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RESPONSE OF DAIRY COWS TO DIETARY STARCH CONCENTRATIONS:
PERFORMANCE, NUTRIENT DIGESTION, AND GAS EMISSIONS

BY

JUAN ISIDRO SANCHEZ-DUARTE

A dissertation submitted in partial fulfillment of the requirements for the

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Specialization in Dairy Science

South Dakota State University

2017

RESPONSE OF DAIRY COWS TO DIETARY STARCH CONCENTRATIONS:
PERFORMANCE, NUTRIENT DIGESTION, AND GAS EMISSIONS

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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To the loving memory of my father Juan Sanchez Martinez

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LIST OF ABBREVIATIONS

°C	Celsius degree
µm	Micrometre
AA	Amino acids
ADF	Acid detergent fiber
ADPG	Adenosine diphosphate glucose
AGP	Alpha-glucan phosphorylase
AIC	Akaike information criterion
aNDFom	Neutral detergent fiber determined on an organic matter basis
ATP	Adenosine triphosphate
BCS	Body condition score
BHB	Beta-Hydroxybutyrate
BIC	Bayesian information criterion
BMR	Brown midrib
BW	Body weight
Ca	Calcium
CH ₄	Methane
CLA	Congugated linolenic acid
CM	Canola meal
CO ₂	Carbon dioxide
CoA	Acetyl coenzyme A
CONV	Conventional corn silage
CP	Crude protein

CS	Corn silage
d	Day
DDGS	Distillers dried grains with solubles
DIM	Days in milk
dL	Deciliter
DM	Dry matter
DMI	Dry matter intake
EAA	Essential amino acids
ECM	Energy-corrected milk
EE	Ether extract
FE	Feed efficiency
FTIR	Fourier transform infrared spectroscopy
g	Grams
GLM	Generalized linear model
GLUT	Basolateral glucose transporter
h	Hour
H	Hydrogen
HCO ₃	Sodium carbonate
iADF	Indigestible acid detergent fiber
IOFC	Income over feed cost
K	Potassium
kg	Kilogram
m	Meters

Mg	Magnesium
mg	Miligrams
MP	Metabolized protein
MUN	Milk urea nitrogen
N	Nitrogen
n	Number of treatments
Na	Sodium
NADH	Nicotinamide adenine dinucleotide
NADH ₂	Hydroxylamine reductase
NDF	Neutral detergent fiber
NDFD-30h	Neutral detergent fiber digestibility at 30 hours
NEAA	Non-essential amino acids
NEFA	Non-esterified fatty acids
NFC	Non-fiber carbohydrates
NFFS	Non-forage fiber sources
NH ₃	Ammonia
NIRS	Near infrared reflectance spectroscopy
OM	Organic matter
P	Phosphorus
PDV	Portal drained viscera
peNDF	Physically effective neutral detergent fiber
pH	Potential hydrogen
PUN	Plasma urea nitrogen

RMSE	Root mean square error
RUP	Rumen undegradable protein
S	Sulfur
SBM	Soybean meal
SCC	Somatic cell count
SCS	Somatic cell score
SD	Standard deviation
SED	Standar error difference
SEM	Standard error of mean
SGLT	Sodium-glucose transporter
SNF	Solids non-fat
St	Starch effect
TMR	Total mixed ration
UDPG	Uridine diphosphate glucose
uNDF	Undigestible neutral detergent fiber
VFA	Volatile fatty acids
VIF	Variance inflation factor
wk	Week

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ABSTRACT

RESPONSE OF DAIRY COWS TO DIETARY STARCH CONCENTRATIONS:
PERFORMANCE, NUTRIENT DIGESTION, AND GAS EMISSIONS

JUAN ISIDRO SANCHEZ-DUARTE

2017

Three studies and a meta-analysis were conducted to determine the performance, nutrient digestion, and gas emissions of dairy cows fed diets with different starch concentrations (19 to 27% of DM). Study 1 evaluated the effects of reducing corn grain starch with non-forage fiber sources (NFFS, soybean hulls and beet pulp) in diets of soybean meal (SBM) or canola meal (CM). In study 1, reducing starch from 27 to 20% with soybean hulls and beet pulp had a negative effect on dry matter intake (DMI), milk yield and energy-corrected milk (ECM), regardless of the crude protein (CP) source. Those effects were explained by a low dry matter (DM) and organic matter (OM) digestibility in cows fed 19% starch. Study 2 explored through a meta-analysis the effects of reducing cereal grain starch with NFFS on the performance, nutrient digestion, and rumen fermentation of dairy cows. The meta-analysis indicated that when dietary starch intake increased from 1 to 9 kg/d, DMI responded quadratically, but milk yield, milk protein concentration, and milk lactose yield increased positively. Milk fat concentration however decreased linearly as starch intake increased in the cows. As dietary starch intake increased in the cows, the concentration of total volatile fatty acids (VFA) and acetate decreased linearly, but propionate, acetate to propionate ratio, isobutyrate, isovalerate, and valerate increased linearly. Increasing starch intake affected quadratically the DM digestibility, linearly CP digestibility, and negatively neutral detergent fiber (NDF) digestibility. Therefore, reducing cereal grains starch with NFFS has a negative

effect on dairy cows performance. Studies 3 and 4 evaluated the effects of diets with conventional (CONV) and Brown midrib (BMR) corn silage with two starch concentrations (19 vs. 25% of DM) on performance and gas emissions, respectively. In study 3, cows fed BMR diets tended to have higher DMI than cows fed CONV corn silage diets. Cows fed BMR-25% had the greatest milk yield and ECM, but cows in BMR-19% produced the same amount of milk and ECM than cows with CONV corn silage in any starch concentration. These effects were explained by the increased digestibility of DM, OM, and CP in response to diets with 25% starch and the increased DMI with BMR corn silage. In study 4, diets did not affect DMI, milk yield, ECM, milk composition, nutrient digestion, and emissions of CH₄, NH₃, and CO₂. However, cows fed BMR corn silage and 25% starch produced less CH₄ and CO₂ per kg of DM, OM, and starch digested than cows in CONV corn silage with any starch concentration, but cows fed 25% starch produced more NH₃ and CO₂ per kg of NDF digested. Overall, reducing starch with increasing NFFS has a negative effect on dairy cow performance and nutrient digestion, but including BMR corn silage improved those effects by increasing nutrient digestion. The combination of BMR corn silage and high starch diets have the potential to reduce gas emissions per kilogram of nutrient digested in lactating dairy cows.

INTRODUCTION

Corn grain has been the main concentrate feedstuff used to satisfy the energy requirements of lactating dairy cows in the United States because of its high starch content. The inclusion rate of corn grain into dairy cow diets, however, has decreased in recent years, with a subsequent reduction in dietary starch from 30 to 25% (Dann and Grant, 2009). This has been in part due to acidosis problems occurring with diets high in starch (greater than 28%; Gott et al., 2015). In addition, the increase in price of corn grain across the years also contributed to this reduction (St-Pierre and Knapp, 2008). Therefore, decreasing starch by partially replacing corn grain in rations of lactating dairy cows is desirable for both animal health and farm economic reasons.

Reducing corn grain by increasing the proportion of non-forage fiber sources (NFFS) in diets has been a valid strategy to deal with high prices. Starch content in NFFS is very low when compared to corn grain, however it contains other fermentable carbohydrates such as sugar, organic acids, fructans, glucans, and pectins (Firkins, 1997; Leiva et al., 2000; Miron et al., 2001; Pereira, and Gonzalez 2004; Bradford and Mullins, 2012). In addition, it also has highly digestible NDF (Dann and Grant, 2009; Bradford and Mullins, 2012) that provides energy for rumen microbes and the cow. Thus far, at least 39 studies have evaluated the effects of reducing corn grain with different NFFS in diets of lactating dairy cows (Table 8). However, limited information has been generated by substituting dietary corn starch with NFFS using different protein sources and corn silage hybrids.

Soybean meal and canola meal (CM) are the main protein sources included in diets of lactating dairy cows. Research information from 122 studies that compared the

feeding value of those protein sources demonstrated greater DMI and milk yield in dairy cows fed CM (Huhtanen et al., 2011). In another study including 27 trials, performance of dairy cows fed soybean meal (SBM) and CM diets was similar (Martineau et al., 2013). On the other hand, CONV corn silage and its isogenic BMR corn silage are the main forages used in diets of lactating dairy cows. Research of 48 feeding trials indicated an increase of 1.1 and 1.5 kg/d in DMI and milk yield, respectively, from cows fed BMR corn silage diets relative to cows fed CONV corn silage diets (Ferrareto and Shaver, 2015). These effects were attributed to the lesser lignin concentration and greater total tract NDF digestibility supplied by BMR corn silage.

Research information of reducing starch with NFFS is currently used in dairy cow diets. Similarly, information comparing diets of SBM with CM and contrasting CONV corn silage versus BMR corn silage diets has been extensively explored. However, the potential effects of reducing dietary starch concentration in combination with SBM and CM, as well as, CONV and BMR corn silage may result in different outcomes that deserve to be investigated. Therefore, the main objective of this research was to investigate the potential effects of reducing dietary starch concentration with NFFS in diets with different protein sources (SBM vs. CM) and corn silage type (CONV vs. BMR corn silage) on the performance and nutrient digestion of lactating dairy cows. A second objective was to evaluate the impact of those diets on gas emissions in dairy cows.

CHAPTER 1: LITERATURE REVIEW

Starch in Feeds

Chemical Composition of Starch

Starch is basically built from six-carbon polymers of the sugar D-glucose. The structure of the D-glucose molecule can be in the form of an open chain or a ring (Thomas and Atwell, 1999). The ring configuration is always referred to as a pyranose, a collective term for a carbohydrate that has a ring consisting of five carbons and one oxygen atom. An example of pyranose is *D-glucofuranose*. The polymers of *D-glucofuranose* from starch are linked together by α -1,4 and α -1,6 glycosidic bonds (BeMiller and Whistler, 2009). The configuration of these glycosidic linkages in starch is determined by the orientation of the hydroxyl group (-OH) on carbon 1 of the pyranose ring (Perez and Bertoft, 2010). This α linkage allows starch polymers to form helical structures essential for the physicochemical properties and susceptibility to certain enzymes when compared to other carbohydrates (Thomas and Atwell, 1999). For example, when compared to the β linkage of cellulose, the α configuration and the helical structure of the starch contributes to make this carbohydrate more susceptible to the hydrolysis by amylase enzymes. Therefore in ruminants, cellulose is less digestible than starch (Van Soest, 1994).

The chemical combination of glucose polymers in starch (α -1,4 and α -1,6 glycosidic bonds) results in two types of molecules, amylose and amylopectin. Amylose is drawn as a linear polymer composed typically by an α -1,4 *D-glucofuranose* chain (Van Soest, 1994; Thomas and Atwell, 1999). However, most starches also contain

branched amyloses (Perez and Bertoft, 2010). Amylose has also been described as a helical structure containing hydrogen atoms in its molecule. Thus, amylose can form molecular compounds with free fatty acids, glycerides, some alcohols, and iodine (Thomas and Atwell, 1999). On the other hand, amylopectin, a branched polymer larger than amylose, is composed of α -1,4 glucose linkages connected to α -1,6-linked branched points (Perez and Bertoft, 2010). In fact, it has been estimated that about 4-6% of the linkages in an amylopectin molecule are α -1,6 (Thomas and Atwell, 1999). This configuration contributes to the amylopectin crystallization and the arrangement of amylopectin molecules within the starch granules (BeMiller and Whistler, 2009). A third component has been investigated called intermediate material located between the linear and branched polymers in some starch species (i.e. 5-7% of the total starch in corn grain; Lansky et al., 1949), but there has been no clear evidence reported of their chemical structure.

Amylose and amylopectin are not free in nature so they appear as semi-crystalline aggregates called starch granules. The size, shape, and structure of starch granules vary among cereal grains, presenting spherical, ovoid, or angular granules with a diameter that varies from less than 1 μm to more than 100 μm (Thomas and Atwell, 1999). The starch granules not only contain amylose and amylopectin, but also comprise very small portions of protein, lipids, and ash (BeMiller and Whistler, 2009). The proportion of amylose and amylopectin varies across cereal grains, but it has been reported that the starch contained in the endosperm of cereal grains in granular form is typically composed of 25-28% amylose and 72-75% amylopectin (Colonna and Buléon, 1992).

Starch Biosynthesis in Plants

Starch is stored in plants to be used as a main source of carbon. In almost all plant tissues and organs (roots, stems, leaves, fruits, and grains), starch is deposited as crystalline granules. Starch synthesis occurs in the plastids of photosynthetic and non-photosynthetic tissues (Geigenberger, 2011). In photosynthetic active cells, such as those found in leaves, the mature chloroplasts provide carbon and energy (ATP, adenosine triphosphate) for starch synthesis in the presence of daylight. However, in non-photosynthetic cells, such as those in roots and seed endosperms, the starch is produced in amyloplasts which depend on carbon and energy synthesized in the cytosol (Bahaji et al., 2014). Therefore, carbon fixed in leaves is retained within the chloroplasts during the day to synthesize starch. Fixed carbon is then mobilized during the night to support the metabolism and growth of the rest of the plant (Sulpice et al., 2009). When changes in day length occur, starch synthesis decreases affecting rate of growth during darkness (Bahaji et al., 2014).

Starch biosynthesis in leaves is a complex process, but some mechanisms have been proposed to describe it as well as its degradation in leaves. Fettke et al. (2011) found that membranes of chloroplasts in mesophyll cells possess a mechanism that allows the incorporation of cytosolic glucose-1-phosphate into the stroma, which is then converted into starch by the stepwise adenosine diphosphate glucose pyrophosphorylase and starch synthase reactions. Bahaji et al. (2014) later indicated that starch is the end-product of a metabolic pathway linked to the Calvin-Benson cycle by the plastidial phosphoglucose isomerase enzyme. First, phosphoglucose isomerase enzyme catalyze the conversion of fructose-6-phosphate from the Calvin-Benson cycle into glucose-6-phosphate. Then,

glucose-6-phosphate is converted into glucose-1-phosphate by the plastidial phosphoglucomutase enzyme. Finally, the adenosine diphosphate glucose (ADPG) pyrophosphorylase enzyme converts glucose-1-phosphate and ATP into inorganic pyrophosphate and ADPG, which are necessary for starch biosynthesis.

Starch biosynthesis in heterotrophic organs (stems, roots, seeds, and fruits) is also a tangled process, but in general, the sucrose synthesized within the cytosol in leaves is used as a carbon source for energy production and starch synthesis. Bahaji et al. (2014) described that sucrose entering to the cytosol of the heterotrophic cells is broken down by sucrose synthase to produce fructose and uridine diphosphate glucose (UDPG). Then, UDPG is converted to glucose-1-phosphate and inorganic pyrophosphate by the UDPG pyrophosphorylase enzyme. Glucose-1-phosphate is later metabolized to glucose-6-phosphate that is converted to starch by sequential activities of plastidial phosphoglucomutase, alpha-glucan phosphorylase (AGP) phosphorylase, and starch synthase in the amyloplasts. Another model of sucrose-starch conversion in heterotrophic organ cells has been proposed (Emes et al., 2003; Bahaji et al., 2014). This mechanism involves the production of ADPG from sucrose and adenosine diphosphate by the sucrose synthase enzyme. In addition, the sucrose synthase enzyme catalyzes de novo production of ADPG from sucrose, which is imported into amyloplasts by the action of plastidial phosphoglucomutase and AGP phosphorylase enzymes for the synthesis of starch. In vitro studies have demonstrated a similar activity of the synthase and AGP phosphorylase enzymes in potato tubers (Baroja-Fernandez et al., 2009), however with a double enzymatic activity for synthase enzyme in developed barley and corn endosperm (Li et al., 2013).

