EVALUATION OF PELLETED PRODUCTS BASED ON COMBINATION OF NEW CO-PRODUCTS FROM BIO-FUEL OR BIO-OIL PROCESSING, PEA SCREENINGS AND LIGNOSULFONATE CHEMICAL COMPOUND FOR RUMINANT DIETS

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By

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ABSTRACT

Brassica carinata meal from bio-fuel processing and canola meal from bio-oil processing have recently become available, but little data is available on their chemical, nutrient profile, bioactive compounds, and nutrient utilization and availability in livestock, especially when carinata meal is blended with other feedstuffs as a pellet in order to optimize nutrient and amino acid supply. The aim of this project was to develop and test blended pelleted products based on combinations of carinata/canola meal, pea screenings, and lignosulfonate at different levels for ruminants. Chemical profile, energy value, rumen degradation kinetics of nutrients, hourly effective rumen degradation ratios/potential N-to-energy synchronization, and intestinal digestion of nutrients were analyzed, then the truly absorbed protein supply to dairy cattle and feed milk values were evaluated using on the DVE/OEB system and the NRC Dairy model. Comparisons were made among blend pelleted products based on carinata meal versus canola meal based pelleted products, addition vs. non addition of lignosulfonate, and low level of inclusion of those co-products with high level of inclusion of pea screenings vs. high level of inclusion of co-products with low level of inclusion of pea screenings. Statistical analyses were performed using PROC MIXED of SAS 9.4 with significance declared at $P < 0.05$. The results showed that all blend pelleted products had safe levels of glucosinolates (3.46 to 5.86 µmol/g) and condensed tannins (maximum of 0.033 % DM), and high pellet durability index (PDI) (88.5 to 97.5 %). Carinata based pelleted products were lower in NDF content (-4.6 % DM), but higher in protein (+3.2 % DM), and in total amino acids (+1.23 % DM) than the canola pelleted products. Canola pelleted products contained higher level of methionine (1.90 vs. 1.70 % CP) than the carinata blend pelleted products. Canola blend pelleted products contained higher lysine ranging from 5.71 to 5.90 % CP than carinata based pelleted products which ranged from 4.20 to 4.43 % CP. Carinata based blend pelleted products contained

higher NE for lactation (NE_L), maintenance (NE_m) and growth (NE_g) (1.99 vs. 1.83, 2.15 vs. 1.98 and 1.47 vs. 1.33 Mcal/kg, respectively) than canola based pelleted products. In terms of protein fractions, carinata based blend pelleted products contain lower true soluble protein (PA2) with a mean of 29.5 % of CP and indigestible protein (PC) with a mean of 1.5 % of CP, but higher ($P < 0.05$) fiber bound-protein (PB2) with a mean of 12.0 % of CP than canola based blend pelleted products. In terms of CHO fractions, carinata based blend pelleted products had lower ($P < 0.05$) indigestible fiber (CC) with a mean of 12.5 % of CHO. According to the hourly effective degradation ratios between rumen available N and CHO (ED_N/ED_CHO), all blend pelleted products had overall degradation ratios above the optimal N to CHO ratio. In addition, carinata based blend pelleted products contain lower ($P < 0.05$) rumen effective degraded protein (221 vs. 245 g/kg DM), higher rumen bypass protein (207 vs. 146 g/kg DM), higher effective fiber degradability of NDF (28.3 vs. 25.4 %), higher intestinal absorbable feed protein (IADP 146 vs. 90 g/kg DM) than canola based blend pelleted products. Furthermore, carinata based blend pelleted products contained higher total protein truly digested in the small intestine (DVE 218 vs. 158 g/kg DM), metabolizable protein (MP 210 vs. 151 g/kg DM) and lower degradable protein balance (OEB and DPB values) (105 vs. 130 and 111 vs. 142 g/kg DM, respectively) than canola based blend pelleted products, which leads them to contain higher ($P < 0.05$) feed milk value (FMV^{DVE} and FMV^{NRC}) (4.43 vs. 3.22 and 4.01 vs. 2.76 g/kg DM, respectively) than canola based blend pelleted products.

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LIST OF ABBREVIATIONS

abs   Absorbance

ADF   Acid detergent fiber

ADICP   Acid detergent insoluble crude protein

ADL   Acid detergent lignin

AMCP   Truly absorbed microbial protein in the small intestine

ARUP   Truly absorbed rumen undegraded protein in the small intestine (NRC Dairy model)

BCP   Rumen bypass feed crude protein (DVE/OEB system)

BDM   Rumen bypass dry matter

BDNDF   Rumen bypass feed neutral detergent fiber

BST   Rumen bypass starch

CA4   Sugar (rapidly degradable carbohydrate fraction)

CB1   Starch (intermediately degradable carbohydrate fraction)

CB2   Soluble fiber (intermediately degradable carbohydrate fraction)

CB3   Digestible fiber (available neutral detergent fiber or slowly degradable carbohydrate fraction)

CC   Indigestible fiber (unavailable neutral detergent fiber)

CHO   Carbohydrate

CP   Crude protein

CT   Condensed tannin

D   Degradable fraction

dBDM   Intestinal digestibility of rumen bypass dry matter

dBNDF   Intestinal digestibility of rumen bypass fiber

dBST   Intestinal digestibility of rumen bypass starch

$DE_{p3\times}$	Digestible energy at a production level ($3\times$ maintenance)
dIDP	Intestinal digestibility of rumen bypass protein
DM	Dry matter
DPB	Degraded protein balance
DVBE	Truly absorbed bypass feed protein in the small intestine
DVE	Total truly digested protein in the small intestine (DVE/OEB system)
DVME	Truly absorbed rumen synthesized microbial protein in the small intestine
ECP	Rumen endogenous protein
ED_CHO	Effectively degraded carbohydrate
ED_N	Effectively degraded nitrogen
EDCP	Effective degraded crude protein
EDDM	Effective degraded dry matter
EDNDF	Effective degraded neutral detergent fiber
EDST	Effective degraded starch
EE	Ether extracts (crude fat)
FMV	Feed milk value
IADP	Intestinal digestible rumen bypass protein
IDBDM	Intestinal digestible rumen bypass dry matter
IDBNDF	Intestinal digestible rumen bypass neutral detergent fiber
IDST	Intestinal digestible rumen bypass starch
iNDF	Indigestible neutral detergent fiber
Kd	Degradation rate of degradable fraction
Kp	Passage rate

MCP$_{RDP}$	Microbial protein synthesized in the rumen based on rumen degraded protein
MCP$_{TDN}$	Microbial protein synthesized in the rumen based on available energy (total digestible nutrients at a production level)
ME	Metabolizable energy
ME$_{p3\times}$	Metabolizable energy at a production level (3× maintenance)
MP	Metabolizable protein (NRC Dairy model)
MREE	Microbial protein synthesized in the rumen based on available energy
MREN	Microbial protein synthesized in the rumen based on rumen degraded feed crude protein
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NE$_g$	Net energy for gain
NE$_{Lp3\times}$	Net energy for lactation at a production level (3× maintenance)
NEm	Net energy for maintenance
NFC	Non-fiber carbohydrate
NPN	Non-protein nitrogen
OEB	Degraded protein balance (DVE/OEB system)
PA2	Soluble true protein (rapidly degradable true protein)
PB1	Insoluble true protein (moderately degradable true protein)
PB2	Fiber bound protein (slowly degradable true protein)
PC	Indigestible protein
PDI	Pellet durability index
RDNDF	Rumen degradable fiber
RDP	Rumen degradable protein

RUDM	Rumen undegradable dry matter
RUNDF	Rumen undegradable neutral detergent fiber
RUP	Rumen undegradable protein
S	Soluble fraction (washable in NDF)
SCP	Soluble crude protein
T0	Lag time
tdCP	Truly digestible crude protein
TDDM	Total digestible dry matter
tdFA	Truly digestible fatty acid
$TDN_{1\times}$	Total digestible nutrients at a maintenance level
TDNDF	Total digestible fiber
tdNDF	Truly digestible neutral detergent fiber
tdNFC	Truly digestible non-fiber carbohydrate
TDP	Total digestible crude protein
TDST	Total digestible starch
U	Rumen undegradable fraction

1. GENERAL INTRODUCTION

Due to the great worldwide demand of oil for human consumption and fuel for industry, new crops became interesting and after processing the seeds, co-products are available. These by-products become a promising feed for animals; this is the case of canola meal from bio-oil processing which is frequently used in ruminant rations (Canola Council, 2015). On the other hand, from bio-fuel processing of *Brassica carinata* seed, grown since 2012, a co-product (carinata meal) is obtained and has become accessible in Canada (Xin and Yu, 2013b). In addition, other co-products from pulse processing industry are available; pea screenings have gained consideration as components of feeds for dairy cattle due to high protein and starch level (Yu et al., 2002). However, in order to use these co-products more effectively, the rate and extent of degradation have to be decreased to improve nitrogen utilization. The rapid rumen degradation (rate and extent) can be decreased using suitable feed processing such as pelleting, and through the use of a feed additive such as lignosulfonate. Pelleting and lignosulfonate improve protein and glucose absorption in the small intestine of dairy cows potentially increasing growth rates and milk yield, due to the rise of rumen undegradable protein to be absorbed. Pelleting these co-products, which each have a unique amino acid profile but not optimal, will result in a blend pelleted product with more balanced and optimal amino acid profile which can meet the requirements for high producing milking cows. However, there is a little information about the nutrient profile of carinata meal, particularly when it is blended with other co-products as a pellet. The aim of this project was to develop and test eight blend pelleted products based on the combination of new co-product of bio-fuel processing of carinata seed, conventional co-product from bio-oil processing of canola seed, pea screenings and lignosulfonate at different levels for ruminants and systemically study the chemical and nutritional characteristics of the blend pelleted products. The first two chapters are

introduction and literature review. The third chapter contains the evaluation on chemical, amino acid, glucosinolates, condensed tannin, energy profiles, and protein and carbohydrate fractions. The fourth chapter covers rumen degradation kinetics, intestinal digestion, and the potential nitrogen to energy synchronization. Finally, the fifth chapter covers investigation of the metabolic characteristics, truly digested nutrient supply and feed milk value of the blend pelleted products in dairy cows.

2. LITERATURE REVIEW

2.1 Utilization of Canola and Carinata Co-Products in North America

2.1.1 Development and Production of Canola and Carinata Co-Products

Rapeseed now termed canola, was cultivated in Europe and Asia as a source of lamp oil and more recently for cooking oil (Australian Government, 2002). Beginning in the 1970s, and with the use of conventional plant breeding methods, canola was developed from rapeseed and nowadays it is one of the most important crops in Canada (Canola Council, 2015; Evans and Callum, 2015). Canola is an offspring of rapeseed (*Brassica campestris/rapa* and *Brassica napus*), which was bred using conventional techniques to obtain oil with low erucic acid (< 2 %), and because the negative repercussion on palatability and toxic properties in humans and livestock, the amount of glucosinolates was reduced (Mailer et al., 2008) to low levels (< 30 µmol/g) in the meal (Australian Government, 2002). The term "canola" (Canadian oil) was created to distinguish it from rapeseed. Canola especially in European countries is known as "double-zero rapeseed" (low glucosinolates and low erucic acid) to identify "canola quality" seed, oil and meal (Canola Council, 2015). Every year, about 8 million hectares of canola are seeded. In 2013, canola production was over 15 million tonnes (Canola Council, 2013; Statistics Canada, 2013). These seeds contain around 44 % oil, which are mainly used as culinary oils. After the oil is obtained, the seed residue solids are processed into a high protein meal which is a useful feed of livestock (Canola Council, 2015; Canola Council, 2009; Downey, 1990). Canola meal (CM) is rich in vitamins B and E and is used in ruminant, turkey, swine and aquaculture feed (Li et al., 2013; Statistics Canada, 2009).

Brassica carinata is also a species of the Brassica family, frequently known as Ethiopian mustard because it was thought to originate from Ethiopia and other areas of East Africa (Rakow, 2004). *Brassica carinata* originated from ancestral hybridization between *Brassica oleracea* and

Brassica nigra (Ban, 2016; Hayward, 2011; Warwick et al., 2006). The increased demand for vegetable-based bio-fuel in the world in order to partially replace fossil fuel, provided the opportunity for a profitable oil crop which can grow in areas with climate limitations, such as semi-arid regions (Agrisoma, 2015). *Brassica carinata* has recently been paid attention and interest is increasing not only for bio-fuel production, but also for its adaptability (Cardone et al., 2003). This is mainly due to its agronomic performance in South Europe and North Africa areas that have negative environmental conditions for the cultivation of canola. Due to its drought and heat tolerance (Malik, 1990; Singh et al., 1988), the crop is now being considered as an alternative to *Brassica napus* and *Brassica juncea* in dry areas of Canada such southern Alberta and southern Saskatchewan, and as a potential oil crop in Spain, India, and Italy (Agrisoma, 2015; Velasco et al., 1999; Rakow, 1995). Carinata is better adapted and more productive than canola in clay and sandy-type soils, semiarid climates and under low cropping system conditions (Cardone et al., 2003). However, carinata has lower oil concentration than canola. But *Brassica carinata* shows a wide range of applications including producing bio-diesel and as a lignocellulose crop to generate heat and power (Bouaid et al., 2005). Hemicelluloses represent a consistent part of the *Brassica carinata* straw, which makes it particularly interesting for energy applications. The remaining is material that can be extracted by solvents for oil and meal production (Stamigna et al., 2012).

Agriculture and Agri-Food Canada (AAFC) developed *Brassica carinata* cultivar which meets the growth requirements in the dry prairie areas (Ban, 2016). Consequently, some regions with semiarid climates, such as the southern prairies of Canada (Alberta, Saskatchewan, Manitoba) and the Northern Plains of the United States, are showing more interest in this vigorous crop for bio-fuel or bio-oil production, resulting in substantial carinata meal left as co-product (Xin and Yu, 2013a). However, information on nutrient profile, nutrient supply and availability of carinata

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meal is rare (Xin and Yu, 2014), and this situation is a real obstacle for its effective utilization in animal feeds specially when this co-product is blended with another feedstuffs (Xin and Yu, 2013c).

2.1.2 Features of Canola and Carinata Meal

Canola is one of the most widely produced crops in Canada. It changes from rapeseed, by containing lower amount of glucosinolates and erucic acid (Canola Council, 2015). The oil extracted is highly appreciated for cooking, and the remaining meal is added to ruminant, swine, poultry and fish diets. Canola meal is the second most extensively traded vegetable protein (Evans and Callum, 2016) and it is a very palatable protein source for ruminant animals. When fed a mash diet, heifers consumed more of canola meal in the first three minutes than those fed soybean meal, demonstrating the highly palatable nature of canola meal (Sporndly and Asberg, 2006). The reasons for the high degree of palatability are not known but may be related to the high sucrose content (Canola Council, 2009). Canadian canola meal guaranteed a minimum crude protein (CP) of 36.0 % (8.5 % moisture basis), even though the actual protein content usually is 36-39 %. Canola meal is considered to have a premium protein quality and has low content of glucosinolates (Theodoridou and Yu, 2013). Canola meal contains a good amino acid profile for animal feeding. Comparable to other vegetable sources of protein it is limiting in lysine, however it is distinguished for its high levels of sulfur amino acids (methionine 1.94 and cysteine 2.37 % CP) (Evans and Callum, 2016; Canola Council, 2009; Christensen, 2006). The seed's oil must contain < 2 % erucic acid and the meal < 30 µmol of four individual glucosinolates per gram in order to be recognized as canola. In some cases the glucosinolate levels in canola meal have been reduced to 11 µmol/g; however sinapine continued at classical levels of 12-15 g/kg (Huang et al., 2015; Mailer et al., 2008; Bell, 1993). A survey was conducted by the Canola Council of Canada in twelve Canadian

meal processing plants. Beginning in 2011, samples were collected three times per year for four consecutive years (Canola Council, 2015). Results of its composition are: CP 41.7 %, lysine 5.92 % CP, methionine 1.94 % CP, histidine 3.39 % CP, acid detergent fiber (ADF) 18.4 %, neutral detergent fiber (NDF) 28.8 %, lignin 5.8 %, fat 3.75 %, linoleic acid 0.76 %, linolenic acid 0.37 %, erucic acid 0.05 %, calcium 0.74 %, phosphorus 1.13 %, glucosinolates 4.2 µmol/g.

Carinata oil is obtained when the seed is smashed like other oilseed crops, such as soybean and canola. However, unlike those oilseeds, carinata is not destined for human consumption; the oil is destined to industrial application, principally bio and jet fuel production. After this crushing process, co-product from brassica carinata seed, carinata meal is obtained (Agrisoma, 2015). Co-products from bio-oil industry are potentially an attractive feedstuff for animals and are extensively used as an outstanding source of protein, such as canola meal which is widely accepted for ruminant diets (Xin and Yu, 2013b). However, nowadays co-product from bio-fuel industry are observed as a potential feedstuff for animals.

Information available on protein nutrient and metabolic supply profiles of *Brassica carinata* meal is very little and this can be a complication for its effective utilization in animal diets (Xin and Yu, 2013a). Nevertheless, it is common to find this basic nutrient composition of carinata meal: DM 88.5 %; CP 44.3 % DM; NDF 23.7 % DM; ADF 16.3 % DM; lignin 5.9 % DM; ether extract 2.1% DM; starch 2.3 % DM; non-fibrous carbohydrate 24.5 % DM; ash 7.6 % DM; glucosinolates 115 µmol/g (Ban, 2016; Anderson, 2015). The amino acid profile of carinata meal showed to be rich in arginine (10.8 % CP), glutamic acid (20.7 % CP) and proline (6.5 % CP), but lower in isoleucine (4.1 % CP), leucine (6.8 % CP), valine (4.9 % CP) and tyrosine (2.5 % CP) compared with canola meal (Ban, 2016). Also, it is found that carinata meal had 1.8 % CP of methionine and 2.0 % CP of cysteine, however, those values are lower than those found in canola

meal (2.1 and 2.4 % CP, respectively) (Canola Council, 2009; Pedroche et al., 2004; Mnzava and Olsson, 1990). Rumen degradability and intestinal digestibility of the carinata and canola meal are different. Studies revealed that for rumen degradable dry matter 63.0 and 50.9 % DM, the rumen degradable protein 70.5 and 52.0 % CP, rumen undegradable protein 29.4 and 48.0 % CP, intestinally digestible protein 80.9 and 70.9 % RUP, intestinally absorbable protein 23.8 and 34.0 % CP, and total digestible protein 94.4 and 86.0 % CP, respectively (Anderson, 2015). Previous data showed that the proportion of ruminally degradable dry matter are higher in carinata, as well as ruminally degradable protein. Carinata meal contained the lowest ruminally undegradable protein and intestinally digestible protein compared with canola meal. However, carinata meal had the lowest intestinally absorbable digestible protein, while the total digestible protein was higher in carinata meal (Ban, 2016; Anderson, 2015).

Actually, there is no study on effects of combination of carinata meal with other feeds as a blend pelleted product; also, there is no study on effect of pelleting on bioactive compounds, glucosinolates and condensed tannins, amino acid profile, chemical and nutrient profiles, as well as nutrient utilization and availability in rumen and intestine in ruminants.

2.1.3   *Utilization and Benefits of Canola Meal in Ruminant Rations*

Most of the studies related to the nutritive and feeding value of canola meal for ruminants have been done with dairy cows. Sanchez and Claypool (1983) found no significant differences in milk production when cows were consuming true protein sources, although milk yields were 3.2 kg/d greater when canola meal substituted soy bean meal in its study. In other study, canola meal vs. soybean meal was compared (Huhtanen et al., 2011). The data consisted of more than two hundred treatment results that had been published over several studies. The studies in which increasing protein in the ration was accomplished by adding canola meal as compared to soybean

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meal were included in the data set. Milk yield rose by 3.4 kg when an additional kilogram of canola meal was fed, and 2.4 kg when an additional kilogram of soybean meal was provided in the diet, showing a 1 kg of milk disadvantage to soybean meal (Canola Council, 2015). A recent study with dairy cows producing ≥40 kg/d (Brito and Broderick, 2007) unquestionably shows that, even at high levels of production, canola meal is a superior protein supplement than soybean meal or cottonseed meal (Canola Council, 2009). Respect to concentrations of amino acids in the plasma (Martineau et al., 2014) conducted a meta-analysis study to compare canola with other protein sources. The results demonstrated that *Brassica napus* meal increased plasma concentrations of total amino acids, total essential as well as all individual essential amino acids. In addition, milk and blood urea–nitrogen was reduced. These data suggest that by feeding canola meal the absorption of essential amino acids rose, therefore milk protein increased, thus protein efficiency was enhanced (Canola Council, 2015).

2.1.4   Available Co-Products for Markets

Australia, China, Canada, the European Union, and India are the major producers of canola meal. In all markets, the use of canola meal is not the same (Canola Council, 2009). Markets and production of canola in Canada has been progressively increasing. Currently production is around 15 million tonnes of canola seed per year. It is targeted that by 2025 it will be a rise to 26 million tonnes per year. This of course as a response to increasing human consumption and thus increasing world demand (Canola Council, 2015). Around fifty percent of the canola seed produced in Canada is exported, while within the country the other part is processed. Importer countries use the most appreciated component of canola seed which is the oil. The seed is processed, then the co-product obtained is destined for animal feed industry application (William and Flad, 2015). Typically sold as mash or pellets, canola meal is widely available and traded, together with rapeseed meal these

protein sources are normally used as ingredients in livestock feeds around the world. Both canola and rapeseed together are the second-most extensively traded protein source, while soybean meal is the first (Statistics Canada, 2009; Canola Council, 2009). Canola meal, that is produced in Canada is sold to the United States and is primarily used by the top dairy producing states in the country. Exported canola is processed and used in pigs, poultry and fish diets. Likewise, the Canadian livestock industry utilize canola meal in dairy, swine and poultry feeds (Canola Council, 2015).

People around the world are observing for new sources of renewable fuel, and carinata may be that opportunity. There is great investment to improve the crop, and researchers selecting and develop appropriate high yielding varieties for production in several different geographies around the world. Canadian Food Inspection Agency (CFIA) approved the use of carinata meal in beef cattle ration in 2014 (Heppner, 2014). Dr. John McKinnon of the University of Saskatchewan found that carinata meal is relatively low in fibre and can be an adequate source of CP readily degradable by rumen bacteria (Personal communication). There is still no published research concerning the nutritional and metabolic effects of new AAFC carinata seeds and carinata meal for dairy cows. However, the rapid development of the bio-fuel industry and increased utilization of carinata seed in Canada and USA contribute to the accessibility of carinata meal. Previous study suggested that co-product from *Brassica carinata* could be added in dairy cattle rations as a promising high protein source (Xin and Yu, 2013a). Considering its digestion features, carinata meal could be a superior protein source for lactating dairy cows compared to canola meal, with a higher predicted milk yield (Ban, 2016). However, further study is required to completely understand the nutritive value, utilization, and availability of carinata meal, especially when it is blended with other feedstuffs, in order to improve its application in dairy cattle.

2.2   Utilization and Benefits of Inclusion of Pulses in Animal Feeds

2.2.1   Development, Production and Features of Pea Screenings

Pea (*Pisum sativum* L.) is a member of the Leguminosae family. Although the exact origin of peas is unidentified, it is mostly accepted that the crop originated in northwest Asia and then spread to Europe (Oelke et al, 1991). European explorers introduced pea into North America at the end of the 15th century. Indigenous people were growing both garden and field pea (dry pea) in Canada. The majority of field pea crops are located in western Canada and have been produced to a defined magnitude since the early 20th century. The rapid surge of field pea production in western Canada initiated in the mid-1980s (The Canadian Encyclopedia, 2015; Hickling, 2003). Today, Canada is the world's leading producer and exporter of pea and lentil. Saskatchewan is the heart of the Canadian pulse industry. The word "pulse" is derived from the Latin words "puls" which means "thick soup". In 2014, Saskatchewan farmers grew 90 % of chickpea crop, and 64 % of the dry pea crop in Canada. Although pulse crops, which include pea, bean, lentil, chickpea, faba bean, and others, all have some similarities, each crop has its own unique features (Saskatchewan Pulse Growers, 2015). Pea screenings are a byproduct obtained after cleaning the grain. The material that is removed after cleaning is referred to as dockage, which includes chaff, broken pea seeds, other grain, weed seeds and pieces of stem. After the dockage is cleaned, three products are obtained, two feed screenings which are relatively high in value and a third refusal one (McKinnon, 2015). Pulses have long been recognized as very nutritious grain because of their high-quality protein (Boye et al., 2010). In western Canada, field pea is the most extensively grown pulse crop. Pea is an outstanding source of protein, fiber, complex carbohydrates, vitamins and minerals. This nutritious legume contains 15 to 35 % protein, and high concentrations of the essential amino acids lysine and tryptophan (Elzebroek and Wind, 2008). Pea is mostly used in

pig feed because of its low content of anti-nutritional factors and high nutritive value. Pea is an energy and protein dense feedstuff. Compared to other ingredients the energy content found in peas is equivalent to that of corn and barley, also as a protein source *Pisum sativum* is comparative to sunflower meal and canola meal. Furthermore, it is demonstrated that peas are highly palatable, therefore increased intake is detected when peas were added to the diet. (Christensen, 2006; Anderson et al., 2002; Hickling et al., 2003). The basic composition of *Pisum sativum* is: CP 25.1 % DM, ether extract 1.5 % DM, ash 3.7 % DM, ADF 9.1 % DM, NDF 18.5 % DM, lignin 0.94 % DM, starch 52.0 % DM, lysine 7.4 % CP, methionine 1.19 % CP (Christensen, 2006; Christensen and Mustafa, 2000). In addition to those positive qualities of peas; they are high in important essential amino acids, particularly high in lysine, showing that *Pisum sativum* contains more lysine than soybean meal. However, peas, like majority of pulses, are low in sulfur amino acids methionine and cysteine (Saskatchewan Pulse Growers, 2015; Pownall et al., 2010; Hickling, 2003). Peas contain about 80 % of the starch content found in barley grain, while other vegetable protein sources such as soybean meal and canola meal contain low levels of starch. Additionally, field peas contain twice as much protein as barley grain, 50 % of the protein found in soybean meal, about 65 % of the protein found in canola meal, and around 40 % of the CP found in rumen microbes. On the amino acid profile, pea protein is very high in lysine (7.4 % CP) compared to cereal grains and most oil seed meals (Christensen, 2006; Christensen and Mustafa, 2000).

2.2.2 *Utilization and Benefits of Pea Screenings in Ruminant Rations*

Studies on the effect of feeding peas to dairy cows are few and the results are not consistent. Because of the higher effective degradability of CP in peas compared to soybean meal (78 vs. 65 %) (Khorasani et al., 2001) and a lower RUP content compared to soybean meal (22 vs. 35 %) milk yield could be reduced in early lactation when the requirements for rumen bypass protein is

high (Corbett et al., 1995). Some studies have confirmed previous data. The decrease in milk yield is accredited to the higher rumen degradation of pea protein (Anderson et al., 2002; Khasan et al., 1989). However, research suggested that when diets based on peas contain the same percentage of RUP as soybean meal and canola meal, peas could substitute canola meal and soybean meal in the rations of early lactation high-producing cows without modifying milk yield (Corbett et al., 1995). Hadsell and Sommerfeldt (1988) conducted a study which demonstrated that peas can completely substitute the concentrate dry matter for dairy cattle rations during early lactation. However, some studies suggested that based on milk protein percentage and feed efficiency, the successful rate of pea inclusion was closer to half the total of the concentrate (Mustafa, 2002). It has been demonstrated that the replacement of peas for soybean meal and barley at levels of 33, 67 and 100 % of the concentrate did not affect dry matter intake, CP intake, milk yield, and duodenal nitrogen factions (Khorasani et al., 2001).

