PHYSIOCHEMICAL, NUTRITIONAL, MOLECULAR STRUCTURAL
CHARACTERIZATION AND DAIRY COW FEEDING VALUE OF OAT GRAIN IN
COMPARISON WITH BARLEY GRAIN:
IMPACT OF VARIETIES AND PROCESSING METHODS

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By

Marcela Tosta

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University of Saskatchewan

116 Thorvaldson Building, 110 Science Place

Saskatoon, Saskatchewan S7N 5C9   Canada

ABSTRACT

The general objectives of this study were to determine the impact of varieties and processing methods on the physiochemical, nutritional and molecular structural characteristics of CDC oat grain, as an alternative to barley grain in dairy cows diets. In the first study, three CDC varieties of oat, CDC Nasser (feed type), CDC Arborg and CDC Ruffian (milling type), were used and compared to CDC Austenson barley grain (feed type). In the second study, commercial oat and barley were tested, the oat was processed using three different methods (dry-rolling, steam-flaking, and pelleting) while barley was dry-rolled. In studies 1 and 2, the chemical profile, energy value, rumen degradation kinetics of nutrients, hourly effective rumen degradation ratios/potential N-to-energy synchronization, and intestinal digestion of nutrients were analyzed, the truly absorbed protein supply to dairy cattle and feed milk values were evaluated using on the DVE/OEB system and the NRC Dairy model, and the protein molecular spectra were analyzed. In study 3, the samples from different processing methods were used in a dairy trial, to evaluate production and milk composition as well as metabolic parameters, such as blood BHBA and urea. In study 1, CDC Nasser showed significantly higher percentage of EE in relation to the other varieties of oat. Degradation of starch and CP in the rumen was higher for all varieties of oat when compared to barley. On the other hand, starch, sugar, and NFC content were higher for CDC Austenson barley grain, that also showed the highest bypass CP and starch. No significant difference was observed between CDC Nasser and CDC Austenson barley on total digestible nutrients (TDN_{1x}), net energy for lactation (NE_L) and intestinal digestibility of bypass CP (dBCP). In study 2, heat processing (steam-flaking and pelleting) increased EE (P<0.01) and tended to decrease uNDF (P=0.09). Steam-flaking increased (P=0.04) the total digestible nutrients (TDN_{1x}), ME, and NE_L and increased (P<0.01) rumen bypass CP (%BCP). Rolled barley showed the lowest (P=0.03) metabolizable protein (MP) and degradable protein balance (DPB) (P<0.01) among the studied treatments. Processing

methods did not significantly change the protein molecular structure of the oat treatments, making the protein related structures hard to separate using PCA or HCLA. In the third study, cows fed dry-rolled oat had the lowest DMI, while increased ($P<0.01$) milk production compared to all other treatments. Milk fat percentage was also higher ($P<0.01$) for rolled-oat when compared to pelleted oat and rolled barley. Acetate concentration in the rumen was lower ($P<0.01$) for cows fed pelleted oat (-3.95 mM). Digestibility of starch was higher for oat grain ($P=0.05$). Based on the data presented by this research, oat grain can be suitable as an energy concentrate for lactating dairy cows in total mixed rations.

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LIST OF ABBREVIATIONS

Abs	Absorbance
ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
ADL	Acid detergent lignin
AMCP	Truly absorbed microbial protein in the small intestine
ARUP	Truly absorbed rumen undegraded protein in the small intestine
AOAC	AOAC Official methods of analysis (2005)
BCP	Rumen bypass feed crude protein (DVE/OEB system)
BDM	Rumen bypass dry matter
BOM	Rumen bypass organic matter
BST	Rumen bypass starch
CA4	Sugar (rapidly degradable carbohydrate fraction)
CB1	Starch (intermediately degradable carbohydrate fraction)
CB2	Soluble fiber (intermediately degradable carbohydrate fraction)
CB3	Digestible fiber
CC	Indigestible fiber
CDC	Crop Development Center
CHO	Carbohydrate
CP	Crude protein
D	Potentially degradable fraction
dBDM	Intestinal digestibility of rumen bypass dry matter
dBOM	Intestinal digestibility of rumen bypass organic matter
dBST	Intestinal digestibility of rumen bypass starch

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

energy (total digestible nutrients at a production level)

ME	Metabolizable energy
$ME_{p3\times}$	Metabolizable energy at a production level (3× maintenance)
MP	Metabolizable protein (NRC Dairy model)
MREE	Microbial protein synthesized in the rumen based on available energy
MREN	Microbial protein synthesized in the rumen based on rumen degraded feed crude protein
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NE_g	Net energy for gain
$NE_{Lp3\times}$	Net energy for lactation at a production level (3× maintenance)
NE_m	Net energy for maintenance
NFC	Non-fiber carbohydrate
OEB	Degraded protein balance (DVE/OEB system)
PA2	Soluble true protein (rapidly degradable true protein)
PB1	Insoluble true protein (moderately degradable true protein)
PB2	Fiber bound protein (slowly degradable true protein)
PC	Indigestible protein
PDI	Pellet durability index
PI	Processing index
RDP	Rumen degradable protein
RUDM	Rumen undegradable dry matter
RUP	Rumen undegradable protein
S	Soluble fraction (washable in NDF)

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

1. GENERAL INTRODUCTION

Oat comes from the genus *Avena* that has approximately 22 species, but the most prevalent one is *Avena sativa*, which is the principal cultivated species in the world (Hareland and Manthey, 2003). In Canada, oat was the most important grain until the 1920's but decreased in popularity once the horse was replaced by machines, and nowadays is the third most important grain cultivated nationally, coming behind only of wheat and barley (Small, 1999; Statistics Canada, 2018). In the last decades, the production of oat has been declining, but studies conducted all over the world have shown the potential of oat to replace barley as a feed grain in cattle production systems which prompted the development of new varieties of oat with different nutritional characteristics (Fuhr, 2006; Zalinko, 2014).

The common practice of feeding cereal grains to cattle in North America arises from the need to supply enough energy for this high producing animal, especially in places where the winter reaches very low temperatures. In Canada, the largest single cost of production facing dairy operations is feed, after the costs with quota. The increasing price of barley grain in western Canada continues to lead farmers to look for an alternative feed grain for dairy cows, in order to reduce feed costs. Lactating dairy cows require a substantial amount of energy to keep a high level of milk production while maintaining normal body processes and physiology (NRC, 2001). In Canada and the United States, common cereal grains fed to dairy cows includes barley, corn, wheat or oat because they are a practical and cost-effective source of energy. However, different cereal grains have unique physicochemical characteristics, which impact the degradation kinetics in the rumen and its intestinal digestion (Herrera-Saldana et al., 1990). For this reason, it is important to know the nutritional value of each grain and its behaviour in the gastro-intestinal tract of dairy cows.

Despite the large use of cereal grains as concentrate for ruminants, the rapid degradation in the rumen can lead to digestive disorders, an imbalance between protein breakdown and

microbial protein synthesis, which can lead to higher nitrogen excretion, and not enough protein and starch bypassing the rumen and being digested in the small intestine (Herrera-Saldana et al., 1990). One way to control this problem and increase the passage rates of nutrients to the small intestine is through processing methods (Chrenkova et al., 2018).

Processing methods can vary depending on the temperature, time, pressure, and moisture that is added, as well as the conditions that the feed was subjected to while in processing can change drastically its effect on ruminants. Rolling is normally used for cereal grains to disrupt the hull and expose the groat to microbial degradation in the rumen (Moran, 1986). To combine feed ingredients, make a mash easier to handle and reduce the number of fine particles, the method of pelleting can be used (Muramatsu et al., 2015). Steam-flaking can disrupt the protein-starch matrix and increase starch digestibility which leads to an increase in milk production and feed efficiency in dairy cows (Kokic et al., 2013).

In order to formulate balanced diets to ruminants, the chemical profile and the nutritional value of the feed ingredients need to be assessed. Methods for determining nutritive value includes chemical analysis, in situ rumen degradation kinetics, and intestinal digestion using the in vitro protocol of Calsamiglia and Stern (1995), as well as modeling systems such as the Cornell Net Carbohydrate and Protein System (CNCPS) and the Dutch DVE/OEB model (Tamminga et al., 1994; 2011). These methods have been studied for several years and deemed reliable, but they can be very time-consuming and several of these methods result in the destruction of the feed sample after analysis. To address these issues, a novel approach has been developed in order to study to reveal the chemical composition and structural make-up/conformation of a feed. Vibrational Fourier transform infrared molecular spectroscopy (FTIR) can be used to reveal the molecular structures and several studies have shown the relationship between molecular structure and nutrient utilization in ruminants (Yang et al., 2014).

The literature review (Chapter 2) in this study includes the global and national production of oat, its place in Canadian history, as well as the studies that have been conducted to evaluate the use of oat grain as a part of the concentrate for dairy cattle nutrition and its impact on milk production. Feed processing methods and their impact on feed quality and nutritional value for ruminants are also described. The methods and techniques of feed evaluation, both conventional, such as chemical analysis, CNCPS model, DVE/OEB model, as well as the novel technique of molecular structure determination (FTIR), that can be used as a non-destructive and fast method, are described.

The objectives of this project are to characterize and compare the physicochemical, molecular structure, energy values, and molecular structure of different varieties of oat grain and compare different processing methods in oat grains, using barley grain as a control, and to compare different processing methods of oat grain in dairy cows production performance. As a hypothesis, we expect that different varieties of oat, feed processing treatments could impact the structural, physicochemical, and nutritional characterization of oat grain grown in western Canada and affect nutrient utilization and availability to dairy cows.

2. LITERATURE REVIEW

2.1 Oat grain production in Canada

The exact location of origin of oat (*Avena sativa*) is yet to be identified, with some authors pointing to the Mediterranean area, while others believe this whole crop came from Europe. Oat was firstly introduced in Canada by European settlers. Up until the 1920's, oat was the main cereal produced in Canada, mainly to feed horses and other livestock, but also for human consumption (Bracken, 1920; Small, 1999). With the increasing automatization of agriculture and less dependence on horses, the oat harvest area was decreased steadily through 1970's (Small, 1999).

Oat is an important cereal grain with annual worldwide production projected for over 22 million metric tonnes in 2018/19 worldwide (USDA, 2018). Canada, with an estimated production of 3.4 million metric tonnes from one million hectares in 2018/19, is the second largest producer in the world, only producing less than Russia (2018). Saskatchewan, Alberta, and Manitoba are the Canadian provinces with the highest production of oat grain, with Saskatchewan being responsible for over 49% of the oat production in 2018 (Hoover, et al., 2003; Statistics Canada, 2018). This high production in Saskatchewan and the prairies is explained by the agro-climate characteristics of oat production.

Oat thrives in cool, moist climates and are particularly sensitive to hot, dry weather from head emergence to maturity. The crop is adaptable to many soil types and produces better on acid soils than other small grains (Hoffman, 1995). In North America, north-central areas are best suited for oat production (Suttie et al., 2004). The long warm days and characteristic of the Canadian prairies coupled with adequate moisture levels and dark soil provides producers with ideal oat-growing conditions. Oat is mainly grown in spring in most parts of the world, and in North America, where winters are long and harsh, short season maturing oat varieties are

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usually grown and oat are seeded early, usually at the end of May (Suttie et al., 2004; Ziesman et al., 2010).

2.2 Cereal grain for dairy cattle nutrition

High producing dairy cows require substantial amounts of energy for maintenance, milk production, fetal development, and growth (NRC, 2001). Cows being raised in milking production systems require an adequate supply of amino acids and glucose that can usually be accomplished using high amounts of concentrates in their rations. In North America, is a common practice to feed concentrates to dairy cows. The main purpose of concentrates is to increase animal performance compared to feeding forage-based feed alone and increase the economic return to dairy farmers since the price of grain relative to forage can be lower.

In western Canada and the United States, dairy cows' diets usually contain corn, barley or oat grain as a concentrate because they are a cost-effective source of digestible energy (Gozho and Mutsvangwa, 2008). Cereal grains differ in their physicochemical properties and rumen degradation patterns (Herrera-Saldana et al., 1990), which results in different nutritional characteristics and feeding value for dairy cows. Knowledge of the unique properties of cereal grains and how they impact the production performance of dairy cows are of paramount importance when formulating diets for a dairy production system.

2.2.1 Barley grain

In western Canada and in the United States, barley is widely used as an energy source for cattle. Studies usually compare the use of barley to the use of corn grain, since both grains are commonly used to feed high producing cows. Differences in the nutritive value of barley and corn can be attributed to the lower starch and higher fiber content of barley: the starch and NDF content in barley has been reported to be 64.3 and 19.5 %DM, respectively, while corn

values were 75.7 and 9.3 % DM, respectively (Herrera-Saldana et al., 1990). Although corn is considered a superior cereal grain to feed to cattle, there are reports of similar animal production performance using barley grain (DePeters and Taylor, 1985; Khorasani et al., 2001; Yang et al., 1997). A meta-analysis by Ferraretto et al. (2013) reported that cows fed corn grain-based diets produced on average 2.5 kg of milk more than cows fed diets containing barley grain, but the milk fat %, protein % and feed efficiency (milk yield/DMI) did not differ between treatments.

Barley grain has showed similar values or advantages compared to other concentrates fed to dairy cows. In a study conducted in Sweden, researchers substituted barley (high starch diet) for fodder beets and raw potato in a proportion of 80:20 (high sugar diet) and found that cows fed the barley grain-based diet produced 1.6 kg of milk and 2.3 kg of energy corrected milk (ECM) more than cows fed beats and potatoes, although the researchers have attributed the lower production to decreased silage intake (Eriksson et al., 2004). Sun and Oba (2014) found no difference in milk or fat production when substituting barley grain with wheat DDG's at a 17% diet inclusion level.

2.2.2 Oat grain

The total world production of oat has been declining for decades. In the 1960's world oat production was estimated at 49.6 MMT (Hoffman, 1995) and it declined more than 55% to this day, which explains the small number of studies that were conducted to assess the effect of feeding oat grain on the production performance of dairy cows. The great majority of studies published focused comparing oat to other grains in complete rations (Fisher and Logan, 1969; Fuhr, 2006; Gozho and Mutsvangwa, 2008; Martin and Thomas, 1988; Moran, 1986; Tommervik and Waldern, 1969; Yu et al., 2010) or as a supplement for cows on pastures (Valentine and Bartsch, 1989). Some studies have focused on naked oat (Fearon et al., 1998;

Fearon et al., 1996; Petit and Alary, 1999), while others have focused on the protein or fat content (Ekern et al., 2003; Schingoethe et al., 1982).

In a study conducted in Norway, Ekern et al. (2003) produced two different concentrates with the same concentration of ingredients, replacing the barley grain of the first concentrate by normal oat grain. The concentrates were given as pellets with wilted, formic acid silage (fed almost at *ad libitum*), beetroot, and ammonia-treated straws. As for production performance, cows in the oat-based diet produced more milk when compared to cows fed barley grain (26.2 and 23.6 kg/d), respectively. Fat and lactose yield did not differ between treatments, but fat % was 6.4 g/kg higher in barley fed cows. ECM did not differ between treatments. Despite the authors not statistically analyzing the intake of the animals in the trial, the fat content of the oat-based diet was much higher than the barley-based one. The higher amount of fat was pointed by the authors as the reason for the higher production of milk by cows fed the oat-containing diet.

In a study conducted in Canada, Fuhr (2006), studying the replacement of CDC Dolly barley grain with Derby oat or a low lignin hull-high oil groat oat (LLH-HOG), conducted an experiment with nine lactating Holstein cows in a triple replicate 3x3 Latin square design and fed them diets as TMR, with grains making up to 33% of the diet. The production trial found no significant difference in dry matter intake (DMI), milk production and 3.5% fat corrected milk (FCM) although milk yield tended to be higher (P=0.08) for cows fed LLH-HOG oat grain when compared to barley grain (42.1 and 40.0 kg/d), respectively.

In a more recent study, Gozho and Mutsvangwa (2008) assessing the performance of dairy cows fed a total mixed ration (TMR) containing barley, oat, corn or wheat grains as the main source of dietary carbohydrates reported no differences in DMI and milk yield for all four treatments. FCM and percentages of fat, protein, and lactose were similar between barley and oat fed cows, even though oat-based diets contained 0.6 kg less grain than barley-based diets.

2.3 Cereal grain processing techniques

Cereal grains can be processed to increase their digestibility, increase the degradation in the rumen by disrupting the fibrous hull and allowing microbial attachment and degradation (Zalinko, 2014) or to enhance the intestinal digestibility of starch that escapes the ruminal degradation (Safaei and Yang, 2017). Processing methods can be physical or chemical and can be done using several settings of temperature, pressure, and moisture. The type of processing to be utilized depends on the type of grain to be fed since different cereal grains possess physical differences in the outer layer of grain kernels and in the different endosperms of cereal grains (Hoseney, 1994).

2.3.1 Rolling

Dry rolling is a mechanical process that involves passing the grain between two steel rotating rolls that are usually grooved in their surface. The two rollers may operate at different speeds, depending on the function and pre-established settings. The higher the speed, the greater the force applied to the grain (McKinney, 2006). Grains passing between the rollers are sheared to break open. It is ideal to control the extent of processing when roller milling feeds for cattle, the processing needs to do enough damage to the outer layer of the grain to allow an easier attachment and degradation by the ruminal microbes while avoiding extensive processing of the kernel, which could produce smaller particles that can rapidly increase rumen degradation and contribute to rumen acidosis (Safaei and Yang, 2017).

The dry-rolling process is very efficient in increasing surface area for degradation and digestion, but it only partly breaks the protein matrix and it is not effective in gelatinizing the starch portion of a feed (Millen et al., 2016). Several studies described the advantages of feeding rolled grains instead of whole grains to cattle (Moran, 1986; Morgan and Campling, 1978;

Nordin and Campling, 1976; Toland, 1976; Valentine and Wickes, 1982). Oat grain has a higher proportion of hull to groat compared to barley, but the pericarp of oat grain is not tightly adhered to the endosperm as barley grains (Zalinko, 2014). This observation corroborates the ideas of Morgan and Campling (1978) that, when feeding young cows (less than 2 years of age), the benefits of rolling oat grain are unlikely to be worth the processing cost, since the higher degree of chewing shown in young animals can damage the hull easily and provide an entrance for microbes to attack the groat.

2.3.2 Flaking

Steam flaking is the process of steaming whole grains, to allow at least 5% moisture uptake (Zinn et al., 2002), and subsequently rolling it into a flake. The two different methods of steam flaking are related to the pressure of steam applied in the grain. The low-pressure method of steaming exposes the grain to low-pressure steam for 30 to 60 minutes, maintaining the temperature between 95 to 99°C. The high-pressure method submits the grain to a pressure of 3.5 kg/cm² for approximately 3 minutes and subsequently allowing it to cool to 95°C before rolling (Boyles et al., 2000).

The quality of steam flaked grains is usually based on physical measurements, such as flake thickness (mm), flake density (kg/L), or measures of the starch content, like starch solubility and enzyme reactivity, and can be largely different depending on the pressure applied, the time inside the steam chest and the roller gap (Zinn et al., 2002). The extent of processing increases as the flake density decreases and a flake density of 0.32 to 0.39 kg/L was suggested to be the optimal (Plascencia and Zinn, 1996).

Several studies researched the impact of steam-flaking grains on ruminant performance, although very few studies have analyzed the impact of this processing method in oat for dairy cattle. Steam-flaking is shown to increase the digestibility of starch in the rumen, and, enhances

the extent of starch digestion in the small intestine (Firkins et al., 2001; Safaei and Yang, 2017). This increase in starch utilization is usually related to the improved feed efficiency seen for feedlot cattle fed steam-flaked grains (Theurer et al., 1999). Zinn et al. (2002) reviewing several studies showed that steam-flaking corn can increase the net energy for gain (NE_g) between 4.5 and 27.2%, when compared to dry processing. Qiao et al. (2015) observed that steam flaking rice grain increased the ME, NE_m and NE_g 0.76, 0.66 and 0.59 MJ/kg of DM, respectively. In dairy cows, steam-flaking of corn and sorghum was previously shown to increase milk production, increase the flow of microbial protein to the duodenum, but slightly decreased milk protein and milk fat percentage (Firkins et al., 2001; Yu et al., 1998).

2.3.3 Pelleting

Pellets are an agglomerate of ground single feed ingredients or combinations of several ingredients that by the use of mechanical pressure, moisture and heat, are bound together (Muramatsu et al., 2015). To make the pellet, the mash of feeds is forced through holes (die) in a metal plate and cut at the preferred length. Pelleting is performed in a wide range of conditioning temperatures and can be accomplished with or without the application of steam. Pellets can be done in a dry-conditioning (at 20-25°C) or heated (usually from 60-90°C).

Pellets must endure packing, transportation and handling on the farm without producing too many fine particles. The quality of a pellet is determined mainly by its ability to withstand handling without fragmentation or abrasion that would generate fine particles (Muramatsu et al., 2015). Pellet durability index (PDI) is the main parameter for determining pellet quality, as the PDI indicates the percentage of pellets that stay intact after physical testing. There are many methods to measure PDI: the tumbling box test, where intact pellets are placed in a box and tumbled for 10 minutes before the percentage of intact pellets and fines are measured with a sieve; Holmen pellet tester, where pellets are moved through tubes using high-velocity air, to

mimic the handling process (Behnke, 2001). Other factors that influence directly the quality of the pellet is particle size, moisture addition, fat inclusion and conditioning (Briggs et al., 1999; Muramatsu et al., 2015).

Previous studies described the benefits of feeding pelleted grain to cattle. In an early study, Bishop et al. (1963) found higher milk yield, similar protein and fat percentage for cows fed a pelleted concentrate instead of a meal made from the same ingredients. Gozho et al. (2008), studying dry-rolled vs. pelleted barley grain for lactating dairy cows, reported lower DMI, similar milk yield, but lower fat percentage for cows in the pellet-based diet. Keyserlingk et al. (1998) showed a higher value of milk yield for lactating Holstein cows fed a pelleted concentrate compared to a textured one, but similar fat suppression was observed. In both studies that observed fat depression, the fat yield was not affected, despite the fat percentage being lower, as a result of the increased milk yield. Keyserlingk et al. (1998) attributed the increase in milk yield to the higher DM and CP degradation in the rumen, which may have allowed higher microbial protein production and thus increased milk yield.

According to Behnke (1994), the improvement in animal performance when fed pellet diets instead of meals, is mainly due to the decrease in food waste (accomplished by the reduction in fines), reduction in selective feeding, decreased ingredient segregation, destruction of pathogens, improved palatability and thermal modification of the starch and protein.

2.4 Impact of processed grains in dairy cattle performance

The tradition of leaving cattle to graze in the fields with little or no supplementation of grain or other concentrates is no longer a common practice in North America. With the increasing world population demanding more available resources, the efficiency of production systems had to increase. Today, high animal performance and production requires larger amounts of feed concentrate that will supply the animal with the required amount of energy.

However, to minimize the costs of feeding concentrates to dairy cows while maximizing the digestibility and energy availability of grains, proper processing is required (Zalinko, 2014).

2.4.1 Milk yield and composition

There is considerable interest in increasing milk yield without decreasing the fat yield, mainly due to the prices of milk in Canada being based on fat content. Processing methods can affect milk production by changing the rate, extent, and site of digestion of cereal grains (Chrenkova et al., 2018; Firkins et al., 2001). Pelleting concentrates before feeding it to dairy cows has been shown to increase milk production but causes depression in milk fat (Gardner et al., 1997; von Keyserlingk et al., 1998). The observed pattern in milk fat depression when feeding pellet concentrates to dairy cow can be attributed to ruminal fermentation changes that result in a decrease in ruminal pH and changes in the biohydrogenation pathways, that can lead to an accumulation of inhibitors of de novo of SCFA in the mammary gland, like $C_{18:1}$ and conjugated linoleic acid isomers (Gozho et al., 2008).

Lactating dairy cows supplemented with steam-flaked corn showed a decrease of 1.3 kg in DMI and an increase of 3.1 kg in milk production, while maintaining milk fat yield similar to cows supplemented ground corn in a diet containing a mix of ryegrass and corn silage (Cooke et al., 2008). The higher milk yield in cows fed steam-flaked grains is attributed to higher digestion of starch in the rumen, that would lead to increased absorption of nutrients available to the mammary gland (Theurer, 1986; Theurer et al., 1999; Yu et al., 1998). Zhong et al. (2008) found similar production of milk and fat for cows fed finely ground or steam-flaked corn, despite the higher total-tract digestibility of steam-flaked corn, which might bring up the question of the possible role intestinal digestion of starch plays in increase milk yield.

2.4.2 Ruminal parameters

Several studies reported the impacts of processing grains on the ruminal parameters of cows. Not only the processing of the grain impacts pH in the rumen but the extent and method of processing will also have an effect. Yang et al. (2000) noticed that despite not finding significant statistical difference among coarse, medium, medium-flat and flat-rolled barley, the hours during which ruminal pH was below 5.8 linearly increased with decreasing processing index (PI %). The effect of pelleting concentrates on ruminal pH is inconsistent throughout the literature. Dos Santos et al. (2011) reported higher ruminal pH when pelleted concentrates were offered as opposed to ground concentrate and hypothesized that increased release of fat in the rumen by pelleted concentrates might be the cause of higher ruminal pH. This hypothesis is supported by experiments done with beef cattle, where steers fed high-fat blended pelleted products had a higher ruminal pH when compared to high starch pellets (Zenobi et al., 2015). On the other hand, Gozho et al. (2008) reported lower ruminal pH for cows fed pellet barley as opposed to ground barley, but in this case, diets were already being supplemented with canola or flax seed.

Ammonia arises from the catabolism of dietary nitrogenous compounds, and in the presence of fermentable energy, this ammonia can be used to synthesize microbial protein (Rodriguez et al., 2007). Ammonia that is not used for microbial growth can overflow from the rumen to the liver, where it will be used to synthesize urea, an energetically costly reaction. Ekinci and Broderick (1997), studying the possibility of feeding ground high moisture ear corn to dairy cows, reported depressed ruminal NH_3 for cows fed the ground-corn diet, and another study by Rust et al. (1980) reported the same depression effect in cows fed steam-flaked corn. The higher digestibility of processed grains most likely contributed to a more extensive degradation of carbohydrates in the rumen, which is positively correlated to the sequestration of ammonia into microbial protein (Chibisa et al., 2015; Russell et al., 1992; Theurer, 1986).

The concentration of SCFA in the rumen can also be impacted by the processing method used on the feed. Ekinci and Broderick (1997) found lower acetate, acetate to propionate ratio and higher propionate for ground corn when compared to whole corn. On the other hand, Knowlton et al. (1998) did not find significant difference between corn ground with a 6.4 mm screen in a hammer mill or corn rolled in a roller mill with an initial gap of 0.58 mm, on SCFA concentration in the rumen.

2.4.3 Digestibility

The digestibility and site of nutrients digestion can also be impacted and altered by processing, and the effect can depend on whether the grains were submitted only to physical processing or to thermal processing. The damage made on the hulls of cereal grains is enough to increase the digestibility of the grains for dairy cows. Grinding grain with a hammer mill reduces the particle size and increases the surface area that bacteria can attach and degrade. This makes the starch more easily digested by the microorganisms in the rumen and rate of starch digestion varies inversely with particle size (Dehghan-banadaky et al., 2007). The starch present in the fecal matter was decreased by 84% just by coarsely rolling oat grain (Moran, 1986) and the apparently total-tract digestibility coefficient of organic matter and starch was increased by 0.167 and 0.490, respectively, when rolling barley and oat (Morgan and Campling, 1978).

Thermal processing methods can add heat only or can be a combination of heat, moisture, and pressure. Submitting feeds to heat treatment without moisture can increase the potentially degradable fraction and the intestinal digestion of RUP, while decreasing the degradation rate in the rumen, depending on the heat intensity and the duration of the process (McNiven et al., 1994; Sadeghi and Shawrang, 2007). The application of moisture to heating processes can increase the nutritive value of a feed by increasing the starch degradability, that

occurs due to starch gelatinization, that occurs when sufficient moisture is added to the grain in an adequate temperature. Different cereal grains posses different starch gelatinization temperatures. Starch gelatinization is a swelling process, in which the crystalline structure of the starch granules are broken and the amylose in the starch granule leaks out and solubilizes outside of the crystal, increasing viscosity (Kokic et al., 2013; Svihus et al., 2005). When steam-flaking is used in cereal grains, the rumen degradable fraction of CP (RDP) was significantly reduced while the rumen undegradable CP (RUP) and the intestinal digestibility of RUP were increased (Chrenkova et al., 2018). Pelleting is another way to decrease the ruminal degradability of starch and CP while increasing the total-tract digestibility of CP and starch (Goelema et al., 1999), although some studies show that pelleting had no effect on apparent total-tract digestibility of grazing cows (Dos Santos et al., 2011).

2.5 Feed evaluation methods

It is important to understand the nutritional value of a feed before offering it to ruminants, especially to high producing dairy cows that require specific nutrients and energy levels to maintain milk production, optimal fetal development and tissues maintenance (NRC, 2001). The nutritive value of a feed is characterized by its nutrient and chemical composition, its nutrient flow through the gastro-intestinal tract and by its inherent molecular structure. To accomplish this evaluation, several methods and techniques can be used.

2.5.1 Chemical analysis

Chemical analysis is a key component in determining the nutritional value of a feed for ruminants. This analysis usually involves the determination of several components, like dry matter (DM), organic matter (OM), carbohydrates (CHO), crude protein (CP), soluble CP (SCP), starch, and fiber. Several components of the chemical profile of a feed are determined

according to the official methods of analysis published by AOAC International (2005). The most widely used methods for determining the fiber portions of a feed are the extraction methods of Van Soest et al. (1991).

The values of each of these components can be further used in the CNCPS model to predict protein and carbohydrate sub-fractions and their degradation rates and extent; and on the summative equations of the NRC 2001 model for prediction of energy content of feeds for dairy cattle.

2.5.2 Cornell Net Carbohydrates and Protein System

In the 1990s a series of papers were published describing the CNCPS model in detail, outlining carbohydrate and protein digestion, microbial growth, amino acid supply and animal requirements (Fox et al., 1992; O'Connor et al., 1993; Russell et al., 1992; Sniffen et al., 1992). The CNCPS model was then created to allow diet formulation, considering the nutritional aspects of feeds available in production systems to enable the formulation of diets that would fulfill the animal's requirements. By accounting for farm-specific characteristics, like management, feed ingredients composition and environment, the CNCPS model allows a more precise prediction of growth, milk production and nutrient excretion by cattle (Fox et al., 2004).

