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EFFECT OF STRATEGIC BLENDING OF FOOD INDUSTRY BY-PRODUCTS WITH PROTEIN SUPPLEMENTS ON PERFORMANCE OF GROWING AND FINISHING BEEF CATTLE

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by

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Submitted in Partial Fulfillment of the Requirements for the
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in the field of Animal Science

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AN ABSTRACT OF THE THESIS OF

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TITLE: EFFECT OF STRATEGIC BLENDING OF FOOD INDUSTRY BY-PRODUCTS WITH PROTEIN SUPPLEMENTS ON PERFORMANCE OF GROWING AND FINISHING BEEF CATTLE

MAJOR PROFESSOR: Dr. Sasidharannair Puthenpurayil

The objective of this research was to evaluate the impact of strategical blending of by-products of pea protein extraction such as pea molasses (PMS) and pea starch and fiber (PSF) with canola meal (CM) or distillers dried grains (DDGS) on the growth performance, rumen fermentation, and total tract nutrient digestibility of beef cattle. Preliminary evaluation (study 1) involved 4 runs of in vitro and two runs of in situ to evaluate the rumen fermentation and nutrient degradation of strategically blended CM. The treatments included regular CM (CM), CM blends containing PMS and PSF at 5% (CM5) and 10% (CM10) levels in CM, 1.5% PMS in CM (CM+PMS) and 1.5% PSF in CM (CM+PSF) (% DM basis). The CM+PMS had greater ($P < 0.05$) DM and CP digestibility in vitro and in situ with significant total gas production, while the CM+PSF had lower methane per gram of DM. In study 2, a 56-d backgrounding and a 145-d finishing trial were carried out to evaluate the growth performance and carcass characteristics of growing and finishing beef steers fed diets containing strategically blended protein supplements. The treatments used were CM (CM), CM+PMS (PMS at 1.5% of CM DM), DDGS, and DDGS+PSF (PSF at 2% diet DM). There was no treatment effect detected during both backgrounding and finishing for overall ADG, DMI or gain:feed. There were numerical improvements in carcass characteristics, indicating likely improvements in carcass traits at a greater level of inclusion in the diets. Study 3 involved a metabolism study using cannulated beef heifers fed the same finishing diets as the feedlot study in a 4 × 4 Latin square design to evaluate

the impact of feeding strategically blended protein by-products on rumen fermentation, total tract nutrient digestibility, and nitrogen balance. There was no variation in total tract nutrient digestibility evaluated. The DM, OM, and CP digestibility were numerically greater for heifers fed CM treatments than those fed DDGS treatments, while the NDF and ADF digestibility were numerically greater for the DDGS treatments. There was no treatment variation in rumen pH measurements. There was also no diet effect on nitrogen balance measured. The results of these studies indicate that the inclusion of PMS and PSF in the diet of beef cattle had no negative influence on the growth performance. Numerical improvements in carcass traits, rumen fermentation, and total tract nutrient digestibility indicate that the growth performance and carcass characteristics may be improved by these food industry by-products at a greater level of inclusion in the beef cattle diets.

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Lastly, I would love to show appreciation to my parents, Mummy's Court and Oga Jude, for their unconditional love and guidance all my life, and to my brothers and sister and friends at SIU who made this process whole.

DEDICATION

This thesis is dedicated to my parents Oga Jude and Mummy's Court whose unconditional love, discipline and spiritual guidance has made me the man I am today and brought me this far in my career. The years of sacrifices and selflessness. I am Everly grateful.

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LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid Detergent Fiber
ADG	Average daily gain
ADG	Average daily gain
ADIN	Acid detergent insoluble nitrogen
Arg	Arginine
B. juncea	*Brassica juncea*
B. nepus	*Brassica nupus*
BSF	By-product feedstuff
BW	Body weight
Ca	calcium
CC	Continuous culture
CCi	Proportion of gas in the sample of the gas being tested.
CCref	Proportion of the reference gas (helium) in the internal standard,
CH4	Methane
Cl	Chlorine
CM	Canola meal
CM+PMS	Canola meal + Pea molases

CM+PSF	Canola meal + Pea starch and fiber
CO2	Carbon dioxide
CP	Crude protein
CPD	Crude protein degradability.
CRD	Completely randomized design
Cu	Copper
DDGS	Distiller’s dried grains with solubles
DFCC	Dual-flow continuous culture
DG	Distillers’ grains
DM	Dry matter
DMD	Dry matter degradability
DMI	Dry matter intake
Fe	Iron
G:F	Gain:Feed
GC	Gas chromatograph
GIT	Gastro-intestinal tract
His	Histidine
IACUC	Institutional animal care and use committee.

K	Potassium
LPS	Lipopolysaccharide stimulation
Lys	lysine
Met	Methionine
Mg	Magnesium
Mn	Manganese
Mo	Molbedium
MP	Metabolizable protein
N	Nitrogen
Na	Sodium
NDF	Nitrogen Detergent Fiber
NDIN	Neutral detergent insoluble nitrogen
Neg	Net Energy Gain
NFC	Non-fiber carbohydrate
NH3	Ammonia
NH3-N	Ammonia nitrogen
OM	Organic matter
OST	Omasal Sampling Technique

P	Phosphorus
PMS	Pea molasses
PPI	Pea protein isolate
PSF	Pea starch and fiber
RDP	Rumen-degraded protein
REC	Rumen epithelial cells
RF	Response factor,
RUP	Rumen undegraded protein
RUSITEC	Rumen Simulation Technique
SBM	Soybean meal
SCFAs	Short-chain fatty acids
sDDGS	Sorghum distiller’s dry grain solubles
Se	Selenium
SFCC	Single-flow continuous culture
TDN	Total Digestible Nutrients
TMR	Total Mixed Ration
TSP	Three-step procedure
VFA (BCVFA)	Branched-chain volatile fatty acid.

VFA	Volatile fatty acid
Zn	Zinc

CHAPTER 1

GENERAL INTRODUCTION

The increasing population and the need to satisfy the expected increase in consumption and nutritional protein requirement are necessities, and with the advancement in technology and various research being carried out, there has been enhancement in agricultural output with improved input. Environmental policies and regulations lead farmers to reconsider conventional production methods and adopt more productive, profitable, and sustainable management systems. Expanding value-added processing in sectors such as cereal grain and oil seed processing has generated ample byproducts at competitive prices. These byproducts, from processes including ethanol production, canola crushing, grain cleaning, and oat processing, possess unique nutritional characteristics, such as high fiber, protein, and minerals. These nutrients are either inherent to byproducts or result from industrial processing. Owing to their unique nutrient content and availability, many of these byproducts are appealing feed sources for cattle producers.

Using byproducts as alternative energy and protein sources in cattle rations have become prevalent because of competitive pricing. However, this trend is driven more by cost than nutritional value. The production of bioenergy through processes such as bio-oil and bioethanol create co-products, including canola meal (CM; *Brassica napus*), carinata meal (*Brassica carinata*), and distillers' dried grains with solubles (DDGS).

Although the nutritional value of these byproducts has been studied to some extent, there are still several unanswered questions regarding their overall feeding value. Research has shown that byproducts from the industrial processing of grains and oil seeds, such as dry and wet distiller grains with solubles, have high energy content owing to their fat content and high levels

of bypass protein (Beliveau & McKinnon, 2000; Beliveau & McKinnon, 2008). Additionally, these byproducts are rich in specific minerals such as phosphorus (McKinnon & Walker, 2008) and sulfur (Corrigan et al., 2009). Walter et al. (2010) found that barley grains in finishing diets could be replaced by wheat and corn DDGS in up to 40% of the total diet without compromising performance and meat quality.

Increased canola production has increased the availability of CM as a high source of protein for ruminants (Harker et al., 2012), which is demonstrated repeatedly in feed trials (Canola et al., 2015). With a crude % protein content of around 40% (DM basis), CM has been included in livestock diets as a protein and energy source (McKinnon et al., 1991; Zinn, 1993; Petit & Veira, 1994; Patterson et al., 1999). The inclusion of CM in the growing diets has been reported to improve beef cattle's nutrient utilization and growth performance (Nair et al., 2015, 2016). CM has been included in livestock diets as a protein and energy source (McKinnon et al., 1991; Zinn, 1993; Petit & Veira, 1994; Patterson et al., 1999). CM has been a tremendous source of protein for beef cattle in the past ten years. Yang et al. (2013) reported that backgrounded steers improved average daily gain (ADG) and G: F when barley was replaced with CM, compared to steers fed wheat-dried distillers' grain with soluble. Pylot et al. (1999) evaluated the performance and rumen function of steers fed varying levels of CM in combination with barley grain. The study concluded that adding CM improved the apparent digestibility of dry matter (DM), crude protein (CP), and fatty acids. CM and DDGS are suitable energy sources and protein for backgrounding cattle; however, they lack sufficient energy for finishing cattle (Pylot et al., 2000b). Similarly, in a recent evaluation of the impact of CM's inclusion level in growing and finishing beef cattle diets, Nair et al. (2015, 2016) indicated that the energy value of CM is lower than cereal grains such as barley in finishing diets.

Therefore, the research aims to evaluate the impact of strategic blending of food industry byproducts with DDGS or CM on the growth performance, rumen fermentation, and total tract nutrient digestibility of beef cattle.

CHAPTER 2

LITERATURE REVIEW

Food industry by-products

Food industry by-products are residual materials formed during food processing and production, and they comprise peels, seeds, shells, bran, stalks, and other wasted or discarded food product components (Lau et al., 2021). These by-products can come from various food industry sectors, including agricultural, livestock production, milling, processing, and packaging. These products are usually not the primary intended result, but they can still be utilized for various uses, such as animal feed, bioenergy generation, or raw materials for further processing.

Types of food industry by-products

By-products are classified into several groups depending on the food sector. For example, fruit and vegetable processing by-products include pomace, peels, cores, and seeds (Gowe, 2015). Byproducts of grain and cereal processing include bran, husks, and germ (Galanakis, 2022). Byproducts of meat and poultry processing include bone, blood, skin, and fat trimming (Irshad and Sharma 2015). Whey, buttermilk, and cheese whey permeate are examples of dairy processing by-products (Barukčić et al., 2019 Rombaut et al., 2017). Spent grains, yeast, and other residues generated during the production of beer and spirits are examples of brewery and distillery by-products. (Costa et al., 2022). Despite being deemed waste within the food industry, many of these by-products contain essential nutritional elements, rendering them valuable for their potential health benefits. For instance, fruit and vegetable pomace, the solid residue remaining after juice extraction or processing, is a rich source of dietary fiber, vitamins, antioxidants, and minerals (Manuel and Mario, 2018). Similarly, grain bran, the outer layer of cereal grains such as rice, wheat, and oats, is abundant in dietary fiber, B vitamins, minerals such

as iron and zinc, and antioxidants (Sharif et al., 2014). Whey, a liquid byproduct derived from the cheese-making process, is notable for its high protein content, essential amino acids, minerals, including calcium, and bioactive peptides (Morya and Danquah-Amoah, 2017; Rocha-Mendoza, 2021). Meat and poultry by-products, including organ meat, bones, skin, and trims, are valuable reservoirs of protein, vitamin B12, minerals such as iron and zinc, and healthy fats (Jayathilakan, 2012). By-products originating from fish and seafood, such as heads, bones, and viscera, are frequently underutilized despite being rich in proteins, omega-3 fatty acids, minerals such as calcium and selenium, and bioactive substances (Anal, 2017).

The recent expansion of the market for plant-based meat alternatives has resulted in an exponential increase in the generation of by-products from the processing of food including soybean (Gizem and Seda 2018), hemp (Wang et. al., 2008), quinoa (Abugoch et al 2009), potatoes (Giuseppin et al 2015), rice (Morita and Kiriyama 1993), maize (Shukla and Cheryan 2001), chickpeas (Paredes López 1991), peas (Sumner et al 1981), sesame, peanuts, walnuts, hazelnuts, wheat, etc. Many of these by-products have minimal industrial use, resulting in underutilization and wastage (Dhillon et al 2016). Pea protein is limited in methionine and cysteine as well as leucine (García Arteaga, 2021) but has a higher level of leucine when compared to soybean 5.7% and 5.0% respectively (Gorissen, 2018). By-products of pea protein extraction such as pea molasses (PMS), pea starch and fiber (PSF), and pea protein isolates have the potential to complement livestock diets. However, the nutrient composition of these by-products is poorly characterized.

Methods of processing of food byproducts

Drying is a common procedure for processing food by-products that involves removing moisture from the substance. This can be accomplished using a variety of processes, including

hot air drying, freeze-drying, and spray drying. Drying extends the shelf life of by-products, reduces their weight and volume, and improves the durability of storage (Bonazzi and Dumoulin 2011).

Grinding and milling are mechanical operations used to break down food byproducts into smaller pieces (Hemery et al 2007). As a result of this method, the by-products are more digestible and more readily utilized, making them suitable for inclusion in animal feeds or for other uses (Rahman et al., 2021).

Extraction and separation techniques isolate specific components or compounds from food by-products. It involves the extraction of oils, proteins, fibers, or bioactive compounds using solvents, water, or other extraction agents. These components, once separated, can be processed, or employed in a variety of ways (Azmir et al., 2013).

Fermentation is a microbial process that transforms food by-products into value-added products. This process involves the controlled growth of microorganisms, such as bacteria or fungi, to alter the by-product composition and characteristics. Verni et al. (2019), found that fermentation significantly improved the nutritional profile, increased digestibility, and enhanced the functional properties of by-products.

Thermal processing involves treating food by-products with heat to improve safety, digestibility, and shelf life. Depending on the desired results, thermal processing methods may include pasteurization, sterilization, blanching, or cooking, and it has the potential to minimize microbial load, deactivate enzymes, and improve the organoleptic qualities of byproducts (van Boekel et al., 2010).

Nutrient composition of food Industry byproducts as animal feed

Nutrition is essential to animal productivity. A balanced diet consists of water, energy, protein, minerals, and vitamins in varying proportions depending on the animals and environmental and management factors (Moran, 2005). There is considerable interest in natural products and healthy foods that can enhance animal welfare, prevent disease, and serve as natural food additives. Secondary feeds, such as those derived from the oil extraction industry, provide valuable nutrients to diets and allow them to be used as feed instead of waste (Panaite et al., 2016). These by-products contain a significant amount of protein, fiber, fat, and minerals in the diet (Eastridge, 2006). Meals, which are raw vegetable materials, are among the by-products of the oil extraction industry that can be utilized in animal feed. These products are highly diverse, complex in composition, and rich in particular nutrients.

Sunflower sprout is a by-product of the edible oil industry. It is high in vegetable proteins as well as other nutrients, such as crude protein, ether extract, crude fiber, and ash (Jabbar, 1998). Wheat germ meal is a significant byproduct of the wheat grinding industry and is regarded as a natural source of highly concentrated nutrients (Ge et al., 2001). Alcaide et al. (2003) reported 90.8% DM, 24% CP, and 10.5% fiber for flax seed meal. Further, flax seed meal contains more polyunsaturated fatty acids, which, according to sultan et al. (2015), when added to diet, can improve the level of Omega 3 polyunsaturated fatty acid (n-3 PUFA) in meat, as it has 53% alpha-linolenic acids (Chow, 1992). It can improve productivity when included in dairy cow feed (De Vries, 2006). Other food industry by-products with this ability include rapeseed, grape seed, flax, buckthorn, and pumpkin meals. With a balanced amino acid composition, rapeseed meal is an important protein source for animal feed (Eastridge, 2006). Rapeseed meal is also a significant source of calcium (0.61%), iron (218 ppm), selenium (0.95 ppm), and phosphorous

(1.88%) with the availability of selenium at 61.3% (Bragg and Seier, 1974). Grape seed meal contains antioxidants and high concentrations of essential amino acids (Nakamura, 2003). McGregor (2000) reported 88.44% DM, 10.64% CP, and 40.66% fiber for grape seed meal. Over 200 bioactive components have been found in buckthorn meal (Lardy et al., 2002). As it is high in carotenoids, xanthophylls, and flavonoids (Burlacu et al., 2002), it has a high antioxidant capacity (Sauro-Calixto, 1998; Noor et al., 2011).

Pumpkin and pumpkin seeds even though considered as agro-industrial-waste (Amin et al., 2019), pumpkin seed are great sources of compounds with bioactive properties with physiological benefits like carotenoids, vitamin E (Abd El-Aziz and Abd El-Kalek, 2011). Pumpkin, its seed and leaves contains polysaccharides, proteins, polyphenols and carotenoids, minerals, and fatty acids (Ceclu et al., 2020). Rapeseeds meal has a high concentration of calcium, iron, selenium, and phosphorus as Enjalbert et al., (2017) discovered concentrations of: 10.4 mg/kg copper, 159.0 mg/kg iron, 1.0 mg/kg selenium and 71.4 mg/kg zinc. Selenium availability is high in the rapeseed meal according to Verite and Geay, (1986) while Pumpkin meal has the highest level of Pb (2.15 mg/kg). In a study by Oeffner et al., 2013 flax meal was determined to have the following levels of minerals: 6.01±0.23mg of iron, 4.43±0.18mg of zinc, 1.90±0.09mg of copper, 236.40±7.26mg of calcium and 2.73±0.10mg of manganese (per 100g). Vasta et al., (2010) analyzed the buckthorn fruits and reported the following levels of minerals: 30.9 mg/kg iron, 1.4 mg/kg zinc, 0.7 mg/kg copper and 1.1 mg/kg manganese. These values reflect the limiting factor for compound feed formulation with these by-products and the stability of the chemical composition.

Effects of feeding food industry by-products livestock performance

Animal productivity depends on nutrition. A balanced diet includes water, energy, protein, minerals, and vitamins in varying proportions based on the animals, environment, and management factors (Moran, 2005). There is a plethora of interest in natural products and healthy foods that can improve animal welfare, prevent disease, and incorporate health-promoting substances into the diet as natural food additives. Secondary feeds add valuable nutrients to diets and allow them to be used as feed rather than being discarded (Panaite et al., 2016), many of these by-products provide significant amounts of protein, fiber, fat, and minerals to the diet (Eastridge, 2006). Meals from the oil extraction industry are highly diverse, with complex compositions rich in specific nutrients, and are used extensively as animal feed.

Studies (Chichlowski et al., 2005, Ollier et al., 2009, and Schmidely & Andrade, 2011) found that inclusion of ground canola seed, intact rapeseed, and rolled canola seed in ruminant diets resulted in higher fat, protein, and lactose yield of milk. Also, Boldea et al. (2021) stated that the rapeseed diet produced a numerical increase in milk yield, milk protein, and fat content than the ground canola seed and rolled canola seed. These similarities (milk yield, milk protein and fat content) may be explained in part by the fact that the dietary energy and protein intakes of the groups were nearly identical. Research by Michelle de Oliveira et al. (2012) found that, despite lowering the concentration of short-chain fatty acids in the rumen, supplementing canola, sunflower, and castor oils at 30 g/kg in diets containing 500 g roughage/kg and 500 g concentrate/kg (DM basis) had no effect on nutrient intake and digestibility in sheep.

Pea and pea by-products for livestock feeding

Pea (*Pisum sativum*) is a significant crop in the Fabaceae family and is known to contain a range of essential nutrients, including protein (20-25%), fat (1.5-2.0%), carbohydrates in the

form of starch (24-49%), and total dietary fiber (60-65%), including 10-15% insoluble fiber and 2-9% soluble fiber (Wang, 2004) Additionally, they contribute non-starch carbohydrates, including sucrose, oligosaccharides, and cellulose. Pea also contains minor constituents such as vitamins, minerals, phytic acid, saponins, polyphenols, and oxalates (Khan et al., 2016: Bajaj et al., 2015: Lu et al 2020: Tulbek et al 2017). Among the mineral elements present in peas, potassium (1.04%) was the most prominent, followed by phosphorous (0.39%), magnesium (0.10%), and calcium (0.08%). Peas are also a good source of water-soluble vitamins, particularly B-group vitamins (Millar et al., 2019: Kumari and Deka, 2021). Furthermore, it contains essential amino acids with high lysine and threonine content. However, it is deficient in sulfur-containing amino acids, including methionine and cysteine (Stone et al. 2015).

Processing of pea for byproducts

Pea protein is offered in diverse forms, including pea flour, concentrates, and isolates. It is primarily utilized in the form of a concentrate that can be fabricated through an acid hydrolysis process (Barac et al 2015; Reinkensmeier et al 2015). Before protein extraction, pea seeds undergo several pretreatment steps, such as cleaning, drying, sorting, dehulling, and splitting, which enable the separation of the hulls and cotyledons from whole pulses. This process facilitates protein extraction without compromising the techno-functional properties (Do Carmo et al 2020).