2.3 Utilization of Feed Additives

2.3.1 *Utilization and Benefits of Lignosulfonate*

Lignosulfonate is a bio-polymer that is completely soluble in water. It often has a sugar component and 'lignin sulfonate' is recognized by AAFCO as source of metabolizable energy (AAFCO, 2013; Morrison, 1968). Lignosulfonate (Calcium Lignosulfonate CaLS) has been used industrially in a diversity of applications. Due to the binding properties demonstrated by lignosulfonate, it is used as a pellet binder in animal feed to improve pellet quality (Corey et al., 2014) therefore, lignosulfonate inclusion significantly improved pellet quality as measured by PDI (Wamsley and Moritz, 2013). In addition, to the binding property lignosulfonate often provides extra lubrication in the processing method, being beneficial for industry equipment (Corey, 2013; Pfost, 1976). On the other hand, soybean meal treated with lignosulfonate efficaciously decreased

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degradation of soybean protein in cultures of rumen contents (Windschitl and Stern, 1988). Also, the effective rumen degradability of CP in canola meal is successfully decreased with 5 % of lignosulfonate and heat, compared with heating without lignosulfonate. Furthermore, increasing the concentration of lignosulfonate to 10 % caused an additional decline of effective rumen degradability of CP in canola meal compared to the treatment with 5 % of lignosulfonate (McAllister et al., 1993).

2.4 Application of Pelleting in the Animal Feed Industry

2.4.1 Introduction of Pelleting

Pelleting is a process where a ground mix of feed ingredients is forced through a metal plate with cylindrical holes, referred to as a die (Rakic, 2012). Pelleting can be defined as "agglomerated feeds formed by extruding individual ingredients or mixtures by compacting and forcing through die openings by any mechanical process" (Behnke and Scott Beyer, 2001). Essentially, pelleting has an objective to take a finely divided, occasionally dusty, unpalatable and difficult-to-handle feed material and, by using moisture, pressure and heat, form larger particles, called pellets. Pellets are more palatable, became easier to handle and frequently as a consequence improved feeding results (Game and Maktos, 2015). In most designs, the die rotates around the fixed rollers, then the feed is obligated through the die due to the pressure caused by the rolls. As feed is forced through the holes, the resulting pressure combined with the temperature which increases as a consequence of friction between the feed and the metal and between different metal parts, will result in chemical changes that cause the feed particles to be glued together (ANAC, 2013; Rakic, 2012; Payne et al., 1994).

2.4.2 Physical Quality of Pellets

Physical pellet quality potentially includes the following characteristics: good appearance, dust free, without cracks, uniform length, hard (sufficient only to withstand pressures during storage) and durable. Durability in handling is the most important characteristic (Payne et al., 1994). Pellet quality determined by the pellet durability index (PDI) and the percentage of fines at the mill or in the farm feeders measured the efficiency of pelleting processing method. One of the methods to measure PDI is the Holmen pellet durability tester, which utilize air to create abrasion of the pellets versus the tumbling action, which take place in the metal box of the Holmen tester. In order to model the handling process which normally take place, pellets are moved through tubes with high speed air (ANAC, 2013; Salas-Bringas et al., 2007; Behnke, 2001). Pellet quality has converted more important in the swine and poultry industries; while other industries continue identifying the worth of feeding high quality pellets (Behnke, 2001).

2.4.3 *Benefits of Pelleting for the Feed Industry and Animal Nutrition*

The cost of animal feed is significant. The total cost of animal production may be increased due to feed processing methods (Nolan et al., 2010). Feed processing methods provide opportunities to increase the value of feedstuffs and therefore animal performance will be improved (Huang, 2015; Abdollahi et al., 2013). One of the forms of feed processing is pelleting animal feed. It is important for improving efficiency in animal feeding and for suitable feed handling. The effect of feed form (meal vs. pellets) on animal performance has been studied. It is considered that feeding pelleted feed improves animal performance and feed conversion compared to feeding meal (Behnke and Scott Beyer, 2001). The physical form of the pellet is related to the improvement in animal performance and according to Behnke (1994), the improvements are due to: decreased feed wastage; reduced selective feeding; decreased ingredient segregation; less energy and time consumed for prehension; destruction of pathogenic organisms; thermal

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modification of protein and starch; improved palatability and allowing larger meals to be eaten in less time. All these factors contribute to optimized feed efficiency (Winowiski, 1995). Historically animal producers have observed a 6 to 8 % improvement in performance when animals are fed pellets (ANAC, 2013). Research has demonstrated that feeding animals with good-quality pellets improved growth performance and feed conversion than feeding animals with pellets with more fines or mash feed (Zatari et al., 1990). In order to survive repeated handling processes and reduce fines by mechanical action during transport good-quality pellets are needed (Mina-Boac et al., 2006; Behnke, 1994). Additionally, pelleting has been used in animal feed processing and it has proved favorable in improving protein digestibility of single and compound feeds (Yu et al., 2002; Thomas and Van der Poel, 1996). Processing methods of the feed can alter the degradation and passage rates of feeds through the digestive system of the animals (Van der Poel et al., 1995). In the feed industry, it is often assumed that pelleting of concentrate mixtures decreases protein degradability due to the heat increment during conditioning and pelleting (Theodoridou and Yu, 2013; Goelema et al., 1999). Therefore, pelleting improves rumen crude protein degradation in dairy cows (Goelema et al., 1999) and also, degradation of resistant starch in the rumen (Tamminga and Goelema, 1995), which consequently resulted in more bypass starch and protein needed to meet nutritional requirements of high production milking cow (Huang et al., 2015).

2.5 Feed Evaluation Techniques and Methods

2.5.1 Glucosinolates Determination

Glucosinolates are a group of sulphur-containing glycosides distributed principally in the family *Brassicaceae* which, after tissue damage, are hydrolysed in a variety of products which show toxic and antinutritive effects, therefore limiting possible utilization of the meal (Velasco et al., 1999; Mithen et al., 1987). Glucosinolates are secondary metabolites recognised for their role

in plant resistance to pathogens and insects (Sønderby et al., 2010). The high level of glucosinolates in Brassica carinata meal prevents the direct use as an animal feed, unless the glucosinolates are previously removed (Cardone et al., 2003). Various technical treatments and methods have been considered in order to diminish the glucosinolate content and increase the nutritional value such as water extraction, heat and copper sulphate treatments (Tripathi and Mishra, 2007; Jensen, 1993). Ruminants are comparatively more tolerant to glucosinolate intake than monogastrics and adults are more tolerant compared to young animals. Reduced palatability, thus less intake and therefore decreased growth and production are the main harmful properties of glucosinolate ingestion in animals (Tripathi and Mishra, 2007). However, microflora in the digestive system of ruminants induce transformation of glucosinolates and/or their metabolites, which are related to the adverse effects (Mandiki et al., 2002; Wallig et al., 2002). Ruminant animals are tolerant to dietary glucosinolates intake, however long-term feeding of diets which contain glucosinolates causes goitrogenicity, decreases thyroxin and elevates thiocyanate levels in the plasma (Vincent et al., 1988). It has been found that a dietary glucosinolate level of 11 µmol/g should be safe for ruminants (Tripathi and Mishra, 2007).

2.5.2 *Condensed Tannin Evaluation*

Condensed tannins are natural plant polyphenolic compounds that bind to proteins via hydrophobic interactions and hydrogen bonding (Mueller-Harvey, 2006). Plant condensed tannins can have beneficial or adverse effects on ruminant production. It depends on their concentration and nature, animal species, physiological state of the animal and composition of the ration (Hymes-Fecht et al., 2013; Patra and Saxena, 2011). Tannins are a group of phenolic compounds which have the ability to form reversible and irreversible complexes mainly with proteins, cellulose, hemicellulose, pectin, nucleic acids and minerals (Frutos et al., 2004). Studies showed that the

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consumption of plant species with high condensed tannin content (generally > 5 % DM) significantly decreases voluntary feed intake, while medium or low consumption (< 5 % DM) seems not to affect it (Waghorn et al., 1994). The decrease of ruminal protein degradation may be the most important effect of condensed tannins (Mueller-Harvey and McAllan, 1992). Literature proposed treatments which can reduce condensed tannin content, such alkali, formalin (Kumar and Singh, 1984) and more recently treatment with polyethylene glycol, polyvinyl-polypyrrolidone, calcium hydroxide. (Makkar, 2001; Ben Salem et al., 2000). The digestive utilization of feed by ruminants is improved when the intake is under (< 5 % DM, between 1 and 4 % of DM), mostly due to the decrease of protein degradation in the rumen, therefore, greater availability of essential amino acids reaching the small intestine to be absorbed (Min et al., 2003; Barry and McNabb, 1999). However, condensed tannin level above 6 % DM in the ration undesirably affect growth rates and milk production (Cannas, 2015).

2.5.3   Amino Acids Determination

The chemical structure of a protein, high molecular weight compound, is made up of about 20 different amino acids (Dalibard et al., 2014). The term amino acid is practically always used to refer to an α-amino carboxylic acid (Wade, 2009). Peptide bonds linked the carboxyl group of one amino acid with the α-amino group of other one (Häffner et al., 2003). These amino acids are main components of animal nutrition, as supplemented individual products, but also as part of a protein-containing diet (Pei et al., 2010). Proteins are needed for dairy production; however, the potential to cover the physiological requirements in terms of amino acids for maintenance and performance determines the quality of protein supply. The amino acid supply for ruminants comes from dietary protein which is not degraded in the rumen, also called by-pass protein, and protein from microbes synthesized in the rumen, called microbial protein (Dalibard et al., 2014; NRC, 2001). The protein

form the diet is broadly degraded within the rumen and is mostly used for rumen bacteria protein synthesis. This microbial protein that reaches the intestine presents the most suitable protein quality for ruminants (Dalibard et al., 2014). Although, the amino acid profile of the microbial protein meets the requirements to synthesize milk protein, the quantity of amino acids which reach the small intestine is not enough to cover the demand of high producing cows, therefore feeding rumen undegraded protein is needed to complement those requirements (Häffner et al., 2003; NRC, 2001). Milking cows use free amino acids in order to synthesize milk protein, as any other protein. Feed rumen bypass protein and rumen microbial protein are the two sources of those amino acids. Those amino acids are digested and absorbed in the small intestine and then circulated to the mammary gland and all other tissues in the blood (Doepel and Lapierre, 2006). There are ten essential amino acids which include arginine (Arg), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val). Essential amino acids cannot be synthesized by the animal or if they are (arginine and histidine), their production is not enough to meet requirements, particularly during the early stages of growth or for high levels of production (NRC, 2001). In usual diets for ruminants methionine and lysine generally are the first limiting amino acids, also histidine is considered as sometimes limiting (Hansen, 2016; Dalibard et al., 2014; Doepel and Lapierre, 2006).

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

At the beginning of the 1990's was introduced the Cornell Net Carbohydrate and Protein System (CNCPS) (Van Amburgh et al., 2015). The updates to the CNCPS described here represent changes that have been made to CNCPS v6.0 (Tylutki et al., 2008) resulting in CNCPS v6.5. Predictions of nutrient requirements and supply are presented. The feed library is described in a companion paper (Higgs et al., 2015). One additional change in the description of feed chemistry

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that affects nutrient supply, the application of unavailable NDF is described in Raffrenato (2011) and Van Amburgh et al., (2015) and it is determined by a 240-h in vitro digestion. The CP and carbohydrate subtractions were partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS). The characterization of the CP fractions as applied in the CNCPS v6.5 system is as follows: fraction PA1 is ammonia and is calculated using the following formula PA1 = ammonia × (SP/100) × (CP/100) and its degradation rate (Kd) is 200 %/h; fraction PA2 which refers to soluble true protein PA2 = SP × CP/100 − PA1 and its Kd range is 10-40 %/h; fraction PB1 is referred to as insoluble true protein and is calculated with the following formula PB1 = CP − (PA1 − PA2 − PB2 − PC); and its Kd range is 3-20 %/h; PB2 fraction refers to fiber-bound protein and is equal to (NDICP − ADICP) × CP / 100, and its Kd range is 1-18 %/h, and PC fraction which is indigestible protein is calculated as PC = ADICP × CP / 100. The carbohydrate fractions are determined as: fraction CB2 soluble fiber which is calculated with the following formula CB2 = NFC − CA1 − CA2− CA3 − CA4 − CB1 and its Kd range is 20-40 %/h; CA fraction refers to volatile fatty acids and is equal to CA1 = Acetic + Propionic + (Butyric + Isobutyric); CA2 refers to lactic acid and its Kd value is 7 %/h; CA3 refers to other organic acids with Kd value is 5 %/h; CA4 water soluble carbohydrates (WSC) and its Kd range is 40-60 %/h; CB1 starch Kd range is 20-40 %/h; CC fraction which is indigestible fiber and calculated as CC = (aNDFom × (Lignin × aNDFom) × 2.4)/100 or, aNDFom × uNDFom and CB3 fraction which is digestible fiber and calculated as follow CB3 = aNDFom – CC, and its Kd range is 1-18 %/h (Higgs et al., 2015; Van Amburgh et al., 2015). After 288 hours of in situ incubation the iNDF were determined. Samples bags (3 grams) were incubated in the rumen using 2 cows. After complete incubation, the bags were washed and cleaned 6 times with cold water and then dry 48 h at 55º C (Huhtanen et al., 1994).

2.5.5   Energy Evaluation in Feeds and Animal Diet Ingredients

National Research Council dairy summative approaches (NRC, 2001) were used to determine values of the truly digestible crude protein (tdCP), the truly digestible fatty acid (tdFA), the truly digestible non-fiber carbohydrates (tdNFC), the truly digestible neutral detergent fiber (tdNDF), the total digestible nutrients at 1x maintenance (TDN_{1x}), the total digestible nutrients at 3x maintenance (TDN_{3x}), the digestible energy (DE_{1x}), the digestible energy at the production level of 3x maintenance (DE_{p3x}), the metabolizable energy at the production level of 3x maintenance (ME_{p3x}), and the net energy at the production level of 3x maintenance (NEL_{p3x}). The NRC beef was used to estimate the metabolizable energy (ME), the net energy for maintenance (NE_m) and the net energy for gain (NE_g) (NRC, 1996).

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

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

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

where R(t) = residue present at t h incubation (%); U = undegradable fraction (%); D = potentially degradable fraction (%); Kd = degradation rate (h−1) and T0 = lag time (h).

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

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

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

where, S stands for soluble fraction (%); Kp stands for estimated passage rate from the rumen (h-1) and was assumed to be 6 %/h for DM, CP and Starch, but 2.5 %/h for NDF. The factor 0.1 in the formula represents that 100 g/kg of soluble fraction (S) escapes rumen fermentation (Tamminga et al., 1994).

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

BDM (BST or BNDF) (g/kg DM) = DM (ST or NDF) (g/kg DM) × % BDM (BST or BNDF)

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

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

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

where 1.11 refers to the regression coefficient between in situ RUP and in vivo RUP (Yu et al., 2002; Tamminga et al., 1994)

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

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

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

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

The effective rumen degradation ratios of N and energy were calculated hourly as modified from Sinclair et al. (1993) as below:

$$\text{Hourly ED ratio N/CHO}_t = 1000 \times (HEDN_t - HEDN_{t-1}) / [(HEDNDF_t - HEDNDF_{t-1}) + (HEDST_t - HEDST_{t-1})],$$

where N/CHO $_t$ = ratio of N to CHO at time t (g N/kg CHO); HEDN $_t$ = hourly effective degradability of N at time t (g/kg DM); HEDN $_{t-1}$ = hourly effective degradability of N 1 h before t (g/kg DM); HEDCHO $_t$ = hourly effective degradability of CHO at time t (g/kg DM); HEDNDF $_t$ = hourly effective degradability of neutral detergent fiber at time t (g/kg DM); HEDNDF $_{t-1}$ = hourly effective degradability of neutral detergent fiber at 1 h before t (g/kg DM); HEDST $_t$ = hourly effective degradability of starch at time t (g/kg DM); HEDST $_{t-1}$ = hourly effective degradability of starch at 1 h before t (g/kg DM). Data reported in previous studies suggested that 32 g N / kg CHO truly digested in the rumen is the optimum ratio to balance microbial protein synthesis and energy cost in regard to rumen fermentation (Sinclair et al., 1993; Tamminga et al., 1990).

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

The estimation of intestinal digestion was determined by a modification of the three-step in vitro procedure described by Calsamiglia and Stern (1995). Briefly, dried ground residues containing 15 mg of N after 12 h ruminal preincubation are deposited into a 50 ml centrifuge tube, after that 10 ml of pepsin (Sigma P-7012) solution (in 0.1 N HCl with pH 1.9) was added, vortexed, and incubated for 1 h at 38 °C in a water bath. After incubation, 0.5 ml 1 N NaOH solution and 13.5 ml of pancreatin (Sigma P-7545) was added, vortexed and incubated at 38 °C for 24 h vortexing every 8 h approximately. Then 3 ml of TCA were added to stop enzymatic hydrolysis. The tubes were vortexed and sit samples for 15 min at room temperature. Then, they were centrifuged for 15 min at 10000 g and analyze supernatant (5 ml) for soluble N by the Kjeldahl method. Intestinal digestion of protein is calculated as TCA-soluble N divided by the amount of N in the rumen residue sample (Gargallo et al., 2006; Calsamiglia and Stern, 1995).

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

2.5.9.1   National Research Council Dairy Model

According to the NRC (2001) model, MP is composed of three major contributory protein sources. Total MP can be calculated as follows:

$$MP \text{ (g/kg DM)} = AMCP^{NRC} + ARUP^{NRC} + AECP,$$

where, AMCP is the absorbable microbial protein, ARUP is the truly absorbable rumen undegraded feed protein, and AECP is the truly absorbable endogenous protein in the small intestine (Theodoridou and Yu, 2013; NRC, 2001).

Degraded protein balance (DBP^{NRC}), based on data from the NRC-2001 model, reflects the difference between the potential microbial protein synthesis based on RDP and the potential microbial protein synthesis based on energy available for microbial fermentation in the rumen. Thus, the DPB^{NRC} was calculated as follows:

$$DPB^{NRC} \text{ (g/kg of DM)} = RDP^{NRC} - 1.18 \times MCP_{TDN}.$$

2.5.9.2   Dutch DVE/OEB Systems

On the basis of the DVE/OEB system provided by Tamminga et al., (1994, 2007), detailed explanations and calculation are given to calculate and predict protein supply to the small intestine of dairy cows. This Dutch DVE/OEB evaluation system calculated two characteristics for each feed: the DVE which refers to the true protein digested in the intestine and the OEB which is the rumen degradable protein balance. DVE represents the protein value of a feed and can be separated into three components: feed CP rumen undegraded but digested in the small intestine (DVBE), microbial true protein synthesized in the rumen and digested in the small intestine (DVME), and endogenous protein lost in the digestive processes (ENDP); while OEB is the difference between the potential microbial protein synthesis on the basis of available rumen degradable protein and

that on the basis of available rumen degradable energy (Van Duinkerken et al., 2011; Tamminga et al., 2009; Tamminga et al., 1994). The DVE value comprises microbial protein, digestible feed protein and an endogenous protein loss correction. The DVE value is calculated as:

$$DVE \ (g/kg \ of \ DM) = DVME + DVBE - ENDP,$$

where, DVME is the absorbable fraction of microbial CP, DVBE is the absorbable fraction of ruminally undegraded feed protein, and ENDP is a correction factor for endogenous protein lost during the digestion process.

The OEB value or degradable protein balance of a feed is the difference between the potential microbial crude protein synthesis based on MREN and the potential microbial crude protein synthesis based on energy extracted from anaerobic fermentation MREE. Therefore

$$OEB \ ^{DVE} \ (g/kg \ of \ DM) = MREN - MREE,$$

where, MREN is calculated as $MREN = CP \times [1 - (1.11 \times RUP \ (\% \ CP)/100)]$. The factor 1.11 in the formula was taken from the French PDI system and represents the regression coefficient of in vivo, on in situ degradation data. $MREE = FOM \times 0.15$ (FOM in g/kg) (Theodoridou and Yu, 2013; Tamminga et al., 1994).

2.5.10 Feed Milk Value Determination in Dairy Cattle

Feed Milk Value (FMV) determined on the basis of metabolic characteristics of protein from the NRC and DVE models, the feed milk values were determined. Protein composition in milk is assumed to be 33 g protein / 1 kg of milk, and the efficiency of use of metabolizable protein for lactation is assumed to be 0.67 (Theodoridou and Yu, 2013; NRC, 2001).

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

Co-products are being used as a protein source in the livestock industry, including canola meal, which is frequently used in ruminant diets (Canola Council, 2009). The new co-product from bio-fuel processing of carinata seed has become accessible in North America (Xin and Yu, 2013b). Pea screenings from pulse processing industry are considered as components of feedstuffs for dairy cattle due to their high starch and protein content (Yu et al., 2002). These co-products have unique amino acid profile, but are not optimal for animals. The amino acid profile could become more balanced and optimal after blending with the new co-product with pea screenings. In addition, the degradation of these co-products should be decreased to be used more efficiently. Rapid feed rumen degradation (rate and extent) can be reduced through both addition of a feed additive (lignosulfonate) and suitable feed processing (pelleting processing method) to improve true protein and glucose accessibility to be absorbed in the small intestine of dairy cows.

2.6.1   Project Hypotheses

- The blend pelleted products as a main concentrate are expected to optimize nutrient and amino acid supply in dairy cattle.

- Pelleting blended ingredients could modify energy and protein characteristics and bioactive compounds levels, while lignosulfonate addition could reduce rumen degradation and increase pellet durability index.

2.6.2   Project Objectives

Long-term:

- To increase knowledge of the optimal nutrient supply to ruminants through feed pellet processing and optimal feed ingredients through blending.

- To increase economic return to pulse and bio-fuel processing, pulse seeds producers and related industries.

Short-term:

- To test and develop blended pelleted products based on combination of co-products from bio-fuel/bio-oil processing (new co-product of carinata meal vs. conventional co-product of canola meal, with high level of protein and unique profile of amino acid), pea screenings (good available energy and unique amino acid profile), and lignosulfonate chemical compound (feed additive: to maximize nutrient utilization and availability) for ruminants for both domestic and international markets.

3. PELLETING AND PROCESSING INDEX, CHEMICAL PROFILES, BIOACTIVE COMPOUNDS, ENERGY AND NUTRIENT FRACTIONS OF BLEND PELLETED PRODUCTS BASED ON COMBINATION OF CO-PRODUCTS FROM BIO-FUEL/BIO-OIL PROCESSING, PEA SCREENINGS AND LIGNOSULFONATE AT DIFFERENT LEVELS FOR RUMINANTS.

3.1 Abstract

The aim of this study was to develop and test eight pelleted products based on combinations of co-products from bio-fuel processing (carinata meal), bio-oil processing (canola meal), pea screenings and lignosulfonate at different levels for ruminants. The eight products include:

BPP1: lignosulfonate 0 % of DM + carinata meal 50 % of DM + pea screenings 50.0 % of DM;

BPP2: lignosulfonate 4.8 % of DM + carinata meal 47.6 % of DM + pea screenings 47.6 % of DM;

BPP3: lignosulfonate 0 % of DM + carinata meal 75 % of DM + pea screenings 25 % of DM;

BPP4: lignosulfonate 4.8 % of DM + carinata meal 71.4 % of DM + pea screenings 23.8 % of DM;

BPP5: lignosulfonate 0 % of DM + canola meal 50 % of DM + pea screenings 50.0 % of DM;

BPP6: lignosulfonate 4.8 % of DM + canola meal 47.6 % of DM + pea screenings 47.6 % of DM;

BPP7: lignosulfonate 0 % of DM + canola meal 75 % of DM + pea screenings 25 % of DM;

BPP8: lignosulfonate 4.8 % of DM + canola meal 71.4 % of DM + pea screenings 23.8 % of DM. Comparisons were made between blend pelleted products based on carinata meal and pelleted products based on canola meal, high or low level of inclusion of co-product (low or high level of inclusion of pea screenings) and inclusion or no inclusion of lignosulfonate in terms of chemical, amino acid, energy, glucosinolate and condensed tannin profiles as well as their protein and carbohydrate fractions. The results showed that pellet durability index (PDI) in all pelleted products ranged from 88.5 to 97.5 %; total glucosinolates from 3.46 to 5.86 µmol/g and condensed

tannins from 0.188 to 0.293 g/kg DM (P< 0.05). Canola pelleted products BPP8 and BPP7 contained higher (P< 0.05) levels of methionine (1.94 and 1.98 % CP) than the other blend pelleted products. Canola blend pelleted products contained higher (P< 0.05) lysine ranging from 5.71 to 5.90 % CP than carinata based pelleted products ranging from 4.20 to 4.43 % CP, while carinata based pelleted product BPP3 provided higher total amino acid content (38.46 % DM) than the other pelleted products. Carinata pelleted products BPP3, BPP4 and canola pelleted product BPP7 had higher (P< 0.05) CP (45.0, 43.1 and 41.0 % DM, respectively). Canola based pelleted product BPP7 had the highest (P< 0.05) content of NDF, ADF and ADL (22.6, 15.2 and 6.7 % DM, respectively) than the other blend pelleted products, except BPP8. Carinata based pelleted product BPP3 had the highest (P< 0.05) NE_L, NE_m and NE_g (2.01, 2.17, 1.49 Mcal/kg DM, respectively) than the other pelleted products. All canola based pelleted products showed higher levels of indigestible protein (PC, P< 0.05, 3.1 vs. 1.4 % of CP) and soluble true protein (PA2, P< 0.05, 40.1 vs. 29.5 % of CP). All blend pelleted products have safe levels of glucosinolates and condensed tannins. Also, carinata meal based blend pelleted products had higher nutritive value and could be used as a potential source of protein and energy for ruminants.

Keywords: Pelleting, Carinata, Canola, Pea, Lignosulfonate

3.2 Introduction

The bio-fuel industry has been growing in North America and thus co-products are left after processing. These co-products from bio-fuel and bio-oil processing of seeds have become promising feeds for animals and are being used as sources of protein in livestock industry, such as canola meal which often is included in ruminant diets (Xin and Yu, 2013b). Brassica carinata, commonly known as Ethiopian mustard, has an oil profile optimized for use in the bio-fuel industry. Carinata crops are suited to grow in semiarid regions and have excellent harvestability

with good lodging and shatter resistance (Agrisoma, 2015). Regions with semiarid climates, such as southern prairies of Canada are showing more and more interest in this crop for bio-fuel production, resulting in substantial carinata meal left as bio-fuel co-product (Xin and Yu, 2013a). This co-product (Carinata meal) from bio-fuel processing of Brassica carinata seed has become accessible (Xin and Yu, 2013b). However, little information is available on glucosinolates, amino acid and nutrient profile. It is expected that this new co-product has different structure and unique amino acid profile. It may be like the conventional co-product (canola meal) from bio-oil processing, whose amino acid profile is not optimized nor balanced for animals. It is estimated that this new carinata meal, similar to other vegetable sources of protein (canola meal) may be limiting in lysine but high in methionine and cysteine (Goihl, 2012; Canola Council, 2009). The pulse processing industry often produces pea screenings (*Pisum sativum*) or pea /lentil screenings (byproduct). Peas have promising and desirable nutritional characteristics for dairy rations, they are palatable and contain over 20 % crude protein (CP) (Christensen, 2006). Also, these low grades of peas and screenings still contain high starch content and thus provide high energy (NDSU, 2002). This byproduct has a unique amino acid profile, high in essential amino acids lysine and tryptophan, but deficient in sulfur amino acids, methionine and cysteine (Pulse Canada, 2003). Our initial study shows that carinata meal has a high rate and extent of degradation of protein, just like conventional co-product of canola seed (Xin and Yu, 2014). Also, the rumen degradation of pea screenings is extremely high (Mustafa, 2002). However, rapid rumen degradation (rate and extent) can be reduced through both addition of a feed additive (lignosulfonate) and suitable feed processing (pelleting processing method) (Thomas et al., 1998; Thomas et al., 1997; Thomas and van der Poel, 1996; Windschitl and Stern, 1988). Much effort is needed to fully understand this new co-product in terms of chemical profile, anti-nutrition factors and nutritive value particularly

when it is blended with other feedstuffs as a blended pelleted feed. For that reason, I decided to conduct this study in which the objective was to develop and test eight blend pelleted products based on the combination of new co-product from bio-fuel processing of carinata seed, conventional co-product from bio-oil processing of canola seed, pea screenings and lignosulfonate at different levels for ruminants in terms of chemical, amino acid, glucosinolate, condensed tannin and energy profile as well as the protein and carbohydrate fractions. Comparisons were made between blend pelleted products based on carinata meal versus canola meal, no lignosulfonate vs. lignosulfonate addition, and low level of inclusion of these co-products vs. high level of inclusion.

