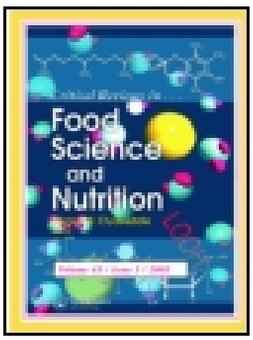

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Contributions To Advances in Blend Pellet Products (BPP) Research on Molecular Structure and Molecular Nutrition Interaction by Advanced Synchrotron and Globar Molecular (Micro)Spectroscopy

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Running head: Contributions to advances in blend pellet products research at a molecular level

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ABSTRACT

ABSTRACT:

To date, advanced synchrotron-based and globar-sourced techniques are almost unknown to food and feed scientists. There has been little application of these advanced techniques to study blend pellet products at a molecular level. This article aims to provide recent research on advanced synchrotron and globar vibrational molecular spectroscopy contributions to advances in blend pellet products research on molecular structure and molecular nutrition interaction. How processing induced molecular structure changes in relation to nutrient availability and utilization of the blend pellet products. The study reviews Utilization of co-product components for blend pellet product in North America; Utilization and benefits of inclusion of pulse screenings; Utilization of additives in blend pellet products; Application of pellet processing in blend pellet products; Conventional evaluation techniques and methods for blend pellet products. The study focus on recent applications of cutting-edge vibrational molecular spectroscopy for molecular structure and molecular structure association with nutrient utilization in blend pellet products. The information described in this article gives better insight on how advanced molecular (micro)spectroscopy contributions to advances in blend pellet products research on molecular structure and molecular nutrition interaction.

Keywords: Molecular Structures, Nutrient Absorption, Pellet Processing, Interactive Association between Structure and Nutrition, Blend Pellet Products

I. UTILIZATION OF BIO-ENERGY CO-PRODUCTS COMPONENTS FOR BLEND PELLET PRODUCT IN NORTH AMERICA

1.1. Development and Production of Blend Pellet Product Components

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 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 of 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 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).

1.2. Unique Features of Blend Pellet Products Components

Canola is one of the most widely produced crops in Canada (Canola Council of Canada, 2015). The oil is extracted, 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. A study demonstrated that 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 of Canada, 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. These suggested that canola meal composition is interesting and can be used as ingredient in ruminant diets.

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 also observed as a potential feedstuff for animals.

Information 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 rumen degradable dry matter is 63.0 and 50.9 % DM, rumen degradable protein is 70.5 and 52.0 % CP, rumen undegradable protein is 29.4 and 48.0 % CP, intestinally digestible protein is 80.9 and 70.9 % RUP, intestinally absorbable protein is 23.8 and 34.0 % CP, and total digestible protein is 94.4 and 86.0 % CP, respectively (Anderson, 2015). Previous data showed that the proportion of ruminally degradable dry matter are higher in carinata than canola meal, as well as ruminally degradable protein, additionally it was showed that carinata meal contained lower 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 or 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.

1.3. Utilization and Benefits of Blend Pellet Product-Component 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. 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 meal were included in the data set. Milk yield rose by 3.4 kg/d when an additional kilogram of canola meal was fed, and 2.4 kg/d when an additional kilogram of soybean meal was provided in the diet, showing 1 kg of milk disadvantage to soybean meal (Canola Council, 2015; Huhtanen et al., 2011). 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 suggested that by feeding canola meal the absorption of essential amino acids was greater, therefore milk protein increased, and protein efficiency enhanced (Canola Council, 2015).

1.4. Available Blend Pellet Product-Components 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 the production will be 26 million tonnes per year. This of course as a response to increasing world demand due to increasing human consumption (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 typically sold as mash or pellets (William and Flad, 2015). 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 a great investment to improve the crop, additionally researchers are selecting and developing 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.

II. UTILIZATION AND BENEFITS OF INCLUSION OF PULSE SCREENINGS AND ADDITIVES IN BLEND PELLET PRODUCTS

2.1. Development, Production and Features of Pulse 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 part which is a 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, peas are 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 dairy cattle diets. (Christensen, 2006; Anderson et al., 2002; Hickling, 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 peas contain 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. 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). 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 and Benefits of Lignosulfonate Additive

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 degradation of soybean protein in cultures of rumen contents (Windschitl and Stern, 1988). Also, the effective rumen degradability of CP in canola meal was successfully decreased with 5 % of lignosulfonate and heat, compared with heating without lignosulfonate (McAllister et al., 1993). 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).

III. RECENT APPLICATION OF PELLET PROCESSING IN BLEND PELLET PRODUCTS

3.1. Pellet Processing

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, the objective of pelleting is to take a finely divided, occasionally dusty and difficult-to-handle feed material and, by using moisture, pressure and heat, form larger particles, called pellets (Game and Maktos, 2015; Payne et al., 1994). 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).

3.2. Physical Quality of Pellets for Blend Pellet Products

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 utilizes air to create abrasion of the pellets versus the tumbling action, which takes 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 become more important in the swine and poultry industries; while other industries continue identifying the worth of feeding high quality pellets (Behnke, 2001).

3.3. Benefits of Pellet Processing for the Feed and Nutrition Industry

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. 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 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 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 during 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 proved favorable effects 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 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).

IV. EVALUATION TECHNIQUES AND METHODS FOR BLEND PELLET PRODUCTS

4.1. Determination of Bio-active Compounds - Glucosinolates in Blend Pellet Products

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, the microflora of the digestive system of ruminants induces 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).

4.2. Evaluation of Condensed Tannin for Blend Pellet Products

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 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).

4.3. Amino Acids Determination in Blend Pellet Products

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 from 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, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. 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).

