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Implications of Recent Research on Microstructure Modifications, through Heat-Related Processing and Trait Alteration to Bio-Functions, Molecular Thermal Stability and Mobility, Metabolic Characteristics and Nutrition in Cool-Climate Cereal Grains and Other Types of Seeds with Advanced Molecular Techniques

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Running head: Protein Research in Cool-Climate Cereal Grains in Molecular Structure and Molecular Nutrition

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ABSTRACT:

The cutting-edge synchrotron radiation based and globar-sourced vibrational infrared microspectroscopy have recently been developed. These novel techniques are able to reveal structure features at cellular and molecular levels with the tested tissues being intact. However, to date, the advanced techniques are unfamiliar or unknown to food and feed scientists and have not been used to study the molecular structure changes in cool-climate cereal grain seeds and other types of bio-oil and bioenergy seeds. This article aims to provide some recent research in cool-climate cereal grains and other types of seeds on molecular structures and metabolic characteristics of carbohydrate and protein, and implication of microstructure modification through heat-related processing and trait alteration to bio-functions, molecular thermal stability and mobility, and nutrition with advanced molecular techniques- synchrotron radiation based and globar-sourced vibrational infrared microspectroscopy in the areas of (1) Inherent microstructure of cereal grain seeds; (2) The nutritional values of cereal grains; (3) Impact and modification of heat-related processing to cereal grain; (4) Conventional nutrition evaluation methodology; (5) Synchrotron radiation-based and globar-sourced vibrational (micro)-spectroscopy for molecular structure study and molecular thermal stability and mobility, and (6) Recent molecular spectroscopic technique applications in research on raw, traits altered and processed cool-climate cereal grains and other types of seeds. The information described in this article gives better insights of research progress and update in cool-climate cereal grains and other seeds with advanced molecular techniques.

Keywords: Molecular Structures, Nutrient Utilization and Availability, Heat-Related Processing, Interactive Association between Structure and Nutrition, Cool-Climate Seeds, Molecular Thermal Stability and Mobility

I. INHERENT MICRORTRUCTURE OF CEREAL GRAIN SEEDS

Cereal grains share many things in common. In general, a cereal grain seed consists of three parts: bran, endosperm and germ. There are two parts in bran: pericarp and seed coat. The endosperm includes the aleurone layer in the outside and starch granules in the middle. The germ, or embryo, includes three parts: plumule, scutellum and radicle. As caryopses and monocots, the fruit coat in cereal grains adheres close to the seed, and there is only one cotyledon in the germ. Starch in endosperm provides nutrient for the seed (Angold, 2012; Rooney et al., 2004).

The structure of starch granules inside the endosperm is one of the crucial parts when studying cereal grains. There are two types of endosperm: the floury endosperm and the vitreous endosperm. The former one is starch granules with very little protein matrix surrounding them, while in the latter one, starch granules are embedded in a protein matrix, making the endosperm more dense and stable (Eckhoff et al., 1996). Using corn as an example, vitreous endosperm has a higher content of lysine than floury endosperm (Gibbon et al., 2003). The two kinds of endosperm granules are also different in shape, with the granules in the vitreous endosperm small, polyhedral and sometimes indented, and those in the floury endosperm large and spherical (Rooney et al., 2004). Both large and small starch granules have concentric layers of starch distribution, which can be observed in a scanning electron micrograph of the endosperm area (Wrigley, 2010).

There are also many differences in structure between different species and varieties of cereal grains, at both the macro and micro levels. Regardless of the difference in appearance such as color, size and shape, cereal grains differ in nature and numbers of aleurone layers (Wrigley, 2010). Wheat, triticale and corn have single-layered aleurone, but they differ in inner structures. Wheat and triticale have a "bi-modal" distribution of starch granules, consisting of both large (A-type: diameter > 10 μm) and small (B-type: diameter < 10 μm) granules. In triticale, higher ratio (both number and weight) of the starch granules are large, compared to wheat and corn (Naguleswaran et al., 2012). Corn is considered to have a "normal" distribution of starch granules, although the starch granules in corn range in size (5-35 μm in diameter) and have irregular shapes (Eckhoff et al., 1996; Rooney et al., 2004; Wrigley, 2010). These differences in different cereal structures result in differences in rate and extent of ruminal fermentation. Generally, wheat and triticale are more rapidly degraded by ruminal microorganisms than corn (McAllister et al., 1993).

Starch granule features can be observed using scanning microscopy. These features include amorphous growth rings, unevenly distributed surface pores and internal channels (Naguleswaran et al., 2012). Differences were found between different cereal grains: more pores and channels were observed in corn than in wheat and triticale. Within the same variety of cereal grain (wheat, triticale or corn), surface pores were more frequently seen on large granules (Naguleswaran et al., 2012). These pores and channels were rich in protein and phospholipids (Naguleswaran et al., 2011). By making some surface areas of wheat and corn more susceptible to amylases, the pores and channels were assumed to be able to affect the pattern of amylase attack (Fannon et al., 1992, 1993). The presence of the proteins and lipids in the channels, however, also blocks binding sites of the enzyme, thus reduces the hydrolysis rate (Naguleswaran et al., 2011). Small granules have relatively larger surface area compared to larger ones, hence they were hydrolyzed faster in the initial stage. The hydrolysis process slows down in later stages due to the denser crystalline lamellae in the small granules. The hydrolysis rates after 24 h was similar between small and large granules (Naguleswaran et al., 2012).