The addition of Pea Protein Isolates (PPI) to ground meat patties has been found to result in the production of softer, tender beef patties that require less compression than those made from pure beef (Banaszek et al., 2019: Peng et al 2016). Similarly, cooked restructured steaks incorporating 8% PPI exhibit increased hardness, chewiness, cohesiveness, and gumminess because of their ability to bind water and fat, as well as their gelling property. Pea starch and

fiber are available as food industry by-products of pea protein extraction. Air classification is the most commonly used commercial method for pea starch isolation, which requires a very high degree of particle size reduction to separate the starch granules from the protein matrix (Czuchajowska et al., 1998). The starch concentrate contains 65% starch (Han and Tyler, 2003). The yield of pure starch, protein content, and ash content of field pea starches range from 35–40%, 0.52–0.70%, and 0.01–0.07%, respectively (Ratnayake et al. 2001; Biliaderis and Grant 1979; Biliaderis and Grant 1981a; Hoover and Sosulski 1991; Davydova et al. 1995). They are used widely for various industrial applications (Pietrasik et al., 2020) and, based on recent findings, have encouraged their usage in the meat industry (Ratnayake et al., 2002; Zhong et al., 2018).

Pea molasses and pea starch and fiber as animal feed

The energy content of peas is comparable to that of corn and wheat, as demonstrated by Nocek (Nocek, James E. 1996) in their study on the site of starch digestion in various feedstuffs. Approximately 78% of the starch in peas is rumen-degradable, with the remaining 22% escaping to the small intestine (NRC, 1989; Aufrere et al., 1994). The starch content of peas ranges from 41 to 54% of the dry matter, with approximately 50% being soluble. The non-soluble rumen-degradable fraction has a slow degradation rate in the rumen (Walhain et al. 1992). Pea starch has a slower rate of degradation when compared to conventional grain sources like wheat, oats, barley but it is like corn (Robinson, 1989). A slow rate of starch degradation in feed would be beneficial for controlling rumen pH, which is especially crucial for animals that consume large amounts of grain. Rumen pH influences fiber digestion, with lower pH levels below 6.0, resulting in reduced dry matter intake, depressed milk fat levels, and increased digestive disturbances. Research has demonstrated a positive effect on rumen pH owing to the relatively

slow starch degradation of peas in cows fed concentrates comprising 70% of the ration dry matter twice daily through a rumen cannula (Valentine and Bartch, 1987). This slow starch degradation may also explain why high-producing cows fed high-grain diets had higher milk fat percentages when peas accounted for a significant proportion of the concentrate (Corbett et al. 1994).

Protein supplements for livestock

Canola meal

The term Canola was coined in 1979 to describe Canadian double-low (low in both erucic acid and glucosinolate) rapeseed types (Bell 1984). Double-low *Brassica (B.) napus* were licensed in Canada in 1974 (Bell, 1984). *Brassica napus*, *B. rapa*, and *B. juncea* are three types of canola that have been developed. *Brassica napus* is the most widely planted cultivar in the United States. Canola meal is a by-product of oil extraction from canola seeds (*Brassica napus* and *Brassica rapa*). It is commonly used as a protein-rich livestock feed additive. CM has a balanced amino acid profile and is rich in essential amino acids such as lysine and methionine.

CM also contains a relatively low amount of crude fiber (about 15-16% of its seed) (Bell and Shires 1982; Mir et al., 2020). CM has been used for a variety of livestock species, including poultry, swine, and ruminants, owing to its high protein content and beneficial amino acid composition.

Canola and rapeseed

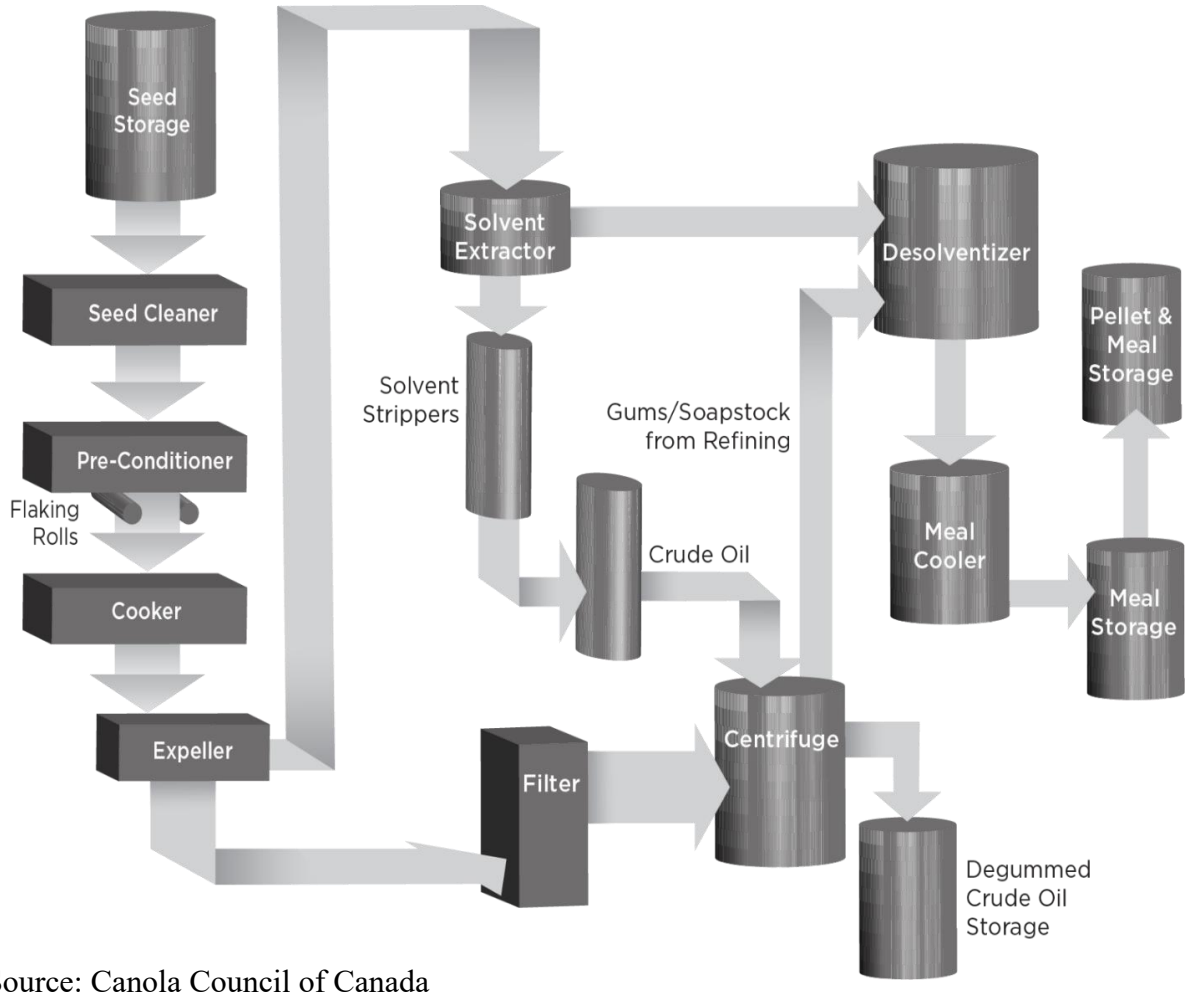
Canola, a derivative of rapeseed (*Brassica napus* and *Brassica campestris/rapa*), was developed using standard plant breeding techniques to reduce the levels of antinutritional factors such as erucic acid (less than 2%) in the oil portion and glucosinolates (less than 30 μmol/g) in the meal portion (Bell. 1993). The total glucosinolate concentration in traditional rapeseed meal

is approximately 50–100 μmol (Bell, 1993). The content of glucosinolates in CM varies depending on the processing method, but it has been reported to contain approximately 2 μmol/g (Newkirk, 2011; Newkirk et al., 2003). High levels of glucosinolates are known to negatively affect thyroid function by inhibiting thyroid hormone production and reducing DMI owing to their bitter taste (Holst and Williamson, 2004).

Processing of canola meal and its effect on nutrients availability

Industrial processing usually involves separating the seeds into oil and meal fractions. Unger (1990) described oil extraction from canola seeds in detail. To minimize breaking and increase oil extraction, canola seed is reconditioned before processing by heating it at 30 to 40°C for 30 to 45 minutes. The canola seeds were then flaked to rupture the oil cells and shatter the cell wall. Following the flaking process, the seed is subjected to steam-heated cooking units (75–85°C for 20–40 min) to enhance its quality. Subsequently, the cooked flakes are processed through screw pressing, which reduces the oil content by 60–70% and produces a press cake.

The remaining oil in the press cake was then extracted using solvents, which were then removed from the solvent-extracted meal in the desolventizer-toaster by heating the extracted meal to 103–107°C for 30–40 min. It is worth noting that heat treatment in the cooker and de-solventizer-toaster can affect the protein quality of the different intermediate canola products at various stages of processing and eventually the quality of the final meal (Mustafa et al 2000).



Source: Canola Council of Canada

Several studies have shown that heat treatment reduces protein solubility and increases the ruminal undegraded protein content in canola seeds (Deacon et al., 1988), canola press cake (Jones, 1993), and CM (McKinnon et al., 1995).

Heat-processing, such as roasting or toasting, is often employed to increase the nutritional quality of CM and reduce antinutritional elements. These treatments improve protein digestibility by denaturing the heat-labile proteins. However, they may also trigger Maillard reactions, resulting in decreased lysine availability (López-Pedrouso et al 2019; Zentek and Boroojeni 2020). The extrusion method is another approach to increasing the nutritional content of CM. Extrusion can improve protein digestion by destroying cell walls, inactivating anti-nutritional

factors, and enhancing enzymatic activity (Mejicanos et al., 2016). Excessive heat and pressure during extrusion, on the other hand, might cause protein denaturation and nutritional losses.

Canola meal blend as a feed ingredient

The deliberate blending of CM with other feed ingredients to generate a balanced and nutritional optimum feed composition for animals is referred to as a CM blend. The blend is intended to improve the overall nutritional value and performance of the feed while accounting for the specific needs of the target animal type. There are various advantages to combining CM with other feed ingredients. Due to its high protein content and well-balanced amino acid profile, CM is utilized as a protein supplement in the livestock industry (Mustafa et al. 1996). It enables the optimization of protein content and amino acid profile, as well as balancing energy sources and other necessary nutrients. NRC (2012), reported that CM combined with complementing ingredients such as cereals, oilseeds, or other protein sources can provide a more complete and balanced diet for animals, meeting their nutritional demands.

CM blends have been shown to improve growth performance, feed efficiency, and nutrient utilization in various livestock species, including poultry, swine, and ruminants Simbaya et al., 1996). These blends not only provide economic benefits but also improve animal health and welfare by providing a nutritionally balanced diet (Wanasundara et al., 2016).

Nutritional composition and feed value of canola meal blends

The nutritional makeup of the CM blend varies based on the ingredients utilized and their inclusion amounts. CM is well known for its high protein content, which normally ranges from 36% to 44% (Mir et al., 2020). It is rich in important amino acids, such as lysine, methionine, and threonine (Bell 1993). Also, CM contains a moderate amount of crude fiber and serves as an energy source for animals. The feed value of a CM blend is determined by its nutrient

composition, amino acid profile, digestibility, and effect on animal performance. CM blends have been shown to improve growth, feed efficiency, and nutrient consumption in various livestock species (Wanasundara et al., 2016).

Distillers' grains with soluble

It is a byproduct of production which is gotten from grain fermentation, with the most common being corn, however other grains can also be used as sources of ethanol like sorghum, wheat, or barley (Schingoethe, 2006). Depending on the processing, the by-product from the ethanol plant could be wet and dry distiller's grain or wet and dry distillers' grain with soluble or condensed distillers soluble with the DDGS being the most used in cattle production (Shurson, 2005). DDGS is commonly used in cattle growing and finishing diets as a rich source of protein. Studies (Ham, 1994; Klopfenstein, 1996) have shown that when compared to dry rolled corn, it is a greater source of energy. Based on its processing which involves the removal of starch from its stock, its nutritional concentration is higher (Belyea, 2004; Nyachoti, 2005). Ruminants fed DDGS have less susceptibility to acute acidosis due to high amount of digestible fiber present resulting from removal of starch (Weigel, 1997), with reported RUP values ranging between 47% - 69% (Schingoethe, 2006) Although it has higher concentration of nutrient, there has been reported instances of variations in nutrient concentration due to the differences in methods of processing and also vary due to the source (Spiehs et al., 2002).

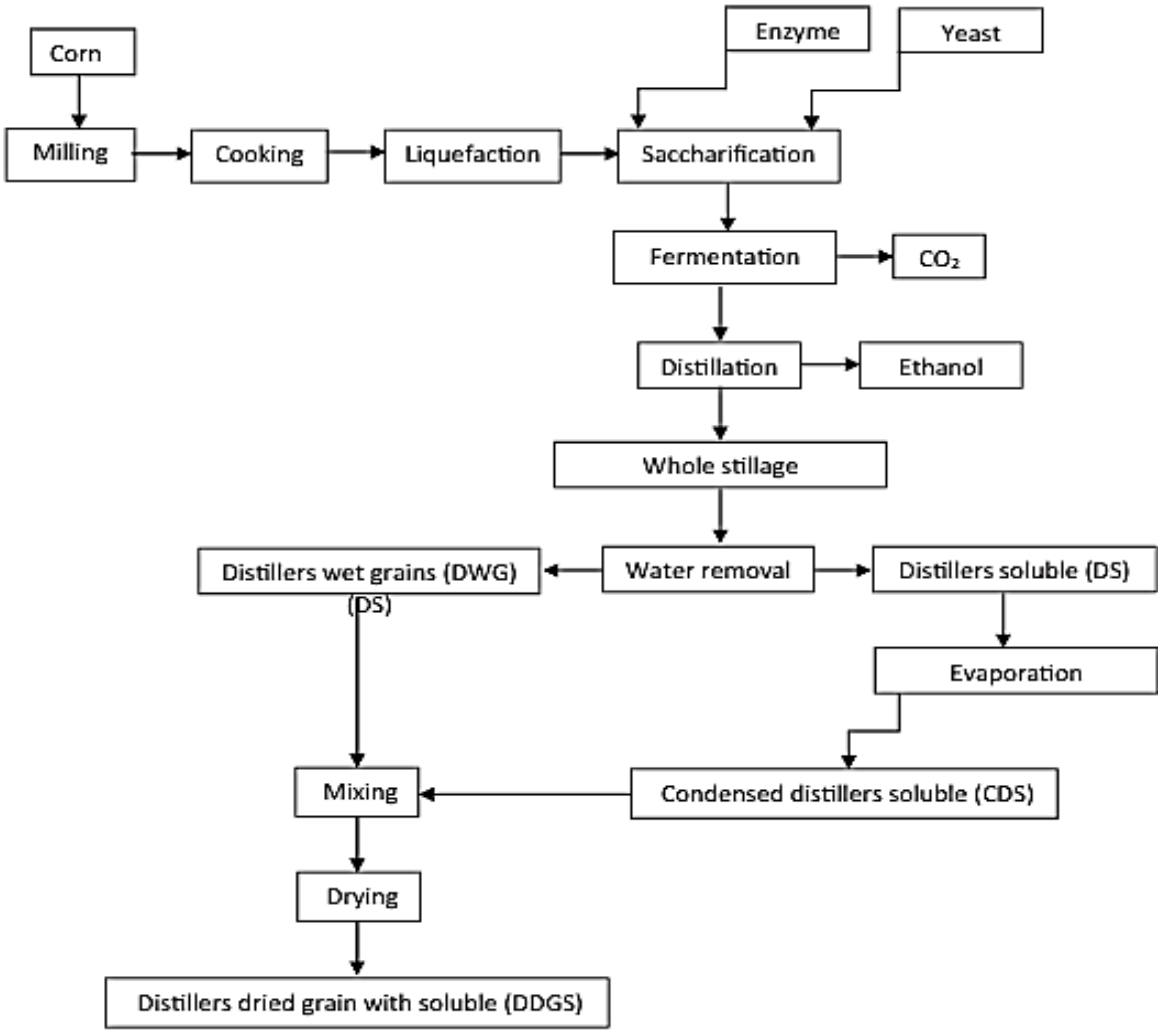
Processing of DDGS

The production process and grain sources are factors (Böttger, 2018) that affect the nutritional composition of DDGS. As such there are different processing methods used which vary between ethanol production plants and the technology used. The general common industrial process used by 82% of bioethanol plants is dry-grinding (Kingsly & Ileleji, 2009) where

supplied corn is ground and water added and mixed into a slurry and then at high temperature (40 – 60°C for pre-mixing and 90– 165°C for cooking), liquified with the addition of α-amylase enzymes at 80– 90°C for 30 mins converting starch into dextrin and through saccharization converted into glucose and fermented at 33°C with a pH of about 4.0 for 48– 72 hr. by yeast into ethanol (Saggi & Dey, 2019) and the non-fermented residues of the grain and yeast are collected and called whole stillage. This mixture is then centrifuged to separate the solid from liquid, the liquid is recycled to make other grain slurry (kim, et al., 2008). The remaining stillage is heated more to remove additional moisture to 30% dry matter (kim et al., 2008). This are then mixed with other unfermented residues as condensed distillers soluble or wet distillers' grain with soluble (WDGS) and dried to 88% dry matter to produce DDGS (Mohammadi Shad, 2021)

Nutrient composition of DDGS

The variability of nutrient composition of DDGS is dependent on the variety of its source and where it was grown (Shurson, 2005) also by the type of grain and production process (Böttger, 2018). The CP of DDGS ranges from 27% - 35% (Belyea et al., 1989). With high quality US corn DDGS having a CP range between 30.6% - 30.9% (Spiehs et al., 2002). It is also rich in Essential Amino Acid (EAA) methionine (0.55%), Lysine (0.85%), leucine (3.55%) and Phenylalanine (1.47%) with the Lysine being the most varying from different sources. (Spiehs, 2002). It also has a high amount of NDF ranging between 28.8 to 40.3% and ADF 10.3 to 18.1% (Cromwell, 1993). A report by Pamp et al. (2006) highlighted an increase in milk yield and its component when dietary RUP was increased in the form of DDGS than against soybean.



Source: (Mohammadi Shad, 2021)

Nutrient composition of protein supplements

Protein

Canola meal has been reported to be a high-protein food, with an average protein content ranging from 36% to 44% depending on processing methods and quality (NRC, 2001; Broderick et al., 2016; Sánchez-Duarte et al., 2019; Paula et al., 2020). Meals derived from yellow seeded *B. juncea* variety contain more protein than the brown-seeded *B. napus* meal variety (Rahman and McVetty 2011; He et al. 2013). Also, CM is reported to contain approximately 41.7% crude

protein (CP), 18% neutral detergent insoluble nitrogen (NDIN), 5% acid detergent insoluble nitrogen (ADIN, as a percentage of N), (53.9-67.7%) rumen-degraded protein (RDP), and (32.3 – 46.1%) rumen undegraded protein (RUP, as a percentage of CP) (Kalscheur, 2017).

Although CM has a lower CP and a higher fiber concentration than SBM (Broderick et al 2015), studies have suggested that CP in CM is used more efficiently used by lactating dairy cows than CP in SBM (Galindo et al. 2015; Harker et al 2012). Additionally, Huhtanen et al. (2011) conducted a meta-analysis that showed that RUP and metabolizable protein (MP) concentrations in CM were like those in SBM. However, Brito et al. (2007) reported that diets containing CM had a greater concentration of RUP compared to SBM. DDGS is said to contain 30.1% CP dry matter weight and 55.0% RUP of CP (Schingoethe 2004). Due to its high energy and protein content, this has made DDGS to be uniquely used for ruminant diets. DDGS is a unique ingredient added to the diet of ruminants as it is a high source of energy (has high fat content) and protein (Belyea, 2010).

Amino acid composition

The amino acid profile of CM is well suited for animal feeding as it contains high levels of methionine and cysteine. However, like many other vegetable protein sources, lysine is limited. The amino acid profile was corrected to a 36% protein basis, which means that the actual amino acid content may be higher than previously stated. It is important to note that the amino acid content varied with the protein content, as reported by Evonik (2018). CM has a higher concentration of sulfur-containing amino acids (methionine and cysteine) but a lower concentration of lysine (Newkirk et al 2003). Approximately 40% of the total AA present in CM are essential AA; CM has approximately 2.52% histidine (His), 4.87% lysine (Lys), 1.88%

methionine (Met), and 5.90% arginine (Arg) (Paz et al 2014; Maxin et al 2013; Newkirk et al 2003). Moreover, NRC (2001) reported a higher Met concentration in CM than that in SBM.

Boila and Ingalls (1992) and Maxin et al. (2013b) reported that CM has a superior amino acid balance compared with SBM and wheat DDGS. Specifically, it contains higher levels of ruminal escape methionine and threonine than SBM, and more essential amino acids than wheatDDGS. Canola meal frequently enhances the amino acid profile of diets compared to SBM and wheat distillers dried grains with soluble due to its higher concentration of rumen escape amino acids, as demonstrated by research conducted by Brito et al. (2007), Mulrooney et al. (2009) and Maxin et al. (2013a). Brito et al. (2007) discovered that the amount of essential amino acids secreted into the small intestine was greater for lactating cows consuming diets supplemented with CM compared to those consuming diets supplemented with SBM, cottonseed meal, and urea.

Energy content and digestibility

The carbohydrate and fiber contents of CM are approximately 29% NDF, 20% ADF, 20% non-fiber carbohydrate (NFC), and 4% starch (% of DM), with a greater NDF and ADF concentration compared to SBM (Broderick et al 2015) and DDGS contains NDF 41.5% ADF 16.1% (% of DM) (Schingoethe 2004). The lignin concentration in CM is approximately 10% (Bell, 1993; Brito and Broderick, 2007). The hull component of canola seeds accounts for up to 30% of the meal and is largely made up of fiber, much of the meal's energy content is determined by the digestibility of the hull fiber fraction (Bell and Shires, 1982).