To better address this objective, several updates were performed since the release of the first version in 1992, that aimed to improve the model's prediction. Research data related to feed analysis, rumen function and metabolism have been incorporated in the mathematical equations of the CNCPS model in the last 15 years. The CNCPS version 6.5 has been available since 2015 (Higgs et al., 2015; Van Amburgh et al., 2015) and it is extensively used in the industry to evaluate and formulate diets through software such as AMTS.Cattle (Agricultural Modeling and Training Systems LLC, Cortland, NY) and NDS (Ruminant Management and Nutrition, Reggio Emilia, Italy). The version 6.5 was upgraded with new predictions of nutrient

requirements and supply, and changes in the feed library (Higgs et al., 2015; Van Amburgh et al., 2015). This use of CNCPS in the industry of several countries makes any improvement in the prediction of nutrient supply and animal requirement's to the model important, not only for research development purposes but also directly impacts production on farm (Van Amburgh et al., 2015).

2.5.3 Energy values

The quantification of the energy value of feeds and diets being offered to cattle is important to determine the amount of feed that needs to be offered daily. Energy is vital for tissue maintenance, growth, milk synthesis and gestation in dairy cows, so the careful assessment of the energy content, needs to be a part of diet formulation (Eastridge, 2002). The NRC 2001 model provides summative equations to predict the energy content of feeds for dairy cows, based on its chemical composition. In this model, the feed energy values are obtained using total digestible nutrients (TDN), determined from composition data rather than being experimentally determined (NRC, 2001). The TDN at maintenance is determined using the total digestible CP, NFC, FA, and NDF, according to the equation:

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

Since the digestibility of diets in dairy cows decreases when DM intake is high, this will reduce the energy value of a diet as intake increases (NRC, 2001). This is particularly important to notice when feeding high producing dairy cows, that can have an intake up to 4 times higher than maintenance levels. For this reason, the NRC model for predicting energy values of diets uses a method to discount the digestibility when calculating digestible energy at a production level of intake (DE_p). The energy required for maintenance and milk production in dairy cows is given by the net energy for lactation (NE_L).

When formulating a diet for cows, it is necessary not only to account for the energy requirements of the animal but also to adjust the requirements based on the level of activity, intake level and environmental stressors, such as temperature (Eastridge, 2002).

2.5.4 In situ rumen degradation kinetics

The rate and extent of degradation of a feed in the rumen is of extreme importance for determining the nutritional value of a feed. Feed ingredients that have fast ruminal degradation can promote digestive disorders in the animal and lead to rumen fermentation disorders (Humer and Zebeli, 2017). On the other hand, feeds that have a slow degradation in the rumen might escape rumen degradation by the microbes and be digested in the small intestine, thereby improving glucose supply to the animal (Humer and Zebeli, 2017). There are two methods of determining this rate and extent of degradation in the rumen; either by determining the amount of a certain nutrient entering the abomasum or by incubating bags of feed in the rumen for a fixed duration of time (Ørskov and McDonald, 1979).

The rumen degradation kinetics determined using the nylon bag technique described by Damiran and Yu (2012) and the equation described by Ørskov and McDonald (1979), and modified by Tamminga (1994) has been proven to be a valuable method to predict the rate and extent of degradation of primary feed components, like DM, OM, CP, starch, and NDF. This method can be used to evaluate the degradation of a feed in the rumen and estimate the rumen bypass part of nutrients, which is important since dairy cows benefit from bypass CP and starch that can be digested in the small intestine (Humer and Zebeli, 2017; Rigout et al., 2002).

2.5.5 Intestinal digestion

The protein that is digested in the small intestine is compromised in part of rumen undegradable CP (RUP) and of microbial protein synthesized in the rumen. The digestibility of

rumen bypass protein varies among feed ingredients (Gargallo et al., 2006). The in vivo methods of determining intestinal digestion of RUP are expensive and very labour demanding, and it required the used of surgically cannulated animals. For this reason, Calsamiglia and Stern (1995) developed an in vitro method of measuring intestinal digestibility of RUP. Later the methodology was updated by Gargallo et al. (2006).

This procedure of determining intestinal digestion using the in vitro technique was deemed a reliable procedure, that can be done routinely in the laboratory and results in a substantial reduction in labour and costs. Moreover, the close simulation that the technique produces, in addition to the ruminal degradation data of individual feed ingredients, can be used as quality control of feed and can determine the value of feed protein for ruminants (Calsamiglia and Stern, 1995).

2.5.6 Prediction of truly digestible protein supply to the small intestine

Models for the prediction of truly digestible protein supply and feed milk values in dairy cows have been used for decades. Based on the characteristics of both systems, the efficiency of a feed can be calculated as the feed milk value (FMV), that represents the amount of milk produced for a given amount of feed DM intake. The Dutch system (DVE/OEB) and the NRC model are useful tools for predicting protein supply for dairy cows. The DVE/OEB system described by Tamminga et al. (1994, 2011) is used in several European countries, while in North America the NRC Dairy is used for research. Both models have similar principles but there are some differences in the concepts and factors used by each (Yang et al., 2013; Yu et al., 2003).

The Dutch system, established in 1991 and revised in 2011, was proposed as a way to improve the predictions of N loss due to an inappropriate intake of feed N and to better describe the digestion and metabolism of N in dairy cows (Tamminga et al., 1994). In this system, the

protein value of feeds and the requirement of dairy cows are indicated as the amount of CP that is truly digested and absorbed in the small intestine. The model is based on two main values: the DVE is the amount of true protein digested in the small intestine and is described as being composed of the amount of undegraded feed CP digested and absorbed in the small intestine as amino acids (DVBE), the microbial protein that flows from the rumen and is digested and absorbed in the small intestine (DVME), while subtracting the endogenous losses resulting from the digestion (DVMFE) (Tamminga et al., 1994). The degradable protein balance (OEB) shows the balance or imbalance between the microbial synthesis that would be possible from available rumen degradable CP and the synthesis that would be possible from energy extracted during fermentation in the rumen. When the OEB value is positive, it indicates loss of N from the rumen, while negative values indicate impaired microbial synthesis in the rumen due to a lack of available N (Yu et al., 2003). Several published studies have focused on using the DVE/OEB system in a North American context (Yang et al., 2013; Yu et al., 2003, 2004).

The NRC dairy system is a TDN based model, similar to the Dutch model in many ways, but differs in some concepts and in the factors used in equations. The metabolizable protein (MP) in the NRC model is defined as the true protein that is digested post-ruminally and absorbed in the small intestine (NRC, 2001). The degraded protein balance is calculated as the difference between the potential microbial protein synthesis based on rumen degraded feed protein and the potential microbial protein synthesized based on available energy (TDN) (Theodoridou and Yu, 2013; Yu and Racz, 2010).

2.5.7 Vibrational Mid-IR molecular spectroscopy

Traditionally, infrared spectroscopy has been used for decades as an important analytical technique for research. This technology is applied in the areas of biology, physics, chemistry and has been proved effective for the area of food technology (Stuart, 2009). The

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advantages of using infrared spectroscopy are many: the method can be applied in a wide range of samples in liquid, solid or gases state, it does not require the use of reagents, the acquisition of data is fast, it is cost-effective, the amount of sample required for the analysis is small and requires little to no processing (Stuart, 2009). In recent years, the acquisition of infrared spectra has been dramatically improved with the use of Fourier-transform infrared (FTIR) spectrometer.

Conventional methods of analysis such as chemical profile and protein and carbohydrates subfractions give us the information about the nutrient composition of a feed, but it cannot detect any inherent molecular structure characteristics. In recent years, it has become important to utilize vibrational FTIR molecular spectroscopy to reveal intrinsic molecular structural changes among feeds that will be used in high producing cows diets (Ismael et al., 2018). This is mainly due to the knowledge acquired from previous studies that reported the correlation between protein and carbohydrate molecular structure profiles and the fermentation feature and the feeding value of a feed for dairy cows (Yang et al., 2014; Zhang and Yu, 2012).

Different statistical approaches can be used to analyze the spectral data collected from the FTIR. The univariate molecular spectral analysis method consists in the evaluation of frequency and intensities of specific functional groups (peak height and area as well as their ratios). This type of analysis can be correlated to the chemical composition of a feed or the pattern of digestion. In the multivariate approach, multiple variables are analyzed at the same time to differentiate the samples and classify them. The most common multivariate analyses are Principal Component analysis (PCA) and Hierarchical Cluster Analysis (HCLA). The PCA is a statistical data reduction method that works by transforming the original data set into a new set of uncorrelated variables called principal components (PC). The purpose of PCA is to derive as small a number of linear combinations (PC's) from the data set as possible, while maintaining as much of the original information as possible (Yu, 2005, 2007). The HCLA is a statistical

method that searches for similarity in the IR spectra's and agglomerates them into clusters that are displayed as dendrograms. Firstly, the HCLA calculates the distance matrix that contains information on the similarity of the spectra, and then the algorithm searches within the distance matrix for similar IR spectra (minimal distance between them) and combines them into a cluster (Yu, 2005, 2007).

2.6 Literature review summary, overall research objectives, and hypothesis

2.6.1 Summary

In Canada, the largest single cost of production facing dairy operations is feed, after the costs with quota. The increasing price of barley grain in western Canada continues to lead farmers to look for an alternative feed grain for dairy cows, in order to reduce feed costs. Oat (*Avena sativa*) was widely used in Canada since its introduction by European settlers as a feed for cattle and horses. In the 1920's, with the replacement of horsepower by machines, oat started losing its place, being replaced by barley and corn. Nowadays, oat has a worldwide production of 22 MMT, and Canada is the second biggest producer in the world, with up to 90% of its production coming from the Prairie region.

The question of whether oat can replace other grains as a feed for dairy cows was raised before. Grains such as corn and barley have higher starch and non-fiber carbohydrates when compared to oat, which means their energy value is higher than oat. However, previous studies conducted in Canada and other countries showed the potential of replacing barley for oat in dairy systems, especially when feed is offered as TMR.

For animal feeding purposes, the highly ligneous hull of the oat grain needs to be broken or removed before feeding it to the animals, otherwise, a large amount of grain will resist degradation and digestibility and end up evacuated whole. However, the readily available and highly ruminal degradable starch present in the oat groat can lead to digestive disorders and an

imbalance between N availability and microbial synthesis in the rumen. In this case, processing methods can be used to reduce nutrient degradation in the rumen and allow higher amounts to bypass to the small intestine.

To determine the nutritive value of a feed and have an idea of how a certain feed ingredient will behave in the animal's digestive tract, several methods and techniques can be applied. The conventional methods of testing feeds can give information about the nutritive composition of a feed but will not detect the molecular structure characteristics that can be related to nutritive value and nutrient utilization. To understand the intrinsic molecular structure of oat grain, the vibrational FTIR molecular spectroscopy method can be applied.

2.6.2 Project hypothesis

In general:

- Different varieties of oat and feed processing treatments will impact the structural, physicochemical, and nutritional characterization of oat grain grown in western Canada.

In details:

- The developed CDC oat varieties (CDC Nasser, CDC Arborg, and CDC Ruffian) will possess distinct molecular structure and nutritional features compared to other oat and barley grain that are already in use in Canada.

- The developed CDC oat varieties (CDC Nasser, CDC Arborg, and CDC Ruffian) will have higher feeding value and increased digestibility compared to other oat and barley grain that are already in use in Canada.

- The processing of CDC oat grain will increase nutrient availability and thus milk production and milk protein or fat yield compared to other oat and barley grain that are already in use in Canada.

2.6.3 Project objectives

Long-term:

- To increase the economic return to dairy farmers and oat producers by exposing the best varieties of oat to be used as animal feed.

- To help create new low-cost feeding strategies to introduce to highly productive dairy cow's systems.

- To improve milk quality and milk yield without degrading cow's health effects through optimal processing and varieties selection.

Short-term:

- To characterize the physicochemical, molecular structure, study energy values, and describe nutrient composition using conventional research techniques for ruminant nutrition assessment: Compare CDC developed varieties of oat (Feed type vs. Milling type vs. Barley as a control) and compare the effects of different processing methods in oat grains (Raw vs. Flaking vs. Pelleting).

- To study ruminal and intestinal degradation and utilization: Compare CDC varieties of oat (Feed type vs. Milling type vs. Barley as a control) and compare different processing methods in oat grains (Raw vs. Flaking vs. Pelleting).

- To study the changes induced by the feed processing techniques in nutrient availability and ruminal digestibility in dairy cows: Compare CDC varieties of oat (Feed type vs. Milling type vs. Barley as a control) and compare different processing methods in oat grains (Raw vs. Flaking vs. Pelleting).

- To identify the difference in feed milk value, nutrients availability, and intestinal digestibility: Compare CDC varieties of oat (Feed type vs. Milling type vs. Barley as a

control) and compare different processing methods in oat grains (Raw vs. Flaking vs. Pelleting).

- Develop effective feeding strategies to implement CDC varieties of oat into high lactation dairy cow's diet and compare different processing methods on milk production (Raw vs. Flaking vs. Pelleting).

3. IMPACT OF VARIETY AND GRAIN TYPE ON PHYSIOCHEMICAL, NUTRITIONAL, MOLECULAR STRUCTURAL CHARACTERIZATION AND DAIRY COW FEEDING VALUE OF OAT GRAIN IN COMPARISON WITH BARLEY GRAIN

3.1 Abstract

The high degradation ratio of protein and starch together with higher prices for barley grains are the leading factor in the search for an alternative grain as a concentrate for dairy cattle's diet. Recently, new varieties of oat were produced for the feed and milling industry, but few studies have been conducted to evaluate what type of grain or variety could be potentially used in dairy cattle nutrition. The main objective of this study was to determine the nutritional and digestive characteristics and the protein related molecular spectral profiles of three different CDC varieties of oat grain [CDC Nasser (feed-type), CDC Arborg (milling-type) and CDC Ruffian (milling-type)] in comparison with CDC barley grain [CDC Austenson (feed-type)]. The results showed that Nasser had higher ($P<0.01$) EE compared to the other varieties. CP content was higher ($P<0.01$) for oat Arborg and Ruffian (15.78 and 14.34%DM), respectively, while starch and NFC content was higher ($P<0.01$) for Austenson barley (58.12 and 65.88%DM), respectively. NDF and ADF were higher ($P<0.01$) for oat in comparison to barley grain, but Nasser oat and Austenson barley had similar ($P>0.05$) content of ADL (1.55 and 0.80%DM), respectively, and uNDF (4.48 and 5.06%DM), respectively. NE_L was similar for Austenson barley and Nasser oat (2.01 and 2.06 Mcal/kg), respectively. Nasser oat and Austenson barley also presented similarly lower values ($P<0.01$) for indigestible fiber (fraction CC) and total ruminally undegraded carbohydrate (TRUCHO) compared to the other oat varieties. Oat presented higher ($P<0.01$) values of effective degraded DM, OM, CP, and starch when taken in a percentage basis (%) when compared to Austenson barley grain. Barley also

had a higher (P<0.01) bypass fraction for CP and starch (25.44 and 14.20%), respectively. Nasser and Ruffian oat maintained the effective degradation ratio between available N and available OM close to the optimal level, 25 g of N/kg of OM, while Austenson barley showed low values for the first 4 hours of degradation. The microbial protein synthesized in the rumen based on available energy (MREE, DVE/OEB system) and the microbial protein synthesis based on available protein (MCP$_{RDP}$, NRC model) were higher (P<0.01) for Austenson barley (105.59 and 198.64 g/kg of DM), respectively. Feed milk value (FMV) was also higher (P<0.01) for Austenson barley, evaluated by both NRC and DVE models. Univariate molecular analysis and principal component analysis (PCA) did not distinguish between Nasser and Ruffian oat in the whole amide spectra.

3.2 Introduction

Barley grain is among the main choices to feed livestock in western Canada, especially for dairy and beef cattle (Yu et al., 2010). However, barley grain has a high indigestible hull content and high rate of ruminal degradation of protein and starch which can cause digestive disorders and an imbalance between protein breakdown and microbial protein synthesis (Yu, et al., 2003). This results in unnecessary loss of nitrogen to the environment and decreased digestibility (Morgan and Campling, 1978). This metabolic imbalance, together with the high price volatility of barley in the market, can lead to serious economic impact on dairy farmers and production losses and has been the driving factor to seek alternatives to barley.

Canada is the third biggest oat producer in the world, with an estimated production of 3.7 million metric tons in 2017 according to Statistics Canada, with western Canada being the most important producer. This makes oat an important candidate to replace barley as an energy source for cattle. Although availability and price are attractive for feeding oat grain to cattle, this cereal tends to have a more fibrous hull and contains substantial amounts of indigestible

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lignin (Arya, 2010), which can decrease metabolizable energy content (Fuhr, 2006). Recently, new varieties of oat produced by the Crop Development Center (CDC), University of Saskatchewan, Canada, have shown promise for usage in dairy farms. CDC Nasser was designed to produce a low-lignin hull with a high-fat content grain (similar to CDC SO-I); CDC Arborg is a milling variety with high-yield and high beta-glucan content; and, CDC Ruffian is a high-yield variety with a defensive stance against crown rust.

Previous studies suggested that partial or total replacement of barley by oat in dairy rations can increase milk yield but can possibly lead to a decrease milk fat yield (Ekern et al., 2003; Yu et al., 2010). However to increase the utilization of glucose present in oat for milk production, it is important to have some protein and starch escaping rumen fermentation and being digested in the small intestine (Noftsger and St-Pierre, 2003; Rigout et al., 2002). However, very limited research has been conducted to determine what variety of oat, milling or feed type, and what variety would best replace barley grain, in order to increase the feed milk value for high producing dairy cows. Therefore, the present study was conducted to test new varieties of oat grain in comparison with barley grain for dairy cattle in terms of chemical composition, energy value, protein partitioning, rumen degradation kinetics, intestinal digestion, potential N to energy synchronization, and molecular structure.

3.3 Material and Methods

3.3.1 Grains Sampling

Three varieties of oat grain, CDC Nasser, CDC Arborg, and CDC Ruffian, and one variety of barley, CDC Austenson, were obtained from different plots by the Crop Development Center (CDC), University of Saskatchewan, Canada. CDC Nasser is a feed type of oat that has on average 27% hull; CDC Arborg and Ruffian are milling types, and both have on average

25% hull. Each variety had three representative samples and CDC Austenson barley had three representative samples (n=12).

3.3.2 Chemical Analysis

The samples were ground through a 1 mm screen (RetschZM200, Retsch Inc., PA, USA) and subsequently analyzed for DM (AOAC official method 930.15), OM, ether extract (EE, AOAC official method 920.39), Ash (AOAC official method 942.05), CP (AOAC official method 984.13) and sugars (AOAC official method 974.06). The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed according to Van Soest et al. (1991) using the filter bag technique from ANKOM Technology. The neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were analyzed according to the procedures described by Licitra et al. (1996). The SCP was determined according to Roe et al. (1990) by incubating samples in borate-phosphate buffer and filtrating it through Whatman filter paper (#54). Starch was analyzed using a Megazyme Total Starch Kit (Megazyme International Ltd., Wicklow, Ireland) Total carbohydrate and non-fiber carbohydrate were determined according to NRC (2001): $CHO = 100 - EE - CP - Ash$, and $NFC = 100 - (NDF - NDICP) - EE - CP - Ash$.

3.3.3 Energy Values

Energy values were determined using the summative approach of the NRC (2001) dairy and NRC (1996) beef. The digestible energy at a production level of intake (DE_{3x}), metabolizable energy at a production level of intake (ME_{3x}), net energy for lactation at a production level of intake (NE_{L3x}), as well as values for truly digestible CP (tdCP), truly digestible NDF (tdNDF), truly digestible NFC (tdNFC), and truly digestible fatty acids (tdFA)

were determined according to NRC-2001. The values of net energy for maintenance (NE_m) and net energy for growth (NE_g) were estimated according to NRC-1996 beef.

3.3.4 Protein and Carbohydrate Profile

The Cornell Net Carbohydrate and Protein System (CNCPS) version 6.5 was used to partition the carbohydrate and protein sub-fractions. Fractions were subdivided considering the rate and extent of degradation in the rumen. Protein was fractioned into PA2= soluble true protein with a Kd ranging from 10 to 40%/h; PB1= insoluble true protein with a Kd of 3-20%/h; PB2= fiber-bound protein with a Kd ranging from 1-18%/h and PC= indigestible protein. The carbohydrates were subdivided into CA4= water-soluble carbohydrates and has a Kd of 40-60%/h; CB1= starch that has a Kd of 20-40%/h; CB2= soluble fiber with a Kd ranging from 20 to 40%/h; CB3= digestible fiber with a Kd of 1-18%/h and CC= indigestible fiber (Higgs et al., 2015; Van Amburgh et al., 2015).

3.3.5 Rumen Incubation Procedures

The University of Saskatchewan Animal Care Committee approved the animal trial under the Animal Use Protocol No. 19910012 and animals were cared for and handled in accordance with the Canadian Council of Animal Care (CCAC, 1993) regulations. The samples used for the in situ animal incubation trial were ground using the Telemecanique Roller Mill (Emerson, Poland) with a roller gap of 0.508 mm at the Canadian Feed Research Centre (CFRC, University of Saskatchewan) located in North Battleford, SK, Canada.

The in situ experiment was carried out in the Rayner Dairy Research and Teaching Facility, University of Saskatchewan, Canada. For the incubation, four Holstein cows fitted with an 88 mm rumen cannula were used. Cows were housed in individual tie stalls with free

access to water and fed a TMR composed of barley silage, alfalfa hay, and lactating pellet twice a day.

The incubation procedure followed a 'gradual addition/all out' schedule according to the protocol by Damiran and Yu (2012). Nylon bags with a 40 μm pore size were used to incubate approximately 7 g per sample per bag for 0, 2, 4, 8, 12 and 24 h with multi-bags (2, 2, 2, 2, 3, 4) for each treatment and incubation time. The incubation procedure was performed for two experimental runs using the same four cannulated cows. After incubation was completed, bags were removed and washed in cold water for six times, to wash out all the rumen fluid, and subsequently dried at 55°C for 48h in a forced-air oven. Samples taken out of the oven were exposed to room temperature and moisture before being weighed and composite by incubation time point and treatment. Pooled samples were then ground through 1 mm screen and analyzed for CP using LECO protein analyzer (Model FP-528, Leco Corp., St. Joseph, MI, USA), DM and OM according to AOAC (2005), and starch was analyzed using a Megazyme total starch kit (Megazyme International Ltd.).

3.3.6 Rumen Degradation Kinetics

Degradation characteristics of DM, OM, CP, and starch were determined following the first-order kinetics degradation model described by Ørskov and McDonald (1979) and modified by Tamminga et al. (1994). The results of rumen degradation kinetics were analyzed using NLIN procedure of SAS (Statistical Analysis System,) version 9.4 with iterative least-square regression (Gausse Newton method).

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

where R(t) was the residue present after t hours of incubation; U was the undegradable fraction (%); D was the potentially degradable fraction (%); Kd was the degradation rate (h^{-1}); and T0 is the lag time.

The percentage of bypass (B) values of nutrients were calculated according to NRC Dairy (2001):

$$\%BDM,\ BOM,\ BCP\ (or\ RUP) = U + D \times Kp/(Kp + Kd)$$

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

where, S=soluble fraction (%); Kp=estimated passage rate from the rumen (h^{-1}) and was assumed to be 6%/h for DM, OM, CP and Starch (Tamminga et al., 1994). The rumen undegradable or bypass DM, OM and starch, in g/kg DM, were calculated as:

$$BDM,\ BOM\ or\ BSt\ (g/kg\ DM) = DM\ (OM\ or\ St)\ (g/kg\ DM) \times \%\ BOM\ (BOM\ or\ BSt),$$

while the rumen bypass CP (BCP) and rumen undegraded CP (RUP) were calculated differently according to the DVE or NRC model:

$$BCP\ DVE\ (g/kg\ DM) = 1.11 \times CP\ (g/kg\ DM) \times \%BCP$$

$$RUP\ NRC\ (g/kg\ DM) = CP\ (g/kg\ DM) \times \%RUP$$

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

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

$$EDDM\ (EDOM,\ EDCP\ or\ EDSt)\ (g/kg\ DM) = DM\ (OM,\ CP\ or\ St)\ (g/kg\ DM) \times \%EDDM$$
$$(EDOM,\ EDCP\ or\ EDSt).$$

3.3.7 Hourly Effective Rumen Degradation Ratio and Potential N to Energy Synchronization

The effective degradation of available N and available OM were calculated according to Sinclair et al. (1993):

$$Hourly\ ED\ (g/kg\ DM) = S + [(D \times Kd)\ /\ (Kp + Kd)] \times 1 - e^{-t \times (Kd + Kp)}$$

The difference in cumulative amounts degraded among successive hours was used to calculate the hourly effective degradation ratio between N and OM (ED_N/ED_OM) following the equation described by Nuez-Ortín and Yu (2010):

$$\text{Hourly ED N/OM}_t = (HEDN_t - HEDN_{t-1}) / (HEDOM_t - HEDOM_{t-1}),$$

where, hourly ED_N/ED_OM was the ratio of N to OM at the time t (gN/kgOM); $HEDN_t$ was the hourly ED of N at the time t (g/kg DM); $HEDN_{t-1}$ was the hourly ED of N 1h before the time t (g/kg DM); $HEDOM_t$ was the hourly ED of OM at the time t (g/kg DM); $HEDOM_{t-1}$ was the hourly ED of OM 1 h before the time t (g/kg DM).

3.3.8 Intestinal Digestion

The intestinal digestion of CP was determined using the three-steps in vitro protocol by Calsamiglia and Stern (1995). Briefly, residues taken out after 12 hours ruminal incubation and containing approximately 15 mg of N were placed in a 50 ml centrifuge tube with 10 ml of pepsin (Sigma P-7000) solution (0.1 N HCl with pH 1.9) and incubated for 1 h at 38°C. After incubation, 0.5 ml of 1 N NaOH solution and 13.5 ml of pancreatin (Sigma P-7545) were added and the solution was incubated for 24 h at 38°C. After the incubation, 3 ml of TCA was used to stop hydrolysis and then centrifuged at 1000g for 15 min and the supernatant was analyzed for soluble N by the Kjeldahl method. Intestinal digestion of protein was calculated as TCA soluble N divided by N present after ruminal incubation.

3.3.9 Nutrient Supply and Feed Milk Value

The DVE/OEB system and the NRC model were used to estimate the nutrient supply and feed milk value. In the Dutch system, the DVE represents the value of a feed protein and it was calculated as:

$$DVE = DVME + DVBE - ENDP,$$

where DVME was the microbial true protein synthesized in the rumen and digested in the small intestine, DVBE was the feed crude protein undegraded in the rumen but digested in the small intestine and ENDP was the endogenous protein lost in the digestive process.

The OEB value was calculated as:

$$OEB = MREN - MREE,$$

where, OEB was the difference between the potential microbial protein synthesis based on available N (MREN) and the potential microbial protein synthesis based on energy extracted from anaerobic fermentation (MREE) (Tamminga et al., 1994).

In the NRC 2001 model, the total metabolizable protein (MP) is constituted by the rumen undegraded feed crude protein (RUP), ruminally synthesized microbial crude protein (MCP) and the rumen endogenous crude protein (ECP), and so MP in this study was calculated as:

$$MP \ (g/kg \ of \ DM) = ARUP + AMCP + AECP,$$

where ARUP was the truly absorbable rumen undegraded CP, AMCP was the truly absorbable ruminal synthesized microbial CP and AECP was the truly absorbable endogenous CP.

The degraded protein balance (DPB) reflects the difference between the potential microbial protein synthesis based on the rumen degradable protein (RDP) and the potential microbial protein synthesis based on energy (TDN) available for microbial fermentation in the rumen. The DPB was calculated as:

$$DPB \ (g/kg \ of \ DM) = RDP - 1.18 \ x \ MCP^{TDN},$$

where RDP was the rumen degradable protein and MCP^{TDN} was the microbial protein synthesis (discounted TDN). Feed milk value was calculated based on metabolizable protein (MP).

3.3.10 Protein Molecular Structures Analysis

Samples were ground through a 0.12 mm screen and subsequently analyzed using a JASCO FTIR-ATR-4200 spectrometer (JASCO Corp., Tokyo, Japan). Right before samples were submitted to spectra collection, the background spectrum was measured with 256 scans to correct the spectra for CO_2 noise. Spectras were collected at the mid-IR region (approximately 4000–700 cm^{-1}) with a spectra resolution of 4 cm^{-1} and using 128 co-added scans (SpectraManager II software, JASCO Corp., Tokyo, Japan). Each sample had five spectra collected as sub-sample replicates.

For univariate molecular spectral analysis, the collected spectrum data related to the protein structure was preprocessed using OMINIC 7.3 software (Spectra Tech, Madison, WI, USA). Each spectrum was normalized, and a second derivative was generated and smoothed, prior to the calculation of peak heights and areas. The primary protein structure, amide I region (at ca. 1720-1577 cm^{-1}) and amide II (at ca. 1577-1486 cm^{-1}), as well as the secondary structures, α-helix (at ca. 1650cm^{-1}) and β-sheets (at ca.1626cm^{-1}) were measured for height and area, and their ratios between Amide I to II and α-helix to β-sheet were determined.

The multivariate molecular spectral analysis was performed to distinguish the inherent structure differences in the whole protein structure among the grains. The whole protein related structures (Amide I and Amide II) were analyzed using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCLA) using Ward's algorithm method. Multivariate spectra analysis was performed using the Unscrambler software v. 10.3 (Camo Software, Norway).

3.3.11 Statistical Analysis

Results were analyzed using the Mixed model procedure in SAS 9.4 (SAS Institute Inc., NC, USA). The detailed chemical profile, protein and carbohydrate subfractions, energy values and protein spectral profile were analyzed according to the model:

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

where Y_{ij} was the observation of the dependent variable ij, μ was the fixed effect of the population mean, T_i was the fixed effect of grains (Nasser, Arborg, Ruffian, and Austenson), and e_{ij} was the random error associated with the observation ij.