II. THE NUTRITIONAL VALUES OF CEREAL GRAINS

Being a good energy source, cereal grains are an essential part of both human food and animal feeds. Grains provide more than 56% of food energy and about 50% of the protein on earth (Cordain, 1999).

Most of the energy contained in the cereals is available in the form of carbohydrate, most of which is starch. As mono-gastric animals are not able to hydrolyze structural carbohydrate, starch is the only polysaccharide that they can utilize (Morris et al., 1996). Cereal grains are thus the primary energy supply for mono-gastric animals such as poultry and swine. About half of the grains are consumed by human beings, while more than one-third of protein in cereal grains is consumed in pig and poultry rations (Cordain, 1999; Morris et al., 1996).

Besides carbohydrate, the endosperm, embryo and aleurone of wheat, triticale and corn also contain protein. Although the total amount only accounts for 10% on average, many kinds of proteins are involved in the grain kernels. Most storage proteins, including prolamins and a small amount of globulins/glutelins, are present in the starchy endosperm. There are also some globulins and oil bodies in the embryo and aleurone layer, and some functional proteins in cell walls, membranes, enzymes and transport systems (Shewry, 1996).

Regardless of their outstanding nutrient values, cereal grains are unsuitable to be used as complete food or feed product because of the lack of some essential amino acids, essential fatty acids, vitamins, and minerals. Low levels of lysine, methionine, tryptophan and threonine in cereals (Figure 1) is not a good indication of feed value for mono-gastric animals or ruminants. Cereals also have low concentration of essential fatty acids such as linoleic acid (except corn: 2.12 in corn vs. 1.2 in wheat vs. 0.95 in rye, g/100g sample) (Cordain, 1999). Vitamin A (except for yellow maize), vitamin C, vitamin B12 and β-carotene and minerals (except for potassium and phosphorus) are also insufficient for a balanced diet (Cordain, 1999; Morris and Rose, 1996).

Although the microbes in the rumen could synthesize all the essential amino acids (EAAs), the amount is not enough for highly productive dairy cows. According to the National Research Council (2001), lysine and methionine are the first-limiting EAAs in metabolizable protein (MP). In dairy cattle, the optimal concentrations of these two AAs are 7.2% and 2.4%, respectively, in order to fulfill the needs of both maintenance and milk production (National Research Council, 2001).

Cereal grains such as wheat, corn and barley are commonly used as the energy source in the concentrate of cattle rations. The amount varies depending on the age, species and physical conditions of the cows. For instance, more than 55% (DM basis) of grains are usually included in the ration of finishing beef cattle (Owens et al., 1997).

Cereal grains are also widely used in the bioethanol industry. In Canada, wheat is used in bioethanol plants in Western Canada and corn is more commonly used in Central Canada (Wheat DDGS, 2010). By having an equivalent nutrient value, high crop yield and competitive price, triticale could be an alternative to wheat and has been processed in some ethanol plants in Western Canada (Government of Alberta, 2014). Research shows that the coproduct, triticale DDGS, also has similar value compared to wheat DDGS and corn DDGS in the beef, dairy and lamb industry (Au et al., 2010; McKeown et al., 2010; Oba et al., 2010; Wierenga et al. 2010).

2.1. Chemical and Nutrient Profile of Cereal Grain-Wheat Seed

Being one of the first domesticated grains, wheat is now grown and commercially consumed worldwide. Based on the different color, texture and the season planted, wheat is classified into eight different market classes in Western Canada, with different kernel sizes, shapes and nutritional values in each market class (Government of Canada, 2015). The protein content of wheat is high compared to other cereal grains, especially in the case of hard and durum wheat. Cheeke (1991) concluded that hard wheat contains about 11-14% protein, while the soft wheat has less protein content (about 8-11%).

The mature wheat grain contains about 85% carbohydrate, around 80% of which is the endosperm starch. The non-starch part includes 7% of mono-, di- and oligo-saccharides and fructans and 12% of polysaccharides in cell walls (Batey, 2010).

The use of wheat in the animal industry is affected by the market price, location and quality (Kent and Evers, 1994). Traditionally, wheat by-products after milling and wheat that is unsuitable for milling due to low quality caused by disease, insects and frost, is used as animal feed. The nutrient value, palatability and digestibility of wheat are equivalent to corn, while the amino acids profile is superior.

However, often the high price of feed wheat in Canada restricts its utilization in the animal industry (Bell, 2003).

The nutritional value of wheat to the animal may vary by cultivar, as well as processing method such as steaming rolling, flaking, pelleting and dry-rolling. Coarse-grinding, dry-rolling of wheat will increase wheat feeding value compared to ground wheat when fed to cattle (Owen et al., 1997). However, the influence of processing varies by animal species and production.