According to He et al. (2013), the fiber digestibility of CM is lower than that of other high-protein by-products, such as SBM or wheat DDGS; hence, its use as an energy source in feedlot diets is limited. CM digestibility can be altered by a variety of factors, including the

presence of anti-nutritional substances, processing, and animal type. Energy and other nutrient digestibility in feed ingredients are often measured using approaches such as in vivo digestibility experiments or laboratory analysis (NRC 2012).

Micronutrients and anti-nutritional factors

Compared to other oilseed meals, CM has been reported to be a good source of essential minerals. The meal has approximately 8% ash (% of DM), and its mineral content is composed of 0.9% Ca, 1.1% P, 0.6% Mg, 1.1% K, 0.23% Na, and 0.11% Cl (% of DM). CM also contains micronutrients (mg/g) such as Cu (5.99), Fe (179), Mn (56.5), Mo (1.27), Se (1.11), and Zn (62.1) (Maesoomi et al., 2006; Mulrooney et al., 2009; Hristov et al., 2011; Moshtaghi Nia and Ingalls, 2010; Paz et al., 2014; Weiss et al., 2015; Paula et al., 2017).

Compared to SBM, CM contains more dietary fiber, glucosinolates, sinapine, phytic acid, phenolic components such as tannins, less consistent AA digestibility, and less than optimal electrolyte balance due to its high sulfur and low potassium contents (Khajali and Slominski, 2012). Among these, fiber, glucosinolates, phytic acid, and sinapine are thought to be the most important antinutritional agents in CM. Canola meal contains 1.5% to 3.0% tannins; cultivars with brown seeds have a higher tannin content than those with yellow seeds. Compared to other plants, CM tannins do not seem to have the same detrimental effects on protein digestibility and palatability with 0.6-1.8% sinapine (Bell 1993; Clandinin, 1961). However, the high content of phytic acid and crude fiber in the meal limits availability of Cu, Zn, and K (Clandinin and Robblee 1980).

Protein supplementation of livestock

Protein is an essential nutrient in the diets of beef cattle, and although it may be a costly addition to feeding programs, it is sometimes necessary to ensure that the animal's nutrient

requirements are met. To support muscle growth in young, growing cattle, they require relatively high levels of CP in their diets. Specifically, creep feeds or forages for nursing calves should contain at least 15% CP. When forage availability is abundant, high-protein creep feeds can be used. Average daily gains in nursing calves tend to increase with higher CP content in creep diets, but the cost of the diet will also increase with higher protein levels. Additional protein and energy are often necessary to balance diets for growing cattle and lactating beef cows on forage-based diets, especially when low-quality stored forages are most of the diet. This is often the case during the winter hay-feeding period after a poor hay production season or with hay produced under low levels of management (NRC, 2001; Moore and Kunle, 1995; Roosi and Silcox, 2007).

CM is a high-quality protein source for animal nutrition. Its typical concentration of CP (%DM) ranges from 38% to 42% (NRC, 2001; Broderick et al., 2016; Gauthier et al., 2019; Sánchez-Duarte et al., 2019; Paula et al., 2020), which is lower than the CP concentration of 49.9% (%DM) for soybean meal (SBM; NRC, 2001). Despite this difference, several studies have demonstrated that CM supports milk production better than soybean meal due to its higher ruminal escape of protein, with RUP values ranging from 44.4% to 52% of CP (Maxin et al., 2013a; Broderick et al., 2016) and a more balanced amino acid profile (Huhtanen et al., 2011; Maxin et al., 2013a; Martineau et al., 2014), while SBM has been reported to contain 29% - 41.9% RUP (Brito et al. 2007; Stanford et al. 1995).

The protein in distillers' grains (DG) is moderately resistant to degradation in the rumen, making it a valuable source of RUP. Firkins et al. (1984) research, has estimated the RUP content of DDGS to be between 47 and 54% of CP. However, Brouk (1994), conducted an in-situ study and found that the RUP content of various distiller grain sources varied, ranging from 53.5 to 87.2% of CP. In both studies, the RUP content of wet distillers' grains (WDG) was found

to be at the lower end of the range, as it does not undergo an additional drying process for DDG. This process may damage a significant portion of the protein, making it unavailable to animals.

When comparing CM to SBM, DDGS, and high-protein dried distillers' grains with soluble in lactating cow rations, no differences were observed in milk yield, energy-corrected milk, or DMI (Maxin et al., 2013a). However, there was a slight decrease in milk fat content in cows fed diets containing WDDGS, possibly because of its higher fat content (Maxin et al. 2013a). This study also showed that CM had the best amino acid profile of all protein sources, with cows fed CM having the highest plasma concentration of all essential amino acids, except leucine, indicating that CM provided a superior profile of essential amino acids (Maxin et al. 2013a). Maxin et al. 2013a determined from their research that CM supplemented diets had the highest metabolizable protein value, while SBM was deficient in methionine and WDDGS was deficient in histidine.

Protein utilization by ruminants

The source of N can vary as seen in a study by (Lapierre and Lobley 2001) where 17% of urea was secreted in saliva of concentrate fed cattle while 36% of saliva urea was from forage fed cattle. The breakdown of degradable protein produces ammonia (NH_3) that is used by rumen microbes to synthesize microbial protein, while the rest is carried to the liver via the bloodstream where it is detoxified before being excreted in the urine. In growing ruminants, there is need for high availability of N as such the recycling of urea-N to the GIT increases N availability from 43 to 130% (Lapierre and Lobley 2001). According to (Lobley, 2000) 3 to 21% of N is lost in the feces while 16 to 70% recycled urea N gets into the GIT and used for further synthesis of protein. Excess protein in the body requires energy to remove, and when there is a surplus of degradable protein or a shortage of energy, the systems for removing NH_3 cannot keep up, leading to toxic

effects in many parts of the body, including the uterus. This can impair fertilization and embryo development, resulting in lower conception rates. Additionally, a reduction in available energy may cause rebreeding problems.

Because CM has a balanced amino acid profile and a high degree of CP degradability, Piepenbrink and Schingoethe (1998) observed that RDP from CM has the potential to promote microbial protein synthesis. Similarly, Krizsan et al. (2017) suggested that the increased ruminal NH_3 in CM-supplemented dairy cows was likely due to the increased proteolysis of a greater amount of available feed protein, leading to increased deamination of amino acids to NH_3. Brito et al. (2007), found no variations in ruminal NH_3 concentrations in dairy cow diets supplemented with CM, cottonseed meal, or SBM. Using an in vitro dual-flow continuous culture system, Paula et al. (2017a) did not observe any variations in ruminal NH_3 concentration when feeding diets containing CM compared with SBM. Compared with SBM, cows fed CM diets had reduced NH_3 levels (Broderick et al. 2015). Similarly, Christen et al. (2010) found no variation in volatile fatty acid (VFA) proportions among different protein supplements, SBM, CM, or DDGS in isonitrogenous diets. Furthermore, no variations in VFA traits were observed in several trials comparing ruminal fermentation parameters in dairy cows fed diets supplemented with CM vs. SBM (Li et al. 2013; Paula et al. 2018; Brandao et al. 2018). Comparing CM and DDGS, Mulrooney et al. (2009) did not find any variation in total or individual VFA concentrations. Krizsan et al. (2017) observed that increasing dietary concentrations of CM in lactating Nordic red cows linearly decreased ruminal pH. Tajaddini et al. (2021) reported that different levels of dietary CM did not impact ruminal pH values in lactating goats at 0 hr and 3 hr post-feeding. Similarly, Broderick et al. (2015) found no differences in ruminal pH values between CM and SBM diets.

Rumen and total tract digestibility of protein supplements

When balancing rations, it is important to not only meet the percentage requirement of CP but also to ensure that the right amounts of soluble, degradable, and undegradable proteins are provided. Soluble protein levels should not exceed 30% of the total CP in the diet and feeds with high levels of non-degradable protein and low levels of degradable and soluble protein may be necessary to balance the high levels of soluble protein found in some forages. On average, the levels of degradable and non-degradable proteins should be 60% and 40% of the total protein, respectively (Karen, 2011).

Canola meal has long been valued in ruminant diets for its high-quality protein. Its amino acid profile was recognized early on to be more suitable for maintenance and milk production than other plant-based proteins (Schingoethe, 1991). However, many feed libraries have inaccurate values for the rumen-undegradable protein (RUP) and rumen-degradable protein (RDP) of feed ingredients, and these are gradually being revised. Historically, soluble protein was assumed to be largely degraded in the rumen, but recent research has shown that the rumen degradability of soluble protein is highly variable. Furthermore, some soluble protein from feed ingredients remains undegraded, and this can vary depending on the protein source. For CM, a significant portion of the soluble fraction remains undegraded. The two main storage proteins in canola are napin and cruciferin. Napin is a low-molecular-weight protein that is soluble, but it appears to be resistant to degradation in the rumen, while cruciferin a 6 complex monomer with varying solubility behavior with changes in pH but unfolds at pH 3 under ambient temperature (Perera et al., 2016),

Although the high dietary CP level may have played a role in ruminal N fermentation, previous studies with 16.5 and 15.7% CP, respectively, reported similar results about ruminal N

metabolism when comparing SBM and CM (Brito and Broderick, 2007; Paula et al., 2016a). This suggests that dietary CP levels play a minor role when comparing these two protein supplements. Lower CP levels would have made it challenging to evaluate ruminal N flow because low CP levels would leave little room for ruminal N differences among treatments. For example, a 16% dietary CP would yield a difference of only 19.2 g of ruminal N/kg of DMI between the two CM diets tested (38 and 50% ruminal N as a percentage of CP) as opposed to 25.2 g of ruminal N/kg of DMI in a 21% dietary CP level. However, high CP levels may have precluded better evaluation of ruminal N fermentation.

Most feed tables (AFRC, 1993; NRC, 2001; INRA, 2007) report greater metabolized protein (MP) for SBM compared with CM because of its lower CP degradability and lower RUP content (Huhtanen et al., 2011). The accuracy of the CM RUP and RDP values in feed tables may be questionable due to the methods used to assess these values. Studies have shown that early methods for reporting the RDP and RUP fractions of CM used in situ methodology, which assumes that all soluble proteins, peptides, and amino acids are completely degraded in the rumen. However, this may not always be the case (Reynal et al., 2007). Additionally, the ruminal degradation rate of the soluble protein fraction of CM is lower than previously thought (Hedqvist and Udén, 2006). Furthermore, a significant portion of the soluble CP fraction in CM may escape rumen degradation (Bach et al., 2008). In situ methodologies may also have physical restrictions on feed within the bags and include microbial contamination in undigested residues (Broderick et al., 1991). Another possible reason for the discrepancy in the RUP and RDP values of CM in feed tables may be due to the differences in seed processing or crushing methods. While NRC (2001) lists only mechanically extracted CM, solvent extraction is the primary method currently being used. The study by Maxin et al. (2013) found that in situ CP degradation was lower for

CM and had greater RUP content compared to SBM. Additionally, Broderick et al. (2015) observed a decrease in ruminal NH_3-N concentration for CM compared to SBM in lactating dairy cows. Brito et al. (2007) estimated the in vivo RUP flows of 29 and 34% (of CP) for SBM and CM diets, respectively.

Protein supplements and rumen volatile fatty acid concentrations

The results of recent studies have demonstrated that CM, when served as the primary source of protein, leads to a reduction in ruminal NH_3-N and branched-chain VFA (BCVFA) concentrations in the rumen, along with decreased methane (CH_4) emissions compared to SBM (Martineau et al., 2013; Broderick et al., 2015; Gidlund et al., 2015). However, the mechanisms behind CM's ability to enhance overall N utilization when replacing SBM are not well understood. While a better AA profile may be involved, the impact of ruminal effects of feeding CM on its effectiveness as a protein supplement for dairy cows is uncertain.

In a study evaluating the Dietary treatments of SBM with 42.6% of RUP as a percentage of CP (NRC, 2001), LCM (38% of RUP; Broderick et al., 2016), and HCM (50% RUP; Broderick et al., 2016), Paula et al. (2017a) reported no impact on the total VFA concentration; the proportions of acetate, propionate, and isobutyrate; and the acetate-to-propionate ratio. These findings are consistent with other studies that compared SBM with CM, as significant differences were not observed for total VFA and VFA molar proportions (Sanchez and Claypool, 1983; Brito and Broderick, 2007). However, the SBM diet showed a trend towards increased total VFA concentration ($P = 0.08$) and a higher proportion of butyrate ($P = 0.06$) compared to the both CM diets This may be due to the greater amount of non-fiber carbohydrates (NFC) in the SBM diet compared to the LCM (Low RUP of CM) and HCM (High RUP of CM) diets (41.3%, 36.0%, and 36.3%, respectively), which could lead to greater microbial fermentation compared to the

CM diets. Brito and Broderick (2007) conducted a study to evaluate the effect of different protein supplements on omasal nutrient flow in lactating dairy cows. They tested SBM and CM at 50.2% and 46.4% levels of inclusion in the diets and found that the diets had differing levels of non-fiber carbohydrates (NFC) at approximately 4 percentage units and did not find any differences in total VFA and molar proportions of acetate and propionate. The results were similar for a follow up study (Broderick et al., 2015) where the NFC levels differed by approximately 3 percentage units (47.8 and 44.5% for SBM and CM, respectively). Given these findings, it is unlikely that the difference in NFC content between SBM and CM played a significant role in ruminal fermentation, despite the difference in NFC between the two types of diets.

Protein supplements and ruminal methane production

Ramin and Huhtanen (2013), stated that there is a positive correlation between the increase in CH_4 production (mol/d) and diet digestibility. Another possible explanation for the larger gas pool for the SBM diet may be the higher concentration of BCVFA in the SBM diet, which may have promoted the growth of cellulolytic and some non-cellulolytic bacteria (Allison, 1969). There are only a limited number of studies on the impact of including CM in the diets of dairy cows on enteric CH_4 emissions (Gidlund et al., 2015; Reynolds et al., 2019). Martineau et al., (2013) reported that incorporating CM into dairy cow diets instead of SBM may result in increased DMI and greater enteric CH_4 production (g/d), with DMI and CH_4 production being positively related (Reynolds et al., 2010; Beauchemin et al., 2020). However, enteric CH_4 yield [g/kg of DMI or as a percentage of gross energy (GE) intake] decreases as DMI increases due to faster passage rates from the rumen (Beauchemin et al., 2020). Additionally, as the milk production increases with the inclusion of CM in the diet, particularly at high inclusion levels

such as 17 to 21% of dietary DM (Broderick et al., 2015; Gidlund et al., 2015), the intensity of enteric CH_4 emission (g of CH_4/kg of milk) is expected to decrease (Beauchemin et al., 2020).

Protein supplements and dry matter intake

Although the effect of replacing SBM with CM in the diets of dairy cows has been inconsistent among studies, with some studies reporting similar performance and others reporting higher dry matter intake (DMI) and milk production for cows fed CM, this inconsistency is due in part to the inclusion rate (Martineau et al., 2013). In fact, at low inclusion rates of CM (10-13% of dietary DM), no difference in dairy cow performance was observed between the two protein sources (Paula et al., 2018; Reynolds et al., 2019; Sánchez-Duarte et al., 2019). However, at high inclusion levels (17-21%), adding CM at the expense of SBM increased the yield. The optimal level of CM dietary inclusion is not well defined, and studies investigating the effect of high percentages (>20%) of CM inclusion in the diet on dairy cow performance are scarce.

In two meta-analysis studies, Huhtanen et al. (2011) and Martineau et al. (2013) reported that diets supplemented with CM increased the daily dry matter intake (DDMI) compared to diets supplemented with SBM. Huhtanen et al. (2011) suggested that CM diets provide better amino acid (AA) supply and support greater milk yield, which increases energy demand and consequently increases DMI.

Supplementation of DDGS frequently in the diet of steers at more than 15% inclusion level has a positive impact on steers growth (Stalker, 2009) in his study where steers were fed with native winter range and supplemented with DDGS 6d/wk performed better with higher digestibility than the steers fed the same diet but supplementation with DDGS was 3 d/wk.

Protein supplements and total tract nutrient digestibility

Brito et al. (2007) found that the ruminal degradability of DM, OM, NDF, and ADF was not significantly different between the SBM and CM treatments, which agrees with the findings of Paula et al. (2017a). In a review and meta-analysis of studies on CM as a protein supplement for dairy cows, Huhtanen et al. (2011) observed that heat-treated CM diets had reduced total-tract true CP digestibility compared to untreated CM diets.

Protein supplements for growing beef cattle

Prior performance and digestibility studies in beef cattle have primarily focused on evaluating the value of CM as a protein source, with dietary inclusion levels ranging from 6 to 15%. Petit et al. (1994) investigated the effect of CM on the growth performance of beef steers when it was used as a protein supplement in a timothy silage-based diet during the growing period. The study found that supplementation with CM increased body weight gain during the overall feeding period. Lardy and Kerley (1994) observed a decrease in feed intake when rapeseed meal was included as a protein supplement in 66% whole-shelled corn and 34% cottonseed hull diets, but with modern CM from canola varieties the intake-reducing effect is less likely to occur. Lardy and Kerley (1994) also reported that the inclusion of CM at 15 and 30% of dietary dry matter (DM) exceeded the recommended dietary crude protein (CP) requirement for growing and finishing beef cattle, as stated by NRC (1996). Body weight gain and carcass quality were not affected when CM was included in barley-based growing or finishing diets at levels up to 30% dietary DM. Unlike CM, the use of wheat DDGS did not result in a decrease in G: F until it accounted for 60% of the dietary DM (Gibb et al., 2008). It has been demonstrated that CM can be used as an effective protein supplement for growing beef

cattle (Newkirk, 2009). However, there are limitations to its use as an energy source in feedlot diets.

Also, (Wood, 2011) in a study on the effect of corn or Sorghum DDGS (sDDGs) on beef steer indicated that inclusion of SDDGS at 200g/kg in a grower or finisher beef diet is permitted and would not negatively affect the growing performance of the steers nor their carcass quality as it took lesser days for steers fed on SDDGS to get to the backfat target of 10mm. Inclusion level of DDGS is also an important factor to consider for growth performance as stated earlier, this is also described in a study by (Gibb, 2008) highlighting that inclusion of DDGS at levels higher than 20% of diet causes a decrease in energy content and digestibility, the study showed that increasing levels of DDGS at 0,20,40 and 60% has a ((P = 0.04) reduction on gain: feed and also a reduction (P = 0.001) on the diet NEg content.

The negative impact of CM on performance parameters compared with wheat DDGS is likely due to the difference in the digestibility of fiber in these two by-products. The fiber in wheat DDGS is highly degradable (Li et al., 2011), which may be associated with the processing and fermentation of wheat during ethanol production. As such, dietary concentration of DDGS can alter the VFA parameter causing a decrease in acetate: propionate ratio by increasing propionate molar percentage and decreasing in acetate molar percentage shown by (Manthey, 2018) where dairy heifers were limit fed DDGS concentrate mix at 0.8% body weight and grass hay ad libitum.

The production of CM does not involve fermentation, and it is well known that the fiber in CM is less degradable in the rumen than fibers from other protein sources, such as DDGS (Heendeniya et al., 2012). Agwa et al., (2023) narrated that replacement of cottonseed meal with 75% CM in growing lamb had no compromising effect on their growth performance and ruminal

fermentation parameters but had a linear decrease in serum total proteins. Similarly, Mustafa et al. (1997) observed a 13% decrease in blood urea N in cows fed CM versus SBM. Martineau et al. (2014) also reported that feeding CM to dairy cattle resulted in lower concentrations of blood urea N, indicating improved whole-body N utilization efficiency by ruminant animals and greater utilization of dietary protein. It is important to note that approximately 10–40% of the N consumed in the feed is recycled back to the digestive tract as urea from saliva, which can be utilized for microbial synthesis (Bach et al., 2005).

Agwa et al., (2023) found that lambs fed diets containing CM had stable metabolic conditions and a moderate level of homeostasis, as evidenced by serum electrolyte, triiodothyronine, and thyroxine levels that were not significantly different among the dietary groups. This is consistent with the findings of Rezaeipour et al. (2016), who reported that varying levels of dietary CM had no significant effect on thyroid hormone secretion in Atabay finishing lambs. Similarly, Maesoomi et al. (2006) found that mid-lactation dairy cows fed diets containing CM had similar levels of plasma thyronines to those fed cottonseed meal. In addition, Newkirk et al. (2003), reported that CM contains approximately 2mol/g glucosinolates. High concentrations of glucosinolates have been known to impair thyroid function by preventing thyroid hormone synthesis (Holst and Williamson, 2004). However, the effect of CM on thyroid function is still a subject of debate. Lardy and Kerley (1994) reported a linear decrease in thyroxin levels in Holstein calves fed diets containing increasing amounts of rapeseed meal, whereas Martineau et al. (2013) found that dairy cows fed CM had increased feed intake and stable thyroid function compared to those fed other frequently fed protein sources. Further research is needed to fully understand the effects of CM on thyroid function in livestock.