The studies of rumen degradation kinetics, hourly effective degradation ratio, nutrient supply and intestinal digestion of rumen undegraded nutrients were conducted and analyzed as randomized complete block design (RCBD) with the experimental run used as a random block, and analyzed with the Mixed model procedure in SAS 9.4, using the model:

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

where Y_{ijk} was the observation of the dependent variable ijk, μ was the population mean, T_i was the effect of grains (Nasser, Arborg, Ruffian, and Austenson) as fixed effect, S_k was the random effect of in situ incubation run and e_{ijk} was the random error associated with the observation ijk.

Prior to the statistical analysis, all outlier data were removed, using the same model, with a criterion of Studentized Residual greater than 2.5. For all statistical analyses, significance was declared at $P<0.05$ and trends at $0.05<P<0.10$. The differences among the treatments were compared using a multiple comparison test following the Tukey method. Contrast statement was used to compare the difference between barley grain and oat grain. The model assumptions of CRD and RCBD were checked using residual analysis. The normality tests were carried out using Proc Univariate with normal plot options.

3.4 Results and Discussion

3.4.1 Chemical Profile

The nutrient profile of the three CDC oat varieties in comparison with barley are shown in Table 3.1. Barley grain had a lower ($P<0.01$) DM when compared to CDC Nasser and CDC Arborg. The concentration of EE was higher ($P<0.01$) for Nasser, followed by Ruffian and

Arborg (6.66, 4.88 and 4.16 % DM), respectively. CDC Nasser is a feed type oat that was bred to contain a high oil (EE) groat and low lignin hull, which might explain the higher EE concentration for this oat variety when compared to Arborg and Ruffian, which are two varieties of milling oat. Barley Austenson had the lowest concentration of fat and ash, with only 1.90 and 2.24% DM, respectively. The higher values of ash for oat grain reduced the OM content of those varieties and made barley Austenson possess higher ($P<0.01$) OM when compared to the other varieties. Niu et al. (2007) reported values of EE, OM, and ash for three varieties of oat grown in Canada, that were similar to the ones found in this study.

In the present study, the crude protein content for oat ranged between 13.8-15.7% of DM, with the higher values being seen in the milling varieties of oat (CDC Arborg and Ruffian). Results for soluble crude protein (SCP) had a higher concentration for Arborg oat and Austenson barley, and barley grain had higher values when contrasted with oat grain (9.04 vs 8.90% DM), respectively. The CP content for oat and barley in this study were similar to the values found by Prates and Yu (2017), although the SCP content was higher in this study. The ADICP and NDICP did not differ among varieties ($P=0.18$ and $P=0.28$), respectively, or between grains ($P=0.55$ and $P=0.32$), respectively.

The groat and hull contribute to different nutritive aspects of whole cereal grains. The hull contains more structural carbohydrates that are low in digestibility, while the groat contains more non-structural carbohydrates, such as starch and sugar. In this case, the proportion of hull in the grain can be related to grain quality (Fuhr, 2006). In barley grain, the proportion of hull falls between 11.1 to 12.9% (Harris, 1949), while oat can have more than 25% hull content (Crosbie et al., 1985). The concentration of carbohydrates in Austenson barley was significantly higher ($P<0.01$) than the three oat varieties. The concentration of aNDF was higher ($P<0.01$) for the three oat varieties when compared to Austenson barley. Acid detergent fiber (ADF) was also higher ($P<0.01$) for the three varieties of oat and for oat grain compared to barley grain

(P<0.01; 12.52 and 5.59% DM), respectively. These higher values of fiber might have arisen from the higher concentration of hull in oat (25-27% in this study). However, the content of lignin (ADL) in CDC Nasser did not differ from that in CDC Austenson (1.55 and 0.80 % DM), respectively. The ungradable fiber (uNDF) was similar for CDC Nasser and Austenson barley (4.48 and 5.06% DM), respectively.

Table 3.1. Chemical profile of different varieties of CDC oat grain in comparison with CDC barley grain.

| Items | Oat varieties (O) | | | Barley variety (B) | SEM | P-value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)			B vs. O
Basic chemical profile							
DM (%)	92.71[a]	92.22[ab]	92.68[a]	91.71[b]	0.166	<0.01	0.04
OM (%DM)	96.92[b]	96.95[b]	97.09[b]	97.76[a]	0.057	<0.01	0.11
Ash (%DM)	3.08[a]	3.05[a]	2.91[a]	2.24[b]	0.057	<0.01	0.11
EE (%DM)	6.66[a]	4.16[c]	4.88[b]	1.90[d]	0.054	<0.01	<0.01
Protein profile							
CP (%DM)	13.82[b]	15.78[a]	14.34[ab]	13.54[b]	0.322	<0.01	0.91
SCP (%DM)	8.59[b]	9.65[a]	8.47[b]	9.04[ab]	0.172	<0.01	0.01
SCP (%CP)	62.20[ab]	61.24[ab]	59.06[b]	66.72[a]	1.55	0.04	0.01
NDICP (%DM)	1.14	1.26	1.01	2.14	0.417	0.28	0.32
NDICP (%CP)	8.24	7.95	7.06	15.82	3.07	0.23	0.33
ADICP (%DM)	0.33	0.51	0.24	0.09	0.130	0.23	0.67
ADICP (%CP)	2.34	3.25	1.69	0.65	0.915	0.30	0.72

Table 3.1. *Cont'd* Chemical profile of different varieties of oat grain in comparison with barley grain.

| Items | Oat varieties (O) | | | Barley variety (B) | SEM | P-value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)			B vs. O
Carbohydrate profile							
CHO (%DM)	76.92[b]	77.33[b]	78.22[b]	82.47[a]	0.297	<0.01	0.08
Starch (%DM)	47.89[b]	47.54[b]	50.52[b]	58.12[a]	0.938	<0.01	0.56
Sugar (%DM)	1.62[b]	1.93[b]	1.92[b]	2.65[a]	0.087	<0.01	0.19
Sugar (%NFC)	2.71[b]	3.37[ab]	3.24[ab]	3.99[a]	0.176	<0.01	0.57
NFC (%DM)	54.69[b]	53.58[b]	55.49[b]	65.88[a]	0.787	<0.01	0.02
NSC (%DM)	49.51[b]	49.46[b]	52.44[b]	60.77[a]	0.994	<0.01	0.50
Fiber profile							
aNDF (%DM)	21.74[a]	23.43[a]	22.38[a]	16.66[b]	0.898	<0.01	0.13
ADF (%DM)	11.64[a]	13.19[a]	12.73[a]	5.59[b]	0.530	<0.01	<0.01
ADL (%DM)	1.55[b]	3.07[a]	3.27[a]	0.80[b]	0.236	<0.01	<0.01
ADL (%NDF)	7.11[ab]	13.10[a]	14.55[a]	4.95[b]	1.472	<0.01	<0.01
uNDF (%DM)	4.48[c]	13.80[b]	15.86[a]	5.06[c]	0.329	<0.01	<0.01
uNDF (%NDF)	20.66[d]	58.91[b]	70.96[a]	30.99[c]	1.341	<0.01	<0.01

SEM: standard error of mean; [a-d] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; DM: dry matter; OM: organic matter; EE: ether extract (crude fat); CP: crude protein; SCP: soluble crude protein; ADICP: acid detergent insoluble crude protein; NDICP: neutral detergent insoluble crude protein; CHO: carbohydrates; aNDF: neutral detergent fiber analyzed with amylase; ADF: acid detergent fiber; ADL: acid detergent lignin; uNDF: undigestible neutral detergent fiber analyzed after 288h in situ incubation; NFC: non-fiber carbohydrate; NSC: non-soluble carbohydrate

3.4.2 Energy Profile

Results for truly digestible nutrients and energy values for the three varieties of oat grain compared to barley grain are shown in Table 3.2. The major storage component of energy in grains is starch, which will be fermented by rumen microbes producing volatile fatty acids (Ørskov, 1986). The content of starch found in barley (58% DM) was lower than the amount typically found in corn, which ranges from 71 to 78% DM (Herrera-Saldana et al., 1990), but higher than in oat grain. The content of truly digestible NDF (tdNDF) was the highest (P=0.02) for CDC Nasser, however, the value was not significantly different from the other two varieties of oat. The highest value of tdCP was obtained for CDC Arborg (15.58% DM), which was similar to CDC Ruffian (14.24% DM). The higher values of EE, ash, and NDF obtained for the oat varieties, decreased the truly digestible non-fiber carbohydrate (tdNFC) value for these grains (P<0.01), resulting in Austenson barley having a greater value (69.27% DM). CDC Nasser oat had significantly (P<0.01) higher tdFA when compared to all the other treatments, and oat grain had higher (P<0.01) values than barley grain (4.23 vs 0.90% DM), respectively. Total digestible nutrients (TDN_{1x}) was higher (P<0.01) for Nasser oat and Austenson barley (88.88 and 87.71% DM).

The nutrient requirements of dairy cattle (NRC, 2001) report a higher energy value for barley grain in comparison to oat grain. In this study, CDC Nasser, which was bred to possess a higher oil content and lower lignin hull, was expected to have a higher energy value compared to other varieties of oat. Nasser oat showed similar values of digestible energy (DE), metabolizable energy (ME), net energy for maintenance (NE_m), net energy for gain (NE_g) and net energy for lactation (NE_L) to Austenson barley grain. CDC Arborg, on the other hand, showed the lowest values for DE, ME and NE_L, being 0.22, 0.21 and 0.17 Mcal/kg of DM lower than Nasser. Similar values of predicted energy for oat grain were reported by Niu et al. (2007). Damiran and Yu (2010), studying a high oil groat, low lignin hull (SO-I), in comparison to two

milling varieties of oat, reported similar high energy for the high oil oat variety (SO-I). The results of DE_{1x}, ME_{3x} and NE_{L3x} found in this study were all higher than the values reported by the NRC (2001), but the difference between barley and oat grain values followed the same pattern as described by the document. Despite the statistical advantage of barley grain, it can be observed that CDC Nasser (feed-type of oat) showed overall higher energy values compared to the milling types of oat. This can be due to the lower content of lignin or the higher fat content of this variety.

Table 3.2. Energy values of different varieties of CDC oat grain in comparison with CDC barley grain.

| Items | Oat varieties (O) | | | Barley variety (B) | SEM | P-value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)			B vs. O
Truly digestible nutrients (%DM)							
tdNDF	12.55^a	11.34^{ab}	10.38^{ab}	9.90^b	0.490	0.02	0.16
tdCP	13.69^b	15.58^a	14.24^{ab}	13.50^b	0.328	<0.01	0.97
tdNFC	56.90^b	55.89^b	57.59^b	69.27^a	0.748	<0.01	<0.01
tdFA	5.66^a	3.16^c	3.88^b	0.90^d	0.054	<0.01	<0.01
Total digestible nutrients (%DM)							
TDN$_{1x}$	88.88^a	82.92^b	83.95^b	87.71^a	0.531	<0.01	<0.01
Predicted energy value (Mcal/kg of DM)							
DE$_{1x}$	3.91^a	3.69^b	3.72^b	3.87^a	0.024	<0.01	<0.01
DE$_{p3x}$	3.60^a	3.39^b	3.41^b	3.55^a	0.022	<0.01	<0.01
ME$_{p3x}$	3.20^a	2.98^b	3.00^b	3.14^a	0.022	<0.01	<0.01
NE$_{Lp3x}$	2.08^a	1.91^b	1.93^b	2.01^a	0.016	<0.01	<0.01
ME	3.21^a	3.03^b	3.05^b	3.17^a	0.019	<0.01	<0.01
NE$_m$	2.20^a	2.05^b	2.07^b	2.17^a	0.016	<0.01	<0.01
NE$_g$	1.52^a	1.39^b	1.41^b	1.49^a	0.014	<0.01	<0.01

SEM: standard error of mean; $^{a\text{-}c}$ Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison using Tukey method; tdNDF: truly digestible neutral detergent fibre; tdCP: truly digestible crude protein; tdNFC: truly digestible non-fibre carbohydrate; tdFA: truly digestible fatty acids; TDN$_{1x}$: total digestible nutrient at one time maintenance. DE$_{13x}$: digestible energy at production level of intake (3×); ME$_{3x}$: metabolizable energy at production level of intake (3×); NE$_{L3x}$: net energy for lactation at production level of intake (3×); ME: metabolizable energy; NE$_m$: net energy for maintenance; NE$_g$: net energy for growth.

3.4.3 Protein and Carbohydrates Subfractions

The Cornell Net Protein and Carbohydrate System (CNCPS) version 6.5 was used to partition the protein and carbohydrates subfractions. The sub-fractions of protein and carbohydrate, as well as the total rumen degradable and undegradable CP and CHO, are represented in Table 3.3. Rapidly degradable fraction (PA2) was higher (P=0.04) for CDC Austenson barley when compared to CDC Ruffian oat (66.72 and 59.06%CP), respectively. Compared to oat grain, barley CDC Austenson showed significantly lower (P<0.01) value of PB1 and higher (P<0.01) values of the slowly degradable fraction (PB2). The unavailable crude protein (PC) ranged from 3.25 to 0.65% CP, but there was no significative difference between treatments (P>0.30) between varieties or grains. Total rumen degradable protein was significantly higher (P<0.01) for CDC Arborg when compared to CDC Nasser and Austenson. These findings are in contrast with previous studies (Prates and Yu, 2017), when comparing two varieties of oat, CDC Nasser and CDC Seabiscuit, with CDC Meredith barley grain, which found only significant difference when comparing unavailable protein fraction. Niu et al. (2007) showed differences between milling and feed type of oat when comparing intermediate degraded protein fractions. In terms of protein fraction, barley contained higher PA2 (P=0.04; +5.88% CP), lower PB1 (P<0.01; -13.62% CP) and higher PB2 (P<0.01; +9.51% CP), when compared to oat grain.

The carbohydrate fraction CA4, which corresponds to sugar, was higher (P<0.01) for CDC Austenson barley when compared to the other treatments. The starch corresponding fraction CB1 ranged from 61.73 to 70.61% CHO, with higher values (P<0.01) being found for barley Austenson. The higher value of CA4 and CB1 for barley Austenson may be explained by the higher values of total carbohydrates (CHO), sugar and starch found in this grain (82.47, 2.65 and 58.12% of DM), respectively. The soluble fiber fraction did not differ among varieties (P=0.77). The fraction CB3 was higher (P<0.01) for CDC Nasser oat when compared to CDC

Ruffian and CDC Austenson barley (23.33, 18.21 and 16.84% CHO), respectively. Despite the total rumen degradable carbohydrate (TRDCHO) being higher (P<0.01) for barley Austenson, the CC fraction and total rumen undegradable carbohydrate (TRUCHO) were similar for CDC Nasser oat and barley Austenson. The hull of a cereal grain is the structure which presents the highest concentration of structural carbohydrates, and oat grain, in general, has a higher proportion of hull to whole grain weight. The lower content of lignin presented by CDC Nasser, when compared to other varieties of oat grain, could be related to the lower indigestible fraction of carbohydrates showed, even though CDC Nasser has an average proportion of hull slightly higher than the other oat grain in this study. Prates et al. (2018) found a similar pattern in their study, with CB1 being significantly lower for oat when compared to barley (442 and 573 g/kg DM, respectively; P<0.01), although the CC fraction was significantly higher for CDC Nasser oat when compared to barley Meredith (82.5 and 36.8 g/kg DM, respectively; P<0.01).

Table 3.3. Protein and carbohydrate sub-fraction, degradable and undegradable fractions of different varieties of CDC oat grain in comparison to CDC barley grain determined according to CNCPS 6.5.

| Items | Oat varieties (O) | | | Barley variety (B) | SEM | P-value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)			B vs. O
Protein sub-fraction (%CP)							
PA2	62.20[ab]	61.24[ab]	59.06[b]	66.72[a]	1.555	0.04	0.04
PB1	29.56[a]	30.82[a]	33.87[a]	17.80[b]	1.569	<0.01	<0.01
PB2	5.90[b]	5.36[b]	4.70[b]	14.83[a]	1.072	<0.01	<0.01
PC	2.34	3.25	1.69	0.65	0.915	0.30	0.60
Carbohydrate sub-fraction (%CHO)							
CA4	2.11[b]	2.49[b]	2.46[b]	3.21[a]	0.117	<0.01	0.32
CB1	62.67[b]	61.73[b]	64.87[ab]	70.61[a]	1.330	<0.01	0.92
CB2	6.75	5.35	3.92	6.19	1.995	0.77	0.38
CB3	23.33[a]	20.35[ab]	18.21[b]	16.84[b]	0.900	<0.01	0.09
CC	5.12[b]	10.06[a]	10.52[a]	3.14[b]	0.687	<0.01	0.05
Rumen degradable fractions (%DM)							
TRDP	10.35[b]	11.73[a]	10.72[ab]	10.13[b]	0.228	<0.01	0.90
TRDCHO	53.40[b]	51.58[c]	52.56[bc]	58.73[a]	0.399	<0.01	<0.01
Rumen undegradable fractions (%DM)							
TRUP	3.47[ab]	4.05[a]	3.61[ab]	3.41[b]	0.134	0.04	0.93
TRUCHO	25.16[b]	27.59[a]	27.59[a]	24.18[b]	0.453	<0.01	<0.01

SEM: standard error of mean. [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; PA2: soluble true protein; PB1: insoluble true protein. PB2: fiber-bound protein; PC: indigestible protein; CA4: sugars; CB1: starches; CB2: soluble fiber; CB3: digestible fiber; CC: indigestible fiber; TRDP: Total rumen degradable protein; TRDCHO: Total rumen degradable carbohydrate; TRUP: Total ruminally undegraded protein; TRUCHO: Total ruminally undegraded carbohydrate.

3.4.4 Rumen Degradation kinetics

The degradation rate (Kd), rumen fractions (S, D, U), rumen bypass DM and effective degradability of DM for oat grain in comparison to barley grain are shown in Table 3.4. CDC Nasser oat had the lowest (P<0.01) soluble (S) and degradable (D) fraction, being significantly lower than CDC Ruffian (-10.03%) and Austenson, (-12.9%) respectively. The degradation rate ranged from 24.53 to 53.28% per hour and it was higher (P<0.01) for all varieties of oat grain. The contrast statement revealed higher (P<0.01) undegradable fraction (U) for oat grain, being 12.79% higher than barley grain. CDC Nasser, Arborg, and Ruffian presented higher (P<0.01) values of BDM (293.10, 303.83 and 280.08 g/kg DM) respectively, and significantly lower (P<0.01) effective degradation (707, 696 and 720 g/kg DM) respectively when compared to barley Austenson. The values for rumen bypass or undegradable DM and EDDM for oat grain were similar to those reported by Damiran and Yu (2010).

Rumen degradation kinetics for OM are presented in Table 3.5. Detailed observation showed that the degradation rate of OM followed the same pattern as the DM Kd, with the three varieties of oat having higher (P<0.01) Kd than barley Austenson. CDC Ruffian showed the highest soluble fraction (P<0.01) of OM (22.47%) and the lowest (P<0.01) degradable fraction (57.14%). Barley showed lower (P<0.01) undegradable fraction (U) compared to oat grain (9.01 vs. 21.48%) respectively. The effective degradability of OM was lower (P<0.01) for all three varieties of oat, with the lowest value for CDC Arborg (-50.48 g/kg DM) when compared to Austenson barley. CDC Ruffian oat and Austenson (273.82 and 250.35 g/kg DM) barley had lower (P<0.01) bypass OM (BOM), compared to Nasser and Arborg oat.

Results from the rumen degradation of CP are shown in Table 3.6. The degradation rate of CP was higher (P=0.01) for oat grain when compared to barley grain (49.44 vs. 17.36% per hour) respectively. Oat grain didn't show any indication of having a lag time (T0), while CDC Austenson barley grain showed approximately half an hour of delay (0.54 hours). Similar to the

results for DM and OM, CDC Ruffian showed the highest (P<0.01) value for the S fraction, but this for CP, the result was not significantly different from the other milling types of oat, CDC Arborg (24.96 and 18.74%) respectively. The degradable fraction was higher (P<0.01) for barley grain in comparison to oat grain (+18.12%). Consequently, the undegradable fraction (U) was lower (P<0.01) for barley grain than for oat grain (2.69 vs. 10.47%) respectively. The content of rumen undegradable CP based on the DVE/OEB system (BCP) showed that oat grain allowed 7.95 g/kg DM less (P<0.01) to be degraded in the small intestine when compared to barley (26.49 vs. 34.45 g/kg DM) respectively. CDC Ruffian showed the lowest (P<0.01) value of rumen undegraded crude protein based on the NRC 2001 model (RUP), followed by CDC Nasser, Arborg and finally Austenson barley (27.99, 29.29, 30.97 and 38.24 g/kg DM) respectively. Similar to this study, Yu et al. (2008) reported intermediate values of BCP and RUP for CDC SO-I, a high oil low lignin variety of oat, similar to the CDC Nasser in this study. The effective degradability of CP (EDCP) showed similar values (P<0.01) for CDC Nasser and Ruffian, while CDC Arborg presented the highest value (129.93 g/kg DM); Austenson barley showed the lowest effective degradability of CP, being 28.98 g/kg DM less than CDC Arborg (100.95 and 129.93 g/kg DM) respectively. Fuhr (2006) reported similar difference of EDCP between oat and barley grain, however, the values for oat grain were higher than the ones found in the present study.

The results for rumen degradation kinetics of starch is presented in Table 3.7. The three varieties of oat grain in this study showed high values (P=0.02) of Kd, however, CDC Arborg and Ruffian were not significantly different from Austenson barley grain. The degradable fraction (D) was lower (P=0.01) for CDC Ruffian when compared to the other varieties, but the contrast statement showed significantly lower (P<0.01) value for oat grain (74.79%), mostly likely due to the low value showed by CDC Ruffian. Nasser oat presented the highest value (P=0.01) for the undegradable fraction (2.76%) and Austenson barley showed the lowest value

(0.63%), with CDC Arborg and Ruffian presenting intermediate values. The rumen undegradable starch (BST) was higher (P<0.01) for CDC Austenson barley (82.59 g/kg DM) when compared to the other three varieties studied, but the contrast did not show a significant difference between barley and oat grain (P=0.26). The effective degradability of starch in the rumen (EDST) showed opposite significances when taken as g/kg of DM or as percentage. The effective degradability (g/kg DM) was lower (P<0.01) for the three varieties of grains when compared to Austenson barley, however, when analyzing the percentage of degradation, the three varieties of oat showed higher values (P<0.01) compared to barley. This difference is most likely caused by the higher content of starch in the barley grain (58.12% DM).

Table 3.4. In situ rumen degradation kinetics of dry matter (DM) of different varieties of CDC oat grain in comparison with CDC barley grain.

| Items | Oat varieties (O) | | | Barley variety (B) | SEM | P-value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)			B vs. O
In situ rumen degradation							
Kd (%/h)	47.42[a]	53.28[a]	41.97[a]	24.53[b]	3.086	<0.01	0.95
T0 (h)	0	0.13	0	0.09	0.070	0.46	0.36
S (%)	12.38[b]	11.38[b]	22.41[a]	12.07[b]	1.582	<0.01	<0.01
D (%)	65.72[b]	65.06[b]	56.73[c]	78.62[a]	1.640	<0.01	<0.01
U (%)	21.90[a]	23.55[a]	20.86[a]	9.31[b]	0.767	<0.01	<0.01
BDM (g/kg DM)	293.10[a]	303.83[a]	280.08[a]	253.10[b]	7.308	<0.01	<0.01
EDDM (g/kg DM)	706.91[b]	696.17[b]	719.92[b]	746.90[a]	7.308	<0.01	0.66

SEM: standard error of mean; [a-b] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; Kd: the degradation rate of D fraction (%h); T0: lag time; S: soluble fraction in the in-situ incubation; D: degradable fraction; U: rumen undegradable fraction; BDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter.

Table 3.5. In situ rumen degradation kinetics of organic matter (OM) of different varieties of CDC oat grain in comparison with CDC barley grain.

| Items | Oat varieties | | | Barley variety | SEM | P-value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)			B vs. O
In situ rumen degradation							
Kd (%/h)	49.12[a]	55.66[a]	43.34[a]	24.81[b]	3.177	<0.01	0.90
T0 (h)	0	0.13	0	0.09	0.070	0.47	0.37
S (%)	12.43[b]	11.45[b]	22.47[a]	11.65[b]	1.616	<0.01	<0.01
D (%)	66.39[b]	66.68[b]	57.14[c]	79.34[a]	1.664	<0.01	<0.01
U (%)	21.18[a]	22.88[a]	20.39[a]	9.01[b]	0.764	<0.01	<0.01
BOM (g/kg DM)	284.34[a]	296.16[a]	273.82[ab]	250.35[b]	7.236	<0.01	0.67
EDOM (g/kg DM)	693.61[b]	682.40[b]	705.07[b]	732.88[a]	7.035	<0.01	0.76
%BOM	28.43[a]	29.61[a]	27.38[ab]	25.04[b]	0.724	<0.01	0.67
%EDOM	71.56[b]	70.38[b]	72.62[ab]	74.96[a]	0.724	<0.01	0.67

SEM: standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; Kd: the degradation rate of D fraction (%h); T0: lag time; S: soluble fraction in the in-situ incubation; D: degradable fraction; U: rumen undegradable fraction; BOM: rumen bypass dry matter; EDOM: effective degradability of dry matter.

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Table 3.6. In situ rumen degradation kinetics of crude protein (CP) of different varieties of CDC oat grain in comparison with CDC barley grain.

| Items | Oat varieties (O) | | | Barley variety (B) | SEM | P-value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)			B vs. O
In situ rumen degradation							
Kd (%/h)	49.53[a]	50.81[a]	47.99[a]	17.36[b]	2.747	<0.01	0.01
T0 (h)	0[b]	0[b]	0[b]	0.54[a]	0.069	<0.01	0.03
S (%)	14.51[bc]	18.74[ab]	24.96[a]	9.06[c]	2.423	<0.01	<0.01
D (%)	74.53[b]	71.19[bc]	64.66[c]	88.25[a]	2.148	<0.01	<0.01
U (%)	10.95[a]	10.07[a]	10.38[a]	2.69[b]	0.571	<0.01	<0.01
BCP (g/kg DM, DVE)	26.38[bc]	27.90[b]	25.21[c]	34.45[a]	0.441	<0.01	<0.01
RUP (g/kg DM, NRC)	29.29[bc]	30.97[b]	27.99[c]	38.24[a]	0.490	<0.01	<0.01
EDCP (g/kg DM)	111.82[b]	129.93[a]	118.19[b]	100.95[c]	2.158	<0.01	0.13
%BCP=%RUP	19.12[b]	17.72[b]	17.59[b]	25.44[a]	0.431	<0.01	<0.01
%EDCP (=%RDP)	80.87[a]	82.28[a]	82.40[a]	74.56[b]	0.431	<0.01	<0.01

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

Table 3.7. In situ rumen degradation kinetics of starch (ST) of different varieties of CDC oat grain in comparison with CDC barley grain.

| Item | Oat varieties (O) | | | Barley variety (B) | SEM | P value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)			B vs. O
In situ rumen degradation							
Kd (%/h)	62.60[a]	59.38[ab]	52.73[ab]	36.55[b]	8.384	0.02	0.97
T0 (h)	0	0.20	0.19	0.46	0.166	0.15	0.83
S (%)	13.52[b]	19.00[b]	36.68[a]	20.11[b]	3.525	<0.01	<0.01
D (%)	83.72[a]	78.75[a]	61.90[b]	79.26[a]	3.504	0.01	<0.01
U (%)	2.76[a]	2.25[ab]	1.42[ab]	0.63[b]	0.440	0.01	0.38
BST (g/kg DM)	43.80[b]	44.94[b]	51.05[b]	82.59[a]	6.364	<0.01	0.26
EDST (g/kg DM)	435.07[b]	430.43[b]	454.15[b]	498.64[a]	8.672	<0.01	0.95
%BST	9.22[b]	9.45[b]	10.11[b]	14.20[a]	1.208	<0.01	0.40
%EDST	90.78[a]	90.54[a]	89.89[a]	85.80[b]	1.208	<0.01	0.40

SEM: standard error of mean; [a-b] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; Kd: the degradation rate of D fraction (%h); T0: lag time; S: soluble fraction in the in-situ incubation; D: degradable fraction; U: rumen undegradable fraction; BST: rumen bypass or undegraded feed starch; EDST: effective degraded starch.

3.4.5 Intestinal Digestion

Estimated intestinal digestibility of DM and OM is shown in Table 3.8. Percentage of intestinal digestibility of bypass or undegradable DM was higher (P<0.01) for barley grain (+26.73% BDM). The higher intestinal digestibility together with the higher rumen effective degradability of DM resulted in Austenson barley grain having a significantly higher (P=0.02) total-tract digestibility of DM (+9.37% DM). The intestinal digestibility of OM (dBOM) was lower (P<0.01) for oat grain (-27.19% BOM) when compared to barley grain. The intestinal digested bypass OM (IDBOM) was lower (P<0.01) for oat grain when compared in a percentage basis, however, when analyzing in a g/kg DM, CDC Nasser showed similar (P>0.10) value to barley Austenson, although the contrast statement showed that barley still had a higher value (P=0.02; +10.74 g/kg DM).

