THE EFFECTS OF EXTRUDING WHEAT DRIED DISTILLERS GRAINS WITH SOLUBLES WITH PEAS OR CANOLA MEAL ON RUMINAL FERMENTATION, MICROBIAL PROTEIN SYNTHESIS, NUTRIENT DIGESTION AND MILK PRODUCTION IN HOLSTEIN DAIRY COWS

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ABSTRACT

The objective of this study was to examine the effects of feeding extruded and non-extruded mixtures of wheat dried distillers grains with solubles with peas (WP) or canola meal (WC) on ruminal fermentation, total tract nutrient digestion and milk production in dairy cows. Eight dairy cows (712 ± 54 kg body weight; 90 ± 31 days-in-milk at the beginning of the experiment) were used in a replicated 4 × 4 Latin square design (28-d periods) with a 2 × 2 factorial arrangement of dietary treatments. Four cows in one Latin square were fitted with rumen cannulas for the measurement of ruminal fermentation characteristics. Treatment diets contained either WP or WC combinations fed in an extruded or non-extruded form (16% of DMI). Diets were isonitrogenous (17.1% crude protein; CP) and contained approximately 53% concentrate and 47% forage (DM basis). Dietary treatment had no significant effect on DMI ($P > 0.10$). Starch intake was higher for cows fed extruded diets compared to those fed non-extruded diets ($P = 0.028$) and was also higher for cows fed WP compared to those fed WC ($P = 0.042$). Cows fed extruded diets had higher apparent ruminal digestion of DM ($P = 0.02$) and a tendency ($P = 0.05$) for a higher OM apparently digested in the rumen compared to those fed non-extruded diets. Total tract digestibilities of organic matter ($P < 0.01$), CP ($P < 0.01$), ether extract ($P < 0.01$) and starch ($P = 0.047$) were higher for cows fed extruded diets compared to those fed non-extruded diets. Total tract digestibility of ether extract was lower ($P = 0.011$) but digestibility of starch was higher ($P < 0.01$) and CP digestibility tended to be higher ($P = 0.08$) for cows fed WP compared to those fed WC. Fecal N excretion was lower in cows fed extruded diets compared to those fed non-extruded diets ($P < 0.01$), but there was no difference in N retention, productive N, RDP or RUP between diets ($P > 0.10$). Ruminal pH was higher for cows fed non-extruded WC compared to those fed extruded WC, but there was no difference between WP diets (interaction; $P = 0.047$). Ruminal

acetate displayed the opposite interaction where concentration was highest for cows fed extruded WC and lowest for those fed non-extruded WC but there was no difference between WP diets (interaction; $P = 0.019$). Ruminal ammonia-N concentration tended to be higher for cows fed WC compared to those fed WP ($P = 0.06$). Ruminal propionate concentration was higher for cows fed extruded diets compare to those fed non-extruded diets ($P = 0.026$). Ruminal isobutyrate concentration was higher for cows fed WC compared to those fed WP ($P < 0.01$). Ruminal butyrate ($P < 0.01$) and isovalerate ($P < 0.01$) concentrations were higher for cows fed extruded WC compared to those fed non-extruded WC, but concentrations decreased for cows fed extruded WP compared to those fed non-extruded WP. Plasma glucose concentration was higher for cows fed WC compared to those fed WP but concentration was highest for cows fed extruded WC but lowest for cows fed extruded WP (interaction; $P < 0.01$). Milk protein yield ($P = 0.047$) was higher and milk yield tended to be higher ($P = 0.06$) for cows fed WP compared to those fed WC diets. Milk protein content was not affected by diet; however, milk fat content ($P = 0.04$) and MUN ($P = 0.011$) were lower, whereas milk yield ($P = 0.030$), 3.5% fat corrected milk yield ($P = 0.027$), milk fat yield ($P = 0.027$), lactose content ($P = 0.011$) and lactose yield ($P < 0.01$) were higher in cows fed the extruded diets compared to those fed non-extruded diets. In summary, these results indicate that extrusion had positive effects on overall milk production and total tract nutrient digestion.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADIN	Acid detergent insoluble nitrogen
ADR	Apparently digested in the rumen
AOAC	Association of Official Analytical Chemists
ATTD	Apparently total tract digestion
BCVFA	Branched chain volatile fatty acids
CLA	Conjugated linoleic acids
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
DDGS	Dried distillers grains with solubles
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
FAB	Fluid associated bacteria
FP	Fluid phase
iNDF	Indigestible neutral detergent fiber

LCFA	Long chain fatty acids
LPP	Large particle phase
MFD	Milk fat depression
MUN	Milk urea nitrogen
N	Nitrogen
NAN	Non-ammonia nitrogen
NANBN	Non-ammonia non-bacterial nitrogen
NDF	Neutral detergent fiber
NDIN	Neutral detergent insoluble nitrogen
NH_3-N	Ammonia nitrogen
OM	Organic matter
OMTDR	Organic matter truly digested in the rumen
OTD	Omasal true digesta
PAB	Particle associated bacteria
PF	Particle phase
PUFA	Polyunsaturated fatty acids
PUN	Plasma urea nitrogen

RDP	Ruminally degradable protein
RUP	Ruminally undegradable protein
SCFA	Short chain fatty acids
SGD	Starch gelatinization degree
SPP	Small particle phase
TMR	Total mixed ration
VFA	Volatile fatty acids
WC	WDDGS-canola meal dietary treatment
WDDGS	Wheat dried distillers grains with solubles
WP	WDDGS-peas dietary treatment

1 GENERAL INTRODUCTION

The expansion of ethanol production in western Canada has brought about an abundant supply of wheat dried distillers grains with solubles (WDDGS), which has become a common protein supplement in Western Canadian dairy cow diets. The renewable fuel standard set by the federal government requires national ethanol production to reach 2 billion litres annually (Canadian Renewable Fuels Association 2010). Approximately 345 million litres of ethanol are produced in Saskatchewan annually, resulting in 400,000 metric tonnes of wheat and corn dried distillers grains with solubles (DDGS; Government of Saskatchewan 2013) which are available to the livestock feed market.

Like corn DDGS in the United States, WDDGS is an economical feed source for ruminants. Corn DDGS offers benefits to dairy cow rations by replacing protein sources such as soybean meal at levels up to 20% (Owen et al. 1991) and partially replacing feed concentrate without negatively impacting feed intake or milk production (Penner et al. 2009). Shingoethe et al. (2009) found that corn DDGS can be included up to 30% without negatively affecting feed intake, milk production, and milk fat and protein content; however, they recommend feeding no more than 20% to reduce nutrient excretion and to avoid underfeeding of other ingredients.

Ethanol production from wheat grain is a more recent development and less information is available on the nutritional value of WDDGS as a feed ingredient compared to corn DDGS. Recent studies have demonstrated that WDDGS can effectively replace corn DDGS without affecting production (Abdelqader et al. 2012; Chibisa et al. 2013; Penner et al. 2009). The crude protein (CP) content of WDDGS is higher than that of corn DDGS (42.3 vs. 29.7%; NRC 2001) and it is more ruminally degradable than corn DDGS (61.07 vs. 47.10% degraded after 24 h and 89.95 vs.

68.14% degraded after 48 h; Nuez Ortin et al. 2009; 62.9 vs. 54.5%; Li et al. 2013); however, post ruminal and total tract protein digestion results have varied (Abdelqader et al. 2012; Boila et al. 1994a; Chibisa et al. 2013; Li et al. 2013). The increased CP content may allow producers to reduce the dietary inclusion of more costly protein sources such as soybean meal or canola meal. Corn DDGS has a high ruminally undegradable protein (RUP; NRC 2001) content, which can be beneficial for dairy cows in peak lactation. Kalscheur et al. (1999) found that with limited dietary CP, increasing dietary RUP content increased milk production in early lactation. Davidson et al. (2003) demonstrated that diets modified to have low CP and high RUP had the ability to reduce N excretion without negatively affecting milk production and milk constituents. The high ruminally degradable protein (RDP) content of WDDGS may be a barrier to the use of this ingredient by limiting the amount at which it can be included in dairy cow diets. Therefore, increasing RUP component of WDDGS could potentially increase the value of the feed.

Processing feed sources by way of heat treating and pressure cooking has been shown to increase RUP content. Extrusion, or a similar process known as expansion, is commonly used in many animal feeds, especially in the pet food industry but is not as common in the dairy industry. By extruding WDDGS, the amount of RUP may increase, thus potentially increasing the metabolizable protein delivered to the animal (assuming that microbial protein production in the rumen is not compromised due to a shortage of RDP).

A second barrier to feeding WDDGS to dairy cows is that the lysine and methionine contents of WDDGS are relatively low (1.55 and 1.41% of CP, respectively; NRC 2001) compared to other protein ingredients, which could potentially limit milk production. Combining WDDGS with other protein sources may improve the amino acid balance by boosting lysine and enhancing diets to the desired 3:1 lysine to methionine ratio. Soybean meal, often considered the “golden

standard" for protein sources, can be a costly ingredient to use in Western Canada due to seasonal supply shortages and transportation costs which are continually on the rise and a barrier to using the ingredient. Although the soybean meal market tends to influence the price of other protein sources, locally produced protein sources such as WDDGS, canola meal or peas may offer a more cost effective alternative.

2 LITERATURE REVIEW

2.1 Dietary protein fractions in terms of rumen utilization

With the push on environmental sustainability of livestock production, practices such as overfeeding protein to achieve maximum milk production are being criticized by the public and by governments. Protein is also one of the most expensive ingredients required in animal diets, thus a reduction in the protein content in dairy diets is of economic benefit if milk production is not negatively affected. This brings to light the exploration of protein sub-fractions used for rumen utilization (RDP) versus intestinal absorption and the study of RUP.

2.1.1 Rumen degradable protein efficiency

Balancing RUP and RDP in a diet is essential, especially in lower protein diets. An adequate amount of RDP is needed to optimize microbial protein production; however, excess RDP can result in additional ruminal protein breakdown without enhancing microbial synthesis, thus causing increased N excretion and reduced efficiency of RDP utilization. Recommended levels of RDP supply for lactating dairy cows are fairly consistent at 9.5-10.5% of dry matter intake (DMI; NRC 2001). At an elevated RDP level, protein will be deaminated by microbes but will not be well utilized for microbial protein production and milk protein production. This is often accompanied by increased ruminal ammonia (NH_3-N) levels and excretion of the deamination products through the milk and urine (Hristov et al. 2004). Reynal et al. (2005) found that ruminal NH_3-N, milk urea-N (MUN), blood urea-N and urine urea-N excretion increased as RDP increased but also observed an increase in true milk protein content, milk protein yield and microbial non-ammonia N indicating an under feeding of RDP in the lower RDP diets. Even though there was a positive impact on milk protein constituents when RDP was overfed, when environmental and

economic considerations are taken into account, the optimal level of RDP (calculated using omasal flow of RUP) was determined to be 11.7% of DMI (Reynal et al. 2005). Conversely, decreasing RDP supply below recommended levels while keeping RUP constant decreased MUN, fecal-N and urine-N excretion (Cyriac et al. 2008). In this study N efficiency was increased due to lower RDP intake (Cyriac et al. 2008); however, milk fat yield linearly declined with decreasing RDP which could have been a negative result because of reduced fermentation products and microbial protein production.

2.1.2 Importance of rumen undegradable protein

Providing an adequate supply of dietary RUP is important in lactating dairy cows because forage CP, especially grass or alfalfa based forages, is comprised of largely RDP (NRC 2001), including soluble protein, supplying rumen microbes an abundant source of CP but little dietary protein for intestinal absorption. Level of recommended RUP for lactating dairy cows is variable at 4.6-10.6% of DMI (NRC 2001). Variability in RUP requirements could be due to differences in breed, DMI, milk production and stage of lactation; thus, experiments studying the effects of RUP supply on performance may not always lead to the similar results. Kalscheur et al. (1999) assessed the impact of increasing RUP level on fresh, mid and late lactation cows. Fresh cows responded positively to increased RUP level with regard to milk yield and fat corrected milk (FCM) with equal dietary CP of 15.2% (Kalscheur et al. 1999). Responses to increasing dietary RUP supply were greater when higher CP (17.4%) diets were fed compared to diets containing lower CP (15.2%) levels (Kalscheur et al. 1999). Mid-lactation cows were not affected by increased RUP supply and late lactation cows had reduced milk constituents with higher RUP (Kalscheur et al. 1999). This study emphasized the differences in RUP requirements between stages of lactation and the importance of RUP in early lactation. Davidson et al. (2003)

demonstrated that cows in early lactation fed diets with increasing levels of RUP while maintaining a similar CP had reduced N excretion without negatively affecting milk production and milk constituent contents or yields when fed the high RUP diets.

Since NRC (2001) RDP requirement is relatively similar throughout lactation, it is important to provide the remainder of CP content as intestinally available protein which will support additional milk yield, milk protein content or protein yield above what is produced from the provision of microbial protein, especially in early lactation. Unfortunately, diet formulations using select protein sources may underfeed RUP when RDP is in excess or RUP may be overfed causing an excessive amount of costly CP in the diet. Santos et al. (1998) criticized the theory of increasing dietary RUP showing that it does not consistently increase milk production or constituents. This was a literature review based on the use of soybean meal completely replaced by other feedstuffs containing more RUP achieving the same protein content; however, in most cases this changed the ingredient composition of the whole diet due to differing CP content of the alternate ingredient. This was not a comparison using an ideal protein balance but an overfeeding of RDP with soybean meal versus overfeeding RUP with the alternative ingredient. Perhaps if ingredients were optimized for RUP and RDP by incorporating both soybean meal and the alternative ingredient and compared to using only soybean meal, the studies in this review would have resulted in more beneficial milk production results. Despite these conflicting results, there is no denying that genetic potential of dairy cows has improved dramatically within the last few decades. The modern dairy cow has additional protein requirements than its predecessors and protein supplied by microbial protein alone is not sufficient in sustaining high milk production that these cows are capable of achieving.

Increasing RUP in the diet often results in the addition of costly ingredients that are not as easy to source as standard CP ingredients. Many studies have looked at the effect of various heat treatments in order to increase RUP content of feeds. Extrusion is a process that has been frequently used to increase RUP in research studies. Processing time of extruded products is relatively quick and can yield high throughput of ingredients which is required for practical application in the dairy industry. The benefit of increased RUP and quick processing of extrusion makes this heat processing ideal for dairy research.

2.2 Extrusion

Extrusion is a high pressure cooking process by way of a screw and barrel. Ingredients are subjected to intense pressure by being moved down the extruder screw which has a narrowing space between its shaft and the barrel in which it is housed. Moisture is often applied either prior to or during the cooking process in order to condition the product and can also act as a lubricator as the product is transferred through the extruder. It is a very quick cooking process, only lasting a matter of seconds between ingredient introduction and product exit; however, extrusion can effectively modify characteristics of several nutrients in this short time. Feeding extruded or heat-treated feedstuffs to dairy cows has been shown to result in many beneficial effects in terms of rumen and total tract digestibility, health of the rumen, milk production (including milk constituents and yields), and environmental sustainability.

2.2.1 Protein alteration from heat treatment

As outlined earlier, increasing RUP in the diet has beneficial effects of reducing nitrogen excretion into the environment and increasing protein utilization for milk protein synthesis,

especially in early lactation. Heat processing such as extrusion can increase the RUP fraction in feedstuffs by way of several chemical and physical alterations.

2.2.1.1 Assessing protein fractions and protein changes due to heat treatment

One of the most pronounced effects of extrusion is its ability to modify N fractions. It is well documented that heat treating protein ingredients can alter protein degradability. To better quantify the degradability of proteins, the Cornell Net Carbohydrate and Protein System (CNCPS) protein fraction system was created. The CNCPS protein fraction system (Sniffen et al. 1992) involves dividing protein into three main groups. Protein A fraction consists of trichloroacetic acid soluble N or non-protein N. Acid detergent insoluble N (ADIN) fraction which is bound to the cell wall and essentially unavailable protein makes up the C fraction. The true degradable protein portion makes up the B fraction which is split up into three separate subdivisions, the rapidly degradable protein which is the buffer soluble fraction (B1 fraction), the intermediate fraction which is insoluble in buffer but soluble in neutral detergent solution (B2 fraction), and the slowly degradable protein fraction (B3 fraction) which is insoluble in buffer and neutral detergent but soluble in acid detergent solution, often referred to as neutral detergent insoluble N (NDIN). This system can reliably determine changes in protein fractions due to heating and other protein structure altering processes which affect rumen and intestinal degradability.

The CNCPS protein fraction system, however, does not explain what happens to the protein on a molecular level when heat treatment is applied. Heating ingredients actually changes the physical structure of its proteins. This denaturation causes unfolding or uncoiling of the protein secondary structures (Khan et al. 2015; Yu et al. 2005) as well as affecting primary structures (Khan et al. 2015). Synchrotron Fourier transform infrared microspectroscopy (S-FTIRM) can be

used to quantify amide bonds within proteins. Vibrations caused by specific bonds are separated along the spectrum of two selected bands, amide I and amide II. Amide I band includes bonds used to characterize secondary structures such as α-helix and β-sheet, whereas the amide II band represents more convoluted amide bonds of the primary structure (Yu 2007). This technology has been used in multiple studies to determine the relationship between protein structure and degradability (Khan et al. 2015; Yu et al. 2005; Yu 2007). Yu et al. (2005) studied raw and heated golden and brown flaxseed under S-FTIRM and observed varietal protein structure differences. They also observed a change in protein structure in golden flaxseed due to heat treatment but no significant change in the brown flaxseed when it was heated. Roasted golden flaxseed had reduced α-helix and increased β-sheets and decreased α-helix:β-sheet ratio, which is related to reduced protein degradability (Yu et al. 2005). This indicates a specific sensitivity of protein structures to heat treatment. These differences in biological value cannot be analyzed by wet chemistry methods which do not take into consideration the actual protein structure and the nutritive aspects associated with those structures including susceptibility to enzymatic hydrolysis (Yu 2007). Comparing the S-FTIRM results to the CNCPS protein fraction system, the heated golden flax had lower A and B1 fractions, but higher B2, B3 and C fractions compared to raw golden flaxseed (Yu 2007). Khan et al. (2015) found that moist heating flax when compared to raw flax decreased soluble protein and increased NDIP; similarly, B1 protein fraction decreased but B2 and B3 protein fractions increased. This change in degradability was accompanied by reduced α-helix and increased β-sheets and a decrease in α-helix:β-sheet ratio (Khan et al. 2015). Moist heat treatment also resulted in a change in Amide I:amide II area ratio, which is likely correlated to crude protein content (Khan et al. 2015). These change indicates a modification of the protein primary structure due to heat treatment.

Other modifications affecting protein degradation or digestibility can occur when heat treating feedstuffs. Heat treatment can destroy anti-nutritional factors such as trypsin inhibitor (Focant et al. 1990; Masoero et al. 2005), although the effect of these factors may not influence ruminal protein degradation. The Maillard reaction (Van Soest 1994) occurs during heat treatment which can bind individual amino acids and make them unavailable. This could cause reduced analytically detectable amino acid content of heat treated ingredients. This reaction will be explained in more detail later in this literature review.