2.2. Chemical and Nutrient Profile of Cereal Grain- Triticale Seed

As a hybrid of wheat and rye, triticale shares the name and traits of both of them. The name “triticale” is derived from *Triticum* (wheat) and *Secale* (rye) (Cheeke, 1991). Triticale has good yield on marginal lands and is tolerant to drought. Its compositional quality remains stable across environments, with nutritional characteristics between wheat and rye. Recently developed triticale varieties have lower protein content (about 13%) than the older varieties (about 17%) (Government of Saskatchewan, 2011). Nevertheless, with the vastly improved grain yield (15-20% higher yield than Canada Prairie Spring wheat), plant breeders increased the protein yield. The nutrient quality is also improved: triticale has intermediate lysine content (higher than wheat, Figure 1) and digestible energy compared to wheat and rye. The starch, lipid, fiber, mineral, and vitamin contents are similar or superior to those of wheat (Government of Alberta. 2014; Government of Saskatchewan, 2011).

Although triticale is not as widely grown as wheat and corn, it is potentially an equivalent feed source for livestock. The digestibility of nutrients such as starch and protein is superior to that of other Canadian cereal grains (Government of Alberta. 2014). As its extent of starch fermentation is similar to barley and oat, triticale may have higher post-ruminal digestibility. As an advantage of having higher lysine content, using triticale in the diets of mono-gastric animals often means a lower requirement of protein supplement. Reports show that triticale is a very successful alternative in swine feed, with the digestible energy (DE) equivalent to wheat and corn when fed to piglets. In the poultry industry, triticale has already been used worldwide and contradictory results were found in research on whether using triticale has a negative effect on production. Despite these differences, a consensus has arrived in economic studies that feeding triticale to mono-gastric animals is a cost-saving approach (Government of Alberta, 2014). For ruminants, there is sparse research on adding triticale in the concentrates for dairy cattle. However, since it is proved that triticale could fully substitute other grains in diets for beef cattle, similar results could be assumed for dairy cattle. Moreover, the substitutive use of triticale is already common in Australia for dairy cows when its price and supply is competitive (Government of Alberta, 2014; Government of Saskatchewan, 2011). One shortcoming of triticale is its susceptibility to ergot and this may be one of the reasons that limited the wide application of the grain (Diana Di Mavungu et al., 2012).

2.3. Chemical and Nutrient Profile of Cereal Grain- Corn Seed

Corn is believed to originate in Central Mexico. It is now the third most popular grain crop in the world, grown all around the world and used as food, feed, seed, and in industrial products. Being the leading cereal in the U.S., about 39.2% of corn is utilized as animal feed (Győri, 2010; Serna-Saldivar et al., 2001; White, 2001).

The kernel of corn averages about 73% starch, 10% protein and 5% oil (DM basis), with about 80-90% of the starch found in the endosperm (Boyer et al., 2001; Eckhoff et al., 1996). The nutrient distribution in corn kernels is similar to other cereal grains. In the pericarp, the main components are fiber, ash and oil (Rooney et al., 2004). The endosperm mainly consists of starch, while oil and protein are concentrated in the germ (Boyer et al., 2001). Comparatively, the peripheral hard endosperm cells are high in protein content (15-50%) and low in starch content, while the center floury endosperm cells are high in starch content and low in protein content (4-5%). Proteins centralized in the germ are mainly albumins and globulins, and those in the endosperm are mostly prolamins. Zein, which was first discovered in 1821 and classified as prolamin in 1924, is the main prolamin in corn and one of the most studied proteins (Anderson and Lamsal, 2011). The cell walls are comprised of cellulose, hemicellulose, ferulic acid and some proteins high in hydroxyproline (Rooney et al., 2004).

Benefiting from being a C4 plant, corn is the cereal grain that produces the most energy per acre. It is also highly palatable, contains no intrinsic toxic or deleterious compounds and has the highest digestible energy content of cereal grains for animals (Cheeke, 1991). Another merit of corn is that it has the highest level of essential fatty acids among all the cereals (Morris et al., 1996).

The biggest limitation of corn is its low concentration of lysine and tryptophan, and the unavailability of niacin (vitamin B3). A type of nutritionally enhanced corn, called quality protein maize (QPM), with high lysine content thus has been developed (Eckhoff et al., 1996; Shewry, 1996).

In western Canada, barley-based diets are commonly fed to feedlot cattle, while corn is used as an alternative grain when barley grain is expensive (Beauchemin and Koenig, 2005). When fed to animals, the corn grain is usually processed in order to increase palatability, reduce particle size, improve digestibility, change the digestion rate, site and extent, or simply for storage purpose (Richards and Hicks, 2007).

III. IMPACT AND MODIFICATION OF HEAT-RELATED PROCESSING TO CEREAL GRAIN

3.1. Involved Mechanism of Heat-Related Processing to Cereal Grain

Heat processing has been used to change the nutrient availability, sterilize samples and deactivate anti-nutritional factors (ANFs) (Goelema, 1999; Van der Poel et al., 1990; Yu et al., 2000). In the animal industry, the most often used heat processing methods are pelleting, extrusion, dry rolling, steam rolling, steam flaking and micronizing (Owens et al., 1997). Moisture is involved in many of these methods, except for dry rolling and micronizing.