Protein supplements for finishing beef cattle and carcass quality

Beef cattle have been shown to find CM to be a palatable feed ingredient. In a recent study, Nair et al. (2015) found that intakes were improved when CM was included in the finishing diets at concentrations of 10 or 20% of the DM. Intakes for beef cattle were higher in backgrounded beef cattle given diets with 10% CM than those containing corn distillers' or wheat DDGS (Li et al., 2013). He et al (2013) determined no reduction in DMI when CM replaced barley grain at 30% of the diet DM during the finishing phase of beef cattle.

The use of CM in diets for growing and finishing beef cattle has been shown to exceed the dietary CP requirement recommended by the NRC (1996). Including CM at 15 and 30% of dietary DM did not affect body weight gain or carcass quality when it was included in barley-based diets. However, substituting 30% CM with barley grain in the finishing diet resulted in a decrease in G: F. When using wheat DDGS, a reduction in G: F was not observed until it accounted for 60% of the dietary DM (Gibb et al. 2008).

Buckner et al. (2007) conducted a study assessing the impact of increasing DDGS levels on the growth performance and carcass characteristics of finishing steers. The study revealed no significant effect of DDGS on dry matter intake, twelfth rib fat depth, loin muscle area, or marbling score. However, there was a quadratic relationship between increasing levels of DDGS and average daily gain (ADG) and hot carcass weight, and a quadratic trend for G: F. The meta-analysis study of Klopfenstein et al. (2008), indicated that the highest ADG was obtained by incorporating 20 to 30% DDGS in the diet, while the maximum G:F ratio was attained by feeding finishing cattle 10 to 20% DDGS diets. For instance, Swanson et al. (2014) demonstrated the positive effects of feeding high levels of DDGS (up to 40 percent) to finishing beef cattle. In their study, yearling steers were fed diets containing 20 or 40% DDGS along with coarsely or

finely ground corn to assess the impact on growth performance and carcass traits. Although final body weight and ADG were not influenced by DDGS inclusion rate or corn particle size, dry matter intake decreased and the G:F ratio increased with an increase in DDGS inclusion rate, Carcass traits were not affected by the inclusion rate of DDGS or the size of dry-rolled corn particles. This suggests that up to 40% of DDGS can be fed to finishing cattle to improve ADG and G: F without compromising carcass quality.

Protein supplements and phosphorus excretion

Canola meal is a rich source of phosphorus, with most of this mineral in the form of phytate phosphorus. Unlike monogastric animals, this form is available to ruminants, due to the presence of bacterial phytases in the rumen that rapidly degrade phytate (Spears, 2003). Research indicates that phytate phosphorus is more effectively utilized by ruminants than non-phytate phosphorus. In a study by Garikipati (2004), dairy cows were fed diets containing approximately half of the phosphorus as phytate, and the overall digestibility of phosphorus was 49%. However, the digestibility of phytate-bound phosphorus was significantly higher (79 %). Similarly, Skrivanova et al. (2004) observed that 10-week-old calves could digest 72% of phosphorus in their diets, with 97% of the phytate portion being digestible.

Corn DDGS is low in calcium but relatively high in phosphorus (P) and sulfur content. Depending on the feeding level, adding distillers' grains to the diet may allow for the complete removal of other supplemental phosphorus sources from the previously fed mineral mixture. Due to the high levels of DDGS, beef cattle feedlot diets contain excess phosphorus relative to their requirements (Grains.org 2018). Owing to the low calcium content in DDGS, it is necessary to provide supplemental calcium sources, such as ground limestone or alfalfa, to maintain a calcium-to-phosphorus ratio between 1.2:1 and no more than 7:1. This is done to prevent

reductions in animal performance and urinary calculi (Tjardes and Wright, 2002). In a study by Geisert et al. (2010), diets containing brewers grits were fed to animals to determine phosphorus digestibility and excretion. The diets provided low phosphorus (0.12% P), medium phosphorus (0.27% P), and high phosphorus (0.42% P), with supplemental monosodium phosphate, dry-rolled corn, and 30% DDGS. The results showed that the addition of 30% DDGS to the diet resulted in a relatively high total phosphorus content and intake, which was approximately 50% digestible. Although DDGS contains more digestible phosphorus than the phosphorus required for finishing cattle, it results in a significant amount of total phosphorus excretion (approximately 54% of the intake). The phosphorus requirement for finishing cattle is lower than the phosphorus content of typical U.S. beef cattle feedlot diets (0.30-0.50%), as estimated by the NRC (2001). As a result, adding supplemental phosphorus to a typical corn-based or DDGS-based diet is unnecessary because the phosphorus requirement for optimal growth performance is less than 0.17% of the dry matter of the diet. Therefore, by eliminating excess phosphorus provided by mineral supplements from feedlot cattle diets, the amount of phosphorus excretion in manure will be reduced to minimize the risk of negative environmental consequences (Grain.org 2018).

In vitro fermentation of protein supplements

In vitro fermentation is a technique utilized in a laboratory setting to replicate the fermentation processes that occur within the digestive system of ruminant animals, specifically in the rumen. This method can be employed to evaluate the degradation and utilization of feed components by bacterial populations in the gastrointestinal tract.

In vitro fermentation principles

Mould et al. (2005), narrated that the goal of all in vitro fermentation systems is to produce an environment that mimics a specific segment of the gastrointestinal tract for any set of parameters. As a result, the inoculum (rumen fluid) is obtained from a donor animal and should reflect the environment in terms of both microbial species and concentration. A vast community of bacteria, protozoa, fungi, and archaea inhabits the fluid and plays an important role in the fermentation process. The substrate is the feed sample or nutrient being analyzed. This may be a single component, or an entire feed mixture and the chemical and physical properties of the substrate determine its fermentation profile and nutrient consumption. The incubation conditions where the in-vitro fermentation occurs under regulated parameters, such as pH, temperature, anaerobic conditions, and incubation time of the substrate. These circumstances are like those observed in the rumen and promote microbial growth and activity. Lastly, the measurement of fermentation parameters includes gas generation (as a sign of microbial activity), pH variation, VFA production, NH_3-N concentration, and nutrient breakdown or utilization (Getachew et al. (2005); Menke & Steingass (1988); Blümmel et al. (1997)).

In-vitro fermentation techniques and models

Pure culture

The use of pure cultures to investigate the characteristics of bacterial species has been widely accepted for decades (Bryant and Burkey, 1953). This approach involves growing a specific strain of bacteria in its optimal media, typically in a culture tube, and then applying a particular treatment to determine changes in the fermentation profile that are unique to that bacterial strain. The initial application of pure culture in ruminant nutrition was to determine the nutritional requirements of specific microorganisms and the end products that they produce from

nutrients (Bryant, 1959; Hungate et al., 1964). Further research has been conducted to develop methods to determine the enzymatic activity of microbial enzymes within a pure culture, which has significantly influenced the trajectory of pure culture research (Joyner and Baldwin, 1966). Because pure culture does not involve interactions between groups of bacteria, it is suitable for studying mechanisms of microbial synthesis, particularly for intermediates that may be devoured or otherwise altered by other microorganisms in a more dynamic system.

Batch culture

The initial report of batch culture incubation methodology for in vitro fermentation of feed ingredients was made by Tilley and Terry in 1963, and it was later updated by Goering and Van Soest in 1970. The history of batch culture was comprehensively reviewed by Yáñez-Ruiz et al. in 2016; however, this methodology involves collecting ruminal fluid, diluting it with a buffer, incubating the mixture in closed bottles with the substrate of interest, filtering the contents after incubation to determine the digestion that occurred, and using it to estimate the degradation of nutrients and the nutritional quality of feed ingredients. Some methodologies utilize the same principles as earlier methodologies but leverage more recent technologies. Holden (1999) compared the use of serum bottles against the Ankom DAISY^{II} (Ankom Technology Corp., Macedon, NY, USA), with the DAISY^{II} being tested using bags containing all the same feed or with different feeds. DAISY^{II} is an incubation cabinet that contains four rotating jars containing buffered ruminal fluid that are used to incubate many samples that have been weighed in nylon bags at the same time. Holden (1999) also compared a different methodology for ten different feeds using a version of the original Tilley and Terry (1963) methodology adapted for use with DAISY^{II} and found that DM degradability was not affected by the methodologies used or the presence of different diets within the same fermentation vessel. This

allowed the authors to evaluate ten different feeds for a fraction of the time, cost, and labor involved in in vivo research.

Batch culture has multiple applications, including gas production, fermentation end products, nutrient degradation, and analysis of microbial communities. The measurement of in vitro gas production has undergone several updates since its introduction in the 1940s (Quin, 1943), with the most recent version of the methodology focusing on automated measurements (Cornou et al., 2013; Muetzel and Tavendale, 2014). However, the initial method largely disregards the extent and rate of fermentation. The current batch culture methodology enables the evaluation of both the quality of fermentation and the extent of nutrient degradation during incubation. This assessment encompasses the evaluation of fermentation profiles and end products, including organic acids, NH_3-N, pH, and microbial ecology, as well as the degradation of nutrients. The methodology has also been developed to analyze different fractions of carbohydrates and proteins, particularly to determine the fractions of RUP, RDP, and the undegradable fractions of fiber (uNDF).

Continuous culture

Hobson first described Continuous Culture Fermentation (CC) in 1965, which involves maintaining an in vitro culture of ruminal fluid for an extended period compared with other in vitro methods. There are two types of CC: single-flow (SFCC) and dual-flow (DFCC). In SFCC, the effluent exits through a single outlet, either by overflowing the vessel contents or by pumping it out at a regulated rate and is a mixture of solid and liquid fractions. One type of SFCC is the Rumen Simulation Technique (RUSITEC). First developed by Czerkawski and Breckenridge in 1977, RUSITEC employs nylon bags filled with feed in a controlled environment of constant agitation, artificial saliva inflow, and ruminal fluid overflow. The use of DFCC was first

described by Hoover et al. in 1976, in which the outflow was separated into solid and liquid fractions. The overflow is the solid fraction, whereas the filtered and pumped outflow is the liquid fraction, providing a more representative in vivo observation than SFCC. A meta-analysis conducted by Brandao et al. in 2020 investigated the relationship between responses reported in vivo using the Omasal Sampling Technique (OST, Huhtanen et al., 1997; Ahvenjärvi et al., 2000) and the DFCC system. OST is a well-known technique for determining ruminal degradability. As a result, the data collected in OST trials should be equivalent to the data acquired in DFCC experiments. Brandao et al. (2020) investigated the association between DFCC and OST outcomes using data from 155 articles (97 DFCC and 58 OST). They discovered that most response variables were identical between DFCC and OST and that any differences were related to differences in the intercept. Similarly, Brandao and Faciola (2019) evaluated the variations observed across different DFCC investigations using another meta-analysis. Scientists examined how dietary composition, specifically CP and NDF, and the amount of feed given each day affected the end products of microbial fermentation. They discovered that estimates of ruminal degradation, VFA concentrations, and N metabolism were comparable among investigations that employed the DFCC approach.

Continuous culture provides meaningful insights into ruminal fermentation but at a fraction of the time and cost of an in vivo experiment. This is because research suggests that in CC experiments, microbial communities stabilize after four days rather than two or more weeks in vivo (Salfer et al., 2018). CC trials have been used to study ruminal fermentation, nutrient degradation, N flow and metabolism, microbial metabolism of nutrients (mainly N), changes in microbial ecology, and gas production. Labeled ^{15}N is often employed as a marker to measure microbial uptake and use of N within the system because of the modest volume of the

fermentation tank (less than 2 L) and ease of application via artificial saliva. Hristov et al. (2012) performed a meta-analysis in which they analyzed CC (both single- and dual-flow), RUSITEC, and in vivo degradability trials to evaluate the differences in variability for ruminal fermentation and nutrient degradation data across different study types. They reported that CC data were more variable than RUSITEC data, which were more variable than in in vivo studies. It is worth noting that the CC data did not separate SFCC and DFCC data, which could be a source of variation, and used a small selection of in vivo data (366 cows from only three Universities) compared to 30 years of CC data (1074 different treatments), which could explain the lack of variation in the in vivo data. CC, to all in vitro methods, CC is constantly being refined. Wenner et al. (2021) modified the original DFCC methodology by rounding the fermenter vessel, increasing the diameter of the impeller, improving the impeller motor, and adding a better liquid flow filtration system, all of which reduced variability when compared to DFCC studies conducted using the original system.

Total tract in-vitro digestibility

While ruminal digestion estimation is relatively simple because ruminal content is easily accessible, collecting inoculum for in vitro digestion from the abomasum or small intestine becomes more complex because collecting content from either is difficult. Thus, the development of in vitro methodologies based on chemicals is critical to provide a reliable methodology for estimating total tract digestibility in vitro. Calsamiglia and Stern (1995) initially developed a three-step procedure (TSP) to determine the total tract digestibility of proteins because other available methodologies, such as the mobile bag technique (Hvelplund, 1985) that uses duodenal cannulated animals or acid detergent insoluble nitrogen (Goering et al., 1972), were highly variable. Calsamiglia and Stern (1995) developed a consistent assay validated using

samples from an in vivo intestinal digestibility study. The first TSP was created to determine protein digestibility and estimate RDP and RUP fractions in feeds (Calsamiglia and Stern, 1995). It included a 16-hour in-situ ruminal fermentation, a 1-hour acid/pepsin incubation, and a 24-hour incubation in a buffered pancreatin solution. Pancreatin is a powdered extract derived from porcine pancreas that contains pancreatic enzymes. Since then, this methodology has been updated several times to improve its validity and scope. Gargallo et al. (2006) were the first to update the method to include the use of the Ankom Daisy^{II}. Ross et al. (2013) further updated the method to eliminate the use of bags, which could potentially inhibit microbial attachment to feed particles, and to provide amounts of individual pancreatic enzymes to use rather than pancreatin, which can vary between batches.

Vinyard et al. (2021) reported additional updates to adapt the methodology to determine lipid digestibility by adding bile and calcium to the TSP's intestinal digestion step. TSP designs vary across the literature and can combine different in vitro and in situ aspects. The initial methodology (Calsamiglia and Stern, 1995) used in situ ruminal incubation and then in vitro for the abomasal and intestinal steps, whereas subsequent methodologies (Gargallo et al., 2006; Ross et al., 2013; Vinyard et al., 2021) only used in vitro. Calsamiglia and Stern (1995) and Gargallo et al. (2006) described methodologies that use bags to contain feeds, which have been shown to limit microbial attachment (Schlau et al., 2021) but also limit the scope of diets that can be investigated (i.e., cannot contain fine particles or liquids). Ross et al. (2013) and Vinyard et al. (2021) used bags to determine digestibility by analyzing digestion end products.

Cell culture

The lack of investigation into physiological impacts is a feature shared by all previously described in vitro methodologies. In recent years, technology has advanced to the point where it

is now possible to grow cultures of epithelial cells from the digestive epithelium in the laboratory. With this advancement, cultured cells can be used to investigate the effect of diet on the epithelial cells themselves, as well as the absorption and bioavailability of digested nutrients. The use of cell culture in ruminant nutrition is of primary interest for two types of epithelia: ruminal and intestinal. Kent-Dennis et al. (2020) investigated the impact of Lipopolysaccharide stimulation (LPS) on rumen epithelium and inflammation in rumen epithelial cells (REC). To accomplish this, they isolated epithelial cells from the rumens of Holstein bull calves and heifers that had already been harvested. After culturing, cells in the first experiment were exposed to varying doses of LPS. The authors discovered that, while LPS did not affect cell viability, it increased the expression of toll-like receptors and pro-inflammatory cytokines. The cells were exposed to LPS at two different doses for varying amounts of time in the second experiment, which included removing the LPS from the media. These results were like those of the first experiment, but they discovered that removing LPS resulted in a return to baseline levels of expression in REC, indicating that REC can recover following LPS exposure. Both experiments conducted by Kent-Dennis et al. (2020) would have required animals to be exposed to high levels of LPS before being euthanized for tissue collection, departing from the three R's of animal research by not reducing the number of animals used or replacing their use with other available methods. Thus, cell culture could be a viable option for these types of studies, especially because animals are slaughtered anyway.

Cell culture methodologies, particularly those involving immortal cell lines, are still in their infancy in ruminant nutrition. As with any new methodology, further examination and repetition are required to refine these methods. Non-immortal cell line cultures, such as those used by Kent-Dennis et al. (2020), are not very efficient for experimental use because they result

in a high percentage of cell death while attempting to culture the cells, as noted by Zhan et al. (2017). Immortal cell lines, on the other hand, are abnormal in nature because of the cancerous properties that make them immortal, making non-immortal cells arguably the best cell lines to provide the most accurate response. Therefore, the current methodology must be improved to improve both the efficiency of the techniques and the breadth of studies using cell culture methodologies. However, cell culture implementation is hampered by the methodologies themselves, which necessitate specialized equipment and care that can be costly and time-consuming, as well as by the animals from which the cells are extracted.

Factors affecting in vitro fermentation.

Although in vitro fermentation can be easily controlled when an experiment is being carried out in the lab, there are still factors that can affect the accuracy of the results from the process, the factors include:

Characteristics of inoculum: considering the rumen fluid used, the source, process of acquisition, and animal status could affect the microbial status or characteristics of the rumen fluid to be utilized for the process (Menke, 1988)

Temperature and pH: for the rumen microbe population to thrive and maintain its characteristics, there is a need to ensure the pH and the temperature is constantly maintained and monitored throughout the process. A study by (Bhatta, 2006) which lasted 17 days showed a decrease in degradation of major nutrients with the increase in temperature from 39°C to 41°C and the altering of the pH from 7 to 6 resulted in a significant negative effect on nutrient degradability.

Size of the particle: particle sizes play a role in ease of access of rumen microbes to degrade the substrate and how they utilize the substrate. (Nsereko, 2000).

Composition of substrate used: Substrates high in soluble fibers readily ferment, producing short-chain fatty acids (SCFAs) like butyrate, propionate, and acetate while substrates high with starch easily ferment by some microbes, rapidly increasing gas production and acidity.

Advantages and limitations of in vitro fermentation techniques

Advantages

Control and reproducibility: In vitro fermentation provides careful control of experimental parameters, such as pH, temperature, and substrate composition, guaranteeing that the results are reproducible (Menke et al., 1988).

Cost and time efficiency: Blümmel et al. (1997), reported that in vitro fermentation is a less expensive and faster alternative than in vivo studies. This enables rapid examination of several feed samples or treatments simultaneously.

Study of specific parameters: Researchers can isolate and examine certain factors of interest by using in vitro fermentation, such as substrate degradation, gas generation, nutrient use, and microbial activity (Getachew et al., 2005).

Limitations:

Lack of host-animal interaction: According to Chaucheyras-Durand, et al. (2014), In vitro fermentation does not involve complex interactions with the host animal like in vivo research does. It does not consider salivary enzymes, host-microbial interactions, or intestinal digestion. Limited representation of in vivo conditions: Hristov et al. (2013), Stated that despite efforts to simulate ruminal conditions, in vitro fermentation may not entirely duplicate the rumen's dynamic and diverse environment, potentially resulting in variations in microbial activity and nutrient breakdown.

Potential interference from laboratory procedures: Williams et al. (2019), explained that in vitro fermentation laboratory methods, such as sample processing and incubation, may add artifacts and change the composition of the substrate, potentially altering the results.

Rate of passage: Microbes experience a variety of substrates and environments as digesta makes its way through the gut in vivo. Conversely, in vitro conditions, where a constant supply of the same substrate is present, can favor the growth of specific microbial populations that may not typically thrive in the dynamic and ever-changing gut environment.

In-situ fermentation

In situ, fermentation is a method for studying ruminal fermentation that involves inserting a sample directly into the rumen of a live animal and enabling microbial fermentation to proceed within the animal's digestive system. In comparison to in vitro approaches, this method provides a more accurate portrayal of ruminal fermentation. The in-situ approach is a common method for characterizing rumen degradability of proteins because of the strong connection and concordance between in vivo and in situ data (Poncet et al., 1995). As a result, this method has been applied to research the digestive processes of the rumen and to forecast how much nutrition would be available for the host animal and rumen microbes (Ørskov et al., 1980). The rates of fermentable organic matter and protein degradation can be estimated and the correlation between energy and nitrogen availability for microbial synthesis in the rumen can be analyzed (Noziere and Michalet-Doreau, 2000).

In situ techniques have also been used to investigate the impact of animal (species, physiological state, level of intake) or dietary (additives, diet composition, fat supplementation) factors on rumen conditions and microbial activity (mainly the fibrolytic activity of ruminal microorganisms) (Ørskov, 2000; Noziere and Michalet-Doreau, 2000). The in-situ technique is

an effective method for measuring the associative effects between forage and fermentable carbohydrates, especially when the basal diet and feed in the bag are considered. Furthermore, the relationship between the degradation rate and rumen fill has allowed for the use of rumen degradation parameters estimated using the in-situ technique to predict the voluntary intake of forage (Hovell et al., 1986; Carro et al., 2009). The in-situ technique is suitable for kinetic studies following the time course of the disappearance of an individual feedstuff and has been used widely to evaluate the rate and extent of degradation in the rumen (Ørskov, 2000). Recently, this technique has been used to estimate the extent of starch degradation in the rumen (Cerneau and Michalet-Doreau, 1991). The kinetics of rumen lipid degradation have also been studied in situ (Perrier et al., 1992).