3.3 Materials and Methods

3.3.1 Ingredients

Different co-products were used to develop the blend pelleted products. Carinata meal (1 sample) from bio-fuel processing of *Brassica carinata* seed was obtained from Agrisoma (Saskatoon, SK Canada). Canola meal (1 sample) from bio-oil processing of canola seed was sourced from Cargill Animal Nutrition (Clavet, SK, Canada). Pea screenings (1 sample) byproduct of pulse production came from ILTA Grain Company (Surrey, BC, Canada) and finally lignosulfonate (1 sample) chemical compound (Ameribond) was used as a feed additive. Eight combinations were developed (40 kg of each combination) with four different levels of inclusion of bio-fuel and bio-oil co-products carinata or canola meal (50.0, 47.6, 75.0 and 71.4 % of DM); four levels of inclusion of pea screenings (50.0, 47.6, 25.0 and 23.8 % of DM) and two levels of inclusion of lignosulfonate (0 and 4.8 % of DM). All the ingredients were obtained through the Canadian Feed and Research Centre (North Battleford, SK, Canada).

3.3.2 Pellet Processing

The combinations were mixed in the Scott Equipment model TSM 363 (New Prague, MN, USA) for a period of two minutes, and then Colorado Mill Equipment ECO-R30 (Cañon City, CO, USA) was used to pellet all different mixtures at 65°C and then pelleted through a 3.6 mm diameter die. Residence time in the die did not exceed 15 seconds. Immediately, after pelleting the temperature of the products was 71°C. Before collect and store the pelleted products, these were cooled to room temperature (21°C). Detailed information of each blend pelleted product (BPP) (Table 3.1.) is as follow. BPP1 = Blend Pelleted Product 1: lignosulfonate 0 % of DM + Low level co-product from bio-energy processing (carinata meal - CR: 50 % of DM), High level of pea screenings (PS: 50.0 % of DM); BPP2 = Blend Pelleted Product 2: lignosulfonate 4.8 % of DM + Low level co-product from bio-energy processing (carinata meal - CR: 47.6 % of DM), High level of pea screenings (PS: 47.6 % of DM); BPP3 = Blend Pelleted Product 3: lignosulfonate 0 % of DM + High level co-product from bio-energy processing (carinata meal - CR: 75 % of DM), Low level of pea screenings (PS: 25 % of DM). BPP4 = Blend Pelleted Product 4: lignosulfonate 4.8 % of DM + High level co-product from bio-energy processing (carinata meal - CR: 71.4 % of DM), Low level of pea screenings (PS: 23.8 % of DM); BPP5 = Blend Pelleted Product 5: lignosulfonate 0 % of DM + Low level co-product from bio-oil processing (canola meal - CN: 50 % of DM), High level of pea screenings (PS: 50.0 % of DM); BPP6 = Blend Pelleted Product 6: lignosulfonate 4.8 % of DM + Low level co-product from bio-oil processing (canola meal - CN: 47.6 % of DM), High level of pea screenings (PS: 47.6 % of DM); BPP7 = Blend Pelleted Product 7: lignosulfonate 0 % of DM + High level co-product from bio-oil processing (canola meal - CN: 75 % of DM), Low level of pea screenings (PS: 25 % of DM); BPP8 = Blend Pelleted Product 8: lignosulfonate 4.8 % of DM + High level co-product from bio-oil processing (canola meal - CN: 71.4 % of DM), Low level of pea screenings (PS: 23.8 % of DM). There were conducted two batches of pellet

processing as replications at the Canadian Feed Research Centre (CFRC) of the University of Saskatchewan.

Table 3.1. Blend pelleted products with different combinations (two levels of lignosulfonate compound, two types of co-products from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings) were produced at CFRC feed processing center

| Blend Pelleted Products (BPP) | Blending | | | |
	Level of Lignosulfonate compound (% of DM)	Co-product from bio-fuel or bio-oil processing	Level of Co-products (% of DM)	Level of Pea Screenings (% of DM)
Blend Pelleted Product 1 (BPP1)	No (0 %)	Carinata meal (CR)	Low level, 50.0 %	High level 50.0 %
Blend Pelleted Product 2 (BPP2)	Add (4.76 %)	Carinata meal (CR)	Low level, 47.6 %	High level, 47.6 %
Blend Pelleted Product 3 (BPP3)	No (0 %)	Carinata meal (CR)	High level, 75.0 %	Low level 25.0 %
Blend Pelleted Product 4 (BPP4)	Add (4.76 %)	Carinata meal (CR)	High level, 71.4 %	Low level, 23.8 %
Blend Pelleted Product 5 (BPP5)	No (0 %)	Canola meal (CN)	Low level, 50.0 %	High level 50.0 %
Blend Pelleted Product 6 (BPP6)	Add (4.76 %)	Canola meal (CN)	Low level, 47.6 %	High level, 47.6 %
Blend Pelleted Product 7 (BPP7)	No (0 %)	Canola meal (CN)	High level, 75.0 %	Low level 25.0 %
Blend Pelleted Product 8 (BPP8)	Add (4.76 %)	Canola meal (CN)	High level, 71.4 %	Low level, 23.8 %

BPP1: (low level of carinata meal, high level of pea screenings and no lignosulfonate); BPP2: (low level of carinata meal, high level of pea screenings and lignosulfonate); BPP3: (high level of carinata meal, low level of pea screenings and no lignosulfonate); BPP4: (high level of carinata meal, low level of pea screenings s and lignosulfonate); BPP5: (low level of canola meal, high level of pea screenings and no lignosulfonate); BPP6: (low level of canola meal, high level of pea screenings and lignosulfonate); BPP7: (high level of canola meal, low level of pea screenings and no lignosulfonate); BPP8: (high level of canola meal, low level of pea screenings and lignosulfonate); CN: canola meal; CR: carinata meal.

3.3.3 Pellet Durability Index

Just after cooling, the Borregaard LignoTech Holmen tester Serial Nº LT 218 (Sarpsborg, Norway) was used to determine the pellet durability index (PDI). A sample of 100 g of each blend pelleted product was placed in the chamber, subjected to a jet of air for 30 seconds, and then weighed. A direct read of pellet durability index expressed on a percentage basis was recorded. During this process, fines were removed (Behnke, 2001; MacMahon and Payne, 1981).

3.3.4 Chemical Analysis

The samples were ground through a 1 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) and analyzed for sugars (AOAC official method 974.06); CP (AOAC official method 984.13); EE (AOAC official method 920.39); Ash (AOAC official method 942.05) (AOAC, 1990); NDICP, ADICP and NPN were analyzed using the methods described in Licitra et al., (1996); SCP was estimated by incubating sample with bicarbonate-phosphate buffer then filtering through Whatman filter paper (Roe et al. 1990); ADF, NDF and ADL were determined using the procedures of (Van Soest et al., 1991); Cellulose and Hemicelluloses were estimated: Hemicellulose = NDF - ADF, and Cellulose = ADF - ADL according to NRC (2001); Total, structural and non-structural CHO were determined using NRC (2001) and Van Soest et al., (1991). Total carbohydrate (CHO) was estimated as: CHO = 100 - EE - CP - ash. Non-fiber carbohydrate was estimated as: NFC = 100 - (NDF - NDIP) - EE- CP - ash, as given by the NRC-2001 (NRC, 2001). Starch was analyzed using the Megazyme Total Starch Assay Kit (Wicklow, Ireland) and by the α-amylase/amyloglucosidase method. (McCleary et al., 1999). All samples were analyzed in duplicate and repeated if chemical analysis error was in excess of 5 %.

3.3.5 Amino Acid Profile

The Complete Amino Acid Profiles (AAP) of the blend pelleted products were determined using the AOAC Official Method 982.30 E (a, b, c), chp. 45.3.05, 2006 at Cumberland Valley Analytical Services (Maugansville, MD, United States of America). The following amino acids were determined: hydroxyproline, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, hydroxylysine and lysine. Also, taurine which is in itself not an amino acid in the scientific sense because it does not contain a carboxy group and lanthionine which is a non-proteinogenic amino acid were determined.

3.3.6 Energy Profile

Energy values, total digestible nutrient (TDN), as well as digestible energy, metabolizable energy, and net energy, are commonly used for estimation of available energy in feedstuffs. Truly crude protein (tdCP), digestible nonfiber carbohydrate (tdNFC), neutral detergent fiber (tdNDF), and fatty acid (tdFA), total digestible nutrient at $1\times$ maintenance (TDN_{1x}), digestible energy at production level of intake (DE_{3x}), metabolizable energy at a production level of intake (ME_{3x}), and net energy at a production level of intake (NE_{L3x}) were determined using a summative approach from the NRC-2001 dairy (NRC, 2001). Net energy for maintenance (NE_m) and net energy for growth (NE_g) were estimated using NRC-1996 beef (NRC, 1996).

3.3.7 Glucosinolates Profile

The official method AOCS Ak 1-92 was used to determine the glucosinolate profile of the blend pelleted products at POS Bio-Sciences (Saskatoon, SK, Canada). This method, adopted from Part 1 of ISO 9167, specifies a procedure for the determination of the content of different glucosinolates by extraction in a methanol solution, then purification and enzymatic desulfation

on ion-exchange resins, followed by determination using reversed-phase high-performance liquid chromatography (HPLC) with gradient elution and ultraviolet detection (AOCS, 2011).

3.3.8   Condensed Tannin Profile

Condense tannins (CT) were analyzed using HCL Butanol method. Briefly, chemicals needed: Concentrated HCl (36 %); Butanol (n-butanol/1-butanol/butyl alcohol) with CAS71-36-3. Reagent: HCl Butanol. Measure 475 ml butanol and 25 ml concentrated HCl. Briefly the procedure was as follow: 1) Weighed 20 - 30 mg finely ground sample into screw-cap tubes in quadruplicates (4 tubes) per sample. 2) Added 5 ml of HCl butanol reagent to all 4 tubes, and seal. 3) Left one tube at room temperature for 60 min (sample blank), rest of 3 tubes from each sample incubated in water bath at 95° C (heat treated) for 60 min. Vortexed all tubes every 10 min. 4) Cooled the tubes to room temperature with cold water. Centrifuged for 10 min at 3000 rpm. 5) Turned on a spectrophometer for 20 min before use. 6) Set wavelength at 550 nm, zero the spectrophotometer with HCl Butanol reagent as reagent blank. 7) Read absorbance (abs) of solutions including sample blanks. 8) Results were expressed as abs at 550 nm per mg of sample (Theodoridou, 2012; Matthäus and Angelini, 2005).

3.3.9   Protein and Carbohydrate Subfractions

The crude protein and carbohydrate subtractions were partitioned according to the Cornell Net Carbohydrate and Protein System (CNCPS). The characterization of the CP fractions as applied in the CNCPS 6.5 system is as follows: fraction PA1 is ammonia and is calculated using the following formula $PA1 = ammonia \times (SP/100) \times (CP/100)$, and its degradation rate (Kd) is 200 %/h; fraction PA2 which refers to soluble true protein $PA2 = SP \times CP/100 - PA1$ and its Kd range is 10-40 %/h; fraction PB1, is referred to as insoluble true protein and is calculated with the following formula $PB1 = CP - (PA1 - PA2 - PB2 - PC)$ and its Kd range is 3-20 %/h; PB2 fraction refers to fiber-

bound protein and is equal to (NDICP − ADICP) × CP / 100) and its Kd range is 1-18 %/h and

PC fraction which is indigestible protein is calculated as PC = ADICP × CP / 100. The

carbohydrate fractions are determined as: fraction CB2 soluble fiber which is calculated with the

following formula CB2 = NFC − CA1 − CA2− CA3 − CA4 − CB1 and its Kd range is 20-40 %/h;

CA fraction refers to volatile fatty acids and is equal to CA1 = Acetic + Propionic + (Butyric +

Isobutyric); CA2 refers to lactic acid and its Kd value is 7 %/h; CA3 refers to other organic acids

with Kd value 5 %/h; CA4 refers to water soluble carbohydrates (WSC) and its Kd range is 40-

60 %/h; CB1 starch Kd range is 20-40 %/h; CC fraction which is indigestible fiber is calculated as

CC = (aNDFom × (Lignin × aNDFom) × 2.4)/100 or, aNDFom × uNDFom and CB3 fraction

which is digestible fiber is calculated as CB3 = aNDFom – CC, and its Kd range is 1-18 %/h

(Higgs et al., 2015; Van Amburgh et al., 2015).

3.3.10 Statistical Analysis

Profiles data of the pelleted products were statistically analyzed using the mixed model

procedure of SAS 9.4. The model was:

$$Y_{ij} = \mu + T_i + e_{ij},$$

where Y_{ij} is an observation of the dependent variable ij; μ is the population mean for the variable;

T_i is the effect of the blend pelleted product (BPP) as a fixed effect, processing batch was used as

replicates (2 batches); and e_{ij} is the random error associated with the observation ij. For all

statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$. Differences among

the treatments were evaluated using a multiple comparison test using the Tukey method. Contrast

statements were used to compare the differences between carinata meal pelleted products and

canola meal pelleted products, high and low level of inclusion of those co-products (low and high

level of inclusion of pea screenings), addition and no addition of lignosulfonate.

3.4 Results and Discussion

3.4.1 Pelleted Products and Pellet Durability Index

Pelleting as a processing method agglomerates smaller particles into larger particles using heat, moisture and pressure (Falk, 1985; Skoch et al., 1981). The benefits of pelleting include improved palatability and hygienic condition of feed, increased feed bulk density and flowability, reduced dust and improved transportation efficiency. Additionally, pelleting method affects the metabolic and digestion characteristics of feeds by decreasing protein and starch rumen degradation (Huang et al., 2015; Thomas and van der Poel, 1996). The feed additive lignosulfonate has been shown to be useful in animal feeds in improving pellet quality as measured by PDI (Corey et al., 2014; Wamsley and Moritz, 2013) as well as pea starch, which is an adequate binder for pellet feeds (NDSU, 2016). Pellet durability index of the blend pelleted products are shown in Table 3.2. Carinata blend pelleted products BPP2, BPP4, and canola blend pelleted products BPP6, BPP8 had higher PDI ($P< 0.05$) (97.0, 96.7, 97.2 and 97.5 %, respectively) than the other blend pelleted products. The main reason of this is that BPP2, BPP4, BPP6, and BPP8 blend pelleted products contain 4.8 % lignosulfonate in their compositions. Blend pelleted products containing lignosulfonate had higher PDI (+4.2 %) than the blend pelleted products without lignosulfonate. This indicates that lignosulfonate not only improves nutrient utilization by decreasing feed rumen degradation but also increases pellet durability. Carinata pellets had lower PDI than the canola blend pelleted products. Lower level of inclusion of pea screenings or higher level of inclusion of co-products also resulted in pellets with lower ($P< 0.05$) PDI (-2.2 %).

Table 3.2. Pellet Durability Index (PDI) of blend pelleted products with different combinations (two levels of lignosulfonate chemical compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

| Items | Blend pelleted products (BPP) | | | | | | | | SEM | P value | Contrast, P value | | |
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Pellet Durability Index PDI (%)	94.8[b]	97.0[a]	88.5[d]	96.8[a]	95.5[b]	97.3[a]	93.0[c]	97.5[a]	0.34	<0.01	<0.01	<0.01	<0.01

SEM: standard error of mean; [a-d] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; CN: canola meal; CR: carinata meal.

3.4.2 Glucosinolates Profile

Glucosinolates are a large group of plant metabolites which contain sulphur and are present in varieties of Brassica family. Generally young animals are more sensitive to glucosinolates than adults. Glucosinolates are known to reduce intake (Hill, 1991), produce thyroid disturbances, depress fertility (Ahlin et al., 1994) and cause mortality (Tripathi and Mishra, 2007). The reduced intake of diets which contain glucosinolates is due to the presence of progoitrin and sinigrin which are associated with bitter taste (Fenwick et al., 1982). In calves, 1.2 to 2.4 µmol/g showed no adverse effect on thyroid and liver function (Anderssen and Sorensen, 1985); in steers 10 to 15 µmol/g showed no detrimental effect on growth and feed conversion (Bush et al., 1978); in dairy cows 11.0 µmol/g induced iodine deficiency (Laarveld et al., 1981), 11.7 to 24.3 µmol/g depressed feed intake and milk production (Waldern, 1973), ≥23.0 µmol/g reduced intake and milk production (Ingalls and Sharma, 1975) and 31.0 µmol/g produced thyroid disturbance and depressed fertility (Ahlin et al., 1994).

POS Bio-Sciences changed the previous Canadian Grain Commission Method for the new AOCS Ak 1-92 Official Method in order to determine glucosinolates on December 2015. The profile of the carinata based pelleted products has glucosinolates which are not the same to the predominant ones in the canola based pelleted products. Glucosinolates profiles of all blend pelleted products are presented in Table 3.3. Canola based pelleted product BPP7 and BPP8 have the highest (P< 0.05) level of progoitrin (2-OH-3-Butenyl) (2.49 and 2.33 µmol/g, respectively) than the other pelleted products. All carinata based pelleted products (BPP1, BPP2, BPP3 and BPP4) showed lower (P< 0.05) level (0.13 µmol/g) of progoitrin than canola based blend pelleted products. BPP7 had the highest (P< 0.05) level of epi-progoitrin (Epi-2-OH-3-Butenyl) (0.05 µmol/g) than the rest of the products. Canola pelleted products had higher levels of progoitrin and

epiprogoitrin (2.02 vs. 0.13 and 0.12 vs. 0.02 µmol/g, respectively) than the carinata blend pelleted products and by adding a lower level of pea screenings or a higher level of co-product results in the BBP products containing higher levels of progoitrin and epiprogoitrin (+0.78 and +0.02 µmol/g). Canola pelleted products have the highest (P< 0.05) level of gluconapoleiferin (2-OH-4-Pentenyl) (0.05 vs. 0.02 µmol/g) than the carinata pelleted products. Carinata based pelleted product BPP3 has the highest (P< 0.05) level of sinigrin (Prop-2-Enyl) (4.86 µmol/g) than the other pelleted products. Canola based pelleted products (BPP5, BPP6, BPP7 and BPP8) showed lower levels of sinigrin (0.03, 0.02, 0.02 and 0.02 µmol/g, respectively). Carinata pelleted products had higher levels of sinigrin (+3.76 µmol/g) than the canola blend pelleted products and adding a lower level of pea screenings or a higher level of co-product results in the BBP products containing higher levels of sinigrin. Canola based pelleted product BPP7 has the highest (P< 0.05) level of glucoalisin (5-CH3-Sulfinyl-Pentyl), gluconapin (3-Butenyl), 4-OH-3-Indolylmethyl, glucobrassicanapin (4-Pentenyl), glucobrassicin (3-Indolymethyl) and Phenethyl (0.49, 1.16, 1.06, 0.12, 0.27 and 0.12 µmol/g, respectively), however those values are not significantly different form the corresponding ones of the BPP8 (0.47, 1.13, 0.99, 0.11, 0.26 and 0.12 µmol/g, respectively). Canola pelleted products had higher levels of glucoalisin (5-CH3-Sulfinyl-Pentyl), gluconapin (3-Butenyl), 4-OH-3-Indolylmethyl, glucobrassicanapin (4-Pentenyl), glucobrassicin (3-Indolymethyl) and phenethyl than the carinata blend pelleted products and by adding a lower level of pea screenings or a higher level of co-product results in the BPP products containing higher levels of glucoalisin (5-CH3-Sulfinyl-Pentyl), gluconapin (3-Butenyl), 4-OH-3-Indolylmethyl, glucobrassicanapin (4-Pentenyl), glucobrassicin (3-Indolymethyl) and phenethyl. Also, adding lignosulfonate reduced the level of gluconapin in the canola based pelleted products. Blend pelleted products with a higher level of co-products (either carinata meal or canola meal) or lower

level of pea screenings (BPP3, BPP7, BPP8) had a higher (P< 0.05) level of total glucosinolates (5.34, 5.86, 5.54 µmol/g, respectively). Previous studies demonstrated that carinata meal had higher levels of glucosinolates than canola meal (Ban, 2016; Anderson, 2015), however possible increment of heating during the bio-fuel processing of carinata seed, or possible increment of time under heating during the process could diminish the glucosinolates content found in carinata meal. Therefore, in this study canola based blend pelleted products have significantly higher levels of total glucosinolates than carinata meal (4.76 vs. 4.28 µmol/g). Also, the non addition of lignosulfonate and higher level of inclusion of co-products, either carinata or canola meal, result in blend pelleted products with higher level of total glucosinolates (+0.33 and +1.7 µmol/g). These results indicate that the levels of glucosinolates in all the blend pelleted products do not cause any significant risk to the health of adult cattle. Additionally, pelleting did not affect the glucosinolate levels in the blend pelleted products.

3.4.3 Condensed Tannin Profile

The presence of condensed tannins in feed ingredients for monogastric animals generally is regarded unfavorably. However, these secondary compounds can be beneficial or detrimental to ruminants, depending on type and amount ingested, the structure of the tannin and the physiology of the consuming animals (Frutos et al., 2004; Hagerman and Butler, 1991). Forages with low to moderate levels of condensed tannins contributed to higher retention of nitrogen in cattle. In these cases, the lower apparent and true digestibility of nitrogen was compensated for by reduced urinary loss of nitrogen (Cannas, 2015). The intake of under 50 g CT/kg DM (10 - 40 g/kg DM) improves the digestive utilization of feed by ruminants, mainly because of a reduction in ruminal protein degradation and, as a consequence, a greater availability of amino acids for absorption in the small intestine (Addisu, 2016; Barry and McNabb, 1999; Schwab, 1995), which result in higher growth

rates and milk yield. On the other hand, even in ruminants, levels of tannins exceeding 5 % in the diet negatively affect growth and milk production (Cannas, 2015). Profiles of condensed tannin (0.019 to 0.033 % DM) are presented in Table 3.3. In this study by adding a lower level of pea screenings or a higher level of co-product results in the BBP products containing higher levels of condensed tannins. Additionally, pelleting did not affect the condensed tannins levels in the blend pelleted products.

Table 3.3. Glucosinolate profile of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

| Items | Blend pelleted products (BPP) | | | | | | | | SEM | P value | Contrast, P value | | |
| | | | | | | | | | | | Co-P CR vs. CN | LSC No vs. Add | Co-P Low vs. High |
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8					
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Glucosinolates Profile (µmol/g)													
2-OH-3-Butenyl (progoitrin)	0.13[c]	0.13[c]	0.13[c]	0.13[c]	1.71[b]	1.55[b]	2.49[a]	2.33[a]	0.035	<0.01	<0.01	0.01	<0.01
Epi-2-OH-3-Butenyl (epi-progoitrin)	0.02[b]	0.02[b]	0.02[b]	0.02[b]	0.02[b]	0.02[b]	0.05[a]	0.03[b]	0.002	<0.01	<0.01	0.02	<0.01
Prop-2-Enyl (sinigrin)	3.17[c]	3.05[c]	4.86[a]	4.32[b]	0.03[d]	0.02[d]	0.02[d]	0.02[d]	0.064	<0.01	<0.01	0.01	<0.01
2-OH-4-Pentenyl (gluconapoleiferin)	0.02[b]	0.02[b]	0.02[b]	0.02[b]	0.04[ab]	0.04[ab]	0.07[a]	0.05[ab]	0.006	<0.01	<0.01	0.23	0.03
5-CH3-Sulfinyl-Pentyl (glucoalisin)	0.18[e]	0.17[e]	0.23[cde]	0.21[de]	0.35[bc]	0.32[cd]	0.49[a]	0.47[ab]	0.023	<0.01	<0.01	0.28	<0.01
3-Butenyl (gluconapin)	0.06[c]	0.06[c]	0.07[c]	0.06[c]	0.79[b]	0.72[c]	1.16[a]	1.13[a]	0.011	<0.01	<0.01	<0.01	<0.01
4-OH-3-Indolylmethyl	0.02[c]	0.02[c]	0.02[c]	0.02[c]	0.69[b]	0.65[b]	1.06[a]	0.99[a]	0.042	<0.01	<0.01	0.34	<0.01
4-Pentenyl (glucobrassicanapin)	0.02[c]	0.02[c]	0.02[c]	0.02[c]	0.08[b]	0.08[b]	0.12[a]	0.11[a]	0.002	<0.01	<0.01	0.02	<0.01
3-Indolymethyl (glucobrassicin)	0.03[c]	0.04[c]	0.03[c]	0.03[c]	0.21[b]	0.19[b]	0.27[a]	0.26[a]	0.009	<0.01	<0.01	0.69	<0.01
Phenethyl	0.02[c]	0.02[c]	0.03[c]	0.03[c]	0.09[b]	0.09[b]	0.12[a]	0.12[a]	0.003	<0.01	<0.01	0.17	<0.01
4-Methoxy-3-IndolylCH3	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.003	0.57	0.20	1.00	0.20
Total	3.57[c]	3.46[c]	5.34[ab]	4.77[b]	4.00[c]	3.67[c]	5.86[a]	5.54[a]	0.106	<0.01	<0.01	<0.01	<0.01
Condensed Tannin													
CT (abs 550 nm / mg)	0.021[b]	0.026[ab]	0.032[ab]	0.036[a]	0.028[ab]	0.030[ab]	0.033[ab]	0.037[a]	0.0023	0.02	0.08	0.08	<0.01

Table 3.3. *Cont'd* Glucosinolate profile of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	Blend pelleted products (BPP)								SEM	P value	Contrast, P value		
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
CT (% DM)	0.019[b]	0.023[ab]	0.028[ab]	0.031[ab]	0.025[ab]	0.026[ab]	0.030[ab]	0.033[a]	0.0022	0.03	0.11	0.11	<0.01
CT (g/kg DM)	0.188[b]	0.227[ab]	0.280[ab]	0.312[a]	0.249[ab]	0.261[ab]	0.293[ab]	0.322[a]	0.0217	0.03	0.09	0.11	<0.01
CT (mg/kg DM)	188[b]	227[ab]	279[ab]	311[a]	249[ab]	261[ab]	293[ab]	321[a]	21.6	0.03	0.09	0.10	<0.01

SEM: standard error of mean; [a-e] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; abs: absorbance; CN: canola meal; CR: carinata meal.

3.4.4 Amino Acid Profile

Rations for dairy cows should be balanced for amino acids instead for protein. Amino acids requirements are not definitively established for all of them. Balancing for metabolizable protein with adequate proportions for at least lysine and methionine seems to be the best alternative (Doepel and Lapierre, 2006). Canola meal is principally well enriched with amino acids methionine and cysteine (1.94 and 2.37 % CP) (Evans and Callum, 2016), also it is an outstanding source of histidine and threonine. The abundance of these amino acids explains the consistent milk yield response found when canola meal is included in dairy cow rations (Canola Council, 2009).