2.2.1.2 Increasing rumen undegradable protein through heat treatment

Rumen undegradable protein has been the focus of many ruminant nutrition studies. It is well documented that extrusion and other heat treatments can increase RUP or, correspondingly, decrease RDP content of several protein ingredients including canola meal (Jones et al. 2001; Moshtaghi Nia et al. 1992; Moshtaghi Nia et al. 1995; Mustafa et al. 2001), canola presscake (Jones et al. 2001) and soybean meal (Reynal et al. 2003; Solanas et al. 2005; Solanas et al. 2008). Protein degradation in the rumen can also be reduced by heat treating grains, pulses and oil seeds such as peas (Focant et al. 1990; Masoero et al. 2005; Mustafa et al. 1998; Petit et al. 1997; Solanas et al. 2005; Solanas et al. 2008; Walhain et al. 1992), lupins (Benchaar et al. 1994; Masoero et al. 2005; Solanas et al. 2005; Solanas et al. 2008), faba beans (Masoero et al. 2005), whole soybeans (Solanas et al. 2005; Solanas et al. 2008), barley (Lund et al. 2008; Solanas et al. 2008), wheat (Lund et al. 2008) and flaxseed (Khan et al. 2015). Extrusion alone has been shown to be responsible for decreased ruminal degradation in several of these ingredients (Benchaar et al. 1994; Masoero et al. 2005; Petit et al. 1997; Solanas et al. 2005; Solanas et al. 2008; Walhain et al. 1992). Khan et al. (2015) observed an increase in RUP content when comparing moist heated flaxseed to raw flaxseed using in situ techniques. This change in degradability resulting in greater RUP

content in addition to a decrease in soluble protein was associated with a decreased α-helix:β-sheet ratio (Khan et al. 2015). Similarly, a reduction in α-helix:β-sheet ratio in conjunction with a reduction in readily rumen degradable protein fractions (A, B1) and an increase in slowly degradable and unavailable protein fractions (B2, B3, C) was previously reported with roasted golden flaxseeds (Yu 2007). This direct correlation between molecular structure and ruminal protein degradability of extruded ingredients solidifies the heat treatment as a valid process for increasing RUP.

Individual amino acids consequently have altered ruminal degradability when heat treated thus increasing RUP compared to untreated amino acids of unaltered peptides. Extrusion of lupin seeds reduced rumen disappearance of all individual amino acids (Benchaar et al. 1994). Lund et al. (2008) reported a numerical decrease in water solubility and degradation rate of total CP, total amino acids, and lysine and methionine of expander treated barley, wheat, maize, rapeseed cake, peas, rye, sunflower meal and guar meal. This did not result in a decrease in rumen protein degradation for all heated feedstuffs. Barley, wheat and guar meal had reduced effective RDP and rumen degradability of individual amino acids when expanded as did rapeseed cake at higher temperatures, whereas maize and sunflower meal did not result in lowered effective RDP or amino acids (Lund et al. 2008). Expanded peas had lowered effective RDP but similar rumen degradability of individual amino acids (Lund et al. 2008). This indicates that feedstuffs that are subjected to heat treatment will be modified differently depending on the chemical structure of their proteins as well as other chemical characteristics of the ingredient.

2.2.2 Starch gelatinization during heat treatment

Starch is very effectively altered when subjected to heat in the presence of moisture. The process of starch gelatinization involves swelling of the starch granules, by way of uptake of water into the starch granules, breaking hydrogen bonds of the crystalline structures of amylose and amylopectin and the exposed hydroxyl groups of those molecules form hydrogen bonds with water molecules (Ratnayake et al. 2002; Svihus et al. 2005). This causes the organized structure of raw starch to become disorganized and loose and allows increased solubility of the starch chains (Ratnayake et al. 2002). The resulting starch is very water soluble and more easily digestible than the raw form. Gelatinization can be limited by water input, causing incomplete gelatinization if there is insufficient water supply (Ratnayake et al. 2002). The temperature required to induce gelatinization is specific to the starch structure or content of amylose and amylopectin (Ratnayake et al. 2002; Svihus et al. 2005; Van Soest 1994). Greater amylose and less amylopectin content in starch requires higher temperatures for gelatinization (Ratnayake et al. 2002; Svihus et al. 2005; Van Soest 1994), which is likely due to the association of amylose with lipids and the hydrophobic nature of these molecules which reduces swelling and solubilisation of this starch (Ratnayake et al. 2002; Svihus et al. 2005).

If there is an overabundance of water in the presence of high temperatures, a process called retrogradation occurs once cooling begins. It is essentially irreversible starch swelling wherein amylose are freed from starch granules and suspended in the solution which, when cooled, can form a gel substance (Ratnayake et al. 2002; Svihus et al. 2005). This gel formation is due to hydrogen bonding between the amylose chains (Ratnayake et al. 2002; Van Soest 1994) whereas amylopectin reassociation is limited (Van Soest 1994) and is a much slower process of aggregation (Svihus et al. 2005). The formation of resistant starch can occur during retrogradation (Ratnayake

et al. 2002, Svihus et al. 2005; Van Soest 1994), which may be but is not always fermented in the rumen (Svihus et al. 2005; Van Soest 1994). In the cooking process of feed ingredients with low or moderate water content or water addition, this process can still occur (Svihus et al. 2005).

Maillard reaction is a potential disadvantage to heat processing such as extrusion which can bind certain proteins and carbohydrates and possibly make them unavailable for digestion. The Maillard reaction is a non-enzymatic process where active carbohydrate degradation products, formed mostly from hemicellulose and soluble carbohydrates, are condensed with available amine groups of amino acids (Van Soest 1994). This process occurs readily at high temperatures but can start to occur at temperatures as low as 60°C (Van Soest 1994). Moisture content is a large factor influencing this process with maximum reaction taking place at 30% moisture (Van Soest 1994), although this process is also dependent on other nutrient factors. The amine group of lysine is very susceptible to the Maillard reaction but the sulfur or amine group of methionine and cystine, and the phenolic group of tyrosine are also reactive (Van Soest 1994). The major issue with the Maillard reaction is that it renders those carbohydrates and amino acids essentially indigestible, with physical and chemical properties similar to lignin (Van Soest 1994). In the case of lysine, which is considered the most limiting amino acid, this reaction can reduce utilizable content and can potentially further limit its availability for intestinal absorption.

2.2.2.1 Heat treated starch in the rumen

In starch rich feed sources such as cereal grains and some pulses, starch degradation and the rate of degradation in the rumen can be increased due to starch gelatinization. Processes where heat and moisture are applied such as extrusion are likely to increase starch gelatinization. Extruding peas has increased the soluble starch portion (Chapoutot et al. 1997; Focant et al. 1990;

Petit et al. 1997), rate of starch degradation (Masoero et al. 2005; Petit et al. 1997; Walhain et al. 1992) and effective degradability of starch (Masoero et al. 2005; Petit et al. 1997), thus indicating that gelatinization of starch has occurred. Starch from field peas is mostly composed of amylopectin (Ratnayake et al. 2002). This indicates that there is less heat required to induce gelatinization and reduced opportunity for retrogradation, thus reducing the opportunity for the formation of resistant starch. Starch gelatinization degree (SGD) was increased when barley, maize and peas were extruded (Solanas et al. 2008) and increased rumen starch degradation was found when expanded barley was fed to cattle (Prestlokken et al. 2001).

On the other hand, chemical barriers such as lipid complexes or protein matrices can reduce ruminal starch degradation (Goelema et al. 1999; Ratnayake et al. 2002; Svihus et al. 2005). Heat treatment such as toasting can decrease rumen degradability of the protein matrix surrounding starch particles, thus reducing ruminal starch degradation (Goelema et al. 1999; Svihus et al. 2005). If heating is too great, Maillard reaction can irreversibly bind proteins and carbohydrates, thus causing them to be undegradable in the rumen and possibly indigestible in the small intestine.

There are also physical barriers such as granule size or differences in mechanical processing that can affect degradation (Goelema et al. 1998; Goelema et al. 1999; Ratnayake et al. 2002; Svihus et al. 2005). Toasting does not necessarily physically alter feed structure because ingredients such as grains and beans are left intact. Thus, compared to heat treatment that causes swelling, decreased particle size or destruction of the original feed form such as extrusion or expansion, the original protective seed structure and coating may still be intact and surface area is not necessarily reduced which could hinder further microbial degradation of the ingredient (Goelema et al. 1998; Goelema et al. 1999; Svihus et al. 2005).

2.2.3 Ruminal characteristics of heat treated feedstuffs

Increasing dietary RUP by heat treatment will result in reduce RDP when those ingredients are fed. As a result, reduced ruminal NH_3-N concentrations can occur (Block et al. 1981; Focant et al. 1990; Hall et al. 2010; Masoero et al. 2006; Prestlokken et al. 2001; Solanas et al. 2007; Soltan 2009). Increasing dietary RUP without increasing dietary CP may also reduce microbial protein production if the initial diet is already limited in RDP. Benchaar et al. (1994) observed a decrease in bacterial amino acids flow in the duodenum when feeding extruded lupin seeds. In a study observing in vitro continuous culture fermentation of a non-extruded treatment, a treatment with extruded cereals, a treatment with extruded proteins or a treatment with extruded cereal plus extruded protein, extruded cereal blend and extruded protein blend both reduced bacterial-N as a percentage of NAN (Solonas et al. 2007). Bacterial-N as a percentage of NAN was further reduced when incubating the extruded cereal plus extruded protein blend but total bacterial-N flow was only reduced by this treatment (Solonas et al. 2007). In that study, bacterial-N flow was hindered by both gelatinized starch and reduced protein available for fermentation. Gelatinized starch would be expected to help with bacterial proliferation; however, it is possible that extruding the cereal blend created resistant starch and reduced the starch available for fermentation and required substrates from VFA for microbial growth. Prestlokken et al. (2001) did not observe any differences in ruminal N degradation or bacterial-N flow to the duodenum when expanded barley replaced pelleted barley in lactating dairy cow diets. This could be because of the pelleting process which can subject ingredients to relatively high temperatures reducing the difference between the two treatments.

On the other hand, gelatinized starch can also contribute to increased microbial protein production. The influence of heat treatment on starch gelatinization can be amplified if rumen

degradable protein is readily available but rumen fermentable carbohydrates are limiting microbial growth in non-heated diets. Focant et al. (1990) found that feeding extruded peas decreased ruminal NH_3-N concentration and increased NAN and bacterial-N duodenal flow compared to feeding ground peas. This was likely a direct effect of the rapidly available starch in the rumen (Focant et al. 1990) from the extruded peas in the diet. When heat treating starch rich feeds such as in this case, reduced ruminal NH_3-N concentration is more likely a result of more efficient incorporation of NH_3-N into bacterial-N rather than from the reduced supply of RDP in the diet (Focant et al. 1990; Prestlokken et al. 2001).

With alterations of ruminal starch degradation rate, ruminal pH and VFA will be altered. Heat treating ingredients containing high levels of starch has been shown to cause decreased ruminal pH (Focant et al. 1990; Prestlokken et al. 2001) and increased total VFA concentrations (Focant et al. 1990; Prestlokken et al. 2001; Solanas et al. 2007). The increased rate of fermentation and, therefore, production of fermentation products are expected with a faster rate of ruminal starch degradation. Extrusion may also result in additional substrates being available for fermentation if resistant starch, due to physical barriers existing in the raw form (Ratnayake et al. 2002), is reduced by processing and heat.

When comparing the results of feeding different heat-treated protein-rich ingredients to ruminants, the effects on ruminal fermentation have not been consistent. Heat treatment has resulted in decreased ruminal pH (Soltan 2009) and increased total VFA (Solanas et al. 2007) but these results are not consistent across studies.

2.2.4 Protein digestibility

In many studies intestinal digestibility or availability of crude protein (Benchaar et al. 1994; Chapoutot et al. 1997; Khan et al. 2015; Kibelolaud et al. 1993; Solanas et al. 2005; Solanas et al. 2008) and RUP supply (Benchaar et al. 1994; Kibelolaud et al. 1993; Solanas et al. 2005; Solanas et al. 2008) increased as a result of moist heat treatment which is an indication that RDP was reduced and RUP quantity was increased without negatively affecting the quality of RUP. These results are not always consistent between different ingredients. For instance, soybean meal in its raw form has very digestible RUP initially (Solanas et al. 2005), so there is little room for improvement. Soybean meal may have already experienced some heating during previous meal processing whereas raw lupins, peas and whole soybeans have lower intestinal digestibility initially so there is a greater chance of improving RUP digestibility through extrusion (Solanas et al. 2005). In some instances, extrusion has increased apparent total tract digestibility (ATTD) of protein, thus increasing retained-N and reducing fecal-N (Petit et al. 1997). Kibelolaud et al. (1993) observed increased protein total tract disappearance of extruded lupins at short rumen retention times but this difference diminished as ruminal retention time increased. In some cases, an increase in intestinal digestibility matched the reduction in ruminal degradation such that protein ATTD did not change significantly (Chapoutot et al. 1997; Prestlokken et al. 2001).

The potential disadvantage to heat treating feedstuffs in an attempt to increase RUP is the risk of overheating the ingredients which can degrade or bind essential amino acids in the Maillard reaction and increase protein C fraction essentially reducing protein digestibility in the rumen as well as in the intestines. Soltan (2009) observed a decrease in several individual amino acids of soybean meal when it was extruded or heat treated which was likely due to amino acids being bound to carbohydrates making them undetectable. Moshtaghi Nia et al. (1992) reported increased

ADIN and NDIN and a decrease in lower gastrointestinal and overall total tract nitrogen disappearance when canola meal heat treatment duration was increased. In this situation, it is quite likely that once degradable or digestible amino acids were rendered indigestible due to prolonged heat treatment.

2.2.5 Dry matter digestibility

The effect of heat treating ingredients on dry matter (DM) ruminal disappearance is directly affected by ruminal protein degradation; a higher RDP fraction will result in higher DM disappearance. Heat treated protein rich feeds have reduced ruminal DM degradability (Jones et al. 2001; Masoero et al. 2005; McKinnon et al. 1991; Moshtaghi Nia et al. 1992). With extreme heat treatment, total tract DM disappearance can be reduced. Moshtaghi Nia et al. (1992) found that canola meal exposed to heat treatment for 45 minutes or longer reduced total tract DM disappearance which is likely associated with increased ADIN and reduced protein digestibility in this study. Reynal et al. (2003) observed reduced DM ATTD when replacing soybean meal with expeller soybean meal which was, in this case, likely a result of the reduced ADF and NDF ATTD which may have been altered during processing. Heat treatment of starchy feed ingredients has resulted in no difference (Prestlokken et al. 2001) or increased DM ATTD (Petit et al. 1997) which may be related to the starch solubility of the raw ingredients.

2.2.6 Starch digestibility

Starch digestibility may be more greatly affected by heat treatment with ingredients that have a greater proportion of resistant starch or with ingredients with fewer physical barriers. Extruding peas increased starch digestibility (Masoero et al. 2005). Goelema et al. (1999) observed an increase in total digestible starch when expanding a pulse mix which was

accompanied by increased SGD, ruminal starch degradation rate and reduced ruminal undegraded intake starch despite no increase in intestinal starch digestion. On the other hand, Prestlokken et al. (2001) found no difference in starch ATTD when cows were fed pelleted or expeller barley. The lack of response could be caused by high pelleting temperature causing some gelatinization of the starch or possibly the formation of resistant starch in the expanding process which could minimize the difference between treatments. Another possible explanation is that the extensive mechanical destruction of the grain during the pelleting process allowed ample microbial degradation of the starch.

Goelema et al. (1998) found that toasting peas and faba beans increased SGD but also increased undegraded intake starch which was accompanied by increased ADIN, thus the decrease in potentially digestible starch was likely due to the Maillard reaction. This study demonstrated that pulses have different starch characteristics and react differently to heat treatment because of differences in starch composition, granule size, physical barriers or chemical barriers that may hinder degradation and differences in mechanical processing (Goelema et al. 1999; Ratnayake et al. 2002; Svihus et al. 2005). Lipid-starch complexes can reduce swelling power and increase gelatinization temperature because of the hydrophobic nature of lipids, moisture resistance in turn reduces enzymatic exposure (Svihus et al. 2005).

2.2.7 Milk production and blood urea-N

The influence of heat treatment on rumen fermentation and intestinal digestibility also has positive results on milk production. Feeding extruded and heat treated ingredients has beneficial effects on milk yield (Jones et al. 2001; Masoero et al. 2006; Reynal et al. 2003; Soltan, 2009), FCM (Titgemeyer et al. 1997) or energy corrected milk (Prestlokken et al. 2001) yields, and feed

efficiency (Masoero et al. 2006; Soltan 2009; Titgemeyer et al. 1997). Individual milk constituents seem to be affected differently by heat treatment when comparing different feedstuffs. These discrepancies in milk constituents are likely also a result of processing method and parameters as well as nutrient supply of the remainder of the diet.

Heat treatment of protein rich feedstuffs has resulted in increased milk fat content (Jones et al. 2001; Soltan 2009), milk fat yield (Titgemeyer et al. 1997; Soltan 2009) and milk lactose yield (Soltan 2009), although the milk fat and lactose yield in some circumstances was a direct result of increased milk yield. Milk protein yield may increase due to heat treatment (Jones et al. 2001) but changes in protein constituents and yield may vary depending on parity (Jones et al. 2001). Decreased milk protein content has been observed when feeding heat treated soybean meal (Hall et al. 2010; Titgemeyer et al. 1997) which can subsequently result in a decrease in milk protein yield (Hall et al. 2010). It is possible that the reduced protein content or yield was due to overheating of the ingredient.

Heat treatment of starch rich feed ingredients has resulted in increased milk fat content (Prestlokken et al. 2001), milk fat yield (Prestlokken et al 2001) milk protein content (Petit et al. 1997; Prestlokken et al. 2001) and milk protein yield (Masoero et al. 2006; Prestlokken et al. 2001). Differing ingredients and heat treatment parameters may be the reason for these inconsistent results. The increased milk fat yield and content was only observed in expanded barley (Prestlokken et al. 2001) indicating a change in fermentation patterns or digested nutrients favouring milk fat production. This may be linked to the difference in starch retrogradation of individual ingredients (Ratnayake et al. 2002) where cereal grain starch retrogradation is lower than in peas or potatoes. Increased milk protein yield and composition were observed in cows fed expanded barley (Prestlokken et al. 2001) and increased milk protein composition (Petit et al.

1997) and yield (Masoero et al. 2006) was observed in cows fed extruded peas which is likely due to the altered fermentation of the gelatinized starch but could also be a result of increased RUP.