General principles

The principles of in situ fermentation include sample placement, which is accomplished by inserting a sample, usually a feed item, into a porous bag or mesh container. The bag is then placed into a living animal's rumen, allowing microbial fermentation to occur on the sample within the rumen environment. The microbial fermentation process is where the sample is fermented by various microbial populations found in the rumen. Bacteria degrade the material, producing gases and metabolites while consuming and converting nutrients. Finally, for sample retrieval and analysis, the sample is extracted from the rumen after a specific incubation period and examined for several characteristics, including nutrient breakdown, fiber digestion, gas production, and VFA production. In situ fermentation is frequently used to investigate ruminal degradation and the use of feed ingredients, particularly to better understand the dynamics of nutrient breakdown and microbial activity within the rumen (Weakley et al., (1983); Menke et al., (1979).

In-situ fermentation techniques

Nylon bag technique: One of the most widely utilized in situ procedures is the nylon bag approach. It entails inserting a tiny bag containing a representative sample of the feed ingredient into the rumen of a living animal for an extended period. Following removal, the bag was rinsed and dried, allowing the extent and rate of deterioration of the feed ingredients to be determined (Ørskov 2000).

Mobile bag technique: the nylon bag technique was modified to create a mobile bag. It entails attaching a weighted bag to a string or other device that allows controlled movement within the rumen into the small intestine and collected either at the ileo-caeca junction or in the feces. This approach provides a more accurate estimate of feed breakdown by stimulating the flow of feed particles in the rumen (McDonald et al., 1991).

Factors affecting in situ fermentation.

Loss of matter from the bag: The particles in the bag must be reduced in size to pass through the pores and exit. However, complete fermentation is not necessary, and particles can escape once they are smaller than the pore size. It has been suggested that particles that leave the bag consist of materials that can be degraded within short incubation times (Setälä, 1983). Nevertheless, the particulate matter lost from the bag also includes particles that have not been previously degraded, which leads to an overestimation of both the immediately soluble fraction and the extent of degradation, and likely an underestimation of the rate of degradation (Huntington and Givens, 1995). The loss of particles from the bag can be attributed mainly to the interaction between the bag pore size and the size of the sample particles. To minimize the impact of such losses on the estimate of degradation, it is desirable to have a standard and appropriate particle size to pore size ratio. The larger the pore size, the greater the loss of

particles and undegraded material. The aperture size of the bag significantly affects the initial rate of degradation; however, its impact on the extent of degradation is relatively minor (Huntington and Givens, 1995). Before incubation, the feed samples are typically ground to facilitate handling, provide a more homogeneous and representative material for incubation, and reduce particle size to simulate the comminution that occurs during mastication and rumination. In the bag, the reduction in particle size is due to microbial fermentation and rubbing forces driven by the movement of the rumen wall and its contents.

Recovery of matter of non-feed origin in the incubation residue: After withdrawal from the rumen, the bags are washed to stop microbial activity and to remove any rumen digesta and microbial matter from the incubation residue or bag. Post-incubation washing procedures have varied, and the rinsing methodology has been reported to have a significant influence on degradability estimates (Cherney et al., 1990; Huntington and Givens, 1995). An influx of small fine particles into the bags allowed for faster inoculation of the samples. The ruminal matter that has infiltrated the bag is usually removed after mild rinsing (Uden and Van Soest, 1984), but complete removal of the microbial mass attached to the feed particles is far more difficult to achieve. Microbial colonization of the feed is required for degradation, but its presence in the residue can lead to substantial underestimation of the extent of degradation. The level of microbial contamination varies depending on the substrate used. According to Michalet-Doreau and Ould-Bah (1992), contamination can significantly affect the estimations of the protein degradability of low-protein forages; however, its effect on other feeds appears to be almost nonexistent. Several techniques have been proposed to minimize residual contamination through microbial detachment (Huntington and Givens, 1995; Michalet-Doreau and Ould-Bah, 1992). Additionally, markers can be used to determine the percentage of microbial matter in incubation

residue. The correction for microbial contamination may result in varying estimates of protein degradability depending on the marker utilized (purines, ^{15}N) and the microbiological pellet recovered (solid- or liquid-associated bacteria).

Confining conditions inside the bag: Regardless of the physical isolation of bag contents from ruminal digesta, conditions inside the bag should be as close to those in the surrounding rumen contents as feasible, therefore selecting a suitable material appears critical. Artificial textile fibers such as polyester, dacron, and nylon are used to make bags. The material should be resistant to microbial degradation. The weave structure of the cloth determines the uniformity of the pore size, with the monofilament weave exhibiting a more precisely defined pore size and less distortion during incubation (Marinucci et al., 1992). Owing to changes in the structure during incubation, repeated use of bags should be prevented. If bags are overfilled with samples, mixing and soaking bag contents with rumen fluid may not be complete (Nocek, 1988; Vanzant et al., 1998). The recommended sample size is expressed in terms of the optimal sample weight-to-bag surface area ratio, which is suggested to be within the range of 15-20 mg/cm^2 (Huntington and Givens, 1995). However, the main bag characteristic considered was the pore size. If the pore size is too small, the exchange of fluids and microorganisms can be restricted. Small pores may become clogged, especially when viscous substrates are incubated. This can inhibit the removal of fermentation end-products from bags with small pores, leading to the accumulation of gas and acidification of the medium inside the bags (Nozière and Michalet-Doreau, 2000). The exchange of fluids between the bag and rumen contents is also influenced by the open surface area of the bag material, specifically the proportion of the total surface area accounted for by the pores (Weakley et al., 1983; Vanzant et al., 1998). Bags with small pore sizes may have different microbial populations than the rumen contents. A pore size of at least 30-40 mm is necessary for

the entry of rumen bacteria, anaerobic fungi, and protozoa into the bag (Lindberg, 1985). Therefore, intermediate pore sizes (35-55 mm) have been recommended to allow for microbial activity in the bags without losing too many fine particles from the feed while still promoting diverse microbial colonization. However, the type and number of microorganisms inside the bag may differ from those in the surrounding rumen digesta.

Advantages and limitations of in situ fermentation
Advantages:

Mimics in vivo conditions: The incubation of feed samples in a rumen-cannulated animal allows for the reproduction of the rumen environment and the interaction of feed particles with rumen bacteria (Huhtanen et al., 2017).

Dynamic and continuous measurement: Ørskov & McDonald (1979), narrated that continuous sampling over time is possible with in situ fermentation, which provides information on the rates of breakdown and nutrient release from feed particles.

Evaluate feedstuff degradability: In situ fermentation allows for the assessment of feedstuff degradation rates, effective degradability, and ruminal digestive characteristics, which aids in the creation of balanced diets for ruminant animals (Diao et al., 2020).

Limitations

Limited to rumen environment: In situ fermentation is only concerned with the rumen environment, with no regard for post-ruminal digestion or the impact of variables, such as saliva and intestinal processes **(**Bach et al., 2005).

Animal variability: Vanzant et al., (1998), stated that individual differences in rumen microbial communities and animal variables may have influenced the results of in situ fermentation studies involving many animals.

Limitations in sampling techniques: Individual variations in rumen microbial populations and animal variables may influence the results of in situ fermentation investigations involving many animals (Hoover 1986).

Summary

Canola meal and DDGS have been extensively used in beef cattle diets. However, studies indicated that the energy value of CM and DDGS is not as high as cereal grains such as barley in finishing diets (Pylot et al., 2000b; Nair et al., 2015, 2016). Strategic blending of food industry by-products such as PMS and PSF to CM and DDGS is an innovative method of fortification of CM and DDGS for making an already protein rich byproduct, energy dense.

We hypothesized that relative to regular CM or DDGS, the strategically blended CM or DDGS will have equal or superior nutrient composition, and will result in improved rumen fermentation, nutrient utilization, and performance of growing and finishing beef steers.

The objectives of the research include:

1. Evaluation of nutrient composition, in vitro and in situ rumen degradability of strategically blended CM relative to regular CM.
2. Evaluate the growth performance and carcass characteristics of growing and finishing beef steers fed diets containing strategically blended proteins supplements.
3. Evaluate rumen fermentation, apparent total tract nutrient digestibility, and nitrogen balance of cannulated beef heifers fed finishing diets.

CHAPTER 3

EVALUATION OF STRATEGIC BLENDING OF FOOD INDUSTRY BY-PRODUCTS WITH CANOLA MEAL ON IN VITRO AND IN SITU NUTRIENT DEGRADATION

Abstract

Four runs of in vitro and two runs of in situ study were conducted to evaluate the fermentation and nutrient degradability of novel blended CM. Both pea molasses (PMS) and pea starch and fiber (PSF) were added as blends to CM at 5% (CM5), 10% (CM10) inclusion, PMS at 1.5% in CM (CM+PMS), and PSF at 1.5% in CM (CM+PSF) (DM basis), with regular CM serving as control (CON). Three ruminally cannulated beef heifers were adapted on 65% grass hay, 20% corn grain, 10% soybean meal, and 5% supplement diet (% DM basis) for two weeks prior to rumen fluid collection. CM treatments were ground, and triplicate (3g) samples per treatment were weighed into Ankom R510 (5 × 10cm) concentrate bags. Ankom RF gas production system was used to measure the total gas produced and to measure fermentation parameters over 24 h. Samples were collected at 0,2,4,8,16,24 h to measure VFA production, and gas samples were collected every 10 mins into tedlar bags. For in situ, 3g CM treatments were weighed into Ankom R510 concentrate bags and placed into laundry bags which were put into the ventral sac of the rumen at 0,2,4,8,16,24 h using the sequential in-all out approach. The results for the in vitro showed a higher ($P<0.05$) DM and CP digestibility for CM+PMS and CM+PSF than CON. VFA production showed a treatment effect for all except valerate, with CM+PSF and CM+PMS having greater ($P<0.05$) acetate and propionate production than CON. The total gas production showed a treatment effect of the CM+PMS and CM+PSF having greater ($P=0.05$) gas production and methane production per gram of DM than CON. The in situ results showed a greater ($P<0.05$) DM and CP degradability for the CM+PMS than CM+PSF. The

results indicate that the inclusion of these by-products as a source of energy could improve rumen fermentation and nutrient utilization. A reduction in methane production per gram of DM for CM+PSF likely indicates the potential impact of these food industry by-products in mitigating CH_4 emissions from beef cattle production.

Introduction

Canola meal has become an increasingly available high-protein feed source for livestock in North America, thanks to the recent expansion of the canola crushing industry in western Canada (Nair et al., 2015) and the United States. The inclusion of CM in the growing beef cattle diets has been reported to improve the nutrient utilization and growth performance of beef cattle (Nair et al., 2015, 2016). However, these studies indicated that the energy value of CM is not as high as cereal grains such as barley in finishing diets. The energy value (TDN or NEg) of CM is relatively lower (NASEM, 2016) than other conventional protein supplements such as dried distillers' grains with solubles (DDGS) or soybean meal (SBM). The strategic blending of food industry by-products with CM is an innovative method of fortification CM for making an already protein-rich by-product energy-dense. Availability of by-products of plant protein extraction is expected to increase exponentially, thanks to increasing interest among consumers for plant-based protein alternatives to replace conventional meat. y-products of pea protein extraction such as pea molasses (PMS) contain oligosaccharides whereas pea starch and fiber (PSF) contain predominantly starch. The non-soluble, rumen-degradable fraction of pea starch has a slow rate of degradation in the rumen, like corn but much slower than wheat, oats, or barley, especially in high-concentrate diets (Walhain et al 1992). A slower rate of starch degradation can help regulate rumen pH, which is especially crucial for animals that consume large amounts of grain. Incorporation of these by-products in CM is expected to increase the energy density of CM thereby improving the protein-energy synchrony in the rumen. Nutrient synchrony, where the energy and protein availability in the rumen are synchronized to maximize ruminal microbial fermentation has been proposed to improve the animal performance (Hall and Huntington, 2008; Zhang et al., 2020). Zhang et al., (2020) indicated that enhancing the degree of synchronization

of energy and nitrogen leads to active ammonia assimilation and greater efficiency of microbial protein synthesis. It is worth evaluating the potential impact of sugars and starches provided by PMS and PSF in this strategically blended fortified CM on rumen fermentation and nutrient degradation.

To the author's knowledge, there have not been any studies conducted to evaluate the impact of strategic blending of PMS and PSF with CM on rumen fermentation and nutrient utilization in vivo or in vitro. Thus, the present study is aimed to explore the impact of these by-product blends on nutrient degradation in vitro and in situ. We hypothesized that relative to regular CM, the strategically blended CM will have equal or superior nutrient composition and will result in improved in vitro rumen fermentation and nutrient degradation.

Materials and Methods

Pea molasses and pea starch and fiber

All CM blends used in the study were proprietary products of Louis Dreyfus Company (Livermore, CA) and were prepared as batches and sent to SIU for analysis. The treatments included regular CM (CM), CM blends containing PMS and PSF at 5% (CM5) and 10% (CM10) levels in CM, 1.5% PMS in CM (CM+PMS) and 1.5% PSF in CM (CM+PSF) (% DM basis).

Animal management

Three ruminally cannulated beef heifers located at the SIU beef center were used for the study. Before sampling, all heifers were housed in a group pen and fed an adaptation diet consisting of 65% grass hay, 20% corn grain, 10% soybean meal, and 5% supplement (% DM basis) for two weeks and had unrestricted access to water. All heifers involved in the study were taken care of as per the guidelines of the Institutional Animal Care and Use Committee (protocol #22-032).

Experiment design

A total of four in vitro batch culture experiments were conducted to determine the in vitro CP degradability, and total gas production with the fermentation parameters of the CM treatments with the use of the Ankom RF gas production measurement system (Ankom Technology inc., Macedon, NY). The CM treatments were arranged as a completely randomized design (CRD) with the five CM treatments (CM, CM5, CM10, CM+PSF, CM+PMS) and three replicates per treatment.

In vitro incubation

Preparation of sample and inoculum

Samples of CM were ground to pass through a 1-mm screen using Wiley Mill (Model 4, Arthur H. Thomas Co, Philadelphia, PA). Ground samples were weighed (3.0g each) into Ankom R510 5 × 10cm concentrate bags, zip tied with a weight was attached to keep the bags immersed in the fermentation medium. Bags were placed into 250 ml Ankom glass bottles. Composite rumen inoculum (~3.0 L) was collected immediately before feeding from the three ruminally cannulated beef heifers during each run. Samples were strained through two layers of cheesecloth and transferred immediately to the laboratory in an insulated, airtight container. In the lab, ruminal fluid samples were strained again through two layers of cheesecloth and placed into a water bath at 39°C with continuous flushing of CO_2. The pH of the ruminal fluid was measured using a portable pH meter and recorded.

Incubation

The in vitro batch culture study was conducted as described by Embaby et al. (2019). McDougall buffer was pre-warmed in a water bath at 39°C and added to the Ankom vials along with ruminal fluid in 3:2 ratio (120 mL buffer medium:80 mL ruminal fluid). Vials were flushed

with oxygen-free CO_2 for 20 seconds and closed with the Ankom gas production module and incubated at 39°C for 24 h. Every two hours, the jars were shaken by hand for approximately 30 seconds. Tedlar gas bags (CEL Scientific Corp., Santa Fe Springs, CA, USA) were connected to the modules for gas collection. The Gas modules were programmed to release the pressure inside the jars every 10 minutes at 0.9 PSI. Cumulative gas pressure was recorded for 24 h and the total gas production measured using the following equation (Ankom RF gas production system, Ankom Technology, 20552 O'Neil Rd, Macedon, NY, USA)

$n = p (V/ RT)$

Where: n = gas produced in moles (mol), P = pressure in kilopascal (kPa), V = head-space volume in the glass bottle in Liters (L), T= temperature in Kelvin (K), and R= gas constant (8.314472 L*kPa*K-1*mol-1).

After the incubation, the nylon bags were removed from the incubation jars and rinsed in cold water for a total of five rinses. The bags were dried in an oven at 55ºC for 48 h and weighed. The residues were analyzed for DM (AOAC, 2000) and CP to estimate DM and CP digestibility.

After 24 h of incubation, collected gas samples were drawn from each bag in duplicate using a 1ml syringe (27G 1 1/4; Fisher Scientific, Chicago, IL, USA) and analyzed for gas composition using a gas chromatograph (GC) (SRI 8610C, Torrance, CA, USA), equipped with TCD detector (6' x1/8' S.S.ShinCarbon) and ST 80/800 column (2 m x2 mm internal diameter). The GC was programmed at 38°C for 5 min, then increased at 5°C/min to 270°C and held for 5 min. Argon was used as a carrier gas, and peaks (CO_2 and CH_4) were identified by comparing the retention times with those of the corresponding standard (Scotty Analyzed Gases 14, Sigma-

Aldrich, St Louis, MO, USA). Gas results were recorded by GC and calculated using the following equation.

$$RF = (CC_i/Area_i) \times (Area_{ref} /CC_{ref});$$

Where, RF is the response factor, CC_i is the proportion of gas in the sample of the gas being tested, $Area_i$ is the area of gas i peak, CC_{ref} is the proportion of the reference gas (helium) in the internal standard, and $Area_{ref}$ is the area of the peak of the reference gas.

After 24 hr of incubation, the jars were removed from the water bath and immersed in ice bath to stop fermentation. Upon opening, nylon bags were removed from the jars, pH of fermentation liquid was measured immediately in duplicate. Samples (1.5 mL) were preserved with 300 μL of 25% (wt/v) metaphosphoric acid for volatile fatty acid analysis and 300 μL of 1% (v/v) aqueous 18.4 M sulfuric acid for ammonia nitrogen (NH_3-N) and stored at -20°C until analysis.

For VFA analysis, samples were centrifuged at 14000 rpm for 15 mins. Approximately 1.5 ml of the centrifuged sample was transferred into GC vials and then analyzed as described by Jenkins (1987), using 2-ethylbutyric acid as an internal standard. A Shimadzu GC-2010 gas chromatograph (Shimadzu Scientific Instruments Inc., Columbia, MD, USA), with a 30-m SP-2560 fused silica capillary column (Restek Stabil WAXDA column, Bellefonte, PA, USA) was used. The GC temperature was programmed to 65°C for 3 min, increased at 12°C/min to a final temperature of 225°Cand held for 9 min. The column temperature was then maintained at 65°C and the flame ionization detector temperature at 225°C. For the analysis of ammonia, samples were centrifuged at 14000 rpm for 15 mins and were analyzed as per Cotta and Russell (1982).

Determination of DM and CP degradation

After incubation, the nylon bags were removed from the incubation jars and rinsed in cold water for a total of five rinses. The bags were rinsed in an oven at 55°C for 48 h and the weights of residues recorded. The DM weights were used to calculate DM degradability (DMD). Residues remaining in the filter bags were further analyzed for CP by Leco method (method 990.03; AOAC 2000) for the determination of CP degradability.

In situ incubation

M and CP degradability of CM blends were determined by in situ incubation of samples using three rumen-cannulated Angus heifers. All heifers were fed the same diet for ad libitum intake with 5% refusal as for the in vitro study. Heifers were adapted to the feed 2 weeks before in situ rumen incubation. All cattle were cared for as per the Institutional Animal Care and Use Committee protocol. The in-situ incubation was carried out as described by Long et al. (2015) and Joy et al. (2021). Briefly, 3g each of the ground CM samples were weighed into triplicate 5 cm × 10 cm nylon bags (Ankom Technology, Macedon, NY) with a pore size of 50 ± 10 µm. Bags were zip-tied and placed into a laundry bag (Pinfox Reusable Nylon Brew 912.6” x 8.66”) with a weight attached and placed in the ventral sac of the rumen of each heifer for 0, 2, 4, 8, 16, and 24 h following sequential in - all-out approach. After removal from the rumen, the bags were rinsed in cold water for five rinses until the rinse water was clear. The bags were then dried in a forced air oven at 55°C for 48 h. The residue remaining in replicate bags at each time point per treatment was analyzed for CP to determine the CP degradability.

Chemical Analysis

All samples and residues were dried at 55°C for 48 h in a forced air oven, ground to pass through a 1.0 mm screen using a Wiley Mill (Model 4, Arthur H. Thomas Co., Philadelphia, PA) and analyzed for DM (Method 930.15; AOAC 2000) and CP (Method 984.13).

Statistics Analysis:

Dry matter and CP degradability were calculated as the difference between the amount of nutrients in the substrate incubated and that in the filter bag residues after 24 h of incubation and after the analysis for residues. Data were analyzed using Mixed Model Procedure of SAS (SAS Institute, Inc. Cary, N.C.) for Completely Randomized Design. Significance was declared at $P < 0.05$.

Results

In Vitro Digestibility

Post incubation analysis showed a significant ($P < 0.01$) dietary effect for DM digestibility (Table 3.1), with CM+PMS and CM+PSF treatments having a greater DMD ($P < 0.01$) than regular CM, with CM5 and CM10 intermediate. It should be noted that PMS contains oligosaccharides which are readily available as an energy source for the rumen microbes. PSF contains starches which are slowly degraded in the rumen.