On the other hand, peas contain high levels of the important essential amino acids, especially lysine. Peas contain higher levels of lysine than soybean meal (Christensen and Mustafa, 2000). Also, peas, like most pulses, are low in methionine and cysteine. However, combining canola meal with peas allows that the high level of lysine in peas to complement the lower content of lysine in canola meal and the high content of methionine and cysteine in canola meal to complement the lower levels found in peas (Martineau et al., 2014; Pulse Canada, 2003). The amino acid profiles of the blend pelleted products as a % of CP are presented in Table 3.4. The contents of taurine, glutamic acid, proline, lanthionine, leucine, tyrosine, phenylalanine and histidine (P> 0.05) were not significant among all the treatments. Canola based pelleted product BPP7 has the numerically higher level of hydroxyproline than the other blend pelleted products. Carinata based pelleted product contains lower (P< 0.05) levels of serine (-0.3 % CP) than the canola based pelleted product. Canola based pelleted product showed higher (P< 0.05) level of alanine than the carinata blend pelleted products (4.17 to 4.35 vs. 3.91 to 4.01 % CP). BPP8 showed the numerically higher (P< 0.05) level of aspartic acid, threonine, glycine and valine (7.18, 4.05, 4.92, 5.08 % CP, respectively) than the other treatments. But, those values are not significantly different from BPP5,

BPP6 and BPP7. Canola based pelleted products had higher levels of hydroxyproline, alanine, aspartic acid, threonine, glycine and valine than the carinata based blend pelleted products. Adding a lower level of pea screenings or a higher level of co-product results in the BBP products containing higher levels of hydroxyproline, aspartic acid, threonine and glycine. Carinata based pelleted products showed higher ($P < 0.05$) level of cysteine than canola based pelleted products (2.14 to 2.31 vs. 1.98 to 2.13 % CP). Adding a lower level of pea screenings or a higher level of co-product results in the BPP products containing higher levels of cysteine (+0.14 % CP). Carinata based pelleted product contains lower levels of isoleucine than the canola based pelleted product. BPP8 showed the highest ($P < 0.05$) level of methionine and hydroxylysine (1.98, 0.31 % CP, respectively) than the other blend pelleted products, however those values are not significantly different form BPP7. Canola based pelleted products contained higher levels of methionine (+0.20 % CP) and hydroxylysine (+0.17 % CP) than the carinata blend pelleted products. Adding a lower level of pea screenings or a higher level of co-product results in the BBP products containing higher levels of methionine (+0.13 % CP). Canola based pelleted products (BPP5, BPP6, BPP7 and BPP8) showed higher ($P < 0.05$) levels of lysine (5.71, 5.74, 5.83, 5.90 % CP, respectively) than the carinata based pelleted products (BPP1, BPP2, BPP3 and BPP4) which showed higher levels of arginine (6.22, 6.25, 6.42 and 6.38 % CP, respectively). Canola pelleted products contained higher levels of lysine (+1.48 % CP) and lower levels of arginine (-0.45 % CP) than carinata based pelleted products. Adding a lower level of pea screenings or a higher level of co-product results in the BBP products containing higher levels of tryptophan (1.08 vs. 1.00 % CP). Adding lignosulfonate reduces the content of tryptophan in the pelleted products (-0.09 % CP).

The amino acid profiles of the blend pelleted products as a % of DM are presented in Table 3.5. Carinata blend pelleted products BPP3 contained the highest (P< 0.05) total amino acid content (38.46 % DM) than the other blend pelleted products followed by BPP4 and BPP7 (36.87, 37.36 % DM). There is no significant difference between BPP4 and BPP7 total amino acid values. Carinata based pelleted products provide higher levels of total amino acids based on DM (+1.23 % DM) than the canola based pelleted products. Adding a lower level of pea screenings or a higher level of co-product results in the BBP products containing higher total amino acid content (+5.65 % DM) and by adding lignosulfonate, pelleted products with lower total amino acid content are obtained (-1.8 % DM).

Table 3.4. Individual and total amino acid composition profiles on crude protein basis of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	Blend pelleted products (BPP)								SEM	P value	Contrast, P value		
											Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8					
Lignosulfonate %DM	No	4.8	No	4.8	No	4.8	No	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product, %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Crude Protein, %DM	38.8^{bc}	36.4^{cd}	45.0^a	43.1^a	35.9^{cd}	33.7^d	41.9^{ab}	38.9^{bc}	0.66	<0.01	<0.01	<0.01	<0.01
Individual Amino Acids Composition on CP basis (% of CP).													
Taurine	0.21	0.19	0.21	0.19	0.21	0.20	0.22	0.21	0.013	0.40	0.19	0.07	0.64
Hydroxyproline	0.18^c	0.27^{bc}	0.37^{abc}	0.37^{abc}	0.43^{ab}	0.38^{abc}	0.56^a	0.50^a	0.060	<0.01	<0.01	0.93	<0.01
Aspartic Acid	6.50^b	6.57^b	6.23^b	6.22^b	7.30^a	7.37^a	7.08^a	7.18^a	0.088	<0.01	<0.01	0.38	<0.01
Threonine	3.52^c	3.57^{bc}	3.64^{bc}	3.64^{bc}	3.82^{abc}	3.83^{ab}	4.02^a	4.05^a	0.054	<0.01	<0.01	0.53	<0.01
Serine	3.50b^c	3.50^{bc}	3.42^c	3.48^c	3.73^{ab}	3.78^a	3.79^a	3.78^a	0.074	0.02	<0.01	0.63	0.87
Glutamic Acid	17.49	17.67	17.70	17.77	17.09	17.13	16.82	16.98	0.228	0.08	<0.01	0.47	0.86
Proline	5.54	5.56	5.53	5.58	5.61	5.63	5.66	5.86	0.070	0.13	0.03	0.16	0.17
Lanthionine	0.13	0.27	0.27	0.28	0.24	0.25	0.13	0.24	0.051	0.32	0.53	0.11	0.87
Glycine	4.33^c	4.40^c	4.54^{bc}	4.54^{bc}	4.63^{abc}	4.65^{abc}	4.82^{ab}	4.92^a	0.064	<0.01	<0.01	0.29	<0.01
Alanine	3.91^c	3.98^{bc}	4.00^{bc}	4.01^{bc}	4.17^{abc}	4.20^{abc}	4.25^{ab}	4.35^a	0.058	<0.01	<0.01	0.22	0.05
Cysteine	2.14^{ab}	2.18^{ab}	2.31^a	2.31^a	2.02^b	1.98^b	2.13^{ab}	2.13^{ab}	0.038	<0.01	<0.01	0.96	<0.01
Valine	4.58^c	4.65^{bc}	4.68^{bc}	4.68^{bc}	4.86^{abc}	4.90^{abc}	4.99^{ab}	5.08^a	0.068	<0.01	<0.01	0.33	0.06
Methionine	1.62^d	1.65^d	1.76^c	1.76^c	1.78^c	1.87^{bc}	1.94^{ab}	1.98^a	0.028	<0.01	<0.01	0.02	<0.01
Isoleucine	3.66^b	3.69^{ab}	3.70^{ab}	3.72^{ab}	3.88^{ab}	3.89^{ab}	3.91^{ab}	3.99^a	0.051	0.02	<0.01	0.35	0.20
Leucine	6.48	6.57	6.52	6.55	6.75	6.77	6.80	6.96	0.090	0.05	<0.01	0.28	0.33
Tyrosine	2.43	2.43	2.43	2.42	2.51	2.53	2.54	2.54	0.039	0.17	0.01	1.00	0.73
Phenylalanine	3.98	4.03	3.89	3.92	4.05	4.07	3.97	4.03	0.053	0.31	0.08	0.32	0.07
Hydroxylysine	0.13^d	0.12^d	0.11^{de}	0.08^e	0.28^{bc}	0.25^c	0.29^{ab}	0.31^a	0.006	<0.01	<0.01	0.03	0.17

Table 3.4. *Cont'd* Individual and total amino acid composition profiles on crude protein basis of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

| Items | Blend pelleted products (BPP) | | | | | | | | SEM | P value | Contrast, P value | | |
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	No	4.8	No	4.8	No	4.8	No	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product, %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Lysine	4.40[b]	4.43[b]	4.20[b]	4.24[b]	5.71[a]	5.74[a]	5.83[a]	5.90[a]	0.072	<0.01	<0.01	0.43	0.64
Histidine	2.37	2.38	2.43	2.44	2.39	2.40	2.48	2.51	0.029	0.07	0.07	0.58	<0.01
Arginine	6.22[ab]	6.25[ab]	6.42[a]	6.38[a]	5.83[b]	5.84[b]	5.85[b]	5.93[ab]	0.087	<0.01	<0.01	0.69	0.11
Tryptophan	1.05[abc]	0.93[c]	1.11[ab]	1.06[ab]	1.06[ab]	0.98[bc]	1.12[a]	1.04[abc]	0.025	0.01	0.33	<0.01	<0.01
Total Amino Acid (% of CP)	84.33[d]	85.28[cd]	85.47[cd]	85.65[bcd]	88.33[abc]	88.61[abc]	89.19[ab]	90.44[a]	1.096	0.03	<0.01	0.41	0.21

SEM: standard error of mean; [a-e] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison: Tukey method; CN: canola meal; CR: carinata meal

Table 3.5. Individual and total amino acid content profiles (on dry matter basis) of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	Blend pelleted products (BPP)								SEM	P value	Contrast, P value		
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	No	4.8	No	4.8	No	4.8	No	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product, %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Amino Acids, %DM													
Taurine	0.08^{ab}	0.07^{b}	0.10^{a}	0.08^{b}	0.07^{b}	0.07^{b}	0.10^{a}	0.08^{b}	0.006	0.02	0.53	0.01	0.01
Hydroxyproline	0.07^{c}	0.10^{bc}	0.17^{abc}	0.16^{abc}	0.15^{abc}	0.13^{bc}	0.23^{a}	0.20^{ab}	0.027	<0.01	<0.01	0.56	<0.01
Aspartic Acid	2.52^{de}	2.40^{f}	2.81^{b}	2.68^{c}	2.62^{cd}	2.48^{ef}	2.97^{a}	2.79^{b}	0.018	<0.01	<0.01	<0.01	<0.01
Threonine	1.36^{de}	1.30^{ef}	1.64^{ab}	1.57^{c}	1.37^{d}	1.29^{f}	1.69^{a}	1.58^{bc}	0.013	<0.01	0.11	<0.01	<0.01
Serine	1.36^{c}	1.28^{d}	1.54^{ab}	1.50^{b}	1.34^{cd}	1.27^{d}	1.59^{a}	1.48^{b}	0.023	<0.01	0.94	0.00	<0.01
Glutamic Acid	6.78^{cd}	6.44^{e}	7.96^{a}	7.65^{b}	6.14^{f}	5.77^{g}	7.05^{c}	6.61^{de}	0.049	<0.01	<0.01	<0.01	<0.01
Proline	2.15^{cd}	2.03^{de}	2.49^{a}	2.40^{ab}	2.01^{de}	1.90^{e}	2.37^{ab}	2.28^{bc}	0.028	<0.01	<0.01	<0.01	<0.01
Lanthionine	0.05	0.10	0.12	0.12	0.09	0.09	0.06	0.09	0.022	0.32	0.29	0.19	0.36
Glycine	1.68^{c}	1.60^{cd}	2.04^{a}	1.95^{ab}	1.67^{c}	1.57^{d}	2.02^{a}	1.92^{b}	0.017	<0.01	0.05	<0.01	<0.01
Alanine	1.52^{c}	1.45^{de}	1.80^{a}	1.72^{b}	1.50^{cd}	1.42^{e}	1.78^{a}	1.69^{b}	0.010	<0.01	0.01	<0.01	<0.01
Cysteine	0.83^{d}	0.79^{d}	1.04^{a}	0.99^{b}	0.72^{e}	0.67^{f}	0.89^{c}	0.83^{d}	0.008	<0.01	<0.01	<0.01	<0.01
Valine	1.78^{c}	1.70^{cd}	2.11^{a}	2.01^{ab}	1.75^{cd}	1.65^{d}	2.09^{a}	1.98^{b}	0.018	<0.01	0.04	<0.01	<0.01
Methionine	0.63^{c}	0.61^{c}	0.79^{ab}	0.76^{b}	0.64^{c}	0.63^{c}	0.82^{a}	0.77^{ab}	0.009	<0.01	0.02	<0.01	<0.01
Isoleucine	1.42^{d}	1.35^{ef}	1.67^{a}	1.60^{bc}	1.40^{de}	1.31^{f}	1.64^{ab}	1.55^{c}	0.011	<0.01	<0.01	<0.01	<0.01
Leucine	2.52^{d}	2.40^{e}	2.94^{a}	2.82^{b}	2.43^{de}	2.28^{f}	2.85^{ab}	2.71^{c}	0.018	<0.01	<0.01	<0.01	<0.01
Tyrosine	0.94^{cd}	0.89^{de}	1.10^{a}	1.04^{ab}	0.91^{de}	0.85^{e}	1.07^{a}	0.99^{bc}	0.013	<0.01	<0.01	<0.01	<0.01
Phenylalanine	1.55^{c}	1.47^{d}	1.75^{a}	1.69^{d}	1.46^{d}	1.37^{e}	1.66^{b}	1.57^{c}	0.009	<0.01	<0.01	<0.01	<0.01
Hydroxylysine	0.05^{c}	0.05^{c}	0.05^{c}	0.04^{c}	0.10^{b}	0.09^{b}	0.12^{a}	0.12^{a}	0.002	<0.01	<0.01	<0.01	<0.01
Lysine	1.70^{f}	1.62^{f}	1.89^{de}	1.83^{e}	2.05^{c}	1.93^{d}	2.45^{a}	2.30^{b}	0.017	<0.01	<0.01	<0.01	<0.01
Histidine	0.92^{d}	0.87^{e}	1.10^{a}	1.05^{b}	0.86^{e}	0.81^{f}	1.04^{b}	0.98^{c}	0.007	<0.01	<0.01	<0.01	<0.01
Arginine	2.41^{cd}	2.28^{e}	2.89^{a}	2.75^{b}	2.09^{f}	1.97^{g}	2.45^{c}	2.31^{de}	0.022	<0.01	<0.01	<0.01	<0.01

Table 3.5. *Cont'd* Individual and total amino acid content profiles (on dry matter basis) of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

| Items | Blend pelleted products (BPP) | | | | | | | | SEM | P value | Contrast, P value | | |
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	No	4.8	No	4.8	No	4.8	No	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product, %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Tryptophan	0.40[b]	0.34[c]	0.50[a]	0.46[a]	0.38[b]	0.33[c]	0.47[a]	0.41[b]	0.011	<0.01	<0.01	<0.01	<0.01
Total amino acids (% of DM)	32.70[d]	31.07[e]	38.46[a]	36.87[b]	31.71[e]	29.86[f]	37.36[b]	35.23[c]	0.255	<0.01	<0.01	<0.01	<0.01

SEM: standard error of mean; [a-g] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; CN: canola meal; CR: carinata meal.

3.4.5 Chemical Profile

The chemical profiles of the blend pelleted products are presented in Table 3.6. The contents of DM, NPN (include peptides, free AA, nucleic acids, amides, amines, and ammonia) and cellulose (P> 0.05) were not significantly different among all the blend pelleted products. However, carinata based pelleted product BPP4 had the highest ash content (7.7 %DM) compared with the other blend pelleted products (P< 0.05). The canola based pelleted product BPP5 is higher (P< 0.05) in OM content (94.4 % DM) than the other blend pelleted products. Canola pelleted product BPP7 had the highest (P< 0.05) level of EE (3.1 %DM) but was not different compared with EE (2.8 % DM) of BPP5. Canola based pelleted products contained higher OM and EE but lower ash than the carinata based pelleted products (93.5 vs. 93.2; 2.5 vs. 1.4 and 6.5 vs. 6.8 % DM, respectively). Adding a lower level of pea screenings or a higher level of co-product results in the BPP products containing lower levels of OM, but higher levels of ash. Adding lignosulfonate increases the ash content in the pelleted products (+0.5 % DM). Canola meal is a highly-concentrated source of protein (42.7 % DM), which is digested mainly in the rumen (Brito and Broderick, 2007; McAllister et al., 1993). Previous research has shown that carinata meal has higher CP (48.8 % DM) than canola meal (Xin and Yu, 2013a). Peas contain approximately two-thirds of protein found in canola meal and twice as much protein as barley grain (Corbett, 2016). Compared to soybean meal and canola meal, respectively, peas contain approximately 24 % CP vs. 49.9 and 40.6 % (Khorasani et al., 2001). Pelleted products BPP3, BPP4 and BPP7 had higher (P< 0.05) CP (45.0, 43.1 and 41.0 % DM, respectively) than the other BPP. This is mainly due to the high level of inclusion of bio-fuel or bio-oil processing co-product in these pelleted products (75, 71.4 % of DM of carinata meal and 75 % of DM of canola meal, respectively). Canola pelleted products contained lower (P< 0.05) CP (-3.2 % DM) than the carinata based pelleted products.

Adding a lower level of pea screenings or a higher level of co-product results in the BBP products containing higher levels of CP (42.2 vs. 36.2 % DM); lignosulfonate reduces the content of CP in the pelleted products. All carinata based pelleted products (BPP1, BPP2, BPP3 and BPP4) showed higher ($P< 0.05$) level of NDICP (13.0, 13.1, 14.4 and 13.4 % CP, respectively) but lower ($P< 0.05$) ADICP (1.4, 1.6, 1.5 and 1.4 % CP, respectively) than canola based pelleted products BPP5, BPP6, BPP7 and BPP8. Previous studies showed that carinata meal has higher levels of soluble CP (SCP) than canola meal (55.6 % vs. 34.8 % CP, Xin and Yu, 2013a). However, in this study canola based blend pelleted product showed higher ($P< 0.05$) level of SCP than the carinata blend pelleted products (+9.4 % CP). Canola pelleted products contained higher ADICP (+1.7 % CP), but lower NDICP (-7.1 % CP) than the carinata based pelleted products. Average starch content of peas is 39 % with a range of 25 to 57 % DM (Christensen, 2006). Blend pelleted products BPP1, BPP2, BPP5 and BPP6 had higher ($P< 0.05$) levels of starch (25.4, 25.3, 26.8, 25.8 % DM, respectively) than the other blend pelleted products. This is mainly due to the high level of inclusion of pea screenings in the composition (50.0, 47.6, 50.0 and 47.6 % of DM, respectively). BPP products contain sugar ranging from 6.3 to 7.8 % DM ($P< 0.05$). Adding a lower level of pea screenings or a higher level of co-product results in the BPP products containing higher levels of sugar and lower levels of starch. Canola meal contains a moderate amount of acid detergent fiber (ADF, 18.4 %) but a relatively low level of neutral detergent fiber (NDF, 28.8 %). This relatively low NDF:ADF ratio may actually benefit the feeding of canola meal to ruminants because high ADF decreases digestible energy levels (Canola Council, 2009). Compared to soybean meal and canola meal, respectively, field peas contain approximately an acid detergent fiber (ADF) content of 9.5 vs. 9.0 and 17.4 %, and a neutral detergent fiber (NDF) content of 19.6 vs. 12.0 and 23.9 % (Fonnesbeck et al. 1984). In our study, it was found that canola based pelleted product BPP7 had

the highest (P< 0.05) content of NDF, ADF and ADL (22.6, 15.2 and 6.7 % DM, respectively) than the other blend pelleted products. However, there is no significance difference between those values and the NDF, ADF and ADL values (21.5, 14.9 and 6.6 % DM, respectively) of the canola based pelleted product BPP8. Carinata based pelleted products contained lower (P< 0.05) fiber (NDF, ADF) (-1.5, -4.6 % DM) and lower (P< 0.05) ADL (-4.1 % DM) than the canola based pelleted products. Adding a higher level of pea screenings or a lower level of co-product results in the BPP products containing lower NDF (-1.7 % DM) and ADL (-1.1 % DM). Adding lignosulfonate results in reduced NDF (-1.1 % DM) content in the blend pelleted products. Hemicellulose content of the blend pelleted product ranging from 6.6 to 11.6 % DM. Carinata based blend pelleted products contain more hemicellulose than canola based pelleted products (10.3 vs. 7.2 % DM). Carinata pelleted products also contained higher NFC than the canola based pelleted products (36.9 vs. 34.7 % DM). Adding a higher level of pea screenings or a lower level of co-product results in the BPP products containing higher NFC (+8.2 % DM) and NSC (+10 % DM), also adding lignosulfonate increased NFC (+3 % DM) in the blend pelleted products.

Table 3.6. Chemical and nutrient composition of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

| Items | Blend pelleted products (BPP) | | | | | | | | SEM | P value | Contrast, P value | | |
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Basic chemical													
DM (%)	87.9	88.3	88.9	88.9	88.0	88.2	88.9	89.1	0.57	0.49	0.94	0.67	0.04
Ash (%DM)	5.9[g]	6.5[e]	7.2[c]	7.7[a]	5.6[h]	6.1[f]	6.9[d]	7.3[b]	0.02	<0.01	<0.01	<0.01	<0.01
EE (%DM)	1.6[d]	1.4[d]	1.5[d]	1.2[d]	2.8[ab]	2.0[c]	3.1[a]	2.4[bc]	0.07	<0.01	<0.01	<0.01	0.09
FA (%DM)	0.6[d]	0.4[d]	0.5[d]	0.2[d]	1.8[ab]	1.1[c]	2.1[a]	1.4[bc]	0.07	<0.01	<0.01	<0.01	0.09
OM (%DM)	94.1[b]	93.6[d]	92.8[f]	92.3[h]	94.4[a]	93.9[c]	93.1[e]	92.7[g]	0.02	<0.01	<0.01	<0.01	<0.01
Protein profile													
CP (%DM)	38.8[bc]	36.4[cd]	45.0[a]	43.1[a]	35.9[cd]	33.7[d]	41.9[ab]	39.0[bc]	0.66	<0.01	<0.01	<0.01	<0.01
SCP (%DM)	10.5[b]	10.2[b]	11.4[ab]	10.2[b]	13.9[ab]	11.7[ab]	15.5[a]	12.2[ab]	1.38	0.01	<0.01	0.01	0.17
SCP (%CP)	27.3[bcd]	28.0[bcd]	25.4[cd]	23.7[d]	38.7[a]	34.7[abc]	37.1[ab]	31.4[abcd]	3.77	<0.01	<0.01	0.07	0.07
NPN (%DM)	13.2	12.9	13.4	12.8	13.1	12.8	12.2	11.6	1.00	0.91	0.39	0.54	0.51
NPN (%CP)	34.0	35.2	29.7	29.8	36.6	37.9	29.0	29.8	2.23	0.11	0.49	0.58	<0.01
NDICP (%DM)	5.1[b]	4.8[b]	6.5[a]	5.8[ab]	2.2[c]	2.0[c]	2.8[c]	2.6[c]	0.20	<0.01	<0.01	0.05	<0.01
NDICP (%CP)	13.0[a]	13.1[a]	14.4[a]	13.4[a]	6.0[b]	6.0[b]	6.7[b]	6.8[b]	0.44	<0.01	<0.01	0.50	0.04
ADICP (%DM)	0.6[d]	0.6[d]	0.7[cd]	0.6[d]	1.0[bcd]	1.0[abc]	1.4[ab]	1.4[a]	0.07	<0.01	<0.01	0.98	<0.01
ADICP (%CP)	1.4[b]	1.6[b]	1.5[b]	1.4[b]	2.7[a]	3.0[a]	3.3[a]	3.6[a]	0.20	<0.01	<0.01	0.28	0.09
Carbohydrate profile													
CHO (%DM)	53.7[bc]	55.7[ab]	46.3[e]	48.0[de]	55.7[ab]	58.2[a]	48.1[de]	51.3[cd]	0.67	<0.01	<0.01	<0.01	<0.01
Starch (%DM)	25.4[a]	25.3[a]	14.6[b]	13.3[b]	26.8[a]	25.8[a]	14.5[b]	14.8[b]	0.57	<0.01	0.07	0.25	<0.01
Starch (%NFC)	64.6[ab]	60.2[bc]	45.7[de]	38.8[e]	71.2[a]	63.8[ab]	51.3[cd]	45.7[de]	1.70	<0.01	<0.01	<0.01	<0.01
Sugar (%DM)	6.5[c]	6.5[bc]	7.2[abc]	7.7[ab]	6.3[c]	6.7[abc]	7.2[abc]	7.8[a]	0.32	0.01	0.99	0.04	<0.01

Table 3.6. *Cont'd* Chemical and nutrient composition of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8	SEM	P value	Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Sugar (%NFC)	16.5[b]	15.5[b]	22.6[a]	22.6[a]	16.8[b]	16.5[b]	25.4[a]	24.0[a]	1.11	<0.01	0.06	0.28	<0.01
NDF (%DM)	19.3[de]	18.4[e]	21.0[bc]	19.5[cde]	20.3[bcd]	19.7[cde]	22.6[a]	21.5[ab]	0.27	<0.01	<0.01	<0.01	<0.01
ADF (%DM)	9.3[c]	9.2[c]	9.4[c]	8.9[c]	12.6[b]	12.6[b]	15.2[a]	14.9[ab]	0.45	<0.01	<0.01	0.51	0.01
ADF (%NDF)	48.2[cd]	50.4[bcd]	44.7[d]	45.8[d]	62.0[abc]	63.6[ab]	67.3[a]	69.5[a]	2.69	<0.01	<0.01	0.36	0.69
ADL (%DM)	1.4[c]	1.6[c]	1.9[c]	1.8[c]	4.9[b]	4.7[b]	6.7[a]	6.6[a]	0.10	<0.01	<0.01	0.34	<0.01
ADL (%NDF)	7.5[c]	8.8[c]	8.8[c]	9.4[c]	24.4[b]	23.9[b]	29.7[a]	30.5[a]	0.51	<0.01	<0.01	0.17	<0.01
Hemicellulose (%DM)	10.0[abc]	9.1[abc]	11.6[a]	10.6[ab]	7.7[bc]	7.2[bc]	7.4[bc]	6.6[c]	0.64	<0.01	<0.01	0.09	0.25
Cellulose (%DM)	7.9	7.6	7.5	7.1	7.6	7.8	8.5	8.4	0.53	0.60	0.17	0.68	0.75
NFC (%DM)	39.4[ab]	42.1[a]	31.8[de]	34.3[cd]	37.6[bc]	40.5[ab]	28.3[e]	32.5[d]	0.63	<0.01	<0.01	<0.01	<0.01
NFC (%CHO)	73.4[ab]	75.6[a]	68.8[db]	71.4[bc]	67.5[d]	69.6[cd]	58.8[f]	63.3[e]	0.44	<0.01	<0.01	<0.01	<0.01
NSC (%DM)	31.9[a]	31.9[a]	21.7[b]	21.0[b]	33.1[a]	32.5[a]	21.7[b]	22.6[b]	0.71	<0.01	0.10	0.80	<0.01

SEM: standard error of mean; [a-f] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; DM: dry matter; EE: ether extracts (crude fat); CP: crude protein; OM: organic matter; SCP: soluble crude protein; NPN: non-protein nitrogen; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: non-fiber carbohydrate; CHO: carbohydrate; NFC: non-fiber carbohydrate; NSC: non-soluble carbohydrate; CN: canola meal; CR: carinata meal.

3.4.6   Energy Profile

The content of starch (54 % DM) found in peas is high (McLean et al., 1974) which is about 80 % of the starch level found in barley grain, while canola and soybean meal contain little or no starch (Christensen and Mustafa, 2000). This makes peas a unique purpose feed, rich in energy. Peas have a slightly higher energy content than barley, but significantly lower energy content than soybean meal (Galmeus, 2012). The major energy storage component in peas consists of starch, which is further fermented to volatile fatty acids by the rumen microorganisms (Bastianelli et al., 1995). The energy profiles of the blend pelleted products are presented in Table 3.7. The level of truly digestible non-fiber carbohydrates (tdNFC) is the highest (P< 0.05) in canola based pelleted product BPP6 (39.7 % DM), however it is not significantly different from the corresponding value of carinata based pelleted product BPP1 (38.6 % DM). Carinata based pelleted product BPP3 showed the highest (P< 0.05) level of truly digestible crude protein (tdCP) (44.7 % DM) than the other blend pelleted products except BPP4 (42.8 % DM). Canola based pelleted product BPP7 has the highest (P< 0.05) level of truly digestible fatty acids (tdFA) (2.1 % DM) than the other treatments except BPP5 (1.8 % DM). Carinata based pelleted product BPP1 showed the highest (P< 0.05) level of truly digestible neutral detergent fiber (tdNDF) (7.5 % DM) than the other blend pelleted products. Also, it has the highest (P< 0.05) total digestible nutrients (TDN$_{1x}$) (79.1 % DM) than the other blend pelleted products. Carinata based blend pelleted products contain higher truly digestible NFC (tdNFC) (+2.1 % DM), crude protein (tdCP) (+3.4 % DM) and fiber (tdNDF) (+1.7 % DM) and lower truly digestible fatty acid (tdFA) (-1.1 % DM) than canola based blend pelleted products, therefore contain a higher TDN value. Adding a higher level of pea screenings or a lower level of co-product results in the BPP products containing higher tdNFC (39.0 vs. 31.1 %

DM), tdNDF (6.4 vs. 5.8 % DM) and lower tdCP (35.9 vs. 41.8 % DM) and higher TDN_{1x} (76.6 vs. 74.1 % DM). Adding lignosulfonate reduced TDN (-0.8 % DM) content in the product.