The effect of heat processing is usually greater with the presence of moisture (Owens et al., 1997). Starch may swell at room temperature when wet, but gelatinization temperature varies according to starch type. With the presence of enough moisture, irreversible swelling of starch may occur when the temperature reaches 60 °C, as the granules swell too much to keep their crystalline structure (Wrigley, 2010). The intermolecular starch and protein bonds are disrupted, resulting in dissolving of the starch granules, leaching of amylose and the forming of a viscous paste or gel. On cooling, the starch retrogradation may take place, reforming amylose to an acid, heat and α-amylase resistant crystalline structure (Bornet, 1993; Cai et al. 2014; Flores-Morales et al., 2012).

Autoclaving is a commonly used method for sterilization in many fields including medicine, microbiology, mycology, and plant science. By combining heat, moisture and pressure, autoclaving is able to change chemical profiles, protein subfractions, rumen degradable nutrients, potential nutrient supply to dairy cattle and the inner structure, such as amide I-to-amid II ratio and α-helix to β-sheet ratio, in flaxseed, camelina seeds, soybeans, yellow and brown canola seeds (Doiron et al., 2009; Peng et al., 2014; Samadi and Yu, 2011; Samadi et al., 2013).

Due to the branched structure, it is harder for amylopectin to undergo both the gelatinization and retrogradation processes. Hence the different amylose / amylopectin ratio in cereal grains may be the reason why they have different responses to heat treatments (Yan et al., 2014, 2018). Richards and Hicks (2007) stated that steam-rolled and steam-flaked starch granules are more susceptible to digestive enzymes after cooling, while McAllister et al. (1991) found that autoclaving could reduce the susceptibility of both the protein matrix and starch granules to microbial attack, especially when the Maillard reaction is involved.

3.2. Impact of Heat Processing on Cereal Grain

Feed cost is one of the largest expenses in the ruminant industry. As cereal grains are not a cheap source of feed, it is important to optimize feed efficiency. Therefore, cereal grains are usually processed before being fed to ruminants.

The major ways of processing include providing heat, moisture and reducing the particle size. When the cows are fed with a high quantity of grain, precautions are needed to avoid acidosis. Besides coarse grinding and using buffering additives, heat processing is another measure that could help prevent acidosis (Government of Alberta, 2014).

The species of grain and the heat processing method used on grains could affect the feeding value, dry matter intake (DMI), as well as production efficiency of the cows. For instance, flame roasting cereal grains at 77-121˚C significantly decreased the ruminal degradation of dry matter (DM) and crude protein (CP) without affecting their digestibility in ruminants (McNiven et al., 1994). Steam flaking increased the body weight-adjusted ME for corn and wheat by 15% and 13%, respectively, compared to dry rolled grain, but it was not as effective when applied to barley and oats. Steam rolling and flaking of corn, wheat and sorghum could maintain average daily gain (ADG) while reducing DMI, thus raise the feed efficiency by 10, 10 and 15%, respectively (Owens et al., 1997). Firkins et al. (2001) indicated the effectiveness of steam-flaking in increasing the starch digestibility in corn, comparing to dry rolling and steam rolling. Richards and Hicks (2007) reported that steam flaking could reduce the degradation rate of wheat and barley but not corn, compared to dry rolling, shift the digestion site of corn from rumen to intestine, compared to high moisture corn (but not dry rolling) and increase the starch digestibility, and increase the feed efficiency of cattle fed corn and sorghum, compared to dry rolling and high moisture. Goelema (1999) summarized that pressure toasting decreased *in situ* rumen degradability of protein and starch while pelleting and expander under mild conditions increased this parameter.

IV. CONVENTIONAL NUTRITION EVALUATION METHODOLOGY

4.1. Cornell Net Carbohydrate and Protein System-Chemical Profile Based Evaluation System

The Cornell Net Carbohydrate and Protein System (CNCPS) was developed by scientists in Cornell University, University of Pennsylvania and Miner Institute, released in 1991, and first published by Russell et al. (1992), Sniffen et al. (1992) and Fox et al. (1992). Based on the principals of feed digestion, rumen function and the physiological state of the animals, the model can evaluate diets and performance of all classes of cattle in their certain living situations, thus to help optimize the feed and management of cattle (Tylutki et al., 2008; Van Amburgh et al., 2013).

The latest updated model, version 6.5, has been available since Mar 20, 2015, on the CNCPS website (http://www.cncps.cornell.edu). The software has a feed library, containing almost all the nutrient values (protein, fiber, volatile fatty acids (VFA), minerals, vitamins, amino acids (AA) profiles, fatty acids (FA) profiles), intestinal digestibility and digestion rates of different protein and carbohydrate fractions of common feeds. These component values and digestion rates are used to compute the available protein, as well as the amount of structural carbohydrate (SC) and non-structural carbohydrate (NSC) in a given feed (Tylutki et al., 2008). Many updates have been made in the version 6.1 of the model, compared to the original one, which separates protein into five fractions and carbohydrates into four fractions. Feed composition in the CNCPS version 6.1 is described by five protein fractions and eight carbohydrate fractions.