Common Starch Sources in Diets of Dairy Cows

Starch is a polysaccharide component contained in the non-fiber carbohydrates (NFC) fraction of the plant. Typical diets for lactating dairy cows contain 70 to 80% carbohydrates on a dry matter basis (Weiss and Firkins, 2007; Hall, 2014). Of these proportions, nearly 35 to 40% is starch, and the rest NDF (40 to 45%), soluble fiber, and simple sugars (20%; Weiss and Firkins, 2007).

Common sources of starch in dairy cow diets include silages. A common average starch content in corn silage is 35%; however, starch concentration depends directly on the hybrid (Ferrareto and Shaver, 2015), maturity at harvest (Khan et al., 2012), proportion of grain in the whole plant (Arias et al., 2003), and chop length (Aoki et al., 2013). Lauer et al. (2015) reported a starch content variation between 26 and 35% from 38 different corn hybrids evaluated within the same region and agronomic management practices. Regarding maturity, Khan et al. (2012) observed an increase from 38 to 43% starch in silage when dry matter in forage corn increased from 31 to 39%. Similarly, starch content of corn silage increased from 22 to 35% as the percentage of grain in the silage increased from 32 to 50% (Mahanna, 1994). On the other hand, the starch concentration in sorghum silage can vary from less than 4 to about 15% (Weiss and Firkins, 2007). Thus, sorghum silage is not used as a source of starch when included in the diets. In small grains, the starch concentration reported changes from 48 to 63% (Ovenell-Roy et al., 1998; Khorasani et al., 2000). However, the starch digestibility of small grain silages is affected by the species (wheat vs. barley vs. oats), maturity (milk stage vs. soft dough vs. hard dough), and mechanical process of the silage during harvest (Weiss and Firkins, 2007).

Grains of corn and sorghum as well as small grains contain substantial amounts of starch, so they are used as starch sources in the diet. Previous research has reported that the greatest starch content is in wheat (72%), followed by 70% for corn and sorghum, and the least starch content is found in barley and oats (57-58% starch) (Aimone and Wagner, 1977; Herrera-Saldana et al., 1990; Hatfield et al., 1993). However, digestion kinetics studies *in vitro* (Lanzas et al., 2007), *in situ* (Herrera-Saldana et al., 1990), and *in vivo* (Galloway et al., 1993) have ranked the starch degradation rate in descending order as wheat > barley > corn > sorghum. These sources can be included as whole grain or more commonly subjected to a processing method before inclusion in the diets. Processing methods include rolling, grinding, cracking, crimping, pelleting, pressure and heat, and high moisture. Processing of the grains improves their digestibility (Huntington, 1999). An extensive review by Theurer (1986) indicated the following effects of processing on starch digestibility in cereal grains: 1) rumen starch digestion of corn was higher in flaked (95%) than ground (88%) and cracked (86%); 2) total tract digestion in sorghum was superior in steam-rolled (95%) compared to finely ground (92%) and coarsely ground (91%); and 3) rumen digestion of barley was similar when it was ground (94%) or rolled (93%). More recently, Oghbaei and Prakash (2016) reported that starch digestion was 3-fold greater in ground wheat than whole wheat. Therefore, not only the grain type needs to be considered when included as a starch source, but also the processing to improve starch utilization. Other by-products such as corn gluten feed, hominy, corn gluten meal, potatoes, bakery waste, potatoes waste, pasta, and unheated starch can all be used as starch sources in diets of lactating dairy cows, but their inclusion will be strictly related to their availability.

Starch Digestion and Metabolism

Starch Digestion in the Rumen

Dietary starch is perhaps the major source of energy for rumen microbes and the host animal. Starch digestion in the rumen can vary from 224 to 942 g/kg starch entering the organ (Moharrery et al., 2014). As with fiber and protein, three-fourths of the starch digestion is accomplished by rumen bacteria (McAllister et al., 1994). The main starch digesting bacteria in the rumen include *Streptococcus bovis*, *Ruminobacter amylophilus*, *Prevotella ruminicola*, *Butyrivibrio fibrisolvens*, *Succinomonas amylolytica*, and *Selenomonas ruminantium* (Cotta, 1988). These bacteria colonize grain particles and through the production of endo- and exo-enzymes hydrolyze the α -1,4 and α -1,6 linkages from amylose and amylopectin (Kotarski et al., 1992). Starch digesting enzymes include phosphorylase, α -amylases, β -amylases, amyloglucosidase, isoamylase, and pullulanase (Selinger et al., 1996). However, research has been focused mainly on α -amylases from *S. bovis* (Walker, 1965; Satoh et al., 1993; Freer, 1993), with little attention on α -amylases from *B. fibrisolvens* (Rumbak et al., 1991). McAllister et al. (1990) indicated a different pattern of amyolytic digestion on starch granules between corn grains and grains of wheat and barley. The authors mentioned that microbial digestion of starch granules in wheat and barley spread from the central point of microbial attachment on the surface of the granules. However, in corn grain amyolytic bacteria tunnel into the interior of starch granules digesting them from inside to out.

Other rumen microorganisms contributing to the starch digestion have been reported. Species of protozoa such as Holotrichs and Entodiniomorphids are capable of digesting up to 50% of the starch in the rumen (Hungate 1950; Jouany and Ushida, 1999),

but findings are confusing because it has been observed that protozoa decrease the digestibility of starch in the rumen of sheep fed high-moisture corn supplemented with sorghum (Mendoza et al., 1995). Considering the positive effect of protozoa on starch digestion, Fondevila and Dehority (2001) stated that the engulfment by protozoa of starch granules is more rapid for granules with a diameter of 3-8 μm than for granules with 9-30 μm . In addition to the starch digestion by protozoa, rhizoids in ruminal fungi can completely digest the enclosed starch granules by penetrating directly through the grain protein matrix (McAllister et al., 1993). Although, starch degradation in the rumen by fungi microorganisms is not well explored. The end products of digested starch in the rumen by rumen microbes comprise of maltose, maltotriose, small amounts of free glucose, and some α -limit-dextrins (Cerrilla and Martinez, 2003).

Not only are rumen microorganisms important to digest starch, but the protein matrix of the cereal grain can also impact the digestibility of the starch in the rumen. The starch granules in the endosperm of cereal grain are embedded within the protein matrix. The protein matrix of cereal grains is constituted of prolamins and other proteins like albumins, globulins, and glutelins (Shewry and Halford, 2002). Of these proteins, prolamins have received more research attention since they have been negatively correlated with the starch digestion in the rumen (Correa et al., 2002). The name prolamins is based on that they are rich in proline and an amide nitrogen derived from glutamine, but the specific name in each cereal grain is different (Shewry and Halford, 2002); gliadin in wheat, hordein in barley, secalin in rye, zein in corn, kafrin in sorghum, and avenin in oats. In comparison to small grains, corn and sorghum grains have greater prolamins content. Therefore, starch in wheat, barley, and oats is more readily fermented

in the rumen than starch in corn, but starch in sorghum is less fermented than corn starch. The zein-subclasses (α , β , γ , and δ) prolamins in corn, which are encapsulating the starch granules, make this grain less susceptible to rumen microbial degradation (Buchanan, et al., 2000). In fact, the content of prolamin-zein protein is significantly greater in vitreous corn endosperm compared to the floury endosperm, making vitreous endosperm less digestible in the rumen (Correa et al., 2002). In this regards, Tylor and Allen (2005a) reported a 22 percentage-unit increase in ruminal starch digestibility in corn floury endosperm than in corn vitreous endosperm in diets of either CONV or BMR corn silage. Similarly, Allen et al. (2008) observed a reduction of 19% starch digestion in the rumen of cows fed corn with 66% vitreous endosperm than in cows fed 25% vitreous endosperm.

The proportion of starch digested in the rumen will also depend on the grain processing method. As a rule, more aggressive processing methods in cereal grains increase starch digestion in the rumen. However, NDF digestibility (NDFD) can be affected as a result of decreased rumen pH (Ferrareto et al., 2013). The main purpose of processing grain is to break the pericarp and expose more surface area for enzymatic degradation of starch granules (Kozakai et al., 2007). Rolling or grinding dry corn grain (Owens, 2009) or processing corn silage through shredlage (Vanderwerff et al., 2015) reduces kernel particle size, increasing starch digestion in the rumen up to 50 and 88%, respectively. Remond et al. (2004) confirmed that starch digestibility was greater when cows were fed ground dry corn (69.8%) than dry rolled corn (53.5%). In the same study, it was also demonstrated that rumen starch digestibility increased 19% when mean particle size in corn was 0.7-1.8 mm compared to 3.7 mm. In general, substantially

greater rumen starch digestion is observed when grinding the grain to a very fine particle size or ripped and cut corn silage; however, starch digestion in the rumen can be even greater in high moisture grain. This processing method can reduce the prolamin-zein proteins up to 2.5% in high moisture corn (Larson and Hoffman, 2009). This can be the result of harvesting at an earlier phenological state (Murphy and Dalby, 1971) or due to prolamin degradation by acid proteolysis during fermentation (Lawton, 2002), which results in increased starch digestion in the rumen. Huntington (1999), in a review of papers published during a nine-year period, found that the greatest rumen starch degradation was for high-moisture corn (89.9%), followed by steam-flaked corn (84.8), steam-rolled corn (72.1%), dry-rolled corn (76.2%), and whole grain (10.65%). In the same study, rumen starch digestibility increased 3.2 and 13.4% in high-moisture sorghum grain, compared to whole and dry-rolled sorghum grain, respectively.

The proportion of the starch digested in the rumen can also be affected by chewing and rumination which depends on bunk management, animal age, feeding frequency, and the amount and type of forages and fiber in the diet. However, the direct effect of these factors on rumen starch digestion have not been well established.

Starch Digestion in the Small Intestine

Starch digestion is initiated in the lumen of the small intestine by the action of α -amylase secreted from the acinar cells of the pancreas (Brannon, 1990). In the lumen of the small intestine this enzyme attacks five adjacent α -1,4 linkages of glucose from starch releasing molecules of maltose, maltotriose, and branched limit-dextrin. Then, these oligosaccharides are hydrolyzed by enzymes such as isomaltase, maltase, and glucoamylase that are produced in the enterocytes (Sushil et al., 2013). Studies with

steers (Russell et al., 1981) and sheep (Janes et al., 1985) demonstrated that α -amylase in the small intestine increases in response to greater starch intake. However, other studies have demonstrated that feeding high starch diets in calves (Kreikemeier et al., 1990; Gilbert et al., 2015) or infusing starch post-ruminally in steers (Taniguchi et al., 1995; Swanson et al., 2002, Swanson et al., 2004), reduced pancreatic α -amylase concentration in the small intestine. Therefore, it is difficult to increase α -amylase concentration in the small intestine by diet formulation (Harmon et al., 2004).

Starch digestion in the small intestine is less than starch digested in the rumen, however it is not well known whether energy efficiency is greater when starch is used in the rumen or the small intestine. A meta-analysis of 184 observations reported that starch digested in the small intestine of lactating dairy cows ranged from 114 to 901 g/kg (Moharrey et al., 2014). Theoretical data indicated that starch digested in the small intestine provides 42% more energy than that digested in the rumen. However, decreasing starch digestion in the rumen reduces the energy available for rumen microorganisms, and the amount of microbial protein available for the animal (Owens et al., 1986). In contrast, no metabolic advantages, energy efficiency, and milk production increases were observed when starch was infused in the rumen or in the small intestine of early- and mid-lactating dairy cows (Reynolds et al., 2001; Arieli et al., 2001), indicating that the energy from infused starch was oxidized or used for tissue metabolism.

Factors limiting starch digestion in the small intestine include the dietary source of this starch and the processing methods discussed above. Other physiological aspects that restrict intestinal starch digestion have been proposed by Owens et al. (1986). First, the activity of enzymes degrading starch in the small intestine (amylase, maltase, and

isomaltase) is reduced as an effect of inadequate production, working conditions, or presence of enzyme inhibitors. Secondly, the absorption of glucose released from the hydrolysis of starch in the small intestine is limited. Third, the residence time for completion of starch digestion can be limiting. Finally, there is insufficient access of enzymes to the starch granules because of their limited solubility. In dairy cows, these effects could be related directly to their high feed intake, which increases the speed at which starch passes through the small intestine reducing the effectiveness of the enzymatic activity. However, measuring the response of starch degrading enzymes in the small intestine as affected by increased feed intake in dairy cows is challenging.

Fates of Digested Starch in the Gastrointestinal Tract

The fate of the digested starch in the rumen is different than that digested in the small intestine. Oligosaccharides such as maltose, maltotriose, free glucose, and α -limit-dextrins are derived through fermentation of more complex carbohydrates. In turn, branching enzymes like pullulanase, iso-amylase or α -limit dextrinase degrade them to glucose (Cerrilla and Martinez, 2003). Glucose molecules are then fermented inside rumen bacteria through the Embden-Meyerhof-pathway and the pentose-phosphate-cycle to produce pyruvate (Fahey and Berger, 1988). A similar fate, but with different intermediates (cellobiose, pentoses, uronic acids, galactose, sucrose, and fructose) has been proposed for the ruminal digestion of other fiber- (fructans) and non-fiber carbohydrates (cellulose, hemicellulose, and pectin; Van Soest, 1994). The conversion of two molecules of pyruvate from one molecule of glucose via the Embden-Meyerhof-pathway later yields 2 ATP and 2 NADH₂ (Hydroxylamine reductase; Fahey and Berger, 1988). Generated ATP is hence the main energy source utilized to support the

maintenance and growth of rumen microorganisms. A comparison of the response of rumen bacteria to the specific energy source has demonstrated that the percentage of total rumen colonies of microbes was superior using starch (88.7%) than glucose (71.9%), cellobiose (68.4%), xylose (77.2%), and pectin (60.4%; Dehority and Grubb, 1976).

Final end-products of microbial starch degradation along with other nutrients are ethanol, CO₂, methane, and VFA including acetate, propionate, butyrate, lactate, valerate, caprate, iso-valerate, and iso-butyrate (Hungate, 1975). From all VFA, acetate, propionate, and butyrate are those in the largest molar proportions with a ratio of 75:15:10 (Bergman, 1990). However, these proportions can vary in response to the diet, feed intake, animal physiology, forage to concentrate ratio, carbohydrate sources, and feed processing. In dairy cows, a representative study of low (23%) and high starch (30%) diets indicated that acetate concentration increased 1.6 mol/100 mol with low starch diets (Silveira et al., 2007). Propionate content however increased 2.1 mol/100 mol in high starch diets, without any changes on butyrate concentrations. It was demonstrated that dietary starch and not dietary fiber is the main driver for propionate production in the rumen.