Carinata based pelleted product showed higher (P< 0.05) energy values than canola based pelleted products. Carinata based pelleted product BPP3 contained the highest (P< 0.05) level of net energy for lactation (NE_{Lp3x}), net energy for maintenance (NE_m) and net energy for gain (NE_g) (2.01, 2.17, 1.49 Mcal/kg DM, respectively) than the other blend pelleted products. However, there is no significant difference between these values and the corresponding ones of BPP1 and BPP4. Carinata based blend pelleted products contain higher truly NE for lactation (+0.16 Mcal/kg DM), maintenance (+0.16 Mcal/kg DM) and growth (+0.14 Mcal/kg DM) than canola based pelleted products and by adding lignosulfonate results in BPP containing reduced NE.

Table 3.7. Energy profile of blend pelleted products with different combinations (two levels of lignosulfonate chemical compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8	SEM	P value	Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Truly digestible nutrient (%DM)													
tdNFC	38.6^{ab}	41.2^{b}	31.2^{de}	33.6^{cd}	36.8^{bc}	39.7^{ab}	27.8^{e}	31.8^{d}	0.62	<0.01	<0.01	<0.01	<0.01
tdCP	38.6^{cd}	36.2^{de}	44.7^{a}	42.8^{ab}	35.5^{de}	33.3^{e}	41.3^{bc}	38.4^{cd}	0.66	<0.01	<0.01	<0.01	<0.01
tdNDF	7.5^{a}	6.8^{b}	7.1^{b}	6.6^{b}	5.7^{c}	5.7^{c}	5.0^{d}	4.7^{d}	0.09	<0.01	<0.01	<0.01	<0.01
tdFA	0.6^{d}	0.4^{d}	0.5^{d}	0.2^{d}	1.8^{ab}	1.0^{c}	2.1^{a}	1.4^{bc}	0.07	<0.01	<0.01	<0.01	0.09
Total digestible nutrient (%DM)													
TDN$_{1x}$	79.1^{a}	78.3^{b}	77.1^{c}	76.5^{c}	75.0^{d}	74.0^{e}	71.9^{f}	71.1^{g}	0.16	<0.01	<0.01	<0.01	<0.01
Energy value (Mcal/kg)													
DE$_{p3x}$, NRC-2001 dairy	3.54^{a}	3.48^{a}	3.55^{a}	3.50^{a}	3.35^{b}	3.28^{bc}	3.30^{bc}	3.23^{c}	0.015	<0.01	<0.01	<0.01	0.04
ME$_{p3x}$, NRC-2001 dairy	3.13^{a}	3.06^{b}	3.13^{a}	3.08^{ab}	2.93^{c}	2.86^{de}	2.88^{cd}	2.81^{e}	0.012	<0.01	<0.01	<0.01	0.01
NE$_{Lp3x}$, NRC-2001 dairy	2.01^{a}	1.96^{b}	2.01^{a}	1.98^{ab}	1.87^{c}	1.82^{de}	1.84^{cd}	1.79^{e}	0.010	<0.01	<0.01	<0.01	0.06
ME, NRC-1996 beef	3.13^{a}	3.06^{b}	3.13^{b}	3.08^{ab}	2.93^{c}	2.86^{de}	2.88^{cd}	2.81^{e}	0.012	<0.01	<0.01	<0.01	0.01
NE$_{m}$, NRC-1996 beef	2.16^{ab}	2.12^{b}	2.17^{a}	2.13^{ab}	2.02^{c}	1.97^{de}	1.99^{cd}	1.94^{e}	0.010	<0.01	<0.01	<0.01	0.06
NE$_{g}$, NRC-1996 beef	1.49^{ab}	1.45^{b}	1.49^{a}	1.46^{ab}	1.37^{c}	1.32^{de}	1.34^{cd}	1.29^{e}	0.009	<0.01	<0.01	<0.01	0.05

SEM: standard error of mean; $^{a-e}$ Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; tdCP: truly digestible crude protein; tdFA: truly digestible fatty acid; tdNDF: truly digestible neutral detergent fibre; tdNFC, truly digestible non-fibre carbohydrate. TDN$_{1\times}$: total digestible nutrient at one times maintenance. DE$_{3\times}$: digestible energy at production level of intake (3×); ME$_{3\times}$: metabolizable energy at production level of intake (3×); NE$_{L3\times}$: net energy

Table 3.7. *Cont'd* Energy profile of blend pelleted products with different combinations (two levels of lignosulfonate chemical compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8	SEM	P value	Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
			Blend pelleted products (BPP)								Contrast, P value		
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					

for lactation at production level of intake (3×); NE$_m$: net energy for maintenance; NE$_g$: net energy for growth CN: canola meal; CR: carinata meal.

3.4.7 Protein and Carbohydrate Subfractions

Crude protein and carbohydrate fractions were partitioned using the Cornell Net Carbohydrate and Protein System (CNCPS 6.5). Protein fractions include PA2, PB1, PB2 and PC; and carbohydrate fractions CA4, CB1, CB2, CB3 and CC. Each of them has different fermentation patterns and degradation rates. The values of protein and carbohydrate fractions of all blend pelleted products are presented in Table 3.8. The contents of insoluble true protein (PB1) ($P> 0.05$) were not different among all the blend pelleted products. Canola based pelleted product BPP5 through BPP8 had higher ($P< 0.05$) levels of soluble true protein (PA2) (35.3 to 44.0 % of CP) than the carinata based pelleted products. Carinata based pelleted products BPP1 to BPP4 showed higher ($P< 0.05$) levels of fiber-bound protein (PB2) (11.5 to 12.9 % of CP) and lower ($P< 0.05$) levels of indigestible protein (PC) (1.4 to 1.6 % of CP) than canola based pelleted products (BPP5, BPP6, BPP7 and BPP8) in which the values of PB2 and PC, respectively are 3.0 to 3.4 % of CP and 2.7 to 3.6 % of CP. In agreement with our findings a previous study found that carinata meal had a lower content of PC fraction ($P< 0.05$), which might be an indication of an improvement of protein nutritional value for ruminants when compared to canola meal (Xin and Yu, 2013a). In terms of protein fraction, carinata based blend pelleted products contain lower PA2 (-10.6 % of CP) and PC (-1.7 % of CP) fractions, but higher PB2 fraction (+8.8 % of CP) than canola based BPP products. Adding a higher level of pea screenings or a lower level of co-product results in the BBP products containing higher PA2 (36.5 vs. 33.0 % of CP). Pelleting processing reduced PA2 fraction and increased PB1 and PC fractions.

The contents of digestible fiber (CB3) ($P> 0.05$) were not significantly different among all the blend pelleted products. Carinata based pelleted products BPP3, BPP4 and canola based pelleted products BPP7, BPP8 had a higher level of degradable carbohydrate CA4. This is due to the high

level of inclusion of bio-fuel or bio-oil processing co-product in these pelleted products (75, 71.4 %

of DM of carinata meal and 75, 71.4 % of DM of canola meal, respectively).

In contrast, BPP1, BPP2, BPP5 and BPP6 had higher ($P< 0.05$) CB1 fraction (starch) (47.4,

45.5, 48.0, 44.4 % of CHO, respectively) than the other blend pelleted products. This is due to the

higher level of inclusion of pea screenings in the blend pelleted product (50.0, 47.6, 50.0 and

47.6 % of DM, respectively). Carinata based pelleted products have higher CB2 fraction (soluble

fiber) (20.4 vs. 13.7 % of CHO) than the canola based pellet products. Canola based pelleted

product BPP7 had the highest ($P< 0.05$) level of CC fraction (indigestible fiber) (25.9 % of CHO)

than the other blend pelleted products, except BPP8 (22.6 % of CHO). Previous study

demonstrated that carinata meal contained more slowly degraded carbohydrate fraction (20.4 vs.

2.0 % of CHO) and less unavailable fiber (18.2 vs. 45.2 % of CHO) compared with canola meal.

Those results showed that carinata meal may not have the same rumen degradation characteristics

as canola meal (Xin and Yu, 2013a). In this study, in terms of CHO fraction profile, carinata based

blend pelleted products had lower CC fraction (-7.3 % of CHO) and higher CB2 (+6.7 % of CHO),

but not different in CA4, CB1 fractions than canola based BBP products. Adding a higher level of

pea screenings or a lower level of co-product results in the BBP products containing lower CA4 (-

3.8 % of CHO), lower CB2 (-7.1 % of CHO) and CC (-6.5 % of CHO) but higher CB1 (+18.8 %

of CHO) fractions. Also, adding lignosulfonate chemical compound reduced CB1 fraction (-2.6 %

of CHO) and increased CB2 fraction (+5.3 % of CHO). Pelleting processing increased CB1

fraction.

Table 3.8. Protein and carbohydrate fractions profiles (that are associated with ruminal and intestinal nutrient supply) in blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings) using CNCPS 6.5 version

Items	Blend pelleted products (BPP)								SEM	P value	Contrast, P value		
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Protein subfraction profile in blend pelleted products													
PA2 (%CP)	31.0[bcd]	31.7[bcd]	28.6[cd]	26.7[d]	44.0[a]	39.4[abc]	41.7[ab]	35.3[abcd]	4.13	<0.01	<0.01	0.07	0.05
PB1 (%CP)	56.0	55.2	57.0	59.9	50.0	54.6	51.7	58.0	4.15	0.13	0.05	0.06	0.11
PB2 (%CP)	11.6[a]	11.6[a]	12.9[a]	12.0[a]	3.3[b]	3.0[b]	3.4[b]	3.1[b]	0.37	<0.01	<0.01	0.18	0.09
PC (%CP)	1.4[b]	1.6[b]	1.5[b]	1.4[b]	2.7[a]	3.0[a]	3.3[a]	3.6[a]	0.20	<0.01	<0.01	0.28	0.09
True Protein (%CP) profile in blend pelleted products													
PA2 (%true protein)	31.4[bcd]	32.2[bcd]	28.0[cd]	27.1[d]	45.2[a]	40.6[abc]	43.1[ab]	36.6[abcd]	4.24	<0.01	<0.01	0.08	0.06
PB1 (%true protein)	56.9	56.1	57.9	60.8	51.4	56.3	53.4	60.2	4.17	0.13	0.12	0.05	0.08
PB2 (%true protein)	11.7[a]	11.7[a]	13.1[a]	12.2[a]	3.4[b]	3.1[b]	3.5[b]	3.2[b]	0.38	<0.01	<0.01	0.19	0.09
Carbohydrate subfraction profile in blend pelleted products													
CHO (%DM)	53.7[bc]	55.7[ab]	46.3[e]	48.0[de]	55.7[ab]	58.2[a]	48.1[de]	51.3[cd]	0.67	<0.01	<0.01	<0.01	<0.01
CA4 (%CHO)	12.1[bc]	11.7[c]	15.5[a]	16.1[a]	11.3[c]	11.5[c]	14.9[ab]	15.2[ab]	0.77	<0.01	0.13	0.71	<0.01
CB1 (%CHO)	47.4[a]	45.5[a]	31.4[b]	27.7[b]	48.0[a]	44.4[a]	30.2[b]	28.9[b]	1.13	<0.01	0.86	0.01	<0.01
CB2 (%CHO)	13.8[bc]	18.4[ab]	21.8[ab]	27.6[a]	8.1[c]	13.8[bc]	13.8[bc]	19.2[ab]	1.88	<0.01	<0.01	<0.01	<0.01
CB3 (%CHO)	16.6	13.5	15.3	15.8	16.7	15.4	15.3	14.1	1.63	0.85	0.93	0.31	0.71
CC (%CHO)	10.1[c]	10.8[c]	16.0[bc]	12.9[bc]	15.8[bc]	15.0[bc]	25.9[a]	22.6[ab]	1.75	<0.01	<0.01	0.23	<0.01

SEM: standard error of mean; [a-e] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; PA2: soluble true protein; PB1: insoluble true protein. PB2: fiber-bound protein; PC: indigestible protein;

Table 3.8. *Cont'd* Protein and carbohydrate fractions profiles (that are associated with ruminal and intestinal nutrient supply) in blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings) using CNCPS 6.5 version

| Items | Blend pelleted products (BPP) | | | | | | | | SEM | P value | Contrast, P value | | |
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					

CHO: carbohydrate; CA4: water soluble carbohydrates; CB1: starch; CB2: soluble fiber; CB3: digestible fiber; CC: indigestible fiber; CN: canola meal; CR: carinata meal.

3.5 Conclusions

All blend pelleted products had high pellet durability index. Both carinata meal based blend pelleted products and canola meal based blend pelleted products have safe levels of glucosinolates and condensed tannins which do not present any risk to the health of ruminants. Carinata meal based blend pelleted products BPP3 and BPP4 provide higher levels of total amino acids on DM basis and higher NE as well, than the canola blend pelleted products (BPP5, BPP6, BPP7, and BPP8). Through a blending strategy of feed ingredient with unique nutrient profile in each ingredient, optimization of nutrient supply could be achieved. Based on these studies it was concluded that carinata meal based blend pelleted products had higher nutritive value and could be used as a good source of protein and energy for ruminants.

4. POTENTIAL NITROGEN TO ENERGY SYNCHRONIZATION, RUMEN DEGRADATION KINETICS, AND INTESTINAL DIGESTIBILITY OF BLEND PELLETED PRODUCTS BASED ON COMBINATION OF CO-PRODUCTS FROM BIO-FUEL/BIO-OIL PROCESSING, PEA SCREENINGS AND LIGNOSULFONATE AT DIFFERENT LEVELS FOR RUMINANTS.

4.1 Abstract

The aim of this project was to develop and test eight different pelleted products based on combination of co-products from bio-fuel (carinata meal) and bio-oil (canola meal), pea screenings and lignosulfonate at different levels for ruminants. The pelleted products include:

BPP1: lignosulfonate 0 % of DM + carinata meal 50 % of DM + pea screenings 50.0 % of DM;

BPP2: lignosulfonate 4.8 % of DM + carinata meal 47.6 % of DM + pea screenings 47.6 % of DM;

BPP3: lignosulfonate 0 % of DM + carinata meal 75 % of DM + pea screenings 25 % of DM;

BPP4: lignosulfonate 4.8 % of DM + carinata meal 71.4 % of DM + pea screenings 23.8 % of DM;

BPP5: lignosulfonate 0 % of DM + canola meal 50 % of DM + pea screenings 50.0 % of DM;

BPP6: lignosulfonate 4.8 % of DM + canola meal 47.6 % of DM + pea screenings 47.6 % of DM;

BPP7: lignosulfonate 0 % of DM + canola meal 75 % of DM + pea screenings 25 % of DM;

BPP8: lignosulfonate 4.8 % of DM + canola meal 71.4 % of DM + pea screenings 23.8 % of DM. Comparisons were made between blend pelleted products based on carinata meal and pelleted products based on canola meal, high or low level of inclusion of co-product (low or high level of inclusion of pea screenings) and inclusion or no inclusion of lignosulfonate in terms of rumen degradation kinetics, iNDF 288h, hourly effective rumen degradation ratios/potential N-to energy synchronization and intestinal digestion of rumen undegraded nutrients. The results showed that carinata based pelleted product BPP3 and BPP4 had the highest (P< 0.05) level of BCP^{DVE} (233

and 239 g/kg DM) and RUP^{NRC} (210 and 216 g/kg DM) than the other blend pelleted products. BPP3, showed higher (P< 0.05) effective degradation of NDF (EDNDF) (64 g/kg DM) than the other blend pelleted products except BPP4 and BPP7 (56 and 61 g/kg DM). iNDF in all blend pelleted products ranged from 5.3 to 12.5 % DM. Intestinal absorbable feed protein (IADP) was higher in BPP4 (168 g/kg DM) than the other pelleted products except BPP3; however, BPP3 showed the highest (P< 0.05) total digestible protein (TDP) (405 g/kg DM) than the other blend pelleted products followed by BPP4 (383 g/kg DM) and canola based pelleted product BPP7 (370 g/kg DM). In addition, BPP3 showed the highest (P< 0.05) total digestible NDF (TDNDF) (139 g/kg DM) than the other blend pelleted products. In conclusion, carinata based pelleted products (BPP1 - BPP4) contain higher RUP (+56 g/kg DM), lower EDCP (-24 g/kg DM), higher EDNDF (+2.9 %), lower iNDF (-4.2 % DM), higher IADP (+55.7 g/kg DM) and higher TDP (+32 g/kg DM) than canola based pelleted products. Carinata based pelleted products BPP3 and BPP4 could be used as an alternative high quality bypass protein supplement for ruminants.

Keywords: Carinata, Canola, Pea, Lignosulfonate

4.2 Introduction

In order to maintain high milk production, dairy cattle require adequate quantities of amino acids and glucose supplied by the rations (Yu et al, 2002; Ferguson, 1975). This can be accomplished with sufficient true protein and glucose accessible to be absorbed in the small intestine. Therefore, high milk producing dairy cows need nitrogen available for microbial protein synthesis in the rumen, digestible dietary bypass protein and bypass non-structural carbohydrates (Chalupa and Sniffen, 1991; Nocek and Tamminga, 1991; Tamminga, 1979). Canola meal which often is used in ruminant diets (Canola Council, 2009) while a comparatively new co-product (Carinata meal) has become accessible in Canada (Xin and Yu, 2013b). In addition, pea screenings

(*Pisum sativum*) have attracted consideration as components of feedstuffs for dairy cows in recent years (Yu et al., 2002). However, studies showed that carinata meal has high degradation rate and extent of protein, similar to the conventional co-product from bio-oil processing of canola seed (Xin and Yu, 2014); rapidly degradable protein content of pea screenings is high (Van Strallen and Tamminga, 1990). Carinata meal had more effectively degraded organic matter and crude protein than canola meal (Ban, 2016). Rapid feed rumen degradation can be reduced through addition of lignosulfonate (Windschitl and Stern, 1988); and suitable pelleting processing method (Huang et al., 2015; Thomas et al., 1997). Degradation in the rumen of resistant starch is improved by 15 % through pelleting (Tamminga and Goelema, 1995) also is improved rumen crude protein degradation in dairy cows (Huang et al., 2015; Goelema et al., 1999). Understanding how rumen degradation kinetics, intestinal digestion characteristics of the blend pelleted product based on combination of carinata meal, canola meal, pea screenings are affected by pelleting and lignosulfonate is essential for these pelleted products evaluation. However, there is little information available on rumen degradation kinetics, intestinal digestibility as well as nitrogen to energy synchronization especially when this new carinata meal is blended with another feedstuff as a pellet. Effort is still required to completely understand this bio-fuel co-product. This study was conducted to test eight blend pelleted products based on the combination of new co-product of biofuel processing of carinata seed, conventional co-product from bio-oil processing of canola seed, pea screenings and lignosulfonate at different levels for ruminants in terms of rumen degradation kinetics, intestinal digestibility and nitrogen to energy synchronization. Comparisons were made between blend pelleted products based on carinata meal and pelleted products based on canola meal, low or high level of inclusion of co-product and inclusion or not of lignosulfonate.

4.3 Materials and Methods

4.3.1 Blend Pelleted Products

Blend pelleted products BPP were used which included: BPP1 = Blend Pelleted Product 1: lignosulfonate 0 % of DM + Low level co-product from bio-energy processing (carinata meal - CR: 50 % of DM), High level of pea screenings (PS: 50.0 % of DM); BPP2 = Blend Pelleted Product 2: lignosulfonate 4.8 % of DM + Low level co-product from bio-energy processing (carinata meal - CR: 47.6 % of DM), High level of pea screenings (PS: 47.6 % of DM); BPP3 = Blend Pelleted Product 3: lignosulfonate 0 % DM + High level co-product from bio-energy processing (carinata meal - CR: 75 % DM), Low level of pea screenings (PS: 25 % DM). BPP4 = Blend Pelleted Product 4: lignosulfonate 4.8 % of DM + High level co-product from bio-energy processing (carinata meal - CR: 71.4 % of DM), Low level of pea screenings (PS: 23.8 % of DM); BPP5 = Blend Pelleted Product 5: lignosulfonate 0 % of DM + Low level co-product from bio-oil processing (canola meal - CN: 50 % of DM), High level of pea screenings (PS: 50.0 % of DM); BPP6 = Blend Pelleted Product 6: lignosulfonate 4.8 % of DM + Low level co-product from bio-oil processing (canola meal - CN: 47.6 % of DM), High level of pea screenings (PS: 47.6 % of DM); BPP7 = Blend Pelleted Product 7: lignosulfonate 0 % of DM + High level co-product from bio-oil processing (canola meal - CN: 75 % of DM), Low level of pea screenings (PS: 25 % of DM); BPP8 = Blend Pelleted Product 8: lignosulfonate 4.8 % of DM + High level co-product from bio-oil processing (canola meal - CN: 71.4 % of DM), Low level of pea screenings (PS: 23.8 % of DM). The Sven Roller Mill manufactured by Apollo Machine and Products Ltd, Saskatoon, SK, Canada was used to roll the samples with a gap of 3.80 mm in the Department of Agricultural Engineering, University of Saskatchewan. Detailed composition of each blend pelleted product is reported in previous chapter.

4.3.2 Animals and Diets

At Rayner Dairy Research and Teaching Facility of the University of Saskatchewan, Saskatoon, Canada, four lactating Holstein cows fitted with a rumen cannula (Bar Diamond Inc, Parma, ID, USA) with an internal diameter of 8.8 cm were used in this study. Cow # 938 − 209 DIM, 2 lactation, 3 years 6 months old, 43 kg milk average, 674 kg bodyweight; Cow # 914 − 223 DIM, 2 lactation, 4 years old, 31 kg milk average, 700 kg bodyweight; Cow # 940 − 228 DIM, 2 lactation, 3 years 6 months old, 28 kg milk average, 692 kg bodyweight and Cow # 920 − 199 DIM, 2 lactation, 3 years 11 months old, 37 kg milk average, 778 kg bodyweight. These cows were kept in the regular boxstall/parlor and fed TMR based on 218 g/kg of barley silage, 157 g/kg of corn silage, 139 g/kg cut alfalfa, 28 g/kg canola meal, 331 g/kg of concentrate, 20 g/kg of barley grain, 102 g/kg of pulp pellets and 5 g/kg of palmitic acid before and while the rumen incubation was conducted. The average daily eating intake was 28 kg of DM and these animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993) and were approved by Animal Research 125 Ethics Board (AREB) at the University of Saskatchewan, Canada with Animal Use Approval Protocol # 19910012.

4.3.3 Rumen Incubation Procedure

Rumen degradation parameters were determined using the in situ method described by Yu et al, (2003). In detail is as follow. 1) Weighed ca. 7 g DM each in a number-coded nylon bag (10 x 20 cm) with multi-bags for each treatment and each incubation time (2, 2, 2, 2, 3, 3 and 4 bags for incubation times 0, 2, 4, 8, 12, 24 and 48 h, respectively). The pore size of nylon bag was ca. 41 μm. These bags are tied about 2 cm below the top, allowing a ratio of sample size to bag surface area of 39 mg/cm2. Recorded bag + string and bag + string + sample weights. 2) The rumen incubations were performed according to the "gradual addition/all out" schedule (the bags are inserted sequentially and retrieved at the same time). Samples were incubated in the rumens for

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48, 24, 12, 8, 4 and 2 h. 3) After incubation, the bags were removed from the rumen and rinsed in a bucket of cold water to remove excess ruminal contents. The bags were then washed with cool water without detergent by hand 6 times with ca. 10 bags each round. The 0 h bags were washed under the same conditions. 4) Washed residues were subsequently dried at 55C for 48 h by placing all bags on stainless steel trays in a forced-air drying oven. All dried bags were exposed to lab room conditions (temperature and moisture) for at least 24 h, then weighed bag + string + residue. Rumen incubation was carried using four fistulated cows with two runs.

4.3.4   Chemical Analysis

Pooled samples of each blend pelleted product residue of each incubation time point were ground through a 1 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) and analyzed for crude protein using Leco protein/N analyzer, Model FP-528 (Leco Corp., St Joseph, MI, USA). Neutral detergent fiber was analyzed using the procedures of Van Soest et al., (1991) combined with Ankom A200 filter bag technique, (Ankom Technology, Fairport, NY, USA). Starch was analyzed using the Megazyme Total Starch Assay Kit (Wicklow, Ireland) and by the α-amylase/amyloglucosidase method (McCleary et al., 1999). All samples were analyzed in duplicate and repeated if chemical analysis error was more than 5 %. Original samples were chemically assessed and reported in the previous chapter.

4.3.5   Rumen Degradation Kinetics

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

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

where R(t) = residue present at t h incubation (%); U = undegradable fraction (%); D = potentially degradable fraction (%); Kd = degradation rate (h−1); and T0 = lag time (h).

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

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

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

where, S stands for soluble fraction (%) or washable fraction (NDF); Kp stands for estimated passage rate from the rumen (h-1) and was assumed to be 6 %/h for DM, CP and Starch, but 2.5 %/h for NDF (Tamminga et al., 1994). The factor 0.1 in the formula represents that 100 g/kg of soluble fraction (S) escapes rumen fermentation.

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

$$BDM\ (BST\ or\ BNDF)\ (g/kg\ DM) = DM\ (ST\ or\ NDF)\ (g/kg\ DM) \times \%\ BDM\ (BST\ or\ BNDF),$$

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

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

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

where 1.11 refers to the regression coefficient between in situ RUP and in vivo RUP (Yu et al., 2002; Tamminga et al., 1994).

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

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

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

$$EDST)$$

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

The effective rumen degradation ratios of N and energy (Sinclair et al. 1993) were calculated hourly as:

$$\text{Hourly ED ratio N/CHO } t = 1000 \times (\text{HEDN}_t - \text{HEDN}_{t-1}) / [(\text{HEDNDF}_t - \text{HEDNDF}_{t-1}) +$$

$$(\text{HEDST}_t - \text{HEDST}_{t-1})],$$

where N/CHO $_t$ = ratio of N to CHO at time t (g N/kg CHO); HEDN t = hourly effective degradability of N at time t (g/kg DM); HEDN $_{t-1}$ = hourly effective degradability of N 1 h before t (g/kg DM); HEDCHO t = hourly effective degradability of CHO at time t (g/kg DM); HEDNDF $_t$ = hourly effective degradability of neutral detergent fiber at time t (g/kg DM); HEDNDF $_{t-1}$ = hourly effective degradability of neutral detergent fiber at 1 h before t (g/kg DM); HEDST $_t$ = hourly effective degradability of starch at time t (g/kg DM); HEDST $_{t-1}$ = hourly effective degradability of starch at 1 h before t (g/kg DM). Previous studies suggested that 32 g N / kg CHO truly digested in the rumen is the optimum ratio to balance microbial protein synthesis and energy cost in regard to rumen fermentation (Sinclair et al., 1993; Tamminga et al., 1990).

4.3.7 Intestinal Digestion of Rumen Undegraded Protein

The estimation of intestinal digestion was determined by a modification of the three-step in vitro procedure described by Calsamiglia and Stern (1995) and Gargallo et al. (2006). Briefly, dried ground residues containing 15 mg of N after 12 h ruminal preincubation were deposited into a 50 ml centrifuge tube, after that 10 ml of pepsin (Sigma P-7012) solution (in 0.1 N HCl with pH 1.9) was added, vortexed, and incubated for 1 h at 38 °C in a water bath. After incubation, 0.5 ml 1 N NaOH solution and 13.5 ml of pancreatin (Sigma P- 7545) was added, vortexed and incubated at 38 °C for 24 h vortexing every 8 hours approximately. Then 3 ml of TCA was added to stop

enzymatic hydrolysis. The tubes were vortexed and sit for 15 min at room temperature, then they were centrifuged for 15 min at 10000 g and analyzed supernatant (5 ml) for soluble N by the Kjeldahl method. Intestinal digestion of protein was calculated as TCA-soluble N divided by the amount of N in the rumen residue sample.