Table 3.1. Digestibility parameters of regular canola meal (CM), 5% CM blend (CM5), 10% CM blend (CM10) CM+ PMS, and CM+PSF after 24 h of in vitro incubation

	Treatments[1]						
Item	CM	CM5	CM10	CM+PMS	CM+PSF	SEM[2]	*P*-value
DM digestibility	72.0b	72.7ab	74.4ab	75.6a	74.1a	0.80	< 0.01
CP digestibility	70.5ab	69.5b	72.1ab	74.5a	73.3ab	0.90	< 0.01

[1]Treatments included regular canola meal (CM), CM blended with 5% (DM basis) inclusion of pea starch and pea molasses (CM5), CM blended with 10% (DM basis) inclusion of pea starch and pea molasses (CM10), CM blended with 1.5% PMS (CM+PMS) and CM blended with 1.5% PSF (CM+PSF)

[2]SEM, pooled standard error of mean ($n = 3$).

Results indicate that both PMS and PSF likely improved the energy available to the rumen microbes, improving the protein-energy synchrony resulting in optimum microbial activity. Similar to DMD, the CPD was greater ($P < 0.01$) for CM+PSF than CM5 with the other treatments intermediate. It is logical to assume that enhancing the degree of synchronization of energy and nitrogen leads to improved ammonia assimilation and greater efficiency of microbial protein synthesis in the rumen.

In vitro fermentation parameters

Proportions of fermentation end products after 24 h of in vitro incubation are presented in Table 3.2. There was a treatment effect for all but proportions of valerate, while the time of sampling was significant for all VFA except for proportions of propionate and butyrate. Treatment × Time was not significant for all but proportions of valerate. Proportions of acetate

were greater (P < 0.0001) for CM+PMS than CM10 while that of propionate was greater for CM+PMS than CM and CM5 after 24 h of in vitro incubation. In contrast, the proportions of butyrate were lower (P < 0.0001) for CM+PMS and CM+PSF than the other treatments. Greater proportions of acetate and propionate for CM+PMS likely indicate they improved microbial activity due to the presence of readily available sources of energy from PMS.

Table 3.2. Fermentation products of regular canola meal (CM), 5% CM blend (CM5), 10% CM blend (CM10), CM+pea molasses (CM+PMS), and CM+pea starch and fiber (CM+PSF) during the 24 h of in vitro incubation

	Treatments[1]						*P*- value		
Item	CM	CM5	CM10	CM+PMS	CM+PSF	SEM[2]	Treat	Time	Treat × Time
Fermentation products, mmol (% of total)									
Acetate	40.8bc	40.0c	39.3d	42.7a	42.2ab	0.64	< 0.0001	<0.0001	0.63
Propionate	28.4b	28.6b	29.4a	29.5a	29.1ab	0.66	<0.0001	0.25	0.38
Butyrate	20.0b	20.6a	20.6a	17.4c	18.2c	0.12	<0.0001	0.31	0.94
Isobutyrate	2.02a	1.93a	1.99a	1.63b	1.85ab	0.026	0.0001	<0.0001	0.72
Valerate	4.3	4.19	4.04	4.5	3.81	0.267	0.76	<0.0001	0.02
Isovalerate	4.76a	4.70a	4.61a	4.20b	4.88a	0.04	<0.01	<0.0001	0.55

[1]Treatments included regular canola meal (CM), canola meal blended with 5% (DM basis) inclusion of pea starch and pea molasses (CM5), canola meal blended with 10% (DM basis) inclusion of pea starch and pea molasses (CM10), CM+pea molasses (CM+PMS), and CM+pea starch and fiber (CM+PSF).

[2]SEM, pooled standard error of mean (n = 3).

Enhanced rumen microbial fermentation activity was reported in literature when the dietary energy was increased (Fernando, 2010; Wang, 2020).

In vitro gas, ammonia, and methane production

Total gas production (mmol) varied between treatments after 24 h or in vitro incubation (Table 3.3), with CM+PMS having the greatest gas production (P = 0.05) than CM with the other treatments intermediate. Similarly, the in vitro ammonia concentration (mg dL^{-1}) was greatest (P = 0.03) for CM+PMS than CM with the other treatments intermediate. The PMS in CM+PMS is mostly oligosaccharides, which are readily available to the rumen microbes as a source of energy.

Table 3.3. Total gas, ammonia, and methane production parameters of regular canola meal (CM), 5% CM blend (CM5), 10% CM blend (CM10), CM+PMS, and CM+PSF after 24 h of in vitro

	Treatments[1]						
Item	CM	CM5	CM10	CM+PMS	CM+PSF	SEM[2]	*P*- value
Total gas, mmol	123.7b	133.4ab	140.4ab	165.9a	147.0ab	7.41	0.05
Ammonia, mg dL^{-1}	4.37b	6.00ab	5.54ab	8.34a	6.90ab	0.789	0.03
Methane, mmol	27.6	28.7	30.9	30.2	29.1	1.05	0.28
Methane, % of total gas	19.2	18.9	18.4	18.2	18.8	0.29	0.18
Methane, g/dm	7.32a	7.12ab	6.89ab	7.13ab	6.80b	0.097	0.03

[1]Treatments included regular canola meal (CM), CM blended with 5% (DM basis) inclusion of PSF and PMS (CM5), CM blended with 10% (DM basis) inclusion of PSF and PMS (CM10), CM+pea molasses (CM+PMS) and CM+pea starch and fiber (CM+PSF).

[2]SEM, pooled standard error of mean (n = 3).

Greater energy content of the diets is reported to improve microbial activity and fermentation in the rumen (Ahmad, 2020; Wang, 2019). Though non-significant, the total gas production proportionately increased from CM to CM10, indicating that the greater inclusion of food industry blends will enhance the rumen microbial activity. Methane production in mmol and as a % of total gas did not vary (P > 0.05) between treatments. In contrast, CH4 production in mmol/g DM showed a significant variation between treatments. CM treatment had the greatest (P = 0.03) CH4 production per g of DM than CM+PSF, with the other treatments intermediate. On average, CH4 (g/dm) was lower for all CM blends than CM, indicating the potential benefits of reducing enteric CH4 emissions from livestock when food industry by-products are added to the diets. Further, the proportion of propionate was greater for the CM blends than for CM (Table 3.2). As propionate production is associated with the utilization of H+ in the rumen, greater proportions of propionate are in general associated with a lower proportion of CH4 (Wang, 2023). Results indicate that irrespective of the food industry blend used, the CH4 production per g of DM decreased. Further research utilizing these food industry by-products in the diets of growing and finishing beef cattle will likely explore the opportunities for the mitigation of CH4 emissions from ruminant production systems.

In situ digestibility

An in-situ study was also done using the five CM treatments as reported in Table 3.4. The DMD was greater for CM, CM5, CM10, and CM+PMS than CM+PSF ($P < 0.002$). Similarly, the CPD was greater ($p = 0.05$) for CM+PMS than CM+PSF with the other treatments intermediate. The greater availability of oligosaccharides provided by PMS likely improved the microbial activity and fermentation, resulting in improved DM and CP digestibility. On the other hand, PSF contains mostly starch (>80%), which is slowly degraded in the rumen.

Table 3.4. Digestibility parameters of regular canola meal (CM), 5% CM blend (CM5), 10% CM blend (CM10), CM+pea molasses (CM+PMS), and CM+pea starch and fiber (CM+PSF) after 24 h of in situ incubation

	Treatments[1]						
Item	CM	CM5	CM10	CM+PMS	CM+PSF	SEM[2]	*P*- value
DM digestibility	70.8a	67.3a	68.7a	72.1a	61.7b	1.12	< 0.001
CP digestibility	68.4ab	66.9ab	68.4ab	70.6a	60.1b	2.13	0.05

[1]Treatments included regular canola meal (CM), CM blended with 5% (DM basis) inclusion of PSF and PMS (CM5), CM blended with 10% (DM basis) inclusion of PSF and PMS (CM10), CM+pea molasses (CM+PMS), and CM+pea starch and fiber (CM+PSF).

[2]SEM, pooled standard error of mean ($n = 3$).

Conclusion

Results of the study indicated that blending of food industry by-products with CM can impact in vitro and in situ nutrient degradation. Greater in vitro and in situ DM and CP degradability for CM+PMS likely indicate that strategic blending of PMS with CM could possibly enhance the nutrient degradation. Greater in vitro gas production for CM+PMS indicate that possible protein-energy synchrony enhanced the microbial activity and fermentation in vitro. Further, a lower CH_4 production (g/dm) for CM+PSF indicates that the incorporation of this food industry by-product with CM can likely reduce CH4 emissions from beef cattle diets by increasing the production or propionate, as propionate is a H+ sink. Further animal feeding studies will provide insights into the function of these food industry by-products on rumen fermentation and growth performance of growing and finishing beef cattle in vivo.

CHAPTER 4

EVALUATION OF THE EFFECTS OF STRATEGIC BLENDING OF FOOD INDUSTRY BY-PRODUCTS WITH PROTEIN SUPPLEMENTS ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF GROWING AND FINISHING BEEF CATTLE

Abstract

A 56-d backgrounding and a 145-d finishing trial were carried out to evaluate the growth performance and carcass characteristics of growing and finishing beef steers fed diets containing strategically blended protein supplements. The treatments included regular CM (CM), CM+PMS (PMS at 1.5% of CM DM), DDGS, and DDGS+PSF (PSF at 2% diet DM). For backgrounding, 64 steers (325.0 ±25kg; mean ± SD) were stratified by weight and randomly assigned to one of 16 feedlot pens, with each pen housing four steers and each pen randomly assigned one of the dietary treatments. Targeted end weight for backgrounding was 400 kg live weight, upon which the steers were transitioned to the finishing diets through a five-step, 15-day step-up process in which the forage contents of the diet gradually decreased while the grain content increased to the formulated levels of the finishing diet. For finishing, the target end was 640 kg live weight. Steers were weighed every two weeks. Orts and TMR samples were collected every two weeks and composited monthly for chemical analysis. Steer performance was analyzed using a completely randomized design with pen as the experimental unit using proc mixed procedure of SAS. The carcass characteristics were analyzed using the GLIMMIX procedure of SAS with a binomial error structure and logit data transformation. For backgrounding, there was no difference between treatments for final BW (405.5±42.3; mean±SD; unshrunk basis), ADG (0.86 kg $d^{-1)}$, DMI (7.7±0.03kg d^{-1}) and G: F across treatments. For finishing, there was no difference

between the treatment for the final BW (633.5±63.1 kg; mean±SD; unshrunk basis), ADG averaged (1.5 kg $d^{-1)}$, DMI (10.75±0.05) and G: F (0.14 ± 0.001) across the treatments. There was no difference between the treatments in the carcass characteristics. However, steers fed CM+PMS showed a numerical increase in marbling % and quality grade % compared to those fed CM. These results showed that the substitution of CM and DDGS with either of these by-products had no negative effect on the growth performance and carcass characteristics of steers fed these growing and finishing diets.

Introduction

Feedlot practice has improved over the years to be more efficient in output resulting from the utilization of new technology for farming, improvement in cattle feed ingredients with varieties of feed byproducts being more available and more research done to show their effectiveness in inclusion in diets. As such there has been the utilization of different protein supplements with common sources such as Canola Meal and DDGS has been used extensively in feedlots across the United States (Schingoethe et al., 2009; Zhang et al., 2010). McKinnon and Walker (2008) showed that growing steers fed diets with increasing levels of DDGS had a linear improvement in growth rate and feed efficiency. Similarly, Petit et al. (1994) reported that increasing the inclusion of CM from 7% to 15% in the diets of growing beef cattle resulted in greater final body weight, ADG, and G: F. However, these authors also reported greater days on feed, and relatively lower final BW, DMI, ADG, and G: F when finishing steers were supplemented with increasing levels of CM in the diet. Similar observations were also reported by Nair et al. (2015, 2016) and Pylot et al. (2000b) for finishing beef cattle fed increasing inclusion of CM and DDGS respectively. These authors reported that the energy value of CM and DDGS is not as high as cereal grains such as barley in finishing diets. The strategic blending of food industry by-products such as pea molasses (PMS) and pea starch and fiber (PSF) to CM and DDGS is expected to increase the starch and sugar content of these protein supplements, likely enhancing the degree of synchronization of energy and nitrogen leads to active ammonia assimilation and greater efficiency of microbial activity in the rumen. We hypothesized that relative to regular CM or DDGS, the strategically blended CM or DDGS will have equal or superior nutrient composition, and will result in improved rumen fermentation, nutrient utilization, and performance of growing and finishing beef steers. The objective of the study

includes the evaluation of growth performance and carcass characteristics of growing and finishing beef steers fed diets containing strategically blended protein supplements.

Materials and Methods

Housing and experimental design

Housing

Sixty-four Angus cross-bred steers (~325. ±25kg; mean ± SD) purchased by the Beef Center of Southern Illinois University were contracted for the study. Upon arrival, all steers were provided with ad lib water and hay and placed in the quarantine barn for 28 d before commencement of the study. All steers used for this study were cared for following the Institutional Animal Care and Use Committee (IACUC) guideline, protocol 23-003.

Experimental design

Steers were weighed for two consecutive days at the beginning of the study and the average of each was taken as the start BW for the trial. The experiment was in a completely randomized design with steers stratified by weight and randomly assigned to one of 16 feedlot pens with each pen housing four steers. Pen was considered as the experimental unit. Each pen was randomly assigned one of the 4 dietary treatments. The target end weight for backgrounding was 400kg live weight, upon which the steers were transitioned to the finishing diets through a five step, 15-day step-up process during which the diet composition changed every 3 d in such a way that the forage contents of the diet was gradually decreased the grain content increased to the formulated levels of the finishing diet. The proportion of protein supplements and food industry by-products remained the same throughout the feeding period. The targeted end period of finishing was 640 kg live weight, upon which the steers were sent as a group for slaughter at Tyson Fresh Meats (Joslin, IL).

Treatments and dietary composition

The food industry byproducts used in the study (PMS and PSF) are the proprietary products of Louis Dreyfus Company (Livermore, CA) and were shipped as batches for incorporation in the feed throughout the study period. On average, PMS contained 14.1% and PSF, 42.9% DM, and were frozen and shipped overnight (Table 4.1). Both the byproducts were stored in a -20°C chest freezer throughout the study period. For each feed mixing, the byproducts were removed from the freezer, thawed overnight, and incorporated into the respective diets. In both the backgrounding and finishing diets, PMS was added in CM+PMS at 1.5% of CM (% DM basis) while PSF was added in DDGS+PSF at 2% of the diet (% DM basis). Both CM and DDGS were purchased as single batches, transported, and stored at the Beef Center of SIU for utilization in the diets. Grass hay corn grain was sourced from the Beef Center of SIU.

The study consisted of backgrounding (56 days) and finishing (145 days). Both backgrounding and finishing phases consisted of four dietary treatments, consisting of diet supplemented with canola meal (CM), a diet supplemented with distillers dried grain soluble (DDGS), a diet supplemented with CM and PMS at 1.5% of CM (% DM basis) (CM+PMS), and diet supplemented with DDGS and PSF at 2% inclusion in the diet (%DM basis) (DDGS+PSF). The composition of backgrounding diets is provided in Table 4.2. All backgrounding diets were formulated to be isonitrogenous and isocaloric with a targeted gain of 1.1 kg d^{-1}. The composition of finishing diets is provided in Table 4.3. Similar to the backgrounding diets, the finishing diets were also formulated to be isonitrogenous and isocaloric, with a targeted gain of 1.5 kg d^{-1}. In both feeding phases, all the diets were formulated to meet or exceed NASEM (2016) nutrient requirements for the targeted level of growth. Calcium: phosphorus ratio was

formulated to range from 1.5:1 to 2:1. Monensin sodium was incorporated in the beef supplement and was formulated to provide 30 mg kg-1 diet DM.

Sampling and data collection

Feed was delivered to each pen daily starting at 0800 and the amount recorded. The steers were fed for ad libitum intake with a targeted 5% leftover. The quantity delivered to each bunk was based on the residual feed in the bunks and the amount fed the previous day. The performance parameters (DMI, ADG, and G: F) of steers were measured based on shrunk body weight (live weight × 0.96). All steers were weighed every two weeks in the morning before being fed through the whole trial, while the bunks were cleaned every two weeks and orts from each pen were weighed and sampled to determine the DM content. Bunk samples of TMR were collected every two weeks from each pen and composited on a treatment basis. Feed ingredients and orts were sampled every two weeks. All samples of feed and TMR were composited monthly, and a representative sample was saved for chemical analysis.

Chemical Analysis

The TMR, bunk, and ort samples were dried in a force air oven at 55º C for 48 h. After drying, samples were ground to pass through a 1mm screen using Thomas Wiley Laboratory mill model 4 (Arthur H. Thomas Company, Philadelphia, PA, USA). Ground samples were sent to Rock River Laboratory (Watertown, WI) for the analysis of DM, OM, CP, ADF, NDF, EE, starch, and ash content.

Carcass traits

Steers were processed at a commercial meat processing plant (Tyson Fresh Meats, Joslin, IL) at an average BW of 633.4±63.1 kg at the end of 145 d of finishing period. Carcass

evaluations included hot carcass weight (HCW), dressing percentage, *L. thoracis* area, grade fat, USDA quality and yield grades, marbling and kidney, pelvic and heart fat measurements.

Statistical Analysis

Backgrounding and finishing performance data were analyzed as completely randomized design with pen as experimental unit and the fixed effect as treatments using the mixed model procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). Denominator degrees of freedom were determined using the Kenward-Roger option. USDA yield and quality data were analyzed using GLIMMIX (SAS software version 9.4; SAS Institute Inc., Cary, NC) with a binomial error structure and logit data transformation. Significant differences and trends were declared at $P < 0.05$ and $P < 0.10$ respectively.

Results and Discussion

Chemical and nutrient profile of diets

The chemical and nutrient profile of feed ingredients and diets are provided in Tables 4.1, 4.2 and 4.3. PMS, which consisted mostly of oligosaccharide, had an average DM content of 14.1±2.1% while the CP concentration averaged 24.2±2.0% during the study (Table 4.1). PSF, consisting mostly of pea starch and fiber, averaged 42.9±1.9% DM and 2.39±0.49% CP. The starch content of PSF was considerably greater (81.3±1.2% vs. 2.75±1.1%) than that of PMS, while PMS had a greater ash content (17.0±1.5% vs. 0.78±0.13%) than PSF.

Table 4.1. Nutrient composition of feed ingredients (n = 4) used the diets of growing and finishing beef steers and cannulated beef heifers

Item[1, 2]	Grass hay	Corn grain	CM	DDGS	PMS	PSF	Supplements
DM	92.3 ± 3.51	89.8 ± 1.27	91.7 ± 0.34	90.6 ± 0.33	14.1 ± 2.06	42.9 ± 0.41	94.5 ± 1.88
OM	91.6 ± 0.66	97.9 ± 0.54	90.7 ± 1.07	93.1 ± 0.58	83.0 ± 1.53	99.2 ± 0.13	72.8 ± 1.03
CP	11.6 ± 0.40	9.58 ± 0.60	39.3 ± 0.68	29.8 ± 1.09	24.2 ± 1.99	2.39 ± 0.49	24.8 ± 1.31
EE	3.71 ± 0.37	4.37 ± 0.35	4.75 ± 0.31	6.81 ± 0.76	0.21 ± 0.10	0.32 ± 0.14	2.03 ± 0.68
ADF	29.2 ± 1.38	5.24 ± 1.12	20.4 ± 0.72	13.8 ± 0.76	0.55 ± 0.15	1.95 ± 0.43	13.8 ± 0.41
NDF	47.1 ± 1.08	13.3 ± 1.33	26.9 ± 1.09	33.9 ± 1.23	0.56 ± 0.14	3.62 ± 0.13	21.1 ± 0.15
Starch	3.61 ± 1.48	63.4 ± 3.88	3.08 ± 1.33	7.74 ± 2.31	2.75 ± 1.09	81.3 ± 1.16	9.46 ± 1.69
Ash	8.45 ± 0.66	2.12 ± 0.54	9.26 ± 1.07	6.92 ± 0.58	17.0 ± 1.53	0.78 ± 0.13	27.2 ± 1.03

[1]CM, canola meal; DDGS, distillers dried grains with solubles; PMS, pea molasses; PSF, pea starch and fiber; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; ADF, acid detergent fiber; NDF, neutral detergent fiber

[2]Analyzed at Rock River Laboratory Inc., Waterton, WI

The CP concentrations of CM, DDGS and grass hay are similar to that reported previously (Yang, 2013). Ingredient and nutrient composition of backgrounding and finishing diets are presented in Tables 4.2 and 4.3. The CP concentrations were similar across backgrounding diets and averaged 11.6±0.36% as the diets were formulated to be isonitrogenous. The diets did not vary in any of the measured nutrients among backgrounding or finishing diets. The fiber fractions (ADF and NDF) and starch concentrations in backgrounding and finishing diets are similar to that reported previously (Good, 2018) who reported 28.4% ADF, and 44.7% NDF in

backgrounding diets and 17.7% ADF and 29.8% NDF in finishing diets of CM supplemented with DDGS.