Table 3.9 shows the intestinal digestibility of crude protein (CP) and starch (ST) for the three varieties of oat grain compared to barley Austenson. Values for the intestinal digestibility of rumen bypass CP (dBCP) were similar (P>0.10) for CDC Nasser oat and Austenson barley (52.03 and 54.00% RUP), but oat grain had lower (P<0.01) values when compared to barley grain (45.52 and 54.00% RUP) respectively. The total tract digestibility of CP was higher (P=0.02) for CDC Nasser when compared to Austenson barley, with CDC Arborg and Ruffian showing intermediate values. Intestinal digestibility (dBST) and intestinal digested starch (IDBST) were higher (P<0.01) for Austenson barley (+2.85% RUP and 32.33 g/kg DM)

Table 3.8. Intestinal digestion of dry matter (DM) and organic matter (OM) of different varieties of CDC oat grain in comparison with CDC barley grain.

| Items | Oat varieties (O) | | | Barley variety (B) | SEM | P-value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	CDC Austenson (Feed Type)			B vs. O
DM intestinal digestion							
dBDM (%BBDM)	38.22[b]	31.40[b]	33.05[b]	60.95[a]	2.423	<0.01	<0.01
IDBDM (%BDM)	11.19[b]	9.58[b]	9.24[b]	15.44[a]	0.792	<0.01	<0.01
IDBDM (g/kg DM)	32.79[ab]	29.27[ab]	25.95[b]	39.21[a]	2.690	0.01	0.02
TDDM (%DM)	81.87[b]	79.19[b]	81.23[b]	90.13[a]	0.823	<0.01	0.02
TDDM (g/kg,DM)	759.06[b]	730.31[b]	752.84[b]	826.64[a]	7.524	<0.01	0.04
OM intestinal digestion							
dBOM (%BOM)	39.05[b]	31.79[b]	33.47[b]	61.96[a]	2.416	<0.01	<0.01
IDBOM (%BOM)	11.09[b]	9.45[b]	9.15[b]	15.53[a]	0.776	<0.01	<0.01
IDBOM (g/kg. DM)	31.52[ab]	28.17[b]	25.13[b]	39.01[a]	2.593	<0.01	0.02
TDOM (%DM)	82.65[b]	79.84[b]	81.77[b]	90.49[a]	0.799	<0.01	0.01
TDOM (g/kg, DM)	801.06[b]	774.07[b]	793.92[b]	884.69[a]	7.812	<0.01	<0.01

SEM: standard error of mean; [a-b] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparison using Tukey method; dBDM: intestinal digestibility of rumen bypass dry matter; IDBDM: intestinal digested rumen bypass dry matter; TDDM: total digested dry matter; dBOM: intestinal digestibility of rumen bypass organic matter; IDBOM: intestinal digested rumen bypass organic matter; TDOM: total digested organic matter.

Table 3.9. Intestinal digestion of crude protein (CP) and starch (ST) of different varieties of CDC oat grain in comparison with CDC barley grain.

| Items | Oat varieties (O) | | | Barley variety (B) | SEM | P-value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	CDC Austenson (Feed Type)			B vs. O
CP intestinal digestion							
dBCP (%RUP)	52.03[ab]	44.61[bc]	39.91[c]	54.00[a]	2.728	<0.01	<0.01
IDP (%RUP)	9.93[b]	7.91[c]	7.01[c]	13.73[a]	0.575	<0.01	<0.01
IDP (g/kg DM)	13.69[b]	12.46[b]	10.05[c]	18.58[a]	0.795	<0.01	<0.01
TDP (%CP)	90.81[a]	90.19[ab]	89.41[ab]	88.28[b]	0.564	0.02	0.58
TDP (g/kg DM)	125.51[bc]	142.39[a]	128.23[b]	119.54[c]	2.178	<0.01	0.72
Starch intestinal digestion							
dBST (%BST)	93.76[b]	91.63[b]	94.46[ab]	97.31[a]	0.960	<0.01	0.83
IDBST (%BCHO)	8.67[b]	8.69[b]	9.55[b]	13.85[a]	1.230	<0.01	0.40
IDBST (g/kg DM)	41.20[b]	41.31[b]	48.22[b]	80.55[a]	6.483	<0.01	0.26
TDBST (%ST)	99.46[ab]	99.24[b]	99.44[ab]	99.65[a]	0.080	0.01	0.91
TDBST (g/kg DM)	476.26[c]	471.74[c]	502.37[b]	579.20[a]	5.931	<0.01	0.34

SEM: Standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; dBCP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digested crude protein; TDP: total digested crude protein; dBST: intestinal digestibility of rumen bypass starch on percentage basis; IDBSTP: intestinal digested bypass starch; TDBST: total digested bypass starch.

3.4.6 Hourly Effective Degradation Ratio between N and OM

The optimal ratio between effective degradability of available N to available organic matter is 25 g N/kg OM (Sinclair et al., 1993). This rate will assure maximal microbial synthesis while preventing N loss. A higher rate of degradation, above the optimal, indicates a potential loss of N or not enough energy available to allow the use of N for microbial protein synthesis, while a lower value than the optimal indicates not enough N to allow microbial growth (Nuez-Ortín and Yu, 2010). The hourly effective degradation ratios between available N and available organic matter (ED_N/ED_OM) at different incubation times of oat grain in comparison to barley grain are presented in Table 3.10. Meanwhile, the ratio curve of the studied grains is shown in Figure 3.1. Results showed that CDC Arborg had higher (P<0.01) overall ratio of ED between available N and available OM, while CDC Austenson showed the lowest value. Despite the tendency to increase the ED_N/ED_OM with increasing incubation time CDC Nasser and CDC Ruffian showed to be close to the optimal rumen fermentation between 0 h and 4 h of incubation time. CDC Arborg showed a significantly higher value at 0 h and 4 h (P<0.01). Although not statistically significant (P=0.30), at 24 h incubation point CDC Austenson barley grain showed the numerically highest value of ED_N/ED_OM (184.42 g/kg). This large difference between grains was mainly caused by a significant difference in hourly effective degradation of N and organic matter. All varieties and grains presented a similar effective degradation of N during 12 h and 24 h incubation, but barley had a larger difference in effective degradation of OM.

Table 3.10 Hourly effective degradation ratios between N and OM of different varieties of CDC oat grain in comparison with CDC barley grain.

| Items | Oat varieties (O) | | | Barley variety (B) | SEM | P value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)			B vs. O
Ratio of N to OM	22.81[bc]	26.05[a]	23.63[b]	22.16[c]	0.334	<0.01	0.91
Ratio of ED_N/ED_OM	25.78[b]	30.42[a]	26.68[b]	22.37[c]	0.409	<0.01	0.30
Ratio at individual incubation hours (g/kg)							
h0	24.91[b]	43.31[a]	26.44[b]	17.58[b]	4.041	<0.01	0.64
h2	25.44[a]	27.97[a]	27.56[a]	19.88[b]	0.769	<0.01	<0.01
h4	25.22[b]	30.43[a]	25.11[b]	22.97[b]	1.023	<0.01	0.36
h8	26.98[ab]	37.32[a]	21.00[b]	31.61[ab]	4.136	0.05	0.03
h12	32.23	47.48	17.74	45.56	9.306	0.09	0.03
h24	89.22	111.48	11.34	184.42	69.134	0.3	0.12

SEM: Standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; N: nitrogen; OM: organic matter; ED: effective degradability.
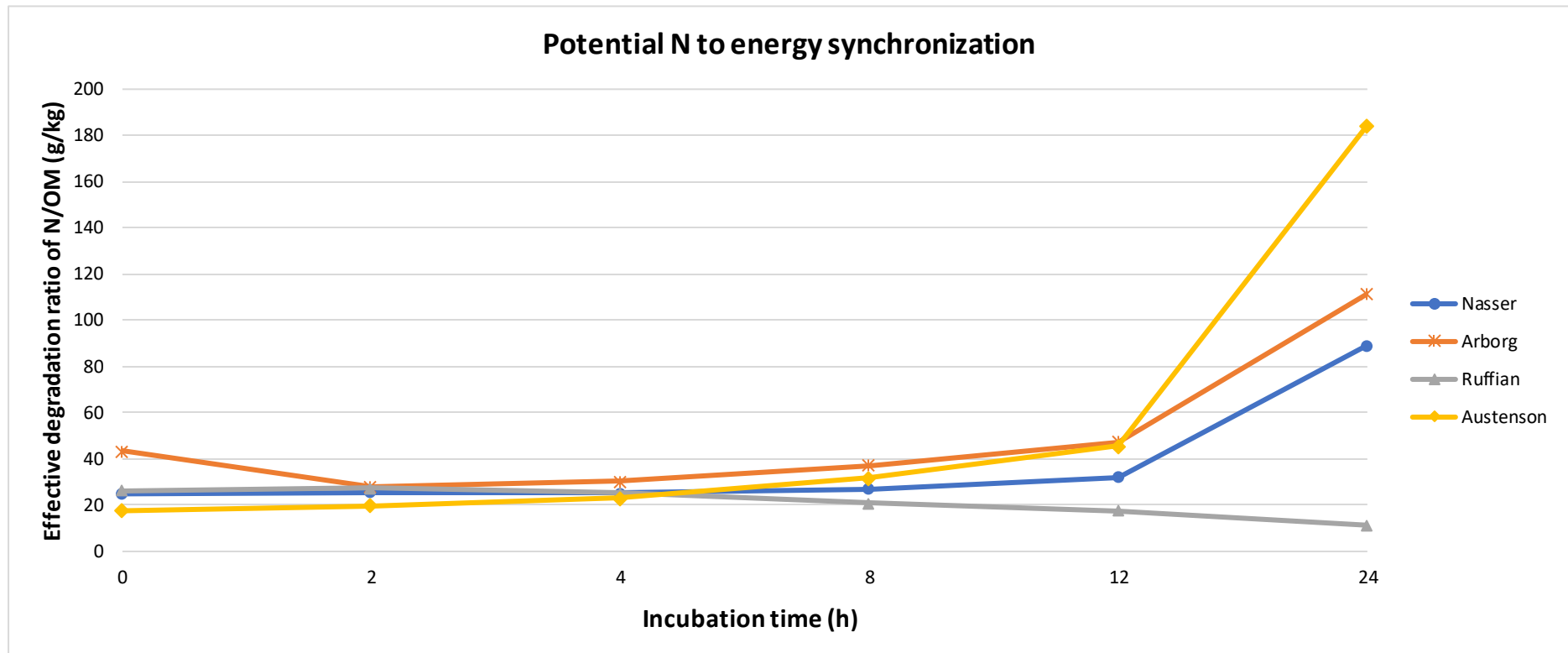
Figure 3.1. Hourly effective degradation ratios between available N and available OM (ED_N/ED_OM) of different varieties of oat grain in comparison with barley grain.

3.4.7 Nutrient Supply and Feed Milk Value

The results for nutrient supply and feed milk value according to the DVE/OEB model are shown in Table 3.11. Bypass crude protein (BCP) was higher (P<0.01) for barley Austenson (123.44 g/kg DM). However, CDC Austenson showed the lowest (P<0.01) microbial protein synthesized in the rumen based on available energy (MREE) and based on available rumen degradable CP (MREN) when compared to the other studied treatments (105.59 and 11.66 g/kg DM) respectively. The total true protein supply absorbed in the small intestine was higher for barley Austenson (P<0.01) when compared to other treatments. DVE ranged from 101.7 to 127.90 g/kg DM. Positive values of degraded protein balance (OEB) signifies the potential loss of N, while negative values can indicate a shortage of N and consequently an impairment on microbial protein synthesis. All treatments in this study presented negative values, ranging from -34.30 g/kg DM in CDC Arborg to -93.92 g/kg DM for CDC Austenson barley. Feed milk value based on the Dutch model varied significantly between treatments (P<0.01), with CDC Austenson barley having the highest value while CDC Ruffian oat had the lowest (2.60 and 2.05 kg of milk/kg of feed) respectively. The results for barley Austenson in this study were different from the data published by Yu (2005b), which obtained much lower DVE and higher OEB using 0.5 mm rolled feed type of barley (Valier). Oat grain in this study showed the same pattern, it had a higher DVE and lower OEB than previous published results for oat (Yu and Niu, 2009).

The metabolic characteristics, true nutrient supply, and feed milk value according to the NRC 2001 system is shown in Table 3.12. The truly absorbed microbial protein in the small intestine (AMCP) was lower for oat grain (P<0.01) when compared to barley (4.8 g/kg DM) and the truly absorbed rumen undegradable protein (ARUP) tended to be lower (P=0.06) for oat grain as well (-28.81 g/kg DM). Consequently, metabolizable protein (MP) was lower for oat grain (P=0.03) compared to barley grain (75.66 vs. 129.62 g/kg DM) respectively. When compared

to the data published by Yu (2005b), that reported a MP 107.4 g/kg DM, barley grain in this study presented higher MP and lower DPB (129.62 g/kg DM). The predicted feed milk value (FMV) was also higher for barley grain when compared to oat grain (2.63 vs. 1.94 kg of milk/kg of DM fed) respectively. The calculation for FMV was based on MP and not energy.

Table 3.11. Metabolic characteristics and truly absorbable nutrient supply (based on non-TDN system: DVE-OEB) of different varieties of CDC oat grain in comparison with CDC barley grain.

| Items | Oat varieties (O) | | | Barley variety (B) | SEM | P-value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)			B vs. O
Truly digestible nutrient supply to dairy cattle (g/kg DM)							
BCP (g/kg DM)	68.57[b]	74.23[b]	78.77[b]	123.44[a]	17.116	<0.01	0.42
EDCP (g/kg DM)	176.13[b]	173.31[b]	165.97[b]	233.70[a]	13.075	<0.01	0.09
MREE (g/kg DM)	115.78[a]	117.90[a]	116.61[a]	105.59[b]	3.308	<0.01	0.10
MREN (g/kg DM)	69.63[a]	83.60[a]	64.63[a]	11.66[b]	17.013	<0.01	0.43
DVME (g/kg DM)	73.81[a]	75.16[a]	74.34[a]	67.31[b]	2.109	<0.01	0.10
DVBE (g/kg DM)	35.61[b]	33.09[b]	31.63[b]	65.44[a]	7.623	<0.01	0.06
Degraded protein balance (OEB) and Total true protein supply (DVE) to dairy cows (g/kg DM)							
DVE (g/kg DM)	103.35[b]	103.05[b]	101.07[b]	127.90[a]	5.652	<0.01	0.06
OEB (g/kg DM)	-46.15[a]	-34.30[a]	-51.97[a]	-93.92[b]	13.812	<0.01	0.56
Feed milk value (kg milk/kg DM fed)							
FMV	2.10[b]	2.09[b]	2.05[b]	2.60[a]	0.114	<0.01	0.06

SEM: Standard error of mean; [a-b] Means with different letters in the same row are significantly different (P<0.05); Multi-treatment comparisons using Tukey method; BCP: bypass crude protein; MREE: microbial protein synthesized in the rumen based on available energy; EDCP: effective degradability of CP; MREN: microbial protein synthesized in the rumen; DVME: rumen synthesized microbial protein digested in the small intestine; DVBE: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV: feed milk value.

Table 3.12. Metabolic characteristics and true nutrient supply (based on TDN system: NRC dairy) of different varieties of CDC oat grain in comparison with CDC barley grain.

Items	Oat varieties			Barley variety			Contrast P-value
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)	SEM	P-value	B vs. O
Truly Digestible Nutrient Supply to Dairy Cattle							
RUP (g/kg DM)	61.77[b]	66.87[b]	70.96[b]	111.48[a]	15.421	<0.01	0.42
MCP_{TDN} (g/kg DM)	98.46[b]	95.06[c]	94.79[c]	103.61[a]	0.382	<0.01	<0.01
MCP_{RDP} (g/kg DM)	149.71[b]	147.31[b]	141.07[b]	198.64[a]	11.891	<0.01	0.09
AMCP (g/kg DM)	63.02[b]	60.84[c]	60.67[c]	66.31[a]	0.245	<0.01	<0.01
ARUP (g/kg DM)	32.08[b]	29.81[b]	28.49[b]	58.95[a]	6.867	<0.01	0.06
ECP (g/kg DM)	10.97[b]	10.95[b]	11.01[a]	10.89[c]	0.005	<0.01	<0.01
AECP (g/kg DM)	4.40[a]	4.38[a]	4.40[ab]	4.35[c]	0.005	<0.01	<0.01
Total metabolizable protein supply and degraded protein balance to dairy cattle							
MP (g/kg DM)	98.38[b]	95.03[b]	93.57[b]	129.62[a]	7.186	<0.01	0.03
DPB (g/kg DM)	59.73[b]	61.13[ab]	54.05[b]	111.44[a]	14.155	0.02	0.16
Feed milk value (kg milk/kg DM fed)							
FMV	2.00[b]	1.93[b]	1.90[b]	2.63[a]	0.146	<0.01	0.03

SEM: Standard error of mean; [a-c] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparisons using Tukey method; RUP: rumen undegradable feed crude protein; MCP_{TDN}: rumen synthesized microbial protein base on available TDN; MCP_{RDP}: microbial protein synthesized in the rumen based on available protein; AMCP: truly absorbed microbial protein in the small intestine; ARUP: truly absorbed rumen undegradable protein in the small intestine; ECP: rumen endogenous protein; AECP: truly absorbed rumen endogenous protein in the small intestine; MP: metabolizable protein; DPB: rumen degraded protein balance; FMV: feed milk value.

3.4.8 Protein Molecular Structures Analysis

Previous findings suggest that protein molecular structure is related to variation in the ruminal and intestinal digestibility of proteins (Damiran and Yu, 2011; Peng et al., 2014). The univariate analysis of the protein molecular structural characteristics of different varieties of oat grain in comparison to barley grain are shown in Figure 3.2 and Table 3.13. In this study Amide I height was significantly higher (P<0.01) for CDC Austenson in comparison to all varieties of oat. The ratio of Amide I to Amide II peak height and area was significantly lower for oat (P<0.01) when compared to CDC Austenson barley grain. The protein secondary structures α-helix and β-sheet showed a diverse range between varieties and grains, with CDC Nasser showing the lowest numbers when compared to CDC Arborg and CDC Austenson (P<0.01). The α-helix to β-sheet ratio was numerically higher for all oat varieties, although CDC Arborg and CDC Ruffian did not show a significant difference from barley grain. Peng et al. (2014) suggested a strong association between Amide II intensity and CP, ruminal degradable protein and intestinal digestibility of RUP, even though this relation could be originated from the CP quantitative value. In this study, CDC Arborg, the variety that had the highest Amide II height, showed the highest value for CP content, however, the values for rumen degradable fraction and intestinal digestibility of RUP were not higher for CDC Arborg when compared to the other varieties.

Figure 3.3 and 3.4 presents the results of the multivariate molecular spectral analysis, PCA and HCLA. The principal component analysis (PCA) was able to group different varieties of oat grain, and compared it to barley grain, by its amide related region. The principal component 1 (PC1) was able to explain 91% of the variation between the spectra data in this protein related region, while PC2 explained 5% of the variation that CDC Arborg oat and Austenson barley were clearly separated from each other and from the other two varieties. On the other hand, CDC Nasser and CDC Ruffian were overlapped, implying that the molecular

structure in the Amide region was similar for these two varieties. The HCLA revealed that the structural makeup between oat varieties was not fully distinguishable in the Amide region. These results suggest that the three varieties of oat were not significantly different in the whole Amide molecular structure.
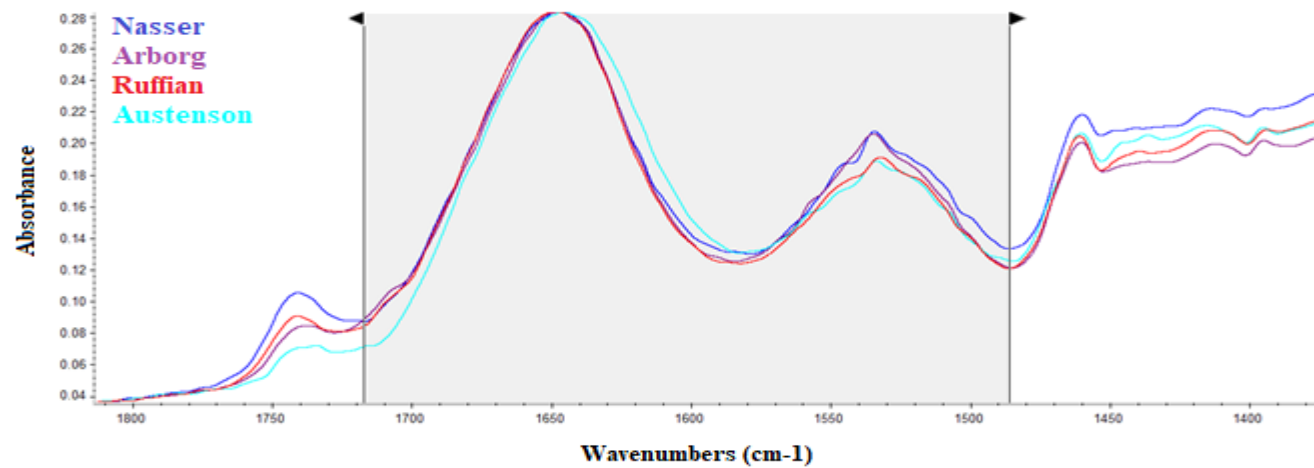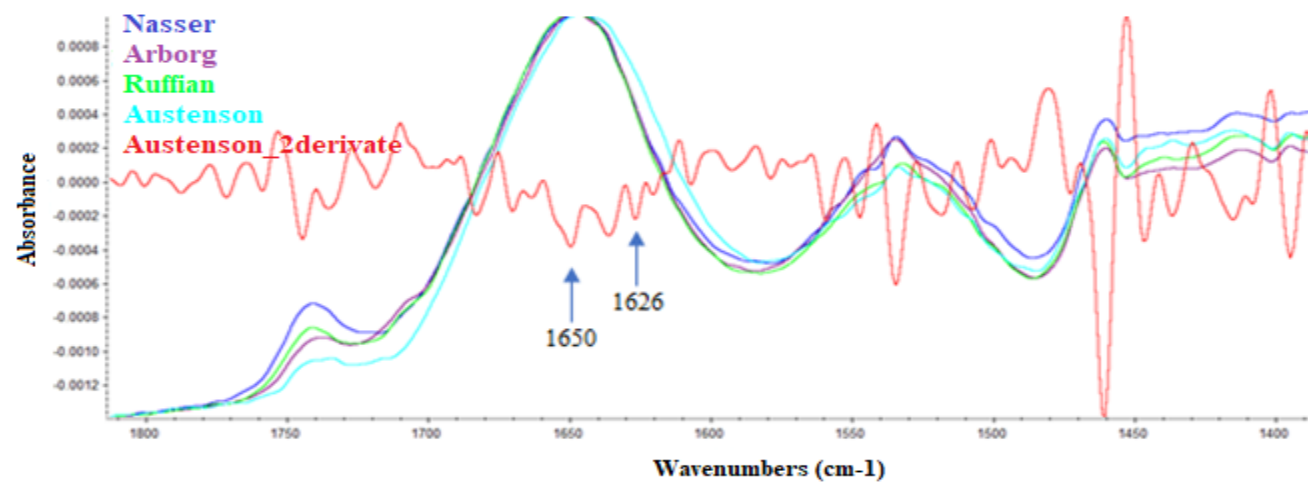
Figure 3.2. (a) Vibrational Fourier transformed infrared attenuated total reflectance (Ft-IR/ATR) biomolecular spectra of different varieties of oat grain in comparison with barley grain of the protein molecular structures, amide I and amide II; (b) Protein secondary structures α-helix and β-sheet heights.

Table 3.13. Protein molecular structure profile of different varieties of CDC oat grain in comparison with CDC barley grain.

| Items | Oat varieties (O) | | | Barley variety (B) | SEM | P-value | Contrast P-value |
	Nasser (Feed-Type)	Arborg (Milling-Type)	Ruffian (Milling-Type)	Austenson (Feed Type)			B vs. O
Amide heights and spectra ratio							
Amide I	0.10[c]	0.12[b]	0.11[bc]	0.14[a]	0.004	<0.01	<0.01
Amide II	0.05[b]	0.06[a]	0.05[b]	0.043[b]	0.003	0.01	0.47
Amide I/Amide II	2.149[b]	2.018[b]	2.25[b]	3.323[a]	0.081	<0.01	0.03
Amide area and spectra ratio							
Amide I	8.69[c]	10.85[b]	9.39[bc]	13.02[a]	0.421	<0.01	0.02
Amide II	2.25[b]	3.32[a]	2.29[b]	2.27[b]	0.218	0.02	0.23
Amide I/Amide II	3.97[b]	3.28[b]	4.10[b]	6.46[a]	0.232	<0.01	0.13
Secondary structure heights and spectra ratio							
α-helix	0.15[c]	0.18[ab]	0.15[bc]	0.18[a]	0.005	<0.01	0.03
β-sheet	0.10[c]	0.12[b]	0.11[bc]	0.14[a]	0.003	<0.01	<0.01
α-helix/β-sheet	1.56[a]	1.45[ab]	1.44[ab]	1.28[b]	0.039	<0.01	0.76

SEM: standard error of mean; [a-c] Means with different letters in the same row a significantly different (P<0.05); Multi-treatment comparison using Tukey method;
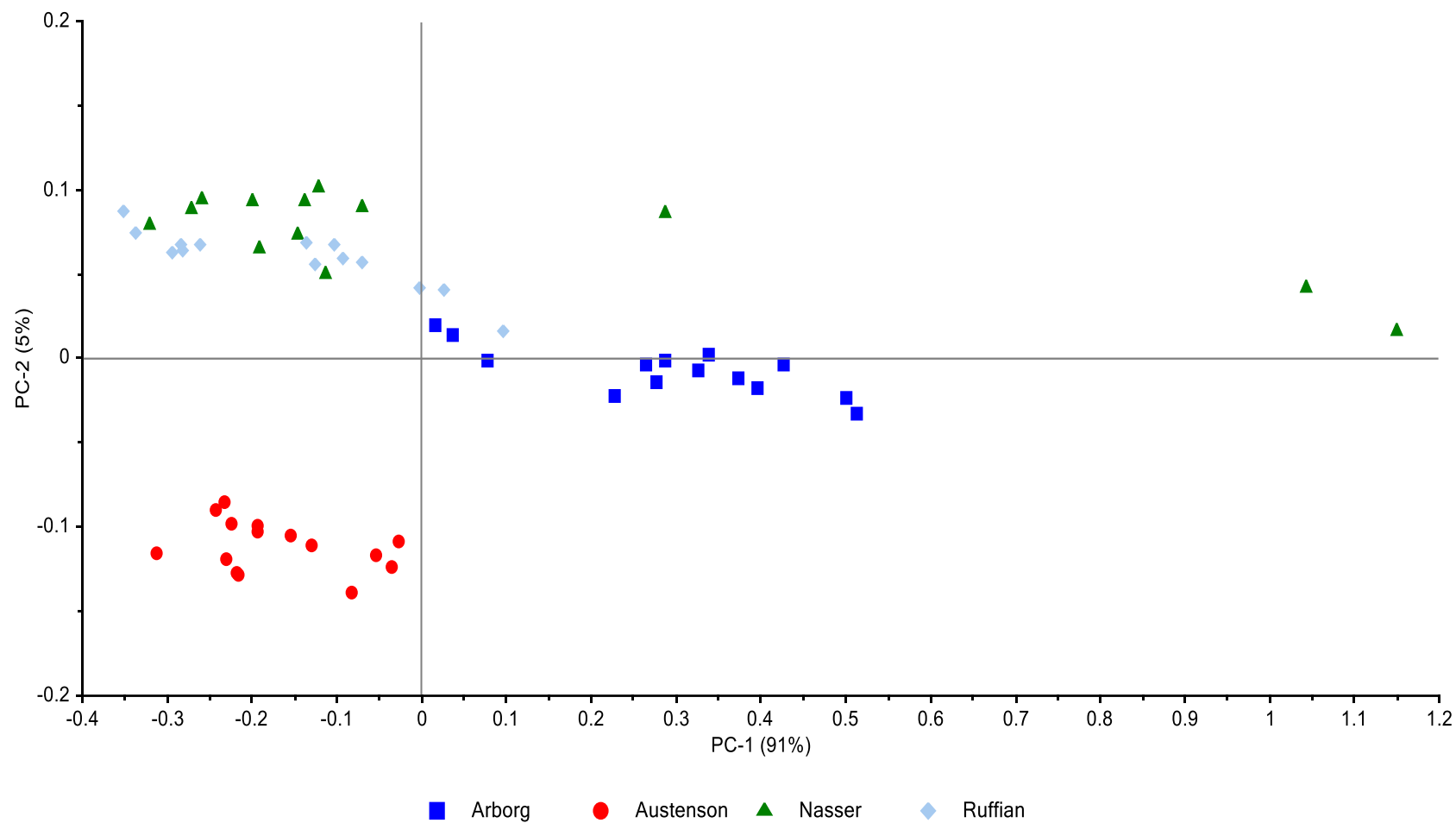
Figure 3.3 Multivariate spectral analyses of different processed oat grain in comparison with barley grain using FTIR vibrational spectroscopy at whole Amide region (ca. 1710-1480 cm^{-1}). PCA (principal component analysis) with a scatter plot of the 1st principal components (PC1) vs. the 2nd principal components (PC2).
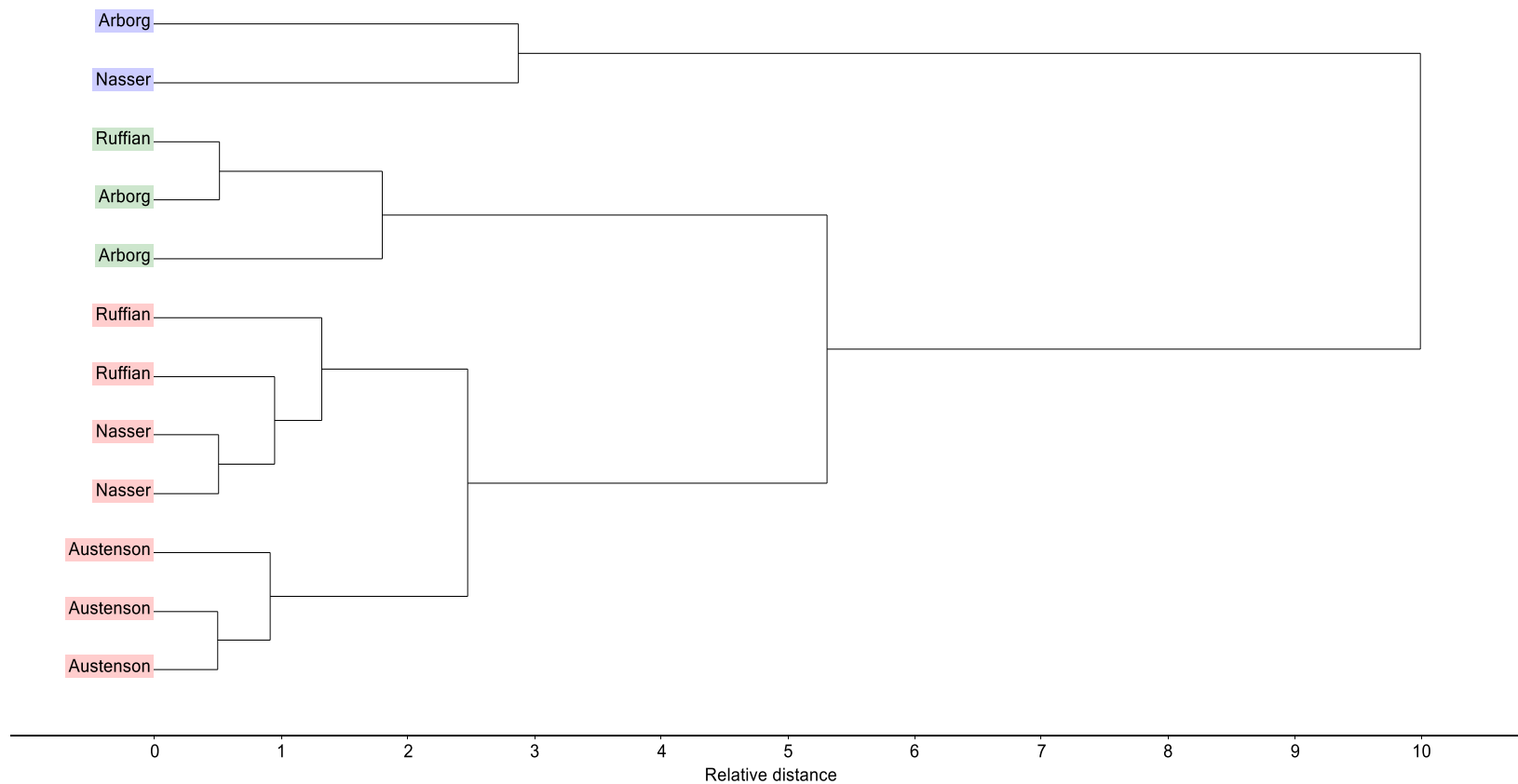
Figure 3.4 Multivariate spectral analyses of different processed oat grain in comparison with barley grain using FTIR vibrational spectroscopy at whole Amide region (ca. 1710-1480 cm^{-1}). CLA (cluster analysis): cluster method (Ward's algorithm) and distance method (Squared Euclidean).