4.3.8 Statistical Analysis

These experiments were designed using the randomized complete block design (RCBD) with run as a random block. The results were statistically analyzed using the Mixed model procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). The model for rumen degradation kinetics, hourly effective degradation ratios between N and CHO and intestinal digestion of rumen undegraded protein was:

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

where, Y_{ijk} is an observation of the dependent variable ijk, μ is the population mean for the variable, T_i is the effect of the blend pelleted product BPP as a fixed effect, S_k is the in situ run effect as a random effect, and e_{ijk} is the random error associated with the observation ijk. For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$. Differences among the treatments were evaluated using a multiple comparison test following the Tukey method. Contrast statements were used to compare the differences between carinata meal pelleted products and canola meal pelleted products, high and low level of inclusion of those co-products (low and high level of inclusion of pea screenings), addition and no addition of lignosulfonate.

4.4 Results and Discussion

4.4.1 In Situ DM Degradation Kinetics

Rate of degradation (Kd), rumen fractions (S, D, U), rumen undegradable dry matter (BDM) and effective degradability of DM (EDDM) of the blend pelleted products (BPP) are presented in Table

4.1. Detailed observation of the data revealed that except for undegradable fraction (U) soluble fraction (S) and degradable fraction (D), were not significantly different (P> 0.05) among all treatments, the rest of rumen degradation characteristics of DM were significantly different (P< 0.05) among all eight blend pelleted products. A higher level of pea screenings in blend pelleted products BPP1, BPP2, BPP5, and BPP6 resulted in higher (P< 0.05) rate of degradation (Kd) (12.76, 14.24, 12.68 and 15.55 %/h, respectively). Carinata based blend pelleted products BPP3 and BPP4 in which the level of inclusion of carinata meal was higher than the other two carinata blend pelleted products BPP1 and BPP2 (75, 71.4 % of DM vs. 50, 47.6 % of DM, respectively), had higher (P< 0.05) %BDM (41.6 and 42.4 %, respectively) than the other blend pelleted products. BPP1, BPP2, BPP5 and BPP6 in which the level of inclusion of pea screenings is higher (50, 47.6, 50 and 47.6 % of DM, respectively) than the other blend pelleted products were higher (P< 0.05) in %EDDM (62.2, 62.7, 63.4, and 63.8 %, respectively) versus %EDDM of BPP3, BPP4, BPP7, and BPP8 (58.4, 57.6, 61.3, and 61.3 %, respectively). Carinata based blend pelleted products contained higher rumen bypassed dry matter (+2.2 %) than canola based blend pelleted products. Adding a higher level of pea screenings or a lower level of co-product resulted in higher Kd (+4.0 %/h), EDDM (+3.4 %), but lower RUDM. Lignosulfonate did not significantly affect these parameters.

Table 4.1. Degradation kinetics of dry matter of blend pelleted products with different combinations (two levels of lignosulfonate chemical compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	Blend pelleted products (BPP)								SEM	P value	Contrast, P value		
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Dry matter													
Kd (%/h)	12.76^{abc}	14.24^{ab}	8.82^c	9.24^c	12.68^{abc}	15.55^a	10.05^{bc}	11.47^{abc}	1.228	<0.01	0.11	0.04	<0.01
Residue at 0 h (%)	82.5	84.0	81.6	83.6	82.5	83.5	79.3	81.7	2.23	0.28	0.21	0.07	0.10
S (%)	17.5	16.0	18.4	16.4	17.1	16.5	20.7	18.3	2.23	0.28	0.21	0.07	0.10
D (%)	66.0	66.7	68.1	68.0	67.8	66.2	64.9	65.5	1.38	0.28	0.15	0.93	0.92
U (%)	16.5	17.3	13.6	15.6	14.7	17.3	14.4	16.2	1.37	0.10	0.90	0.02	0.04
%BDM=%RUDM	37.7^{bc}	37.3^{bc}	41.6^a	42.4^a	36.6^c	36.2^c	38.8^b	38.7^b	0.44	<0.01	<0.01	0.96	<0.01
RUDM (g/kg DM)	377^{bc}	373^{bc}	416^a	424^a	366^c	362^c	388^b	387^b	4.4	<0.01	<0.01	0.96	<0.01
%EDDM	62.3^{ab}	62.7^{ab}	58.4^c	57.6^c	63.4^a	63.8^a	61.2^b	61.3^b	0.44	<0.01	<0.01	0.96	<0.01
EDDM (g/kg DM)	622^{ab}	627^{ab}	584^c	576^c	634^a	638^a	612^b	613^b	4.4	<0.01	<0.01	0.96	<0.01

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

4.4.2   In Situ CP Degradation Kinetics

Previously published studies (Ban, 2016) showed that carinata meal had similar Kd, T0, lag time and U fraction of crude protein to canola meal, while carinata meal contained more S fraction and less D fraction of CP. The rumen undegraded CP was lower in carinata meal (115 g/kg based on NRC Dairy), so carinata meal was greater in EDCP than canola meal (370 vs. 235 g/kg DM) (Ban, 2016). Mainly due to the high in situ rapidly soluble fractions found in peas, they are highly degraded in the rumen. In order to decrease ruminal degradability of peas and therefore rise the RUP available for intestinal absorption processing methods which include heat treatment can be used (Mustafa, 2002). Compared to soybean meal and canola meal, respectively, field peas contain approximately 24 % CP vs. 49.9 and 40.6 %, a RUP content of 22 vs. 35 and 28 % (Khorasani et al., 2001; Fonnesbeck et al. 1984). In this study, parameters of rumen degradation kinetics of crude protein of the blend pelleted products showed significant difference (P< 0.05) among all the eight blend pelleted products (Table 4.2). Canola based pelleted products (BPP5, BPP6, BPP7, BPP8) had higher (P< 0.05) Kd of crude protein than carinata based pelleted products (10.4 vs. 7.9 %/h). However, previously published study showed that carinata meal has higher Kd of crude protein (0.33 vs. 0.17 /h) than canola meal (Xin and Yu, 2014). Canola based pelleted products also had higher (P< 0.05) soluble fraction of crude protein (S) (+2.7 %) than the carinata based pelleted products. The degradable fraction (D) of crude protein of carinata based pelleted products is lower (P< 0.05) than the canola based pelleted products (68.4 vs. 73.2 %). All carinata based pelleted products (BPP1, BPP2, BPP3 and BPP4) showed higher (P< 0.05) levels of undegradable fraction (U) of crude protein (16.4, 18.0, 13.9 and 13.9 %, respectively) than canola based pelleted products (BPP5, BPP6, BPP7 and BPP8 6.6, 10.0, 6.2 and 8.3 %, respectively). BPP3 and BPP4 had the highest (P< 0.05) level of BCP^{DVE} (233 and 239 g/kg,

DM) and RUP^{NRC} (210 and 216 g/kg, DM) than the other blend pelleted products. There is no significant difference in BCP^{DVE} and RUP^{NRC} values between BPP3 and BPP4. However, the RUP^{NRC} numerical values are slightly lower than BCP^{DVE} values. Previously published study suggested that the effective degradable fraction of CP (EDCP) tended to be higher in carinata meal than canola meal (Xin and Yu, 2014). However, in this study, it was found that canola based pelleted products had higher EDCP than carinata based pelleted products (245 vs. 221 g/kg DM) with BPP7 having the highest (P< 0.05) EDCP (278 g/kg DM) among all treatments. In summary carinata based blend pelleted products contain lower degradation rate (-2.5 %/h), lower soluble (-2.7 %) and potential degradation fraction (-4.8 %), but higher undegraded fraction (+7.7 %), lower rumen effective degraded protein (-24 g/kg DM) and higher rumen bypass protein (+56 g/kg DM) than canola based blend pelleted products. Adding a higher level of pea screenings or a lower level of co-product results in higher degradation rate (10.2 vs. 8.1 %/h), higher soluble crude protein (+1.4 %), lower D fraction (-3.6 %), and higher U fraction (+2.2 %), therefore lower rumen bypass protein (-37 g/kg DM). Also, the results showed that adding lignosulfonate did not change degradation rate of crude protein, but reduced soluble fraction (-2.9 %), therefore increased percentage of rumen bypass protein (+2.9 %).

Table 4.2. Degradation kinetics of primary nutrient (crude protein) of blend pelleted products with different combinations (two levels of lignosulfonate chemical compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8	SEM	P value	Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Crude Protein													
CP (g/kg DM)	388[c]	364[d]	450[a]	430[b]	359[d]	337[e]	419[b]	390[c]	4.9	<0.01	<0.01	<0.01	<0.01
Kd (%/h)	9.54[abc]	9.27[abc]	6.95[bc]	5.96[c]	10.4[ab]	11.60[a]	9.93[abc]	9.70[abc]	0.919	<0.01	<0.01	0.91	<0.01
Residue (0 h, %)	82.1[bc]	83.7[ab]	83.8[ab]	86.0[a]	79.2[c]	82.2[abc]	79.0[c]	84.0[ab]	1.15	<0.01	<0.01	<0.01	0.03
S (%)	17.9[ab]	16.3[bc]	16.3[bc]	14.0[c]	20.8[a]	17.8[abc]	21.0[a]	16.1[bc]	1.15	<0.01	<0.01	<0.01	0.03
D (%)	65.8[b]	65.7[b]	69.9[ab]	72.2[a]	72.6[a]	72.2[a]	72.7[a]	75.6[a]	1.31	<0.01	<0.01	0.22	<0.01
U (%)	16.4[ab]	18.0[a]	13.9[abc]	13.9[abc]	6.6[d]	10.1[bcd]	6.2[d]	8.3[cd]	1.71	<0.01	<0.01	0.11	0.05
%BCP=%RUP	41.9[c]	44.1[bc]	46.7[b]	50.1[a]	33.2[e]	35.7[de]	33.7[e]	37.2[d]	0.60	<0.01	<0.01	<0.01	<0.01
BCP (g/kg DM, DVE)	181[b]	178[b]	233[a]	239[a]	132[d]	134[d]	157[c]	161[c]	3.5	<0.01	<0.01	0.18	<0.01
RUP (g/kg DM, NRC)	163[b]	161[b]	210[a]	216[a]	119[d]	120[d]	141[c]	145[c]	3.1	<0.01	<0.01	0.18	<0.01
%EDCP=%RDP	58.1[c]	55.9[cd]	53.3[d]	49.9[e]	66.8[a]	64.3[ab]	66.3[a]	62.8[b]	0.60	<0.01	<0.01	<0.01	<0.01
EDCP=RDP (g/kg DM)	225[cd]	204[e]	240[bc]	215[de]	240[bc]	217[de]	278[a]	245[b]	3.6	<0.01	<0.01	<0.01	<0.01

SEM: standard error of mean; [a-e] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; Kd: the rate of degradation of D fraction (%/h); U: undegradable degradable fraction; D: potentially degradable fraction; T0: lag time (all zero); S: soluble fraction in the in situ incubation; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded of crude protein; CN: canola meal; CR: carinata meal.

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4.4.3 In Situ NDF Degradation Kinetics

The results of NDF rumen degradation parameters and iNDF at 288h incubation are presented in Table 4.3. Detailed observation of the data exposed that T0 ranged from 0.20 to 2.92 h. All canola based pelleted products (BPP5, BPP6, BPP7, BPP8) showed higher (P< 0.05) rate of degradation of NDF (Kd) (+ 3 %/h) than carinata based blend pelleted products. Carinata based blend pelleted products (BPP1, BPP2, BPP3 and BPP4) had higher (P< 0.05) degradable fraction of NDF (D) (85.2, 86.0, 88.9 and 99.6 %, respectively) than canola based pelleted products (BPP5, BPP6, BPP7 and BPP8 57.1, 48.7, 51.4, 52.4 %, respectively). Undegradable fraction of NDF (U) of canola based pelleted products (BPP5, BPP6, BPP7 and BPP8 42.0, 51.3, 48.6 and 47.6 %, respectively) were higher (P< 0.05) (+ 37.4 %) than carinata based pelleted products. Rumen undegradable neutral detergent fiber value (RUNDF) of canola based pelleted products was higher (P< 0.05) (150 to165 g/kg DM) than the carinata blend pelleted products (135 to 145 g/kg DM). Partially in agreement and partially in contrast to our findings, the previously published study showed that among the co-products, canola meal had the highest higher effective fiber degradability (EDNDF) followed by carinata meal (Ban, 2016). Carinata based blend pelleted products contain lower Kd (2.9 vs. 5.9 %/h), longer lag time (T0) (1.85 vs. 0.57 h), higher D (89.9 vs. 52.4 %), lower U (9.9 vs. 47.4 %) and higher effective fiber degradability (28.3 vs. 25.4 %) than canola based blend pelleted products. Adding a higher level of pea screenings or a lower level of co-product resulted in lower lag time of fiber degradation and lower rumen undegradable NDF (-0.89 h and -8 g/kg DM). Also, adding lignosulfonate reduced EDNDF (-5 g/kg DM). In addition, canola based pelleted products possessed higher iNDF than the carinata blend pelleted products (10.4 vs. 6.2 % DM).

Table 4.3. Degradation kinetics of primary nutrient (fiber: NDF) and iNDF (at 228 h based on 2015-CNCPS6.5) of blend pelleted products with different combinations (two levels of lignosulfonate chemical compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

| Items | Blend pelleted products (BPP) | | | | | | | | SEM | P value | Contrast, P value | | |
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Fiber (NDF) Degradation													
NDF (g/kg DM)	193^e	184^f	210^{bc}	195^e	203^{cd}	197de	226^a	215^b	1.7	<0.01	<0.01	<0.01	<0.01
Kd (%/h)	3.07^{bc}	2.74^{bc}	3.42^{abc}	2.37^c	4.41^{abc}	6.63^a	6.78^a	5.78^{ab}	0.725	<0.01	<0.01	0.94	0.47
T0 (h)	1.20^{abc}	0.91^{bc}	2.40^{ab}	2.92^a	0.20^c	0.77^{bc}	1.01^{bc}	0.33^c	0.643	0.04	0.01	0.94	0.05
Residue (0h, %)	100.0	100.0	100.0	99.6	99.0	100.0	100.0	100.0	0.40	0.48	0.60	0.60	0.60
S (%)	0.0	0.0	0.0	0.4	1.0	0.0	0.0	0.0	0.40	0.48	0.60	0.60	0.60
D (%)	85.2^a	86.0^a	88.9^a	99.6^a	57.1^b	48.7^b	51.4^b	52.4^b	4.55	<0.01	<0.01	0.70	0.16
U (%)	14.8^b	14.0^b	11.1^b	0.0^b	42.0^a	51.3^a	48.6^a	47.6^a	4.47	<0.01	<0.01	0.74	0.18
%BNDF=%RUNDF	72.7^{ab}	73.5^{ab}	69.2^b	71.4^{ab}	74.9^a	75.9^a	73.0^{ab}	74.6^a	1.00	<0.01	<0.01	0.06	<0.01
RUNDF (g/kg DM, NRC)	141^{cde}	135^e	145^{cde}	139de	152^{bc}	150^{bcd}	165^a	160^{ab}	2.6	<0.01	<0.01	0.01	<0.01
%EDNDF=%RDNDF	27.3^{ab}	26.5^{ab}	30.8^a	28.6^{ab}	25.1^b	24.1^b	27.0^{ab}	25.4^b	1.00	<0.01	<0.01	0.06	<0.01
EDNDF=RDNDF (g/kg DM)	53^{bc}	49^c	64^a	56^{abc}	51^c	48^c	61^{ab}	54^{bc}	1.9	<0.01	0.16	<0.01	<0.01
iNDF (288 h, CNCPS 6.5) (% DM)	5.3^c	6.1^c	7.4^{bc}	6.2^c	8.8^{abc}	8.8^{abc}	12.5^a	11.6^{ab}	0.82	<0.01	<0.01	0.55	0.01

SEM: standard error of mean; $^{a-e}$ Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; Kd: the degradation rate of D fraction; T0: lag time; S: washable fraction; D: degradable fraction; U: rumen undegradable fraction; BDNDF or RUNDF: rumen bypass or undegraded feed neutral detergent fiber; EDNDF or RDNDF: effective degraded neutral detergent fiber. iNDF: indigestible neutral detergent fiber; CN: canola meal; CR: carinata meal.

4.4.4 In Situ Starch Degradation Kinetics

The results of starch rumen degradation parameters according to the DVE model (Tamminga et al., 1994) are presented in Table 4.4. Value of rate of degradation of starch (Kd) ranged from 18.01 (BPP7) to 28.62 %/h (BPP2). Soluble fraction of starch (S) ranged from 18.9 (BPP2) to 36.7 % (BPP7). Degradable fraction of starch (D) ranged from 63.4 (BPP7) to 81.1 % (BPP2). BPP3, BPP4, BPP7 and BPP8 had lower (P< 0.05) values of rumen undegradable starch (BSt) (28, 22, 28 and 24 g/kg DM, respectively) than the other blend pelleted products. Carinata based pelleted products BPP1, BPP2 and canola based pelleted products BPP5, BPP6 had higher (P< 0.05) levels of effective degradability of starch (EDST) (210, 212, 219 and 213 g/kg, DM, respectively) than the other blend pelleted products. Based on this study there is no difference between carinata based blend pelleted products and canola based blend pelleted products in terms of %BSt and %EDSt. Adding a higher level of pea screenings or a lower level of co-product resulted in higher D fraction (76.0 vs. 69.0 %), but lower S fraction (-7.0 %), therefore higher BSt (45 vs. 26 g/kg DM) and EDSt (214 vs. 11 g/kg DM). This is mainly due to higher starch content in BPP with a higher level of pea screenings or a lower level of co-product. Adding lignosulfonate increased Kd of starch (26.1 vs. 20.9 %/h) and insoluble but potentially degradable fraction (D) (+5.9 %), but decreased soluble fraction (S) (-6.0 %), BSt (-2.0 %) and increased EDSt.

Table 4.4. Degradation kinetics of primary nutrient (starch) (Dutch Model) of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	Blend pelleted products (BPP)								SEM	P value	Contrast, P value		
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
St (g/kg DM)	254^a	253^a	146^{bc}	133^c	268^a	258^a	145^{bc}	148^b	3.3	<0.01	<0.01	0.04	<0.01
Kd (%/h)	24.01^{abc}	28.62^a	18.78^{bc}	27.10^{ab}	22.81^{abc}	23.64^{abc}	18.01^c	25.32^{abc}	2.379	<0.01	0.10	<0.01	0.07
S (%)	24.8^{ab}	18.9^b	35.3^a	19.9^b	25.0^{ab}	27.1^{ab}	36.7^a	32.0^{ab}	5.59	<0.01	0.01	0.01	<0.01
D (%)	75.2^{ab}	81.1^a	64.7^b	80.1^a	75.1^{ab}	72.9^{ab}	63.4^b	68.0^{ab}	5.59	<0.01	0.01	0.01	<0.01
%BSt	17.5^{ab}	16.2^a	19.4^a	16.5^a	18.2^{ab}	17.6^{ab}	19.6^a	16.4^a	0.83	0.02	0.33	<0.01	0.28
BSt (g/kg DM)	44^{ab}	41^b	28^c	22^c	49^a	45^{ab}	28^c	24^c	1.5	<0.01	0.01	<0.01	<0.01
%EDSt	82.5^{ab}	83.9^a	80.6^a	83.5^a	81.8^{ab}	82.4^{ab}	80.4^a	83.7^a	0.83	0.02	0.33	<0.01	0.28
EDST (g/kg DM)	210^a	212^a	117^b	111^b	219^a	213^a	117^b	124^b	4.0	<0.01	0.05	0.80	<0.01

SEM: standard error of mean; $^{a-c}$ Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; Kd: the degradation rate of D fraction; T0: lag time; D: degradable fraction; U: rumen undegradable fraction; BSt: rumen bypass or undegraded feed starch; EDST: effective degraded starch; CN: canola meal; CR: carinata meal.

4.4.5 Hourly Effective Degradation Ratios between Available N and Available CHO

In order to achieve maximum microbial synthesis and minimum N loss the optimal ratio between effective degradability of N and energy is 25 g of N/kg of OM or 32 g of N/kg of CHO for dairy cattle (Tamminga et al., 1994, 2007; Sinclair et al., 1993). It has been shown that higher ratio N/CHO or N/OM than the optimal indicates a potential nitrogen loss from the rumen or not enough energy supply to the microbes in the rumen. On the other hand, a lower ratio than the optimal implies a shortage of nitrogen needed for microbial growth (Nuez-Ortin and Yu, 2010). The hourly effective degradation ratios between available N and available carbohydrates (ED ratio of N/CHO) at different incubation times of the blend pelleted products is shown in Table 4.5. The ratio curve of the carinata blend pelleted products is shown in Figure 4.1, while the ratio curve of the canola blend pelleted products is shown in Figure 4.2. Detailed observation of the data revealed that canola blend pelleted products had higher (P< 0.05) overall ratio of ED_N/ED_CHO than carinata blend pelleted products (165 vs. 141 g/kg). Canola pelleted product BPP7 showed the highest (P< 0.05) overall ratio of ED_N/ED_CHO (215 g/kg) while carinata blend pelleted product BPP2 had the lowest overall ED_N/ED_CHO (110 g/kg) than the other blend pelleted products. In contrast with our findings, previously published study with pure carinata and canola meals, demonstrated that the effective degradation ratio of N to OM was significantly higher in carinata meal (95 g N/kg OM) than in canola meal (77 g N/kg OM) (Ban, 2016). Adding a higher level of pea screenings or a lower level of co-product resulted in BPP with lower (-66 g/kg) overall ratio of ED_N/ED_CHO. Also, adding lignosulfonate decreased the overall ratio of ED_N/ED_CHO in the blend pelleted products (P< 0.05) (-12 g/kg). The largest effective degradation ED_N/ED_CHO ratios at individual incubation times (h) of the blend pelleted products without significant (P> 0.05) difference among them were seen at beginning of

incubation (h0). At individual incubation times h1, h2, h3, h4 and h6 canola blend pelleted product BPP7 had higher (P< 0.05) effective degradation ED_N/ED_CHO ratios (202, 208, 213, 217 and 222 g/kg, respectively) than the other blend pelleted products. At h10 incubation time, effective degradation ED_N/ED_CHO ratio of BPP8 reached the highest point in the curve (244 g/kg)., while BPP7 reached at h6 (222 g/kg). Blend pelleted products BPP1, BPP2, BPP5 and BPP6 reached the highest effective degradation ED_N/ED_CHO ratio (164, 177, 166 and 165 g/kg, respectively) in the curve at time point h12. BPP3 reached the highest effective degradation ED_N/ED_CHO ratio (198 g/kg) at incubation time h8, while BPP4 reached the highest effective degradation ED_N/ED_CHO ratio (231 g/kg) at incubation time h10. Canola based pelleted products showed greater ratio of ED_N/ED_CHO at h1 (+36 g/kg), h2 (+34 g/kg), h3 (+31 g/kg), h4 (+27 g/kg) and h6 (+19 g/kg) than the carinata based pelleted products. Adding a higher level of pea screenings or a lower level of co-product resulted in BPP with lower ratio of ED_N/ED_CHO at h8 (153 vs. 219 g/kg), h10 (165 vs. 222 g/kg), h12 (168 vs. 216 g/kg) and h16 (148 vs. 187 g/kg). Also, adding lignosulfonate increased the ratio of ED_N/ED_CHO at h10 (+17 g/kg), h12 (+58 g/kg) and h16 (+16 g/kg) in the blend pelleted products.