Depending on production and turnover rate, the absorption of VFA across the rumen and reticulum wall account for 65-85% of those produced in these organs (Dijkstra et al., 1993). The absorption of VFA in the ionized form occurs by simple diffusion (involving Na⁺/H⁺ exchange), and the non-ionized form by facilitated diffusion (anion-exchange of HCO₃⁻ (Sodium carbonate); Nozière et al., 2010). Volatile fatty acids that escape the reticulum and rumen pass to the omasum and then the abomasum where are potentially absorbed. In situations where there is excessive intake of rapidly fermentable

carbohydrates, such as starch, the concentration of VFA and lactic acid increase in the rumen as a consequence of low effective fiber, reduced chewing, saliva production, and the rumen capacity to handle H^+ , leading to rumen acidosis (Plazier et al., 2009).

In the brush border of the small intestine, oligosaccharides (maltose, maltotriose, and branched limit-dextrin) are hydrolyzed by oligosaccharidases to produce glucose (Nozière et al., 2010). Glucose is then linked to sodium-glucose transporters (SGLT1) by exchanging two molecules of sodium that allows entrance to the epithelial cells. Glucose is later carried into the interstitium by the basolateral glucose transporters (GLUT2) that allows it to enter the bloodstream (Deckardt et al., 2013). When available starch exceeds the hydrolytic and absorptive capacity of the small intestine, it reaches the large intestine to be fermented or excreted in feces. In dairy cows, the analysis of starch concentration in feces has been proposed as a good indicator to estimate the total tract starch digestibility, therefore adjusting dietary starch concentration may improve the efficiency of milk production (Fredin et al., 2014).

Volatile Fatty Acids and Glucose Metabolism

The metabolism of the absorbed VFA and glucose takes place mainly in the tissues of the portal drained viscera (PDV) and liver, which deliver nutrients to the mammary gland and metabolites for milk synthesis (Reynolds et al., 1994). Acetate and butyrate are completely oxidized to CO_2 in the PDV, entering the Krebs cycle via Acetyl-CoA and the production of ketones via acetoacetyl-CoA. Propionate enters into the Krebs cycle via succinyl-CoA or via malate before being oxidized to CO_2 or metabolized into pyruvate, lactate or alanine (Nozier et al., 2010). Quantifying the metabolism of VFA into the PDV is difficult, but an *in vivo* study with sheep and steers demonstrated that it

was possible to recover into the portal vein 107% acetate, 93% propionate, and 101% isobutyrate (Kristensen and Harmon, 2006). The authors of this trial used the washed rumen technique to separate the metabolism of VFA by the rumen microbes and the absorption by the ruminant. More recently, Su et al. (2014) found a higher propionate portal flux in dairy goats fed 43% corn grain than in those fed 29% (74 mmol/h), indicating that more propionate was absorbed by PDV in high grain diets. Measured as net energy absorption by the PDV, total VFA and BHBA (β - Hydroxybutyrate acid) in dairy cows accounted for 78%, with 64% as energy flux, and 53% as ME, respectively (Reynolds et al., 1988). Therefore, the net rate of absorption of VFA by the PDV contributes to the energy budget in ruminants. It has been observed that mesenteric drained viscera from PDV uses up to 70% of glucose uptake (Reynolds et al., 1988). Based on starch digestion, Nozière et al. (2010) estimated that the average basal use of arterial glucose by PDV was 0.103 mmol/h/kg BW. Thus, increasing peripheral supply of starch increases the metabolism of glucose by the PDV.

In the liver, non-ionized forms of VFA (acetic, propionic, *n*-butyric, isobutyric, *n*-valeric, isovaleric and *n*-caproic) are absorbed passively through the hepatocyte membranes. Short chain fatty acids then enter from the cytosol to the mitochondria through a monocarboxylate carrier or “mitochondrial carnitine transporter” to be esterified as CoA esters by different synthetases (Zammit, 1990). The uptake of acetate and butyrate by the liver is different. They are channeled through ketogenesis converted to acetyl-CoA before entering the Krebs cycle where they are oxidized (Nozière et al., 2010). The proportion of acetate taken by the liver is less than butyrate, but acetate is more efficient to produce ATP. Butyrate is predominantly driven to ketogenesis

(Heitmann et al., 1987). In the case of propionate, it takes a different enzymatic pathway. Propionate is converted by propionyl-CoA carboxylase to oxaloacetate in the mitochondria as part of the Krebs cycle. Oxaloacetate is later metabolized by phosphoenolpyruvate carboxykinase to phosphoenolpyruvate, yielding glucose or serving as an acetyl-CoA acceptor in the Krebs cycle (Aschenbach et al., 2010). Other important precursors of hepatic gluconeogenesis, such as lactate and the major glucogenic amino acids (Ala, Gln, and Gly), are converted to pyruvate in the cytosol before being converted to oxaloacetate by the mitochondrial pyruvate carboxylase.

Glucose is synthesized in the liver from carbon precursors of propionate, glucogenic amino acids, lactate, glycerol, and potentially glycogen stored in the liver. The merging point for entry of most glucogenic substrates into gluconeogenesis is mitochondrial oxaloacetate, as explained before (Aschenbach et al., 2010). Liver uptake of hepatic gluconeogenesis substrates follows this order: propionate (60-74%), amino acids (8-40%; 3-5% alanine), L-lactate (5-7%), and glycerol (0.5-5%; Nozière et al., 2010; Aschenbach et al., 2010). Therefore, propionate is the major substrate for gluconeogenesis. Without accounting for the glucose absorption in the PDV, it has been reported that 7.4 kg/d of glucose absorbed in a dairy cow with a milk production of 90 kg/d, of which 4.4 kg will be used for lactose synthesis in mammary gland (Aschenbach et al., 2010). Using the complete CO₂ oxidation of palmitate ([1-¹⁴C]), Andersen et al. (2002) showed a greater capacity for palmitate conversion to CO₂ in the liver of early lactating dairy cows fed more propiogenic diets (26.7% starch), than the liver of cows fed less propiogenic diets (17.8%), indicating that feeding high starch diets promote more

complete oxidation of acetyl CoA derived from mitochondrial β -oxidation of fatty acyl CoA.

Starch Concentration in Diets of Dairy Cows

Non-forage Fiber Sources to Replace Starch from Corn Grain

Non-forage fiber sources are byproducts that result from the extraction of starch, sugar, or other non-fiber components from various crops. Due to their price, availability, and rapidly fermentability, NFFS have traditionally been used as a concentrate feedstuff when formulating diets for dairy cows (Firkins, 1997; Bradford and Mullins, 2012). As general characteristics, NFFS have typically very small particle size, they are low in lignin and starch, with highly digestible fiber, and variable soluble fiber and sugar concentrations (Bradford and Mullins, 2012). In fact, the highly digestible NDF of NFFS can supply substantial amounts of ruminal fermentable organic matter resulting in a more constant production of VFA compared to high-starch concentrate diets (Stock et al., 2000).

The most common NFFS used in the formulation of diets for dairy cows include soybean hulls, beet pulp, citrus pulp, corn gluten feed, and distillers grains. Soybean hulls are low in lignin (2.59%) and high in NDFD (82.2%) and glucose precursors (39.5%). In fact, approximately 75% of polymers of glucose are recovered in the NDF fraction (Miron et al., 2001). Feeding soybean hulls in replacement of corn resulted in a positive associative effect on fiber digestion, which was reported to be independent of increases in rumen pH (Ipharraguerre and Clark, 2003). Beet pulp contains approximately 41% NDF with similar NDFD (80%) as soybean hulls, but with higher content of NFC (45.7%). However, beet pulp has a high concentration of soluble NDF, especially pectins that are

degraded more rapidly than cellulose and hemicellulose in the rumen (Pereira and Gonzalez, 2004). The dilution of NFC with beet pulp NDF resulted in a low rate of ruminal fermentation and reduced acid load, which lessened the risk of ruminal acidosis (Teimouri Yansari , 2013). Citrus pulp may contain 20% sugar, with similar NDFD (79%) as beet pulp, and higher NFC (59%) than soybean hulls. The digestible energy of citrus pulp is similar to ground corn, but the ruminal fermentation of dried citrus pulp resulted in greater acetic to propionic acid ratio compared with ground corn. Therefore, on an energy basis, dried citrus pulp may replace corn in dairy diets (Wickes and Bartsch, 1978). Corn gluten feed contains approximately 25% CP, 12% starch, 13% sugar, and 1.6% lignin. The energy in wet corn gluten feed is on average 93% of the energy value of ground shelled corn (Firkins et al., 1985) and can be a source of highly digestible carbohydrates to supply energy when included in dairy cow diets (Miron et al., 2001; NRC, 2001; Schroeder, 2003). Distillers dried grains with solubles (DDGS) are traditionally used as a protein source (31.4% CP) when formulating diets for dairy cows; however, due to their high concentration of digestible NDF (54%) and fat content (12%), DDGS have been reported to be an excellent source of energy for dairy cattle (Schingoethe et al., 2009). Overall, the variation in nutrient composition among NFFS, the high fiber digestibility, and in some NFFS, the high sugar or fat content allow to partially replace corn starch in dairy cows diets. Furthermore, the substitution by NFFS with cereal grains could represent an opportunity to reduce feed costs when formulating diets, while still maintaining energy density.

Low and High Starch Diets in Dairy Cows

As the cow does not have specific starch requirement, its concentration in diets of lactating dairy cows can vary from 15% to more than 30%. However, formulating high starch diets (>28%) when corn grain price is high, negatively affects the profitability of the farm (Grant et al., 2013). Based on six years (2003-2008) feed and milk prices, Ranathunga et al. (2010) estimated an income over feed cost (IOFC) of 7.02, 7.40, 7.98, and 8.44 \$/cow/day when ground corn was reduced with DDGS and soybean hulls to formulate diets with 29, 26, 23, and 20% starch, respectively. In this study, DMI decreased linearly in response to decreased starch concentration in the diets, without affecting milk production, milk composition, and blood measurements (glucose and BHB). It was demonstrated that IOFC can be higher in low starch diets. In addition, high starch diets have negative effects on cow health by increasing the risk of subclinical and clinical rumen acidosis. Gott et al. (2015) demonstrated that cows fed diets to induce milk fat depression (29% starch and 32% NDF) and diets to induce rumen acidosis (32% starch and 30% NDF) altered milk fatty acid profile and resulted in milk fat depression compared to cows fed normal starch diets (24% starch and 35% NDF). Therefore, formulating high starch diets can increase feeding costs and increase the incidence of acidosis in the herd.

On the other hand, low starch diets reduce acidosis while maintaining milk production and composition. There were no acidosis episodes in lactating dairy cows fed diets with 18.2% starch (Fredin et al., 2015b) or 21.5% starch (Dann et al., 2015) compared to cows fed on average 26% starch. In these studies, DMI, milk production and milk composition were similar between treatments. However, these effects were not

observed when the stage of lactation or milk production was factored in the analysis. Milk production and milk composition were not altered when cows received high (25.9%) or low starch (20.1%) diets in early lactation. Nonetheless, when cows in late lactation were fed high starch diets (24.7%) milk production (0.9 kg/d) and milk protein (0.08 kg/d) increased when compared to cows fed low starch diets (15.3%; Piccioli-Cappelli et al., 2014). The authors of these studies stated that undetected milk production response could be related to the limited capacity of the mammary gland to absorb glucose from the blood during early lactation. When the level of milk production was considered, Boerman et al. (2015) showed that cows producing more than 30 kg/d milk responded positively to high starch diets (30%), but cows producing less than this amount maintained milk production even when fed a diet with 12.2% starch. This indicated that high producing dairy cows have greater glucose requirements than low producing cows. Therefore, dairy cow productivity in response to dietary starch concentration can vary in the same cow depending on the stage of lactation.

Recommended Dietary Starch Concentration During Lactation

Due to the physiological changes during lactation, dietary starch recommendation in dairy cows differs between the fresh period, early to mid-lactation, and late lactation. Fresh cows need glucose precursors because their intake is low and the glucose supply for milk production is reduced. As a result, starch concentrations of 24-26% are recommended immediately after calving (Lean et al., 2013). McCarthy et al. (2015) evaluated high (26.2%) and low starch (21.5%) diets from day 1 to 21 postpartum. Feed intake and milk production were 0.8 kg/d and 1.2 kg/d higher, respectively, in cows on high starch diets than in those on low starch diets; percentages of milk fat, milk protein,

milk lactose, and total solids, however, were lower in cows on high starch diets. In addition, cows fed high starch diets lost less body condition and had greater plasma concentration of glucose and insulin and lesser non-esterified fatty acids (NEFA), compared to cows fed low starch diets. This suggests high starch diets contributed to improved energy balance in early lactation cows. Grains with floury starch with high rate of fermentation such as wheat, barley, and high moisture corn should be used with caution to avoid acidosis. Rather, dry ground corn is recommended to increase the starch level in fresh cow diets (Allen and Piantoni, 2014).

Similar to the fresh period, cows in early- to mid-lactation require high glucose supply to support high milk production. At this stage of lactation cows have a positive response to starch availability so starch concentrations of 25 to 30% are recommended (Allen and Piantoni, 2014). Voelker and Allen (2003a) evaluated mid-lactation diets with four starch concentrations (35, 31, 27, and 18%) using high moisture corn and beet pulp to reduce dietary corn. The authors reported a linear decrease in DMI and a quadratic response in milk production as dietary starch decreased from 35 to 18%. Similarly, Batajoo and Shaver (1994) formulated diets for mid-lactating dairy cows with four starch levels (30, 26, 21, and 15) using wheat middlings, brewer's dried grains, and soybean hulls, in replacement of dry ground corn. Dry matter intake and milk protein percentage and yield decreased linearly as starch was reduced with those NFFS, while milk fat percentage was reduced in higher starch diets. Therefore, high starch diets during mid lactation can have a positive response on milk production but can compromise milk fat percentage.

When compared to cows in early- and mid-lactation, cows in late-lactation required less glucose for milk production, as a result a range of 18 to 22% starch has been recommended in late lactation diets (Allen and Piantoni, 2014). Gheman et al. (2006) found similar DMI and milk production response when dairy cows in late lactation grazing ryegrass (1.8% starch) were supplemented with three different starch concentrations (38.6, 29.2%, and 20.3%). Milk protein percentage (2.80%) and yield (0.81 kg/d) tended to be greater for cows fed diets formulated at the 38.6 and 29.2% starch concentrations, compared to cows fed the diet formulated to 20.3% (2.70% and 0.80 kg/d). More recently, Piccioli-Cappelli et al. (2014) demonstrated similar DMI between late lactation dairy cows fed high- (24.7%) and low-starch diets (15.4%), however, milk production was 0.9 kg/d greater in cows fed the high starch diet. In this experiment, milk fat percentage decreased 0.22 percentage units in cows fed high starch, whereas milk protein percentage was 0.11% greater. Therefore, it appears that diets formulated to a very low starch content (less than 18% starch as reported by Allen and Piantoni, 2014), will compromise milk production in cows in late lactation, needing diets formulated with 20% starch (Gehman et al., 2006).