When feeding heat treated fat rich feed ingredients, milk fatty acid profile can also be modified. Heat treatment has been shown to increase milk fat content (Jones et al. 2001; Prestlokken et al. 2001; Soltan 2009; Tymchuk et al. 1998) or decrease milk fat content (Block et al. 1981; Neves et al. 2009) and milk fat yield (Block et al. 1981). The inhibition of milk fat can be a result of increased unprotected fat in the rumen (Neves et al. 2009) depressing fermentation or it may be due to increased milk fat depressing poly unsaturated fatty acids (PUFA). *Trans*-10 C18:1 (Griinari et al. 1998) and *trans*-10 *cis*-12 conjugated linoleic acid (CLA; Bauman et al. 2001) content in the milk are correlated with milk fat depression (MFD). These *trans* C18 fatty acids can be produced in the rumen from the incomplete biohdydrogenation of PUFA (Bauman et al. 2001, Bauman et al. 2006) and accumulate in the milk. This process is known as the "biohydrogenation theory" which involves the production of these *trans* C18 fatty acid intermediates in the rumen. An overabundance of rumen available PUFA or a state of depressed rumen pH can cause a disturbance in the biohydrogenation pathway (Bauman et al. 2006) leading to the fatty acid intermediates remain incompletely hydrogenated. *Trans*-10 *cis*-12 CLA has been shown to be a powerful inhibitor of de novo milk fat synthesis (Bauman et al. 2006, Baumgard et al. 2000, deVeth et al. 2004) potentially by reducing of mRNA of mammary enzymes that are involved in milk fat synthesis regulation (Griinari et al. 2006). In order for heat treatment to cause MFD by way of CLA intermediates, there would need to be a modification of the physical structure of the ingredient allowing greater access to fatty acids or a modification of the chemical structure to the existing ingredient fatty acids which has not been well proven in the literature.

The modified protein degradation and digestibility in cows fed heat treated ingredients can also reduce the excretion of MUN (Hall et al. 2010; Prestlokken et al. 2001; Jones et al. 2001) offering an energy savings to the cow by way of reduced handling and alteration of deamination products. Reduced blood urea-N (BUN) in cows fed heat treated ingredients (Block et al. 1981; Hall et al. 2010; Prestlokken et al. 2001; Soltan 2009) may also reflect the reduced protein degradation in the rumen and a more efficient utilization of dietary protein.

2.3 Nutrient characteristics of Peas, Canola meal and WDDGS

Protein sources within a diet can enhance or inhibit the efficiency of nutrient utilization the ration. Ingredients are not just composed of a single nutrient that acts alone but their dietary usefulness can be influenced by other nutrients of that particular ingredient as well as nutrients from other ingredients in the ration especially with respect to carbohydrate sources and composition (Hall et al. 2010). Canola, peas and WDDGS are all grown or produced in the prairie provinces and can be sourced relatively easily. Canola meal and WDDGS are protein sources that offer a significant amount of protein, whereas, peas are a dual functioning ingredient, contributing a significant amount of starch as well as some protein. There are many functions of protein ingredients that can make it a more suitable match to the other feed sources in a diet, such as its individual amino acid content, protein fraction composition and rumen degradability as well as other functions such as carbohydrate composition, ash and ether extract (EE) content.

2.3.1 Protein fractions and amino acids of peas and canola meal

Peas contain up to 25.6% CP (DM basis; Hickling 2003; NRC 2001). They can be used to fortify the dietary protein concentration and, specifically, lysine in a dairy cow diet (Table 2.1). Peas contain 7.17% of CP content as lysine and 1.0% of CP content as methionine, contributing a

7:1 ratio of lysine to methionine. Canola meal is a locally processed and commonly used protein source included in dairy rations in Western Canada. It contains 40.9% CP but it has a lower lysine concentration at 5.62% of CP content (Table 2.1). Canola meal has more beneficial amino acid balance for dairy cow dietary requirement with methionine concentration 1.87% of CP resulting in a lysine to methionine ratio of 3:1. Canola meal will not be the only contributor to the amino acid content in dairy cow diets so other protein sources will alter this well balanced ratio. The combination of either of these two ingredients with WDDGS (1:1 lysine:methionine ratio, Table 2.1) would, therefore, result in very different combined ratios. The amino acid balance of a supplement, however, is only beneficial when the whole diet is composed of a ratio that optimizes protein utilization once absorbed in the intestines. Balancing the lysine:methionine ratio can be advantageous but only if the optimal ratio is not altered by rumen fermentation and is delivered to the intestines in a digestible form.

Canola meal, WDDGS and peas have a substantial amount of soluble protein. Crude protein A fraction of canola meal, peas and WDDGS are much higher than soybean meal, therefore, the locally produced ingredients have much lower B fraction (Table 2.1). Rate of B fraction degradation of these three ingredients is also greater than soybean meal (Table 2.1), resulting in a quicker and more extensive rumen protein utilization suggesting lower RUP values. Although RUP can be analyzed and calculated for individual ingredients, protein degradation may be altered by the nutrients in the total diet as well as characteristics that influence passage rate and microbial diversity affecting fermentation and nutrient utilization within the rumen. Individual ingredient RUP values, although giving insight into the ingredient's protein potential, have limited application in ration formulation thus RUP content of individual ingredients are not always reported (NRC 2001). Total dietary RUP, however, is an important measurement that is useful in

Table 2.1 Nutrient composition of soybean meal, canola meal, peas and wheat dried distillers grains with solubles (WDDGS).

	Soybean meal[1]	Canola meal[2]	Peas[3]	WDDGS[4]
DM (%)	89.5	88.0	90.0	8.2
CP (% of DM)	53.8	40.9	25.6	38.9
Lysine (% of CP)	6.29	5.62[1]	7.17[1]	1.55[1]
Methionine (% of CP)	1.44	1.87[1]	1.00[1]	1.41[1]
N Fractions				
A Fraction (% of CP)	15.0	23.2[1]	55.5[1]	21.1[1]
B Fraction (% of CP)	84.4	70.4[1]	44.4[1]	76.9[1]
C Fraction (% of CP)	0.6	6.4[1]	0.1[1]	2.0[1]
Kd of B Fraction (% / hour)	7.5	10.4[1]	16.7[1]	26.1[1]
ADF (% of DM)	6.2	19.1	9.1	15.0
NDF (% of DM)	9.8	23.5	18.6	32.8
Lignin (% of DM)	0.5	5.8	0.6	6.5
Ash (% of DM)	6.4	6.9	3.7	5.3
EE (% of DM)	1.1	4.0	1.6	5.1
Starch (% of DM)	2.8[5]	5.8	51.1	4.2
Sugars (% of DM)	9.3[5]	7.6	5.1	-

[1] Nutrient values from NRC (2001).
[2] Nutrient values from Newkirk (2009).
[3] Nutrient values from Hickling (2003).
[4] Nutrient values from University of Saskatchewan (2010).
[5] Nutrient values from U.S. Soybean Export Council (2012)

determining the protein efficiency of the diet and the ability to support high levels of milk production. A more applicable use of RUP data is that which is gathered from the whole ration which can be used to determine the effectiveness of similarly balanced rations.

2.3.2 Ash

Ash does not directly contribute as an energy supplying nutrients of a ration although minerals contained in the ash fraction are utilized for important metabolic functions. High ash values can indicate an unnecessary overabundance of one or more minerals and can be a detriment to a dairy cow diet by decreasing energy value of the ration because of the reduced organic matter. The ash value of peas is much lower than soybean meal, canola meal or WDDGS (Table 2.1). This may give diets that include peas a slight advantage in organic matter content and dry matter digestibility.

2.3.3 Carbohydrates

Several plant based protein sources are derived from oilseeds which contain very little starch or sugars and content in the respective by-products are still quite minimal. In protein sources derived from cereal grains processed for ethanol or brewery production, most of the starch is removed by the yeast fermentation process also resulting in a low starch and sugar content of the by-products. Canola meal and WDDGS have relatively low starch; however, raw peas contain a large amount of starch (Table 2.1).

Most protein rich plant by-products have relatively high fiber content due to the plant cell wall components which are concentrated (e.g., ADF and NDF) during processing. Although fiber is not the largest component of these by-products, it limits the protein content of these ingredients, causing an inverse relationship between NDF and protein. Standard solvent extracted soybean

meal is low in ADF and NDF (Table 2.1), contributing to the ingredient's reputation of being the protein standard to which all other proteins are compared. Peas have an intermediate level of ADF and NDF but canola meal and WDDGS have higher fiber content. Lignin is especially important because it represents the fiber content that essentially cannot be utilized in the rumen and therefore does not contribute to the utilizable energy value to the feed. In forages, lignin contributes physically effective fiber in the rumen but in by-products, the particles are likely too small to contribute to physically effective fiber. Lignin in soybean meal and peas is quite low (Table 2.1) but it is substantially higher in canola meal and WDDGS.

2.4 Conclusion

There has been ample research on extruding individual feedstuffs and feeding different protein sources or protein combinations although WDDGS has not been studied in a modified form. The current research study is unique in that there is very little information on the effects and interactions of extrusion on peas versus canola meal in combination with WDDGS on ruminal fermentation characteristics, omasal flow, nutrient digestibility and milk production parameters. This study was, therefore, of interest in determining the use of these locally produced protein sources on ruminal health and production benefits in the western Canadian dairy industry.

The hypothesis for this thesis research was that feeding combinations of WDDGS-canola meal and WDDGS-peas would result in differences in ruminal N utilization, microbial protein production, omasal nutrient flow, and production performance of dairy cows, and that these effects would be more pronounced with extruded diets. Therefore, the objective was determine the effects of extruding WDDGS-peas or WDDGS-canola meal combinations as part of the concentrate on

milk production and composition, feed intake, ruminal fermentation characteristics, microbial protein synthesis and nitrogen excretion.

3 THE EFFECTS OF EXTRUDING WHEAT DRIED DISTILLERS GRAINS WITH SOLUBLES WITH PEAS OR CANOLA MEAL ON RUMINAL FERMENTATION, MICROBIAL PROTEIN SYNTHESIS, NUTRIENT DIGESTION AND MILK PRODUCTION IN HOLSTEIN DAIRY COWS

3.1 Introduction

The majority of ethanol production in western Canada uses wheat as the fermentation ingredient. Recently, the rapid expansion of ethanol production has resulted in an abundant supply of WDDGS that can be used as a protein source for dairy cows. Several recent studies have demonstrated that WDDGS can be used as a replacement for traditional protein supplements like canola meal (Abdelqader et al. 2012; Chibisa et al. 2012; Chibisa et al. 2013; Maxin et al. 2013) and corn DDGS (Abdelqader et al. 2012; Chibisa et al. 2013; Penner et al. 2009) without compromising milk production. In western Canada and parts of the USA, dairy cow diets are typically formulated with canola meal as the major protein source because it is a readily available, high quality protein supplement (Hickling 2008; Mulrooney et al. 2009). It is well-established that major differences exist in the chemical compositions and ruminal degradabilities of canola meal and WDDGS (Boila et al. 1994b). When compared to canola meal, the available data indicates that WDDGS is similar in CP content (38.9 vs. 40.9%; University of Saskatchewan 2010; Newkirk 2009, respectively), but is lower in methionine (1.41 vs. 1.87% of CP; NRC 2001) and, particularly, lysine (1.55 vs. 5.62% of CP; NRC 2001) contents. Although WDDGS protein is more ruminally degradable than corn DDGS (Li et al. 2013; Nuez Ortin et al. 2009), ruminal N degradability of WDDGS has been shown to be lower compared to canola meal using the in situ technique (Boila et al. 1994b). In vivo measurements using the omasal sampling technique also

indicated that RPD supply decreased whereas RUP supply increased when WDDGS replaced canola meal in lactating cow diets (Chibisa et al. 2012). Peas (*Pisum sativum*) contain relatively high levels of CP (24 to 28%; Petit et al. 1997; NRC 2001) and starch (51%; Hickling 2003). Peas can been used as a cheaper replacement for more expensive protein and energy sources in ruminant diets. Lactating dairy cow diets can utilize peas without negatively affecting milk yield and composition (Petit et al. 1997; Khorasani et al. 2001; Masoero et al. 2006). When compared to WDDGS and canola meal, peas contain less CP which is more ruminally-degradable (78% RDP as a % of CP); however, peas contain greater levels of lysine compared to WDDGS (NRC 2001). Canola meal, peas and WDDGS could be blended to result in a favourable protein combination due to the differences in chemical composition and ruminal degradability between those individual ingredients. Combining WDDGS with canola meal, and WDDGS with peas would supply sufficient amounts of RDP to meet microbial N requirements, while also potentially providing adequate amounts of RUP with a good amino acid (lysine and methionine) balance to optimize milk production in high-producing dairy cows.

Altering the physical structure of proteinaceous feedstuffs through heat processing methods such as extrusion may help to protect dietary protein from ruminal degradation, thus increasing the post-ruminal supply of digestible RUP (Benchaar et al. 1994; Chapoutot et al. 1997; Kibelolaud et al. 1993; Prestlokken et al. 2001; Solanas et al. 2005; Solanas et al. 2008) that could benefit high-producing dairy cows (NRC 2001; Kalscheur et al. 1999). Heat processing decreases the extent of ruminal protein degradation primarily by lowering the solubility of dietary proteins through protein denaturation (Khan et al. 2015; Yu et al. 2005), and the formation of carbohydrate-protein (Van Soest 1994) and protein-protein cross-linkages (NRC 2001). Numerous studies (Benchaar et al. 1994; Masoero et al. 2005; Focant et al. 1990; Petit et al. 1997; Solanas et al.

2005; Solanas et al. 2008; Walhain et al. 1992) have reported decreased ruminal protein degradation when proteinaceous feedstuffs like lupins, peas, soybeans and soybean meal have been subjected to extrusion processing. The differences in chemical composition and ruminal degradability among WDDGS, canola meal, and peas, as indicated previously, may make the judicious combinations of WDDGS with canola meal and WDDGS with peas good candidates for extrusion processing. Extrusion of these combinations could be of benefit in terms of improving ruminal N utilization and post-ruminal RUP supply. To our knowledge, comparative studies do not exist that have investigated the effects of feeding non-extruded or extruded combinations of WDDGS-canola meal and WDDGS-peas on ruminal digestion, N utilization, and production performance in dairy cows. Therefore, the specific objective of this study was to determine the interactive effects of feeding non-extruded or extruded mixtures of WDDGS-canola meal and WDDGS-peas on ruminal fermentation characteristics, microbial protein production, omasal nutrient flow, and production performance of high-producing dairy cows. The hypothesis of this research was that feeding combinations of WDDGS-canola meal and WDDGS-peas would result in differences in ruminal N utilization, microbial protein production, omasal nutrient flow, and production performance of dairy cows, and that these effects would be more pronounced with extruded diets.

3.2 Materials and Methods

3.2.1 Animals, Experimental Design and Dietary Treatments

Eight multiparous lactating Holstein cows (712 ± 54 kg body weight, 90 ± 31 days in milk) that were housed in individual tie-stalls at the University of Saskatchewan Greenbrae Dairy Research Facility were used in this study. The experimental design was a replicated 4 × 4 Latin

square design with a 2 × 2 factorial arrangement of dietary treatments. Each experimental period was 28-d in length, consisting of 13-d of diet adaptation and 15-d of sample and data collection. One Latin square had four ruminally cannulated cows which were used in a metabolism study. Treatments were either 8% WDDGS-7% peas (WP) or 8% WDDGS-7% canola meal (WC) combinations fed in either an extruded or non-extruded form. Supplement ingredients were ground through a hammer mill using a #10 (2-mm) screen prior to weighing and mixing ingredients for 2 minutes using a ribbon mixer. Extruded diets were processed at O&T Farms (Regina, SK) using an Insta-Pro International 2500 series extruder (Des Moines, IA) using a custom 5-hole (5/16 inch hole) nose cone. The extruder averaged 600 rpm creating 400 lbs pressure with the motor averaging 12 Amps for the WP treatment and 10 Amps for the WC treatment. Water was added to the extruder at 15-21 L per tonne of supplement. Extruding temperature for extruded WP and WC treatments were 161ºC (range 131 to 184ºC) and 160ºC (range 142 to 179ºC), respectively. The four experimental diets (Table 3.1) were fed as a TMR with approximately a 47:53 forage to concentrate ratio. Due to the large protein content difference between WC and WP supplements, soybean meal and corn gluten meal were added to make diets isonitrogenous. Cows were fed twice daily at 0900 and 1600 h for *ad libitum* intake. Animals were cared for and handled according to guidelines set by the Canadian Council of Animal Care (1993). Experimental procedures used were approved by the University of Saskatchewan Animal Care Committee.

Table 3.1 Feed ingredients and chemical composition of the four experimental diets

	Extruded		Non-extruded	
	WP[1]	WC[1]	WP[1]	WC[1]
Ingredient (% of diet DM)				
Barley silage	31.6	31.8	31.6	31.8
Alfalfa hay	14.6	15.1	14.6	15.1
Barley grain	30.0	31.8	30.0	31.8
WDDGS[1,2]	7.9	7.9	7.9	7.9
Canola seed[2]	0.8	0.8	0.8	0.8
Pea grain[2]	7.2	-	7.2	-
Canola meal[2]	-	7.2	-	7.2
Molasses	0.2	0.2	0.2	0.2
Corn gluten meal	1.98	0.20	1.98	0.20
Soybean meal	1.09	0.08	1.09	0.08
Vitamin premix[3]	1.8	1.8	1.8	1.8
Limestone	0.05	0.05	0.05	0.05
Golden flake	1.58	1.79	1.58	1.79
Salt	0.20	0.20	0.20	0.20
Sodium bicarbonate	0.87	0.88	0.87	0.88
Dynamate	0.16	0.16	0.16	0.16
Chemical composition				
DM (%)	57.7	57.7	57.5	57.3
OM (% of DM)	92.1	91.8	92.0	91.6
CP (% of DM)	17.1	17.0	17.3	17.2
ADF (% of DM)	14.7	15.6	14.2	15.2
NDF (% of DM)	27.4	29.1	26.6	28.5
EE (% of DM)	3.9	4.2	3.9	4.2
Starch (% of DM)	23.3	21.3	22.2	21.1
NE_L (Mcal/kg of DM)[4]	1.68	1.65	1.68	1.65
Calcium (% of DM)[4]	0.69	0.75	0.69	0.75
Phosphorus (% of DM)[4]	0.51	0.56	0.51	0.56

[1] WDDGS = wheat dried distillers grains with soluble, WP =WDDGS-peas treatment combination, WC = WDDGS-canola meal treatment combination.
[2] Ingredients included in the processed treatments.
[3] Containing (per kg of DM premix): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1,000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg Zn, 1,500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.
[4] NE_L, calcium and phosphorus calculated using CPM Dairy V3.0.

3.2.2 Data and sample collection

3.2.2.1 Feed intake and milk production data and sample collection

Feed intake and milk yield of all eight cows were recorded daily during the 15-d collection period. All cows were milked three times daily at 0430, 1230 and 1900 h. Milk samples were taken at each milking on d 17, 18 and 19. Samples were preserved with Brotab "10" (2-bromo-2-nitro-propane-1-3-diol), pooled per cow per day (proportionally based on milk yield) and stored at 4 ºC until they were sent to Central Milk Testing Laboratory (Edmonton, AB) for analysis. Representative 1.5 kg samples of TMR were taken on d 17, 18, 19, 25, 26 and 27 and stored at -20ºC to be used for feed nutrient analysis.