3.5 Chapter summary and conclusions

Chemical profiles were different among varieties and between grains. CDC Nasser had lower lignin content despite having a higher percentage of hull compared to the other oat varieties (27 vs. 25%). The content of carbohydrate, starch, and sugar for the barley variety was 82.47, 58.12 and 2.65, respectively, and it was higher than any variety of oat. Despite the differences in chemical profile, CDC Nasser oat and CDC Austenson barley showed similar energy values. Oat grain had a NE_L of 1.96 Mcal/kg and TDN_{1x} 84.89% DM. Oat had a higher PB1 and a lower PB2, while the PC sub-fraction did not differ between grains. Despite that, CDC Austenson barley showed a higher FMV (2.63 kg of milk/kg of feed) when compared to all three varieties of oats. The three oat varieties presented a higher Kd for DM, OM, and CP, and oat grain had a higher effective degradability of CP in the rumen (81.85%). The results of this study indicate that oat varieties have singular chemical and nutritional characteristics. In addition, CDC Nasser and Ruffian can provide higher effective degradability of CP in the rumen without affecting the effective degradability of N/OM and so contribute to the maximum microbial production while preventing N loss.

4. IMPACT OF PROCESSING METHODS ON PHYSIOCHEMICAL, NUTRITIONAL MOLECULAR STRUCTURAL CHARACTERIZATION AND DAIRY COW FEEDING VALUE OF OAT GRAIN IN COMPARISON WITH BARLEY GRAIN

4.1 Abstract

Processing cereal grains can lead to an improvement in nutrient digestibility and have an impact on the rate and site of grain nutrients digestion. However, there are several methods of grain processing and it is important to understand which processing method provides the best results for dairy cows ration with oat grain. The main objective of this study was to determine the impact of processing methods (Rolling, Steam-Flaking, Pelleting) on the nutritional and digestive characteristics and the protein related molecular spectral profiles of oat grain in comparison to barley grain. Results showed that heat treating oat (steam-flaking and pelleting) did not alter the DM, OM, and SCP of oat grain, but it did increase the EE (+0.50% DM). Rolled barley had higher ($P<0.01$) starch and NFC content when compared to the other treatments. Steam-flaking increased the energy content (DE, ME and NE_L) and the intermediate degradable protein fraction PB1 (+13.68% CP), while reduced PA2 (-17.19% CP) fraction when compared to rolled oat. Steam-flaking also increased bypass CP (+14.71%BCP) while decreasing the EDCP in the rumen (-14.71%). Rolled barley showed higher values of intestinal digestibility and total-tract digestibility for DM and OM. In the DVE/OEB system, steam-flaked oat and pelleted oat presented lower values of OEB when compared to rolled oat, but they were higher than the value for rolled barley.

Univariate analysis of the protein molecular structure features showed only changes in the protein beta-sheet height, with flaked oat presenting the higher value (0.05), pelleted oat showing the lowest value (0.02) and rolled barley and oat showing intermediate values (0.04 and 0.03). There was overlap among treatments when analyzed with PCA, implying similar molecular structure among the treatments.

4.2 Introduction

Oat (*Avena sativa*) is one of the most important cereal grains produced in Canada. In recent years, with the increase in demand by the international market, oat production has grown more than 10% in the last two years, which promoted an increase in availability of this grain for Canadian farms (Statistics Canada, 2018). Although oat seems to be a good replacement for other cereal grains for high producing dairy cattle, oat grain generally has a high proportion of hull, accounting for up to 25% of the whole oat weight (Crosbie et al., 1985). This high content of fiber protecting the groat is known to decrease the total-tract digestibility of the grain and increases the loss of whole grains in feces (Beauchemin et al., 1994; Morgan and Campling, 1978).

Processing methods have been shown to improve nutrient digestibility and the rate and site of grain digestion (Chrenkova et al., 2018; Prates et al., 2018). Grains can be physically processed by the application of several combinations of heat, moisture, time and pressure. So, it is important to understand which kind of processing method is more adequate to optimize overall dairy cattle performance and milk production. It is also important to understand how the molecular structure of grains is affected by heat processing methods and how the changes in protein molecular structure can affect nutrient profile and availability for dairy cattle (Yu, 2007). Recently, ATR-FT/IR

molecular spectroscopy has been used as a non-invasive and non-destructive technique to rapidly characterize the feed molecular structure and use this data to predict nutrient profile and utilization.

Therefore, the present study was conducted to evaluate three different processing methods for oat grain in comparison to barley grain in relation to nutrient profile, energy values, protein sub-fractions, rumen degradation kinetics, intestinal digestibility, nitrogen to energy synchronization and protein molecular structure, in order to determine the most efficient processing method to enhance truly absorbable nutrient supply for high production dairy cattle.

4.3 Material and Methods

4.3.1 Grains Collection and Processing

Samples of CDC Ruffian oat and barley grain used in this study were obtained from commercial sellers by Canadian Feed Research Centre (CFRC, University of Saskatchewan). Processing of the grains was also conducted at the Canadian Feed Research Centre (CFRC), University of Saskatchewan, North Battleford, Canada. Two different production batches were made for all treatments (n=8). Pellets were made at 62°C in a pellet mill (UAS-Muyang Model: MUZL350II) with a die inside diameter of 350 mm and hole area of 4 mm die (PDI=66%). For steam-flaking, samples were steamed for 25 min at atmospheric pressure and subsequently flakes were made at approximately 100°C (AT Ferrell 18×39 Dual Drive), before being transferred to the flaker cooler (Geelen Model VK 28 × 28 KL). Rolled samples (rolled oat and rolled barley) were made using a roller grinder (G.J. Vis Triple Pair 12" x 20"). The grains ended up with a processing index of 50.9 for oat and 73.4 for barley.

4.3.2 Chemical Analysis

The samples were ground through a 1 mm screen (RetschZM200, Retsch Inc., PA, USA) and subsequently analyzed for DM (AOAC official method 930.15), OM, EE (AOAC official method 920.39), Ash (AOAC official method 942.05), CP (AOAC official method 984.13) and sugars (AOAC official method 974.06). The NDF, ADF, and ADL were analyzed according to Van Soest et al. (1991) using the filter bag technique from ANKOM Technology. The NDICP and ADICP were analyzed according to the procedures described by Licitra et al. (1996). The SCP was determined according to Roe et al. (1990) by incubating samples in the borate-phosphate buffer and filtrating it through Whatman filter paper (#54). Starch was analyzed using a Megazyme Total Starch Kit (Megazyme International Ltd., Wicklow, Ireland). Total carbohydrate and non-fiber carbohydrate were determined according to NRC (2001): $CHO = 100 - EE - CP - Ash$, and $NFC = 100 - (NDF - NDICP) - EE - CP - Ash$. All samples were analyzed in duplicate and repeated if chemical analysis error was in excess of 5 %.

4.3.3 Energy Values

The energy values of grains were determined using the summative approaches of the NRC (2001) dairy and NRC (1996) beef. The digestible energy at a production level of intake (DE_{3x}), metabolizable energy at a production level of intake (ME_{3x}), net energy for lactation at a production level of intake (NE_{L3x}), as well as values for truly digestible CP (tdCP), truly digestible NDF (tdNDF), truly digestible NFC (tdNFC) and truly digestible fatty acids (tdFA) were determined according to the equations of NRC-2001 dairy. The values of net energy for maintenance (NE_m) and net energy for growth (NE_g) were estimated according to the equations of NRC-1996 beef.

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4.3.4 Protein and Carbohydrate Profile

The Cornell Net Carbohydrate and Protein System (CNCPS) version 6.5 was used to partition the carbohydrate and protein sub-fractions. Fractions were subdivided considering the rate and extent of degradation in the rumen. Protein was fractioned into PA2= soluble true protein with a Kd ranging from 10 to 40%/h; PB1= insoluble true protein with a Kd of 3-20%/h; PB2= fiber-bound protein with a Kd ranging from 1-18%/h and PC= indigestible protein. The carbohydrates were subdivided into CA4= water-soluble carbohydrates and has a Kd of 40-60%/h; CB1= starch that has a Kd of 20-40%/h; CB2= soluble fiber with a Kd ranging from 20 to 40%/h; CB3= digestible fiber with a Kd of 1-18%/h and CC= indigestible fiber.

4.3.5 Rumen Incubation

The University of Saskatchewan Animal Care Committee approved the animal trial under the Animal Use Protocol No. 19910012 and animals were cared for and handled in accordance with the Canadian Council of Animal Care (CCAC, 1993) guidelines. The in situ experiment was carried out in the Rayner Dairy Teaching and Research Facility, University of Saskatchewan, Canada. For the incubation, four Holstein cows fitted with an 88 mm cannula were used. Cows were housed in individual tie stalls with free access to water and fed a TMR diet composed of barley silage, alfalfa hay, and a lactating pellet twice a day.

The incubation procedure followed a 'gradual addition/all out` schedule according to the protocol by Damiran and Yu (2012). Nylon bags with a 40 μm pore size were used to incubate approximately 7 g per sample per bag for 0, 2, 4, 8, 12 and 24 h with multi-bags (2, 2, 2, 2, 3, 4) for each treatment and incubation time as well as each experiment run. The incubation procedure was performed for two experimental runs using the same four cannulated cows. The two batches

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of each treatment were used in this experiment (n=8). After incubation was completed, bags were removed and washed in cold water for six times, to wash out all the rumen fluid, and subsequently dried at 55°C for 48h in a forced-air oven. Samples taken out of the oven were exposed to room temperature and moisture before being weighted and composite by incubation time point and treatment. Pooled samples were then ground through 1 mm screen and analyzed for CP using LECO protein analyzer (Model FP-528, Leco Corp., St. Joseph, MI, USA), DM and OM according to AOAC (2005), and starch was analyzed using a Megazyme total starch kit (Megazyme International Ltd.).

4.3.6 Rumen Degradation Kinetics

Degradation characteristics of DM, OM, CP, and Starch were determined following the first-order kinetics degradation model described by Ørskov and McDonald (1979) and modified by Tamminga et al. (1994). The results of rumen degradation kinetics were analyzed using NLIN procedure of SAS (Statistical Analysis System,) version 9.4 with iterative least-square regression (Gausse Newton method).

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

where R(t) was the residue present after t hours of incubation; U was the undegradable fraction (%); D was the potentially degradable fraction (%); Kd was the degradation rate (h^{-1}); and T0 was the lag time.

The percentage of bypass (B) values of nutrients were calculated according to NRC Dairy (2001):

$$\%BDM, \ BOM, \ BCP \ (or \ RUP) = U + D \times Kp/(Kp+Kd)$$

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

where, S=soluble fraction (%); Kp=estimated passage rate from the rumen (h^{-1}) and was assumed to be 6%/h for DM, OM, CP and Starch (Tamminga et al., 1994). The rumen undegradable or bypass DM, OM and Starch, in g/kg DM, were calculated as:

BDM, BOM or BSt (g/kg DM) = DM (OM or St) (g/kg DM) × % BOM (BOM or BSt),

while the rumen bypass CP (BCP) and rumen undegraded CP (RUP) were calculated differently according to the DVE or NRC model:

BCP DVE (g/kg DM) = 1.11 × CP (g/kg DM) × %BCP

RUP NRC (g/kg DM) = CP (g/kg DM) × %RUP

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

%EDDM (EDOM, EDCP or EDSt) = S + D × Kd/(Kp + Kd),

EDDM (EDOM, EDCP or EDSt) (g/kg DM) = DM (OM, CP or St) (g/kg DM) × %EDDM (EDOM, EDCP or EDSt).

4.3.7 Hourly Effective Rumen Degradation Ratio and Potential N to Energy Synchronization

The effective degradation of available N and available OM were calculated according to Sinclair et al. (1993):

Hourly ED (g/kg DM) = S + [(D × Kd)/ (Kp + Kd)] × 1 − $e^{-t \times (Kd+Kp)}$.

The difference in cumulative amounts degraded among successive hours was used to calculate the hourly effective degradation ratio between N and OM (ED_N/ED_OM) following the equation described by Nuez-Ortín and Yu (2010):

Hourly ED N/OM$_t$ = (HEDN$_t$ − HEDN$_{t-1}$)/ (HEDOM$_t$ − HEDOM$_{t-1}$),

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where, hourly ED_N/ED_OM was the ratio of N to OM at the time t (gN/kgOM); $HEDN_t$ was the hourly ED of N at the time t (g/kg DM); $HEDN_{t-1}$ was the hourly ED of N 1h before the time t (g/kg DM); $HEDOM_t$ was the hourly ED of OM at the time t (g/kg DM); $HEDOM_{t-1}$ was the hourly ED of OM 1 h before the time t (g/kg DM).

4.3.8 Intestinal Digestion of Rumen Undegradable Protein

The intestinal digestion of CP was determined using the three-steps in vitro protocol by Calsamiglia and Stern (1995). Briefly, residues taken out after 12 hours ruminal incubation and containing approximately 15 mg of N were placed in a 50 ml centrifuge tube with 10 ml of pepsin (Sigma P-7000) solution (0.1 N HCl with pH 1.9) and incubated for 1 h at 38°C. After incubation, 0.5 ml of 1 N NaOH solution and 13.5 ml of pancreatin (Sigma P-7545) were added and the solution was incubated for 24 h at 38°C. After the incubation, 3 ml of TCA was used to stop hydrolysis and then centrifugated at 1000g for 15 min and the supernatant was analyzed for soluble N by the Kjeldahl method. Intestinal digestion of protein was calculated as TCA soluble N divided by N present after ruminal incubation.

4.3.9 Nutrient Supply and Feed Milk Value

The DVE/OEB system and the NRC model were used to estimate the nutrient supply and feed milk value. In the Dutch system described by Tamminga et al. (1994, 2010), the DVE represents the value of a feed protein and it is calculated as:

$$DVE = DVME + DVBE - ENDP,$$

where DVME is the microbial true protein synthesized in the rumen and digested in the small intestine, DVBE is the feed crude protein undegraded in the rumen but digested in the small

intestine and ENDP is the endogenous protein lost in the digestive process. The OEB value is calculated as:

$$OEB = MREN - MREE,$$

where OEB is the difference between the potential microbial protein synthesis based on available N (MREN) and the potential microbial protein synthesis based on energy extracted from anaerobic fermentation (MREE)

In the NRC 2001 model, the total metabolizable protein (MP) is constituted by the rumen undegraded feed crude protein (RUP), ruminally synthesized microbial crude protein (MCP) and the rumen endogenous crude protein (ECP), and so MP is calculated as:

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

where ARUP is the truly absorbable rumen undegraded CP, AMCP is the truly absorbable ruminal synthesized microbial CP and AECP is the truly absorbable endogenous CP.

The degraded protein balance (DPB) reflects the difference between the potential microbial protein synthesis based on the rumen degradable protein (RDP) and the potential microbial protein synthesis based on energy (TDN) available for microbial fermentation in the rumen. The DPB is calculated as:

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

where RDP is the rumen degradable protein and MCP^{TDN} is the microbial protein synthesis (discounted TDN). Feed milk value was calculated based on MP.

4.3.10 Protein Molecular Structures Analysis

Samples were ground through a 0.12 mm screen and subsequently analyzed using a JASCO FTIR-ATR-4200 spectrometer (JASCO Corp., Tokyo, Japan). Right before samples were

submitted to spectra collection, the background spectrum was measured with 256 scans to correct the spectra for CO_2 noise. Spectra were collected at the mid-IR region (approximately 4000–700 cm^{-1}) with a spectra resolution of 4 cm^{-1} and using 128 co-added scans (SpectraManager II software, JASCO Corp., Tokyo, Japan). Each sample had five spectra collected as sub-sample replicate.

For univariate analysis, the collected spectrum data related to the protein structure was preprocessed using OMINIC 7.3 software (Spectra Tech, Madison, WI, USA). Each spectrum was normalized, and a second derivative was generated and smoothed, prior to the calculation of peak heights and areas. The primary protein structure, amide I region (at ca. 1718-1584 cm^{-1}) and amide II (at ca. 1584-1485 cm^{-1}), as well as the secondary structures, α-helix (at ca. 1647cm^{-1}) and β-sheets (at ca.1628cm^{-1}) were measured for height and area, and their ratios between Amide I to Amide II and α-helix to β-sheet were determined.

The multivariate spectral analysis was performed to distinguish the inherent differences in the whole protein structure between the grains. The whole protein related structures (Amide I and Amide II) were analyzed using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCLA) using Ward's algorithm method. Multivariate spectra analysis was performed using the Unscrambler X software v. 10.3 (Camo Software, Norway).

4.3.11 Statistical Analysis

Results were analyzed using the Mixed model procedure in SAS 9.4 (SAS Institute Inc., NC, USA). The detailed chemical profile, protein and carbohydrate subfractions, energy values and protein spectral profile were analyzed according to the model:

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

where Y_{ij} was the observation of the dependent variable ij, μ was the fixed effect of the population mean, T_i was the fixed effect of treatment and e_{ij} was the random error associated with the observation ij.

Rumen degradation kinetics, hourly effective degradation ratio, nutrient supply and intestinal digestion of rumen undegraded nutrients were analyzed as randomized complete block design (RCBD) with experimental run used as a random block, and analyzed with the Mixed model in SAS 9.4, using the model:

$$Y_{ijk} = μ + T_i + S_k + e_{ijk},$$

where Y_{ijk} was the observation of the dependent variable ijk, μ was the population mean, T_i was the effect of treatment as fixed effect, S_k was the random effect of in situ incubation run and e_{ijk} was the random error associated with the observation ijk.

Prior to the statistical analysis, all outlier data were removed, using the same model, with a criterion of Studentized Residual greater than 2.5. For all statistical analysis, significance was declared at $P<0.05$ and trends at $0.05<P<0.10$. The differences among the treatments were compared using a multiple comparison test following the Tukey method. Contrast statement was used to compare the difference between barley grain and oat grain. The model assumptions were checked using research analysis. The normality test was carried out using Proc Univariate with Normal and Plot option.

4.4 Results and Discussion

4.4.1 Chemical Profile

Results for the impact of processing method on the chemical profile of oat in comparison to barley grain is shown in Table 4.1. Different processing methods and grain types did not affect

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dry matter (DM), organic matter (OM) or ash content (P≥0.20). Significant difference was observed (P<0.01) for EE between rolled and heat processed oat. The apparent increase in crude fat when grains are submitted to heat processing methods has been observed in other studies (Doiron et al., 2009) and can be related to the lipid composition of oat and protein denaturation, which could affect the oleosins that cover the oil bodies, exposing the inside of these oil bodies to one another and allowing them to coalesce (Doiron et al., 2009; Huang, 1996; Huang, 1992). In this study, CP (P=0.09) and ADICP (P=0.73) did not differ among treatments, while SCP was lower (P=0.02) for flaked oat when compared to rolled oat (3.49 and 5.77% DM, respectively) and NDICP was higher (P<0.01) for flaked oat (1.24% DM). For the carbohydrate profile, CHO, NFC, and starch content were higher (P<0.01) for rolled barley when compared to rolled oat grain, and a significant difference was found between heat processed treatments and rolled oat for starch (P<0.01) and NFC (P=0.01). These findings agree with Rahman et al. (2016), that reported an increase of more than 5% DM in starch by dry roasting oat grain. Total CHO was not significantly impacted by heat processing, and this can be related to the balance between increased EE and lower ash, which may have balanced the CHO equation. Both dry-rolled oat and steam-flaked oat had the groat separated from the hull after processing, but a large sample was collected and evenly ground through a 1mm screen. The acid detergent fiber (ADF) was significantly lower for flaked oat when compared to rolled oat (8.43 and 10.29% DM, respectively). Qiao et al. (2015), studying effects of steam flaking in three different types of cereal grains, reported no difference between NDF content for raw and steam-flaked grains, while ADF was increased for steam-flaked maize when comparing to the raw grain (4.07 and 3.05%DM, respectively), but it did not differ between the other two grain types. On the other hand, Doiron et al. (2009), studying Vimy flaxseed, described similar decrease in ADF concentration when the samples were submitted to autoclave treatment at 120°C, showing

a reduction of up to 24%. These results indicate that different grain types and different processing methods react differently to heat processing methods.

Table 4.1. Effect of heat processing methods on chemical profile of oat grain in comparison with barley grain.

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
Basic chemical profile									
DM (%)	87.73	86.70	88.37	85.78	0.984	0.37	0.63	0.96	0.25
Ash (%DM)	3.56	2.92	3.34	2.68	0.256	0.20	0.40	0.55	0.52
EE (%DM)	3.95^a	4.60^a	4.31^a	1.21^b	0.272	<0.01	0.01	<0.01	<0.01
OM (%DM)	96.44	97.08	96.66	97.31	0.256	0.21	0.40	0.55	0.52
Protein profile									
CP (%DM)	13.48	13.64	13.02	11.76	0.401	0.09	0.13	0.10	0.46
SCP (%DM)	5.77^a	3.49^b	4.29^{ab}	3.98^{ab}	0.312	0.02	0.03	0.02	0.20
SCP (%CP)	42.75^a	25.56^b	30.03^{ab}	33.87^{ab}	1.876	0.01	<0.01	<0.01	0.08
ADICP (%DM)	004	0.02	0.02	0	0.024	0.73	0.93	0.94	0.98
ADICP (%CP)	0.31	0.18	0.15	0.02	0.189	0.76	0.91	0.93	0.96
NDICP (%DM)	1.02^b	1.24^a	0.73^c	0.78^c	0.037	<0.01	<0.01	<0.01	0.02
NDICP (%CP)	7.62^{ab}	9.12^a	5.64^c	6.62^{bc}	0.341	<0.01	<0.01	<0.01	0.02
Carbohydrate profile									
CHO (%DM)	79.00^b	78.83^b	79.31^b	84.34^a	0.677	<0.01	0.06	0.03	0.05
Starch (%DM)	48.91^b	52.59^b	47.55^b	66.58^a	1.726	<0.01	0.43	0.07	<0.01
Sugar (%DM)	1.95	1.75	1.96	2.10	0.145	0.48	0.21	0.20	0.72
NFC (%DM)	55.09^b	59.99^b	58.42^b	71.86^a	0.951	<0.01	0.18	0.04	0.01
NFC (%CHO)	69.75^b	76.10^b	73.65^b	85.20^a	1.491	<0.01	0.96	0.49	0.10
NSC (%DM)	50.87^b	54.35^b	49.51^b	65.68^a	1.398	<0.01	0.57	0.08	<0.01

Table 4.1. *Cont'd* Effect of heat processing methods on chemical profile of oat grain in comparison with barley grain.

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
Fiber profile									
aNDF (%DM)	23.91[a]	18.84[ab]	23.91[a]	12.48[b]	1.309	<0.01	0.87	0.71	0.17
ADF (%DM)	10.29[a]	8.43[b]	9.93[ab]	4.36[c]	0.312	<0.01	0.55	0.04	<0.01
ADF (%NDF)	43.18	45.09	47.54	34.98	3.001	0.14	0.41	0.18	0.08
ADL (%DM)	2.72[a]	2.04[ab]	2.11[ab]	0.75[b]	0.281	0.03	0.60	0.42	0.34
ADL (%NDF)	11.49	10.92	10.04	6.04	1.883	0.30	0.47	0.40	0.61
uNDF (%DM)	15.36[a]	13.76[a]	13.41[ab]	3.11[b]	1.772	0.04	0.13	0.07	0.09

SEM: standard error of mean; [a-d] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; DM: dry matter; OM: organic matter; EE: ether extract (crude fat); CP: crude protein; SCP: soluble crude protein; ADICP: acid detergent insoluble crude protein; NDICP: neutral detergent insoluble crude protein; CHO: carbohydrates; NFC: non-fiber carbohydrate; NSC: non-soluble carbohydrate; aNDF: neutral detergent fiber analyzed with amylase; ADF: acid detergent fiber; ADL: acid detergent lignin; uNDF: undigestible neutral detergent fiber analyzed after 288 h in situ incubation.

4.4.2 Energy Profile

Cereal grains store their energy as starch (Hoseney, 1994). The energy value of barley was higher than the oat grains, with NE_L being 0.09 Mcal/kg higher (NRC, 2001). However, in this study, the oat contained higher values of EE and fat contains more energy per unit (+2.25) than protein and carbohydrate (Fuhr, 2006). Results for the impact of processing method on predicted energy values and truly digestible nutrients of oat grain in comparison to barley grain are shown in Table 4.2. The tdNDF (P=0.08) and tdCP (P=0.10) showed no significant differences between grains or processing methods. Rolled barley showed the highest value (P<0.01) for truly digestible NFC, while rolled oat showed the lowest value and was significantly lower (P=0.02) than heat-processed oat (flaked and pelleted). Results related to the truly digestible FA showed significantly higher (P<0.01) values for oat grain when compared to barley, and the heat-processing methods increased the tdFA when compared to dry-rolled oat (+0.5% DM). The total digestible nutrients (TDN_{1x}) was lower (P=0.02) for rolled oat when compared to flaked oat and rolled barley (82.12, 86.47 and 86.87, respectively). Results related to digestible energy at maintenance (DE_{1x}) and production (DE_{p3x}), metabolizable energy (ME_{p3x}), net energy for maintenance (NE_m) and net energy for gain (NE_g) were significantly lower (P≤0.04) for rolled oat when compared to flaked oat and rolled barley. Net energy for lactation (NE_{Lp3x}) was higher (P=0.04) for flaked oat when compared to rolled oat (+0.12 Mcal/kg). Steam flaking corn and sorghum can lead to an increase of 20% in NE_L (Theurer et al., 1999), while in this study the steam flaking process only increased by 6.4%. The energy value for rolled oat in this study was higher than the values determined by NRC dairy (2001), probably because of the high values of fat for these grain (Fuhr, 2006).

Table 4.2. Effect of heat processing methods on truly digestible nutrients, total digestible nutrients and predicted energy values of oat grain in comparison with barley grain.

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
Truly digestible nutrients (%DM)									
tdNDF	12.86	10.58	11.55	8.06	0.955	0.08	0.84	0.92	0.40
tdCP	13.46	13.38	13.01	11.76	0.391	0.10	0.23	0.18	0.45
tdNFC	56.14[b]	61.39[b]	59.53[b]	73.24[a]	1.081	<0.01	0.27	0.07	0.02
tdFA	2.95[a]	3.60[a]	3.31[a]	0.36[b]	0.204	0.01	<0.01	<0.01	<0.01
Total digestible nutrients (%DM)									
TDN_{1x}	82.12[b]	86.47[a]	84.56[ab]	86.87[a]	0.670	0.02	0.06	0.07	0.94
Predicted energy values (Mcal/kg)									
DE_{1x}	3.63[b]	3.81[a]	3.73[ab]	3.81[a]	0.031	0.04	0.07	0.08	0.85
DE_{p3x}	3.33[b]	3.50[a]	3.42[ab]	3.49[a]	0.028	0.03	0.06	0.07	0.78
ME_{p3x}	2.92[b]	3.09[a]	3.01[ab]	3.08[a]	0.029	0.04	0.07	0.07	0.74
NE_{Lp3x}	1.87[b]	1.99[a]	1.93[ab]	1.98[ab]	0.021	0.04	0.06	0.06	0.71
ME	2.97[b]	3.12[a]	3.05[ab]	3.12[a]	0.027	0.04	0.08	0.08	0.89
NE_m	2.01[b]	2.13[a]	2.07[ab]	2.13[a]	0.022	0.04	0.07	0.08	0.93
NE_g	1.35[b]	1.46[a]	1.41[ab]	1.46[a]	0.019	0.04	0.08	0.08	0.92

SEM: standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; tdNDF: truly digestible neutral detergent fibre; tdCP: truly digestible crude protein; tdNFC: truly digestible non-fibre carbohydrate; tdFA: truly digestible fatty acids; TDN_{1x}: total digestible nutrient at one time maintenance. DE_{l3x}: digestible energy at production level of intake (3×); ME_{3x}: metabolizable energy at production level of intake (3×); NE_{L3x}: net energy for lactation at production level of intake (3×); ME: metabolizable energy; NE_m: net energy for maintenance; NE_g: net energy for growth.