Interaction of Starch with Other Nutrients

Dietary Starch and Crude Protein

Synchronization of starch and CP in diets has a large impact on rumen function optimization, efficiency of energy and nitrogen use, and productive response of dairy cows. The availability of MP (metabolized protein; used for maintenance, growth, fetal growth, and milk production) in the small intestine depends mainly on the microbial protein synthesis in the rumen and the dietary RUP (rumen undegradable protein; NRC,

2001). Rumen microbial protein can contribute between 50-79% of the total MP used by the cow, but its yield is driven by the availability of nitrogen and energy from carbohydrates (Block, 2006). Of the carbohydrates, starch has been demonstrated to have more influence (85.6 mg/mg OM (organic matter)) on microbial protein synthesis than NDF (40.4 TCACP mg/mgOM), sucrose (73.3 mg/mg OM), and pectin (75.4 mg/mg OM; Hall and Herejk, 2001). Failure to synchronize starch and CP degradation in the rumen results in a large amount of free ammonia-N, which is absorbed across the rumen wall, contributing to the urea synthesis in the liver and later excreted in urine and milk. As a result, a decrease in the energy used for microbial protein synthesis (Mutsvangwa, 2011), leads to a decline in the MP supply and the productivity of the animal.

Being more specific, the availability of AA (amino acids) in the small intestine increases because of microbial protein synthesis is increased in response to the optimal synchronization of dietary starch and CP. In fact, rumen microbial protein represents the major source of amino acids to the animal and it has been estimated that the composition of bacteria and protozoa is 104.7 g/100 g AA of EAA (essential amino acids) and 95.6 g/100 g AA of NEAA (non-essential amino acids), respectively (Orskov, 1982). At similar dietary CP intakes, Čerešňáková et al. (2006) reported a greater passage of total AA to the duodenum in non-lactating dairy cows fed corn meal (EAA = 127.2% intake and NEAA = 135.1% intake) compared to cows fed wheat meal (EAA = 104.5% intake and NEAA = 89.2% intake). McCarthy et al. (1989) found that the flow of total AA to the duodenum in lactating dairy cows, was 194 g/d more in dairy cows fed 45% starch from corn than in those fed 40% starch from barley at similar CP (14.75%). In a recent experiment by Fanchone et al. (2013) dairy cows fed starch-based diets (30.7% starch)

had a higher flow of EAA (948.5 g/d) and NEAA (1457.5 g/d) to the duodenum compared to those fed fiber-based diets (15.2% starch; EAA = 847.5 g/d and NEAA = 1300 g/d) in diets formulated with either, 11% or 14.75% CP. The high starch diets were formulated using corn, wheat, and barley, and the low starch diets by increasing soybean hulls. In this study, milk protein concentration was greater with high starch diets indicating that the increased supply of AA to the small intestine can contribute to milk protein synthesis. This effect was confirmed by Cantalapiedra-Hijar et al. (2014) when formulating diets differing in CP (12 and 16%) and starch content (4.4 and 34.5%) by varying the proportions of grass silage, grass hay, dehydrated corn plant pellets, corn, barley, wheat, wheat bran, soybean hulls, citrus pulp, beet pulp, tannin-treated soybean meal, and urea. High starch diets increased the microbial N (nitrogen) flow to the duodenum and the recovery of total AA in the portal vein, with no differences in hepatic use. This resulted in increased AA release (22%) to the splanchnic tissue and increased milk protein yield (7%). The authors indicated that the greater transfer of N from feed to milk in high starch diets resulted from the lower energy requirement by the PDV and the higher microbial flow to the duodenum. Therefore, optimal synchronization of dietary starch and CP allows improvement of the energy and N use through their incorporation into rumen microbial protein used for milk protein synthesis.

Dietary Starch and Fiber

The starch-fiber interaction contributes also to an optimal rumen function and dairy cow productivity. The negative interaction between starch and ruminal NDF digestion is well known; it has even been estimated that NDF degradation declines from 65% with no starch intake to 30% with an intake of 10 kg of starch per day (Bannink and

Tamminga, 2005). This is the result of starch fermentation to VFA and greater organic acid production. This surpasses the cow's buffering capacity, which results in decreased rumen pH which restricts the growth of cellulolytic bacteria. When rumen pH is less than 5.2, the fermentation shifts to lactic acid fermentation leading to sub-clinical acidosis, which can then progress to acute ruminal acidosis concurrent with milk fat depression (Slyter et al., 1970; Nocek, 1997; Enemark et al., 2004). These effects are not only observed with low dietary NDF and high starch concentration, but also when in high starch diets, peNDF (physically effective neutral detergent fiber) is less than 9% (Beauchemin, 2007). In addition, the source of starch plays an important role, when those with higher degradation rates (i.e. wheat vs. corn) are used when formulating diets for dairy cows (Gulmez and Turkem, 2007).

The fiber to starch ratio is another possible explanation for the interaction starch-NDF in dairy cows. Beckman and Weiss (2005) formulated diets with different NDF:starch ratios (0.74, 0.95, and 1.25) by increasing soybean and cottonseed hulls. Digestion of NDF was unaffected, but starch digestibility increased linearly as the NDF:starch ratio increased without reducing rumen pH and milk production parameters. Zhao et al. (2016) used greater ranges of NDF to starch ratios (0.86, 1.18, 1.63, and 2.34) by increasing hay and reducing corn grain. The authors reported a linear decrease of total nutrient digestion (DM, OM, NDF, and CP), DMI, and milk production as the NDF:starch ratio increased. Milk fat percentage and rumen pH increased as the NDF:starch ratio went from 0.86 to 2.63, indicating that lower NDF:starch ratios caused rumen acidosis. Therefore, the optimal NDF:starch ratio for dairy cows could be in the range of 0.95-1.63; however, the effect of the NDF and starch sources was not evaluated.

The interaction of starch with fiber of different degradation, such as NDF from BMR varieties, can be different. Cell walls of corn BMR hybrids contain less lignin (7 g/kg; Sattler et al., 2010) and reduced syringyl-type lignin than CONV corn silage (guaiacyl-type lignin; Jung and Allen, 1995), which contributes to improve cell wall digestion. Moreover, the digestion of starch and NDF are 30 and 15 g/kg, respectively, greater in BMR corn silage than CONV corn silage (Sattler et al., 2010). In a meta-analysis comparing CONV corn silage with BMR corn silage, Ferraretto and Shaver (2015) reported that total tract NDFD was 2.6% greater in dairy cows fed BMR corn silage diets (24 treatment means) than those fed CONV corn silage diets (38 treatment means). Total tract starch digestion however was 1.4% greater when cows were fed CONV corn silage diets. A direct effect of starch on NDF digestibility from BMR corn silage has not been investigated.

Methane Production in Response to Dietary Starch and Fiber

One of the main factor affecting CH₄ emission in dairy cows is the type of carbohydrate included in the diet. The fermentation of sugars to VFA in the rumen produce metabolic H₂ in form of reduced equivalents (i.e. NADH; Nicotinamide adenine dinucleotide), which is later converted to CH₄ by Archaea species (Knapp et al., 2014). Compared to acetate and butyrate, the synthesis of propionate consume more reduced equivalents, as a result diets stimulating higher proportions of propionate in the rumen can reduce CH₄ production (Van Soest and Nisbet, 1996). Hattar et al. (2015) formulated diets with slowly fermentable starch (275 and 518 g/kg of concentrate DM) and rapidly fermentable starch (303 and 542 g/kg of concentrate DM) by using normal and gelatinized corn grain and beet pulp resulting in four starch concentrations (11.0,

20.7, 12.1, and 21.7% starch). Increasing the rate of starch fermentation and its concentration in the diets reduced CH₄ production per unit of rumen fermentable organic matter. However, CH₄ emission per unit of feed dry matter intake or per unit of milk produced was similar among treatments. In this study, there was a greater proportion of propionate when rapidly fermentable starch was fed, which can be related to the reduced CH₄ production.

Methane production increases 2-5 times more with the fermentation of cellulose and hemicellulose than the fermentation of NFC (Moe and Tyrrell, 1979). This might be related to the higher proportions of acetate and butyrate produced in the rumen with high fiber diets. This in turn has a large impact on the DMI and the NDF digestibility in dairy cows. Thus, high quality forages with less lignified NDF that increases fiber digestibility and allows for greater DMI were related to reduced CH₄ per unit of intake (Beauchemin and McGinn, 2006, Archimède et al., 2011). Hammond et al. (2016) designed an experiment to evaluate the effects of diets formulated with corn silage low (34.2%) and high (40.1%) and diets with grass silage low (36.6%) and high (39.8%) NDF on CH₄ emission in dairy cows. Chopped barley straw and soybean hulls were used to increase dietary NDF, and CH₄ emissions were measured by using either Green Feed or respiratory chambers techniques. Methane emissions were similar between Green Feed and the respiratory chamber methods, with 24% lower CH₄ emissions in cows fed corn silage-based diets compared to grass silage-based diets. The addition of NDF with barley starch and soybean hulls to both types of forage diets increased CH₄ emissions and reduced DMI and milk production. In addition, there was a trend for an interaction between forage type and NDF treatment. Yields of CH₄ tended to increase when NDF

was included in corn silage-based diets, but not in grass silage-based diets. Diets without NDF addition were formulated with 21% starch, and diets with added NDF had 15.1% starch. The authors speculated that those effects are related to the starch concentrations in the diets and the NDF type between forage sources. Therefore, the effects of the interaction between NDF type and starch concentration on CH₄ emissions in lactating dairy cows needs further research.

Conclusions

Starch is a polymer carbohydrate synthesized in leaves but stored mainly in seeds. As most cereal grains are rich in starch, they are used as the main energy source in dairy cow diets. Available energy from cereal grains depends on the source, processing method, and animal behavior, which improves digestion and ultimate VFA production and glucose supply by the animal. Regarding the source, corn grain is the main cereal grain used for diet formulation in U.S. dairy farms. Nevertheless, as corn is highly demanded for human and other animal species consumption as well for the ethanol production and sweetener industries, its price fluctuates often, stimulating research for less expensive alternatives.

Reducing dietary starch from corn grain by including NFFS in dairy cow diets has been proposed as an alternative to cope with high corn grain prices. So far, the scientific dairy community has explored and recommended a variety of NFFS such as soybean hulls, beet pulp, citrus pulp, corn gluten feed, and DDGS to substitute in part corn grain in the diets. Studies have demonstrated promising positive effects on the performance of lactating dairy cows of replacing starch from cereal grains with NFFS. However, the potential effects of reducing dietary starch with NFFS in diets of dairy cows with

different protein sources and corn silage with altered NDF digestibility needs more research. In addition, there is limited information on gas emissions in lactating dairy cows as affected by fiber digestion from different corn silages and dietary starch.

The objectives of this research were to evaluate the effects on dairy cow performance of reducing starch from corn grain in diets formulated with soybean hulls, beet pulp, SBM, CM, and CONV and BMR corn silage. The impact of dietary starch and fiber digestibility from corn silage type on nutrient digestibility and gas emissions in lactating dairy cows was also assessed.

CHAPTER 2:
**EFFECTS OF STARCH CONCENTRATION IN DIETS WITH SOYBEAN OR
CANOLA MEAL ON THE PERFORMANCE OF LACTATING DAIRY COWS**

Abstract

This study was designed to evaluate the effects of substituting corn grain starch with non-forage fiber sources in diets containing SBM or CM as the primary protein sources. Sixteen Holstein cows were assigned to a 4×4 Latin square design with 4 periods of 28 d. Treatments were arranged as a 2×2 factorial with two protein sources (SBM and CM) and two dietary starch concentrations (21 and 27% DM). Diets were formulated to contain 16.5% CP and the lesser starch concentration was obtained by replacing corn grain with soybean hulls and beet pulp. Protein source \times starch interactions ($P < 0.05$) were observed for DMI, milk fat and protein concentrations, milk protein yield, MUN, and FE. Cows fed the 27% starch with any protein source had more DMI than cows fed the CM-21% starch ($P=0.03$). Milk fat concentration was reduced in cows fed CM-27% starch ($P=0.003$). Milk protein concentration and yield, and milk lactose concentration were least for CM-21% starch compared with the other three diets ($P < 0.05$), but FE was greater in cows fed CM-21% starch ($P=0.03$). Milk urea nitrogen was least for cows fed CM-27% starch compared with the other 3 diets ($P=0.03$). There was a starch effect on milk yield and ECM. Cows fed diets with 27% starch produced on average 2.5 kg/d milk and 1.9 kg/d ECM more compared to cows fed 21% starch. Rumen fermentation parameters were affected by the interaction CP source \times starch. Acetate concentration was the least in cows fed CM-21% starch, but similar to cows fed CM-27% starch regardless of the protein source ($P=0.01$). However, propionate concentration was

the highest in CM-21% starch, but similar to cows with CM-27% starch regardless of the protein source ($P=0.03$). Isobutyrate concentration was the highest for cows in CM-21% starch ($P=0.04$), but acetate to propionate ratio was the lowest in these cows, being similar to cows fed CM-27% starch ($P=0.01$). Those differences in rumen organic acids explain partially the effects of dietary starch on milk fat concentration. Apparent total tract nutrient digestibility differed with starch concentration and CP sources. In cows fed 21% digestibility of DM and OM starch was reduced by 2.7% ($P=0.001$) and 2.6% ($P=0.002$), respectively. Digestibility of NDF and ADF was higher in diets with SBM than those with CM ($P<0.05$). Reduced DM and OM digestibility explain mainly the negative effects observed of reduced dietary starch on DMI, milk yield, and ECM. These results demonstrated that reducing dietary starch by replacing corn grain with soybean hulls and beet pulp had a negative effect on lactating dairy cow performance, regardless of the CP source in the diet.

Keywords: dietary starch, crude protein source, cow performance

Introduction

In diets of lactating dairy cows, SBM is the main protein source whereas corn grain is used as the main energy source. When commodity prices are high, CM can replace SBM (Broderick et al., 2015), and NFFS can be used to reduce corn grain in the diet (Dann and Grant, 2009). As a result, the potential effects of reducing corn grain with NFFS in diets of SBM and CM deserved to be investigated.

A comprehensive literature review showed that SBM as a source of dietary RUP in dairy cows milking more than 30 kg per day was able to maintain DMI, milk production and composition, as well as FE (Santos and Huber, 1995; Huber and Santos, 1996; Santos et al., 1998). Increases in SBM prices have prompted the substitution of this protein source with CM without negatively affecting dairy cow performance. An evaluation of 122 studies comparing the feeding value of SBM and CM in dairy cows demonstrated a greater DMI (26 g/kg increase in CP intake) and milk yield for CM (3.4 kg/kg/d increase in CP intake) compared with SBM diets (11 g/kg and 2.4 kg/kg/d incremental CP; Huhtanen et al., 2011). More recently, a meta-analysis by Martineau et al. (2013) found positive responses in DMI (0.24 kg/cow/day), fat corrected milk (0.85 kg/cow/day), efficiency of corrected milk (0.84 kg/cow/day), milk protein (48 g/cow/day) and fat yield (28 g/cow/day) when CM replaced different protein sources including SBM.