3.2.2.2 Omasal sampling for microbial protein production and nutrient flow

Continuous marker infusion of the four ruminally cannulated cows began on d 14. Microbial protein flow to the omasum was measured using $(^{15}NH_4)_2SO_4$ (10 atom percent excess [APE] ^{15}N; Cambridge Isotope Laboratories, Andover, MA) as a marker (Reynal et al. 2005). Omasal nutrient flow was measured using the triple marker method (France et al. 1986) using $YbCl_3$ (Siddons et al. 1985), indigestible NDF (iNDF; Huhtanen et al. 1994) and Cr-EDTA (Uden et al. 1980) as markers for the small particle phase (SPP), large particle phase (LPP) and fluid phase (FP), respectively. Prior to infusion, a 400-mL sample of omasal digesta was collected to determine the background levels of bacterial ^{15}N. A 1-L priming dose (equal to half the daily infusion) was given just before the initiation of the continuous marker infusions. Markers were infused at daily rates of 3.35 g Yb (Brito et al. 2006), 2.77 g Cr (Binnerts et al. 1968) and 0.22 g of ^{15}N (Brito et al. 2006). A total marker solution of 2 L was continuously infused daily during the 8-d infusion period using a Watson and Marlow Model 205U peristaltic pump (Cornwall, UK).

Containers holding the marker solutions were weighed daily and recorded to determine the exact amount infused. Omasal samples were collected on three consecutive days to represent a 24-h feeding cycle (d 20 at 0900, 1500 and 2100 h; d 21 at 0300, 1200, 1800 and 2400 h; and d 22 at 0600 h). At each sampling, a 600 mL sample of omasal digesta was collected using methods developed by Huhtanen et al. (1997) but modified for manual sampling. The omasal digesta was thoroughly mixed and divided into three separate fractions. A 300 mL sub-sample was stored at -20ºC and pooled per cow per period for an accumulated 2.4 L sample. A 100-mL sub-sample was stored at -20ºC and pooled per cow per period to serve as a reserve sample. The remaining 200 mL was held on ice and pooled after every other sampling to yield a 400-mL sample which was then immediately processed to isolate bacteria as described by Reynal et al. (2005).

3.2.2.3 Total collection of urine and feces

Urine and feces were collected for 4 d to determine output (Plaizier et al. 2000). On d 24, cows were fitted with indwelling bladder catheters (Bardex Foley 75cc 2 way ribbed balloon, lubricious coated 27Fr bladder catheters; C. R. Bard Inc, Covington, GA). At 0900 h on d 25, steel trays were placed behind each cow for fecal collection. Bladder catheters were then attached to tubing which was connected to 20-L Nalgene bottles containing 150 mL concentrated HCl. The acid served to acidify the urine and prevent NH_3-N volatilization. Urine was collected in these containers and after each 24-h interval, urine was weighed and a representative 5% sub-sample was taken and stored at -20ºC. These samples were pooled per cow per period. The feces collected from the 24 h interval were mixed, weighed and a representative 2.5% sub-sample was taken and stored at -20ºC.

3.2.2.4 Blood sampling

Blood was collected from the coccygeal vein of all eight cows at 1100 h on d 25 using a 10-mL lithium heparin-coated vacutainer vial (Becton Dickinson, Franklin Lakes, NJ). Blood was centrifuged at 1,500 x *g* at 4ºC for 15 minutes. Plasma was extracted and frozen at -20ºC.

3.2.2.5 Ruminal digesta collection for pH, VFA and NH_3-N

Starting on d 27, ruminal digesta samples were collected at 0900, 1200, 1500, 1800, 2100, 2400, 0300 and 0600 to represent a 24-h feeding cycle. Ruminal digesta samples were collected from the cranial dorsal, cranial ventral, ventral and caudal ventral regions. Ruminal fluid was obtained by straining ruminal digesta through 4 layers of cheesecloth. Ruminal fluid pH was measured immediately using a Model 265A portable pH meter (Orion Research Inc., Beverly, MA). A 10 mL ruminal fluid sub-sample was mixed with 2 ml of 25% meta-phosphoric acid (HPO_3) and stored at -20ºC for later determination of VFA. A second 10 mL sample was mixed with 2 mL of 1% sulphuric acid (H_2SO_4) and stored at -20ºC for later determination of NH_3-N.

3.2.3 Sample analysis

3.2.3.1 Milk analysis

Milk samples were sent to Central Milk Testing Laboratory (Edmonton, AB) for infrared analysis of milk urea-N (MUN), fat, protein and lactose content using a Milkoscan FT6000 (Foss Electric, Denmark).

3.2.3.2 Urine, fecal and feed sample analysis

Feed and fecal samples were thawed overnight at room temperature, dried at 55ºC for 72-h and then ground with a Christy-Norris hammer mill through a 1-mm screen (Christy and Norris

Ltd., Chelmsford, UK). Ground feed and fecal samples were composited per cow in each experimental period. Composited feed and fecal samples were analyzed for DM (method 930.15; AOAC 1990), OM (method 942.05; AOAC 1990), EE (method 920.39; AOAC 1990), CP (macro-Kjeldahl procedure, method 976.05; AOAC 1990), total starch (Megazyme Total Starch K-TSTA kit, DMSO method 996.11; AOAC 1990), ADF and NDF (Van Soest et al. 1991). Amylase and sodium sulphite were used for NDF analysis.

Urine samples were analyzed for N (macro-Kjeldahl procedure, method 976.05; AOAC 1990) using a Kjeltec 2400 auto-analyzer (FOSS Analytical, Hillerod, Denmark). Urine was analyzed for urea-N using a diacetyl monoxime-based method (Stanbio Urea Nitrogen BUN kit, Procedure No. 0580; Stanbio Laboratory, Boerne, TX).

3.2.3.3 Bacterial pellet isolation and analysis

Bacterial isolation was performed after every other omasal collection on the chilled, pooled 400-mL sample using a modified procedure of Reynal et al. (2005). Background ^{15}N omasal samples were also processed using the same procedure. Briefly, the 400-mL sample was filtered through one layer of cheesecloth (collecting the filtrate). The filtrand within the cheesecloth was rinsed with 320 ml 0.85% NaCl solution and excess solution was collected. The filtrate and rinse solution were combined and centrifuged at 1,000 x *g* at 5ºC for 5 minutes using a Beckman Coulter Avanti J-E Centrifuge (Indianapolis, IN). The supernatant, used for fluid associated bacteria (FAB) extraction, was decanted and set aside. The remaining filtrand from the cheesecloth was combined with 280 mL of a solution containing 0.85% NaCl and 0.1% Tween-80 and held on ice. Once the pellet from the centrifugation was isolated, it was added to the filtrand mixture which was then blended for 30 seconds and refrigerated at 4ºC for 24 h. This mixture was later used to

extract the particle associated bacteria (PAB). In the meantime, the FAB solution was centrifuged at 11,300 x *g* at 5ºC for 30 minutes. The pellet was collected (discarding the supernatant) and mixed with 75 mL of McDougall's buffer (McDougall 1948) and centrifuged again at 11,300 x *g* at 5ºC for 30 minutes. The supernatant was again discarded and the FAB pellet was collected in an aluminium tray, stored at -20ºC and pooled per cow per period with subsequent FAB pellets. Similarly, after chilling for 24 h, the PAB solution was mixed and filtered through two layers of cheesecloth. The PAB filtrate was centrifuged at 1,000 x *g* at 5ºC for 5 minutes. The PAB supernatant was collected and was processed similarly to the FAB solution (this time discarding the pellet) by centrifuging at 11,300 x *g* at 5ºC for 30 minutes, collecting the PAB pellet and mixing it with 75 mL of McDougall's buffer for the second centrifugation. The isolated PAB pellet was also stored at -20ºC and pooled per cow per period with the subsequent PAB pellets. Both frozen FAB and PAB samples were freeze-dried, ground with a mortar and pestle and analyzed for DM and OM as previously described. For analysis of non-ammonia nitrogen (NAN) and ^{15}N enrichment of FAB and PAB samples, NH_3-N was volatilized using a modified procedure from Reynal et al. (2005). Briefly, 2 mg (approximately 150 μg N) was weighed into 5 x 9 mm tin capsules (Elemental Microanalysis Limited, Okehampton, UK), treated with 75 μL of 72 m*M* K_2CO_3 and dried in a forced air oven at 60ºC for 24 h. Samples were then analyzed for ^{15}N using isotope ratio-mass spectrometry.

3.2.3.4 Omasal sample analysis

The pooled 2.4 L omasal sub-sample collected for omasal nutrient flow was thawed overnight at room temperature and separated into three phases (Reynal et al. 2005). Briefly, omasal digesta was strained through one layer of cheesecloth. The filtrand remaining was kept as the LPP. The filtrate was then centrifuged at 1,000 x *g* at 5ºC for 5 minutes. The resulting supernatant was

kept as the FP and the pellet was kept as the SPP. The three separated phases were frozen at -20ºC and then freeze dried and ground with mortar and pestle (FP and SPP) or ground to 1 mm (LPP) using a Retsch ZM100 grinder (Haan, Germany). The content of iNDF in TMR, SPP and LPP samples was determined using a 12-d ruminal incubation procedure (Huhtanen et al. 1994) with 3, 3.5 and 1.5 g samples, respectively, in 6 μm pore 5 x 10 cm nylon bags (Ankom Technology, Macedon, NY). The incubated samples were analyzed for NDF using the procedure described previously. All three phases were combusted and digested with nitric acid according to Lopez Molinero et al. (1988) and analyzed for Yb concentration using atomic emission and Cr concentration using atomic absorption on a Varian SpectrAA 220 Atomic Absorption Spectrometer (Varian Analytical Instruments, Mulgrave, Australia). An infusate sample taken from each infusion was also analyzed for Cr and Yb concentration. The concentrations of iNDF (LPP marker), Yb (SPP marker) and Cr (FP marker) in the specified phases were used to reconstitute the omasal true digesta (OTD) using the triple marker method of France et al. (1986). The OTD was then analyzed for DM, OM, CP, total starch, ADF and NDF using procedures described previously. Concentration of OTD NH_3-N was determined by adding 10 mL of 0.07 *M* sodium citrate solution (pH 2.2) to a 0.5-g sample then placed in a forced air oven at 39ºC for 30 minutes. Samples were centrifuged at 18,000 × *g* at 4ºC for 15 minutes and then immediately analyzed for NH_3-N using the phenol-hypochlorite assay (Broderick et al. 1980) using a Helios Delta Spectrophotometer (Thermo Electron Corporation, England). A particle phase (PF) was also reconstituted from LPP and SPP using marker concentration. Both PF and FP samples were ground using a ball mill, treated to volatilize NH_3-N (as previously described) and then analyzed for NAN and ^{15}N enrichment using isotope ratio-mass spectrometry.

3.2.3.5 Plasma analysis

Plasma was thawed at room temperature and analyzed for glucose using a glucose oxidase method (Stanbio Glucose LiquiColor kit, Procedure No. 1070; Stanbio Laboratory, Boerne, TX) and for plasma urea-N (PUN) using a diacetyl monoxime-based method (Stanbio Urea Nitrogen (BUN) kit, Procedure No. 0580; Stanbio Laboratory, Boerne, TX).

3.2.3.6 Ruminal fluid sample analysis

Thawed ruminal samples previously acidified with HPO_3 were vortexed and centrifuged using a Beckman Coulter Avanti J-E Centrifuge (Indianapolis, IN) at 3,655 x *g* at 4ºC for 15 minutes. From the supernatant, 120 µL of sample was added to 500 µL of 1mM trimethyl acetic acid (used as an internal standard) and 880 µL of acetonitrile in a micro-centrifuge tube being kept on ice. The solution was then vortexed and centrifuged at 16,100 x *g* at 4ºC for 5 minutes using an Eppindorf 5415R Centrifuge (Westbury, NY). From the supernatant, 1 mL was transferred into a GC vial. Samples were analyzed for VFA (Erwin et al. 1961) using an Agilent 6890 Series Gas Chromatography System with FID (Wilmington, DE) and an Agilent 7683 Series injector using a Zebron ZB-FFAP High Performance GC Capillary Column (30.0 m × 320 µm × 0.25 µm, Phenomenex, Torrance, CA).

Thawed ruminal samples previously acidified with H_2SO_4 were vortexed, centrifuged at 14,000 × *g* at 4ºC for 10 minutes and analyzed using the phenol-hypochlorite assay for NH_3-N concentration (Broderick et al. 1980) as previously described.

3.2.4 Calculations

Apparent digestion of nutrients in the rumen (ADR) was calculated as follows:

$$\text{Nutrient ADR} = \text{nutrient intake} - \text{nutrient omasal flow}$$

Bacterial NAN, DM and OM flow and non-NH_3-N non-bacterial nitrogen (NANBN) flow to the omasum as well as RDP supply and OM truly digested in the rumen (OMTDR) was calculated as per Brito et al. (2009). The following equations assume that FAB is representative of bacteria flowing within FP and PAB is representative of bacteria flowing within PF:

$$^{15}\text{N APE } (^{15}\text{N enrichment}) = {}^{15}\text{N atom \%} - \text{background } {}^{15}\text{N atom \%}$$

$$\text{Omasal phase (FP or SPP) NAN flow} = \text{Omasal phase flow} \times \text{NAN in Omasal phase}$$

$$\text{FAB NAN flow} = \text{FP NAN flow} \times (\text{FP } {}^{15}\text{N APE} \div \text{FAB } {}^{15}\text{N APE})$$

$$\text{PAB NAN flow} = \text{PF NAN flow} \times (\text{PF } {}^{15}\text{N APE} \div \text{PAB } {}^{15}\text{N APE})$$

$$\text{Total bacterial NAN flow} = \text{FAB NAN flow} + \text{PAB NAN flow}$$

$$\text{NANBN flow} = \text{total NAN flow} - \text{total bacterial NAN flow}$$

$$\text{RDP supply} = \text{total CP intake} - (\text{NANBN flow} \times 6.25)$$

$$\text{Bacterial (FAB or PAB) DM flow} = \text{Bacterial NAN flow} \div (\text{\% Bacterial NAN} \div 100)$$

$$\text{Bacterial (FAB or PAB) OM flow} = (\text{Bacterial DM flow} \times \text{\% Bacterial OM}) \div 100$$

$$\text{Total bacterial OM flow} = \text{FAB OM flow} + \text{PAB OM flow}$$

$$\text{OMTDR} = \text{OM intake} - (\text{omasal OM flow} - \text{total bacterial OM flow})$$

3.2.5 Statistical analysis

Data on DMI, milk yield and composition, PUN and plasma glucose were analyzed as a replicated 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments using the PROC MIXED procedure of SAS (SAS Institute 2002) using the following model: $Y_{ijklm} = \mu + S_i + P_{j(i)} + C_{k(i)} + \alpha_l + \beta_m + (\alpha\beta)_{lm} + E_{ijklm}$ where Y_{ijklm} is the dependent variable, μ is the overall mean, S_i is the fixed effect of square i, $P_{j(i)}$ is the fixed effect of period j (within square i), $C_{k(i)}$ is the random effect of cow k (within square i), α_l is the fixed effect of treatment factor 1 (supplement protein combination, SC) l, β_m is the fixed effect of treatment factor 2 (supplement extrusion, SE) m, $(\alpha\beta)_{lm}$ is the interaction between treatment factor 1 and factor 2 lm, and E_{ijklm} is the random residual error. Data on nutrient digestibility, omasal flow and ruminal digestion of nutrients, ruminal microbial-N production and nitrogen balance was analyzed as a 4 x 4 Latin square with a 2 × 2 factorial arrangement of treatments using the PROC MIXED procedure of SAS (SAS Institute 2002) using the following model: $Y_{ijkl} = \mu + P_i + C_j + \alpha_k + \beta_l + (\alpha\beta)_{kl} + E_{ijkl}$ where Y_{ijkl} is the dependent variable, μ is the overall mean, P_i is the fixed effect of period i, C_j is the random effect of cow j, α_k is the fixed effect of treatment factor 1 (SC) k, β_l is the fixed effect of treatment factor 2 (SE) l, $(\alpha\beta)_{kl}$ is the interaction between treatment factor 1 and factor 2 kl, and E_{ijkl} is the random residual error. Ruminal pH, NH_3-N and VFA were analyzed as repeated measures by including time, factor 1 × time interaction, factor 2 × time interaction and factor 1 × factor 2 × time interaction as fixed effects along with period, factor 1 and factor 2 as fixed effects and cow as a random effect. Significance of treatment effects was chosen as $P \leq 0.05$ and tendencies when $0.05 < P \leq 0.10$.

3.3 Results

3.3.1 Experimental diet and supplemental treatment chemical composition

Chemical composition of the four experimental diets were similar (Table 3.1) with the exception of ADF, NDF and EE which were all numerically higher in the WC diets compared to the WP diets. Starch content was numerically higher in the WP diets compared to the WC diets (Table 3.1).

The WC supplemental treatment had numerically higher CP content compared to the WP treatments (Table 3.2). Various protein supplements were included purposefully for extruded vs. non-extruded WP or WC diets; however, in order to make diets containing WP or WC isonitrogenous (Table 3.1), it was necessary to include variable amounts of soybean meal and corn gluten meal due to the differing CP content of WP and WC treatments. Even though the inclusion levels of soybean meal and corn gluten meal across diets were different, it should be noted that all diets contained the same supplemental sources of protein which would provide similar profiles of amino acids.

Supplemental starch and OM content were numerically higher (Table 3.2) in WP treatments compared to the WC treatments. Similar to the complete diets, ADF and NDF were numerically higher in WC treatments (Table 3.2) compared to the WP treatments; however, EE was numerically higher in the non-extruded treatments compared to the extruded treatments.

Table 3.2 Chemical composition of the extruded and non-extruded supplemental treatments

	Extruded		Non-extruded	
	WP[1]	WC[1]	WP[1]	WC[1]
DM (%)	90.9	90.8	89.9	91.2
OM (% of DM)	95.6	93.9	95.9	93.9
CP (% of DM)	30.8	39.2	31.3	39.4
ADF (% of DM)	8.6	14.5	7.8	14.5
NDF (% of DM)	20.4	31.7	20.9	31.5
EE (% of DM)	6.0	5.8	7.1	7.1
Starch (% of DM)	21.5	2.6	21.0	1.9

[1] WDDGS = wheat dried distillers grains with solubles, WP =WDDGS-peas treatment combination, WC = WDDGS-canola meal treatment combination.

3.3.2 DMI, milk yield and milk components

DMI was not affected ($P > 0.05$) by dietary treatment (Table 3.3). Milk yield was higher ($P = 0.030$; Table 3.3) in cows fed extruded diets compared to those fed non-extruded diets (42.9 vs. 40.8 kg/d), and milk yield of cows fed WP diets tended to be higher ($P = 0.06$) than those fed WC diets (43.5 vs. 40.2 kg/d; Table 3.3). Milk fat content was higher ($P = 0.040$) in cows fed non-extruded diets compared to those fed extruded diets (3.58 vs. 3.53%), but milk fat yield ($P = 0.027$; 1.50 vs. 1.44 kg/d) and fat corrected milk yield ($P = 0.027$; 42.9 vs. 41.2 kg/d) were higher in cows fed extruded diets compared to those fed non-extruded diets (Table 3.3). Milk protein content was not affected ($P > 0.05$) by diet but protein yield was higher ($P < 0.05$) in cows fed WP diets compared to those fed WC diets (1.38 vs. 1.29 kg/d; Table 3.3). Milk lactose content was higher ($P = 0.011$) in cows fed extruded diets compared to those fed non-extruded diets (4.55 vs. 4.48%; Table 3.3). Cows fed non-extruded diets had higher MUN ($P = 0.011$) compared to those fed extruded diets (15.7 vs. 14.9 mg/dL; Table 3.3).