Carinata based pelleted products BPP1, BPP2 and canola based pelleted products BPP5 and BPP6 reached lower (P< 0.05) effective degradation ED_N/ED_CHO ratios (17, 17, 21 and 28 g/kg, respectively) than the optimal 32 g of N/kg of CHO at incubation time h40, while BPP3, BPP4, BPP7 and BPP8 reached that lower (P< 0.05) ratio (21, 22, 27 and 23 g of N/kg of CHO, respectively) at incubation time h48. This may be due to the BPP3, BPP4, BPP7 and BPP8 had higher levels of crude protein that the other blend pelleted products because the higher levels of inclusion of bio-fuel and bio-oil co-product in their compositions. The results showed that due to high levels of SCP of canola based blend pelleted products than the carinata based blend pelleted

products the ED_N/ED_CHO ratios did not decrease at a lower point as much as carinata based pelleted products did at h1 incubation time. Also, the levels below the optimal reached at different incubation times due to the available CP content in their compositions. Higher protein pelleted products reached a lower ED_N/ED_CHO ratio than the optimal at longer incubation time.
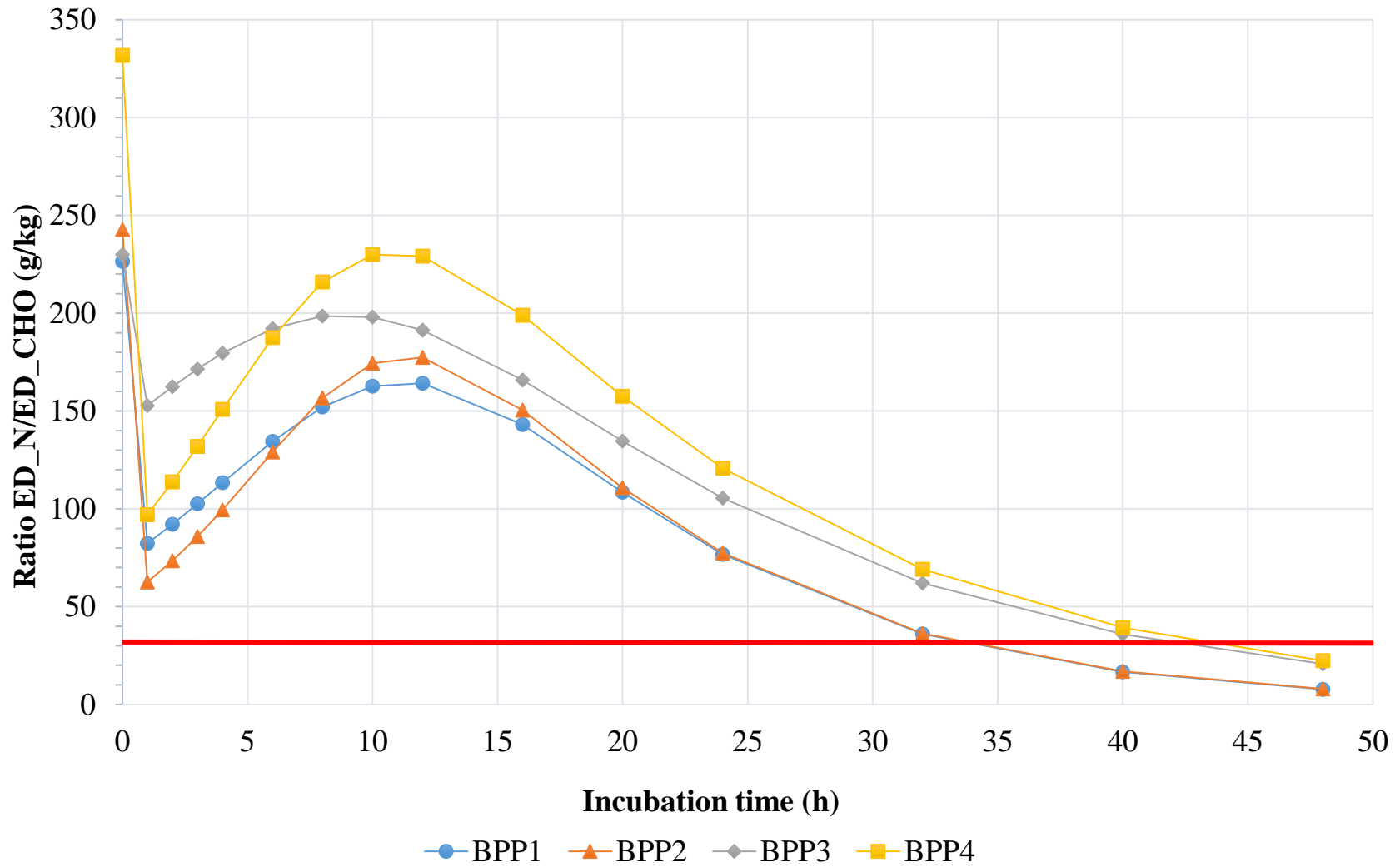
Figure 4.1. Hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of carinata based blend pelleted products. Optimum ratio = 32 EN/ECHO g/kg.
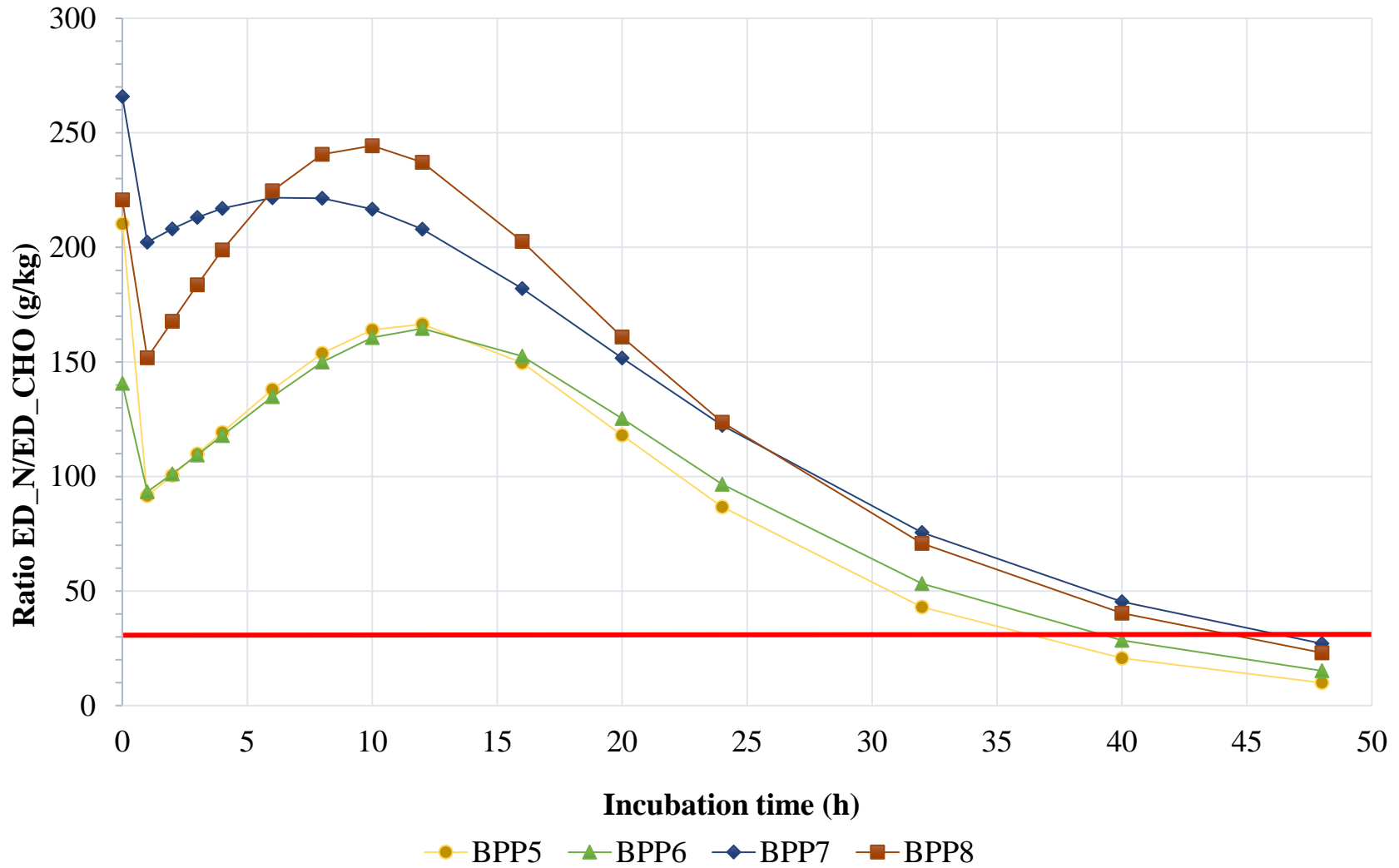
Figure 4.2. Hourly effective degradation ratios (ED_N/ED_CHO) between available N and available CHO of canola based blend pelleted products. Optimum ratio = 32 EN/ECHO g/kg.

Table 4.5. Potentially available N to available CHO synchronization of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8	SEM	P value	Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Ratio of N/CHO (g/kg)	139^c	134c	203^a	210^a	122^d	118^d	181^b	172^b	2.4	<0.01	<0.01	0.07	<0.01
Ratio of ED_N/ED_CHO (g/kg)	119de	110e	170^c	165^c	128^d	121de	215^a	190^b	3.2	<0.01	<0.01	<0.01	<0.01
Ratio at individual h (g/kg)													
h0	226	243	230	332	211	141	266	221	51.3	0.15	0.09	0.97	0.05
h1	82^{cd}	63^d	153^b	97^c	92^{cd}	93^{cd}	202^a	152^b	10.1	<0.01	<0.01	<0.01	<0.01
h2	92^{cd}	73^d	162^b	114^c	100^{cd}	101^{cd}	208^a	168^b	8.8	<0.01	<0.01	<0.01	<0.01
h3	103de	86^e	171^b	132^c	110^{cd}	109^{cd}	213^a	184^b	7.3	<0.01	<0.01	<0.01	<0.01
h4	113de	99^e	180^b	151^c	119^d	118de	217^a	199^a	5.7	<0.01	<0.01	<0.01	<0.01
h6	134^c	129^c	192^b	188^b	138^c	135^c	222^a	225^a	3.6	<0.01	<0.01	0.34	<0.01
h8	152^c	157^c	198^b	216^{ab}	154^c	150^c	221^{ab}	241^a	5.9	<0.01	0.01	0.02	<0.01
h10	163^d	174^{cd}	198^{bc}	231^{ab}	164^d	161^d	217^{ab}	244^a	8.7	<0.01	0.30	<0.01	<0.01
h12	164^c	177^{bc}	191^{bc}	229^a	166^c	165^c	208^{ab}	238^a	9.9	<0.01	0.51	<0.01	<0.01
h16	143^c	150^{bc}	166^{bc}	199^a	150^{bc}	152^{bc}	182^{ab}	203^a	9.1	<0.01	0.16	<0.01	<0.01
h20	108^d	111^d	135^{abcd}	158^{ab}	118^{cd}	125^{bcd}	152^{abc}	161^a	8.0	<0.01	0.04	0.05	<0.01
h24	77^c	77^c	105^{abc}	121^{ab}	87^{bc}	97^{abc}	122^a	124^a	7.6	<0.01	0.03	0.20	<0.01
h32	36^c	36^c	62^{abc}	69^{ab}	43^{bc}	53^{abc}	76^a	71^{ab}	6.4	<0.01	0.04	0.48	<0.01
h40	17^c	17^c	36^{abc}	39^{ab}	21^{bc}	28^{abc}	45^a	40^{ab}	4.7	<0.01	0.06	0.64	<0.01
h48	8^c	8^{bc}	21^{abc}	22^{abc}	10^{bc}	15^{abc}	27a	23^{ab}	3.3	<0.01	0.09	0.74	<0.01

SEM: standard error of mean; [a-e] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; ED: effective degradability; CHO: carbohydrates; CN: canola meal; CR: carinata meal.

4.4.6 Intestinal Availability of Rumen Bypass Nutrients

Intestinal digestible rumen bypass and total digestible DM, CP, NDF and Starch of the blend pelleted products are presented in Table 4.6. Detailed observation of the data revealed that the intestinal digestibility of DM (dBDM) was higher ($P< 0.05$) in carinata based blend pelleted products (BPP1, BPP2, BPP3 and BPP4 66.4, 66.3, 70.5 and 71.5 %, respectively) than canola based pelleted products (BPP5, BPP6, BPP7 and BPP8 62.9, 59.2, 62.0 and 62.7 %, respectively). However, there is no significant difference among intestinal digestibility of BDM in all carinata based pelleted products, and there is no significant difference among all canola based pelleted products.

Carinata based pelleted product BPP4 had the highest ($P< 0.05$) intestinal digestible rumen bypass DM (IDBDM) (306 g/kg DM) than the other blend pelleted products except BPP1 and BPP3 (252 and 301 g/kg DM). As to total digestible DM (TDDM) carinata based pelleted products had higher value than canola based pelleted products (+22 g/kg DM).

Carinata based pelleted product had the higher ($P< 0.05$) intestinal digestibility of CP (dIDP) than the canola based blend pelleted products (78.0 vs. 69.0 %), but it was found no significant difference among the values (dIDP) of all carinata based pelleted products. Intestinal absorbable feed protein (IADP) is higher in the carinata based pelleted product BPP4 (168 g/kg DM) and BPP3 (164 g/kg DM) than the other blend pelleted products. This may be due to the higher level of inclusion of the bio-fuel co-product carinata meal in their compositions (75.0 and 71.4 % of DM), also the IADP value of BPP4 may be due to the inclusion of lignosulfonate (4.8 % DM) into its composition which reduced soluble fraction of CP and increased bypass protein to be digested in the small intestine. The lowest ($P< 0.05$) value of intestinal absorbable feed protein was found in canola pelleted product BPP5 (83 g/kg DM), BPP6 (84 g/kg DM) and BPP7 (91

g/kg DM). A previous study showed, in contrast with our findings, that canola meal had an advantage in providing more intestinal digested crude protein (97 g/kg DM) compared to carinata meal (71 g/kg DM) because of less amount of RUP passing from the rumen provided by carinata meal (Ban, 2016). However, this study showed that canola based pelleted products contained lower IADP (90 vs. 146 g/kg DM) than the carinata based pelleted products. Carinata based pelleted product BPP3 showed the highest (P< 0.05) total digestible protein (TDP) (405 g/kg DM) than the other blend pelleted products followed by BPP4 (383 g/kg DM) and BPP7 (369 g/kg DM). Canola based pelleted products contained lower TDP (335 vs. 367 g/kg DM) than the carinata based pelleted products. In agreement with our findings the previous study demonstrated that carinata meal had higher total digested feed protein (TDP) than canola meal (441 vs. 332 g/kg DM) (Ban, 2016).

Intestinal digestibility of rumen undegraded starch ranged from 96.5 to 98.7 %. Also, digestible rumen bypass starch was higher (P< 0.05) in canola blend pelleted products BPP5 (48 g/kg DM), BPP6 (45 g/kg DM) and carinata blend pelleted products BPP1 (44 g/kg DM), BPP2 (40 g/kg DM). Total digestible starch was higher (P< 0.05) in carinata blend pelleted products BPP1, BPP2 and canola pelleted products BPP5, BPP6 (253, 252, 267 and 257 g/kg DM, respectively) than the other blend pelleted products. This is mainly due to the higher level of inclusion of pea screenings in their composition (50.0, 47.6, 50.0 and 47.6 % of DM, respectively).

Carinata based pelleted products BPP1 to BPP4 showed higher (P< 0.05) level of intestinal digestibility of NDF (dBNDF) than the canola based blend pelleted products (48.1 vs. 25.2 %). In addition, digestible rumen bypass NDF (IDBNDF) was higher (P< 0.05) in carinata based pelleted

products (62 to 74 g/kg DM). Carinata based pelleted product BPP3 showed the highest ($P < 0.05$) total digestible NDF (TDNDF 139 g/kg DM).

Carinata based blend pelleted products contained higher TDDM (+19 g/kg DM), TDP (+32 g/kg DM), TDNDF (+30 g/kg DM) and higher %dBDM (+7.1 %), %dIDP (+8.9 %), %dNDF (+23.0 %), but lower TDST (-9 g/kg DM) than canola based blend pelleted products. By adding a higher level of pea screenings or a lower level of co-product resulted in BPP with higher TDST (257 vs. 142 g/kg DM), lower TDNDF (100 vs. 116 g/kg DM) and lower TDP (326 vs. 376 g/kg DM). Also, adding lignosulfonate in the composition results in BPP with higher IADP (+24 g/kg CP) and lower IDBST (-4 g/kg DM).

Table 4.6. Intestinal digestibility and total tract digestion of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	Blend pelleted products (BPP)								SEM	P value	Contrast, P value		
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Dry matter													
%dBDM	66.4[ab]	66.4[ab]	70.5[a]	71.5[a]	62.9[b]	59.2[b]	62.0[b]	62.6[b]	1.91	<0.01	<0.01	0.65	0.02
%IDBDM	25.2[abc]	24.4[c]	30.1[ab]	30.6[a]	22.5[c]	20.7[c]	24.6[bc]	25.0[bc]	1.60	<0.01	<0.01	0.62	<0.01
IDBDM (g/kg DM)	252[abc]	244[c]	301[ab]	306[a]	225[c]	207[c]	246[bc]	250[bc]	16.0	<0.01	<0.01	0.62	<0.01
%TDDM	87.4[a]	87.7[a]	87.5[a]	87.8[a]	86.8[ab]	85.8[ab]	84.9[b]	85.2[b]	0.45	<0.01	<0.01	0.94	0.07
TDDM (g/kg DM)	874[a]	877[a]	875[a]	878[a]	868[ab]	858[ab]	849[b]	852[b]	4.6	<0.01	<0.01	0.95	0.07
Crude protein													
% dIDP	79.1[a]	76.7[ab]	78.3[a]	78.0[a]	69.8[cd]	69.9[c]	64.7[d]	71.9[bc]	1.50	<0.01	<0.01	0.15	0.43
IADP (g/kg DM)	129[b]	123[b]	165[a]	168[a]	83[d]	84[d]	91[d]	104[c]	2.4	<0.01	<0.01	0.08	<0.01
IADP (g/kg CP)	332[c]	338[bc]	366[ab]	391[a]	232[e]	250[de]	218[e]	267[d]	7.2	<0.01	<0.01	<0.01	<0.00
TDP (g/kg DM)	354[cd]	327[e]	405[a]	383[b]	323[e]	301[f]	369[bc]	349[d]	3.23	<0.01	<0.01	<0.01	<0.01
TDP (g/kg CP)	913[a]	897[ab]	899[ab]	890[b]	900[ab]	893[ab]	881[b]	896[ab]	7.1	0.01	0.04	0.21	0.01
%IADP (%CP)	33.2[c]	33.8[bc]	36.6[ab]	39.0[a]	23.2[e]	25.0[de]	21.8[e]	26.8[d]	0.72	<0.01	<0.01	<0.01	<0.01
%TDP (%CP)	91.3[a]	89.7[ab]	89.9[ab]	89.0[b]	90.0[ab]	89.3[ab]	88.1[b]	89.6[ab]	0.71	0.01	0.04	0.21	0.01

SEM: standard error of mean; [a-f] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison: Tukey method; dBDM: intestinal digestibility of rumen bypass dry matter; IDBDM: intestinal digested rumen bypass dry matter; TDDM: total digested dry matter dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digested crude protein; TDP: total digested crude protein. CN: canola meal; CR: carinata meal.

Table 4.6. *Cont'd* Intestinal digestibility and total tract digestion of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8	SEM	P value	Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Starch													
%dBST	98.4[ab]	98.4[ab]	97.0[cd]	96.5[d]	98.7[a]	98.4[ab]	98.2[ab]	97.7[bc]	0.21	<0.01	<0.01	0.02	<0.01
% IDBST	17.2[ab]	15.9[b]	18.8[a]	15.9[b]	17.9[ab]	17.3[ab]	19.2[a]	16.0[b]	0.83	0.02	0.23	<0.01	0.46
IDBST (g/kg DM)	44[ab]	40[b]	27[c]	21[c]	48[a]	45[ab]	28[c]	24[c]	1.5	<0.01	0.01	<0.01	<0.01
%TDST	99.7[a]	99.7[a]	99.4[b]	99.4[b]	99.8[a]	99.7[a]	99.7[a]	99.6[a]	0.03	<0.01	<0.01	0.67	<0.01
TDST (g/kg DM)	253[a]	252[a]	145[bc]	132[c]	267[a]	257[a]	145[bc]	148[b]	3.3	<0.01	<0.01	0.04	<0.01
Fiber (NDF)													
%dBNDF	45.4[a]	45.9[a]	51.0[a]	50.21[a]	26.6[b]	23.6[b]	25.4[b]	25.2[b]	2.20	<0.01	<0.01	0.49	0.05
%IDBNDF	33.1[a]	33.7[a]	35.3[a]	35.85[a]	20.0[b]	18.0[b]	18.5[b]	18.9[b]	1.81	<0.01	<0.01	0.90	0.37
IDBNDF (g/kg DM)	64[a]	62[a]	74[a]	70[a]	41[b]	35[b]	42[b]	41[b]	4.0	<0.01	<0.01	0.17	0.01
%TDNDF	60.4[b]	60.2[b]	66.1[a]	64.5[ab]	45.1[c]	42.1[c]	45.5[c]	44.2[c]	1.42	<0.01	<0.01	0.07	<0.01
TDNDF (g/kg DM)	117[bc]	110[cd]	139[a]	126[b]	91[ef]	83[e]	103[de]	95[ef]	3.5	<0.01	<0.01	<0.01	<0.01

SEM: standard error of mean; [a-f] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; dBST: intestinal digestibility of rumen bypass starch; IDBST: intestinal digested rumen bypass starch; TDST: total digested starch. dBNDF: intestinal digestibility of rumen bypass neutral detergent fiber; IDBNDF: intestinal digested rumen bypass neutral detergent fiber; TDNDF: total digested neutral detergent fiber; CN: canola meal; CR: carinata meal.

4.5 Conclusions

Based on these studies, carinata based pelleted products can provide higher amounts of rumen bypass protein, intestinal absorbable feed protein and total digested protein as well as total digestible NDF than the canola blend pelleted products. In addition, the BPP3 and BBP4 products provide higher rumen bypass protein, intestinal absorbable feed protein and higher total digested NDF than the other blend pelleted products. For these reasons, it was concluded that carinata based pelleted products BPP3 and BPP4 could be used as an alternative high quality bypass protein supplement for ruminants.

5. METABOLIC CHARACTERISTICS, TRULY DIGESTED NUTRIENT SUPPLY, AND FEED MILK VALUE OF BLEND PELLETED PRODUCTS BASED ON COMBINATION OF CO-PRODUCTS FROM BIO-FUEL/BIO-OIL PROCESSING, PEA SCREENINGS AND LIGNOSULFONATE AT DIFFERENT LEVELS FOR DAIRY CATTLE.

5.1 Abstract

The aim of this study was to develop and test eight different pelleted products based on combination of co-products from bio-fuel processing (carinata meal), bio-oil processing (canola meal), pea screenings and lignosulfonate at different levels for ruminants. The eight products include:

BPP1: lignosulfonate 0 % of DM + carinata meal 50 % of DM + pea screenings 50.0 % of DM;

BPP2: lignosulfonate 4.8 % of DM + carinata meal 47.6 % of DM + pea screenings 47.6 % of DM;

BPP3: lignosulfonate 0 % of DM + carinata meal 75 % of DM + pea screenings 25 % of DM;

BPP4: lignosulfonate 4.8 % of DM + carinata meal 71.4 % of DM + pea screenings 23.8 % of DM;

BPP5: lignosulfonate 0 % of DM + canola meal 50 % of DM + pea screenings 50.0 % of DM;

BPP6: lignosulfonate 4.8 % of DM + canola meal 47.6 % of DM + pea screenings 47.6 % of DM;

BPP7: lignosulfonate 0 % of DM + canola meal 75 % of DM + pea screenings 25 % of DM;

BPP8: lignosulfonate 4.8 % of DM + canola meal 71.4 % of DM + pea screenings 23.8 % of DM. Comparisons were made between blend pelleted products based on carinata meal and pelleted products based on canola meal, high or low level of inclusion of co-product (low or high level of inclusion of pea screenings) and inclusion or no inclusion of lignosulfonate in terms of metabolic characteristics, truly digested nutrient supply and feed milk value. According to the DVE-OEB System the results showed that carinata based pelleted products BPP3 and BPP4 had the highest

(P< 0.05) values of true protein digested in the small intestine (DVE 236 and 240 g/kg DM). Canola pelleted product BPP7 had the highest (P< 0.05) degradable protein balance (OEB 164 g/kg DM), followed by BPP8, BPP5 and BPP3 (130, 125 and 125 g/kg DM, respectively). Those findings were also supported by the NRC 2001 in which, metabolizable protein (MP) content was the highest (P< 0.05) in carinata based pelleted products BPP3 and BPP4 (228 and 231 g/kg DM) and the lowest values were found in BPP5, BPP6 and BPP7 (145, 145 and 151 g/kg DM, respectively). Rumen degraded protein balance (DPB) was the highest (P< 0.05) in BPP7 (177 g/kg DM) and lowest (P< 0.05) in BPP2 (93 g/kg DM) and BPP4 (107 g/kg DM). The FMV based on the DVE system was highest (P< 0.05) in carinata based pelleted products BPP3 and BPP4 (4.80 and 4.86 kg milk/kg DM feed), based on the NRC 2001 model the pattern is the same only it shows slightly lower numerical values than those in the DVE system with FMV of 4.34 and 4.34 kg milk/kg DM feed, respectively. In conclusion BPP3 and BPP4 provided the highest DVE and MP than the other blend pelleted products, which lead to highest feed milk values.

Keywords: Carinata, Canola, Pea, Lignosulfonate, Truly digestible nutrient supply.

5.2 Introduction

Animal productivity and production efficiency can be improved by enhancing ruminant nutrition and methodically evaluating the nutritive value of each different feed ingredient of a diet (Theodoridou and Yu, 2013). Modern models are able to calculate nutrient requirements and feed utilization in production situations. In terms of protein nutrition, the quantity of digestible protein reaching the small intestine and the level of microbial protein synthesized in the rumen are significant elements of the response and efficiency with which nutritional nitrogen is used for milk production (Tamminga et al., 2007; Yu et al., 2002). Information on chemical profiles, digestive behaviors of feeds used in dairy cattle as well as prediction systems make it possible to feed protein

more accurately according to the actual requirements of the animals and avoid unnecessary nitrogen losses (Tamminga et al., 1994). In order to predict metabolizable protein supply to small intestine, the DVE/OEB and NRC 2001 dairy are modern and advanced protein evaluation systems that presently are being used broadly in some countries around the world. In Canada, various co-products are used in the livestock industry such as canola meal which is considered an excellent protein source (Canola Council, 2009). Also, pea screenings are used because of its high content of protein and starch (Yu et al., 2002) and in addition, the new carinata meal available after bio-fuel processing is now available. However, little information about this co-product is available. Previous study demonstrated that this new co-product can be a potential protein source, but it has high degradation rate and extent of protein, similar to canola meal (Xin and Yu, 2014). The addition of lignosulfonate and pelleting can be used to achieve reduced rumen degradation (Tamminga and Goelema, 1995; Windschitl and Stern, 1988). In order to evaluate a specific feed, it is important to comprehend how metabolic characteristics and nutrient supply to dairy cattle are affected by blending, pelleting processing and addition of lignosulfonate as a feed additive. Also, the level of microbial protein synthesis and the quantity of digestible protein in the intestine are important to determine the response and efficiency of feed nitrogen used for milk production. However, there is insufficient information accessible on metabolic characteristics, nutrient supply and feed milk value of this new co-product particularly when it is blended with another feedstuff as a pelleted product. In order to completely understand this bio-fuel co-product much effort is still needed. This research was conducted to test eight blend pelleted products based on the combination of new co-product of biofuel processing of carinata seed, conventional co-product from bio-oil processing of canola seed, pea screenings and lignosulfonate at different levels for ruminants. The metabolic characteristics, true nutrient supply and feed milk value evaluated based on the advanced

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evaluation systems, the NRC-2001 model, and the DVE/OEB System were determined. Comparisons were made between blend pelleted products based on carinata meal and pelleted products based on canola meal, low or high level of inclusion of co-product and inclusion or no inclusion of lignosulfonate.

5.3 Materials and Methods

5.3.1 Nutrient Supply with the DVE/OEB System

The DVE/OEB, Dutch protein evaluation system, calculated two characteristics for each feed: true protein digested in the intestine (DVE) and the rumen degradable protein balance (OEB). DVE represents the protein value of a feed and can be separated into three components: feed crude protein undegraded in the rumen but digested in the small intestine (DVBE), microbial true protein synthesized in the rumen and digested in the small intestine (DVME), and endogenous protein lost in the digestive processes (ENDP); while OEB is the difference between the potential microbial protein synthesis (MPS) on the basis of available rumen degradable protein and that on the basis of available rumen degradable energy (Van Duinkerken et al., 2011; Tamminga et al., 2007, 1994). The DVE value comprises digestible feed protein, microbial protein, and an endogenous protein loss correction. The DVE value was calculated as:

$$DVE\ (g/kg\ of\ DM) = DVME + DVBE - ENDP,$$

where, DVME is the absorbable fraction of microbial crude protein, DVBE is the absorbable fraction of ruminally undegraded feed protein, and ENDP is a correction factor for endogenous protein lost during the digestion process.

The OEB value of a feed is the difference between the potential microbial protein synthesis based on MREN and the potential microbial protein synthesis based on energy extracted from anaerobic fermentation MREE. Therefore

$$OEB \text{ (g/kg of DM)} = MREN - MREE,$$

where, MREN was calculated as MREN = CP × [1 − (1.11 × RUP (% CP)/100)]. The factor 1.11 in the formula was taken from the French PDI system and represents the regression coefficient of in vivo, on in situ degradation data. MREE = FOM × 0.15 (FOM in g/kg) (Tamminga et al., 1994).

5.3.2 Nutrient Supply with the NRC-2001 Model

According to the NRC (2001) model, MP is composed of 3 major contributory protein sources. Total MP can be calculated as follows:

$$MP \text{ (g/kg DM)} = AMCP + ARUP + AECP,$$

where, AMCP is the absorbable microbial protein, ARUP is the truly absorbable rumen undegraded feed protein, and AECP is the truly absorbable endogenous protein in the small intestine (NRC, 2001)

Degraded protein balance (DPB), based on data from the NRC-2001 model, reflects the difference between the potential microbial protein synthesis based on RDP and the potential microbial protein synthesis based on energy available for microbial fermentation in the rumen. Thus, The DPB^{NRC} was calculated as follows:

$$DPB \text{ (g/kg of DM)} = RDP^{NRC} - 1.18 \times MCP_{TDN}.$$

5.3.3 Feed Milk Value

Feed Milk Value was determined on the basis of metabolic characteristics of protein from the DVE system and NRC model. The efficiency of use of metabolizable protein for lactation is assumed to be 0.67 (NRC, 2001), and protein composition in milk is assumed to be 33 g protein / 1 Kg of milk.

5.3.4 Statistical Analysis

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These experiments were designed using the randomized complete block design (RCBD) with pellet processing run as a random block effect. The results were statistically analyzed using the mixed model procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). The model for nutrient supply and feed milk value with the DVE System and NRC-2001 Model was:

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

where, Y_{ijk} is an observation of the dependent variable ijk, μ is the population mean for the variable, T_i is the effect of the blend pelleted product BPP as a fixed effect, S_k is the run effect as a random effect, and e_{ijk} is the random error associated with the observation ijk. For all statistical analyses, significance was declared at $P < 0.05$ and trends at $P \leq 0.10$. Differences among the treatments were evaluated using a multiple comparison test following the Tukey method. Contrast statements were used to compare the differences between carinata meal pelleted products and canola meal pelleted products, high and low level of inclusion of those co-products (low and high level of inclusion of pea screenings), addition and no addition of lignosulfonate.