4.4.3 Protein and Carbohydrates Subfractions

Results of the protein and carbohydrate fractions according to CNCPS 6.5 are presented in Table 4.3. Steam-flaking decreased the soluble true protein fraction (PA2), which is rapidly degraded in the rumen, by 59%. Rolled oat showed lower values of PB1 (P=0.02) when compared to flaked oat. Sub-fraction PC is bound to lignin, tannins and to protein complexes of Maillard products (Sniffen et al.,1992). Although heat processing methods can increase the Maillard reaction, the results of this study showed no significant influence of heat processing methods on the PC fraction (P=0.76). Total rumen degradable protein did not differ between treatments (P=0.28), but total rumen undegradable protein was significantly higher for flaked oat (P=0.01) when compared to barley (4.35 and 3.30% DM, respectively).

Rodriguez Espinosa (2018) described the impact of steam pressure and microwave irradiation on cool-season adapted faba beans grown in western Canada and reported that the heat-processing methods decreased the rapidly degradable fraction, increased the intermediate degradable fraction and reduced the undegradable fraction of CHO. In this study, heat-processing methods had no effect (P=0.71) on the rapidly degradable fraction (CA4), however steam-flaking and pelleting increased (P<0.01) the fraction related to starches (CB1) when compared to rolled oat (63.32 vs. 61.89% CHO, respectively). Despite the increase, rolled barley still presented significantly higher (P<0.01) values of CB1 when compared to the other treatments. The intermediate degradable fractions CB2 and CB3 did not differ between treatments or grain types (P=0.21 and P=0.07, respectively). The indigestible fibre was higher (P=0.02) for rolled oat when compared to rolled barley, while the heat-processed treatments showed intermediate values of CC. The heat processing (steam-flaking + pelleting) increased (P<0.01) the total digestible CHO

(TRDCHO) when compared to dry-rolled oat (+2.11% DM) but had no impact on total undegradable CHO (TRUCHO) (P=0.16).

Table 4.3. Effect of heat processing methods on protein and carbohydrates subfraction according to CNCPS 6.5 of oat grain in comparison with barley grain.

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
Protein subtractions (%CP)									
PA2	42.75[a]	25.56[b]	33.03[ab]	33.87[ab]	1.88	0.01	<0.01	<0.01	0.08
PB1	49.63[b]	63.31[a]	61.32[ab]	59.50[ab]	2.08	0.02	0.02	0.01	0.06
PB2	7.31[ab]	8.94[a]	5.49[b]	6.60[b]	0.33	<0.01	<0.01	<0.01	0.02
PC	0.31	0.18	0.15	0.02	0.19	0.76	0.92	0.93	0.97
Carbohydrate subfractions (%CHO)									
CA4	2.48	2.23	2.46	2.49	0.18	0.71	0.30	0.31	0.93
CB1	61.89[b]	66.71[b]	59.94[b]	78.93[a]	1.74	<0.01	0.92	0.16	<0.01
CB2	5.37	7.16	11.25	3.78	2.12	0.21	0.89	0.38	0.06
CB3	21.60	17.25	19.74	12.54	1.73	0.07	0.74	0.94	0.27
CC	8.64[a]	6.64[ab]	6.59[ab]	2.26[b]	0.86	0.02	0.46	0.32	0.34
Rumen degradable fractions (%DM)									
TRDP	9.77	9.30	9.16	8.37	0.282	0.10	0.56	0.53	0.81
TRDCHO	53.48[c]	55.88[b]	55.30[bc]	64.56[a]	0.360	<0.01	0.01	<0.01	<0.01
Rumen undegradable fractions (%DM)									
TRUP	3.78[ab]	4.35[a]	3.86[ab]	3.30[b]	0.109	0.01	<0.01	<0.01	0.07
TRUCHO	25.78[a]	23.18[ab]	24.27[a]	20.06[b]	0.637	0.01	0.80	0.76	0.16

SEM: standard error of mean. [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; PA2: soluble true protein; PB1: insoluble true protein. PB2: fiber-bound protein; PC: indigestible protein; CHO: carbohydrates; CA4: sugars; CB1: starches; CB2: soluble fiber; CB3: digestible fiber; CC: indigestible fiber; TRDP: Total rumen degradable protein; TRDCHO: Total rumen degradable carbohydrate; TRUP = Total ruminally undegraded protein; TRUCHO: Total ruminally undegraded carbohydrate.

4.4.4 Rumen Degradation Kinetics

Results for the impact of processing method on ruminal degradation kinetics of dry matter are shown in Table 4.4. The results showed that rumen undegradable dry matter varied (P<0.01) among treatments and flaked oat showed similar values to rolled barley (345 and 378 g/kg, respectively). Oat products showed higher values of effective degradability of dry matter, however, similar to BDM, flaked oat showed similar values to rolled barley. For organic matter degradation, significance followed the same pattern as dry matter. Bypass organic matter (BOM) showed lower values (P<0.01) for rolled and pelleted oat while flaked oat showed similar values to rolled barley (340 and 371 g/kg, respectively).

Rumen degradation of OM is presented in Table 4.5. Heat treatments were previously shown to decrease the degradability of DM and OM in the rumen and consequently increasing the bypass DM and OM to the small intestine (Prates et al., 2018; Rahman et al., 2016). Pelleted oat showed the highest (P<0.01) degradation rate being 55.42%/h higher than rolled barley (11.97%/h). Degradable content (D) was higher for rolled barley (P<0.01) when compared to the other treatments. Heat-processing did not impact (P=0.14) the undegradable fraction (U). The highest values (P<0.01) for bypass OM (BOM) were found for rolled barley (371 g/kg DM and 37.14%), followed by flaked oat (340 g/kg DM and 33.97%), however, flaked oat did not differ from other oat treatments. Rolled barley showed the lowest (P<0.01) values for rumen effective degradability of OM.

In situ degradation of crude protein is shown in Table 4.6. Higher values (P<0.01) of rumen undegradable crude protein (RUP) are seen in flaked oat and rolled barley (48.15 and 43.44, respectively). Chrenkova et al. (2018) reported similar results for flaked wheat, maize, and barley, showing higher RUP (40.1, 67.6 and 49.2 % CP, respectively) and lower rumen degradable protein.

The increase of NDICP (slowly degraded in the rumen), major constituent of RUP (Sniffen et al., 1992), for flaked oat presented by the detailed chemical analysis may be directly related to the increase in RUP shown by the flaking process. Rolled oat presented the highest value (P<0.01) of rumen effective degradable crude protein (112 g/kg DM) followed by pelleted oat (102 g/kg DM). Ljøkjel et al. (2003) found no significant differences between untreated and pelleted oat when comparing EDCP and EDST, which is consistent with the results found in the present study. When the results are accounted in a percentage basis, rolled oat and pelleted oat did not significantly differ, probably because of the lower content of protein in pelleted oat when compared to rolled oat.

The values for bypass starch ranged from 34 g/kg DM (pelleted oat) to 156 g/kg DM (rolled barley), as seen in Table 4.7. BST was significantly impacted by grain type (P<0.01), but it did not differ between rolled oat and heat processed oat (P=0.42). Effective degradability of starch (EDST) was similar for flaked oat and rolled barley when measured in a g/kg DM basis and they showed higher values when compared to the other oat's treatments. However, rolled barley contained the highest amount of starch (66.58% DM) for the studied treatments, followed by flaked oat (52.59 %DM), which may have impacted the high amount of the ruminal effective degradable starch (510 and 470 g/kg DM, respectively). The values for EDST taken in a percentage basis showed that barley truly had a smaller amount of rumen degradable starch when compared to the oat grain treatments (P<0.01). In the present study, no difference was observed for BST when comparing rolled and pelleted oat. Goelema et al. (1999) reported a significant reduction of 51% in BST when a feed mixture of broken peas, lupin, and faba beans was submitted to pelleting conditions at 80°C. The difference between results could have been raised by the incomplete gelatinization of the starch obtained by pelleting of peas, which had a much higher starch gelatinization temperature when compared to oat grain, 50% in 55°C on average (Hoseney, 1994).

Table 4.4. Effect of heat processing methods on in situ rumen degradation kinetics of dry matter (DM) of oat grain in comparison with barley grain.

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
In situ rumen degradation									
Kd (%/h)	49.83[ab]	33.51[bc]	64.86[a]	11.88[a]	7.591	<0.01	<0.01	<0.01	0.01
T0 (h)	0.13	0	0.18	0.25	0.137	0.63	0.37	0.58	0.37
S (%)	17.99[a]	20.74[a]	11.13[ab]	3.98[b]	3.134	<0.01	<0.01	<0.01	0.09
D (%)	59.06[b]	52.92[b]	64.98[b]	90.61[a]	3.651	<0.01	<0.01	<0.01	0.06
U (%)	22.94[a]	26.33[a]	23.88[a]	5.41[b]	1.615	<0.01	<0.01	<0.01	0.17
BDM (g/kg DM)	299.08[b]	345.23[ab]	296.34[b]	377.86[a]	13.292	<0.01	<0.01	<0.01	0.01
EDDM (g/kg DM)	700.92[a]	654.78[ab]	703.66[a]	622.14[b]	13.292	<0.01	<0.01	<0.01	0.01

SEM: standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; Kd: the degradation rate of D fraction (%h); T0: lag time; S: soluble fraction in the in-situ incubation; D: degradable fraction; U: rumen undegradable fraction; BDM: rumen bypass or undegraded feed dry matter; EDDM: effective degraded dry matter.

Table 4.5. Effect of heat processing methods on in situ rumen degradation kinetics of organic matter (OM) of oat grain in comparison with barley grain.

Items	Oat (O) Rolled (R)	Flaked (F)	Pellet (P)	Barley (B)	SEM	P-value	Contrast P-value B vs. O	B vs. FP	R vs. FP
In situ rumen degradation									
Kd (%/h)	51.76[ab]	34.78[bc]	67.21[a]	11.97[c]	8.250	<0.01	<0.01	<0.01	0.01
T0 (h)	0.14	0.00	0.17	0.25	0.137	0.63	0.36	0.57	0.37
S (%)	17.92[a]	20.75[a]	11.04[ab]	3.99[b]	3.158	<0.01	<0.01	0.01	0.10
D (%)	59.57[b]	53.26[b]	65.47[b]	91.26[a]	3.653	<0.01	<0.01	<0.01	0.06
U (%)	22.50[a]	25.98[a]	23.48[a]	4.75[b]	1.549	<0.01	<0.01	<0.01	0.14
BOM (g/kg DM)	292.99[b]	339.72[ab]	291.37[b]	371.44[a]	13.486	<0.01	<0.01	<0.01	0.01
EDOM (g/kg DM)	681.92[a]	641.01[ab]	684.94[a]	610.56[b]	13.500	<0.01	<0.01	<0.01	0.02
%BOM	29.30[b]	33.97[ab]	29.14[b]	37.14[a]	1.35	<0.01	<0.01	<0.01	0.01
%EDOM	70.70[a]	66.03[ab]	70.86[a]	62.85[b]	1.348	<0.01	<0.01	<0.01	0.01

SEM: standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; Kd: the degradation rate of D fraction (%h); T0: lag time; S: soluble fraction in the in-situ incubation; D: degradable fraction; U: rumen undegradable fraction; BOM: rumen bypass dry matter; EDOM: effective degradability of dry matter.

Table 4.6. Effect of heat processing methods on in situ rumen degradation kinetics of crude protein (CP) of oat grain in comparison with barley grain.

| Items | Oat (O) | | | Barley (B) | SEM | P-value | Contrast P-value | | |
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
In situ rumen degradation									
Kd (%/h)	40.66[ab]	18.03[bc]	41.18[a]	10.06[c]	6.402	<0.01	<0.01	<0.01	<0.01
T0 (h)	0.00	0.27	0.16	0.53	0.207	0.36	0.13	0.10	0.46
S (%)	31.58[a]	23.35[ab]	17.26[b]	16.37[b]	3.095	0.01	0.04	0.04	0.76
D (%)	59.17[b]	61.37[b]	70.05[b]	82.02[a]	3.114	<0.01	<0.01	<0.01	0.33
U (%)	9.24[b]	15.28[a]	12.69[ab]	1.61[c]	1.306	<0.01	<0.01	<0.01	0.02
BCP (g/kg DM)	23.02[b]	43.38[a]	27.90[b]	39.14[a]	2.034	<0.01	<0.01	<0.01	<0.01
RUP (g/kg DM)	25.55[b]	48.15[a]	30.97[b]	43.44[a]	2.257	<0.01	<0.01	<0.01	<0.01
EDCP (g/kg DM)	111.77[a]	93.09[c]	102.33[b]	78.57[d]	1.541	<0.01	<0.01	<0.01	<0.01
%BCP=%RUP	17.06[b]	31.77[a]	21.33[b]	33.20[a]	1.343	<0.01	<0.01	<0.01	<0.01
%EDCP	82.94[a]	68.23[b]	78.67[a]	66.80[b]	1.343	<0.01	<0.01	<0.01	<0.01

SEM: standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; Kd: the degradation rate of D fraction (%h); T0: lag time; S: soluble fraction in the in-situ incubation; D: degradable fraction; U: rumen undegradable fraction; BCP: rumen bypassed crude protein in DVE/OEB system; RUP: rumen undegraded crude protein in the NRC Dairy 2001 model; EDCP: effectively degraded of crude protein.

Table 4.7. Effect of heat processing methods on in situ rumen degradation kinetics of starch (ST) of oat grain in comparison with barley grain.

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
In situ rumen degradation									
Kd (%/h)	54.77[ab]	47.05[ab]	93.85[a]	18.09[b]	15.364	0.03	0.02	0.01	0.17
T0 (h)	0.20[b]	0.00[b]	0.11[b]	0.98[a]	0.171	<0.01	<0.01	<0.01	0.46
S (%)	24.71	19.98	9.39	19.24	7.111	0.48	0.88	0.80	0.73
D (%)	72.50	71.37	86.09	79.17	7.053	0.45	0.76	0.99	0.38
U (%)	2.79[b]	8.65[a]	4.52[ab]	1.58[b]	1.029	<0.01	<0.01	0.13	<0.01
BST (g/kg DM)	50.44[b]	55.75[b]	34.44[b]	156.16[a]	13.148	<0.01	<0.01	<0.01	0.42
EDST (g/kg DM)	438.72[b]	470.16[ab]	441.04[b]	509.65[a]	13.80	0.01	<0.01	<0.01	0.10
%BST	10.38[b]	10.63[b]	7.29[b]	23.30[a]	2.021	<0.01	<0.01	<0.01	0.48
%EDST	89.61[a]	89.36[a]	92.70[a]	76.70[b]	2.021	<0.01	<0.01	<0.01	0.48

SEM: standard error of mean; [a-b] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; Kd: the degradation rate of D fraction (%h); T0: lag time; S: soluble fraction in the in-situ incubation; D: degradable fraction; U: rumen undegradable fraction; BST: rumen bypass or undegraded feed starch; EDST: effective degraded starch.

4.4.5 Intestinal Digestion

Intestinal digestion of dry matter and organic matter, as shown in Table 4.8, were significantly higher (P=0.02) for heat processed oat when compared to rolled oat. In this study heat treatments (steam-flaking and pelleting) changed the intestinal digestibility of DM and OM. Heat-processed oat showed higher (P=0.02) values of intestinal digestibility of DM and OM (34.56 %BDM and 34.87 %BOM, respectively) when compared to rolled oat (25.08 % BDM and 25.55 % BOM) respectively. The total-tract digestibility was higher for barley grain when compared to oat grain; however, no significant difference was found between dry-rolled oat and heat-processed oat grain.

Studying the effects of dry roasting and microwave irradiation on oat grain, Rahman et al. (2016) did not notice a significant difference on the intestinal digestibility of RUP of the grains submitted to dry roasting, but microwave irradiation numerically increased the RUP degradation in the small intestine, which could be related to a shift in protein sub-fractions, but also a lower degradability of CP in the rumen. In this study, flaking and pelleting numerically increased the intestinal digestion of bypass crude protein, although values did not significantly differ from rolled oat (P=0.87). Results for intestinal digestibility of CP and ST are presented in Table 4.9. The numerical increase in intestinal digestibility of RUP seen in flaked oat could have been caused by the increased PB2 sub-fraction. The intestinal digestion of rumen bypass starch was significantly lower for flaked oat (P<0.01, 70.74 %BST) when compared to pelleted oat and rolled barley (87.59 and 89.33 %BST, respectively), but it was similar to rolled oat (78.78 %BST). Despite the intestinal digestibility of starch being higher for pelleted oat when on a percentage basis, changing the unit to g/kg of DM showed higher (P<0.01) intestinal digestibility and total-tract digestibility for rolled barley (160 and 650 g/kg DM, respectively).

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Table 4.8. Effect of heat processing methods on intestinal digestion of dry matter (DM) and organic matter (OM) of oat grain in comparison with barley grain.

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
DM intestinal digestion									
%dBDM (%BBDM)	25.08[b]	34.86[b]	34.27[b]	63.27[a]	2.949	<0.01	0.11	0.03	0.02
IDBDM (%BDM)	7.64[b]	12.05[b]	10.15[b]	23.46[a]	1.384	<0.01	0.28	0.05	<0.01
IDBDM (g/kg DM)	23.34[b]	41.70[b]	30.09[b]	101.62[a]	9.087	<0.01	0.36	0.09	0.01
TDDM (%DM)	77.73[b]	77.53[b]	80.52[b]	86.31[a]	0.789	<0.01	0.01	<0.01	0.15
TDDM (g/kg DM)	681.87[b]	672.25[b]	711.53[a]	734.73[a]	5.131	<0.01	<0.01	<0.01	0.63
OM intestinal digestion									
dBOM (%BOM)	25.55[b]	35.33[b]	34.41[b]	71.49	4.293	<0.01	0.11	0.03	0.02
IDBOM (%BOM)	7.63[b]	12.02[b]	10.02[b]	23.69[a]	1.378	<0.01	0.27	0.05	<0.01
IDBOM (g/kg DM)	22.87[b]	40.95[b]	29.20[b]	100.61[a]	9.125	<0.01	0.36	0.09	0.01
TDOM (%DM)	78.33[b]	78.05[b]	80.89[b]	87.34[a]	0.828	<0.01	<0.01	<0.01	0.09
TDOM (g/kg DM)	755.39[b]	757.73[b]	781.81[b]	848.39[a]	7.523	<0.01	<0.01	<0.01	0.06

SEM: standard error of mean; [a-b] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; dBDM: intestinal digestibility of rumen bypass dry matter; IDBDM: intestinal digested rumen bypass dry matter; TDDM: total digested dry matter; dBOM: intestinal digestibility of rumen bypass organic matter; IDBOM: intestinal digested rumen bypass organic matter; TDOM: total digested organic matter.

Table 4.9. Effect of heat processing methods on intestinal digestion of crude protein (CP) and starch (ST) of oat grain in comparison with barley grain.

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
CP intestinal digestion									
dIDP (%RUP)	42.18[b]	54.17[ab]	52.99[ab]	65.28[a]	3.524	<0.01	0.87	0.92	0.87
IDP (%RUP)	7.14[c]	17.25[ab]	11.46[bc]	21.76[a]	1.413	<0.01	0.04	0.13	0.11
IDP (g/kg DM)	9.61[b]	23.55[a]	15.10[b]	25.66[a]	1.980	<0.01	0.01	0.03	0.32
TDP (%CP)	90.08[a]	85.48[b]	90.13[a]	88.56[a]	0.688	<0.01	<0.01	<0.01	0.35
TDP (g/kg DM)	121.39[a]	116.65[a]	117.43[a]	104.23[b]	2.253	<0.01	0.39	0.19	0.12
ST intestinal digestion									
dBST (%BST)	78.78[ab]	70.74[b]	87.59[a]	89.33[a]	3.533	<0.01	<0.01	<0.01	0.28
IDBST (%BCHO)	8.16[b]	7.56[b]	6.52[b]	20.89[a]	1.989	<0.01	0.06	<0.01	<0.01
IDBST (g/kg DM)	39.66[b]	39.68[b]	30.78[b]	159.65[a]	8.132	<0.01	<0.01	<0.01	<0.01
TDBST (% ST)	97.77[ab]	96.9[b]	99.23[a]	97.59[ab]	0.436	0.02	0.03	0.18	0.01
TDBST (g/kg DM)	478.39[b]	509.85[b]	471.82[b]	649.77[a]	11.027	<0.01	0.09	<0.01	<0.01

SEM: Standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; dIDP: intestinal digestibility of rumen bypass protein on percentage basis; IDP: intestinal digested crude protein; TDP: total digested crude protein; dBST: intestinal digestibility of rumen bypass starch on percentage basis; IDBSTP: intestinal digested bypass starch; TDBST: total digested bypass starch.

4.4.6 Hourly Effective Degradation Ratio between N and OM

The hourly effective degradation ratio between available N and available OM at different times for different processing methods of oat grain in comparison to barley grain are shown in Table 4.10. The analysis of the data showed that overall ED_N/ED_OM were higher (P<0.01) for oat products when compared to rolled barley (24.66 and 20.47 g/kg, respectively). Rolled oat showed the highest value of ED_N/ED/OM (26.24 g/kg), while rolled barley showed the lowest value. Although not statistically significant (P=0.43), data analysis showed higher values of ED_N/ED_OM for rolled barley at the beginning of the incubation time (0h), followed by a rapid decrease that made this treatment have the lowest ED_N/ED_OM in all the subsequent time points analyzed. At individual incubation times 4 h, 8 h 12 h and 24 h, pelleted oat showed the highest ratio of degradation between available N and available OM (31.52, 72.06, 168.74 and 2420.78 g/kg, respectively), although the values were not statistically different from the other treatments for oat. So, the highest point in the degradation curve for oat grain was reached at 24 h incubation, while rolled barley had its highest point at the beginning of the incubation period (0h). Huang et al. (2015) noted that increasing temperature during processing can lead to a decrease in ED_N/ED_OM when studying different temperatures and times of pelleting canola meal. In the present study, pelleted had the lowest conditioning temperature of the two heat processing methods. It showed higher hourly values of degradation in all the time points when compared to flaked oat.

Table 4.10. Effect of heat processing methods on hourly effective degradation ratios between N and OM of oat grain in comparison with barley grain.

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
Ratio of N to OM	22.37[a]	22.49[a]	21.56[a]	19.47[b]	0.421	<0.01	0.02	0.01	0.25
Ratio of ED_N/ED_OM	26.24[a]	23.69[b]	24.05[b]	20.47[c]	0.480	<0.01	0.86	0.59	0.27
Ratio at individual incubation hours (g/kg)									
h0	40.74	26.12	38.93	72.92	18.004	0.43	0.26	0.2	0.45
h2	21.23	16.93	21.06	15.52	1.367	0.04	0.17	0.42	0.15
h4	27.03[a]	23.14[a]	31.52[a]	12.77[b]	3.015	<0.01	0.82	0.29	<0.01
h8	34.48[bc]	46.34[ab]	72.06[a]	13.52[c]	7.798	<0.01	0.45	0.03	<0.01
h12	99.66[ab]	101.19[ab]	168.74[a]	14.33[b]	39.497	0.04	0.85	0.27	0.01
h24	1232.26	1572.16	2420.78	17.19	1003.44	0.24	0.71	0.35	0.09

SEM: Standard error of mean; [a-c] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparison using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; N: nitrogen; OM: organic matter; ED: effective degradability.
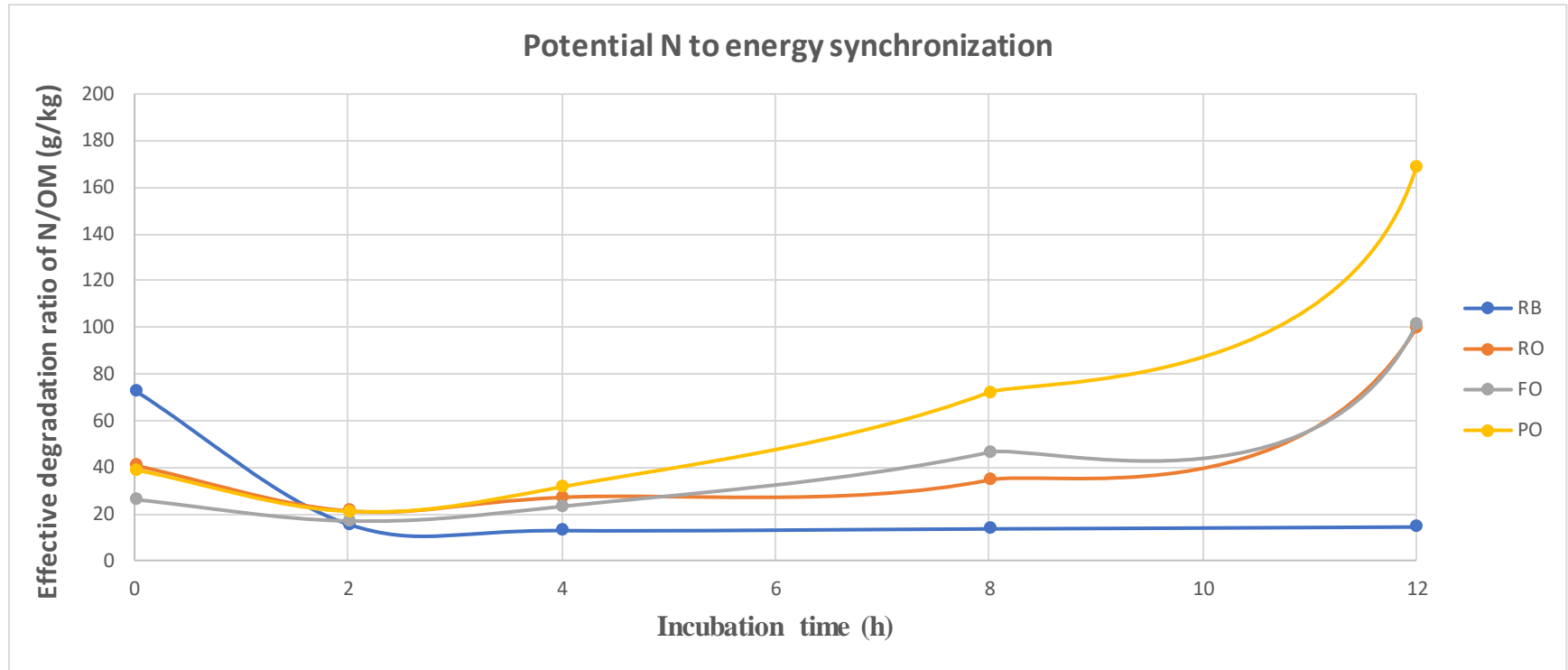
Figure 4.1. Hourly effective degradation ratios between available N and available OM (ED_N/ED_OM) of different varieties of oat grain in comparison to barley grain.

4.4.7 Nutrient Supply and Feed Milk Value

Metabolic characteristics and true nutrient supply based on the DVE/OEB system are presented in Table 4.11. The microbial protein synthesized in the rumen based on available energy (MREE) did not show any difference (P=0.14) between treatments. On the other hand, the potentially synthesized microbial protein based on available nitrogen (MREN) showed a significant decline (P<0.01) when oat was submitted to heat treatment (steam-flaking and pelleting) (-15.45). The total true protein degradable and absorbed in the small intestine (DVE) was higher (P<0.01) for rolled barley (80.80 g/kg DM), followed by flaked oat (66.95 g/kg DM). The degraded protein balance was higher (P<0.01) for rolled oat when compared to the other treatments, with flaked oat and pelleted oat showing intermediate levels and rolled barley showing the lowest value. According to Tamminga et al. (1994), the optimal value for OEB is zero or slightly above, and in this case, oat submitted to heat processing had the best value, suggesting that these treatments would have a lower loss of N and adequate supply of energy to the rumen for microbial protein growth. The predicted feed milk value was higher (P<0.01) for rolled barley, followed by flaked oat (1.64 and 1.36 kg milk/kg DM fed, respectively). Doiron et al. (2009) found a similar increase of DVE and decrease in OEB when flaxseed was autoclaved, despite none of their treatments reaching a negative value.

Data for the metabolic characteristics and true nutrient supply based on the NRC model are shown in Table 4.12. The microbial protein synthesized based on available TDN (MCP_{TDN}) was higher (P<0.01) for rolled barley and flaked oat (103.72 and 103.24 g/kg DM, respectively) and lower for pelleted oat (-2.76 g/kg DM) and rolled oat (-5.67 g/kg DM). Heat treatments significantly reduced (P<0.01) the amount of microbial protein that could be potentially synthesized based on rumen degradable protein (MCP_{RDP}). The degraded protein balance was

significantly reduced with heat-treatment, steam-flaking and pelleting (-24.81 and -12.88 g/kg DM, respectively). The predicted feed milk value was not impacted by treatments (P=0.06), processing methods (P=0.57) or grain type (P=0.10).

Table 4.11. Effect of heat processing methods on metabolic characteristics and truly absorbable nutrient supply (based on non-TDN system: DVE-OEB) of oat grain in comparison with barley grain

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
Truly digestible nutrient supply to dairy cattle (g/kg DM)									
BCP	25.55[b]	48.15[a]	30.97[b]	43.44[a]	2.257	<0.01	<0.01	<0.01	0.22
EDCP	111.17[a]	93.09[c]	102.33[b]	78.57[d]	1.541	<0.01	0.03	0.29	<0.01
MREE	95.97	91.17	100.99	98.67	2.858	0.14	0.04	0.10	0.31
MREN	109.24[a]	88.32[c]	99.26[b]	74.23[d]	1.606	<0.01	<0.01	0.11	<0.01
DVME	61.18	58.12	64.38	62.90	1.822	0.14	0.04	0.10	0.31
DVBE	15.14[b]	25.57[a]	13.10[b]	26.29[a]	2.148	<0.01	0.01	0.09	0.01
Degraded protein balance (OEB) and Total true protein supply (DVE) to dairy cows (g/kg DM)									
DVE	59.71[b]	66.95[ab]	62.74[b]	80.80[a]	3.542	<0.01	0.85	0.46	0.11
OEB	13.26[a]	-2.86[b]	-1.73[b]	-24.44[c]	2.708	<0.01	0.65	0.43	0.27
Feed milk value (kg milk/kg DM fed)									
FMV	1.21[b]	1.36[ab]	1.27[b]	1.64[a]	0.071	<0.01	0.85	0.45	0.10

SEM: Standard error of mean; [a-c] Means with different letters in the same row are significantly different (P<0.05); Multi-treatment comparisons using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; BCP: bypass crude protein; MREE: microbial protein synthesized in the rumen based on available energy; EDCP: effective degradability of CP; MREN: microbial protein synthesized in the rumen; DVME: rumen synthesized microbial protein digested in the small intestine; DVBE: truly absorbed bypass protein in the small intestine; DVE: truly digested protein in the small intestine; OEB: degraded protein balance; FMV: feed milk value.