Table 3.3 Dry matter intake, milk yield, milk composition and component yield, 3.5% fat corrected milk (FCM) and milk urea nitrogen (MUN) in cows fed the four experimental diets[1]

	Extruded		Non-extruded			*P* value		
	WP[2]	WC[2]	WP[2]	WC[2]	SEM	PROT[2]	EXT[2]	PROT × EXT[2]
DMI (kg/d)[3]	28.4	29.1	28.1	27.6	1.1	0.95	0.18	0.36
Milk yield (kg/d)	44.2	41.6	42.8	38.8	2.0	0.06	0.030	0.42
3.5% FCM (kg/d)	43.7	42.0	42.6	39.9	1.7	0.17	0.027	0.38
Milk fat (%)	3.48	3.59	3.54	3.61	0.24	0.26	0.040	0.35
Milk protein (%)	3.21	3.22	3.20	3.21	0.08	0.74	0.79	0.96
Milk lactose (%)	4.57	4.52	4.47	4.50	0.03	0.71	0.011	0.13
Milk fat yield (kg/d)	1.53	1.47	1.49	1.40	0.06	0.17	0.027	0.38
Milk protein yield (kg/d)	1.38	1.32	1.37	1.26	0.06	0.047	0.14	0.30
Milk lactose yield (kg/d)	2.02	1.88	1.91	1.75	0.10	0.06	0.009	0.62
MUN (mg/dL)	15.1	14.8	15.5	16.0	0.8	0.80	0.011	0.19

[1] Data was obtained from all 8 cows in 2 Latin squares.

[2] WDDGS = wheat dried distillers grains with solubles, WP =WDDGS-peas treatment combination, WC = WDDGS-canola meal treatment combination, PROT = treatment protein combination factor, EXT = treatment extrusion factor, PROT × EXT = interaction between PROT and EXT.

[3] DMI was calculated using intake records throughout the 15-d sample and data collection.

3.3.3 Ruminal fermentation characteristics

Ruminal pH was higher in cows fed extruded WP compared to those fed extruded WC; however, in cows fed non-extruded diets, ruminal pH was higher in those fed WC compared to those fed WP (interaction, $P = 0.044$; Table 3.4). Ruminal NH_3-N concentration tended ($P = 0.06$) to be higher in cows fed WC diets compared to those fed WP diets (11.80 vs. 10.47 mg/dL; Table 3.4). For cows fed extruded diets, ruminal acetate concentration was higher in cows fed WC compared to those fed WP; however, for cows fed non-extruded diets, ruminal acetate concentration was lower for cows fed WC compared to those fed WP (interaction, $P = 0.019$; Table 3.4). Ruminal propionate concentration was higher ($P = 0.026$) in cows fed extruded diets compared to those fed non-extruded diets (22.7 vs. 19.1 m*M*); however, the acetate:propionate ratio was not affected by dietary treatment ($P > 0.05$; Table 3.4). Ruminal butyrate (interaction, $P < 0.01$) and isovalerate (interaction, $P < 0.01$) concentrations were similar among cows fed non-extruded diets; however, cows fed extruded WC had higher ruminal concentrations of butyrate and isovalerate than those fed extruded WP (Table 3.4). Ruminal valerate concentration tended to be higher in cows fed extruded WC compared to those fed extruded WP; however, ruminal concentration of valerate in cows fed non-extruded diets was similar (interaction, $P = 0.07$; Table 3.4). Ruminal isobutyrate concentration was higher ($P < 0.01$) in cows fed WC diets compared to those fed WP diets (0.85 vs. 0.79 m*M*; Table 3.4). Total VFA was not affected by diet ($P > 0.05$; Table 3.4). All ruminal fermentation characteristics were affected by time ($P < 0.01$); however, there were no interactions between time and treatments (Figures 3.1, 3.2, 3.3, 3.4, 3.5, 3.6).

Blood PUN concentration tended to be lower in cows fed extruded diets compared to those fed non-extruded diets ($P = 0.10$; Table 3.4). Plasma glucose concentration was higher in cows fed WC diets compared to those fed WP diets (Table 3.4); however, the difference in plasma

glucose concentration between WC and WP was greater in cows fed extruded diets compared to those fed non-extruded diets (interaction, $P < 0.01$).

Table 3.4 Ruminal fermentation characteristics[1] and blood metabolites[2] in cows fed the four experimental diets

	Extruded		Non-extruded			*P* value		
	WP[3]	WC[3]	WP[3]	WC[3]	SEM	PROT[3]	EXT[3]	PROT × EXT[2]
Ruminal Fermentation Characteristics								
Ruminal pH	5.92[ab]	5.81[b]	5.91[ab]	6.10[a]	0.10	0.56	0.06	0.044
Ruminal NH_3-N (mg/dL)	10.08	12.32	10.85	11.28	0.61	0.06	0.84	0.19
VFA concentration (m*M*)								
Acetate	61.3[ab]	64.9[a]	60.8[ab]	58.1[b]	2.0	0.72	<0.01	0.019
Propionate	20.7	24.6	19.7	18.5	2.2	0.30	0.026	0.08
Butyrate	10.7[b]	12.6[a]	12.4[ab]	11.0[ab]	0.8	0.59	0.90	<0.01
Isobutyrate	0.78	0.87	0.80	0.83	0.02	<0.01	0.79	0.11
Valerate	1.42	1.93	1.64	1.57	0.14	0.14	0.65	0.07
Isovalerate	1.12[b]	1.27[a]	1.21[ab]	1.19[ab]	0.05	0.028	0.78	<0.01
Total VFA	96	106	97	91	9.6	0.64	0.18	0.15
Acetate:Propionate ratio	3.10	2.71	3.23	3.23	0.26	0.30	0.10	0.28
Blood Metabolites								
Plasma urea-N (mg/dL)	14.0	14.8	15.4	14.8	0.7	0.93	0.10	0.11
Plasma glucose (mg/dL)	52.2[d]	59.2[a]	53.1[c]	57.7[b]	1.4	<0.01	0.023	<0.01

[1] Data was obtained from the four ruminally cannulated cows.

[2] Data was obtained from all 8 cows in 2 Latin squares.

[3] WDDGS = wheat dried distillers grains with solubles, WP =WDDGS-peas treatment combination, WC = WDDGS-canola meal treatment combination, PROT = treatment protein combination factor, EXT = treatment extrusion factor, PROT × EXT = interaction between PROT and EXT.

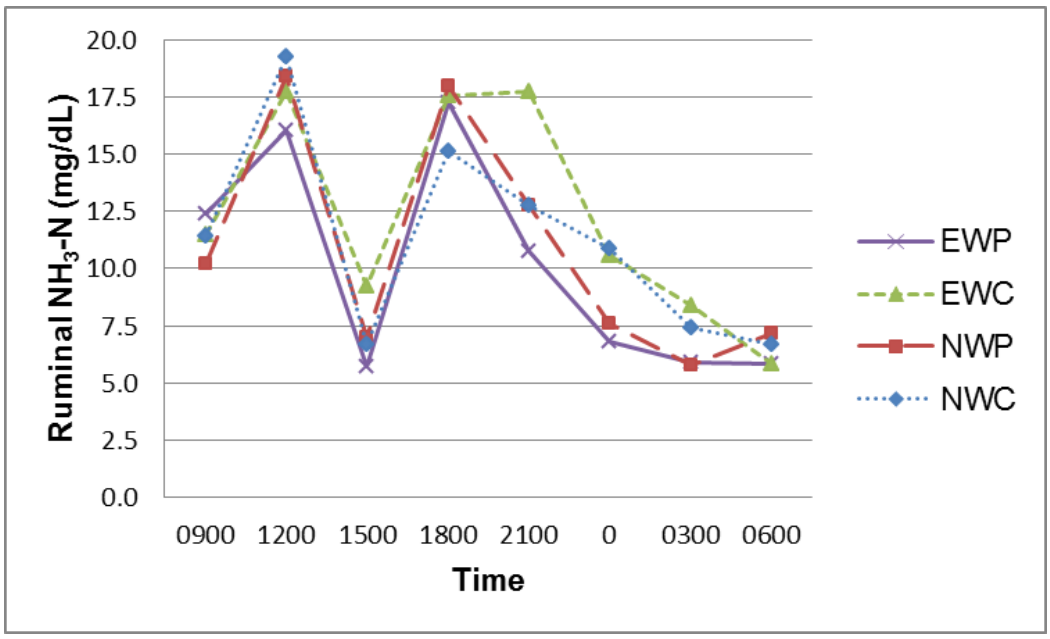

Figure 3.1 The effect of feeding extruded WDDGS-peas (EWP), extruded WDDGS-canola meal (EWC), non-extruded WDDGS-peas (NWP) and non-extruded WDDGS-canola meal (NWC) diets on ruminal NH_3-N concentration over a 24 h period. Cows were fed at 0900 and 1600 h.

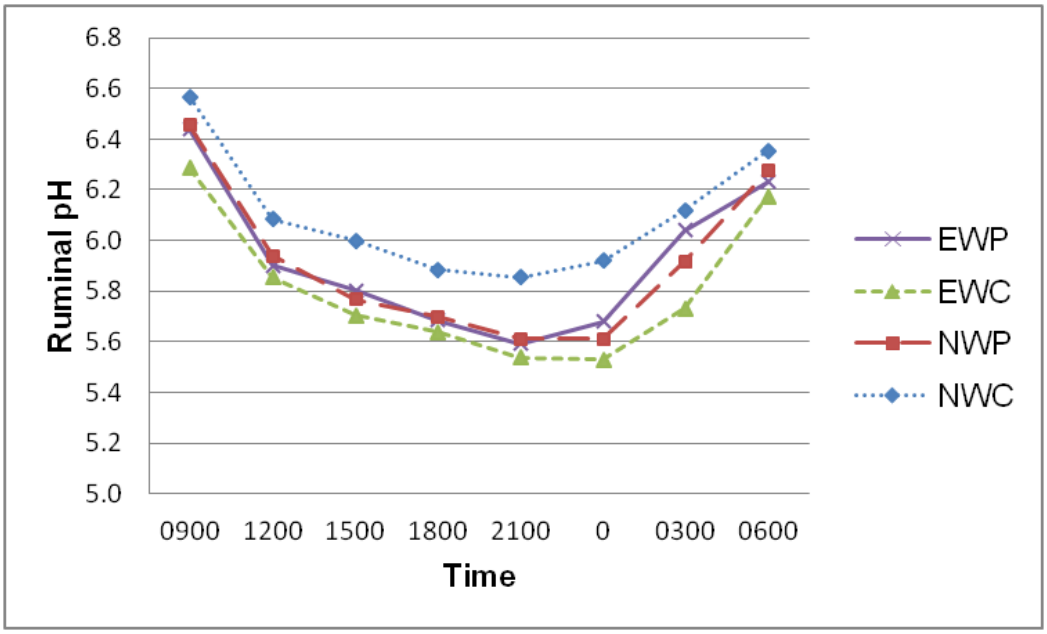

Figure 3.2 The effect of feeding extruded WDDGS-peas (EWP), extruded WDDGS-canola meal (EWC), non-extruded WDDGS-peas (NWP) and non-extruded WDDGS-canola meal (NWC) diets on ruminal pH over a 24 h period.

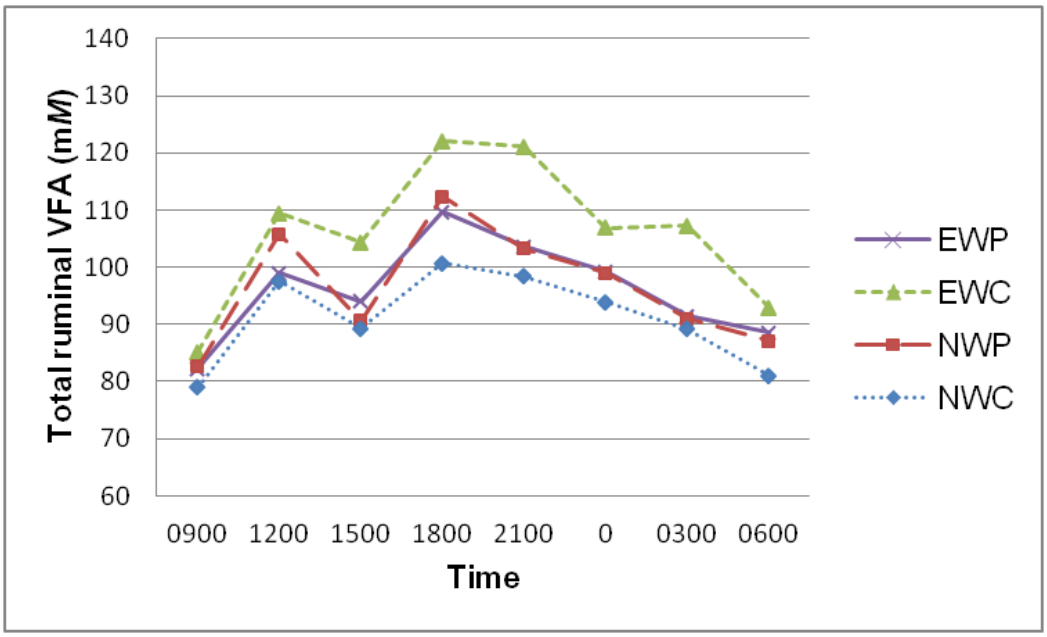

Figure 3.3 The effect of feeding extruded WDDGS-peas (EWP), extruded WDDGS-canola meal (EWC), non-extruded WDDGS-peas (NWP) and non-extruded WDDGS-canola meal (NWC) diets on a) total ruminal VFA concentration over a 24 h period.

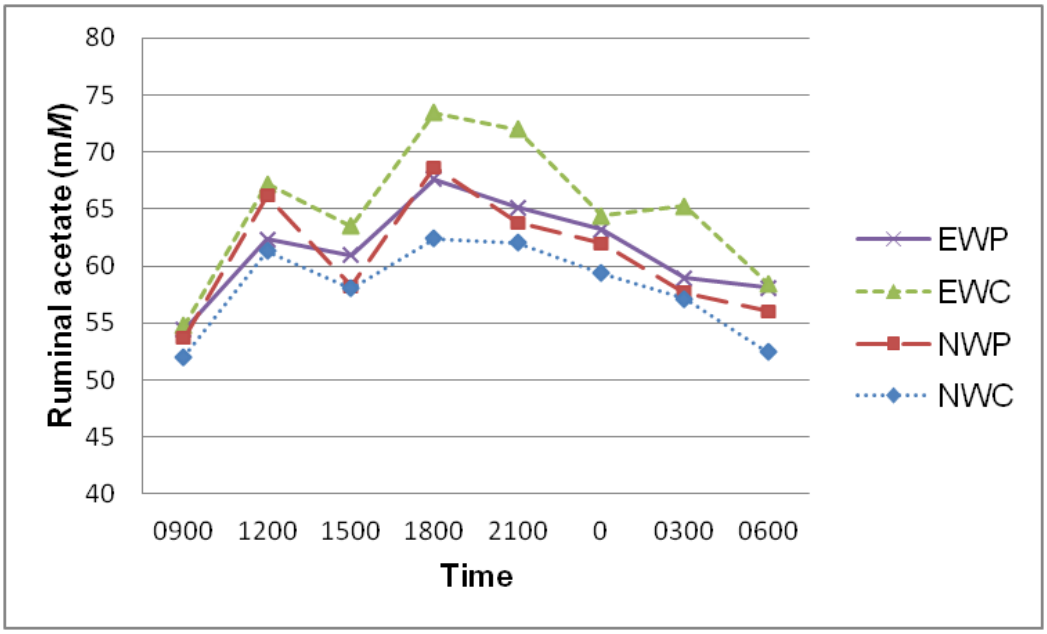

Figure 3.4 The effect of feeding extruded WDDGS-peas (EWP), extruded WDDGS-canola meal (EWC), non-extruded WDDGS-peas (NWP) and non-extruded WDDGS-canola meal (NWC) diets on ruminal acetate concentration over a 24 h period.

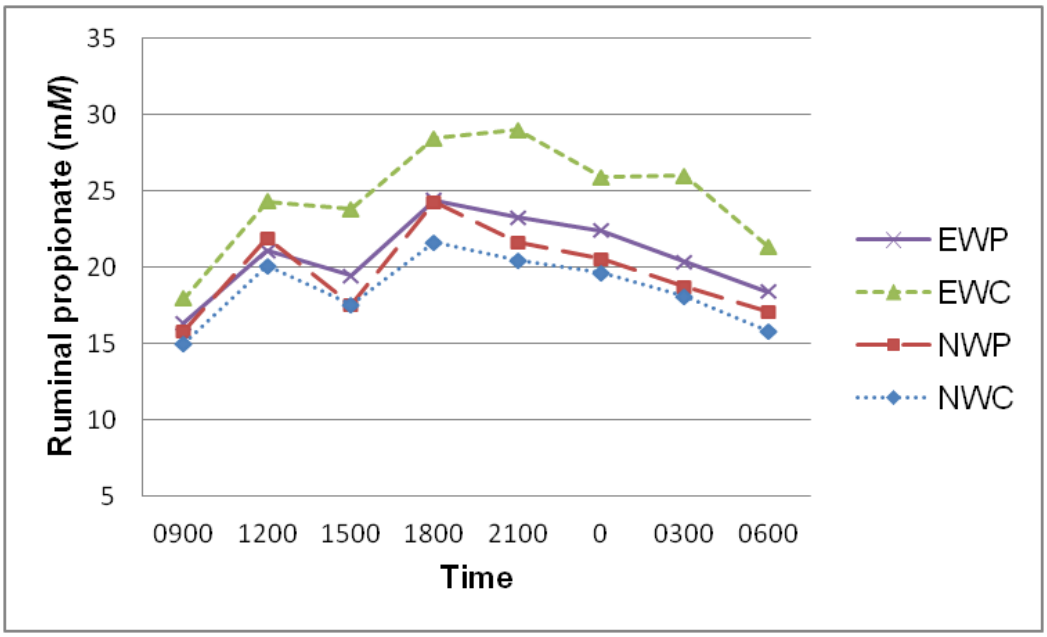

Figure 3.5 The effect of feeding extruded WDDGS-peas (EWP), extruded WDDGS-canola meal (EWC), non-extruded WDDGS-peas (NWP) and non-extruded WDDGS-canola meal (NWC) diets on ruminal propionate concentration over a 24 h period.