The number of protein pools doesn’t change between versions, but the pool size of PA fractions is different. In CNCPS version 6.1, the PA fraction was redefined as ammonia because some small peptides and free amino acids (AA) could escape rumen degradation (Higgs et al., 2012). Therefore, old method (Licitra et al., 1996) of analyzing non-protein nitrogen (NPN) has been abandoned due to the large pore size of Whatman #54 filter paper (20μm) (Tylutki, 2010; Van Amburgh et al., 2010). In some research, a colorimetric method (AOAC 967.07) was used to measure NPN in animal feed (Haig et al., 2002). The PB part is known as "true protein", among which, the PB1 fraction is soluble in borate phosphate buffer but can be precipitated by trichloroacetic acid (TCA) and the PB3 fraction, which represents "fiber bound protein", is insoluble in neutral detergent but soluble in acid detergent solution. The PC fraction is insoluble even in acid detergent and the PB2 fraction is computed by difference (Van Amburgh et al., 2013). Further updates (version 6.5) rename PA as PA1 and PB1 as PA2 since they are both soluble, whilst PB2 is renamed as PB1 and PB3 as PB2 (Van Amburgh et al., 2013; Higgs et al., 2012).

The number of carbohydrate (CHO) pools is increased from four to eight. The former CA fraction (fast degraded: sugar) was further divided into four sub-fractions: CA1 (acetic, propionic and butyric acids), CA2 (lactic acid), CA3 (organic acids) and CA4 (sugars). The CB fraction was partitioned into three sub-fractions instead of two. The new fraction is CB2 (soluble fiber), which was previously partitioned together with starch in CB1 (version 5). The CB1 fraction remains as starch, while the former CB2 fraction (slow degraded: available cell wall) is now CB3 fraction (available NDF). Meanwhile, The CC fraction is still unavailable NDF (Sniffen et al., 1992; Lanzas et al., 2007a; Tylutki et al., 2008).

Besides the expanded scheme, the passage rate equations were also updated according to Seo et al. (2006). The soluble pools (CA, PA and PB1) are re-assigned to the liquid passage rate equation (6-12%/h), instead of the solid passage rate (4%/h). Degradation rate (K_d) for some protein and CHO fractions are adjusted downward, to be more appropriate in accordance with the biology of the cattle. For example, K_d for sugar was 200-300% /h in previous versions, but now it is set as 40-60%/h in version 6.1. The degradation rate for NPN is also adjusted from 10,000%/h to 200%/h and K_d for PB1 regulated from 130-300%/h to 10-40%/h (Lanzas et al., 2007b; Van Amburgh et al., 2010). There are also other important changes: for instance, the physical effective NDF (peNDF) adjustment factor is no long used, because sodium sulfite is now routinely added when analyzing NDF in the feed in most labs. In the 6.5 version, NDF is further adapted to ash corrected NDFom.

Maintenance requirements are calculated using equation summarized by Fox et al. (2004), in which factors such as breed, physiological state and environmental effects are taken into consideration.

According to information such as body weight, rate of body weight gain, chemical composition of gain and mature weight, energy and protein requirements for growth are predicted. Requirement of energy and protein for lactation is calculated based on actual milk production and composition. Pregnancy requirement and shrunk body weight (SBW) are computed from growth of gravid uterus depending on expected birth weight and day of gestation. CNCPS also has equations on amino acid requirements, in accordance with tissue and milk protein content of amino acids (Tylutki et al., 2008).

The new CNCPS was proved to be more biologically correct and more accurate on feed chemistry and rumen fermentation characteristics (Van Amburgh et al., 2010), thus it can be used to formulate the diets to effectively reduce the feed cost, as well as the impact of ruminant husbandry on the environment.

4.2. Methodology to Measure Bio-Energy Values in Cereal Grains

Energy is an expensive nutrient in animal industry. Thus precise estimation is required to guarantee that the energy supply in the feed meets the needs of the animal. Bomb calorimeters are commonly used to measure the Gross Energy (GE) by burning the sample to ash. However, to directly measure Digestible Energy (DE), Metabolizable Energy (ME) and Net Energy (NE), large efforts are required. By contrast, using theoretically-based models to calculate DE, ME and NE is more convenient.

Weiss et al. (1992) developed a mathematical model to estimate the total digestible nutrient (TDN) of feeds, in which, the nutrients were partitioned into four fractions: CP, NDF, ether extract (EE) and non-fiber carbohydrate (NFC). By estimating the energy values in each part, we could add up the TDN and DE of the feed at maintenance using equations in National Research Council (2001).

Today's high producing dairy cattle actually consume about 3 to 4 times of the maintenance requirement. As the digestibility of feed declines with increasing level of feed intake, a discount needs to be applied to calculate DE at productive levels of intake (DE_p). The ME at actual intake (ME_p) is estimated according to DE_p and NE_L at actual intake (NE_{Lp}) is estimated based on ME_p (National Research Council, 2001; Robinson, 2007; Tyrrell et al., 1975). The net energy for maintenance (NE_m) and net energy for gain (NE_g) are determined according to National Research Council (1996).

4.3. Methodology of Rumen Kinetics Determination Using In situ Technique

The *in situ* technique was first introduced by Quin et al. (1938) and it has since been used to estimate the feed degradation in the rumen. Mehrez and Ørskov's (1977) report raised the interest in this technique by proving it as a simple and useful guide to determine the nutrient (such as protein and carbohydrate) disappearance in the rumen. After years of modification and development, the *in situ* technique has now become a reliable and rapid way to estimate the rate and extent of degradation of feedstuff in the functioning rumen (Ørskov et al., 1979; 1980).