For energy sources, the greater values of total tract digestibility (>90%; Galvayan et al., 1979; Owens et al., 1986; Overton et al., 1995) and the in vitro microbial protein synthesis (Hall and Herejk, 2001) from corn starch are the main factors that explain sustaining high milk production in lactating dairy cows. Recent price increases in corn grain have prompted scientists to evaluate its substitution with NFFS, which results in

rations with much lower starch content. Ipharraguerre and Clark (2003) indicated that rumen fermentation, nutrient digestion, and the performance of mid- to late lactation dairy cows were not negatively affected in high-grain diets where soyhulls replaced corn grain to supply about 30% of the dry matter. Guo et al. (2014) did not report changes in DMI, milk yield, and feed efficiency when ground corn was substituted by beet pulp in dairy cow diets, with the benefits of improved fiber digestion and reducing sub-acute rumen acidosis (Guo et al., 2013).

Replacing SBM with CM and reducing starch concentration of the diet by replacing corn grain with NFFS are good alternatives when commodity prices are high. Currently, no information is available that evaluated the substitution of SBM and corn grain with CM and NFFS, respectively, within a single study. Therefore, the objective of this study was to evaluate the replacement of starch with NFFS using either SBM or CM as the primary protein sources on milk production, rumen fermentation, and nutrient digestion of lactating dairy cows. The hypothesis was that the use of soybean hulls and beet pulp as sources of NFFS to reduce corn grain in CM diets would similarly support milk production, rumen fermentation, and nutrient digestion when compared to SBM diets.

Materials and Methods

Cows, Treatments, and Diets

The experiment was conducted at the Dairy Research and Training Facility at South Dakota State University and all procedures were approved by the South Dakota Institutional Animal Care and Use Committee. Sixteen Holstein cows [4 primiparous (135±17 DIM (days in milk) at the beginning of the study and 629±99 kg of BW) and 12 multiparous (111±46 DIM at the beginning of the study and 733±71 kg of BW)] were used in a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments. Each experimental period consisted of 28-d separated into 14-d for adaptation and 14-d for sampling and data collection. Dietary treatments consisted of two protein sources (SBM and CM) and two starch concentrations (27% and 21%). The proportions of SBM and CM were maintained similar within their respective dietary starch treatments, and the reduced starch concentration was achieved by replacing 9.0 percentage units of ground corn with soybean hulls and dry beet pulp (Table 1). All other ingredients were maintained at the same proportions across diets. Diets were formulated to meet the requirements of a mature Holstein cow with 703 kg of BW and a BCS of 3.0 at 90 DIM and producing 45 kg of milk containing 3.5% fat and 3.0% protein (NRC, 2001). Diets were fed as TMR once daily (0800 h; Calan Broadbent feeder door system, American Calan Inc., Northwood, NH) adjusting to allow for ad libitum intake (10% orts) with unlimited access to water.

Measurements and Sampling

Feed intake was measured individually in all cows during the entire experiment by recording feed offered and orts daily. Forage samples were collected once a week to adjust for DM. Samples of TMR, corn silage, and alfalfa haylage were collected twice a

week during wk 3 and 4. Samples of SBM, CM, ground corn, soybean hulls, and dry beet pulp were collected during wk 4 every period during the whole experiment. All of these samples were stored at -20°C until analyzed. Additionally, 500 g TMR samples were taken twice during wk 3 and 4 to determine particle size distribution (Heinrichs, 2013).

Rumen fluid samples were collected with an esophageal tube and a hand-operated pump approximately 3 h after feeding on the last 2 consecutive days of wk 4 in each experimental period. Approximately the first 200 mL of sampled fluid were discarded to reduce potential contamination with saliva. Rumen fluid pH was measured immediately and then 2 aliquots of 10 mL each were acidified with either 200 μL of 50% (vol/vol) sulfuric acid or 2 mL of 25% (wt/vol) metaphosphoric acid for their respective analysis of ammonia and VFA. Samples were stored at -20°C until analysis.

Blood samples were collected in all cows by venipuncture of the tail (coccygeal) vein on 2 consecutive days during wk 4 in each period, approximately 3 h after feeding. Blood was drawn into 10 mL vacutainer tubes containing lithium heparin for plasma urea N and BHBA analysis, and 7 mL vacutainer tubes containing sodium fluoride-potassium oxalate for glucose analysis (Becton, Dickinson, and Company, Franklin Lakes, NJ). Blood samples were centrifuged at $2,400 \times g$ for 20 min at 5°C and then the plasma stored at -20°C for later analysis.

Fecal samples were collected in all cows on 3 consecutive days during wk 4 in each experimental period to estimate total tract nutrient digestion. In total, 12 fecal samples (from 6 to 8 h intervals) per cow with approximately 20 g each were collected directly from the rectum or spontaneous release in all animals, and then composited by cow and experimental period.

Cows were milked thrice daily at 0600, 1400, and 2100 h, with milk production recorded daily. Individual milk samples from each milking were collected 2 consecutive days in wk 3 and wk 4 and sent to a commercial laboratory for milk components analysis (Dairy Herd Improvement Association (DHIA); MQT Lab Services, Kansas City, MO).

Body weight of each cow was recorded for three consecutive days approximately 3 h after feeding at the beginning and end of each experimental period. On weighing day BCS was evaluated by 5 individuals according to Wildman et al. (1982).

Laboratory Analysis

Samples of forages, TMR, feces, and individual feedstuffs were dried at 55°C for 48 h in a Despatch oven (style V-23, Despatch Oven Co., Minneapolis, MN). Samples of TMR and individual samples were further dried at 105°C for 3 h in a Precision oven (Model 28, Precision Scientific CO, Chicago Illinois). All dried samples were ground to a 4-mm particle size (Wiley mill, model 3, Arthur H. Thomas Co., Philadelphia, PA), and then further ground to 1-mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). Ground samples were then analyzed for CP, NDF, ADF, ether extract, starch, and ash. Crude protein percentages were determined by analyzing total N using a combustion assay (Leco FP-2000 N Analyzer, Leco Instruments Inc., St. Joseph, MI). The analysis of NDF was determined by using sodium sulfite and α -amylase (Van Soest et al., 1991), and ADF was analyzed sequentially by using an Ankom fiber analyzer (Ankom Technology Corp., Macedon, NY). Ether extract was analyzed with the Ankom extractor using petroleum ether as solvent (920.39; AOAC International, 1998). Starch was analyzed on sub-samples ground through a 1-mm screen of an abrasion mill (Udy Corp., Fort Collins, CO) using the methodology proposed by

Hall (2015). Samples were incubated in screw cap tubes with thermostable α -amylase in 30 mL sodium acetate (pH 5.0) for 1 h at 100°C with periodic mixing (initial vortex, and then vortexed at 10, 30, and 50 min) to gelatinize and partially hydrolyze α -glucan. Amyloglucosidase was then added to the samples, and the reaction mixture incubated in a water bath at 50°C for 2 h then hand mixed once (vortexed after the first hour). After incubation, 20 mL of distilled water was added and tubes were inverted $\pm 10\times$ to mix completely. Approximately 2 mL of the solution was centrifuged at $1000 \times g$ for 10 min and then 0.1 mL working test solution and standards (in duplicate) were added into 16×100 mm glass tubes. Finally, 3.0 mL of glucose oxidase–peroxidase was added to each glass tube, and the tubes were vortexed, covered with plastic film to seal, and incubated in a 50°C water bath for 20 min. Absorbance was read at 505 nm. Samples of TMR, forages, and individual feeds were corrected for free glucose. Ash concentration was analyzed by heating 1 g of sample for 8 h at 450°C in a muffle furnace (942.05; AOAC International, 1998). Organic matter (OM) was calculated as $100 - \% \text{ ash}$. Non-fibrous carbohydrates were calculated based on nutrient analysis as $100 - (\% \text{ CP} + \% \text{ NDF} + \% \text{ EE} + \% \text{ ash})$ according to the NRC (2001). Individual forage and feedstuff analysis along with the proportion of each ingredient in the ration were used to calculate the chemical composition of experimental diets. In addition, TMR samples were also analyzed to validate the calculated chemical composition of the diets. Mineral analysis (Ca, P, Mg, K, and S) in TMR samples was performed using wet chemistry by Dairyland Laboratories, Inc. (Arcadia, WI). Particle size distribution was determined by using the 4-screen Penn State Particle Separator in fresh TMR samples replacing the 1.9 mm screen with 8 mm screen (Heinrichs, 2013).

Rumen fluid samples were thawed and vortexed to mix the contents. From samples reserved for VFA analysis, 2 mL of rumen fluid were centrifuged at $10,000 \times g$ for 20 min at 10°C in a micro-centrifuge (model A-14, Jouan Inc., Winchester, VA). Volatile fatty acid concentrations were measured using an automated gas chromatograph (model 6890, Hewlett-Packard, Palo Alto, CA) equipped with a 0.25 mm i.d. \times 15 m column (Nukol 24106-U, Supelco Inc., Bellefonte, PA). The flow rate was set at 1.3 mL/min of helium, maintaining the column and detector temperatures at 140 and 250°C , respectively. The internal standard used was 2-ethylbutyrate. Samples collected for ammonia N were centrifuged at $10,000 \times g$ for 10 min at 4°C then analyzed according to Chaney and Marbach (1962).

Plasma metabolites were analyzed with commercial enzymatic or colorimetric kits using a micro-plate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA). Serum glucose was analyzed by the glucose oxidase reagent (Cat. No G7521. Pointe Scientific Inc., Canton, MI) as described by Trinder (1969). Plasma urea N was analyzed with the methodology diacetylmonoxime (Stanbio Laboratory, Boerne, TX). Serum BHB was determined by the β -Hydroxybutyrate reagent (Cat. No H7587-58. Pointe Scientific Inc., Canton, MI) according to Williamson et al. (1962).

Total tract nutrient digestion was determined *in situ* using iADF by incubating bags (pore size of $25 \mu\text{m}$) inside the rumen of two cows during 288-h (Huhtanen et al., 1994). Analysis of DM, OM, CP, NDF, ADF, and starch in TMR and fecal samples along with the internal marker were utilized to estimate the total tract nutrient digestibilities following the equation used by Ferrareto et al. (2015): apparent total tract nutrient

digestibility (%) = $100 - [100 * (\text{TMR marker concentration} / \text{fecal marker concentration}) \times (\text{fecal nutrient concentration} / \text{TMR nutrient concentration})]$.

Milk samples were sent to Heart of America DHIA Laboratory (Manhattan, KS) for analysis of fat, protein, lactose, TS, MUN, and SCC. Milk fat, protein, and lactose concentrations were analyzed at that laboratory using midinfrared spectroscopy (AOAC International, 2006; Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Concentration of MUN was analyzed using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments). Somatic cell counts were determined using a flow cytometer laser (Somacount 500, Bentley Instruments), and then we converted to a linear SCS. Nitrogen fractions in milk were analyzed according to the method described in AOAC (2006), which considered total milk protein (method 991.20), non-protein nitrogen (method 991.21), and non-casein nitrogen (method 998.05). True protein and casein nitrogen were calculated using the methods 991.23 and 998.07, respectively.

Statistical Analysis

All data were analyzed by the MIXED procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC) for a Latin square design. The statistical analysis considered TMR particle size, daily DMI, and daily means for milk production and milk components concentration and yield from the last two weeks of each experimental period. The analysis for rumen fermentation and blood variables considered the average of two days from week 4 in each period. Nutrient digestion variables included averages from the 3 days of sampling in week 4. Body weight and BCS were analyzed considered the

measurements per experimental period. The effect of dietary treatments was evaluated with the following model:

$$Y_{ijklm} = \mu + PS_i + St_j + (PS \times St)_{ij} + Sq_k + (PS \times Sq)_{ik} + (St \times Sq)_{jk} + (PS \times St \times Sq)_{ijk} + P_l + C_{m(Sqk)} + e_{ijklm}$$

Where: Y_{ijklm} = dependent variable, μ = overall mean, PS_i = effect of protein source i ($i = 1$ to 2), St_j = effect of starch concentration j ($j = 1$ to 2), $(PS \times St)_{ij}$ = interaction between protein source i and starch concentration j , Sq_k = effect of square k ($k = 1$ to 4), P_l = effect of period l ($l = 1$ to 4), $C_{m(Sqk)}$ = effect of cow m ($m = 1$ to 4) nested within square k , and e_{ijklm} = random residual error. The experimental design used cow as experimental unit and cow (square) as the random variable. Data were reported as least square means and the Tukey's test was used to separate treatment means if there is an interaction between protein source and starch concentration. Interactions that were found not significant ($P \geq 0.05$) were removed from the model. Statistical significance for all analysis was declared at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

Results and Discussion

Nutrient Composition of Diets, Feeds, and Particle Size of Diets

Nutrient composition of diets and individual feeds are indicated in Table 1 and 2, respectively. Crude protein content was similar across diets, but NDF and ADF concentrations were greater in diets with 21% starch than in those with 27% starch regardless of the protein source. This likely reflects of the substitution of dietary starch with soybean hulls and beet pulp. There was on average an actual difference of 6.35% starch concentration between diets with 27 and 21% starch; all other nutrient concentration were comparable across the diets.

Particle size of the diets did not differ by varying the starch concentration suggesting the addition of soyhulls and beet pulp in substitution for part of the ground corn did not alter the physical form of the diets (Table 3; $P>0.05$). It has been demonstrated that particle size distribution is not affected when soybean hulls (Akins et al., 2014) or beet pulp (Poorkasegaran and Yansari, 2014) substitute for corn grain in the diets. However, in the present study, SBM diets had a greater proportion of particles retained in the upper pan of the particle separator compared to CM diets ($P=0.008$).

Performance, Rumen Fermentation, Plasma Metabolites, and Nutrient Digestion

Lactation responses of dairy cows fed different protein sources and starch concentrations are in Table 4. Significant interactions ($P<0.05$) between protein source and starch concentration were observed for DMI, concentrations of milk fat, protein, and lactose, as well as milk protein yield, feed efficiency, and MUN. Cows fed the SBM or CM diets formulated at 27% starch consumed greater DMI (27.2 kg/d) than cows fed CM-21% starch (24.7 kg/d). Other researchers reported similar results for DMI when beet pulp (Voelker and Allen, 2003a) or soybean hulls (Batajoo and Shaver, 1994; Ipharraguirre et al., 2002b; Aikman et al., 2006) were used to reduce starch from corn grain. In cows fed beet pulp, factors such as high rumen digesta volume and weight, and water-holding capacity cause rumen distension and can limit DMI (Voelker and Allen, 2003b). For diet with soybean hulls, decreased DMI was related to increased NDF concentration (Batajoo and Shaver, 1994) and dietary bulk as indicated by the increased eating time and associative effects on gut fill (Aikman et al., 2006).

Milk fat percentage was the least (3.86%) in cows fed CM-27% starch (Table 4), however, this effect was not observed for the same starch concentration in SBM diets.

Increasing dietary carbohydrates may have a negative effect on milk fat concentration because of the increase in ruminal organic acids, which consequently reduce ruminal pH (Robinson et al., 1987; Sievert and Shaver, 1993a; Batajoo and Shaver, 1994). No differences, however, were detected for rumen pH when increasing the starch in the diets of this study (Table 5; $P>0.05$). The reduced milk fat content in cows fed CM-27% starch could be partially explained by the low concentration of acetate, isobutyrate, and acetate to propionate ratio in the rumen of cows affected by the interaction of protein source \times starch level ($P<0.05$). On the other hand, high milk fat concentration in cows fed SBM-21% starch can be partially explained by high rumen isobutyrate content in these cows as an effect of the interaction protein source \times starch (Table 5; $P=0.04$).