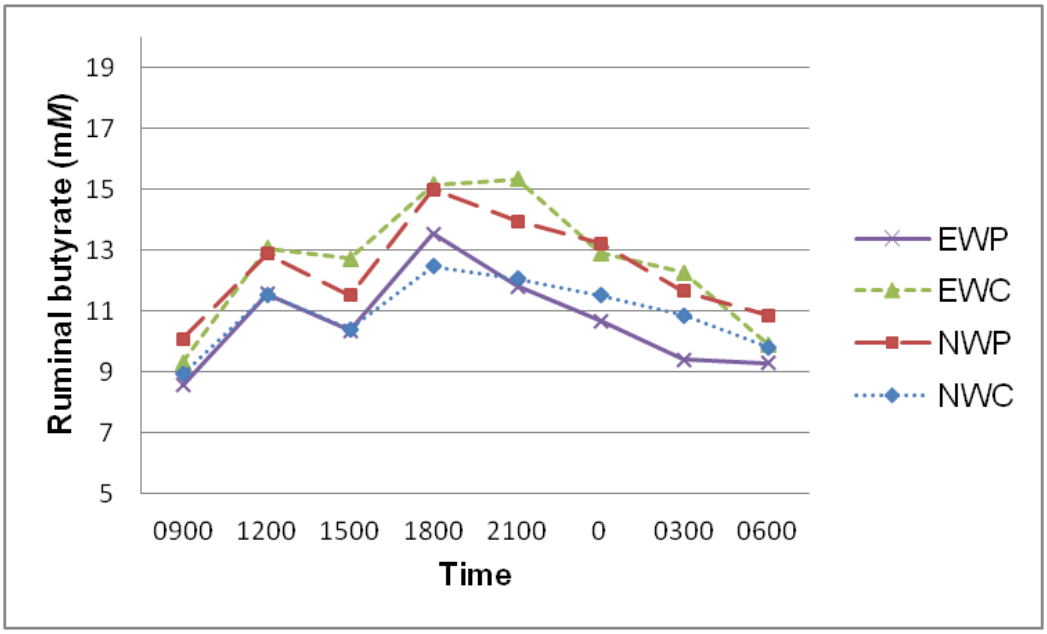

Figure 3.6 The effect of feeding extruded WDDGS-peas (EWP), extruded WDDGS-canola meal (EWC), non-extruded WDDGS-peas (NWP) and non-extruded WDDGS-canola meal (NWC) diets on ruminal butyrate concentration over a 24 h period.

3.3.4 Intake, omasal flow and ruminal digestion of nutrients

Intake of DM and DM omasal flow were not affected by diet ($P > 0.05$; Table 3.5); however, percent of DM apparently digested in the rumen was greater ($P = 0.020$) in cows fed extruded diets compared to those fed non-extruded diets (34.6 vs. 30.3%). Intake of OM was higher ($P = 0.011$; Table 3.5) in cows fed extruded diets compared to those fed non-extruded diets (27.4 vs. 24.3 kg/d) but OM flow and OM apparently digested in the rumen did not differ among diets ($P > 0.05$). Organic matter truly digested in the rumen (OMTDR) amount did not differ between diets and OMTDR as a percent of DM intake did not differ between cows fed WP diets (Table 3.5) but tended to be higher in cows fed extruded WC compared to those fed non-extruded WC (interaction, $P = 0.06$). Intake of NDF and ADF, and their apparent ruminal digestion were not different among diets ($P > 0.05$; Table 3.5); however, omasal flows of NDF ($P = 0.026$; 4.25 vs. 3.96 kg/d) and ADF ($P = 0.029$; 2.13 vs. 1.89 kg/d) were higher in cows fed WC diets compared to those fed WP diets. Starch intake was higher in cows fed extruded diets compared to those fed non-extruded diets ($P = 0.028$; 6.46 vs. 5.91 kg/d) and was also higher in cows fed WP compared to those fed WC ($P = 0.042$; 6.43 vs. 5.94 kg/d). Omasal flow of starch was similar in cows fed non-extruded diets but those fed extruded WP diets tended to have higher starch omasal flow than those fed extruded WC diets (interaction, $P = 0.09$; Table 3.5). Starch ADR amount ($P = 0.06$; 5.70 vs. 5.11 kg/d) tended to be higher in cows fed extruded diets compared to those fed non-extruded diets (Table 3.5) but when compared to starch intake there was no difference between diets.

Table 3.5 Intake, omasal flow and ruminal digestion of nutrients in cows fed the four experimental diets[1]

	Extruded		Non-extruded			*P* value		
	WP[2]	WC[2]	WP[2]	WC[2]	SEM	PROT[2]	EXT[2]	PROT × EXT[2]
DM								
Intake (kg/d)	29.1	28.8	27.6	27.3	1.4	0.79	0.21	1.00
Omasal flow (kg/d)	19.0	19.4	18.8	18.7	0.6	0.76	0.49	0.67
Apparent digestion								
kg/d	10.1	9.4	8.8	8.6	1.0	0.52	0.22	0.77
% of DM intake	35.4	33.7	30.9	29.7	1.7	0.23	0.020	0.80
OM								
Intake (kg/d)	27.3	27.5	24.5	24.0	1.0	0.25	0.011	0.10
Omasal flow (kg/d)	14.5	14.9	13.9	14.1	0.5	0.50	0.15	0.81
Apparent digestion								
kg/d	12.3	11.7	11.4	10.7	0.9	0.41	0.22	0.96
% of OM intake	45.8	44.2	44.5	42.8	1.5	0.28	0.38	0.97
OMTDR (kg/d)	17.7	17.6	16.7	16.1	1.0	0.69	0.15	0.76
OMTDR (% of DM intake)	65.9	66.7	65.8	64.2	0.6	0.48	0.05	0.06
ADF								
Intake (kg/d)	4.27	4.49	3.92	4.15	0.22	0.25	0.10	0.97
Omasal flow (kg/d)	1.92	2.18	1.86	2.09	0.10	0.029	0.43	0.89
Apparent digestion								
kg/d	2.35	2.31	2.06	2.06	0.17	0.93	0.15	0.91
% of ADF intake	54.8	51.7	52.2	49.5	1.9	0.15	0.23	0.94
NDF								
Intake (kg/d)	8.0	8.4	7.3	7.8	0.4	0.24	0.10	0.97
Omasal flow (kg/d)	3.90	4.39	4.02	4.11	0.20	0.026	0.42	0.08
Apparent digestion								
kg/d	4.01	4.08	3.36	3.59	0.29	0.62	0.10	0.81
% of NDF intake	47.8	52.2	41.7	48.8	1.4	0.08	0.12	0.53
Starch								
Intake (kg/d)	6.77	6.14	6.08	5.74	0.26	0.042	0.028	0.49
Omasal flow (kg/d)	0.86	0.66	0.74	0.85	0.08	0.58	0.64	0.09
Apparent digestion								

kg/d	5.92	5.49	5.34	4.89	0.30	0.14	0.06	0.96
% of starch intake	87.3	89.2	87.8	85.0	1.6	0.79	0.29	0.18

[1] Data was obtained from the four ruminally cannulated cows using intake records during omasal sampling.

[2] WDDGS = wheat dried distillers grains with solubles, WP =WDDGS-peas treatment combination, WC = WDDGS-canola meal treatment combination, PROT = treatment protein combination factor, EXT = treatment extrusion factor, PROT × EXT = interaction between PROT and EXT.

3.3.5 Apparent total tract nutrient digestibilities

Intake of DM and apparent total tract DM digestibility were not affected ($P > 0.05$) by dietary treatment (Table 3.6). Total tract ADF ($P = 0.09$; 46.5 vs. 43.3%) and NDF ($P = 0.09$; 49.6 vs. 46.4%) digestibility tended to be higher in cows fed extruded diets compared to those fed non-extruded diets (Table 3.6). Total tract OM digestibility was similar in cows fed non-extruded diets, but those fed extruded WP had higher total tract OM digestibility than those fed extruded WC (interaction, $P < 0.01$; Table 3.6). Total tract CP digestibility was higher ($P < 0.01$) in cows fed the extruded diets compared to those fed non-extruded diets (71.6 vs. 69.9%) and tended to be higher ($P = 0.08$) in cows fed WP diets compared to those fed WC diets (71.3 vs. 70.3%; Table 3.6). Total tract EE digestibility was higher ($P = 0.011$) in cows fed WC diets compared to those fed WP diets (79.8 vs. 76.5%) and it was also higher ($P < 0.01$) in cows fed extruded diets compared to those fed non-extruded diets (81.1 vs. 75.2%; Table 3.6). Total tract EE digestibility in cows fed extruded diets was similar; however, cows fed non-extruded WC had higher EE digestibility than those fed non-extruded WP (interaction, $P = 0.08$). Total tract starch digestibility was higher ($P < 0.01$) in cows fed WP diets compared to those fed WC diets (93.1 vs. 90.7%) and it was also higher ($P = 0.047$) for cows fed extruded diets compared to those fed non-extruded diets (92.5 vs. 91.3%, Table 3.6).

Table 3.6 Apparent total tract nutrient digestibilities in cows fed the four experimental diets[1]

	Extruded		Non-extruded			*P* value		
	WP[2]	WC[2]	WP[2]	WC[2]	SEM	PROT[2]	EXT[2]	PROT × EXT[2]
DMI (kg/d)[3]	27.6	28.3	27.4	27.0	2.1	0.94	0.68	0.76
Digestibility (%)								
DM	70.1	68.1	68.0	67.7	0.9	0.19	0.16	0.31
OM	72.4[ab]	70.6[ac]	68.5[cd]	68.8[bd]	0.8	0.34	<0.01	<0.01
CP	72.5	70.8	70.1	69.8	0.8	0.08	<0.01	0.19
ADF	46.6	46.5	44.2	42.4	1.6	0.62	0.09	0.62
NDF	48.6	50.7	46.7	46.1	1.6	0.66	0.09	0.47
EE	80.4	81.8	72.6	77.7	1.3	0.011	<0.01	0.08
Starch	93.7	91.3	92.4	90.2	0.98	<0.01	0.047	0.80

[1] Data was obtained from the four ruminally cannulated cows.

[2] WDDGS = wheat dried distillers grains with solubles, WP =WDDGS-peas treatment combination, WC = WDDGS-canola meal treatment combination, PROT = treatment protein combination factor, EXT = treatment extrusion factor, PROT × EXT = interaction between PROT and EXT.

[3] DMI was calculated using intake records during total collection of urine and feces.

3.3.6 Nitrogen balance

Dietary treatment had no effect ($P > 0.05$) on N intake and excretion of total N and fecal-N (Table 3.7); however, fecal-N when expressed as a percentage of N intake was higher ($P = 0.01$) in cows fed non-extruded diets compared to those fed extruded diets (30.1 vs. 28.4%), and it tended to be higher ($P = 0.08$) in cows fed WC diets compared to those fed WP diets (29.7 vs. 28.7%; Table 3.7). Urinary N excretion was not affected by dietary treatment ($P > 0.05$; Table 3.7). Urinary urea-N excretion was higher in cows fed extruded WC compared to those fed extruded WP (Table 3.7); however, with non-extruded diets, urinary urea-N excretion was higher in cows fed WC compared to those fed WP (interaction, $P = 0.011$). Urea-N when expressed as a percent of urinary N was not affected by dietary treatment ($P > 0.05$; Table 3.7). Milk N, N retention and productive N were not affected ($P > 0.05$) by dietary treatment (Table 3.7).

Table 3.7 Nitrogen balance in cows fed the four experimental diets[1]

	Extruded		Non-extruded			*P* value		
	WP[2]	WC[2]	WP[2]	WC[2]	SEM	PROT[2]	EXT[2]	PROT × EXT[2]
N intake (g/d)[3]	714	728	720	707	55	0.99	0.88	0.78
Total N excretion (g/d)	416	470	423	428	22	0.11	0.32	0.17
Fecal-N								
g/d	196	212	214	214	15	0.57	0.52	0.59
% of N intake	27.5	29.2	29.9	30.2	0.8	0.08	<0.01	0.19
% of total N excretion	46.8	45.6	50.5	50.4	3.1	0.83	0.20	0.85
Urinary N								
g/d	223	255	206	217	19	0.25	0.16	0.55
% of N intake	31.5	35.2	29.5	31.2	4.1	0.53	0.48	0.81
% of total N excretion	53.2	54.4	49.5	49.6	3.1	0.83	0.20	0.85
Urea-N								
g/d	150	151	136	132	12	0.89	0.18	0.83
% of urinary N	65.3[a]	65.6[a]	63.3[b]	62.6[b]	3.9	0.39	<0.01	0.036
Milk N (g/d)	233	216	213	199	18	0.30	0.21	0.92
% of N intake	33.1	29.6	29.5	28.2	1.6	0.13	0.12	0.47
N retention								
g/d	66	42	84	80	20	0.72	0.48	0.80
% of intake N	7.8	6.0	11.0	10.4	3.0	0.83	0.50	0.91
Productive N[4] (g/d)	299	258	297	279	46	0.52	0.83	0.80
% of N intake	40.9	35.6	40.5	38.6	4.3	0.42	0.77	0.70

[1] Data was obtained from the four ruminally cannulated cows.

[2] WDDGS = wheat dried distillers grains with solubles, WP =WDDGS-peas treatment combination, WC = WDDGS-canola meal treatment combination, PROT = treatment protein combination factor, EXT = treatment extrusion factor, PROT × EXT = interaction between PROT and EXT.

[3] N intake was calculated using intake records during total collection of urine and feces.

[4] Productive-N calculated as sum of milk secreted N and N retention.

3.3.7 Apparent and true digestibility and omasal flow of nitrogen fractions and microbial protein synthesis

Omasal flow of N, NH_3-N, NAN, NANBN, FAB NAN and PAB NAN, as well as N intake, N apparently digested in the rumen, RUP, RDP and microbial efficiency did not differ between diets ($P > 0.05$; Table 3.8). Although the amount of N truly digested in the rumen did not differ between diets ($P > 0.05$; Table 3.8), N truly digested in the rumen as a percentage of N intake extruded WC tended to be higher than extruded WP. Total bacterial NAN flow to the omasum tended to be higher in cows fed WC diets compared to those fed WP ($P = 0.05$; 548 vs. 519 g/d) and also tended to be higher in cows fed extruded diets compared to those fed non-extruded diets ($P = 0.10$; 541 vs. 526 g/d) (Table 3.8) however, when expressed as a percentage of NAN, total bacterial N did not differ between diets ($P > 0.05$).

Table 3.8 Apparent and true digestibility and omasal flow of nitrogen fractions and microbial protein synthesis in cows fed the four experimental diets[1]

	Extruded		Non-extruded			*P* value		
	WP[2]	WC[2]	WP[2]	WC[2]	SEM	PROT[2]	EXT[2]	PROT × EXT[2]
N intake[3] (g/d)	792	780	765	750	36	0.66	0.36	0.97
Omasal N flow								
g/d	784	785	763	768	27	0.92	0.49	0.93
% of N intake	99.3	100.8	99.9	102.4	2.5	0.19	0.45	0.70
Apparent N digestion								
g/d	8.3	-4.2	2.2	-16.9	18.7	0.21	0.43	0.78
% of N intake	0.68	-0.76	0.13	-2.42	2.48	0.19	0.45	0.70
N truly digested in the rumen								
g/d	530	564	531	500	31	0.94	0.26	0.25
% of N intake	66.6	72.1	69.3	66.8	2.2	0.50	0.57	0.10
RDP supply								
g/d	3525	2715	3496	3325	207	0.96	0.27	0.33
% of DM intake	12.1	12.4	12.6	12.9	0.2	0.23	0.13	0.88
RUP supply								
g/d	1427	1163	1287	1386	136	0.52	0.82	0.24
% of DM intake	4.97	4.08	4.66	4.98	0.43	0.52	0.51	0.19
Flow at omasal canal								
NH_3-N (g/d)	34.7	30.3	29.1	32.2	2.9	0.81	0.48	0.17
NAN[4]								
g/d	750	754	734	736	25	0.90	0.52	0.95
% of N intake	95.0	96.9	96.1	98.1	2.5	0.17	0.39	0.98
NANBN[5]								
g/d	228	186	206	219	22	0.52	0.82	0.24
% of NAN flow	29.4	27.8	28.9	26.8	1.2	0.26	0.46	0.79
% of N intake	29.1	24.1	26.9	28.9	2.4	0.56	0.60	0.18
% of DM intake	0.80	0.65	0.75	0.80	0.07	0.52	0.50	0.18
FAB[6] NAN								
g/d	288	309	305	267	28	0.76	0.65	0.32
% of total bacterial NAN	55.2	54.8	57.1	51.3	3.7	0.42	0.84	0.49

PAB[7] NAN								
g/d	233	260	224	250	21	0.25	0.67	0.99
% of total bacterial NAN	44.8	45.2	42.9	48.7	3.7	0.42	0.84	0.49
Total bacterial NAN								
g/d	529	553	509	544	10	0.05	0.10	0.24
% of NAN	70.6	72.2	71.1	73.2	1.2	0.26	0.46	0.79
Microbial efficiency								
g of microbial N/kg OMTDR[8]	29.6	32.4	31.9	32.3	1.6	0.28	0.45	0.41

[1] Data was obtained from the four ruminally cannulated cows.
[2] WDDGS = wheat dried distillers grains with solubles, WP =WDDGS-peas treatment combination, WC = WDDGS-canola meal treatment combination, PROT = treatment protein combination factor, EXT = treatment extrusion factor, PROT × EXT = interaction between PROT and EXT.
[3] N intake was calculated using intake records during omasal sampling.
[4] NAN = non-NH_3 nitrogen.
[5]NANBN = non-NH_3 non-bacterial N.
[6]FAB = fluid associated bacteria.
[7]PAB = particle associated bacteria.
[8]OMTDR = organic matter truly digested in the rumen.

3.4 Discussion

3.4.1 Diet composition

The WC diets had numerically higher ADF and NDF compared to WP diets, which is partially due to the higher fiber content in canola meal compared to peas (Hickling 2003; Newkirk 2009; NRC 2001). This difference in fiber content between the ingredients was also reflected in the WC and WP treatments. The WC diets also had slightly more forage content than WP diets; as forages typically contain more fiber than concentrates, this would have also contributed to the numerically greater fiber content of WC diets compared to WP diets. Previous studies have observed increases (Moshtaghi Nia et al. 1992) or decreases (Chapoutot et al. 1997; Kibelolaud et al. 1993; Masoero et al. 2005; Prestlokken et al. 2001; Walhain et al. 1992) in fiber content due to heat processing. Those studies reporting a decrease in fiber content have attributed the change to an increase in soluble fibers (Kibelolaud et al. 1993; Walhain et al. 1992) that are not necessarily captured when analyzing NDF or ADF. Block et al. (1981) observed an increase in ADF and a decrease in NDF when extruding soybeans, whereas Walhain et al. (1992) reported a decrease in crude fiber content of extruded peas at moderate extrusion temperatures (140 and 180°C), but at a high extrusion temperature (220°C) fiber content was similar to the non-extruded control. The increase of fiber content of the high extrusion temperature treatment could be an indication that the formation of protein-carbohydrate complexes of the Maillard reaction were occurring thus increasing ADF quantity upon analysis. In this study there was a slight numerical increase in ADF content of the extruded WP treatment compared to non-extruded WP which was likely due to the Maillard reaction.