5.4 Results and Discussion

5.4.1 Nutrient Supply with the DVE/OEB System

Metabolic Characteristics and True Nutrient Supply based on the DVE/OEB System are presented in Table 5.1. Bypass crude protein (BCP) was highest (P< 0.05) in carinata blend pelleted product BPP3 and BPP4 (233 and 239 g/kg DM). Canola blend pelleted product BPP5 and BPP6 contained the lowest (P< 0.05) amount of BCP (132 and 134 g/kg DM) than the other blend pelleted products.

However, these canola products had the highest (P< 0.05) microbial protein synthesised in the rumen based on available energy (MREE) (102 and 103 g/kg DM). Canola pelleted product BPP7 had the highest (P< 0.05) microbial protein synthesised in the rumen based on the available rumen

degradable CP (MREN) (262 g/kg DM) than the other blend pelleted products. Partially in agreement with our findings, previous research showed that carinata meal had more truly absorbed rumen synthesized microbial protein (DVME), but less absorbed bypass protein (DVBE) in the small intestine compared with canola meal (Ban, 2016). This study showed that BPP products had minimal differences in rumen synthesised microbial protein digested in the small intestine (DVME) ranging from 58 to 66 g/kg DM. Carinata based pelleted product BPP3 and BPP4 showed the highest truly absorbed bypass protein in the small intestine (DVBE 183 and 187 g/kg DM, respectively) than the other pelleted products. Previous study concluded that truly absorbed protein in the small intestine (DVE) were similar for both carinata and canola meals (Xin and Yu, 2014). However, in this study, after blending, carinata based pelleted products BPP3 and BPP4 had the highest ($P < 0.05$) values of protein truly digested in the small intestine (DVE 236 and 240 g/kg DM), while canola based pelleted products BPP5, BPP6, BPP7 and BPP8 had the lowest values (151, 153, 158 and 172 g/kg DM, respectively).

Positive OEB means potential nitrogen loss from the rumen whereas a negative value indicates potential shortage of nitrogen supply in the rumen (Tamminga et al.,1994). Canola pelleted product BPP7 had the highest ($P < 0.05$) degradable protein balance (OEB 164 g/kg DM), with lowest value in carinata blend pelleted product BPP2 (87 g/kg DM). Canola pelleted products have higher OEB value (+25 g/kg DM) due to the higher level of RDP and lower level of energy content. This study suggested that carinata based blend pelleted products contained higher total DVE (+59 g/kg DM) and lower OEB (-25 g/kg DM) than canola based blend pelleted products. Adding a higher level of pea screenings or a lower level of co-product resulted in lower DVE (-26 g/kg DM) and OEB (-24 g/kg DM) values. Also, including lignosulfonate in the composition did not significantly affect DVE, but significantly reduced degraded protein balance OEB (-25 g/kg DM) value.

5.4.2   Nutrient Supply with the NRC-2001 Model

Metabolic Characteristics and True Nutrient Supply based on the NRC-2001 model are shown in Table 5.1. BPP7 and BPP8 contained the highest (P< 0.05) ruminally synthesized microbial protein based on availability of RDP (MCP$_{RDP}$) (236 and 208 g/kg DM). Ruminally synthesized microbial protein based on TDN$_{3x}$ (MCP$_{TDN}$) and truly absorbed rumen synthesized microbial protein in the small intestine (AMCP) were minimal different with MCP$_{TDN}$ ranging from 85 to 94 g/kg DM and AMCP from 54 to 60 g/kg DM. Rumen undegradable protein (RUP) (210 and 216 g/kg DM) and truly absorbed rumen undegraded feed protein (ARUP) (164 and 168 g/kg DM) was highest (P< 0.05) in BPP3 and BPP4. In this study, it was found that carinata based pelleted products contain higher AMCP and higher ARUP than canola based pellet products. Partially in agreement with previous studies, carinata meal showed higher AMCP but a lower ARUP compared with canola meal; (Ban, 2016). In contrast to our findings, Ban (2016) found a higher MP value in canola meal than carinata meal. However, in this study, metabolizable protein (MP) content was highest (P< 0.05) in carinata based pelleted products BPP3 and BPP4 (228 and 231 g/kg DM). Rumen degraded protein balance (DPB) was highest (P< 0.05) in BPP7 (177 g/kg DM) and ranged from 93 to 107 g/kg DM. Carinata based blend pelleted products contained higher MP value and lower DPB value than canola based blend pelleted products (210 vs. 151 and 111 vs. 142 g/kg DM, respectively). Adding a higher level of pea screenings or a lower level of co-product result in lower MP (-26 g/kg DM) and DPB (-27 g/kg DM) values. Including lignosulfonate in BPP compositions did not significantly affect MP, but significantly reduce protein degraded balance DPB value (-25g/kg DM).

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Table 5.1. Metabolic characteristics and true nutrient supply of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings), determined based on TND-based and non-TDN based ruminant nutrient supply systems

Items	Blend pelleted products (BPP)								SEM	P value	Contrast, P value		
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Truly digestible nutrient supply to dairy cows based on non-TDN system: DVE system													
BCP (g/kg DM)	181[b]	178[b]	233[a]	239[a]	132[d]	134[d]	157[c]	161[c]	3.5	<0.01	<0.01	0.18	<0.01
MREE (g/kg DM)	99[c]	99[bc]	92[d]	91[d]	102[ab]	103[a]	99[c]	99[c]	0.6	<0.01	<0.01	0.44	<0.01
MREN (g/kg DM)	208[cd]	186[e]	217[bc]	191[de]	227[b]	203[cd]	262[a]	229[b]	3.7	<0.01	<0.01	<0.01	<0.01
DVME (g/kg DM)	63[c]	63[bc]	59[d]	58[d]	65[ab]	66[a]	63[c]	63[c]	0.3	<0.01	<0.01	0.44	<0.01
DVBE (g/kg DM)	143[b]	137[b]	183[a]	187[a]	92[d]	93[d]	101[c]	116[c]	2.6	<0.01	<0.01	0.08	<0.01
Degraded protein balance (OEB of BPP products) and Total true protein supply (DVE of BPP products) to dairy cows													
DVE (g/kg DM)	201[b]	196[b]	236[a]	240[a]	151[d]	153[d]	158[d]	172[c]	2.5	<0.01	<0.01	0.07	<0.01
OEB (g/kg DM)	109[cd]	87[e]	125[bc]	100[de]	125[b]	101[de]	164[a]	130[b]	3.6	<0.01	<0.01	<0.01	<0.01
Truly digestible nutrient supply to dairy cows based on TDN system: NRC dairy													
RUP (g/kg DM)	163[b]	161[b]	210[a]	216[a]	119[d]	120[d]	141[c]	145[c]	3.1	<0.01	<0.01	0.18	<0.01
MCP$_{TDN}$ (g/kg DM)	94[a]	93[b]	92[c]	91[d]	90[e]	88[f]	86[g]	85[h]	0.2	<0.01	<0.01	<0.01	<0.01
MCP$_{RDP}$ (g/kg DM)	192[cd]	173[e]	204[bc]	183[de]	204[bc]	184[de]	236[a]	208[a]	3.1	<0.01	<0.01	<0.01	<0.01
AMCP (g/kg DM)	60[a]	60[b]	59[c]	58[d]	57[e]	57[f]	55[g]	54[h]	0.1	<0.01	<0.01	<0.01	<0.01
ARUP (g/kg DM)	129[b]	123[b]	164[a]	168[a]	83[d]	84[d]	91[d]	104[c]	2.4	<0.01	<0.01	0.08	<0.01
Degraded protein balance (DPB of BPP products) and Total metabolizable protein supply (MP of BPP products) to dairy cows													
MP (g/kg DM)	193[b]	187[b]	228[a]	231[a]	145[d]	145[d]	151[d]	163[c]	2.4	<0.01	<0.01	0.17	<0.01
DPB (g/kg DM)	114[c]	93[d]	131[b]	107[cd]	134[b]	112[c]	177[a]	145[b]	3.6	<0.01	<0.01	<0.01	<0.01

Table 5.1. *Cont'd* Metabolic characteristics and true nutrient supply of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings), determined based on TND-based and non-TDN based ruminant nutrient supply systems

Items	Blend pelleted products (BPP)								SEM	P value	Contrast, P value		
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					

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

5.4.3 Feed Milk Value

Feed Milk Values are presented in Table 5.2. The FMV based on the DVE system was highest (P< 0.05) in carinata based pelleted products BPP3 and BPP4 (4.80 and 4.86 kg milk/kg DM feed), while canola based pelleted products BPP5, BPP6 and BPP7 had the lowest Feed milk values (3.07, 3.11and 3.21 kg milk/kg DM feed, respectively). According to The NRC 2001 model (NRC, 2001), the pattern is the same, only it shows slightly lower numerical values than the DVE system with carinata based blend pelleted products BPP3 and BPP4 containing the highest (P< 0.05) FMV (4.34 and 4.34 kg milk/kg DM feed) and canola based pelleted products (BPP5, BPP6, BPP7 and BPP8) containing the lowest (P< 0.05) values ranging from 2.69 to 2.93 kg milk/kg DM feed. Feed Milk Value based on NE_L were higher (P< 0.05) in all carinata based pelleted products (BPP1, BPP2, BPP3 and BPP4) with values ranging from 2.82 to 2.88 kg milk/kg DM feed, while the lowest value was observed in the canola based pelleted product BPP8 (2.56 kg milk/kg DM feed). Carinata based blend pelleted products contained higher FMV than canola based blend pelleted products (+1.22 based on DVE; +1.25 based on NRC and +0.23 kg milk/kg DM feed based on NE_L). Adding a higher level of pea screenings or a lower level of co-product resulted in lower FMV based on true protein value (DVE or MP, -0.53 or -0.40 kg milk/kg DM), but tended to be significance (P= 0.06) based on NE_L energy value. Adding lignosulfonate reduce slightly FMV based on NE_L value (-0.07 kg milk/kg DM).

Table 5.2. Feed Milk Value of the blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	Blend pelleted products (BPP)								SEM	P value	Contrast, P value		
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Based on Total Truly Absorbable Protein Value													
FMV (kg milk/kg DM BPP)	4.09[b]	3.97[b]	4.80[a]	4.86[a]	3.07[d]	3.11[d]	3.21[d]	3.50[c]	0.051	<0.01	<0.01	0.07	<0.01
Based on Total Metabolizable Protein													
FMV (kg milk/kg DM BPP)	3.76[b]	3.62[b]	4.34[a]	4.34[a]	2.69[c]	2.67[c]	2.75[c]	2.93[c]	0.343	<0.01	<0.01	0.94	<0.01
Based on net energy NE_L for lactation													
FMV (kg milk/kg DM BPP)	2.88[a]	2.82[a]	2.88[a]	2.84[a]	2.68[b]	2.61[cd]	2.64[bc]	2.56[d]	0.014	<0.01	<0.01	<0.01	0.06

SEM: standard error of mean; [a-d] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison: Tukey method; The efficiency of use of metabolizable protein for lactation is 0.67 (source NRC, 2001), and protein composition in milk is assumed to be 33 g protein/1000 g milk; CN: canola meal; CR: carinata meal.

5.5 Conclusions

Based on this study carinata based blend pelleted products contained higher total true protein digested in the intestine (DVE) and metabolizable protein (MP) values and lower degraded protein balance (OEB and DPB) values than canola based blend pelleted products. In addition, carinata based blend pelleted products contained higher feed milk values (FMV) than canola based blend pelleted products. BPP3 and BPP4 provided highest true protein value (DVE and MP) as well provided highest Feed Milk Values than the other blend pelleted products. These results suggest that carinata based pelleted products BPP3 and BPP4 could be used as an adequate protein supplement feed for high producing dairy cows.

6. GENERAL DISCUSSION, OVERALL CONCLUSION AND IMPLICATIONS

Canola meal is an adequate feed for animals which often is used in ruminant diets because it is considered as an excellent protein source for livestock industry (Canola Council, 2015). In addition to canola meal, the option of carinata meal is available in Canada. It has high crude protein and low fiber, which seems to make it a satisfactory protein and energy source in animal feed; possibly better than canola meal. However, the nutritional values and bioavailability of carinata co-product is not substantial and there is no information when it is blended with other feedstuffs as a pellet for dairy rations. This project investigated the nutritional values of eight different blend pelleted products based on combination of canola meal, carinata meal, pea screenings and lignosulfonate for dairy cows. Chemical profiles, bioactive compounds, energy values, protein and carbohydrate fractions, rumen degradation kinetics, hourly effective degradation ratios of ED_N to ED_CHO, intestinal nutrient digestion, predicted truly absorbed protein supply and FMV based on NRC and DVE systems were determined.

The first section of the studies showed that adding lignosulfonate improved the pellet durability index measured with the Holmen tester. Canola blend pelleted products and higher level of inclusion of pea screenings or lower level of inclusion of co-products resulted in blend pelleted products with higher pellet durability index. Although the predominant glucosinolates in carinata pellets and canola pellets are different, canola pelleted products have significantly higher levels of total glucosinolates than carinata meal, also no lignosulfonate and higher level of inclusion of co-products resulted in pellets with higher level of total glucosinolates. Condensed tannins are very low in all the blend pelleted products and by adding a lower level of pea screenings or a higher level of co-product results in the BBP products containing higher levels of condensed tannins. The amino acid profile as a % of CP showed that canola pelleted products contained higher levels of

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methionine and lysine but lower levels of arginine than the carinata blend pelleted products. Adding a lower level of pea screenings or a higher level of co-products resulted in the BBP products containing higher levels of methionine. However, carinata based pelleted products provided greater levels of total amino acids based on DM than the canola based pelleted products. Adding a lower level of pea screenings or a higher level of co-product resulted in the BBP products containing higher total amino acid content and by adding lignosulfonate, pelleted products with lower total amino acid content are obtained. The chemical results showed that canola pelleted products contained lower CP than the carinata based pelleted products. Adding a lower level of pea screenings or a higher level of co-product resulted in the BBP products containing higher levels of CP. The energy value study demonstrated that carinata based blend pelleted products contained higher truly NE for lactation, maintenance and growth than canola based pelleted products and by adding lignosulfonate the result is BBP containing reduced NE. According to the CNCPS 6.5 model, in terms of protein fractions carinata based blend pelleted products contained lower PA2 and PC fraction, but higher PB2 fractions than canola based BBP products. Adding a higher level of pea screenings or a lower level of co-product resulted in the BBP products containing higher PA2. In terms of CHO fraction profile, carinata based blend pelleted products had lower CC fraction, but no difference in CA4, CB1 and CB2 fractions compared to canola based BBP products. Adding a higher level of pea screenings or a lower level of co-product resulted in the BBP products containing lower CA4 and CC but higher CB1 fractions. Also, adding lignosulfonate reduced CB1 fraction and increased CB2 fraction.

Based on the second section of the studies the results showed that carinata based blend pelleted products contain higher rumen bypass dry matter, lower rumen effective degraded protein, higher rumen bypass protein, higher effective fiber degradability (NDF) than the canola based blend

pelleted products. In addition, canola based pelleted products possessed higher iNDF than the carinata blend pelleted products. Adding a higher level of pea screenings or a lower level of co-product resulted in higher EDDM, BSt, EDSt and lower rumen bypass protein. The intestinal results indicated that carinata based blend pelleted products contained higher TDDM, TDP, TDNDF and higher %dBDM, %dBCP, %dNDF, but lower TDST than canola based blend pelleted products. In addition, canola based pelleted products showed greater ratio of ED_N/ED_CHO at H1, H2, H3, H4 and H6 than the carinata based pelleted products.

The third section of studies suggested that the carinata based blend pelleted products contained higher total DVE, MP and lower OEB, DPB values than the canola based blend pelleted products. Adding a higher level of pea screenings or a lower level of co-product resulted in lower MP and DPB values. Including lignosulfonate in BPP did not significantly affect MP, but significantly reduced protein degraded balance value. Carinata based blend pelleted products contained higher FMV than canola based blend pelleted products Adding a higher level of pea screenings or a lower level of co-product resulted in lower FMV based on true protein value.

In conclusion, the carinata based blend pelleted products differed in chemical, amino acid, glucosinolates, condensed tannin and energy profiles, protein and carbohydrate fractions, potential nitrogen to energy synchronization, rumen degradation kinetics, intestinal digestion, metabolic characteristics, truly digested nutrient supply and feed milk value from the canola based pelleted products. Carinata based pelleted products provided higher CP, less NDF, higher levels of total amino acids based on DM, higher net energy of lactation, higher rumen bypass protein, higher effective fiber degradability (EDNDF), higher MP or DVE consequently higher FMV than the canola pelleted products. Based on all studies, carinata based pelleted products BPP3 and BPP4 showed to have superior feed quality and can be used as a potentially high value feed for dairy

cows. Furthermore, these blend pelleted products based on pea screenings, co-products and lignosulfonate can be used as future marketable products in Canada and worldwide.

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8. APPENDIX

8.1 Chemical composition of the ingredients of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Item	Pea Screenings	Carinata Meal	Canola Meal
DM %	85.7	90.1	89.9
ASH (%DM)	3.1	8.5	8.1
OM (%DM)	96.9	91.5	91.9
EE (%DM)	1.6	1.3	3.1
FA (%DM)	0.6	0.3	2.1
CP (%DM)	22.5	52.3	49.8
CHO (%DM)	72.8	37.9	39.0
NDF (%DM)	19.0	23.2	24.7
NDFn (%DM)	18.1	14.3	21.4
ADF (%DM)	7.7	9.3	17.3
ADL (%DM)	1.0	2.2	8.7
Hemicellulose (%DM)	11.2	13.9	7.4
Cellulose (%DM)	6.7	7.1	8.7
Starch (%DM)	48.0	1.9	2.0
NFC (%DM)	54.8	23.6	17.6
NDICP (%DM)	0.9	8.8	3.3
ADICP (%DM)	0.2	0.8	1.6
SCP (%DM)	12.7	13.8	18.9
NPN (%DM)	6.6	12.7	14.0
Starch (%DM)	48.0	1.9	2.0
Sugar (%DM)	3.4	7.4	7.5
NSC (%DM)	51.4	9.2	9.5
NDICP (%CP)	4.0	16.9	6.5
ADICP (%CP)	0.7	1.5	3.3
SCP (%CP)	56.4	26.4	38.0
NPN (%CP)	29.2	24.2	28.1
NFC (%CHO)	75.2	62.2	45.1
Starch (%NFC)	87.7	8.0	11.5
Sugar (%NFC)	6.2	31.3	42.5
ADF (%NDF)	40.8	40.0	70.2
ADL (%NDF)	5.5	9.4	35.1
Glucosinolates (µmol/g)	0.10	6.35	8.10
Condensed Tannin (% DM)	0.02	0.04	0.03

8.2 Amino acid profile of the ingredients of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Item	Pea Screenings	Carinata Meal	Canola Meal
Amino Acids, %DM			
Taurine	0.05	0.11	0.10
Hydroxyproline	0.00	0.18	0.58
Aspartic Acid	1.91	3.11	3.32
Threonine	0.77	1.92	1.98
Serine	0.89	1.77	1.80
Glutamic Acid	4.21	9.19	7.38
Proline	1.33	2.93	2.67
Lanthionine	0.01	0.13	0.16
Glycine	0.94	2.41	2.34
Alanine	0.93	2.09	2.03
Cysteine	0.37	1.26	1.05
Valine	1.07	2.44	2.44
Methionine	0.28	0.96	0.99
Isoleucine	0.89	1.92	1.91
Leucine	1.58	3.40	3.27
Tyrosine	0.65	1.25	1.21
Phenylalanine	1.07	1.99	1.90
Hydroxylysine	0.05	0.05	0.18
Ornithine	0.01	0.02	0.01
Lysine	1.31	2.09	2.79
Histidine	0.50	1.30	1.21
Arginine	1.43	3.38	2.75
Tryptophan	0.20	0.57	0.58
Total	20.47	44.50	42.64

8.3 Degradation kinetics of primary nutrient (starch) of blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Items	Blend pelleted products (BPP)								SEM	P value	Contrast, P value		
	BPP1	BPP2	BPP3	BPP4	BPP5	BPP6	BPP7	BPP8			Co-P CR vs. CN	LSC No vs. Add	Co-P Low vs. High
Lignosulfonate %DM	no	4.8	no	4.8	no	4.8	no	4.8					
Type of Co-Product	CR	CR	CR	CR	CN	CN	CN	CN					
Level of Co-Product %DM	50	47.6	75	71.4	50	47.6	75	71.4					
Pea Screenings %DM	50	47.6	25	23.8	50	47.6	25	23.8					
Starch													
St (g/kg DM)	254[a]	253[a]	146[bc]	133[c]	268[a]	258[a]	145[bc]	148[b]	3.3	<0.01	<0.01	0.04	<0.01
Kd	28.65[ab]	31.67[a]	20.24[ab]	32.92[a]	24.95[ab]	28.76[ab]	14.05[b]	30.04[a]	3.355	0.01	0.11	<0.01	0.09
Residue (0 h, %)	75.2[ab]	81.0[a]	64.7[b]	80.1[a]	75.1[ab]	72.9[ab]	63.4[b]	68.0[ab]	5.59	<0.01	0.01	0.01	<0.01
S (%)	24.8[ab]	18.9[b]	35.3[a]	19.9[b]	25.0[ab]	27.1[ab]	36.7[a]	32.0[ab]	5.59	<0.01	0.01	0.01	<0.01
D (%)	72.4[abc]	78.3[a]	63.7[bc]	75.2[ab]	72.8[abc]	70.1[abc]	60.5[c]	64.6[abc]	4.89	<0.01	0.02	0.03	<0.01
U (%)	2.9[a]	2.1[a]	1.0[a]	4.8[a]	2.3[a]	2.8[a]	2.9[a]	3.4[a]	1.39	0.57	0.97	0.16	0.68
%BSt	15.4[a]	15.6[a]	15.7[a]	16.5[a]	16.6[a]	15.5[a]	26.1[a]	14.5[a]	3.80	0.40	0.36	0.25	0.35
BSt (g/kg DM)	43[a]	44[a]	25[bc]	24[c]	49[a]	44[a]	42[ab]	24[c]	6.6	0.01	0.18	0.16	<0.01
%EDSt	84.6[a]	84.4[a]	84.3[a]	83.5[a]	83.4[a]	84.6[a]	73.9[a]	85.5[a]	3.80	0.40	0.36	0.25	0.35
EDST (g/kg DM)	215[a]	214[a]	123[b]	110[b]	223[a]	218[a]	107[b]	127[b]	6.3	<0.01	0.47	0.93	<0.01

SEM: standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison: Tukey method; Kd: the degradation rate of D fraction; T0: lag time; S: soluble fraction; D: degradable fraction; U: rumen undegradable fraction; BSt: rumen bypass or undegraded feed starch; EDST: effective degraded starch. CN: canola meal; CR: carinata meal.

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8.4 Effect of pelleting on some nutrients and bioactive compounds of the blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

Theoretical Calculation

	%DM			%CP				%CHO			mg/kg DM	µmol/g
	CP	ADICP	NDICP	PA2	PB1	PB2	PC	CA4	CB1	CB2	CT	GS
BPP1	37.4	0.5	4.9	41.4	48.2	9.3	1.1	12.1	35.5	8.9	273	3.23
BPP2	35.6	0.5	4.6	39.4	45.8	8.9	1.1	11.5	33.8	8.5	260	3.07
BPP3	44.9	0.6	6.8	33.9	52.4	12.3	1.3	15.8	20.2	11.7	326	4.79
BPP4	42.7	0.6	6.5	32.3	49.9	11.7	1.2	15.0	19.2	11.1	310	4.56
BPP5	36.2	0.9	2.1	47.2	47.5	3.3	2.0	11.9	35.6	7.9	237	4.10
BPP6	34.4	0.9	2.0	44.9	45.3	3.1	1.9	11.3	33.9	7.5	226	3.90
BPP7	43.0	1.3	2.7	42.6	51.5	3.3	2.7	15.5	20.4	10.1	273	6.10
BPP8	40.9	1.2	2.5	40.5	49.0	3.1	2.5	14.8	19.4	9.6	260	5.81

Actual Values After Pelleting

	CP	ADICP	NDICP	PA2	PB1	PB2	PC	CA4	CB1	CB2	CT	GS
BPP1	38.8	0.6	13.0	31.0	56.0	11.6	1.4	12.1	47.4	4.4	188	3.57
BPP2	36.4	0.6	13.1	31.7	55.2	11.6	1.6	11.7	45.5	9.8	227	3.46
BPP3	45.0	0.7	14.4	28.6	57.0	12.9	1.5	15.5	31.4	7.8	279	5.34
BPP4	43.1	0.6	13.4	26.7	59.9	12.0	1.4	16.1	27.7	15.6	311	4.77
BPP5	35.9	1.0	6.0	44.0	50.0	3.3	2.7	11.3	48.0	4.3	249	4.00
BPP6	33.7	1.0	6.0	39.4	54.6	3.0	3.0	11.5	44.4	10.3	261	3.67
BPP7	41.9	1.4	6.7	41.7	51.7	3.4	3.3	14.9	30.2	8.0	293	5.86
BPP8	39.0	1.4	6.8	35.3	58.0	3.1	3.6	15.2	28.9	14.1	321	5.54

Difference

	CP	ADICP	NDICP	PA2	PB1	PB2	PC	CA4	CB1	CB2	CT	GS

8.4 *Cont'd* Effect of pelleting on some nutrients and bioactive compounds of the blend pelleted products with different combinations (two levels of lignosulfonate compound (LSC), two types of co-products (Co-P) from bio-energy or bio-oil processing with two levels of each type, and two levels of pea screenings)

	CP	ADICP	NDICP	PA2	PB1	PB2	PC	CA4	CB1	CB2	CT	GS
BPP1	-1.4	-0.1	-8.1	10.4	-7.9	-2.3	-0.3	0.0	-12.0	4.5	85	-0.35
BPP2	-0.8	-0.2	-8.5	7.7	-9.4	-2.7	-0.5	-0.2	-11.8	-1.3	33	-0.39
BPP3	-0.1	-0.1	-7.6	5.3	-4.6	-0.6	-0.2	0.3	-11.2	3.9	47	-0.55
BPP4	-0.4	0.0	-6.9	5.6	-10.0	-0.3	-0.2	-1.1	-8.5	-4.5	-1	-0.21
BPP5	0.3	-0.1	-3.9	3.2	-2.5	0.0	-0.7	0.6	-12.4	3.6	-12	0.10
BPP6	0.7	-0.1	-4.0	5.5	-9.3	0.1	-1.1	-0.2	-10.5	-2.8	-35	0.23
BPP7	1.1	-0.1	-4.0	0.9	-0.2	-0.2	-0.7	0.6	-9.8	2.1	-20	0.24
BPP8	1.9	-0.2	-4.3	5.2	-9.0	0.0	-1.1	-0.4	-9.5	-4.5	-61	0.27
P value												
	CP	ADICP	NDICP	PA2	PB1	PB2	PC	CA4	CB1	CB2	CT	GS
	0.65	0.01	<0.01	0.01	<0.01	0.11	<0.01	0.81	<0.01	0.92	0.79	0.50

BPP1: (low level of carinata meal, high level of pea screenings and no lignosulfonate); BPP2: (low level of carinata meal, high level of pea screenings and lignosulfonate); BPP3: (high level of carinata meal, low level of pea screenings and no lignosulfonate); BPP4: (high level of carinata meal, low level of pea screenings and lignosulfonate); BPP5: (low level of canola meal, high level of pea screenings and no lignosulfonate); BPP6: (low level of canola meal, high level of pea screenings and lignosulfonate); BPP7: (high level of canola meal, low level of pea screenings and no lignosulfonate); BPP8: (high level of canola meal, low level of pea screenings and lignosulfonate). Level of significance (P < 0.05); CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; PA2: soluble true protein; PB1: insoluble true protein. PB2: fiber-bound protein; PC: indigestible protein; CHO: carbohydrate; CA4: water soluble carbohydrates; CB1: starch; CB2: soluble fiber; CT: condensed tannins; GS: glucosinolates.