According to the protein source \times starch interaction, the lowest values of milk protein percentage (3.15%) and yield (1.5 kg/d), and lactose percentage (4.08%) were observed in cows fed CM-21% starch (Table 4; $P<0.05$). Other studies have reported similar results in that feeding cows low starch diets showed a negative impact on milk protein content and yield (Batajoo and Shaver, 1994; Aikman et al., 2006; Almeida et al., 2014), along with lactose concentration (Cabrita et al., 2007). These effects may be related to the decrease of microbial growth and subsequently protein synthesis in response to a shortage of available starch in the rumen (Hall and Herejk, 2001) and glucogenic nutrients to the cow (Cabrita et al., 2007). However, it was not possible in this study to detect lack of glucogenic nutrients in cows fed CM-21% starch as measured by plasma glucose concentration as indicator of energy supply to the cow (Table 6; $P>0.05$).

Feed efficiency (ECM/DMI) was greatest (1.66) in cows fed CM-21% starch and least (1.53) in cows fed CM-27% starch (Table 5; $P=0.03$). High feed efficiency has been

reported in reduced starch diets (Aikman et al., 2006; Nelson et al., 2011), and it has been associated with lower DMI (Shaver, 2010). This was similar to the low DMI intake and high feed efficiency (Table 4) observed in cows fed CM-21% starch in this study. It has been observed that body tissue mobilization can contribute to increased feed efficiency (Vallimont et al., 2011); however, in the present study, BW and BCS (Table 4), together with BHB concentrations, were not different between diets (Table 6; $P>0.05$); there was only a trend observed for loss of BW (1.21 kg/d; $P=0.09$) in cows fed CM-21% starch.

Milk urea nitrogen values ranged from 11.20 to 12.81 mg/dL across the treatments with the least value in cows fed CM-27% starch (Table 4; $P=0.03$). Similar results have been reported in the literature in cows fed high starch diets (Gencoglu et al., 2010; Nelson et al., 2011). This can be explained as an effect of an improved carbohydrate and protein balance in the rumen of cows fed CM-27% starch that would allow for better protein utilization by the animal (Butler 1998; Faciola and Broderick, 2014). However, it was not possible to detect effects of dietary treatments on $\text{NH}_3\text{-N}$ as an indicator of an improvement in protein utilization by the animal (Table 5; $P>0.05$). Those effects are in line to a certain degree with the lowest values of PUN in cows fed CM-27% starch regardless of the protein source (Table 6; $P<0.05$). This would suggest that more dietary nitrogen was incorporated into microbial protein synthesis and thus less nitrogen was available for ureagenesis, which decreased blood urea nitrogen in this treatment (Kohn, 2007).

On the other hand, milk yield and ECM were affected by dietary starch concentration (Table 4; $P<0.05$). Cows fed 27% starch diets produced more milk (2.5 kg/d) and ECM (1.9 kg/d) compared to cows fed 21% starch diets. Using soybean hulls to

reduce starch content in dairy cows diets decreased DMI without any negative effects on milk yield (Batajoo and Shaver, 1994; Ipharraguirre et al., 2002b; Aikman et al., 2006). This suggests that milk yield can be maintained when highly digestible fiber (soybean hulls, brewer's dried grains, and wheat middlings) replaces starch as a source of energy in lactating dairy cow diets. In the present study, the reduction of milk yield and ECM in cows fed CM-21% starch paralleled the lower DMI observed in these animals. It seems that cows fed CM-21% starch mobilized fat to support milk production, since there was a trend observed to decrease BW in these animals (Table 4; $P=0.09$).

Lactose and total solids yields differed by starch concentration, whereas percentage of total solids differed by protein source. Lactose yield was higher in cows fed 27% starch diets (1.83 kg/d) compared to cows in 21% starch diets (1.78 kg/d).

Lemosquet et al. (2010) indicated that milk lactose and protein yields in dairy cows increased in response to increasing supply of intestinal protein or glucogenic nutrients. Similar effects were observed in cows fed 27% starch diets in this study, however more information about metabolism could help explain these results. Cows fed 27% starch diets yielded more total milk solids than cows on 21% starch diets. No statistical differences for BCS, BCS change, and milk fat percentage were observed in this study among treatments.

Apparent total tract nutrient digestibility is shown in Table 7. Starch by protein source interaction significantly affected CP intake, indicating the highest intake (4.36 kg/d; $P=0.04$) in cows fed CM-27% starch; however, CP digestibility was similar between treatments ($P=0.69$). Higher DM and OM intakes were observed in cows fed SBM-27% starch and CM-27% starch regardless of the protein source ($P=0.04$). Their

digestibility was affected by protein source and dietary starch concentration. Cows fed SBM had a higher average DM (2.8%) and OM (2.9%) digestibility ($P<0.05$) than cows fed CM diet. Also cows fed 27% starch diets showed greater DM (2.7%) and OM (2.9%) digestibility compared to cows on 21% starch ($P<0.05$). Intake of NDF and ADF tended to differ because of the protein source by starch ($P=0.08$) interaction. Their digestibility's however were significantly affected by protein source ($P<0.05$), indicating a lower digestibility for cows fed CM diets when compared to SBM diets. Cows fed 27% starch had higher starch intake (2.1 kg/d) and starch digestibility (1.98%) compared to cows fed 21% starch diets ($P<0.05$). The greater DM, OM and starch digestibilities in cows fed the 27% starch diets, rather than different protein sources, also explains the positive effect of these diets on DMI, milk yield, and ECM observed in cows in the present study. Batajoo and Shaver (1994) found a decreasing linear effect of DM digestibility in response to the gradual decrease of dietary starch (32.9, 28.5, and 24%) as the proportion of soybean hulls, brewer's dried grains, and wheat middlings in the diet increased. van Vuuren et al. (2010) reported increased duodenal flow of microbial OM in high starch diets formulated with corn grain compared to low starch diets formulated with dry bet pulp. Therefore, decreasing starch from corn grain with soybean hulls and beet pulp reduced DM and OM digestibility, which has a negative effect on dairy cow performance.

Conclusions

The use of soybean hulls and beet pulp to replace a portion of starch from corn grain in either SBM and CM diets negatively affected DMI, milk yield, ECM, milk protein and lactose concentrations, and total solids yield. Increasing starch concentrations in CM diets decreased milk fat concentrations, which is partially explained by a low concentration

of acetate, isobutyrate, and acetate to propionate ratio in the rumen. However, decreasing starch concentration in CM diets improved FE. Increased dietary starch concentration in CM diets seems to improve protein balance in the cow because less MUN was observed. Moreover, increased dietary starch improved DM and OM digestibility regardless of the protein source, which supports the positive effect of high starch diets on the performance of lactating dairy cows.

Table 1. Ingredient and nutrient composition of experimental diets containing SBM and CM with different starch concentrations

Item	SBM		CM	
	21%	27%	21%	27%
Ingredients, % of DM				
Corn silage	31.46	31.46	31.46	31.46
Alfalfa haylage	20.58	20.58	20.58	20.58
Whole cottonseed	4.08	4.08	4.08	4.08
Soybean meal (47.5% CP)	9.01	9.01	-	-
Canola meal	-	-	12.93	12.93
Ground corn grain	13.10	22.11	13.10	22.11
Soybean hulls	7.18	6.02	4.97	2.93
Beet pulp	10.58	2.55	9.18	2.04
Rumen-inert fat ¹	1.53	1.53	1.53	1.53
Urea	0.05	0.05	0.05	0.05
Rumen-protected met ²	0.07	0.07	0.07	0.07
Limestone	0.51	0.68	0.51	0.68
Dicalcium phosphate	0.51	0.51	0.20	0.20
Salt, white	0.34	0.34	0.34	0.34
Mineral and vitamin premix ³	0.26	0.26	0.26	0.26
Magnesium oxide	0.19	0.19	0.19	0.19
Sodium bicarbonate	0.56	0.56	0.56	0.56
Nutrients, % of DM				
DM, % of diet	57.57	57.28	57.57	57.29
CP	15.45	15.38	15.20	15.37
NDF	29.82	26.87	30.63	27.50
NDF from forages	17.17	17.21	17.12	17.15
ADF	18.60	16.51	19.01	16.74
Starch	20.10	26.34	19.84	26.30
NFC ⁴	42.93	46.59	42.18	45.72
Ether extract	5.16	5.43	5.04	5.29
Ash	8.13	7.26	8.09	7.29
Ca	0.93	0.93	0.85	0.88
P	0.39	0.36	0.40	0.40
Mg	0.37	0.36	0.39	0.39
K	1.38	1.33	1.23	1.24
S	0.20	0.19	0.21	0.22

SBM = soybean meal; CM = canola meal; 21% and 27% = starch concentrations in the diets.

¹Energy Booster 100 (Milk Specialties Global, Co., Dundee, IL).

²Smartamine M (Adisseo Inc. Alpharetta, GA)

³Contained: Vitamin A, 3,740,000 IU/kg; vitamin D3, 935,000 IU/kg; vitamin E, 12,155 IU/kg; Menadione, 18.7 Mg/kg; Choline, 622.6 Mg/kg; Iron, 0.49%; Zinc, 3.49%; Manganese, 3.48%; Copper, 7,507 mg/kg Iodine, 499 mg/kg; Cobalt, 327 mg/kg; Selenium, 165 mg/kg (Ridley Feed Ingredients, Mendota, IL).

⁴NFC = 100 - (% NDF + % CP + % EE + % ash).

Table 2. Nutrient composition of forages and feeds used in diets containing SBM and CM with different starch concentrations

Nutrient, % of DM (unless noted)	Corn silage	Alfalfa Haylage	Canola meal	Soybean meal	Ground corn	Beet pulp	Soybean hulls	Cottonseed
DM	39.74	46.35	91.03	89.95	87.13	92.22	91.46	91.90
CP	6.26	24.26	38.83	51.34	7.54	6.76	11.42	21.40
NDF	35.52	29.16	27.68	8.59	9.36	39.01	61.01	52.04
ADF	19.02	23.54	16.60	4.35	2.37	22.73	43.88	38.01
Starch	34.52	0.21	2.39	1.31	69.34	0.94	0.69	0.11
Ether extract	1.85	2.16	2.67	0.91	2.66	0.04	1.28	15.00

Table 3. Particle size distribution of diets containing SBM and CM with different starch concentrations

Screen ² , % retained in each sieve	SBM ¹		CM		SEM	Effect ³ (<i>P</i> > <i>F</i>)		
	21%	27%	21%	27%		PS	St	PS × St
Upper (>19.0 mm)	3.83	3.97	3.15	3.30	0.20	0.008	0.47	1.00
Middle (19.0-8.0 mm)	37.88	38.60	36.58	37.1	0.65	0.06	0.36	0.88
Lower (8.0-4.0 mm)	14.73	15.00	14.15	14.73	1.05	0.70	0.70	0.89
Bottom pan (<4 mm)	43.60	42.45	46.15	44.88	1.34	0.10	0.39	0.96

^{ab}Means across rows with different superscripts differ significantly (*P*<0.05).

¹SBM = soybean meal; CM = canola meal; 21% and 27% = starch concentration in diets.

²Particle size distribution measured according to Heinrichs (2013).

³PS = protein source effect (SBM vs CM); St = dietary starch effect (21% vs 27%); PS × St = interaction protein source by starch concentration.

Table 4. Lactation performance of cows fed diets containing SBM and CM with different starch concentrations

Item	SBM ¹		CM		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	21%	27%	21%	27%		PS	St	PS × St
DMI, kg/d	26.2 ^{ab}	26.8 ^a	24.7 ^b	27.7 ^a	0.90	0.57	0.001	0.03
Milk, kg/d	36.5	38.5	36.6	39.7	1.30	0.36	0.002	0.49
Fat, %	4.08 ^{ab}	4.14 ^a	4.21 ^a	3.86 ^b	0.16	0.24	0.02	0.003
Fat, kg/d	1.49	1.57	1.53	1.51	0.06	0.82	0.41	0.24
Protein, %	3.28 ^a	3.26 ^a	3.15 ^b	3.26 ^a	0.06	0.05	0.15	0.03
Protein, kg/d	1.20 ^{ab}	1.24 ^a	1.15 ^b	1.28 ^a	0.04	0.82	0.001	0.03
Lactose, %	4.79 ^a	4.77 ^{ab}	4.68 ^b	4.78 ^a	0.03	0.07	0.13	0.02
Lactose, kg/d	1.84	1.74	1.72	1.91	0.06	0.67	0.002	0.34
Total solids, %	13.07	13.00	12.86	12.74	0.19	0.01	0.31	0.78
Total solids, kg/d	4.76	4.98	4.69	5.03	0.15	0.93	0.006	0.55
MUN, mg/dL	12.81 ^a	12.38 ^a	12.62 ^a	11.20 ^b	0.53	0.005	0.001	0.03
SCS ³	4.72	4.47	4.73	4.46	0.37	0.99	0.28	0.97
ECM, ⁴ kg/d	39.8	41.8	40.0	41.8	1.23	0.88	0.02	0.92
ECM/DMI	1.53 ^b	1.57 ^{ab}	1.66 ^a	1.53 ^b	0.06	0.27	0.26	0.03
BW, kg	716.7	663.3	716.8	724.1	33.4	0.30	0.43	0.30
BW change, kg/d	8.84	13.54	-1.21	13.59	5.80	0.38	0.37	0.09
BCS ⁵	3.05	2.89	3.07	3.10	0.14	0.34	0.55	0.43
BCS change	0.45	0.28	0.48	0.57	0.12	0.20	0.76	0.29

^{ab}Means across rows with different superscripts differ significantly (*P* < 0.05).

¹SBM = soybean meal; CM = canola meal; 21% and 27% = starch concentration in diets.

²PS = protein source effect (SBM vs CM); St = dietary starch effect (21% vs 27%); PS × St = interaction protein source by starch concentration.

³SCS = log(SCC).

⁴ECM = [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein yield (kg)].

⁵Body condition score: 1 = emaciated to 5 = obese (Wildman et al., 1982).

Table 5. Rumen fermentation of cows fed diets containing SBM and CM with different starch concentrations

Item	SBM ¹		CM		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	21%	27%	21%	27%		PS	St	PS × St
pH	6.78	6.91	6.89	6.84	0.07	0.81	0.52	0.19
NH ₃ -N, mg/dL	9.81	9.06	8.44	8.23	0.91	0.11	0.47	0.69
Total VFA, mM	98.32	97.28	84.54	94.61	3.9	0.04	0.24	0.15
VFA (Molar % total)								
Acetate	63.36 ^a	61.94 ^{ab}	60.83 ^b	61.88 ^{ab}	0.63	0.008	0.70	0.01
Propionate	21.15 ^b	22.60 ^{ab}	24.21 ^a	23.12 ^{ab}	0.72	0.003	0.76	0.03
Butyrate	10.64	10.51	9.88	10.15	0.28	0.04	0.80	0.48
Isobutyrate	1.77 ^b	1.79 ^b	1.94 ^a	1.82 ^b	0.03	0.01	0.14	0.04
Valerate	1.36	1.46	1.43	1.44	0.04	0.57	0.25	0.33
Isovalerate	1.70	1.66	1.70	1.60	0.07	0.67	0.32	0.46
Acetate to propionate	3.08 ^a	2.87 ^{ab}	2.55 ^c	2.76 ^{bc}	0.10	0.001	0.95	0.01

^{abc}Means across rows with different superscripts differ significantly (*P* < 0.05).