Starch content of WP treatments was numerically higher than WC treatments. This is due to the high starch content of peas compared to canola meal, thus WP diets were numerically higher in starch despite a higher inclusion of barley grain in WC diets. The WC treatments had numerically higher CP content than WP treatments, which was expected because of the difference in protein content between peas and canola meal. Diets were formulated to be isonitrogenous and, overall, dietary CP was similar due to a larger inclusion of corn gluten meal and soybean meal in WP diets. Ether extract was numerically higher in WC diets compared to WP diets, which may be a result of slightly higher fat inclusion in WC diets.

3.4.2 Effects of supplement treatments on DMI and milk production parameters

Intake of DM was not affected by dietary treatment, which is in agreement with other studies which have reported no effect of extrusion (Masoero et al. 2006; Neves et al. 2009; Petit et al. 1997; Soltan 2009) or other heat treatments (Hall et al. 2010; Jones et al. 2001; Prestlokken et al. 2001; Soltan 2009) on DMI. Cows fed extruded diets had increased milk yield which is in agreement with Masoero et al. (2006) who found that milk yield increased in cows fed extruded peas compared to those fed raw peas; however, Petit et al. (1997) observed that cows fed extruded or non-extruded peas did not differ in milk yield, which contradicts results of the present study. In the study by Petit et al. (1997), cows were fed a high fiber diets yet all diets contained low forage content which could have been lacking physically effective fiber and may have impeded milk production. Feeding extruded products consisting of ingredients other than peas to cows has resulted in increased milk yield (Soltan 2009) but this is not always the case as several studies did not observe any increase in milk yield (Block et al. 1981; Hall et al. 2010; Jones et al. 2001; Neves et al. 2009; Prestlokken et al. 2001). The differences in milk yield responses among studies is likely due to differences in feed ingredients used as well as heat processing parameters which can

affect the chemistry of several nutrients and, thus, alter rumen degradability and total tract digestibility. Milk yield differences between diets could also be affected by cow variables such as breed or genetics, level of DMI, stage of lactation and parody. In this study, extrusion resulted in a positive effect on OM, CP, EE and starch ATTD, which could have increased nutrient absorption from the gastrointestinal tract and, as a result, a greater nutrient supply post-absorptively could have increased milk yield.

Milk fat content was decreased in cows fed extruded diets compared to those fed non-extruded diets. Other studies investigating the effects of extrusion on milk fat content have reported increased (Jones et al. 2001; Prestlokken et al. 2001; Soltan 2009), decreased (Block et al. 1981; Neves et al. 2009), or no change (Hall et al. 2010; Jones et al. 2001; Masoero et al. 2006) in milk fat content when extruded diets were fed to dairy cows compared to those fed non-extruded diets. Hall et al. (2010) observed that carbohydrate source had an impact on milk fat content whereas RUP content modified by feeding a heat treated soybean meal did not affect milk fat content. The milk fat results in the current study may be due to a change in the carbohydrate degradability or digestibility. Extrusion may have caused a shift in fiber fractions and increased soluble fiber (Kibelolaud et al. 1993; Walhain et al. 1992) which is more readily available for digestion. All supplements contained WDDGS which contains a low level of starch as does canola meal in WC supplement; however, peas in WP supplement contain a substantial amount of starch available to be altered by extrusion. Consequently, extrusion of WP caused a greater decrease in milk fat content when compared to extrusion of WC.

A current theory of the cause of milk fat depression (MFD) is the "biohydrogenation theory" which involves the production of fatty acid intermediates in the rumen from the incomplete biohdydrogenation of polyunsaturated fatty acids (PUFA; Bauman et al. 2001). These fatty acid

intermediates remain incompletely hydrogenated as a result of an overabundance of rumen available PUFA or when rumen pH is depressed and the rumen environment is altered causing a disturbance in the biohydrogenation pathway (Bauman et al. 2006). *Trans*-10 C18:1 (Griinari et al. 1998) and *trans*-10 *cis*-12 CLA (Bauman et al. 2001) content in the milk are correlated with MFD. These *trans* C18 fatty acids can be produced in the rumen (Bauman et al. 2001; Bauman et al. 2006) and accumulate in the milk and *trans*-10 *cis*-12 CLA has been shown to be a powerful inhibitor of de novo milk fat synthesis (Bauman et al. 2006; Baumgard et al. 2000; deVeth et al. 2004). The presence of *trans*-10 *cis*-12 CLA has been specifically identified as being responsible for the reduction of mRNA of mammary enzymes that are involved in milk fat synthesis regulation (Griinari et al. 2006). In the present study, cows fed extruded diets had a lower ruminal pH compared to cows fed non-extruded diets. Although we did not measure the extent of ruminal biohydrogenation of dietary PUFA and post-ruminal flow of biohydrogenation intermediates, it is plausible that the more acidic ruminal environment in cows fed extruded diets could have shifted ruminal biohydrogenation pathways (Bauman et al. 2006), thus resulting in a greater ruminal production and post-ruminal flow of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA. If that was the case, this could partly explain the decrease in milk fat content that was observed in cows fed extruded diets. Neves et al. (2009) found that feeding extruded canola seed increased C18:1 *trans*-10 *cis*-12 CLA in the milk, indicating incomplete ruminal biohydrogenation and greater post-ruminal flow of this biohydrogenation intermediate which would influence mammary fat synthesis; however, milk fatty acid composition was not analyzed so this MFD theory due to fatty acid composition cannot be verified in the current study.

In the current study, milk fat yield was increased despite the decreased fat content in the extruded diets. The increased fat yield is attributed to the increased milk yield of extruded diets.

In previous studies, heat treatment of various ingredients has resulted in increased milk fat yield (Prestlokken et al. 2001; Soltan 2009; Titgemeyer et al. 1997); however, in some of these studies milk fat content increased and directly affected fat yield.

Milk lactose is a highly conserved milk constituent that does not often change from one diet to the next. In the current study there was an increase in milk lactose content and lactose yield when feeding extruded diets which is an indication that there was a greater amount of lactose precursors available for mammary utilization. Ruminal propionate, a glucose precursor and, thus, also a lactose precursor, was higher in concentration when feeding extruded diets compared to non-extruded diets; however, there was no subsequent response of increased plasma glucose in those diets which cannot be fully explained. Contrary to these results, Masoero et al. (2006) observed a decrease in milk lactose content of FCM in cows fed expanded peas compared to those fed raw peas but there was no difference between raw and extruded pea diets. Neves et al. (2009) noted an increase in lactose yield but not lactose content when feeding cows extruded canola seeds which may be attributed to a numerical, but not significant, increase in milk yield.

A greater supply of pre-formed long chain fatty acids (LCFA) in the blood can be used directly for milk fat synthesis and it has been postulated that an overabundance of LCFA reduces the requirement for milk short chain fatty acids (SCFA) and may actually inhibit mammary de novo SCFA synthesis (Grummer 1991) leaving the precursors, which comprise of other preformed fats and VFAs, free for other purposes. One reducing agent involved in SCFA synthesis is NADPH, which can be produced by the oxidization of glucose; however, in the presence of abundant LCFA and its inhibitory effect on SCFA synthesis, glucose may be spared, thus available for other purposes (Palmquist et al. 1980). Because there was not only an increase in lactose yield but also an increase in lactose content in cows fed extruded diets in the current study, it is likely

that there was a greater availability of glucose for the mammary gland. In this case, milk yield may have actually been limited from further increases in production by milk constituents other than lactose in cows fed extruded diets.

There was no effect of feeding extruded diets on milk protein content or yield; however, previous studies have observed positive effects of extrusion on milk protein content (Block et al. 1981; Petit et al. 1997) and yield (Jones et al. 2001; Masoero et al. 2006; Soltan 2009) as well as negative effects on milk protein content (Hall et al. 2010; Titgemeyer et al. 1997) when feeding heat treated protein sources. The effectiveness of heat treatment, such as extrusion, to increase milk protein may be variable depending on the type of protein source and its protein solubility and rumen degradability. Peas have a higher soluble protein fraction than canola meal (NRC 2001), soybean meal (Khorasani et al. 2001; Masoero et al. 2005; NRC 2001) and WDDGS (NRC 2001; University of Saskatchewan 2010). As a result, WP had a greater potential to be modified by heat treatment to reduce rumen degradation than WC. By reducing RDP, there would be a greater potential for increased retained-N and productive-N; however, there was no effect of feeding extruded diets on N retention, productive-N or milk protein content or yield. The effectiveness of heat treatments to modify protein fractions is also variable depending on temperature or cooking parameters. Soluble protein is easily reduced by heat treatment (Focant et al. 1990; Moshtaghi Nia et al. 1992; Moshtaghi Nia et al. 1995; Mustafa et al. 1998; Titgemeyer et al. 1997) but results quantifying the indigestible protein portion such as ADIN are not always measured. By increasing the time or temperature of heat treated protein sources, ADIN can be increased (Moshtaghi Nia et al. 1992; Moshtaghi Nia et al. 1995; Mustafa et al. 1998; Mustafa et al. 2001) so there can be negative effects on protein availability if protein sources are over processed. Other factors including moisture content, pH and carbohydrate content of feedstuffs can affect the optimal

processing parameters of heat treatment (Mustafa et al. 2000). It may be possible that in the current study, the extrusion mean temperatures of 161ºC and 160ºC for extruded WP and WC treatments, respectively, may have shifted a greater proportion of the RUP to ADIN, or protein C fraction, causing no additional benefit for milk protein production.

Cows fed extruded diets had lower MUN than those fed non-extruded diets, which is in agreement with results from Jones et al. (2001) who reported a decrease in MUN when feeding heated canola presscake to primiparous cows, and Hall et al. (2010) who reported a tendency for a decrease in MUN when soybean meal was partially replaced with expeller heat treated soybean meal in cow diets indicating a more efficient protein utilization. Milk urea-N is derived from PUN which tended to be lower in cows fed extruded diets. Increased PUN can result from two scenarios; an increase in ruminal NH_3-N concentration causing greater absorption from the rumen and increased hepatic ureagenesis of the excess nitrogen or excess amino acid availability in the blood which is derived from intestinal absorption of RUP. Ruminal NH_3-N was not affected by extrusion so it is unlikely that the decrease in MUN and PUN was a result of ruminal NH_3-N. There was also no significant change in RUP between diets but there was a tendency for increased microbial protein flow for cows fed extruded diets. It is possible that the increased microbial protein of extruded diets delivered a more balanced, intestinally available amino acid concentration, thus providing more utilizable protein to the body and reducing deamination products in the blood and milk.

The increased milk protein yield for cows fed WP compared to WC was attained due to the increased milk production of WP. Milk lactose yield also tended to increase when feeding WP. There was no difference in DMI although starch intake was higher for WP. Starch ATTD was greater and CP ATTD tended to be greater for WP which likely provided an extra supply of

substrates for milk production. WP seemed to have subtle influence on ruminal protein utilization including tendencies for reduced ruminal NH_3N, decreased total bacterial NAN and decreased fecal-N excretion as a percentage of N intake; however, WP and WC had similar nutrient ADR which indicates that the accelerated digestion occurred in the intestines instead of the rumen.

Another factor that effects milk protein production is the ability of a protein source to be used for microbial protein synthesis which is influenced by carbohydrate degradation and, therefore, microbial energy supply in the rumen. Hall et al. (2010) demonstrated that it is not only protein source or type but the protein carbohydrate synchrony that affects milk production and milk protein yield. This balance can be influenced by starch type and degradability. There are differences in amylopectin chain length between cereal and pea starch which affects enzymatic hydrolysis potential of the ingredients (Ratnayake et al. 2002). Peas are mostly composed of amylopectin (Ratnayake et al. 2002). High amylopectin, or low amylose containing starchy ingredients can gelatinize at lower temperatures compared to high amylose containing ingredients. Solanas et al. (2008) reported higher SGD for peas compared to barley which is expected with higher amylopectin containing peas. Starch composed of high amylopectin is also less likely to reassociate (Van Soest 1994) in the retrogradation process resulting in decreased formation of resistant starch (Ratnayake et al. 2002). This indicates that peas have the ability to gelatinize to make starch more available but there is less likeliness that resistant starch will occur with heat treatment thus there is a difference in starch chemistry between pea and barley starch which could have affected starch digestion. The protein carbohydrate synchrony of WP may have interacted more successfully compared to WC resulting in the higher milk production, protein yield and lactose yield of WP.

3.4.3 Rumen metabolism, pH, VFA production and NH_3-N

Non-extruded WC was the only diet that maintained a mean ruminal pH above 6 whereas extruding WC caused a significant drop in ruminal pH. Ruminal pH was intermediary for WP diets but extruding WP did not depress pH more than non-extruded WP. Cellulolytic bacteria can be suppressed at a pH as high as 5.8. Since mean ruminal pH of cows fed extruded WC was 5.81 but pH dipped much lower than 5.8 after meals (Figure 3.2), it is possible that sub-acute ruminal acidosis was prevalent when feeding this diet. Previous studies have reported a drop in ruminal pH when feeding heat treated ingredients (Focant et al. 1990; Prestlokken et al. 2001; Soltan 2009) which in some cases was accompanied by an increase in total ruminal VFA concentration (Focant et al. 1990; Prestlokken et al. 2001). It would be expected to see rumen pH decrease when starchy feeds are heated due to gelatinization because of increased energy substrate availability; however, ideal processing has to occur in order to increase readily available carbohydrate sources for fermentation which does not always occur. Total ruminal VFA concentration was not significantly different in the current study but was numerically greatest in extruded WC which may have driven the drop in pH, whereas, both WP diets had intermediate concentrations and non-extruded WC had the lowest VFA concentration. A similar pattern of OMTDR was observed between diets. The different levels of ruminal pH and VFA may be related to the increased starch intake and tendency for greater starch ADR amount of extruded diets. The greater starch ADR in extruded diets was also supported by higher propionate concentrations in those diets. The increased ruminal fermentation carried through to all VFA causing the highest acetate, propionate, butyrate, isobutyrate, valerate and isovalerate concentrations when extruded WC was fed.

Ruminal NH_3-N tended to be lower in cows fed WP compared to those fed WC, which is likely due to the higher dietary starch content and, as a result, higher starch intake in cows fed WP

compared to those fed WC. The greater starch intake in cows fed WP compared to those fed WC could have provided more ruminally-fermentable energy to support microbial sequestration of ruminal NH_3-N, thus resulting in lower ruminal NH_3-N concentrations with WP. Since protein supply of WP was not solely provided by the supplemental treatment, changes in ruminal NH_3-N due to differences in rumen protein degradation between WP and WC diets cannot be attributed solely to the dietary supplements. Peas are a good source of RDP with a large portion of the protein being A fraction (NRC 2001) but soybean meal and corn gluten meal were added to the WP diet which may have added more RUP or slowly degradable protein. The inclusion of these ingredients could have potentially reduced the total rumen protein degradation and resulted in the tendency for lower ruminal NH_3-N in WP.

Ruminal acetate concentration is often negatively correlated with ruminal pH and acetate production generally increases when forage or fiber level is increased in the diet; however, in this study acetate concentration inversely mimicked ruminal pH and acetate concentration was greatest in extruded WC and lowest in non-extruded WC despite having the same fiber and forage content. Hall et al. (2010) reported a change in acetate when feeding different non-fiber carbohydrates sources, in particular sugar versus neutral detergent soluble fiber sources, without any significant changes in ruminal pH. Perhaps extrusion of the WC treatment shifted the carbohydrate fractions so that there were more substrates, such as soluble fiber, available for certain acetate producing microbes.

The greater ruminal propionate production in extruded diets was likely due to greater starch intake and tendency for increased amount of starch ADR observed in those diets. Increasing starch fermentation typically results in an increased proportion of ruminal propionate (Van Soest 1994).

As a precursor to glucose, the increased propionate could have been partially responsible for the increase in milk lactose content and lactose yield in cows fed the extruded diets.

Ruminal butyrate concentration decreased with extrusion of WP compared to non-extruded WP which is in agreement with Van Soest (1994) who identified that butyrate concentration typically decreases with an increase in starch fermentation. On the other hand, the opposite response occurred with WC where butyrate concentration increased when the treatment was extruded. Block et al. (1981) observed an increase in butyrate concentration when feeding extruded soybean meal. Hall et al. (2010) found that butyrate levels increased with ingredients with higher sugar content. It may be possible that sugar availability may have been altered in a different manner when extruding the WC and WP treatments making sugar more accessible in WC while possibly binding sugar for reduced ruminal degradation in WP when extruded.

Branched chain VFA (BCVFA) such as isobutyrate and isovalerate are substrates for cellulolytic bacteria along with valerate and NH_3-N (Hoover 1986). These BCVFA are formed from the breakdown of branched amino acids, specifically valine, leucine and isoleucine (Hoover 1986; Allison et al. 1962). It is possible that the greater BCVFA and valerate concentrations in extruded WC may be due to those cows experiencing sub-acute ruminal acidosis thus negatively affecting cellulolytic bacterial populations and reducing BCVFA catabolism. Cellulolytic bacteria can be suppressed at a ruminal pH as high as 5.8. Severe drops in pH cause a depression in fiber degradation although ruminal fiber digestion was not impeded in extruded WC. Since mean ruminal pH of cows fed extruded WC was 5.81, and pH dipped lower, it is possible that sub-acute acidosis was prevalent when feeding this diet. This is not the sole explanation for the differences in BCVFA since the lowest concentrations of isobutyrate, isovalerate and valerate were observed in extruded WP which had an intermediate ruminal pH level yet non-extruded WC had the highest

ruminal pH level and intermediary concentration of VFA. Fermentation of fibrous material would be expected to be greatest with the non-extruded WC diet based on ruminal pH and thus a greater cellulolytic bacterial population which could reduce the concentrations of isobutyrate, valerate and isovalerate. There was a tendency for increased NDF ADR when feeding WC diets which conflicts with the theory of sub-acute ruminal acidosis in cows fed extruded WC. Despite the NDF ADR result, NDF intake tended and NDF omasal flow was significantly higher in WC so the increased NDF fermentation may have been due to increased fiber supply.

Diets comprised of a large amount of rapidly fermentable carbohydrates, such as in the current study, may also indirectly inhibit cellulolytic bacteria by reducing cellulolytic bacterial substrates. Amylolytic bacteria have a requirement for preformed amino acids which can potentially diminish the availability of protein for ruminal degradation by other microbes and reduce the production of ruminal NH_3-N (Hoover 1986) or BCVFA. Starch intake was significantly higher in cows fed extruded diets and was also higher in cows fed WP diets which resulted in extruded WP exhibiting the highest starch intake. The amount of starch ADR also tended to be higher in cows fed extruded diets which may have essentially reduced availability of rumen degradable amino acids by means of amylolytic bacterial protein utilization and thus reduced the BCVFA production in the rumen especially in cows fed extruded WP. Hall et al. (2010) found that BCVFA concentration decreased when cows were fed increased sugar content in the ration and also noted that ruminal NH_3-N levels did not follow the same pattern indicating that BCVFA concentrations can be altered independent from NH_3-N. In this study ruminal NH_3-N had an inverse relationship with BCVFA results but this was only a numeric relationship. It is possible that the BCVFA are also incorporated into microbial fatty acids (Allison et al. 1962) not just reformed into microbial amino acids with the presence of NH_3-N which would contribute to

the loss of BCVFA. Extrusion of WP may have ramped up the starch degradability by gelatinization or by the physical destruction of the pea grain structure allowing more access to starch and sugars and created a preferential environment for amylolytic bacteria and the resulting low levels of BCVFA.