Table 4.12. Effect of heat processing methods on metabolic characteristics and truly absorbable nutrient supply (based on TDN system: NRC dairy) of oat grain in comparison with barley grain.

	Oat (O)						Contrast P-value		
Items	Rolled (R)	Flaked (F)	Pellet (P)	Barley (B)	SEM	P-value	B vs. O	B vs. FP	R vs. FP
Truly digestible nutrient supply to dairy cattle (g/kg DM)									
RUP	23.02[b]	43.38[a]	27.90[b]	39.14[a]	2.034	<0.01	<0.01	<0.01	0.23
MCP_{TDN}	98.05[c]	103.24[a]	100.96[b]	103.72[a]	0.464	<0.01	<0.01	<0.01	0.90
MCP_{RDP}	95.00[a]	79.13[c]	86.98[b]	66.78[d]	1.309	<0.01	0.03	0.29	<0.01
AMCP	60.80[a]	50.64[c]	55.67[b]	42.74[d]	0.837	<0.01	0.03	0.29	<0.01
ARUP	13.64[b]	23.04[a]	11.80[b]	23.68[a]	1.935	<0.01	0.01	0.09	0.01
ECP	10.41[ab]	10.29[ab]	10.49[a]	10.19[b]	0.068	0.04	0.38	0.93	0.04
AECP	4.17[ab]	4.12[ab]	4.19[a]	4.07[b]	0.026	0.03	0.39	0.94	0.04
Total metabolizable protein supply and degraded protein balance to dairy cattle (g/kg DM)									
MP	78.61[a]	77.80[a]	71.66[a]	70.50[a]	2.081	0.03	0.10	0.23	0.28
DPB	-3.92[a]	-28.73[c]	-16.80[b]	-43.82[d]	1.200	<0.01	<0.01	<0.01	<0.01
Feed milk value (kg milk/kg DM fed)									
FMV	1.58	1.58	1.47	1.43	0.041	0.06	0.10	0.16	0.57

SEM: Standard error of mean; [a-d] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparisons using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; RUP: rumen undegradable feed crude protein; MCP_{TDN}: rumen synthesized microbial protein base on available TDN; MCP_{RDP}: microbial protein synthesized in the rumen based on available protein; AMCP: truly absorbed microbial protein in the small intestine; ARUP: truly absorbed rumen undegradable protein in the small intestine; ECP: rumen endogenous protein; AECP: truly absorbed rumen endogenous protein in the small intestine; MP: metabolizable protein; DPB: rumen degraded protein balance; FMV: feed milk value.

4.4.8 Protein Molecular Spectra

Results for the impact of processing method on protein molecular structure of oat grain in comparison to barley grain is shown in Table 4.13. The different processing methods did not significantly affect the Amide I height (P=0.17), Amide II height (P=0.11) or Amide I/Amide II height ratio (P=0.5) when comparing all treatments, however, rolled oat was significantly different from heat-processed oat for Amide II height and area (P=0.03). Amide I area tended to be significantly different between treatments (P=0.09) and significantly differ for rolled oat when compared to heat-processed oat (P=0.04). Xu et al. (2018) found a strong positive correlation between Amide I and Amide II peak area and rumen degradable protein (RDP), but in the present study, higher values of Amide I and II area were found for flaked oat (4.26 and 1.15, respectively) and are presented together with lower EDCP and a higher RUP. Analysis of the protein secondary structure profile revealed that α-helix did not significantly differ between treatments (P=0.18), although it tended to differ between rolled oat and heat-processed oat (P=0.07). β-sheet height showed significant difference between rolled oat and heat-processed oat (P=0.02) with flaked oat being 40% higher than rolled oat (0.0489 and 0.0292, respectively). The heat processing did not impact the α-helix to β-sheet ratio among treatments (P=0.52). The lack of significant difference between treatments was similar to findings reported by Huang et al. (2015), but these are in contrast with the molecular changes induced by heat processing methods described in other studies (Prates et al., 2018; Rodriguez Espinosa, 2018).

The principal component analysis (PCA) was able to group different processing methods of oat and barley grain by its whole Amide related region, however none of the treatments was clearly separated from the other, being possible to see overlaps, and implying similar molecular structure in terms of protein make up in some degree (Figure 4.3). Principal component one (PC1)

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explained 74% of the variation between spectra data while PC2 explained 18% of the variation. The same overlap could be seen in the hierarchical cluster analysis (HCLA), with pelleted and rolled oat being grouped into one cluster, while rolled barley and flaked oat was cluster into another group (Figure 4.4).
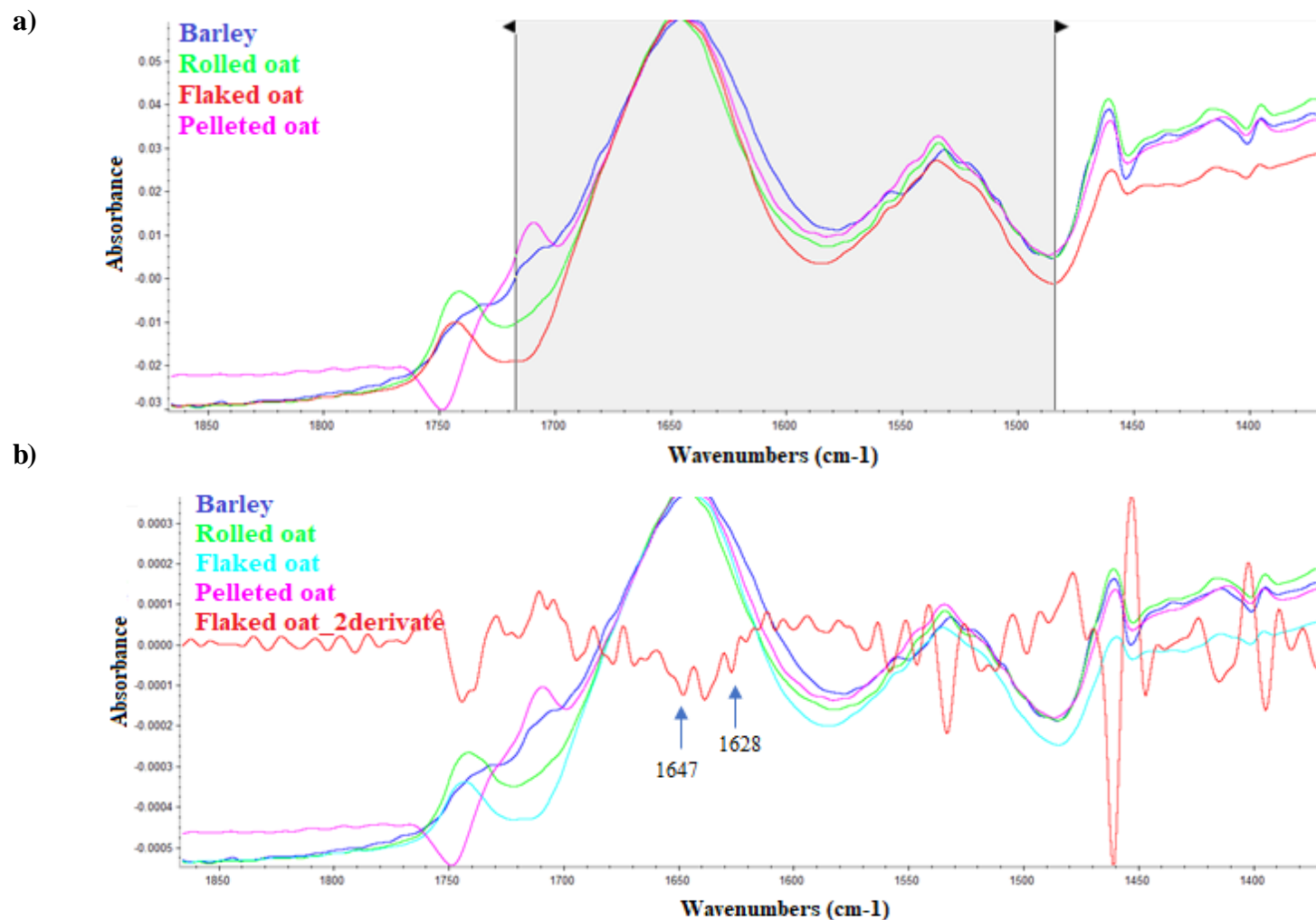
Figure 4.2. (a) Fourier transformed infrared attenuated total reflectance (Ft-IR/ATR) biomolecular spectra of different processed oat grain in comparison with barley grain of the protein molecular structures, amide I and amide II; (b) Protein secondary structures α-helix and β-sheet heights.

Table 4.13. Effect of heat processing methods protein molecular structure profile of oat grain in comparison with barley grain.

	Oat (O)						Contrast P-value		
Items	Rolled (R)	Flaked (F)	Pellet (P)	Barley (B)	SEM	P-value	B vs. O	B vs. FP	R vs. FP
Amide heights and spectra ratio									
Amide I	0.04	0.06	0.03	0.04	0.008	0.17	0.80	0.59	0.07
Amide II	0.02	0.02	0.01	0.01	0.003	0.11	0.94	0.35	0.03
Amide I/Amide II	2.57	2.38	2.57	3.47	0.500	0.5	0.71	0.51	0.35
Secondary structure heights and spectra ratio									
α-helix	0.04	0.06	0.03	0.04	0.008	0.18	0.84	0.58	0.07
β-sheet	0.03^{ab}	0.05^{a}	0.02^{b}	0.04^{ab}	0.004	0.03	0.30	0.92	0.02
A-helix/β-sheet	1.46	1.24	1.48	1.16	0.096	0.18	0.21	0.31	0.52
Amide area and spectra ratio									
Amide I	2.45	4.27	1.15	2.95	0.621	0.09	0.66	0.62	0.04
Amide II	0.56	1.16	0.18	0.38	0.218	0.12	0.97	0.35	0.03
Amide I/Amide II	4.41	3.70	9.89	17.28	6.855	0.54	0.5	0.34	0.3

SEM: standard error of mean; $^{a-b}$ Means with different letters in the same row are significantly different (P<0.05); R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat.

Figure 4.3. Multivariate spectral analyses of different processed oat grain in comparison with barley grain using FTIR vibrational spectroscopy at whole Amide region (ca. 1710-1480 cm^{-1}). PCA (principal component analysis) with a scatter plot of the 1st principal components (PC1) vs. the 2nd principal components (PC2). RB: rolled barley; RO: rolled oat; FO: flaked oat; PO: pelleted oat.
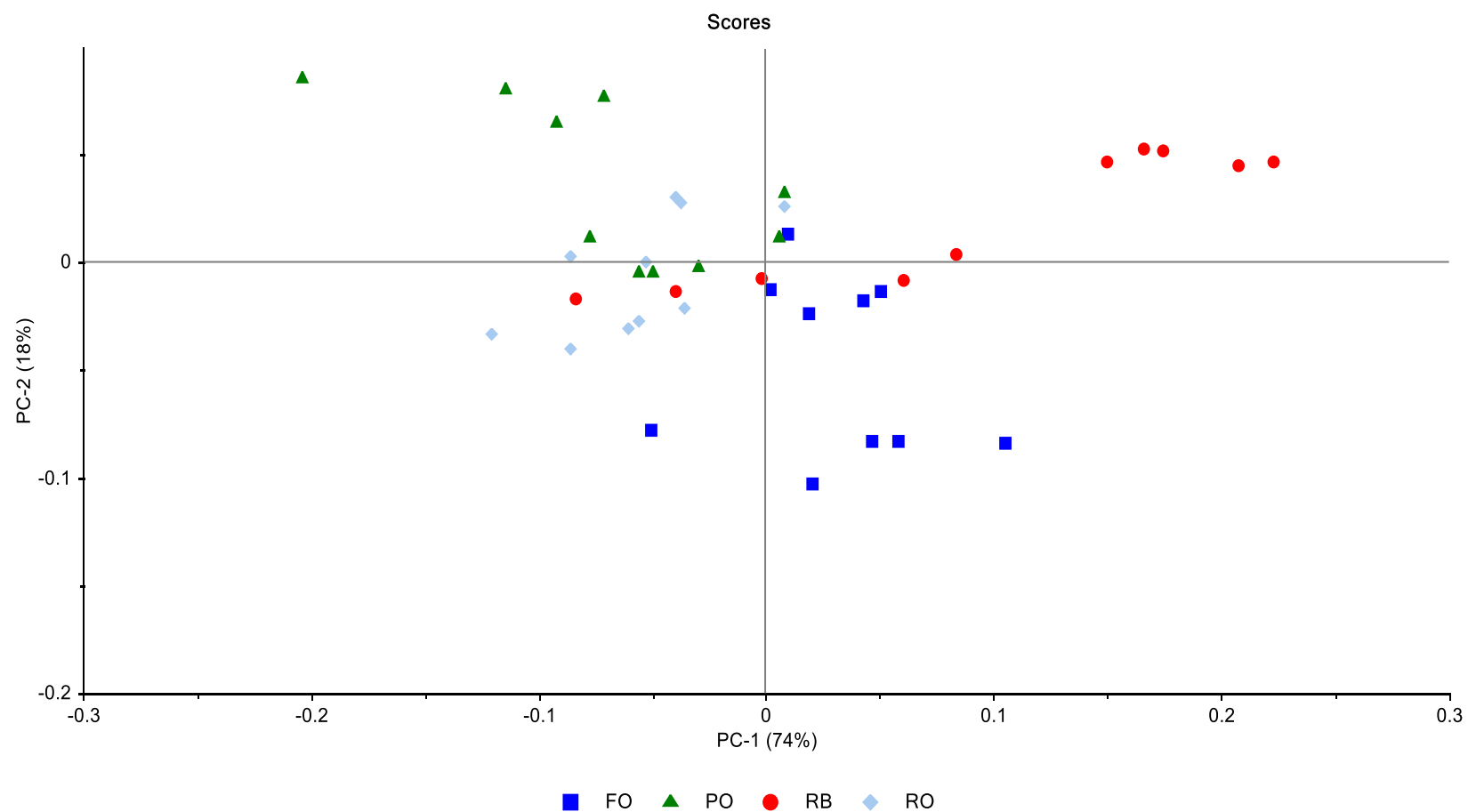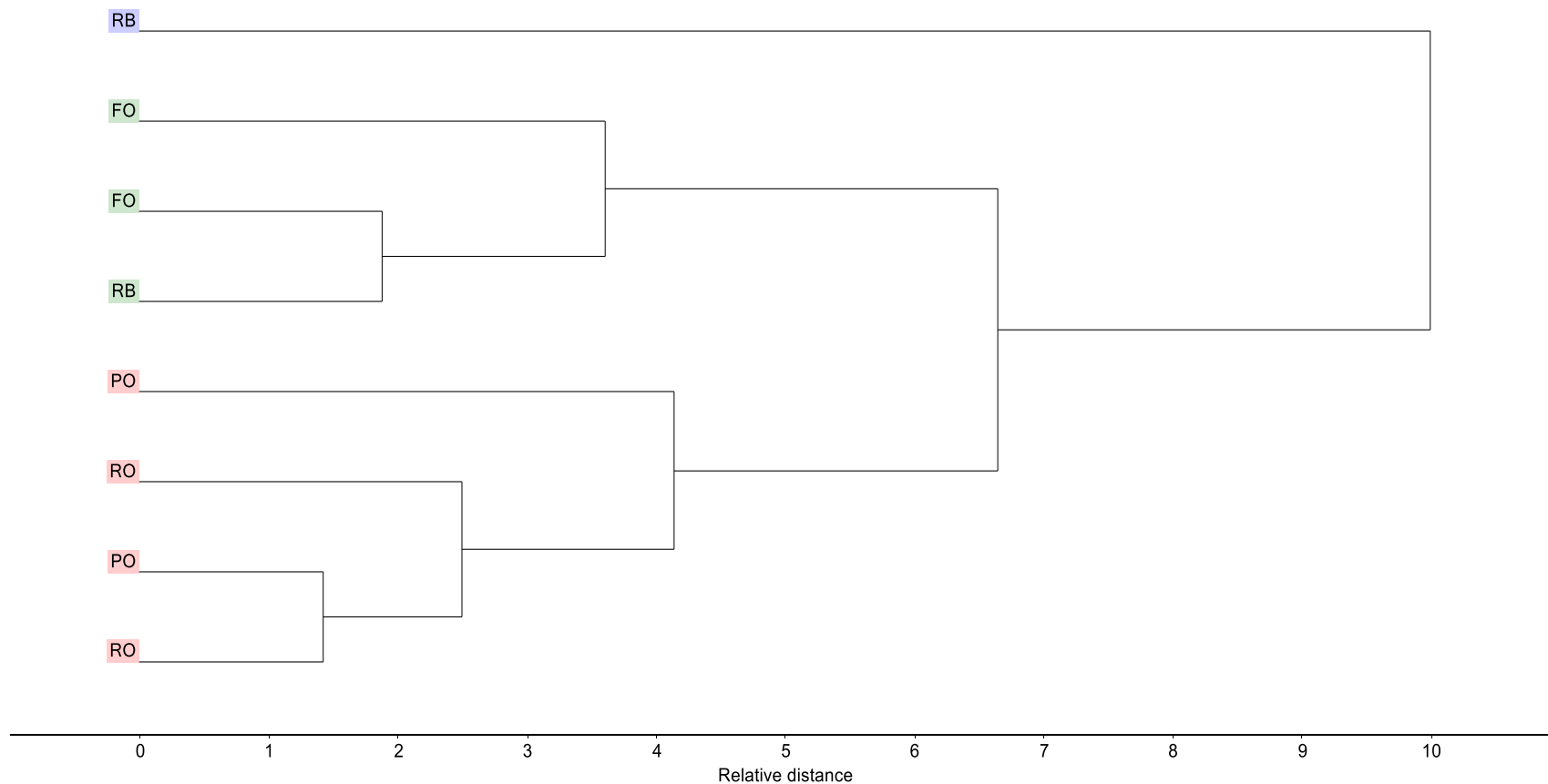
Figure 4.4. Multivariate spectral analyses of different processed oat grain in comparison with barley grain using FTIR vibrational spectroscopy at whole Amide region (ca. 1710-1480 cm^{-1}). CLA (cluster analysis): cluster method (Ward's algorithm) and distance method (Squared Euclidean). RB: rolled barley; RO: rolled oat; FO: flaked oat; PO: pelleted oat.

4.5 Chapter summary and conclusions

In conclusion, the present study showed that heat processing methods altered nutritional and metabolical characteristics of oat grain, however, the effects caused by steam-flaking and pelleting were different. Both heat-treatments increased the ether extract of oat grain (+0.50%DM), while only steam-flaking increased the total digestible nutrients. Energy values were higher for flaked oat when compared to rolled oat, and although pelleting increased numerically the energy value, it was not significantly different from dry-rolled oat. Steam-flaking also reduced the PA2 fraction by 40.12%, while increasing the PB1 fraction (-13.68% CP). Steam-flaking and pelleting increased the rumen undegradable CP while decreasing the effective degradability of crude protein in the rumen. Rolled barley showed higher total tract digestibility of DM and OM while having a lower total tract digestibility of CP and starch when compared to oat grain. The three treatments for oat showed the closest ratio between N and OM degradation ratio to the optimum value pointed by Sinclair et al. (1993). Rolled oat showed a higher degraded protein balance while rolled barley showed a low OEB; oat submitted to heat-treatments showed values of OEB close to 0, characterized as the optimal ratio (Tamminga et al., 1994). Heat treatment did not seem to have substantially modified the protein molecular structure, but pelleted oat had a significant decrease in β-sheet height when compared to flaked oat. The principal component analysis showed an overlap between all the treatments in this study, suggesting a similar molecular structure in terms of protein make-up of the grains and processing methods studied.

5. IMPACT OF PROCESSING METHODS ON DAIRY COWS PRODUCTION PERFORMANCE AND METABOLIC PARAMETERS

5.1 Abstract

Several processing techniques can be used to slow degradation rate in the rumen and thus provide more bypass CP and starch to the small intestine. The aim of this study was to evaluate the effect of processing methods on oat grain compared to dry rolled barley grain when fed as TMR for lactating dairy cows. Eight lactating Holstein cows were used in a replicated 4×4 Latin square design with 21-day periods and fed TMR's with one of four treatments (dry-rolled oat, steam-flaked oat, pelleted oat or dry-rolled barley), using the same treatments as chapter 4. DMI ranged from 28.19 to 31.61 kg/d and was lower for rolled oat when compared to pelleted oat; despite the nutrient intake being higher for pelleted oat fed cows, rolled oat was responsible for the highest milk production and milk fat percentage (49.23 kg/d and 4%, respectively), and significantly higher than pellet oat and rolled barley. Ruminal fermentation characteristics were similar across treatments with only notable differences in acetate (lowest value for pelleted oat) and total SCFA (highest value for rolled barley) concentration and a lower pH for flaked oat at the 9 h and 12 h point. Dietary treatments did not affect total-tract digestibility of DM, OM, and CP; digestibility of starch was lower for rolled barley (89.04%). Measured blood metabolites, urea, glucose, and BHBA, were not affected by dietary treatment. Purine derivatives and microbial N supply were also unaffected by dietary treatments. Cows fed flaked oat-based TMR showed the lowest N excretion in milk, however, the lack of difference between diets on urinary N and fecal N excretion resulted in no significant changes in N balance between diets. Therefore, rolled oat allow cows to have higher milk production with lower DMI.

5.2 Introduction

Lactating dairy cows require a high energy intake to maintain milk production and drive microbial protein synthesis. Typically, dairy cow rations contain 40-50% concentrates, that are used to supply starch as an energy source. In western Canada, barley grain is widely used as a concentrate in dairy farms, however, the prairies region of Canada is responsible to produce more than 90% of oat in the country (Statistics Canada, 2018), and with a lower price per tonne, oat can be a cost-effective source of digestible energy. Although oat grain has lower digestible energy and net-energy for lactation when compared to barley grain (NRC, 2001), several studies showed no negative impact of replacing barley by oat grain in production performance (Ekern et al., 2003; Fuhr, 2006; Gozho and Mutsvangwa, 2008).

Despite cereal grains such as barley and oat being widely used in ruminants diets, the whole cereal grains is resistant to digestion by ruminants mainly due to the protective seed coat and the ligneous hull that shields the groat from microbial degradation in the rumen (Morgan and Campling, 1978). Therefore, processing techniques are required to break the hull and expose the grain to microbial attachment and subsequent digestion. Processing techniques can also be applied to manipulate ruminal rate and extent of degradation and hence increase the availability of nutrients for the rumen and small intestine (Chrenkova et al., 2018; Svihus et al., 2005).

Therefore, the objective of this study was to evaluate the effect of replacement of rolled barley grain with three types of processed oat (dry-rolled, steam-flaked and pelleted) on the performance, total-tract digestibility, N-balance, ruminal fermentation characteristics and metabolic parameters in lactating dairy cows.

5.3 Material and Methods

5.3.1 Animals and Diets

Eight lactating multiparous Holstein dairy cows (average of 715 kg BW; 79±12 DIM; parities 2.5±1.5, average initial milk yield 51±6.9 kg/d) were used in a replicated 4 × 4 Latin square design with 21 days periods (17 days of dietary adaptation and 4 days of sample collection). Four cows in one Latin square were ruminally cannulated to allow the measurement of ruminal fermentation characteristics. All cows were housed in individual tie stalls in the Rayner Dairy Teaching and Research Facility (University of Saskatchewan, Saskatoon, Canada) with free access to water. A 7 days adaptation period was established prior to the beginning of the experimental period in order to get the animals accustomed to the new environment. The University of Saskatchewan Animal Care Committee approved the animal trial under the Animal Use Protocol No. 19910012 and animals were cared for and handled in accordance with the Canadian Council of Animal Care (CCAC, 1993) regulations.

The cows were fed diets containing 54% forage and 46% concentrate containing grains pertaining to one of the four treatments: R=Rolled oat, F=Flaked oat, P=Pelleted oat or B=Rolled barley, as shown in Table 5.1. Grains processing methods are described in chapter 4, with estimated prices for dry rolling, steam-flaking and pelleting of $6, $10 and $24 (grinding+pelleting) per ton, respectively. The diets were formulated using NDS Professional (Version 3, RUM&N-NDS Professional, Reggio Nell'Emilia, Emilia-Romagna, Italy). Cows were fed daily at 0800 as a TMR, refusals were collected every morning before feeding and weighted to estimate dry matter intake (DMI). Dry matter intake reported was calculated based on the 4 days of the collection period. Feed offerings were adjusted daily to allow for a 5-10% refusal (on as fed basis). Samples of alfalfa hay and barley silage were collected twice a week and DM content was determined, allowing adjustments to the diet to maintain the forage to concentrate ratio. Feed ingredients and orts were sampled in the last four days of each period

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and pooled together by cow and by period before being ground through a 1 mm screen and submitted to chemical analysis.

Table 5.1. Ingredient composition of experimental diets fed to lactating Holstein cows as total mixed ration (TMR).

| | Diets | | | |
| | | Oats | | |
Items	Barley	Flaked	Rolled	Pelleted
Ingredient composition, %				
Barley silage	36.82	36.82	36.82	36.82
Alfalfa hay	17.33	17.33	17.33	17.33
Rolled barley	15.54	-	-	-
Flaked oat	-	15.54	-	-
Rolled oat	-	-	15.54	-
Pelleted oat	-	-	-	15.54
RP10 Palmitic	1.3	1.3	1.3	1.3
Canola meal	8.63	8.63	8.63	8.63
Corn grain	6.62	6.62	6.62	6.62
Soybean meal	5.13	5.13	5.13	5.13
Peas	3.98	3.98	3.98	3.98
Soybean hulls	1.74	1.74	1.74	1.74
Urea	0.11	0.11	0.11	0.11
Tallow	0.80	0.80	0.80	0.80
Premix[1]	1.04	1.04	1.04	1.04
PotMag Sulfate	0.21	0.21	0.21	0.21
Sodium Bicarbonate	0.46	0.46	0.46	0.46
Limestone	0.13	0.13	0.13	0.13
Rumensin	0.01	0.01	0.01	0.01
Ameribond	0.15	0.15	0.15	0.15
Chemical composition				
Dry matter	53.16	52.62	54.41	53.80
Crude protein, %DM	16.96	16.56	16.75	17.03
Starch, %DM	15.90	15.68	16.96	15.82
Ether extract, %DM	4.75	4.72	4.93	4.80
Neutral detergent fibre, %DM	34.47	35.55	33.65	34.51
Acid detergent fibre, %DM	23.02	23.95	22.40	22.91
NE_L, Mcal/kg of DM	1.65	1.63	1.67	1.65

Diets: B= Rolled Barley, F= Flaked Oat, R= Rolled Oat and P= Pelleted Oat; Mineral-Vitamin Premix contained: 16% DM Ca, 7% DM P, 7% DM Mg, 2% DM K, 10% DM Cl, 1.25% DM S, 1507 ppm Mn, 678 ppm Cu, 1005 ppm Fe, 2513 ppm Zn, 80 ppm I, 30 ppm Co, 20 ppm Se, 251 256 IU/kg Vit. D3, 2010 IU/kg Vit E.

5.3.2 Milk Production and Composition

Cows were milked three times a day at 0500, 1200 and 2000 h. Milk yield was recorded in the first three days of every collection period (days 18, 19 and 20), and milk samples were collected at every milking during the same period, preserved with potassium dichromate, and sent to CanWest DHI (Edmonton, AB) to be tested for milk fat (%), protein (CP %), lactose (%), total solids (%), milk urea N and somatic cell count (SCC, determined using near infrared analyzer, Foss System 4000, Foss Electric, Hillerød, Denmark). Yield of milk fat, protein, and lactose were calculated multiplying the item percentage by the milk yield. Fat-corrected milk (FCM) was determined as: 3.5% FCM = (0.434 × kg of milk) + (16.216 × kg of milk fat). Energy-corrected milk was calculated as ECM = (0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.2 × kg of milk protein) (Chibisa et al., 2015). Feed efficiency was calculated as milk yield/DMI, FCM/DMI, and ECM/DMI.

5.3.3 Ruminal Parameters

Ruminal parameters were analyzed on the last day of sampling (day 21), every three hours, starting right after feeding (0800) and ending right before the next morning feeding time, so the collected samples represented 24 h feeding cycle. The digesta were collected from three different areas of the rumen (cranial ventral, rumen ventral, and caudal ventral) and filtered through 4 layers of cheesecloth. The pH was measured with a pH meter (Accumet AP110, Fisher Scientific) and 10 mL was taken and immediately mixed with 2 mL of metaphosphoric acid at 25% wt/vol (H_2PO_4) and stored at -20°C for later determination of SCFA by gas-chromatography using the TRACE 1310 (Thermo Fisher Scientific, Waltham, MA, USA) at the Ministry of Agriculture Strategic Feed Research Chair Lab (Dept. Animal and Poultry Sciences, University of Saskatchewan, Canada).

5.3.4 Total Collection of Urine and Feces

For determination of apparent total-tract digestibility and N balance, a 3-day total collection of urine and feces were conducted from 0700 on day 18 to 0700 on day 20. Total urine output was collected using Foley bladder catheters (26 Fr, 75-mL ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA). Catheters were inserted at 0900 h on day 17 and were connected to a urine collection container using a hose at 0800 on day 18. Urine output was weighted, volume was quantified, and samples were collected at 1300, 1900, 0100 and 0700, daily. At the end of each sampling day, samples were pooled by cow, by day, and a 10 mL aliquot was mixed with 40 mL of H_2SO_4 and stored at -20°C for analysis. Total N was measured using the Kjeldahl method, uric acid, and creatinine content were quantified using a uric acid assay kit (Item No. 700320) and a creatinine assay kit (Item No. 500701), respectively, by Cayman Chemical (Cayman Chemical, Michigan, USA). The concentration of allantoin was quantified using the colorimetric method described by Cheng and Gomes (1992). Total purine derivative (PD) excretion per day was used to estimate microbial N yield as described by Cheng and Gomes (1992). Nitrogen retained was calculated as intake N – fecal N – urinary N – milk N. Milk N was determined as milk CP ÷ 6.38.

