy value for beef cattle (NRC 1996)					
ME (Mcal/kg DM)	1.91^c	2.66^b	2.91^b	3.30^a	0.090
NE_m (Mcal/kg DM)	1.07^c	1.74^b	1.96^b	2.27^a	0.077
NE_g (Mcal/kg DM)	0.52^c	1.12^b	1.31^b	1.58^a	0.068

$^{a, b, c, d,}$ Means with the same superscripts in the same row are not significantly different (P>0.05) by LSD test

SEM Standard error of mean

Table 4. Predicted values of potential protein supply to dairy cattle from fibre-protein in comparison with commercial canola meal and soy meal using the Dutch system and NRC-2001 dairy model.

	Feed Ingredient			SEM
	Fibre-Protein	Canola meal	Soy Meal	
Using the Dutch (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
Using 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