3.4.4 Blood plasma glucose and urea-N

Cows fed extruded diets tended to have decreased PUN compared to those fed non-extruded diets which has been observed in previous studies using heat treated ingredients (Block et al. 1981; Soltan 2009). Similarly to MUN, a decreased PUN indicates that extrusion may have effectively reduced excessive or rapid protein degradation in the rumen or the reduced the deamination of proteins that have been absorbed into the body. Blood samples were collected only once per sample day, taken two hours after the first daily feeding to represent the peak post meal PUN indicating the spike in deamination or waste N products in the blood. The PUN results may not be an accurate representation of mean daily PUN concentration. The response in lowered MUN and PUN for cows fed extruded diets was not supported by a significant change in ruminal NH_3-N or nitrogen flows including RUP and bacterial N through the omasal canal. If mean daily PUN was lower in extruded diets, it was likely due to changes in protein absorbed from the intestines.

Plasma glucose concentration was highest for cows fed extruded WC yet it was lowest for cows fed extruded WP; however, blood sampling was only a snap shot value and not representative of the mean daily plasma glucose. Masoero et al. (2006) did not find any differences in blood glucose between cows fed extruded, expanded and raw peas. In the current study, concentration of ruminal propionate was numerically higher at all sampling times in cows fed extruded WC

compared to all other diets, which may have contributed to the elevated plasma glucose content in cows fed this diet.

In a summary of glucose utilization in cattle, Reynolds (2005) suggests that a shift in starch digestion from ruminal to post ruminal digestion does not result in a net gain in glucose supply due to increased glucose utilization by tissues of the portal-drained viscera. If ruminal degradation of starch was slowed down or reduced in cows fed extruded WP, which could occur if resistant starch was formed, it is possible that the peak post meal blood glucose level was effectively delayed or reduced for this diet. Reynolds (2005) also noted that greater insulin responses can occur with increased intestinal absorption of glucose. It may be possible that when feeding cows WP diets, it triggered a greater insulin response, thus lowering plasma glucose concentration compared to cows fed WC diets although this was not substantiated with any increase in nutrient storage such as N retention which is expected with an insulin response (Reynolds 2005). Although analysis of experiments do not show any benefit in post ruminal glucose absorption on glucose status because of decreased endogenous glucose synthesis (Reynolds 2005) this may be a result of the short term exposure to increased post ruminal glucose supply often supplied by ruminal infusion. If enough time is given for adaptation to post ruminal glucose absorption, these initial metabolic processes may subside (Reynolds 2005), allowing for more endogenous glucose synthesis from precursors such as propionate, lactate and glucogenic amino acids despite a greater glucose influx from intestinal absorption.

3.4.5 Nutrient intake, ruminal digestion and apparent total tract digestibility

3.4.5.1 DMI, OM and EE

Dry matter intake was not significantly different between diets. Despite no change in DMI, extruded diets had higher OM and starch intake and numerically higher ADF and NDF intake. There was a tendency for increase OMTDR in cows fed extruded diets which contributed to the greater ADR of DM in those diets. The difference between non-extruded and extruded WC OMTDR was much greater than the difference between non-extruded and extruded WP. Processing the WC diet may have pulverized the supplementary treatment thus reducing particle size and allowing a greater microbial utilization of OM.

An increase in OM ATTD in the extruded diets was observed and also resulted in an interaction where OM ATTD was highest in cows fed extruded WP but lowest in cows fed non-extruded WP. This increase in OM ATTD is likely a direct reflection of the increased ATTD of CP, EE and starch as well as a tendency for increased ADF and NDF ATTD in extruded diets. The increased digestibility of individual nutrients and collectively as OM ATTD may also be due to the physical as well as chemical alteration of the ingredients that occur during extrusion allowing for greater digestion.

Prestlokken et al. (2001) and Neves et al. (2009) observed numerical increases in EE total tract digestibility with heat treatment of barley and canola seeds, respectively, which is in agreement with the increased EE ATTD of extruded diets in this study. This may be due to the physical alteration of ingredients during extrusion potentially increasing accessibility to lipids. The WC diets also had greater EE ATTD which could be associated with the greater proportion of

added fat of these diets. This fat source was not associated or bound to plant material and thus may have been more accessible for digestion than the fats contributed by other ingredients.

3.4.5.2 ADF and NDF

Content of NDF in WC was greater than in WP but this difference was not reflected in NDF intake. The extra NDF content would have been contributed by the canola meal in WC supplement as well as the slight increase in forage content of WC compared to WP supplement and WP diets. Apparent ruminal digestion of NDF tended to be greater for WC which may be related to the NDF composition of canola meal which could contain more degradable fiber than fiber contained in peas or from fiber originating predominantly from forages which supplied a greater proportion of the fiber in WP. The WC supplement may have also be more rumen degradable due to physical particle size compared to the coarse ground peas in WP or the extruded nuggets formed from extrusion of WP.

Apparent total tract digestibility of ADF and NDF tended to be greater in cows fed extruded diets compared to those fed non-extruded diets. Kibelolaud et al. (1993) also found that in sacco intestinal disappearance of ADF and NDF was increased when lupin seeds were extruded at 130°C or higher but total tract digestibility was increased only for NDF at varying extrusion temperatures and rumen incubation times. It is possible that the Maillard reaction occurred in extruded diets shifting carbohydrates to nitrogen-carbohydrate complexes but remained potentially digestible in the total gastrointestinal tract. Intake of ADF and NDF tended to be greater in extruded diets and numerical increases in NDF and ADF ADR amounts were observed in cows fed extruded diets compared to those fed non-extruded diets. This increased amount rumen digested fiber plus greater

intestinal availability may have been the reason ATTD of ADF and NDF also tended to be higher in cows fed extruded diets compared to those fed non-extruded diets.

3.4.5.3 Starch

Starch content of WP was greater than that of WC so it is expected to see a greater starch intake as was seen in the WP diets in this study. The extruded diets had a tendency for greater starch ADR amount which may be due to increased starch intake or starch gelatinization of the extruded diets (Chapoutot et al. 1997; Prestlokken et al. 2001; Solanas et al. 2008) and subsequent increase in ruminal degradability which has been observed in previous studies (Chapoutot et al. 1997; Petit et al. 1997; Prestlokken et al. 2001). Goelema et al. (1998) observed an increase in SGD and in starch degradation rate when peas were toasted; however, overall rumen undegraded starch fraction increased with toasting. The difference in results may be due to moisture or temperature differences causing more of the gelatinized starch to be retrograded and thus become less available for rumen degradation in the study by Goelema et al (1998).

Starch ATTD was greater when feeding extruded diets which may have been due to the physical alteration of the ingredients during processing. Greater starch exposure due to the smaller particle size would allow for greater microbial utilization in the rumen as well as the greater potential for residual starch to be digested for intestinal absorption. Starch can be encased in a protein matrix that reduces digestibility of unprocessed ingredients (Goelema et al. 1999; Ratnayake et al. 2002; Svihus et al. 2005). It is possible that rumen degradation of starch was increased incrementally but processing actually aided digestion in the lower gastrointestinal tract.

3.4.5.4 Nitrogen fractions, ruminal degradation, total tract digestibility, utilization and microbial protein production

Diets in the current study were formulated to be isonitrogenous so it was expected that heat processing would decrease ruminal N degradation and increase RUP fraction if protein had been effectively protected from the rumen. Contrary to these assumptions, there was no increase in RUP or decrease in RDP due to extrusion. This may be related to the particular protein sources that were extruded. Prestlokken et al. (1999) found that when expanding rapeseed meal, ruminal CP degradation was not altered until high temperature treatment was used (190°C) at which point ruminal CP degradation was reduced. Other studies using dry heat (McKinnon et al. 1991; Mustafa et al. 2001) and moist heat (Moshtaghi Nia et al. 1992) on canola meal have demonstrated reduced ruminal CP degradation at lower temperatures than the study by Prestlokken et al. (1999). By-product ingredients, such as canola meal or WDDGS, can vary in processing procedures (for example, solvent extracted versus mechanically extracted canola meal) thus there may be variation in protein degradability of a single ingredient from different sources or even from batch to batch from the same source (Nuez Ortin et al. 2009). This processing prior to heat treatment of the experiment may alter the effectiveness of heat treatment and diminish the changes in N utilization. In the case of extruded WP, there are many studies that demonstrate reduced ruminal disappearance or effective degradability of extruded peas (Focant et al. 1990; Masoero et al. 2005; Solanas et al. 2005; Solanas et al. 2008; Walhain et al. 1992); however, differing ruminal retention times between a dietary treatment in a metabolism trial versus in sacco or in situ experiments could result in differences in degradability. In these aforementioned studies, the retention times may not be adequate (several had a maximum retention time between 12 and 24 hours) to match the retention in this study which would explain why these studies found a significant reduction in RDP

compared to no change in RDP in the current study. By increasing retention time of an in sacco or in situ treatment, results may have indicated slower ruminal protein degradation but no actual reduction in RDP due to heat treatment. Solanas et al. (2008) found that in situ protein degradation differences between extruded and non-extruded feedstuffs was diminished at extended rumen retention times of 48 hours as well as some feedstuffs at 24 hours. Heat treatment did not have a significant effect on RUP in this study which may have also been from the use of other protein sources in the diets reducing the effect of the supplemental treatments.

Omasal flow of bacterial NAN amount tended to be higher in cows fed extruded diets which indicates a greater utilization of ruminal N but bacterial NAN was not significant when expressed as a percentage of total NAN. It would be expected that reducing the protein availability in the rumen, as is thought to happen in heat processes such as extrusion, would reduce bacterial protein production and allow more dietary protein to escape the rumen. Brito et al. (2007) did not see a difference in total microbial NAN flow when cows were fed diets of differing plant protein sources. In the study by Brito et al. (2007), all diets had RDP supply above 11.2%, greater than NRC (2001) recommendations of 9.5-10.5% RDP supply in order to maintain necessary microbial protein synthesis. In the current study RDP supply was well above this level, ranging from 12.1 to 12.9% of DMI, so protein supply for microbial growth was likely more than adequate in all diets thus there was no significant difference in bacterial NAN omasal flow between cows fed extruded and non-extruded diets.

The tendency for a greater amount of bacterial NAN omasal flow of WC could be related to the increased ruminal fermentation. There was a tendency for increased NDF ADR for WC which would supply more useable, slow release carbohydrates for energy production. Increased fermentation substrates were also observed with greater ruminal VFA concentrations of extruded

WC. The analysis procedure of NDF does not eliminate nitrogen that is bound to NDF fiber so an increase in NDF fermentation could have provided an extra, albeit small, source of nitrogen for bacterial protein synthesis. Overall bacterial NAN when expressed as a percentage of total NAN was not different between diets but was numerically greater in WC.

Apparent total tract digestibility of CP was increased when feeding extruded diets which is in agreement with Petit et al. (1997). Several other studies have observed increased intestinal digestibility (Benchaar et al. 1994; Solanas et al. 2005; Solanas et al. 2008) or intestinal availability (Benchaar et al. 1994; Chapoutot et al. 1997; Masoero et al. 2005) of RUP when legumes were extruded. Intestinal digestibility of CP in the aforementioned studies and in the present study was not compromised but was, in fact, enhanced by extrusion. There was no significant difference in RDP between diets therefore greater CP ATTD of the extruded diets positively affected CP hindgut digestibility. The increased CP utilization in extruded diets was reflected in a reduced fecal-N excretion as a percentage of N intake in those diets. Petit et al. (1997) observed similar total tract and fecal-N results when feeding extruded peas to cows compared to feeding raw peas.

Total tract CP digestibility of WP was greater than WC. Fiber content of the WP treatment was much lower than that of the WC treatment, therefore, there could be more nitrogen associated with the fiber of WC likely making that CP more resistant to ruminal degradation and intestinal absorption. Reported values of ADIN and NDIN for canola meal (Moshtaghi Nia et al. 1992; Mustafa et al. 2001) are much higher than for peas (Goelema et al. 1998; Masoero et al. 2005; Mustafa et al. 1998). This indicates there is more CP bound to fibrous fractions in canola meal compared to peas, even when peas are heat treated. This gap in N availability may be the difference in CP ATTD between WC and WP diets.

3.5 Summary

In summary, extrusion had positive effects on milk production, milk fat yield and FCM. Ruminal propionate concentrations were greater in extruded diets which contributed to the increased milk lactose concentration and yield in those diets. Extrusion also increased apparent total tract digestibility of all analyzed nutrients. There was little effect on protein utilization in the rumen of either treatment factorial which was unanticipated since one of the expectations of extruding the treatments was that processing would increase RUP. In spite of this, extrusion appeared to reduce absorbed nitrogen excretion and deamination products in the form of MUN and PUN. One potentially negative effect of extrusion, especially for extruded WC was greater ruminal fermentation, resulting in higher ruminal VFA concentrations and lower pH posing a risk to cow health. Milk production and milk protein yield were increased when feeding WP. Positive effects were also observed for apparent total tract digestibility of CP, EE and starch when feeding WP. Plasma glucose concentration was lower in WP diets compared to WC diets which may require more experimentation to determine whether this drop in glucose was a benefit or detriment to cow energy status.

4 GENERAL DISCUSSION

Feeding extruded WDDGS and peas or canola meal to dairy cows could potentially be of value to Saskatchewan dairy producers. The increase in milk production and fat yield would be beneficial to producers in the Canadian dairy industry because of the milk pricing structure. Quota is based on kilograms of milk fat shipped so a higher fat yield results in more efficient production with fewer cows needed to fill quota. Milk fat also has the highest economic value compared to other milk components, such as protein, so as more milk fat is shipped, the producer's economic return increases as long as processing cost of extrusion does not exceed the benefit of added production.

Extruding these particular supplements resulted in greater total tract digestibility of several nutrients. The increased DM ADR and OMTDR as well as increased total tract apparent digestibility of nearly all nutrients shows the potential benefit of extrusion and its suitability within the whole TMR in this study. The enhanced nutrient digestibility is a desirable feed trait on farm especially if there is an opportunity to reduce feed costs. Lowering costly and, in some cases, oversupplied ingredients such as protein sources with the assurance that intake of digestible nutrients is not hindered would be a substantial benefit to producers. Also if an added feedstuff has the ability to increase digestibility or utilization of protein, fiber or starch provided by on farm ingredients, this would also prove beneficial in the supply of nutrients for milk production. The benefit of increased nutrient digestibility of this dietary treatment, however, may be short lived if fed with different feedstuffs that are not as well suited to the supplement.

The positive effect on ruminal propionate concentrations and, subsequently, milk lactose content and yield with the extruded diets indicate a greater utilization of glucose in cows fed these

diets. This attribute could result in many more benefits besides added milk production, especially if it aids in overcoming negative energy balances in early lactation by providing needed glucose during this period of short supply. The benefits may also include a shorter duration of negative energy balance and it is possible that reproduction could be positively influenced as well. Further studies could include feeding groups of early lactation cows extruded and non-extruded WC and WP diets along with a control to determine potential benefits on intake, milk production, measurable energy balance characteristics such as non-esterified fatty acids and β-hydroxybutyric acid, and reproductive efficiency.

Extrusion of the WC treatment had greater ruminal fermentation than the other treatments and had more influence on ruminal characteristics. All individual VFA as well as total VFAs were numerically increased when feeding extruded WC compared to all other dietary treatments. This resulted in a decrease in ruminal pH, potentially causing other negative repercussions on the rumen environment such as acidosis. The ramification of feeding this particular diet may not outweigh the production benefits for the long term health and productivity of the cow.

In regards to the WP treatment, it appears that the protein combination of this particular supplement could have positively influenced nitrogen metabolism although results only showed subtle effects on NH_3-N, CP ATTD and fecal-N excretion. Perhaps feeding a greater proportion of the dietary treatments would have resulted in a more prominent effect of the WP combination on nitrogen efficiency. Treatments were included at 16% of the ration. It is possible including the supplementary treatments at 20 to 25% of the diet or gradually replacing one supplement with the other making up 25% of the diet would result in greater differences in nutrient omasal flow including nitrogen fractions of RUP, RDP and microbial protein than in the current study. The downfall of increasing feeding rate of these particular supplementary treatments is the difference

in protein and starch content between WC and WP which vary substantially and would require more supplementation of these nutrients in the rest of the ration. A potential solution to overcome the greater protein content and lower starch in WC compared to WP would be to replace a portion of the canola meal in WC with a starchy grain source such as barley. This would help balance the nutrient profiles of the treatment combinations, thus reducing the variation of the other ingredients in the ration. This dilution of the WC treatment would also likely have a positive effect on processing quality of the extruded WC treatment by binding the ingredients together.

Feeding extruded treatments to lactating dairy cows in this study demonstrated positive results on milk production parameters and in the case of extruded WP treatment there was no negative impact on ruminal fermentation characteristics or apparent nutrient digestibilities in the rumen or total gastro intestinal tract using conventional Western Canadian forage and grain sources. The positive effects of extrusion on ruminal propionate and subsequent blood glucose balance indicate that this product may have specific beneficial application to early lactation cows which is often the most critical stage of lactation where the stress of energy balance can play a major role in the cow's health and milk production of that lactation cycle.

There is potential for refining a product for retail distribution based onthese results; however, processing the feed ingredients has to be economically feasible. To quantify the economic feasibility of extruding these protein combinations, production costs including labour, utilities, maintenance depreciation and other production incurred expenses could be budgeted at $48.00 per metric tonne (E. Ange, personal communication, January 11, 2016) not including product margin. The milk income to Saskatchewan dairy producers in November, 2015 for milk component yields were $11.22, $8.22 and $1.23 per kilogram for milk fat, milk protein, and other milk solids (calculated using lactose content plus 0.94% for minerals and other milk solids; D. A.

Christensen, personal communication, January 12, 2016), respectively (SaskMilk 2015). The net revenue based on milk component content and resulting milk yield less processing cost based on treatment DMI for the extruded diets in this study works out to $30.99 vs. $29.84 per cow per day when feeding the non-extruded diets. Although there would be some requirement for margin in order to make feed processing enticing for a manufacturer, there is the potential for increased producer income with the supplemental treatments.

5 GENERAL CONCLUSIONS

Results presented in this thesis indicate that extrusion of WP and WC combinations can increase milk production and milk fat yield and had positive effects on nutrient digestibility although the specific expectation of increasing RUP was not achieved. The processing advantage of increased milk production and milk fat yield could be widely adopted in the industry. Although extrusion of the same ingredients provided an economic advantage in this study above the cost of processing, a more broad assessment of feeding these particular processed ingredients compared to the feed cost and milk production results of using other commonly included ingredients is needed. There is also evidence that the WP treatment provides a benefit of additional milk production when compared to WC treatment. Sourcing locally grown peas provides producers with the advantage of having an alternate starch source when quality cereal grains are in limited supply or during times of high protein ingredient prices when there is value in incorporating alternate protein sources to alleviate feed costs.

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