Despite the merits of the *in situ* method, there are also some limitations. As mentioned by Ørskov et al. (1980), the samples used in the technique are not influenced by the chewing and rumination. The particles in the artificial bags could not leave the rumen unless broken down, not necessary to simple chemical compounds, but to a size that is smaller than the pore size. Therefore, the dimensions and pore size of the artificial bags, particle size and amount of feed in each bag, incubation time in the rumen are all critical factors that affect the outcome. The pore size was suggested to be between 15-40 μm to neither restrict microbial colonization and trap fermentation gases, nor lose solubles and undegradable particles (Mohamed and Chaudhry, 2008).

The degradation of feeds in the rumen is largely related to the colonization of rumen microorganisms to the plant tissues and the microbial colonization could significantly impact the estimation of ruminal protein degradation, especially for forages that are high in fiber and low in nitrogen content (Wanderley et al., 1999).

4.4. Modeling Truly Protein Supply in Small Intestine and Metaboloc Characteristics

Several nutrition models were developed in the period from 1970 to 2010 and used to predict the true protein supply to the small intestine and feed milk protein production (Nuez-Ortín, 2010). The DVE/OEB Model and the NRC Dairy 2001 are two of these models. Both of them are modern models based on similar principles, but there are also differences due to the slight differences in conceptions and the use of different factors in the formulas, including the determination of endogenous protein losses, microbial protein synthesis and rumen bypass protein (Yu et al., 2003a, b).

The DVE/OEB Model was first developed in 1991 and introduced and revised by Tamminga et al. (1994, 2007), mainly used in European countries. The most updated version is available in Van Duinkerken et al. (2011). According to the results from chemical analysis, ruminal kinetics and protein digestibility, the DVE value is calculated as: DVE = ABCP +AMP – ENDP, where ABCP is truly absorbed rumen bypass protein in small intestine; AMP is truly absorbed microbial protein in small intestine; ENDP is endogenous protein losses in the digestive tract, which is related to the amount of undigested DM extracted in the feces. The OEB value is the balance between microbial protein synthesized from available rumen degradable protein and that from the energy extracted from the anaerobic fermentation in the rumen. Detailed equations could be found in Tamminga et al. (1994, 2007) and Yu et al. (2003a, b).

In contrast, the NRC Dairy Model is developed in 1985 and has been regularly updated. The newest version is the 7th revision in 2001. The NRC Dairy model is comparatively popular in research areas in North America and Asia. Information on TDN values of the feed is required when using this model. The major concept in this model is metabolizable protein (MP), defined as “truly digested and absorbed protein in small intestine”. The MP value is calculated as: MP = ARUP + AMP + ENDP, where ARUP is truly absorbed rumen undegraded protein in small intestine; AMP is truly absorbed microbial protein in the small intestine; ENDP is endogenous protein that contributes to duodenal protein. The sources of

ENDP include saliva, respiratory tract, mouth, esophagus, reticulo-rumen, omasum and abomasum (National Research Council, 2001). More detailed comparison between the two models could be found in Yu et al. (2003a,b), Yu (2005a), Gamage and Yu (2013) and Theodoridou and Yu (2013).

V. CUTTING-EDGE SYNCHROTRON- AND GLOBAR-SOURCED VIBRATIONAL (MICRO)SPECTROSCOPY FOR MOLECULAR STRUCTURE STUDY

Traditional “wet” chemical methods often result in destruction of the intrinsic chemical make-up of a biological tissue during processing for analysis. Recently, advanced synchrotron radiation-based Fourier transform infrared microspectroscopy (SR-IMS) has been developed as a rapid, direct, non-destructive and bioanalytical technique. In contrast to traditional “wet” chemical methods, this technique, taking advantages of synchrotron light brightness (million times brighter than sunlight) and small effective source size, is capable of exploring the molecular chemistry within microstructures of a biological tissue without destruction inherent structures at ultra-spatial resolutions (Yu, 2004, 2010, 2011; Yu et al., 2007). To date there has been very little application of this advanced technique to study tissue chemical make-up and image molecular chemistry which are modified by physical, chemical, biological treatments such as heat-related processing and gene-transformation.

VI. RECENT MOLECULAR SPECTROSCOPIC TECHNIQUE APPLICATIONS IN RAW, TRAITS ALTERED AND HEAT-RELATED PROCESSED CEREAL GRAIN AND OTHER TYPE OF SEED RESEARCH

6.1. On a Molecular Basis, Type of Food and Feeds Explored by Molecular Spectroscopy – SR-IMS, ATR-Ft/IRs and DRIFT Techniques

Recently, the advanced Synchrotron-Radiation based molecular technique- SR-IMS and SR-X and Globar-Sourced Molecular Spectroscopy - Ft/IRs, ATR-Ft/IRs and DRIFT Techniques have been used to study the molecular structures of protein, lipid (Xin et al., 2014; Thedoridou et al., 2015; Yu and Yu, 2015; Yu, 2012), carbohydrates, fibers and their structural changes in relation to nutrient availability and utilization.