Feces were collected in large steel trays, which were positioned to cover the gutter behind each stall. Daily fecal output was measured by weight, taken at the same time points as urine sampling, mixed thoroughly before 3% was sampled for each time point. At the end of each collection day, samples of feces were pooled by cow by day and dried at 60°C for 48 h (AOAC 1990, official method 930.15) and ground through a 1 mm screen for analysis of DM, OM, CP, NDF, and starch (AOAC, 2005)

5.3.5 Blood Sampling

On the last day of sampling (day 21), blood samples were collected every six hours at 0800, 1400, 2000, 0200 and 0800 in the next morning right before feeding, via a jugular vein catheter into a 10 mL vacutainer tube containing heparin (Becton Dickinson, NJ, USA). Blood samples were placed in ice water immediately after the collection until samples for all animals were obtained and then centrifuged at $1200 \times g$ for 15 min at 4°C. Plasma was subsequently stored at -20°C until analysis. Beta-hydroxybutyrate (BHBA) was determined according to Williamson et al. (1962), blood urea nitrogen (BUN) was determined using the method of (Fawcett and Scott, 1960) and plasma glucose concentration was determined using a glucose oxidase/peroxidase enzyme and dianisidine dihydrochloride method as described by Oba et al. (2010).

5.3.6 Statistical Analysis

The data was analyzed using the Procedure Mixed of SAS 9.4 (SAS Institute, NC). Milk yield and composition, apparent total-tract digestibility, urinary, and blood parameters and ruminal SCFA were analyzed following the model:

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

where Y_{ijk} was the observation of the dependent variable, μ was the population mean, T_i was the fixed effect of treatment i, P_j was the fixed effect of period j, C_k was the random effect of the cow and e_{ijk} was error associated with the observation. Prior to analysis the best variance and covariance structure model was selected based on the AIC and BIC values.

Prior to the statistical analysis, all outlier data were removed, using the same model, with a criterion of Studentized Residual greater than 2.5. For all statistical analyses, significance was declared at P<0.05 and trends at 0.05<P<0.10. The differences among the treatments were compared using a multiple comparison test following the Tukey method. Contrast statements

were used to compare the difference between barley grain and oat grain, rolled barley and heat-processed oat grains, and rolled oat and heat-processed oat.

5.4 Results and Discussion

5.4.1 DMI, Milk Production and Composition

Dry matter intake, milk yield, milk composition, and feed efficiency are shown in Table 5.2. Dry matter intake was higher (P=0.01) for cows fed pelleted oat-based diet than cows on the rolled oat based TMR. Cows on rolled barley, flaked oat and rolled oat consumed 1.90, 2.06 and 3.42 kg less, respectively, than cows fed pelleted oat diet. However, milk yield was significantly higher (P<0.01) for cows fed rolled oat-based TMR when compared to the other diets, but the contrast statement showed no significant difference (P=0.39) between rolled oat and heat-processed oat (steam-flaked and pelleted). The milk yield values measured were different from the feed milk value (FMV) prediction reported on study 2. This difference arises from the fact that, in study 2, only the FMV of grains were being reported, while in this study, cows were fed a TMR containing several different ingredients. Overall cows fed oat-based diets produced an average of 1.17 kg milk more milk (P<0.01) than cows fed barley-based TMR. Several comparative studies investigated production responses in trials comparing grain types and different processing methods (Ekern et al., 2003; Fuhr, 2006; Gozho and Mutsvangwa, 2008; Safaei and Yang, 2017). Additionally, some of the studies in the literature showed variable production responses. Gozho and Mutsvangwa (2008) reported no differences in DMI and milk yield for cows being fed TMR's containing barley grain, corn grain, wheat or oat grain. Ekern et al. (2003) on the other hand reported higher milk yield for cows being fed oat grain.

Fat percentage ranged from 4.00 to 3.62%, with cows fed pelleted oat-based TMR showing the lowest (P<0.01) fat percentage. Milk fat depression has been previously reported

for cows being fed a pelleted concentrate (Dos Santos et al., 2011). Increased ruminal release of fat from pelleted oat could modify FA metabolism and change biohydrogenation pathways. Dos Santos et al. (2011) also showed that pelleting increased trans-C18:1 FA concentration in milk, which is correlated with milk fat depression (Harvatine, 2016). Despite that, fat yield and FCM were not significantly different (P>0.10) for all the treatments. Protein percentage was higher (P<0.01) for barley-based TMR and lower for flaked oat-based diet. In contrast, Cooke et al. (2008) reported higher milk protein production per day, while maintaining protein and fat percentage, and fat production. Similarly, Zhong et al. (2008) reported similar fat production for cows fed finely ground corn or steam-flaked corn-based TMR, but cows being fed steam-flaked corn produced 0.21% of milk protein more. Milk urea N (MUN) had no significant difference between treatments (P=0.27). Somatic cell count (SCC) ranged from 32.56 to 39.35 10^3 cell ml^{-1} and showed no difference (P=0.83) between treatments. The lower DMI of cows fed a rolled oat-based diet impacted feed efficiency parameters in this study. Milk yield/DMI and FCM/DMI were both higher for rolled oat (P≤0.02) when compared to flaked oat.

Table 5.2. Dry matter intake, average daily gain, milk yield and milk composition in lactating Holstein cows fed diets containing rolled barley (B), rolled oat (R), flaked oat (F) or pelleted oat (P).

| Items | Oat (O) | | | Barley (B) | SEM | P-value | Contrast P-value | | |
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
DM Intake, kg/d	28.19[b]	29.55[ab]	31.61[a]	29.71[ab]	1.010	0.01	0.70	0.43	<0.01
Milk yield, kg/d	49.23[a]	46.55[b]	47.32[b]	46.53[b]	1.970	<0.01	<0.01	0.01	0.39
3.5% FCM, kg/d	51.54	48.17	50.47	50.09	1.778	0.17	0.05	0.05	0.79
ECM, kg/d	50.38	47.30	49.51	49.23	1.681	0.15	0.04	0.04	0.80
Fat, %	4.00[a]	3.69[ab]	3.62[c]	3.93[bc]	0.165	<0.01	0.04	0.02	<0.01
Fat yield, kg/d	1.89	1.74	1.78	1.86	0.078	0.11	0.06	0.02	0.11
Protein, %	2.94[b]	2.92[c]	2.95[b]	3.03[a]	0.088	<0.01	<0.01	<0.01	<0.01
Protein yield, kg/d	1.42[a]	1.34[b]	1.41[ab]	1.39[ab]	0.045	0.03	<0.01	<0.01	0.69
Lactose, %	4.42	4.35	4.40	4.43	0.029	0.06	0.01	0.02	0.29
Lactose yield, kg/d	2.15[a]	1.99[b]	2.15[ab]	2.06[ab]	0.087	0.02	<0.01	0.01	0.26
MUN, mg/dL	18.81	18.75	18.28	18.59	0.603	0.27	0.54	0.90	0.33
Total solids, %	12.37[ab]	12.18[b]	12.01[c]	12.47[a]	0.314	<0.01	0.05	<0.01	<0.01
SCC, 10^3 cell ml^{-1}	39.35	32.56	38.39	33.97	10.18	0.83	0.49	0.53	0.79

Table 5.2. Cont'd Dry matter intake, average daily gain, milk yield and milk composition in lactating Holstein cows fed diets containing rolled barley (B), rolled oat (R), flaked oat (F) or pelleted oat (P).

| | Oat (O) | | | | | | Contrast P-value | | |
Items	Rolled (R)	Flaked (F)	Pellet (P)	Barley (B)	SEM	P-value	B vs. O	B vs. FP	R vs. FP
Feed efficiency									
Milk yield/DMI	1.70[a]	1.52[b]	1.64[ab]	1.55[b]	0.049	<0.01	<0.01	<0.01	0.76
FCM/DMI	1.81[a]	1.64[b]	1.66[b]	1.67[ab]	0.058	0.02	0.09	0.03	0.08
ECM/DMI	1.73[a]	1.62[ab]	1.64[ab]	1.64[b]	0.049	0.01	0.08	0.09	0.22
ADG (kg/d)	0.14	0.20	-0.21	-0.22	0.261	0.53	0.29	0.39	0.55

SEM: Standard error of mean; [a-d] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparisons using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; MUN: milk urea nitrogen; SCC: somatic cell count' ADG: average daily gain.

5.4.2 Ruminal Parameters

Ruminal short-chain fatty acid (SCFA) concentration is presented in Table 5.3. Individual SCFA concentration were not affected by dietary treatment, with the exception of the concentration of acetate which was significantly lower ($P<0.01$) for cows fed the pelleted oat-based diet when compared to rolled oat and barley. Increased acetate supply is linked to milk fat production (Urrutia and Harvatine, 2017) and the decreased amount of acetate concentration in cows fed the pellet oat-based diet may explain the lower milk fat percentage caused by this diet. Total SCFA concentration was higher ($P=0.01$) for cows fed rolled barley when compared to rolled and pelleted oat.

Mean ruminal pH over a 24 h period was unaffected ($P=0.63$) by dietary treatment, with mean values of 6.17, 6.14, 6.21 and 6.13 for rolled oat, flaked oat, pelleted oat, and barley, respectively (Figure 5.1). At the 9 h and 12 h post-feeding time points, however, flaked oat exhibited the lowest pH, while pelleted oat and rolled barley showed the highest values (at 9 and 12 h, respectively; $P=0.03$ and $P=0.04$, respectively). The pH decline was faster for the heat-processed oat (steam-flaking and pelleting) up until 6 h post-feeding, but the recovery for cows fed pelleted oat was faster and higher when compared to flaked oat and rolled barley. Previous studies also reported higher ruminal pH for cows fed pelleted concentrates (Dos Santos et al., 2011). Oat grain present a higher starch degradation rate and extent in the rumen compared to barley grain, however, no difference in pH was observed between grains in this study, which is similar to the results obtained by Gozho and Mutsvangwa (2008). This might have resulted from the high concentrations of NDF across all dietary treatments that can result in high ruminal buffering from the saliva. Dietary NDF is inversely related to ruminal pH since it promotes less acid production and stimulates chewing and saliva production (NRC, 2001).
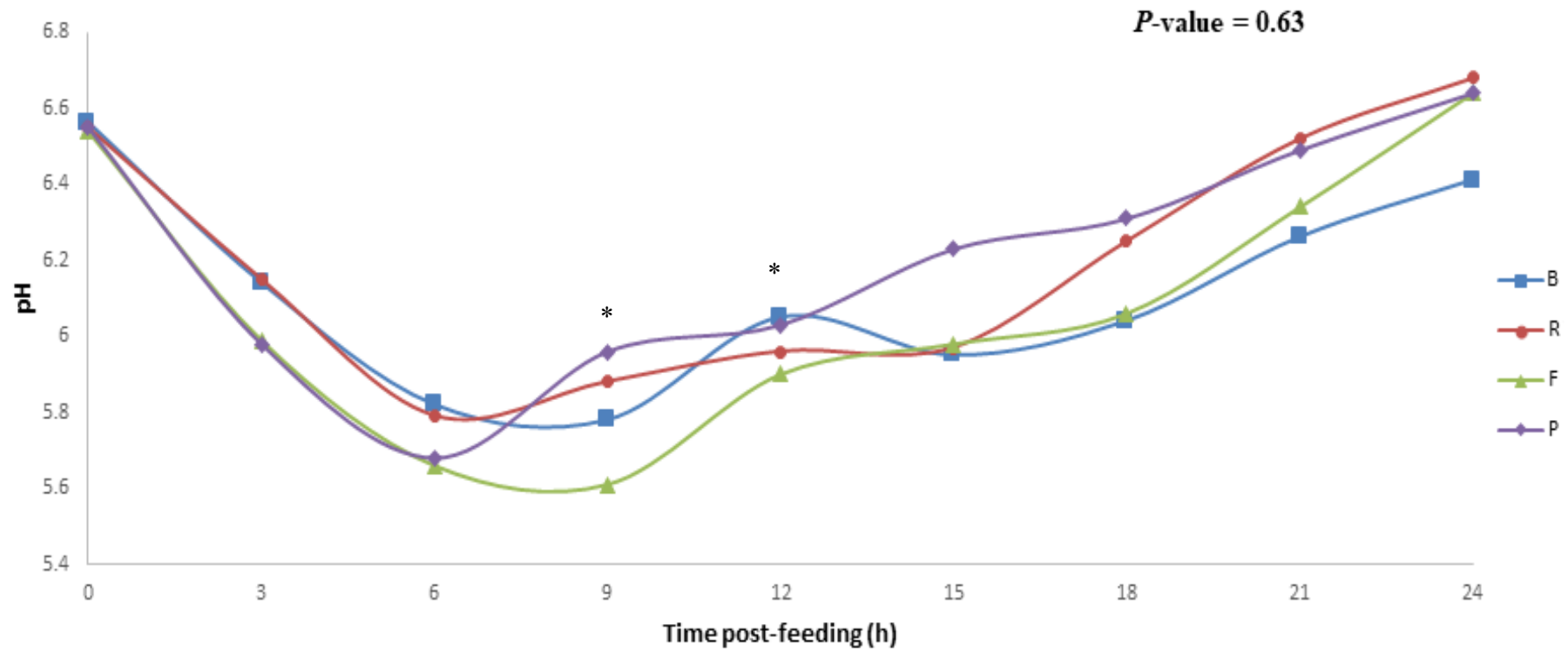
Figure 5.1. Ruminal pH of lactating Holstein cows fed diets containing rolled barley (B), rolled oat (R), flaked oat (F) or pelleted oat (P). Cows were fed just before the 0 (zero) hour collection. * represents a statistically significant difference between treatments.

Table 5.3. Ruminal fermentation characteristics in lactating Holstein cows fed diets containing rolled barley (B), rolled oat (R), flaked oat (F) or pelleted oat (P).

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
SCFA concentrations, mM									
Acetate	58.98[a]	58.79[ab]	54.84[b]	61.11[a]	2.304	<0.01	0.59	0.18	<0.01
Propionate	21.96	23.86	23.11	22.86	1.629	0.32	0.22	0.14	0.43
Butyrate	10.45	10.71	10.97	10.57	0.502	0.08	0.81	0.23	0.02
Isobutyrate	0.78	0.75	0.74	0.82	0.039	0.15	0.21	0.09	0.07
Valerate	1.31	1.27	1.42	1.39	0.098	0.04	0.03	0.08	0.14
Isovalerate	1.37	1.28	1.23	1.34	0.171	0.06	0.44	0.11	0.02
Caprionic	0.39	0.37	0.46	0.48	0.049	0.28	0.20	0.23	0.53
Total SCFA	93.62[b]	96.71[ab]	92.86[b]	100.45[a]	3.976	0.01	0.43	0.79	0.01
Acetate:Propionate	2.59	2.54	2.44	2.75	0.128	0.42	0.71	0.42	0.18

SEM: Standard error of mean; [a-b] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparisons using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; SCFA: short chain fatty acids; SCFA: short-chain fatty acids.

5.4.3 Nutrient Digestibility

Apparent total tract digestibility of DM, OM, CP, NDF, and starch are shown in Table 5.4. Total-tract digestibility of DM, OM and CP were not affected by dietary treatment (P≤0.14). Apparent total tract digestibility of CP tended to be lower (P=0.09) for rolled oat when compared to heat-processed oat (3.24 vs. 3.56 kg/d, respectively). Total-tract NDF digestibility ranged from 54.16 to 59.92% of NDF intake and it was lower (P=0.03) for rolled oat compared to pelleted oat and rolled barley-based diets.

Apparent total-tract starch digestibility was lower (P<0.01) for rolled barley when compared to oat grain-based diets and the contrast also showed significant difference between rolled oat and heat-processed oat-based TMR (P=0.02). In dairy cows, the main site for cereal grain starch digestion is the rumen, and with oat grain showing a faster and more extensive starch degradation in the rumen compared to barley (Herrera-Saldana et al., 1990) it was expected that oat-based TMR would have a higher starch digestibility. Moran (1986) also described a higher digestibility of starch for cows fed complete diets with rolled oat when compared to rolled barley or wheat-based diets.

Table 5.4. Apparent total-tract nutrient digestibility of lactating Holstein cows fed diets containing rolled barley (B), rolled oat (R), flaked oat (F) or pelleted oat (P).

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
Apparent DM digestion, % of DMI	63.96	66.5	66.08	66.27	1.078	0.25	0.37	0.24	0.38
Apparent DM digestion, kg of DM/d	18.40	20.02	20.19	19.93	0.763	0.23	0.54	0.34	0.22
Apparent OM digestion, % of OM intake	62.55	65.18	64.33	64.66	1.193	0.35	0.31	0.22	0.54
Apparent OM digestion, kg of OM/d	15.10	16.46	16.43	16.27	0.631	0.30	0.46	0.30	0.29
Apparent CP digestion, % of CP intake	69.13	71.37	71.20	70.36	0.988	0.26	0.32	0.16	0.18
Apparent CP digestion, kg of CP/d	3.24	3.50	3.62	3.50	0.139	0.14	0.77	0.40	0.09
Apparent NDF digestion, % of NDF intake	54.16[b]	57.49[ab]	59.56[a]	59.92[a]	1.365	0.03	0.77	0.72	0.07
Apparent NDF digestion, kg of NDF/d	5.06	5.91	5.96	6.03	0.665	0.09	0.53	0.34	0.25
Apparent ST digestion, % of ST intake	92.38[a]	92.79[a]	92.52[a]	89.04[b]	0.588	<0.01	0.05	0.01	0.02
Apparent ST digestion, kg of ST/d	4.46	4.42	4.44	4.22	0.212	0.65	0.80	0.68	0.60

SEM: Standard error of mean; [a-b] Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparisons using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; ST: starch.

5.4.4 Urine Parameters, N balance, and microbial N flow

Urinary purine derivatives excretion and microbial N supply to the small intestine are shown in Table 5.5. Allantoin output did not differ between treatments (P=0.16), but contrast statement showed lower values for cows fed the heat-processed oat-based diet (steam-flaked and pelleted) when compared to dry-rolled oat (P=0.03), suggesting that cows being fed TMRs containing heat-processed oat have a lower microbial production compared to dry-rolled oat. Uric acid excretion was higher (P=0.05) for cows fed the pelleted oat-based TMR when compared to rolled-barley (18.67 vs. 16.46 mmol/d, respectively), however, there was no difference for total PD or allantoin excretion between these two treatments. Gozho et al. (2008) also showed no significant difference in allantoin and PD excretion between cows fed rolled or pelleted barley. Total purine excretion did not differ between treatments (P=0.17), however, the contrast statement showed lower values (P=0.03) for heat-processed oat when compared to dry-rolled oat. Consequently, the calculated intestinal flow of microbial N was also unaffected by dietary treatment (P=0.16), but the contrast statement also showed a higher value for cows being fed dry-rolled oat. Theurer et al. (1999) pointed that increasing ruminal starch digestibility increased microbial N supply, however, in this study, the extra 200 g of starch consumed by cows fed pelleted oat did not increase microbial N production.

Nitrogen intake was similar for cows across all diets (Table 5.6). However, as a percentage of N intake, urinary N excretion was lower for cows fed rolled barley when compared to oat (P=0.03) or heat-processed oat (P=0.04). Total urinary excretion in g/d did not differ between treatments (P=0.87). Fecal N excretion did not show any significant difference between the treatments studied. Milk N excretion was higher for cows fed the rolled oat-based TMR when compared to flaked oat (P=0.03), and as a percentage of N intake, rolled oat-based diet also showed

a higher excretion (P<0.01). The higher milk N excretion was expected for rolled oat-based diet since cows in this diet presented a higher milk yield (49.23 kg/d). The N balance (N intake – N excretion) varied between 1.61 to 67.79 g/d, however, despite the great numerical difference, no statistical difference was found between treatments, grains or processing methods (P≥0.11).

5.4.5 Blood parameters

The measured blood metabolites are shown in Table 5.5. Plasma concentrations of urea nitrogen (PUN) did not differ between treatments (P=0.58). In dairy cows, the resulting NH_3-N that is not used for microbial protein synthesis in the rumen is absorbed into portal blood and subsequently converted to urea in the liver (NRC, 2001). So, despite the higher EDST and lower EDCP, and consequently a lower DPB, for rolled barley when compared to oat grain (Chapter 4), the difference in fermentation patterns wasn't enough to lead to a difference in ureagenesis. Plasma glucose did not respond to dietary treatment (P=0.82). Generally, due to the extensive degradation of starch in the rumen, glucose being absorbed in the small intestine is limited, so glucose in the plasma mainly arises from gluconeogenesis in the liver. Rumen derived propionate is the major precursor to glucose formation in the liver (Gozho and Mutsvangwa, 2008). In this study, rumen propionate concentrations were not different between dietary treatments, which may explain the lack of difference in plasma glucose. Blood concentration of the ketone body BHBA (β-hydroxybutyrate) were not affected by treatment (P=0.10).

Table 5.5. Blood metabolites, urine output, urinary purine derivatives excretion and microbial N supply of lactating Holstein cows fed diets containing rolled barley (B), rolled oat (R), flaked oat (F) or pelleted oat (P).

Items	Oat (O)			Barley (B)	SEM	P-value	Contrast P-value		
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
Urinary parameters									
Output (kg/d)	36.33	36.37	37.45	38.38	1.555	0.19	0.27	0.31	0.92
Allantoin (mmol/d)	341.86	335.08	294.47	327.96	26.164	0.16	0.45	0.99	0.03
Uric acid (mmol/d)	17.77^{ab}	17.55^{ab}	18.67^{a}	16.46^{b}	2.049	0.05	0.89	0.43	0.02
Purine Derivatives (mmol/d)	360.12	350.38	313.11	345.99	28.106	0.17	0.56	0.88	0.03
Microbial N supply (g/d)	262.88	255.57	222.02	251.23	23.441	0.16	0.51	0.92	0.03
Blood metabolites									
BUN (mg/dL)	19.13	18.48	18.75	17.97	0.605	0.58	0.83	0.91	0.78
Plasma glucose (mg/dL)	46.57	47.06	45.86	45.39	1.249	0.82	0.46	0.48	0.94
Plasma BHBA (mg/dL)	8.76	9.52	8.94	8.98	0.287	0.10	0.02	0.02	0.8

SEM: Standard error of mean; $^{a-b}$ Means with the different letters in the same row are significantly different (P < 0.05); Multi-treatment comparisons using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; BUN: blood urea nitrogen; BHBA: beta-hydroxybutyrate.

Table 5.6. Nitrogen balance in lactating Holstein cows fed diets containing rolled barley (B), rolled oat (R), flaked oat (F) or pelleted oat (P).

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| Items | Oat (O) | | | Barley (B) | SEM | P-value | Contrast P-value | | |
	Rolled (R)	Flaked (F)	Pellet (P)				B vs. O	B vs. FP	R vs. FP
N intake (g/d)	746.28	769.81	816.21	797.38	31.141	0.13	0.46	0.89	0.10
Urinary N excretion									
N (g/d)	296.40	292.26	294.68	302.03	15.89	0.87	0.64	0.54	0.68
N (% N intake)	38.48	33.31	38.53	36.19	1.632	0.10	0.03	0.04	0.24
Fecal N excretion									
N (g/d)	230.56	220.03	233.78	234.81	11.724	0.36	0.10	0.10	0.87
N (% of N intake)	30.85	28.34	28.80	29.67	1.042	0.26	0.27	0.14	0.20
Milk nitrogen									
Milk (g/d)	222.99[a]	210.76[b]	221.41[ab]	219.22[ab]	7.094	0.03	<0.01	<0.01	0.69
Milk (% of N intake)	29.05[a]	27.06[c]	28.39[b]	28.47[b]	0.847	<0.01	<0.01	<0.01	<0.01
Nitrogen balance									
N balance (g/d)	1.61	48.52	67.79	35.56	28.593	0.28	0.69	0.38	0.11

SEM: Standard error of mean; [a-c] Means with the different letters in the same row are significantly different ($P < 0.05$); Multi-treatment comparisons using Tukey method; R: rolled oat; F: flaked oat; P: pelleted oat; B: rolled barley; B vs. O: contrast between barley and oat grain; B vs. FP: contrast between barley grain and heat-processed oat; R vs. FP: contrast between rolled oat and heat-processed oat; N: nitrogen.

5.5 Chapter summary and conclusions

This study showed that feeding rolled oat, rolled barley or flaked oat as a concentrate in a total mixed ration resulted in similar levels of DMI but that feeding pelleted oat can increase DMI. Despite the higher nutrient consumption by cows fed pelleted oat-based TMR, production of milk and fat percentage was depressed, however milk fat yield was not affected by dietary treatment, mainly because the higher numerical production of milk by cows being fed the pelleted oat-based diet when compared to flaked oat and rolled barley helped decrease the gap caused by the lower milk fat percentage. Rumen acetate concentration was lower for pelleted oat-based diet compared to rolled oat, which might explain the fat depression caused by this diet. Other SCFA did not significantly differ between dietary treatments and daily mean pH was also unaffected, although flaked oat showed lower pH values at 9-h and 12-h post-feeding. Digestibility of DM, OM, and CP was similar across all diets; however, starch digestibility was higher for oat-based diets than barley based. This was expected since oat grain has a higher rumen and total-tract digestibility compared to other cereal grains. Measured blood metabolites (urea, glucose, and BHBA) were similar across all treatments. Purine derivative excretion and microbial N supply were not affected by dietary treatments. Overall, the lower production cost for dry-rolled oat grain ($6/ton) together with the higher milk yield production suggests that rolled oat is the most cost-effective treatment to be fed as a concentrate for dairy cows in North America, if the price of purchase of the oat grain is lower than barley.

6. Research discussion and conclusions

Oat was a common grain used in ruminant and horse nutrition until the earlier twenty century, but after the horsepower was replaced by machinery, the use of oat in cows diet declined. Oat grain is an adequate feed for ruminants being considered a good protein source, however, the low content of starch and high fiber content makes oat grain a less desirable grain when compared to highly used grains, like barley or corn. To improve the quality of oat, its yield, and nutritional value, the Crop Development Centre (CDC) of the University of Saskatchewan has a program to develop new varieties. Three of these varieties were used in this study to determine their nutritive value for dairy cows. Moreover, several studies reported that processing methods can alter the site and extent of digestion, which can lead to a nutritional improvement of the feed. For this reason, this study focused on investigating the nutritional value of different varieties of CDC oat grain and the impact of different processing methods on the nutritive value of the grain for dairy cows.

The first chapter of this study showed that despite the higher hull content, CDC Nasser (feed type of oat) presented a lower lignin and undegradable NDF (uNDF) when compared to other varieties of oat, and the values rivaled the ones presented by barley grain. However, CDC Austenson barley grain still provided greater levels of starch and sugar on a DM basis. CDC Nasser was bred to provide a high oil groat (high in EE) while having a low lignin hull. These characteristics resulting in CDC Nasser having a higher energy value when compared to other oat varieties and showing similar values to barley grain. According to the CNCPS 6.5 model, Nasser oat also showed a low level of CC and TRUCHO, while for protein fraction it did not differ from the other oat varieties. The rumen degradation kinetics values obtained from an in situ study showed that all varieties of oat had higher degradation kinetics of DM, OM, and CP when compared to Austenson barley grain. Synchronizing the degradation of N and OM (ED_N/ED_OM) is important to ensure reduction in the loss of N and maximize microbial

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protein synthesis, for this a value of 25 g N/kg OM is considered optimal (Sinclair et al., 1993). In this circumstance, CDC Nasser presented optimal values up until 8 h of incubation, while the other oat varieties presented higher values and CDC Austenson barley showed lower values (<23). When the protein molecular structure was analyzed, CDC Austenson was revealed as having the highest β-sheet height, this is important since studies related the β-sheet content of a feed with lower protein digestibility (Yu, 2007b).

The results of the second study showed that heat-treatment of oat grain (steam-flaking and pelleting) did not produce any changes in DM and CP content, but it did increase the crude fat of oat. Steam-flaking reduced acid detergent fiber (ADF), decreased PA2 fraction while increased the NE_L (+0.12 Mcal/kg). The heat-processing methods showed increased bypass fraction of DM, OM, and CP, while reduced the effective degradability of the same nutrients in the rumen; despite that, no difference was observed for ruminal degradation of starch. Heat treatments did not impact the intestinal digestion of DM and OM but steam-flaking increased the digestion of CP in the small intestine when compared to dry-rolled oat. The synchronization between N and OM degradation in the rumen was closer to optimal in dry-rolled oat, between the 2-h and 4-h incubation points, while dry-rolled barley showed below optimal results for all time points after 2 h. This shows that, despite the high nutritional value and FMV (DVE/OEB model), rolled-barley presents a lack of available energy in the rumen to allow optimal microbial protein synthesis. This is further outlined by the results of DPB of the NRC 2001 model, showing the lowest value for rolled-barley (-43.82 g/kg DM). Processing methods did not impact significantly the protein molecular structure, making the treatments hard to separate using multivariate spectral analysis and implying similar molecular structure in terms of protein make-up for all the treatments studied.

The third study suggests that rolled oat was the best concentrate to put into dairy cows ration since it had the lowest dry matter intake (DMI) but highest milk production per day with

a high-fat percentage. As a consequence of the lower DMI, cows fed rolled oat also had the highest feed efficiency. It is interesting to observe that, even though not statistically significant, the amount of microbial N supplied to the small intestine was much higher for rolled oat when compared to the other treatments. This implies, as suggested in the second study, that rolled oat shows a closer to optimal synchronization between available N and available energy degradation in the rumen.

In conclusion, different varieties of oat grain differ in their chemical constitution, energy profile, protein and carbohydrates fractions, potential N to energy synchronization, rumen degradation kinetics, metabolic characteristics, and protein molecular structure features. Based on the second study results, steam-flaking oat grain increased the bypass of DM, OM, CP and starch to the small intestine. However, the heat processing at the current conditions was not enough to cause dramatic changes in the protein molecular structure and one could not distinguish treatments in a multivariate spectral analysis. Based on the third study, dry-rolled oat was shown to have a superior response in milk production and milk fat percentage, while it did not show any negative repercussions on the animals' metabolites. In this case, dry-rolled oat can be used as a high-value grain for high producing lactating dairy cows in total mixed rations.

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