4.4. Application of Updated Cornell Net Carbohydrate and Protein System V6.5 in Blend Pellet Products Study

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 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).

4.5. Energy Evaluation for Blend Pellet Products

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).

4.6. Assessing Fermentation and Degradation Kinetics for Blend Pellet Products

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 (2001): %BDM; BCP or BNDF = U + D × Kp / (Kp + Kd), %BST = 0.1 × S + D × 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} (g/kg DM) = 1.11 × CP (g/kg DM) × RUP (%) and RUP^{NRC} (g/kg DM) = CP (g/kg DM) × 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 × Kd / (Kp + Kd) and EDDM (CP, NDF or ST) = DM (CP, NDF or ST) (g/kg DM) × %EDDM (EDCP, EDNDF or EDST)

4.7. Hourly Effective Degradation Ratios/Potential N-to-Energy Synchronization in Blend Pellet Products

The effective rumen degradation ratios of N and energy were calculated hourly as modified from Sinclair et al. (1993) as below: Hourly ED ratio N/CHO_t = 1000 × ($HEDN_t$ – $HEDN_{t-1}$) / [($HEDNDF_t$ – $HEDNDF_{t-1}$) + ($HEDST_t$ – $HEDST_{t-1}$)], where N/CHO_t = ratio of N to CHO at time t (g N/kg CHO); $HEDN_t$ = hourly effective degradability of N at time t (g/kg DM); $HEDN_{t-1}$ = hourly effective degradability of N 1 h before t (g/kg DM); $HEDCHO_t$ = hourly effective degradability of CHO at time t (g/kg DM); $HEDNDF_t$ = 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).

4.8. Evaluation of Intestinal Digestibility of Blend Pellet Products 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).

4.9. Prediction of Truly Digestible Protein Supply to Small Intestine from Blend Pellet Products

4.9.1. National Research Council Model

According to the NRC (2001) model, MP is composed of three major contributory protein sources. Total MP can be calculated as follows: MP (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} (g/kg of DM) = RDP^{NRC} − 1.18 × MCP_{TDN}.

4.9.2. The 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., 2007; 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 × [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) (Theodoridou and Yu, 2013; Tamminga et al., 1994).

4.10. Feed Milk Value Determination for Blend Pellet Products

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).

V. RECENT APPLICATIONS OF VIBRATIONAL MOLECULAR SPECTROSCOPY FOR MOLECULAR STRUCTURE ASSOCIATION WITH NUTRIENT UTILIZATION IN BLEND PELLET PRODUCTS

5.1. Application of Vibrational Molecular Spectroscopy in Protein Spectral Intensities in the Different Blend Pelleted Products (BPP)

Recently, Ismael et al. (2018) applied advanced vibrational molecular spectroscopy to study protein spectral profiles for the different blend pelleted products based on canola meal and carinata meal with different level of lignosulfonate and pea screenings. This spectral are related to protein primary structures and protein secondary structures. It was found that the blend pelleted products differed in amide I peak height and peak areas, the ratio of amide I to amide II and the secondary structures such as α–helix and *β*–sheet and their ratio. The protein spectral profiles were highly sensitive to the changes in the blend pelleted products composition and nutrient utilization and availability such as metabolizable protein (MP) and degraded protein balance in ruminants.

5.2. Application of Vibrational Molecular Spectroscopy to Study Interaction Association between Protein Molecular Spectral Features and Nutrients Absorption in the Blend Pelleted Products

Ismael et al. (2018) reported the correlation analysis between protein spectral structure and protein profiles, protein subfractions and predicted energy values for the combination of the blend pelleted products. It was found that CP component had correlation with amide I spectral feature and total amide area and the ratio of α–helix to *β*–sheet. Both NDICP and ADICP had correlation with amide II spectral profiles and the ratio of amide I to amide II height. NPN also had association woth amide I area, amide I height, amide I to amide II area ratio, amide I to amide II height ratio, β-sheet height and α–helix height and the ratio of α–helix to β sheet. Truly digestible nutrient study (Ismael et al., 2018) showed that tdCP had correlation with amide I area, amide I height, the amide area in the blend pelleted products. Protein subfraction study also showed that the slowly degradable protein (PB2 fraction), the PC fraction had high corrections with amide spectral profiles, but no significant correction with the ratio of α-helix to *β*-sheet in the blend pelleted products. In the rumen and intestine phase, Ismael et al. (2018) reported the Kd for the slowly degradable fraction of CP, EDCP and in vitro digestion of BCP (dIDP) and total intestinal digestibility of CP (IADP) were correlated with the amide spectral profiles in the blend pelleted products. In the modeling nutrient supply study from the blend pelleted products, Ismael et al. (2018) found that AMCP, ARUP, MP and DPB as well as FMV values had relationship to various amide spectral profiles.

5.3. Application of Vibrational Molecular Spectroscopy to Determine Most Important Protein Spectral Parameters Used to Predict Protein Utilization and Absorption

Recently, Ismael et al (2018) reported the multiple regression analysis to choose best unique spectra to predict the rumen degradation and intestinal digestion of CP in the blend pelleted products. This study indicated that protein spectral profiles (amide I and II, α–helix, *β*-sheet and their ratio) can be used to predict EDCP and Kd. In the modeling truly nutrient supply study, Ismael et al (2018) reported through selection of the best protein spectral profile, it is promised that the nutrient supply and feed milk value (ARUP, AMCP, MP, DBP and FMV) of the blend pelleted products could be estimated based on BPP products molecular spectral profiles.

In summary, the current studies indicated that the molecular structures and processing induced molecular structure changes related to the protein nutrient absorption in the blend pelleted products could be revealed by the advanced molecular (mirco)spectroscopy.

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iv.

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