¹SBM = soybean meal; CM = canola meal; 21% and 27% = starch concentration in diets.

²PS = protein source effect (SBM vs CM); St = dietary starch effect (21% vs 27%); PS × St = interaction protein source by starch concentration.

Table 6. Plasma metabolites of cows fed diets containing SBM and CM with different starch concentrations

Item	SBM ¹		CM		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	21%	27%	21%	27%		PS	St	PS × St
Glucose, mg/dL	73.82	72.71	73.70	72.60	1.53	0.92	0.33	0.99
BHB, mmol/L	0.93	0.92	0.89	0.89	0.05	0.19	0.92	0.68
PUN, mg/dL	17.26 ^b	16.01 ^b	19.91 ^a	16.24 ^b	0.72	0.02	0.001	0.05

^{ab}Means across rows with different superscripts differ significantly (*P*<0.05).

¹SBM = soybean meal; CM = canola meal; 21% and 27% = starch concentration in diets.

²PS = protein source effect (SBM vs CM); St = dietary starch effect (21% vs 27%); PS × St = interaction protein source by starch concentration.

Table 7. Apparent total tract nutrient digestibility of cows fed diets containing SBM and CM with different starch concentrations

Item	SBM ¹		CM		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	21%	27%	21%	27%		PS	St	PS × St
Intake, kg/d								
DM	25.94	26.16	24.74	28.35	1.00	0.58	0.04	0.06
OM	25.28	25.49	24.14	27.52	0.98	0.60	0.04	0.07
CP	4.0 ^{ab}	4.02 ^{ab}	3.76 ^b	4.36 ^a	0.15	0.74	0.03	0.04
NDF	7.73	7.03	7.57	7.80	0.29	0.23	0.34	0.08
ADF	4.82	4.32	4.69	4.74	0.18	0.35	0.15	0.08
Starch	5.21	6.89	4.94	7.45	0.24	0.50	<0.001	0.06
Digestibility, %								
DM	67.38	68.79	63.28	67.32	0.87	0.001	0.001	0.10
OM	68.07	69.29	63.79	67.78	0.85	0.001	0.002	0.08
CP	67.38	69.78	67.63	67.09	1.21	0.27	0.39	0.69
NDF	51.84	48.46	47.90	47.11	1.40	0.04	0.11	0.31
ADF	51.74	48.74	47.14	45.87	1.39	0.004	0.08	0.47
Starch	93.78	95.12	93.77	96.38	0.66	0.34	0.004	0.32

^{ab}Means across rows with different superscripts differ significantly (*P*<0.05).

¹SBM = soybean meal; CM = canola meal; 21% and 27% = starch concentration in diets.

²PS = protein source effect (SBM vs CM); St = dietary starch effect (21% vs 27%); PS × St = interaction protein source by starch concentration.

CHAPTER 3:
EVALUATION OF REDUCING CEREAL GRAINS STARCH WITH
NONFORAGE FIBER SOURCES IN DIETS OF DAIRY COWS: A META-
ANALYSIS

Abstract

The objective of this meta-analysis was to evaluate the effects of reducing dietary starch from cereal grains by including NFFS on the productive response of lactating dairy cows. Thirty-nine studies were selected that used NFFS to replace a portion of dietary cereal grains starch and that reported one or more of the following: cow performance, rumen fermentation, or total tract nutrient digestion. Data were analyzed through regression analysis by the mixed effect models procedure of R using the study as a random effect. The variance explained by the models was evaluated calculating marginal $R^2_{(m)}$ and conditional $R^2_{(c)}$. The heteroscedasticity and normality of the models were evaluated with residuals and Q-Q plots. Cow performance evaluation showed that when dietary starch intake increased, DMI and milk fat yield responded quadratically; milk yield, milk protein concentration, and milk lactose yield increased linearly, whereas milk fat concentration decreased linearly. No effect of starch intake on milk lactose concentration was observed. As dietary starch intake increased total volatile fatty acids (VFA) and acetate concentration in rumen fluid decreased linearly, but propionate, acetate to propionate ratio, isobutyrate, isovalerate, and valerate increased linearly. No significant effects were found for rumen pH and NH_3 concentration. Increased dietary starch intake affected nutrient DM digestibility quadratically, increased CP digestibility linearly, and reduced NDF digestibility linearly. Dietary starch intake did not affect the

digestion of OM or starch. Higher values of $R^2_{(m)}$ and $R^2_{(c)}$ were observed in significant models compared to non-significant ones, indicating a better goodness-of-fit of significant models. Residuals and Q-Q plots of the models obtained were symmetrical and their errors were at least normally distributed, with the exception of milk fat and acetate concentration models. Additionally, intake of DM, CP and NDF, as well as DIM contributed to the variation of the models. It is important to highlight that diets formulated with NFFS ($25.94 \pm 11.52\%$ as DM basis) had lower DMI, milk yield, and milk protein concentration than cows fed diet high in cereal grains ($27.48 \pm 11.52\%$ as DM basis). Cows on NFFS however may present higher milk fat percentage. Therefore, all those factors should be taken into account when NFFS are used to reduce starch from cereal grains in lactating dairy cow diets.

Keywords: meta-analysis, starch intake, milk production.

Introduction

Starch concentration reduction in dairy cow diets is warranted when cereal grain prices increase or simply when there is a risk of causing acidosis problems. Lower dietary inclusion of costly grains can improve IOFC (St-Pierre and Knapp, 2008; Ghebremichael et al., 2009) and potentially spare cereal grains for other more profitable enterprises (CAST, 2013). Non-forage fiber sources have been traditionally recommended to reduce cereal grain starch in the diet since they contain monosaccharides (Miron et al., 2001) and highly digestible fiber that can maintain or even improve the performance of dairy cattle (Bradford and Mullins, 2012). However, the effects of decreasing dietary starch concentration by partially replacing cereal grains with NFFS on the productivity of lactating dairy cows remains debatable.

Research has evaluated the replacement of cereal grains with different NFFS on the performance of lactating dairy cows. Combining soybean hulls with brewer's dried grains and wheat middlings (Batajoo and Shaver, 1994) or with DDGS (Ranathunga et al., 2010) to reduce corn grain starch concentration, indicated a similar effect on milk production than with high starch diets, although DMI was reduced in both experiments when NFFS were included. But, DMI increased linearly when soybean hulls and cottonseeds were increased to reduce wheat starch (Beckman and Weiss, 2005). Other NFFS such as beet and citrus pulp have also been investigated. Reducing starch in the diet by partially replacing barley with beet pulp resulted in similar DMI and milk production (Silveira et al., 2007). When beet pulp was used however replacing high moisture corn, DMI decreased linearly without affecting milk production (Voelker and Allen, 2003a). Almeida et al. (2014) found that when formulating diets with increased

amounts of citrus pulp to reduce starch from finely ground corn, cows reduced DMI and milk production. However, when citrus pulp was combined with soybean hulls and corn germ meal to replace finely ground corn, DMI and milk production were not significantly affected (McCarthy et al., 2015).

Some relevant reviews have described nutritional approaches when NFFS are incorporated to diets of lactating dairy cows. Firkins (1997) described the digestion kinetics of NFFS and determined that dietary NDF from NFFS had more contribution (two-thirds) to the total tract NDF digestibility when compared to forage NDF. In addition, this author stated that replacing starch with NFFS increases fiber digestibility as a result of reduced negative associative effects. More recently, Bradford and Mullins (2012) concluded that when NFFS replace forages, DMI in cows increased, but physical effectiveness of the diet decreased. The authors specified that the partial replacement of starch with NFFS can optimize nutrient utilization in the cows without compromising animal health.

Although these review papers mentioned the potential of NFFS to replace starch in diets of lactating dairy cows, this effect has not been evaluated under a meta-analytic procedure and did not specifically address the starch reduction from cereal grains. Moreover, information generated assessing the effects of the partial dietary substitution of cereal grains with NFFS on dairy cow performance has been inconsistent. Therefore, the objective of this meta-analysis was to evaluate the effects of reducing dietary starch from cereal grains by including NFFS on the productive response of lactating dairy cows. It was hypothesized that productive response would be at least similar between cows fed reduced starch diets with NFFS as cows fed high starch diets

Materials and Methods

Data Search

A relevant literature search was conducted mainly in two steps. First, peer-review manuscripts were identified through Web of Science (<https://apps.webofknowledge.com/>), PubMed (<https://www.ncbi.nlm.nih.gov/pubmed/>), Scopus (<https://www.scopus.com/home.uri>), and Google Scholar (<http://scholar.google.com/>) using a combination of the keywords “dairy cows”, “starch”, “low-starch” or “high-starch”, “non-forage fiber sources” or “NFFS”, “non-fiber carbohydrates” or “NFC”, “non-soluble carbohydrates” or “NSC”. Additionally, some of the previous keywords along with the specific NFFS were also utilized; for instance, “dairy cows and citrus pulp”. Second, once a certain number of papers were identified, specific published papers cited in their references section were identified and then searched directly in the journals or in the web.

Selection Criteria and Data Extraction

Papers for conducting the meta-analysis were selected based on the following criteria: (1) the information should have been published only in peer-reviewed manuscripts, (2) experiments should have used lactating dairy cows as experimental units, (3) manuscripts should have reported all or either, DMI, milk production and composition, rumen fermentation parameters, and total tract nutrient digestibility, (4) experiments should have been designed to replace dietary starch with one or a blend of different NFFS, (5) diets within each experiment should have maintained the same proportion of forage across treatments; when two levels of forage and different dietary starch concentrations were evaluated within the same experiment, they were treated as

separate trials, (6) experiments should not have evaluated cows under grazing conditions, and (7) cows fed the experimental diets should have been producing more than 15 kg/d milk. After the screening procedure, 39 manuscripts published from 1982 to 2016 were selected for conducting the meta-analysis (Table 8).

Data extraction consisted of collecting information related to diet, cow performance, rumen fermentation, and total tract nutrient digestion. Diet information considered percentages of each ingredient and DM, OM, and individual nutrient composition (CP, NDF, ADF, EE, NFC, starch, and ash). When dietary starch was not reported, it was then estimated using the proportion of each ingredient in the diet and 16 year-average (2000-2016) starch concentration of each ingredient reported in the Interactive Feed Composition Library of Dairy One laboratory (<http://dairyone.com/>). Cow performance included DMI, BW, BCS, milk yield, FCM, ECM, feed efficiency, and percentages and yields of milk fat, protein, and lactose. Intakes of OM and individual nutrients (CP, NDF, ADF, EE, and starch) were also calculated based on nutrient composition of the diets, and DMI. Nutrient digestion considered the digestibility of DM, OM, CP, NDF, ADF, and starch. Rumen fermentation measurements included rumen pH and NH₃-N total VFA, concentrations of acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, and acetate to propionate ratio. Almost all cow variables in the data set considered their respective standard error of the mean. All variables were not available in all manuscripts so the number of observations used in the meta-analysis varied depending on their availability.

Statistical Analysis

Variables of cow performance, total tract nutrient digestion, and rumen fermentation measurements were weighed according to their standard error (St-Pierre, 2001). When papers reported SED, the standard error of the mean was calculated as $SEM = SED/\sqrt{2}$ (Roman-Garcia et al., 2016). It has been reported that SEM in mixed models has a tendency to be higher than the SEM obtained with General Linear Models (Littel et al., 1998, Littell et al., 2000). To prevent overweighing data with very low SEM obtained from those models, high SEM were trimmed at half or to one-fourth of the mean SEM across trials. Then, the reciprocals of the SEM were calculated as $1/SEM$, which avoid giving too much weight for data derived from Latin square designs (Roman-Garcia et al., 2016). Trimming processes and calculation of reciprocals were done separately for each model procedure (GLM and mixed models). Finally, to center weighing factors to 1 the reciprocals were standardized to the mean of their respective distributions (Roman-Garcia et al., 2016).

Reduction of dietary starch with NFFS was evaluated by the mixed-effect models approach of R (2015). Linear, quadratic, and cubic effects of starch intake on cows' productive response were modeled using study as a random effect (St-Pierre, 2001) according to the following model:

$$Y_{ij} = \beta_0 + T_i + \beta_1 St_{ij} + \beta_2 St_{ij}^2 + \beta_3 St_{ij}^3 + b_i St_{ij} + e_{ij}$$

Where:

Y_{ij} = dependent variables representing performance, rumen fermentation or total tract nutrient digestibility variables at the level j of the variable S in the study i .

β_0 = overall fixed intercept across studies.

β_1 , β_2 , and β_3 = the overall (linear, quadratic, and cubic) fixed regression coefficients of Y on St across studies.

T_i = random effect of study i .

St_{ij} = independent variable representing starch intake (kg/d) at the level j of the continuous variable St in study i .

b_i = random effect of study i on the regression coefficient of Y on St in study i .

e_{ij} = unexplained residual error.

To explore other potential factors affecting response variables (cow performance, total tract nutrient digestion, and rumen fermentation measurements) a stepwise regression analysis was conducted. Intakes of DM, CP, and NDF as well as DIM were including in the model as independent variables and all the possible two-way interactions with starch intake as well as linear, quadratic, and cubic effects were evaluated. Intakes of OM, ADF, EE, and NFC were not considered in the model because data was limited. Some response variables such as BW, BCS, FCM, ECM, feed efficiency, and ADF digestibility were not included either in the meta-analysis due to incomplete data. Nonsignificant interactions and main effects ($P > 0.05$) were removed sequentially from the model during the stepwise selection procedure. When one or more independent variables were tested in the model along with starch intake, the existence of multicollinearity was quantified with the variance inflation factor (VIF). A VIF value greater than 10 was considered to remove variables from the model. Additionally, the best fit model was chosen based on the lowest Akaike information criterion (AIC) and Root mean square error (RMSE). As the analysis was performed using the mixed models procedure, it was necessary to quantify the goodness-of-fit of the models by estimating

marginal $R^2_{(m)}$ (proportion of variance explained by the fixed factor) and conditional $R^2_{(c)}$ (proportion of variance explained by both fixed and random effects; Nakagawa and Schielzeth, 2013). Pan and Lin (2005) indicated that evaluating goodness-of-fit of a model generated by the mixed model procedure with the simple R^2 does not account for the proofs of the asymptotic distribution of the cumulative sum and the consistence of the tests derived from the random effects. Stepwise regression analysis, the assessment of multicollinearity, and goodness-of-fit of a model were evaluated by the mixed-effect models approach of R (2015) using the nlme package (Pinheiro *et al.*, 2016). The heteroscedasticity of final models was evaluated by checking the residual vs. fitted values plot and the normality through the Q-Q plot. Significance was set at $P < 0.05$ for all variables evaluated, and trends were established at $0.05 < P \leq 0.10$.

Results and Discussion

Data Description

A summary of descriptive statistics is in Table 9. The total number of observations in the variables evaluated varied according to what was reported in selected papers. Starch intake averaged 5.10 kg/d across studies with a range between 0.32 and 9.08 kg/d. Intake of CP also varied, but the minimum and maximum values of NDF intake indicated higher variations in this variable. Wide range of starch and NDF intakes is an effect of the inclusion of NFFS to reduce the starch concentration in the diets. Substantial ranges were also observed for cow, rumen fermentation, and total tract nutrient digestion variables. Similar mean and median values for all evaluated variables indicated that data was normally distributed.