The food and feed studied including different types of cool-climate cereal grains, pulse seeds, bio-oil seeds and bio-energy seeds, forage and transgenic grain forage as well as the mycotoxin levels in various feeds and food: Oat (Prate et al., 2017a,b; 2018), hulled and hulless barley with CHO trait alteration and grain silages (Refat et al., 2017; Yang et al., 2013, 2014; Sun et al., 2017; Yan et al., 2014, 2018), cool-climate wheat grain and its DDGS products (Ying and Yu, 2017), cool-climate corn (Xu et al., 2018; Abeysekar et al., 2017; Refat et al., 2017; Ying and Yu, 2017), cool-climate triticale and its DDGS products (Ying and Yu, 2017; Gamage et el., 2014), pulse seeds- peas (Yu et al., 2015), bio-oil and bio-energy seeds (Doiron et al., 2009; Khan et al., 2015; Ban et al, 2017; Thedoridou et al., 2014, 2015; Peng et al., 2014a,b), mycotoxin in feeds and food (Shi and Yu, 2017a,b) and forage and transgenic forage (Aufrère et al., 2014; Jonker and Yu, 2017). These molecular techniques also have been used for molecular thermal stability and molecular energy transition and mobility in cereal grain structure modified by heat-related processing (Khan and Yu, 2013).

6.2. Association of Molecular Structure Spectral Features with Nutritive Absorption in Cool-Climate Cereal Grains with Alteration of CHO Traits, Revealed by Molecular Spectroscopy – SR-IMS, ATR-Ft/IRs and DRIFT Techniques

Scientists in Crop Development Center (University of Saskatchewan, Canada) used modern breeding techniques to alter several carbohydrate traits in cool-climate hulless barley grain varieties (Yang et al. 2013a,b; 2014; Sun et al., 2017; Yan et al., 2014, 2018), it was found that with manmade alteration of carbohydrate traits, the predicted truly absorbed protein supply from hulless barley cultivars (*Hordeum vulgare L.*) were also significant changed. Yang et al (2014) did further linear relationship study to characterize the molecular structure features of newly developed hulless barley cultivars with altered carbohydrate traits (*Hordeum Vulgare l.*) by Globar-sourced infrared spectroscopy in relation to nutrient utilization and availability. It is interesting to find that some molecular structure spectral parameters had linear correlation with nutrient availability and some did not have linear relationship. No curvilinear relationship were carried out in this study (Yang et al., 2014).

The question we asked was: if there is no linear relationship between the molecular structure spectral parameters and nutrient availability, did these molecular structure spectral parameters have curvilinear relationship with nutrient availability?

Therefore, a further study (Sun et al., 2017) did was carried out on curve-Linear relationship between altered carbohydrate traits and molecular structure and truly absorbed nutrient supply from Hulless Barley *(Hordeum Vulgare L.).* It was found that although no linear relationship existed in some molecular structure spectral parameters, they had curvilinear relationship with various nutrient supply (Sun et al., 2017). These studies indicated that when we study relationship between the molecular structure features and nutrient availability, we should investigate not only linear relationship but also curvilinear relationship in cereal grains (Yang et al., 2013, 2014, Sun et al., 2017; Yan et al., 2014, 2018).

6.3. Implication of Modified Molecular Structure through Heat-Related Process, Revealed by Molecular Spectroscopy – SR-IMS, ATR-Ft/IRs and DRIFT Techniques

Food and feed industries often used various food and feed processing technology to improve bio-functions and nutrient utilization and availability, such as dry heating (Prate et al., 2017a,b; 2018), moisture heating, pressure toasting (Yu et al., 2000), dry roasting (Doiron et al., 2009), steam flaking (Xu et al., 2017), extrusion, pelleting (Huang et al., 2015), microwave irradiation (Yan et al., 2014, 2018) etc. In conventional nutrition studies, we always use traditional "wet" chemistry method to determine the impact of processing on chemical and nutrient availability. However, this wet chemistry method cannot be used to determine inherent structure make-up in the feed and food, mainly because it usually destroys the internal structure during the processing for the "wet" chemistry analysis. So it cannot be used to study the molecular structure conformation or molecular make-up in relations to nutrient availability and absorption in the digestive tract. Recently, the molecular spectroscopy – SR-IMS, Ft/IRs, ATR-Ft/IRs and DRIFT techniques have been further developed. These techniques could be used to study an interactive association between structure and nutrition (Prate et al., 2017a,b; Yang et al., 2013, 2014; Sun et al., 2017; Yan et al., 2014, 2018) and make great contributions to advance in food, feed and nutrition sciences.