Table 4.2. Ingredient and chemical composition of the experimental diets used for the evaluation of the inclusion of PMS and PSF for growing beef cattle during backgrounding

	Treatments[1]					
Item	CM	DDGS	CM+PMS	DDGS+PSF	SEM[2]	*P*- value
Diet composition (% DM basis)						
Grass hay	61.7	67.6	61.3	66.6	-	-
Corn grain	24.6	14.8	24.7	13.4	-	-
CM	9.8	-	9.8	-	-	-
DDGS	-	13.6	-	13.9	-	-
PMS	-	-	0.1	-	-	-
PSF	-	-	-	2.0	-	-
Supplement	3.9	4.0	4.1	4.1	-	-
Backgrounding diets nutrient composition (n = 4; % DM basis)						
OM	92.2	92.6	92.4	92.9	0.59	0.83
CP	11.9	11.4	11.9	11.2	0.56	0.74
EE	3.09	2.92	2.96	2.97	0.482	1.00
ADF	24.8	27.3	26.0	25.7	3.99	0.97
NDF	42.5	45.0	44.3	43.6	4.78	0.98
Starch	20.6	18.9	20.5	21.5	6.14	0.99
Ash	7.83	7.36	7.56	7.08	0.587	0.83

[1]Treatments included CM, diets containing regular CM, DDGS, diets containing regular DDGS, CM+PMS, diets containing 1.5% (DM basis) inclusion of pea molasses in CM and DDGS+PSF, diets containing pea starch and fiber in the diet at 2% inclusion

[2]SEM, pooled standard error of mean, n = 16 steers per treatment.

[3]Shrunken BW calculated as 96% of live weight (NRC 2000).

[4]Calculated based on performance (Zinn and Shen 1998; Zinn et al. 2002).

Table 4.3. Ingredient and chemical composition of the experimental diets used for the evaluation of the inclusion of PMS and PSF in the diets of beef steers during finishing and cannulated beef heifers during the metabolism study

	Treatments[1]					
Item	CM	DDGS	CM+PMS	DDGS+PSF	SEM[2]	*P*- value
Diet composition (% DM basis)						
Grass hay	8.8	15.0	8.7	13.9	-	-
Corn grain	77.2	67.2	77.3	65.9	-	-
CM	10.0	-	9.9	-	-	-
DDGS	-	14.0	-	14.0	-	-
PMS	-	-	0.1	-	-	-
PSF	-	-	-	2.0	-	-
Supplement	4.0	3.8	4.0	4.2	-	-
Backgrounding diets nutrient composition (n = 4; % DM basis)						
OM	94.6	94.5	94.7	94.3	0.35	0.88
CP	14.1	13.8	14.2	14.0	0.25	0.72
EE	4.18	4.26	4.31	4.18	0.121	0.84
ADF	14.5	15.2	13.2	14.8	1.03	0.55
NDF	26.6	27.0	24.0	27.5	1.74	0.50
Starch	38.1	38.2	40.1	37.3	1.59	0.67
Ash	5.40	5.48	5.28	5.67	0.035	0.88

[1]Treatments included CM, diets containing regular CM, DDGS, diets containing regular DDGS, CM+PMS, diets containing 1.5% (DM basis) inclusion of pea molasses in CM and DDGS+PSF, diets containing pea starch and fiber in the diet at 2% inclusion

[2]SEM, pooled standard error of mean, n = 16 steers per treatment.

[3]Shrunken BW calculated as 96% of live weight (NRC 2000).

[4]Calculated based on performance (Zinn and Shen 1998; Zinn et al. 2002).

Performance of backgrounding steers

Growth performance parameters of beef steers during the 56-day backgrounding period are presented in Table 4.4. The average start trial BW was 354.3±40.2 (mean±SD; unshrunk basis) and the end of backgrounding BW was 405.5±42.3 (mean±SD; unshrunk basis) across treatments. The blending of PMS to CM or PSF to DDGS did not result in any negative impact on the growth performance of growing beef steers as the steers had similar final shrunk BW, ADG, DMI, and G: F (Table 4.4) across treatments. The ADG averaged 0.86 kg d-1 across treatments which was slightly lower than the targeted ADG of 1.1 kg d-1. The CP concentration

was not limiting in any of the diets as the CP concentration averaged 11.6±0.36% across diets which is sufficient to meet the requirement (NRC, 2000) of growing beef cattle (~9.9% for 350 kg growing/finishing beef cattle). However, the DMI of steers during backgrounding averaged 7.7±0.03kg d-1 which is relatively lower than the DMI requirement of ~9.7 kg d-1. It is possible that the greater NDF content of the diets (43.9±1.1% across treatments) likely reduced the DMI of steers impacting the growth performance during backgrounding. Reduced DMI for growing beef steers fed diets containing greater NDF concentrations has been reported previously (Nair et al., 2016). These authors reported an average DMI of 7.68 kg d-1 for steers fed diets containing 41.8% NDF while diets containing 36.6% NDF resulted in a DMI of 8.3 kg d-1. The average DMI (7.7±0.03kg d-1) and the NDF concentration of diets (43.9±1.1%) are similar to that reported by Nair et al. (2016). These authors also reported 0.93% NDF intake (as a % of BW) for steers-fed diets containing 41.8% NDF. The NDF intake as a % of BW in the present study was 0.93±0.20% across treatments during backgrounding (data not shown). It is logical to assume that the DMI was negatively impacted by the greater NDF concentration of diets. A review of the literature indicated that when the NDF intake reaches 1.2% to 1.5% (Mertens 1985; Murphy 2004) expressed as % of BW, the DMI is negatively impacted in dairy cattle. This relationship between NDF intake and DMI is not clearly defined in beef cattle. However, it is logical to assume that the limit for NDF intake as a % of BW impacting DMI in growing beef cattle is likely lower than that of dairy cattle due to differences in rumen size, DMI, and passage rate, etc.

Table 4.4. Effect of inclusion of food industry by-products in backgrounding diets on the performance of growing beef cattle

	Treatments[1]					
Item	CM	DDGS	CM+PMS	DDGS+PSF	SEM[2]	*P*- value
Number of steers	16	16	16	16	-	-
Number of pens	4	4	4	4	-	-
Initial shrunk BW[3], kg	340.5	342.2	338	339.9	17.78	1.00
Final shrunk BW[3], kg	388.7	389.5	387.0	387.2	17.83	0.99
ADG, kg d^{-1}	0.86	0.85	0.88	0.85	0.103	1.00
DMI, kg	7.76	7.72	7.73	7.69	0.054	0.79
DMI as % BW	2.14	2.12	2.15	2.13	0.102	0.99
G:F	0.111	0.109	0.113	0.110	0.0134	1.00
NE_m[4], Mcal kg^{-1} DM	1.56	1.56	1.58	1.56	0.096	0.99
NE_g[4], Mcal kg^{-1} DM	0.96	0.95	0.97	0.95	0.084	0.99

[1]Treatments included CM, diets containing regular CM, DDGS, diets containing regular DDGS, CM+PMS, diets containing 1.5% (DM basis) inclusion of CM+PMS and DDGS+PSF, in the diet at 2% inclusion

[2]SEM, pooled standard error of mean, n = 16 steers per treatment.

[3]Shrunken BW calculated as 96% of live weight (NRC 2000).

[4]Calculated based on performance (Zinn and Shen 1998; Zinn et al. 2002).

Performance of finishing steers

Growth performance parameters of beef steers during the 145-day finishing period are presented in Table 4.5. The end of finishing BW was 633.5±63.1 kg (mean±SD; unshrunk basis) across treatments. Similar to backgrounding, blending of PMS to CM or PSF to DDGS did not result in any negative impact on the growth performance of finishing beef steers as the steers had similar final shrunk BW, ADG, DMI, and G: F (Table 4.5) across treatments. The ADG averaged 1.5 kg d^{-1} across treatments. The ADG is like that of finishing beef steers fed similar diets and to the same end trial BW (Nair et al., 2016).

Table 4.5. Effect of inclusion of food industry by-products in diets on the performance of finishing beef cattle

	Treatments[1]					
Item	CM	DDGS	CM+PMS	DDGS+PSF	SEM[2]	*P*- value
Number of steers	16	16	15	16	-	-
Number of pens	4	4	4	4	-	-
Initial shrunk BW[3], kg	388.7	389.5	387.0	387.2	17.83	0.99
Final shrunk BW[3], kg	606.0	608.2	606.8	605.7	22.39	0.99
ADG, kg d^{-1}	1.50	1.51	1.51	1.51	0.060	0.99
DMI, kg	10.5	10.7	11.1	10.7	0.46	0.84
DMI as % BW	2.12	2.16	2.15	2.24	0.091	0.81
G:F	0.143	0.141	0.142	0.136	0.0034	0.48
NE_m[4], Mcal kg^{-1} DM	1.94	1.91	1.92	1.85	0.057	0.70
NE_g[4], Mcal kg^{-1} DM	1.29	1.26	1.27	1.27	0.049	0.70

[1]Treatments included CM, diets containing regular CM, DDGS, diets containing regular DDGS, CM+PMS, diets containing 1.5% (DM basis) inclusion of CM+PMS and DDGS+PSF, in the diet at 2% inclusion

[2]SEM, pooled standard error of mean, n = 16 steers per treatment.

[3]Shrunken BW calculated as 96% of live weight (NRC 2000).

[4]Calculated based on performance (Zinn and Shen 1998; Zinn et al. 2002).

Overall performance

The overall performance of beef steers when measured from the beginning of backgrounding to end of finishing did not vary between treatments. As for backgrounding and finishing, blending of PMS to CM or PSF to DDGS did not result in any negative impact on the growth performance of finishing beef steers as the steers had similar final shrunk BW, ADG, DMI, and G: F (Table 4.6).

Table 4.6. Effect of inclusion of food industry by-products in the diets on overall performance of beef cattle

	Treatments[1]					
Item	CM	DDGS	CM+PMS	DDGS+PSF	SEM[2]	*P*- value
Number of steers	16	16	15	16	-	-
Number of pens	4	4	4	4	-	-
Initial shrunk BW[3], kg	340.5	342.2	338.0	339.9	17.78	0.99
Final shrunk BW[3], kg	606.0	608.2	606.8	605.7	22.39	0.99
ADG, kg d^{-1}	1.32	1.32	1.34	1.32	0.048	0.99
DMI, kg	9.8	9.93	9.88	10.19	0.34	0.86
DMI as % BW	2.08	2.10	2.10	2.16	0.084	0.90
G:F	0.135	0.133	0.135	0.130	0.0024	0.43
NE_m[4], Mcal kg^{-1} DM	1.86	1.84	1.85	1.80	0.049	0.79
NE_g[4], Mcal kg^{-1} DM	1.22	1.20	1.21	1.16	0.042	0.76

[1]Treatments included CM, diets containing regular CM, DDGS, diets containing regular DDGS, CM+PMS, diets containing 1.5% (DM basis) and DDGS+PSF, diet at 2% inclusion

[2]SEM, pooled standard error of mean, n = 16 steers per treatment.

[3]Shrunken BW calculated as 96% of live weight (NRC 2000).

[4]Calculated based on performance (Zinn and Shen 1998; Zinn et al. 2002).

Carcass characteristics

Carcass characteristics showed numerical changes across treatments, though the changes were not significant. L. thoracis area for steers fed CM+PMS and DDGS+PSF was 1.2% and 1.7% greater respectively than for steers fed the corresponding CM and DDGS diets. Similarly, YG2 and YG3 were similar or greater while YG2 was lower for steers fed CM+PMS and DDGS+PSF than for steers fed the corresponding CM and DDGS diets. Greater yield grades are desirable as yield grade is a measure of cutability or yields of boneless, closely trimmed retail cuts from the primal cuts. Prime quality grade was 13 units greater for steers fed CM+PMS than those fed CM diets. A corresponding increase in marbling was also noticed for steers fed CM+PMS than those fed CM diets. It should be noted that the level of inclusion of PSF was 1.5% of CM DM (0.1% of diet DM) while PSF was incorporated in the diet at 2% of diet DM. Numerical improvement in yield and quality grade for steers fed CM+PMS and DDGS+PSF

likely indicates that these treatments could impact carcass characteristics at a greater level of inclusion in the diets.

Table 4.7. Effect of inclusion of food industry by-products in finishing diets on carcass characteristics of feedlot cattle

	Treatments[1]					
Item	CM	DDGS	CM+PMS	DDGS+PSF	SEM[2]	*P*- value
Hot carcass weight (kg)	374.1	372.1	372.9	372.1	15.03	1.00
Dressing percentage (%)	58.7	58.5	59.4	59.1	0.54	0.60
L. thoracis area (cm^2)	80.1	83.0	81.1	84.4	1.67	0.31
Grade fat (cm)	1.59	1.63	1.57	1.48	0.116	0.83
Yield grade (%)						
YG 1	0.0	0.0	0.0	0.0	-	-
YG 2	18.8	25.0	13.3	25.0	4.91	0.86
YG 3	50.0	50.0	60.0	68.8	6.27	0.65
YG 4	31.2	18.8	26.7	15.9	5.48	0.47
YG 5	0.0	6.3	0.0	0.0	-	0.43
Quality grade (%)						
Prime	6.3	6.3	20.0	6.3	3.87	0.53
Choice	87.5	87.5	66.7	81.2	4.87	0.57
Select	6.3	6.3	13.3	12.5	3.54	0.87
Marbling (%)						
Slightly abundant	6.3	6.3	20.0	6.3	3.87	0.53
Moderate	25.0	25.0	26.7	37.5	5.51	0.80
Modest	37.5	12.5	13.3	18.8	4.93	0.29
Small	12.5	50.0	26.7	25.0	5.44	0.34
Slight	18.8	6.3	13.3	12.5	4.17	0.79
Kidney, pelvic and heart fat (KPH)						
1.50%	31.2	37.5	33.3	37.5	5.98	0.97
2%	62.5	56.2	60.0	62.5	6.19	0.98
2.50%	6.3	6.3	6.7	0.0	1.530	0.79

[1]Treatments included CM, diets containing regular CM, DDGS, diets containing regular DDGS, CM+PMS, diets containing 1.5% (DM basis) inclusion and DDGS+PSF, diet at 2% inclusion

[2]SEM, pooled standard error of mean, n = 16 steers per treatment.

Yield grades range from 1-5, with 5 indicating the highest carcass yield (more available cuts)

Quality grades include Prime, Select and Choice, with Prime> Choice > Select

Marbling range from slightly abundant>Moderately abundant>Modest>Small>Slight

KPH - lower is preferable with 1.5% > 2.0% > 2.5%

Conclusion

The study evaluated the impact of the strategic blending of food industry byproducts with protein supplements on the growth performance and carcass characteristics of beef cattle. Combining protein supplements with either PMS, having a greater proportion of oligosaccharides, or PSF having >80% starch concentration would be an innovative method of fortifying these protein supplements energy-dense for improved nutrient synchrony in the rumen. Results indicated that there was no negative impact on growth performance or carcass characteristics of steers fed these blends relative to steers fed the corresponding regular protein supplements. Numerical improvement in carcass characteristics indicates that these treatments could impact growth performance and carcass characteristics at a greater level of inclusion in the diets.

CHAPTER 5

EVALUATION OF THE EFFECTS OF STRATEGIC BLENDING OF FOOD INDUSTRY BY-PRODUCTS WITH PROTEIN SUPPLEMENTS ON RUMEN FERMENTATION AND TOTAL TRACT NUTRIENT DIGESTIBILITY OF FEEDLOT HEIFERS FED FINISHING DIETS.

Abstract

The objective of this study was to evaluate the impact of the inclusion of food industry by-products in the diet of finishing beef cattle on rumen fermentation, total tract nutrient digestibility, and nitrogen balance. Four ruminally cannulated beef heifers (501 ± 9 kg, mean ± SD) were housed in individual pens of a floor space of $9m^2$ area. Each pen had a self-fill water bowl, rubber floor mat, and an individual feed bunk. The diet used was the same as the finishing diet of the feedlot study. The study lasted for 100 days with four periods and 25 days each. The first 7 days of each period were used for dietary adaptation, and voluntary intake was measured from days 8-12. From 13^{th} to 15^{th} day of each period, indwelling pH probes were placed inside the rumen for pH measurements every 1 min over 72 h. Rumen samples were collected on the 16^{th} day every three hours over 24 h for measuring VFA proportions. From the 20^{th} to the 25^{th} day, urine and fecal samples were collected to measure N balance. Feed ingredients were analyzed for DM, OM, CP, ADF, NDF, starch, and ash content. Proc mixed model of SAS for Latin Square design was used to analyze the nitrogen balance, indwelling pH probe measurements, and the total tract digestibility. The results showed no treatment effect (P>0.05) for the rumen pH across the treatments, while steers fed DDGS+PSF had the lowest duration below all pH thresholds with 281.5min d^{-1}, 418.5 min d^{-1} and 594.5min d^{-1} for pH parameters 5.2, 5.5 and 5.8 respectively than those fed the CM diets. The diets did not impact (P>0.05) the

total tract nutrient digestibility. The DM, OM, and CP digestibility were numerically greater for heifers fed CM treatments than those fed DDGS treatments, while the NDF and ADF digestibility were numerically greater for those fed the DDGS treatments. There was no dietary effect ($P>0.05$) for N balance measured. These results indicated that that inclusion of PMS at 1.5% (% of CM DM) and PSF (2% of diet DM) in the diets of beef heifers did not adversely affect the rumen fermentation, total tract nutrient digestibility, or N balance measures.

Introduction

In a bid to reduce pollution, most large companies convert their process residues into raw materials known as BSF which are derived from the processing of commercial crops, ethanol production, fiber production, and from food processing industry (Mirzaei-Aghsaghali, 2008) the digestibility of these by-products depends on their varying composition of the diet which would affect the energy value to be supplied to the ruminant (Denek, 2006; Pirmohammadi, 2007). Improvement in the processing of these by-products can help to improve their nutritive value as most are high in fiber, and low in N and nutrient density (Reddy, 1992; Aregheore, 1994). However, due to the adaptative physiological nature of the rumen, ruminants can utilize these BSFs to meet their maintenance, growth, and reproduction requirements (Aregheore, 2000).

Food industry by-products are increasingly available thanks to the exponential increase in the demand for plant-based proteins as an alternative animal protein. Pea protein extraction results in the production of PMS and PSF as potential by-products that can be incorporated into livestock diets. However, the use of these food industry by-products is less explored and nutrient composition and digestibility less defined in ruminant diets. A preliminary in vitro and in situ evaluation indicated that the inclusion of CM with PMS could enhance nutrient degradation likely by improving protein-energy synchrony and enhancing the microbial activity in the rumen.

Further, blending CM+PSF was found to reduce CH4 emission in vitro. Likely, the incorporation of these by-products could potentially impact the rumen microbial activity, nutrient digestibility, and nitrogen balance in vivo. We hypothesized that strategic blending of PMS and PSF to protein supplements such as CM and DDGS can impact the rumen fermentation and nutrient utilization by beef cattle. The objectives of the study include the evaluation of rumen

fermentation, total tract nutrient digestibility, and nitrogen balance as impacted by the inclusion of strategically blended protein supplements in beef cattle finishing diets.

Materials and methods

Animal Housing

Four cannulated beef heifers (501 ± 9 kg, mean ± SD) housed at the beef center of SIU were used for the study. The heifers were housed in individual indoor pens, with each pen having a floor space of $9m^2$ area. Each pen had a self-fill water bowl, rubber floor mat and an individual feed bunk. All heifers were cared for as per the guideline of IACUC protocol 22-014.

Experimental design

The experiment was designed as a 4×4 Latin square design. Before the commencement of the study, all heifers were transitioned to a finishing diet by a 15 d, five-step step-up program where the dietary composition changed every three days. During this period, the heifers transitioned from an all-forage diet to the final finishing dietary composition.

The study lasted for 100 days with four periods and 25 days each. The first 7 days of each period were for diet adaptation, voluntary intake from the 8th till the 12th day, followed by the insertion of the indwelling pH probes to measure rumen pH from day 13 to day 15. Post removal of the pH probe, rumen fluid was collected during the 16th and 17th days after which the heifers were fed at 95% voluntary intake for proper feed consumption until the end of each period. On day 20, a urinary catheter (Bard Catheter, C. R Bard. Inc, Covington, GA 30014 USA) was inserted into each heifer for total urine and fecal collection which were carried out on days 20-25.

Treatment and dietary composition

The diets used in the present study were of similar composition to the diets used in the finishing feedlot trial as described in chapter 4. The four diets were formulated to meet or exceed CP requirements of the heifers. The diet was fed in two equal proportions at 0800 and 1600h throughout the trial. Each heifer had unlimited access to water. Each morning before feeding, the pens and bunks were cleaned, and orts were weighed and sampled during total collection to determine the DM content and for chemical analysis. Each heifer was weighed at the beginning and end of phase to calculate DM as a percentage of body weight.

The food industry byproducts used in the study (PMS and PSF) are the proprietary products of Louis Dreyfus Company (Livermore, CA) and were shipped as batches for incorporating in the feed throughout the study period. Both CM and DDGS were purchased as single batches, transported, and stored at the Beef Center of SIU for utilization in the diets. Grass hay corn grain was sourced from the Beef Center of SIU.