In the literature, several new studies were found on the effect of processing-induced structure changes on nutrient utilization and availability using the molecular techniques- SR-IMS, Ft/IRs, ATR-Ft/IRs, and DRIFT (Doiron et al., 2009; Prate et al., 2017a,b; 2018; Yan et al., 2014, 2018; Xu et al., 2018; Gamage et el., 2014; Thedoridou et al., 2014, 2015; Peng et al., 2014a,b; Khan and Yu, 2013). For example, Yan et al. (2014, 2018) carried out interesting studies on the effect of durations of microwave irradiation (3 and 5 min) on truly absorbable nutrient supply of newly developed hulless barley varieties (*Hordeum vulgare L.*) in comparison with conventional hulled barley variety under a cool climate condition in western Canada. It was found that the microwave irradiation increased the levels of intestinal degradable protein, total digestible protein, intestinal digestible rumen bypass carbohydrates and starch in the *Hordeum vulgare L* barley. The microwave treatment had positive impact and improved the truly absorbed protein supply to dairy cattle. The microwave irradiation-induced changes in protein molecular structures of barley grains had significant relationship with changes in protein nutrient profiles as well as digestion in dairy cows (Yan et al., 2014, 2018). In another recent study (Ying and Yu, 2017) on cool-climate cereal grains–triticale (CST), wheat (CSW) and corn (CSC) that grown in western Canada, it was found that heat-related processing significantly impacted structural spectral features of protein and carbohydrate (eg. amide I, amide II, α-helix, β-sheet and their ratios) among different types of cool-climate cereal grains, indicting protein and carbohydrate structure alteration by processing. They also found the response and sensitivity of grain seed tissue to different processing methods differed.

6.4. Molecular Thermal Stability and Molecular Mobility of Heat-Induced Cool-Climate Cereal Grains, Revealed with Raman Molecular Mirospectroscopy and Differential Scanning Calorimetry

The molecular thermal stability and molecular mobility of heat-induced cereal grains, could be revealed with Raman molecular mirospectroscopy together differential scanning calorimetry (Khan and Yu, 2013). In this study, they found that the thermal degradation behavior of cereals was significantly changed after moist and dry heat treatments. The position of the major endothermic peak of dry heated cereals shifted toward higher temperature, from 132.7 to 133.5 °C, suggesting the high thermal stability of dry heated cereals. In contrast, the endothermic peak position was slightly decreased to 131.5 °C in case of moist autoclaved heating. These internal thermal stability and mobility changes on a molecular level indicated a dramatic impact on biobarrier, biofunction and biodegradation.

6.5. Summary

Here is summary of these studies (eg. Yan et al., 2014, 2018; Ying and Yu, 2017; Prate et al., 2017a,b; 2018; Thedoridou et al., 2014, 2015), the trait alteration (Yang et al., 2013, 2014; Sun et al., 2017) and heat-related process significantly modified inherent protein and carbohydrate molecular structure (lipid, protein, carbohydrate) or chemical make-up and nutritive values. Moist heat-related process had a dramatic impact on molecular structure and nutrient profiles. The type and varieties of cereal grain, pulse and oil seeds responded independently to different heat-related process (eg. dry heating, moisture heating, microwave irradiation). There was an interactive association between molecular structure modification and molecular thermal stability and mobility with nutrient utilization and availability.

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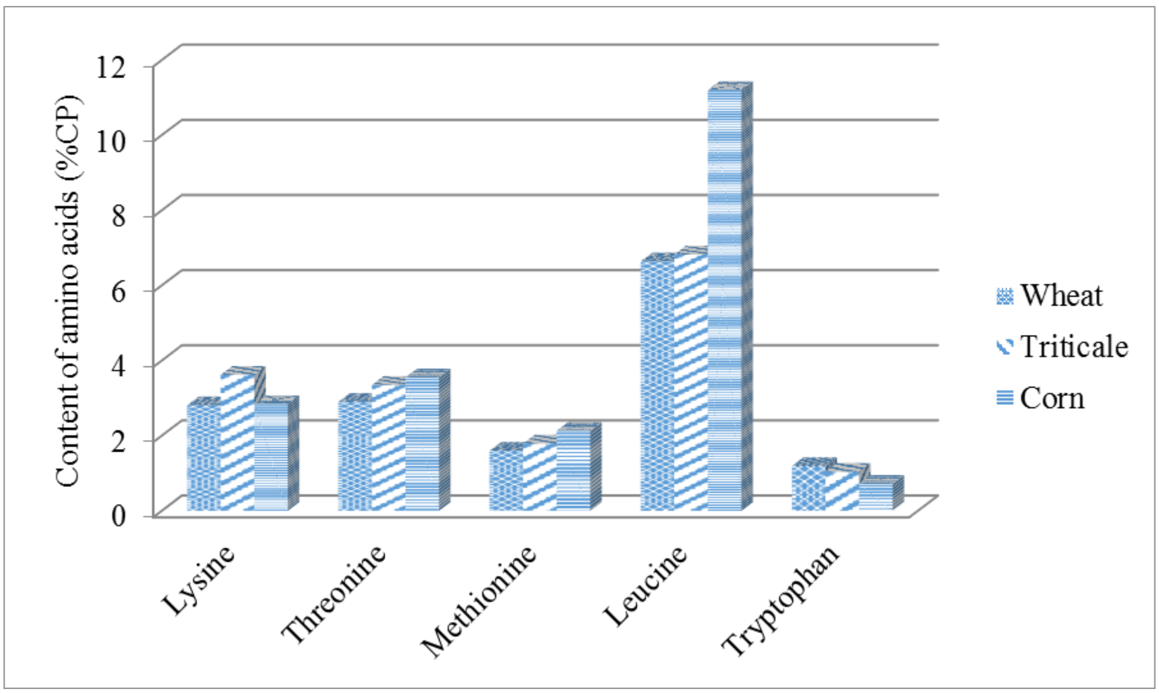

Figure 1. Amino acid content (g/100g crude protein) of wheat, triticale and corn

(Data from National Research Council, 2001)