Rumen Fermentation

In-dwelling rumen pH measurement

For each period starting day 13, indwelling rumen pH probes were attached to data loggers and inserted into the ventral sac of rumen for the continuous measurement of the pH as described by Penner et al, (2006). Prior to introduction, the pH probes were calibrated and standardized using standard buffers (pH 4 and 7). The probes were programmed to record the rumen pH readings every 1 minute for 72 h from day 13 through 15. At 0800 h on day 15, the pH probes were removed from the rumen of each heifer, washed, cleaned and the logged data downloaded to the system.

The recorded data were averaged for daily mean, minimum and maximum pH measurements. The pH thresholds for calculating the duration and area were 5.8, 5.5 and 5.2 as described previously (Nair et al., 2016). For categorizing ruminal acidosis as mild (pH < 5.8), moderate (pH < 5.5) and severe (pH < 5.2) pH thresholds of 5.5 to 5.8 was used to determine duration and area under categorizing SARA and 5.0 to 5.2 for ARA (Nocek 1997; Penner et al. 2007).

Rumen fluid collection

For each period, rumen fluid was collected on day 16 and 17 every 3hrs starting from 0800 hr. During each collection, approximately 250 ml of rumen fluid was collected from the rumen from four different regions (ventral, posterior, anterior and rumen mat), the collected samples were then strained through two layers of cheese cloth and then the solid part left is discarded. After straining, the pH was immediately measured twice using an Accumet Portable AAP115 Laboratory pH (Fisher scientific) and the values recorded. Samples (10 ml) were collected into tubes containing 2 ml of 25% metaphosphoric acid for VFA analysis, and tubes containing 2 ml of 1% sulfuric acid for ammonia analysis. A third sample (10 ml) was collected into tubes without any preservatives and served as spare. All samples were stored at -20°C until analyzed.

Volatile fatty acid analysis

For VFA analysis, the frozen samples were thawed overnight at 4°C. The contents were then thoroughly mixed centrifuged at 12000 rpm for 15 min at 4°C. About 2ml of the supernatant was transferred into microcentrifuge tubes and centrifuged at 14000 rpm for 15 min at 4°C with a 5425R Centrifuge. After centrifugation, 1 ml of supernatant was pipetted into GC vial (Fisher brand, 2ml screw thread autosampler vials) containing 0.1 ml of internal standard (Ethyl butyric Acid). The internal standard was freshly prepared by mixing 20ml of 25% metaphosphoric acid

with 300µL of isocaproic acid and made up to a volume of 100 ml with double distilled water. VFA were analyzed by GC (Shimadzu GC-2010 gas chromatograph, Shimadzu Scientific Instruments Inc., Columbia, MD, USA), with a 30-m SP-2560 fused silica capillary column (Restek Stabil WAXDA column, Bellefonte, PA, USA). The GC temperature was programmed to 65°C for 3 min, increased at 12°C/min to a final temperature of 180°C with over all 12 minutes running time for each sample. A mixed standard, containing known amounts of acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic and isocaproic acids were used to construct a calibration curve for analysis of unknown samples. The VFA concentration was analyzed by comparing their peaks with that of the internal standard, Ethyl Butyric Acid.

Rumen ammonia

Collected rumen fluid samples for ammonia were thawed overnight to 4°C. The contents of each tube were thoroughly mixed and centrifuged at 14 000 rpm for 15 mins. The supernatant was transferred into 2ml centrifuge tubes and centrifuged at 14 000 rpm for 15 mins in a 5425R Centrifuge. The supernatant was used to determine the concentration of ammonia by colorimetric method using the phenol-hypochlorite procedure stated by Broderick and Kang (1980). Briefly, 200 µL of sample was added to a 5 ml tube, then 1.25 ml of phenol reagent and 1 ml of hypochlorite were also added. The test tubes were then immersed in water at 37°C for 10 mins for colorimetric interaction. After incubation, 100 µL microliters of each sample were pipetted in duplicates into the 96-plate reader (Bio Tek Synergy HT Multi-Mode Microplate reader Winooski, VT) and ran through the system for the ammonia concentration.

Total tract collection

Urine and feces collection was carried out for each phase from day 20 till 25 starting from 0800 hr. Before collection, each heifer was fitted with an indwelling bladder catheter (Bard Catheter,

C. R Bard. Inc, Covington, GA 30014 USA). The heifers were then holstered to their pen but were provided with adequate space to eat, drink, and lie down. The urinary catheters were attached to Nalgene plastic tubes which were connected to 20L Nalgene plastic containers containing 500ml of 4N sulfuric acid to prevent volatilization of urinary ammonia, Lopez et al. (1998).

From day 21, the total volume of urine is weighed daily for the 24 h urine output. The container was mixed thoroughly and 500ml was collected into a 4L container and stored in a freezer at -20°C. For each period, urine samples were collected and added over the five days of total collection for a composite sample to serve as the representative sample per heifer at the end of each period. The composite urine sample was thawed mixed properly, and sub-sampled into a 500 ml container for each animal at each period at -20°C for urinary N analysis.

Total tract fecal collection was done by observing the pen every 3 h from 0600 to 2200 h. From 2200 to 0600 h, the pens were checked every 4 h. At every observation, manure when present is scraped off the floor and stored in Rubbermaid plastic containers with lids. From day 21, total feces collected over 24 h was weighed, subsampled at 2.5% of daily weight stored in Ziploc bags, and stored in a freezer at -20°C. at the end of each period, total collected feces for each heifer were thawed and mixed thoroughly for a representative fecal sample per period per heifer. and the samples were dried in a forced air oven at 55º C for 120 h for analysis.

Chemical Analysis

Individual feed samples including crack shelled corn, hay, CM, PMS, PSF and supplement were all sampled during the beginning of every phase. Also, orts were sub sampled during total collection along with TMR for each period and were dried in a forced air oven at 55º C for 48 h. All dried samples were ground using Thomas Wiley Laboratory mill Model 4 (Arthur

H. Thomas company, Philadelphia, PA, USA) through a 1 mm screen. All processed samples were sent to Rock River Laboratory Inc., Waterton, WI for analysis according to the Association of Official Analytical Chemists (2000).

Statistical Analysis

Rumen fermentation data, including indwelling pH probe measurements, nitrogen balance data and total tract digestibility data were analyzed using Mixed Model Procedure of SAS (SAS Institute, Inc. Cary, N.C.) for Latin Square Design with the heifers as random effect and treat and period as fixed effects. Rumen VFA concentration and proportion, ammonia and spot pH data were analyzed as repeated measure with the fixed effect of time (day) and treatment x time (day) interaction included in the model. Significance was declared at $P < 0.05$.

Results and Discussion

Diet composition

The dietary composition was similar to that of feedlot finishing diets (Table 4.2). Treatments were formulated to be isonitrogenous and isocaloric and did not vary between treatments.

Rumen pH (In-dwelling and spot sample rumen pH)

Rumen pH measurements did not vary between treatments for the majority of parameters evaluated except for a trend for DDGS+PSF having a greater minimum rumen pH than CM. Spot rumen pH measurements indicated a numerically greater mean rumen pH for diets containing DDGS than for the treatment containing CM.

Also, the area (pH *min) and duration (min) were classified by (Penner et al. 2007) as under pH threshold 5.2 (severe acidosis) and 5.5 (moderate acidosis) and 5.8 (mild acidosis) did not vary between treatments ($P > 0.05$). Overall, heifers fed the DDGS+PMS diet had the lowest duration below all pH thresholds for acidosis with 281.5min, 418.5min, and 594.5min for pH parameters

5.2, 5.5, and 5.8 respectively than those fed the CM diets.

Table 5.1. Effect of inclusion of food industry by-products in the finishing diets on ruminal pH of beef heifers

	Treatments[1]					
Item	CM	DDGS	CM+PMS	DDGS+PSF	SEM[2]	*P*- value
Spot rumen pH	6.02	6.32	6.21	6.35	0.189	0.70
Rumen pH parameters using indwelling pH probes						
Mean daily rumen pH	5.89	6.10	6.02	6.23	0.418	0.95
Minimum rumen pH	4.94	5.14	4.92	5.16	0.160	0.07
Maximum rumen pH	6.49	7.05	6.93	6.98	0.223	0.33
Rumen pH parameters 5.8 or lower (mild acidosis)						
Total duration (min d^{-1})	932.7	979.7	843.3	594.5	257.34	0.73
pH area (pH × min)	464.1	437.7	488	306.0	204.00	0.92
Rumen pH parameters 5.5 or lower (moderate acidosis)						
Total duration (min d^{-1})	672.4	672.5	689.9	418.5	275.4	0.88
pH area (pH × min)	220.4	188.6	255.8	155.8	128.7	0.95
Rumen pH parameters 5.2 or lower (severe acidosis)						
Total duration (min d^{-1})	328.7	285.3	472.9	281.5	248.14	0.95
pH area (pH × min)	74.2	46.0	79.8	50.9	51.90	0.96

[1]Treatments included CM, diets containing regular CM, DDGS, diets containing regular DDGS, CM+PMS, diets containing 1.5% (DM basis) inclusion of pea molasses in CM and DDGS+PSF, diets containing pea starch and fiber in the diet at 2% inclusion

[2]SEM, pooled standard error of mean, n = 4 heifers.

Digestibility

The diets did not vary (P > 0.05) in any of the total tract nutrient digestibility evaluated (Table 5.3). Overall, The DM, OM, and CP digestibility was numerically greater for heifers fed CM treatments than those fed DDGS treatments while the NDF and ADF digestibility were numerically greater for the DDGS treatments. Starch digestibility was similar across all treatments.

Table 5.2. Effect of inclusion of food industry by-products in the finishing diets of beef heifers on apparent total tract nutrient digestibility

	Treatments[1]					
Item	CM	DDGS	CM+PMS	DDGS+PSF	SEM[2]	*P*- value
Dry matter intake						
kg d^{-1}	12.2	10.5	12	12.3	1.17	0.35
% of BW	2.28	2.05	2.22	2.28	0.119	0.32
Apparent nutrient digestibility coefficients (%)						
DM	78.8	75.1	78.7	75.5	2.41	0.59
OM	82.1	78.4	81.8	78.4	2.12	0.45
CP	70.4	68.1	71.6	67.6	3.61	0.84
EE	50.9	37.7	46.2	45.7	6.78	0.64
NDF	54.9	62.5	56.0	60.7	5.73	0.75
ADF	43.6	52.5	48.8	54.7	4.71	0.40
Starch	98.6	97.0	98.2	98.1	0.65	0.40

[1]Treatments included CM, diets containing regular CM, DDGS, diets containing regular DDGS, CM+PMS, diets containing 1.5% (DM basis) inclusion of PMS in CM and DDGS+PSF, diets containing PSF in the diet at 2% inclusion

[2]SEM, pooled standard error of mean, n = 4 heifers.

Nitrogen balance

The diets did not vary (P>0.05) in any of the nitrogen balance measures evaluated (Table 5.3) Overall, the fecal output (kg DM/d) was greater for heifers fed the DDGS diets than those fed CM diets. Similarly, the total N intake, total N excreted, and fecal N excreted were relatively greater for heifers fed DDGS+PSF diets than those fed CM diets, indicating the greater DM intake of those heifers. A numerically lower fecal N as a percent of total N excreted and greater urinary N as a percentage of total N excreted for CM treatments likely reflect the numerically greater CP digestibility for the CM treatments than DDGS treatments. N efficiency did not vary between treatments. Apparent N retained (g d^{-1}) ranged from 41.1 to 69.7 g d^{-1} which is within the range reported for heifers fed finishing diets (Walter et al. 2012; Nair et al. 2016) containing graded levels of high protein supplements. However, a review of literature indicated that these N retention values are greater than would be expected for normal lean tissue deposition (Spanghero and Kowalski 1997; Kohn et al. 2005). N losses during the drying of fecal samples, and

volatilization of N from urine and feces cold impact N retention. However, measures were taken to minimize N losses during sampling. Urine samples were collected into 20L Nalgene plastic containers containing 500ml of 4N sulfuric acid to minimize volatilization of ammonia while the fecal and urine samples were stored at -20°C until analysis.

Table 5.3. Effect of inclusion of food industry by-products in the finishing diets on nitrogen (N) balance of beef heifers

	Treatments[1]					
Item	CM	DDGS	CM+PMS	DDGS+PSF	SEM[2]	*P*- value
Fecal output (kg DM d^{-1})	2.19	2.66	2.18	3.01	0.40	0.14
Urine output (kg d^{-1})	21.6	19.6	20.2	20.7	3.88	0.98
Nitrogen (g d^{-1})						
Total N intake	208.9	229.0	202.6	248.6	22.98	0.26
Total N excreted	154.6	161.8	162.5	194.9	22.24	0.34
Fecal N	62.6	73.2	60.1	83.6	12.03	0.20
% of total N excreted	39.9	46.7	36.6	42.2	4.94	0.55
Urinary N	92.1	88.3	102.3	111.1	16.63	0.64
% of total N excreted	60.1	53.3	63.4	57.8	4.94	0.55
Apparent total N retained	55.4	69.7	41.1	55.2	9.41	0.36
N retained as a % of intake	25.4	31.7	22.4	23.8	4.17	0.44

[1]Treatments included CM, diets containing regular CM, DDGS, diets containing regular DDGS, CM+PMS, diets containing 1.5% (DM basis) inclusion of PMS in CM and DDGS+PSF, diets containing PSF in the diet at 2% inclusion

[2]SEM, pooled standard error of mean, n = 4 heifers.

Conclusion

The result indicated that inclusion of PMS at 1.5% (% of CM DM) and PSF (2% of diet DM) in the diets of beef heifers did not adversely affect the rumen fermentation, total tract nutrient digestibility, or N balance measures. The inclusion of PSF may likely improve the rumen fermentation measures as indicated by a tendency for a higher minimum rumen pH and lower duration and area under various pH thresholds. Further evaluation of food industry byproducts at various inclusion rates in beef cattle diets may provide an opportunity for improving the protein-energy synchrony in the rumen and rumen function.

CHAPTER 6

GENERAL DISCUSSION

An expansion in ethanol production and canola crushing industries in the last few decades has made the availability of high-protein by-products such as DDGS and CM the major source of protein for livestock feeding. The recent expansion of the market for plant-based meat alternatives has resulted in an exponential increase in the generation of by-products of pea protein extraction such as PMS and PSF. These by-products have the potential to complement livestock diets, though the nutrient composition of these by-products is poorly characterized. The present research focused on strategical blending of the by-products of plant protein extraction with high-protein by-products such as CM and DDGS and the impact of such blends on nutrient composition, in vitro and in situ nutrient degradability, growth performance of growing and finishing beef cattle and rumen fermentation, total tract nutrient degradability and N balance of beef heifers.

The in vitro and in situ evaluation of different combinations of PMS and PSF with CM indicated that the DM and CP degradability was greater for the blends than regular CM. Results indicated that both PMS and PSF likely improved the energy available to the rumen microbes, improving the protein-energy synchrony and resulting in optimum microbial activity. It is logical to assume that enhancing the degree of synchronization of energy and nitrogen leads to active ammonia assimilation and greater efficiency of microbial protein synthesis in the rumen. Improved fermentation was also reflected by the greater total gas production in vitro for the blends than regular CM.

In vitro fermentation showed a greater proportion of propionate for the blends than regular CM and a concurrent decrease in the proportion of CH4 as a % of total gas. Moreover,

CH4 in mmol/g DM was lower for CM+PSF than CM, likely indicating that the strategic blending of food industry by-products could potentially minimize CH4 production in ruminants. It is logical to assume that a greater proportion of H+ was redirected to propionate production than CH4 production for the blends. The in vitro and in situ results were encouraging to further expand the research to feeding studies to evaluate the biological significance of such blends in the diets of growing and finishing beef cattle. Favorable results from animal feeding studies will be a win-win situation for the livestock and by-products industry. The food industry by-products sector will benefit from the added value of the by-products in livestock diets while the livestock sector will benefit from sustainable (thanks to a reduction in CH4 production) performance improvement (thanks to greater nutrient degradation and propionate concentrations).

A previous study by Nair et al., 2016 indicated that though high protein by-products in backgrounding diets of growing beef cattle resulted in improved growth performance, the energy value of CM and DDGS is not as high as cereal grains such as barley in finishing diets. Strategic blending of food industry by-products such as PMS and PSF to CM and DDGS is expected to increase the starch and sugar content of these protein supplements, likely enhancing the degree of synchronization of energy and nitrogen leading to active ammonia assimilation and greater efficiency of microbial activity in the rumen. The level of PMS was fixed at 1.5% (% DM) of treatment supplemented with CM where 1.5% of CM was replaced with PMS. The level of PMS in the total diet was 0.15% (%DM). The inclusion of PSF was fixed at 2% (% DM) of the diet supplemented with DDGS, replacing parts of hay and corn grain in the diet. The levels of these food industry by-products were strategically fixed for future licensing purposes.

The growth performance of backgrounding and finishing steers did not vary between treatments during the study. The results indicated that blending of food industry by-products in

beef cattle diets and replacing parts of protein supplements, forage or grain did not negatively impact animal performance. Similarly, the metabolism study using cannulated beef heifers indicated that rumen fermentation, total tract nutrient digestibility, and nitrogen balance parameters did not vary between treatments. The results somewhat contrast the findings of in vitro and in situ studies where there was significant improvement in DM and CP degradability. It should be noted that the treatments used in the in vitro and in situ studies are somewhat different than those used in the growth performance and metabolism studies. The blends used in in vitro and in situ utilized only CM as the protein source while the growth performance and metabolism studies utilized both CM and DDGS as the source of protein supplement. Further, the blends used in the in vitro and in situ studies included a mix of PMS and PSF at 5% and 10% levels, along with PMS (CM+PMS) and PSF (CM+PSF) at 1.5% inclusions in CM.

Further, though non-significant, carcass characteristics showed numerical changes across treatments. *L. thoracis* area for steers fed CM+PMS and DDGS+PSF was 1.2% and 1.7% greater respectively than for steers fed the corresponding CM and DDGS diets. Similarly, YG2 and YG3 were similar or greater while YG2 was lower for steers fed CM+PMS and DDGS+PSF than for steers fed the corresponding CM and DDGS diets. Greater yield grades are desirable as yield grade is a measure of cutability or yields of boneless, closely trimmed retail cuts from the primal cuts. Prime quality grade was 13 units greater for steers fed CM+PMS than those fed CM diets. A corresponding increase in marbling was also noticed for steers fed CM+PMS than those fed CM diets. Numerical improvement in yield and quality grade for steers fed CM+PMS and DDGS+PSF likely indicates that these treatments could impact carcass characteristics at a greater level of inclusion in the diets.

It is logical to assume that further fine-tuning of the protein supplement (CM or DDGS) and the food industry by-product (PMS or PSF) and the levels of inclusion in the diets could optimize the protein-energy synchronization resulting in optimum microbial activity and nutrient utilization in the rumen. Further, PMS and PSF used in the study were frozen and shipped overnight as batches throughout the study. These by-products were stored in a freezer at -20°C and thawed to be incorporated into the diets throughout the study period. Alternate processing of these by-products, including drying or freeze-drying could add consistency in nutrient composition and feed formulation.

In short, the results of the study indicated that there is potential for the use of food industry by-products such as PMS and PSF as a component of ruminant diets and did not negatively impact the growth performance of growing and finishing beef cattle when these by-products replaced the protein supplement, forage, or grain sources.

CHAPTER 7

GENERAL CONCLUSION

The objectives of the study were to evaluate the effects of the inclusion of strategically blended food industry by-products in the diets of beef cattle on growth performance, carcass characteristics, rumen fermentation, total tract nutrient digestibility, and nitrogen balance. In vitro and in situ studies indicated that nutrient degradability and fermentation were greater for the blended CM than regular CM, likely indicating enhanced protein-energy synchrony for optimum ruminal microbial activity. Further, when the by-products replaced the protein supplement, forage, or grain sources in the diets of growing and finishing beef cattle, there were no negative effects on growth performance, rumen fermentation, or total tract nutrient digestibility. Though non-significant, numerical improvements in carcass traits such *as L. thoracis* area, yield grade, quality grade, and marbling for steers fed blended protein supplements than those fed regular protein supplements. It is logical to assume that these treatments could impact carcass characteristics at a greater level of inclusion in the diets. The lack of negative effects when the by-products replace the protein supplement, forage, or grain sources in the diets of growing and finishing beef cattle will encourage the inclusion of these food industry by-products in ruminant diets for sustainable animal agriculture.

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APPENDIX A

INSTITUTIONAL CARE AND USE COMMITTEE (IACUC) APPROVALS

For Chapter 3 (Evaluation of strategic blending of food industry by-products with canola meal on in vitro and in situ nutrient degradation):

The IACUC approved protocol is #22-032.

For Chapter 4 (Evaluation of the effects of strategic blending of food industry by-products with protein supplements on growth performance and carcass characteristics of growing and finishing beef cattle):

The IACUC approved protocol is #23-003.

For Chapter 5 (Evaluation of the effects of strategic blending of food industry by-products with protein supplements on rumen fermentation and total tract nutrient digestibility of feedlot heifers fed finishing diets):

The IACUC approved protocol is #23-014.

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Research Paper Title:

Effect of Strategic Blending of Food Industry By-Products with Protein Supplements on Performance of Growing and Finishing Beef Cattle

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