Cow Performance

Results of linear and quadratic regression of dairy cow performance to different dietary starch intake are indicated in Table 10. Dry matter intake responded quadratically ($P=0.01$) to the increase of dietary starch intake, but milk yield increased linearly ($P<0.0001$) with starch intake. Increasing dietary starch intake reduced linearly milk fat concentration ($P<0.0001$) but increased milk protein concentration linearly ($P<0.0001$). Milk fat yield responded quadratically ($P=0.03$) and yields of milk protein ($P=0.0003$) and milk lactose ($P=0.02$) increased linearly with increased dietary starch intake. Responses of milk components yield result from the combination of increased milk yield and their respective milk component percentage. Milk lactose percentage was not affected by starch intake ($P=0.91$). There was a stronger relationship in significant models ($R^2_{(m)} = 0.003-0.04$; $R^2_{(c)} > 0.90$) compared to non-significant models ($R^2_{(m)} = 0.00004$; $R^2_{(c)} = 0.88$).

Figures 1 and 2 show a graphic representation of the relationship between dietary starch intake and production variables. Higher starch intake corresponded to cows fed diets with more grain (average inclusion: $27.48 \pm 11.52\%$ as DM basis) compared to those fed NFFS (average inclusión: $25.94 \pm 11.52\%$ as DM basis). However, as dietary starch intake increased DMI, decreased (Figure 1a), which could be because of subclinical/clinical acidosis (Oetzel, 2003). However, in this meta-analysis, it was not possible to detect changes in rumen pH by increasing dietary starch intake (Table 12). It is important to point out the effect of diets with NFFS to the parameters in the graphs. Cows fed diets with high NFFS ($25.94 \pm 11.52\%$ as DM basis) to reduce dietary starch had the lowest DMI (Figure 1a), milk yield (Figure 1a), and milk protein concentration

(Figure 2a), whereas the same diets increased milk fat concentration (Figure 2b). Limited DMI and milk yield have been observed when sources of NFFS such as wet corn gluten feed (Staples et al., 1984), soybean hulls plus brewer's dried grains (Batajoo and Shaver, 1994), and DDGS (Schingoethe et al., 1999) were used to replace highly digestible carbohydrates. Similar to the effect in the present study, the replacement of corn grain with NFFS improved milk fat concentration (Weiss, 2012). The positive response of dietary starch on microbial protein synthesis has been well-documented (Herrera-Saldan et al., 1990; Clark et al., 1992). Cows in high dietary starch intake produced a high amount of microbial protein, which contributed to increase milk yield and milk protein concentration.

Although productive performance variables were significantly related to dietary starch intake, there are other dietary nutrients and animal variables contributing to cow performance (Table 11). Intake of DM, CP, NDF, and starch \times NDF, as well as DIM, and starch \times DIM affected feed intake, milk production, and milk composition. The proportion of variance explained by the fixed factors was better ($R^2_{(m)} = 0.11-0.81$) for these equations than for equations using only starch intake. The proportion of variance explained by both fixed and random factors however, was similar between equations ($R^2_{(c)} > 0.90$). Therefore, the intake of those nutrients and cow production parameters must be considered beyond dietary starch when including NFFS to reduce grains in dairy cow diets.

Rumen Fermentation

Table 12 shows the response of rumen fermentation variables to dietary starch intake. Rumen pH and NH_3 concentration were not affected by increasing dietary starch

intake, but the increase of starch intake tended to reduce linearly total VFA ($P = 0.09$) and acetate to propionate ratio ($P = 0.06$). Similarly, acetate concentration in the rumen decreased linearly as dietary starch intake increased ($P = 0.0004$). In contrast, increasing starch intake resulted in a linear increase of the concentrations of propionate ($P = 0.01$), isobutyrate ($P = 0.03$), isovalerate ($P = 0.002$), and valerate ($P = 0.04$). The statistical differences and trend effects, as well as marginal (0.004-0.03) and conditional R^2 (>0.90) values in the present meta-analysis indicated that after adjusting for differences between studies, dietary starch intake contributed to changes in the rumen fermentation parameters. It is well known that starch fermentation increases the concentration of propionate (Raun, 1961; Rémond et al., 1995) but reduces acetate concentration in the rumen (Smith, 1961; Rémond et al., 1995; Gao and Oba, 2016). The increased propionate concentration might have affected DMI since it has been suggested to play an important role in feed intake regulation by affecting satiety and hunger (Oba and Allen, 2003). The increased propionate, valerate, and isobutyrate may explain the increase in milk yield in the current meta-analysis. These metabolites are glucogenic precursors for the net synthesis of glucose (Reynolds et al., 2003; Larsen and Kristensen, 2009) used to synthesize lactose, the main determinant of milk yield (Aschenbach et al., 2010). On the other hand, low rumen acetate along with high propionate concentrations may be associated with the decrease of milk fat concentration as it has been demonstrated to increase *trans*-10, *cis*-12 CLA isomer in the rumen, which in turn reduce milk fat synthesis in the mammary gland (Bauman and Griinari, 2001).

Multiple regression equations demonstrated that rumen $\text{NH}_3\text{-N}$ was affected linearly by CP intake. Total VFA concentrations were negatively affected by NDF intake,

and DMI was positively contributing to total VFA, acetate, and isobutyrate (Table 13).

An empirical approach to estimate production of total and individual organic acids in the rumen of lactating dairy cows found that DMI explained changes of the molar proportion of total and individual VFA (Nozière et al., 2010).

Total Tract Nutrient Digestibility

The relationship of dietary starch intake and total tract nutrient digestion variables is shown in Table 14. The digestion of DM ($P = 0.04$) and CP ($P = 0.01$) responded quadratically to dietary starch intake. The digestion of NDF decreased linearly ($P < 0.0001$) with the increase of starch intake. No relationship was observed between dietary starch intake and digestion of OM and starch. The best prediction was confirmed with a higher relationship of significant models ($R^2_{(m)}$ 0.03-0.24; $R^2_{(c)}$ 0.84-0.94) than non-significant ones ($R^2_{(m)} = 0.00009-0.005$; $R^2_{(c)} = 0.80-0.90$). The quadratic effect of DM digestibility along with rumen propionate concentrations explain the quadratic effect of DMI as dietary starch increased. In addition, the quadratic response of CP intake contributed partially to the increase of milk yield when dietary starch intake increased. The negative effect of increasing starch intake on NDF digestibility is well known (Mertens and Loften, 1980), and it is the result of decreasing the fibrolytic activity in the rumen with low pH as dietary starch increases (Hoover, 1986; Lechartier and Peyraud, 2011). Since fiber degrading bacteria are the main acetate producers, this inverse relationship between dietary starch intake with rumen acetate concentration and NDF digestibility was confirmed in the current meta-analysis, although no effects of starch intake on rumen pH was detected.

Intakes of DM and CP contributed significantly to the variation of OM and NDF intake and affected negatively the digestibility of DM, OM, and NDF (Table 15). However, the interaction of starch \times NDF intake influenced positively NDF digestibility. It is clear that intake of NDF explained better the total tract nutrient digestibility than other nutrients. This is the result of dietary starch of the diets being replaced with highly digestible NDF from NFFS which contributed to total NDF intake in the cows.

Heteroscedasticity and Normality

The diagnostic of the heteroscedasticity and normality for the variables evaluated are in Figure 3. Residuals vs. fitted plots for performance, rumen fermentation, and total tract nutrient digestion are symmetrical and their errors are at least normally distributed. In addition, Q-Q plots of these models confirmed that errors belong to a normal distribution. However, residuals vs. fitted plots and Q-Q plots of milk fat and rumen acetate concentration are asymmetrical. Therefore, those models should be interpreted with caution.

Conclusions

This meta-analysis demonstrated that reducing starch from cereal grain with NFFS had significant effects on cow performance, rumen fermentation, and total tract nutrient digestion. As dietary starch intake increased, DMI and milk fat yield responded quadratically, but milk yield, milk protein concentration, and milk lactose yield increased linearly. In addition, milk fat concentration decreased linearly as dietary starch intake increased. The effect of DMI may be explained by the quadratic and linear response of DM digestibility and propionate concentration in the rumen as starch intake increased, respectively. Milk yield and milk protein concentration might be the result of increasing

rumen propionate, isobutyrate, isovalerate, and valerate along with the increase of CP digestion and potentially microbial protein synthesis as dietary starch intake increased. Reduced milk fat concentration can be an effect of reduced NDF digestion and rumen acetate as starch intake increased, which could increase *trans*-10, *cis*-12 CLA isomer in the rumen and affect milk fat synthesis in the mammary gland. However, those last relationships should be interpreted with caution because of the variability of these parameters across the range of dietary starch intakes. It is important to mention that intakes of DM, CP, and NDF, as well, as DIM contributed significantly to the variation of all variables measured. Moreover, it was illustrated that cows fed diets with NFFS ($25.94 \pm 11.52\%$ as DM basis) had lower DMI, milk yield, and milk protein concentration than cows fed diets high in cereal grains ($27.48 \pm 11.52\%$ as DM basis), although those cows produced high milk fat concentration. Therefore, all those factors should be taken into consideration simultaneously when NFFS are used to reduce cereal grains starch in the diets of lactating dairy cows.

Table 8. Studies included in the meta-analysis

Authors and year	Journal (volume:pages)	Cereal grain source	NFFS
Grings et al., 1982	J. Dairy Sci. (75:1946-1953)	Ground corn	DDGS, beet pulp Hominy feed, potato pulp, wheat bran, DDGS
MacGregor et al., 1983	J. Dairy Sci. (66:39-50)	Ground corn, oats grain	DDGS
Robinson et al., 1986	Livest. Prod. Sci (15:173-189)	Corn hominy feed, tapioca	Soybean hulls, dried beet pulp
Sutton et al., 1987 (comparison 1)	J. Agric. Sci. Camb. (109:375-386)	Barley, wheat, cassava	Beet pulp, citrus pulp, wheat feed
Sutton et al., 1987 (comparison 2)	J. Agric. Sci. Camb. (109:375-386)	Barley, wheat, cassava	Beet pulp, citrus pulp, wheat feed
Nakamura and Owen, 1989	J. Dairy Sci. (72:988-994)	Corn	Soybean hulls
Sievert and Shaver, 1993 ^a	J. Anim. Sci. (71:1032-1040)	Shelled corn	Wheat middlings, brewer's dried grains Soybean hulls, beet pulp, brewer's dried grains
Sievert and Shaver, 1993 ^b	J. Dairy Sci. (76:245-254)	Shelled corn	Soybean hulls, wheat middlings, brewer's dried grains
Batajoo and Shaver, 1994	J. Dairy Sci. (77:1580-1588)	Shelled corn	
Mansfield and Stern, 1994	J. Dairy Sci. (77:1070-1083)	Ground corn	Soybean hulls
O'Mara et al., 1997	J. Dairy Sci. (80:530-540)	Ground corn, ground wheat	Beet pulp
Leiva et al., 2000 (trial 1)	J. Dairy Sci. (82:2866-2877)	Corn hominy feed	Beet pulp
Leiva et al., 2000 (trial 2)	J. Dairy Sci. (82:2866-2877)	Corn hominy feed	Beet pulp
Boddugari et al., 2001	J. Dairy Sci. (84:873-884)	Ground corn	Wheat corn gluten feed
Broderick et al, 2002 (trial 1)	J. Dairy Sci. (85:1767-1776)	High moisture ear corn, cracked shelled corn	Citrus pulp
Broderick et al, 2002 (trial 2)	J. Dairy Sci. (85:1767-1776)	High moisture ear corn, cracked shelled corn	Citrus pulp
Ipharraguerre et al., 2002 ^a	J. Dairy Sci. (85:2890-2904)	Ground shelled corn	Soybean hulls
Ipharraguerre et al., 2002 ^b	J. Dairy Sci (85:2905-2912)	Ground shelled corn	Soybean hulls
Voelker and Allen, 2003 ^a	J. Dairy Sci. (86:3542-3552)	High moisture corn	Beet pulp

Voelker and Allen, 2003b	J. Dairy Sci. (85:2905-2912)	High moisture corn	Beet pulp
Beckman and Weiss, 2005	J. Dairy Sci (88:1015-1023)	Ground corn	Soybean hulls, cottonseed hulls
Aikman et al., 2006	Livest. Sci. (104:23-32)	Wheat	Soybean hulls
Cabrita et al., 2007	J. Dairy Sci. (90:1429-1439)	Corn grain	Citrus pulp
Silveira et al., 2007	J. Dairy Sci. (90:2860-2869)	Barley grain, corn grain	Beet pulp
van Vuuren et al., 2010	J. Anim. Physiol. Anim. Nutr. (94:319-329)	Corn grain	Dried beet pulp
Gencoglu et al., 2010	J. Dairy Sci. (93:723-732)	Dried ground shelled corn	Soybean hulls
Ranathunga et al., 2010	J. Dairy Sci. (93:1086-1097)	Ground corn	Soybean hulls, DDGS
Hall et al., 2010	J. Dairy Sci. (93:311-322)	Ground corn	Citrus pulp
Zhang et al., 2010	J. Dairy Sci. (93:3231-3242)	Rolled barley	Beet pulp
Akins et al., 2014	J. Dairy Sci. (97:1-13)	Dry ground shelled corn	Soybean hulls pellets
Poorkasegaran and Yansari, 2014	J. Anim. Sci. Biotechnol. (5:6)	Barley grain, corn grain	Beet pulp
Sun and Oba, 2014	J. Dairy Sci. (97:1594-1602)	Rolled barley grain	Beet pulp, barley DDGS
Dann et al., 2014	J. Dairy Sci. (97:7151-7161)	Corn meal	Wheat middlings, DDGS, beet pulp
Pirondini et al., 2015	J. Dairy Sci. (98:357-372)	Corn meal	Soybean hulls
Almeida et al., 2015	Trop. Anim. Health Prod. (47:179-184)	Fine ground corn	Citrus pulp
McCarthy et al., 2015	J. Dairy Sci. (98:3335-3350)	Fine ground shelled corn	Citrus pulp, soybean hulls, corn germ meal
Dann et al., 2015	J. Dairy Sci. (98:4041-4054)	Corn meal	Pelleted beet pulp, wheat middlings, DDGS
Fredin et al., 2015 ^a	J. Dairy Sci. (98:554-565)	Ground corn	Soybean hulls
Fredin et al., 2015 ^a	J. Dairy Sci. (98:541-553)	Ground corn	Soybean hulls
Boerman et al., 2015	J. Dairy Sci. (98:4698-4706)	Ground corn	Soybean hulls
Gao and Oba, 2016	J. Dairy Sci. (99:291-300)	Rolled corn grain	Beet pulp

Table 9. Descriptive statistics of the diet and parameters used in the meta-analysis

Variable	n ¹	Mean	Median	SD	Minimum	Maximum
Nutrient intake (kg/d)						
Starch	123	5.10	5.43	1.93	0.32	9.08
CP	119	3.87	3.86	0.74	0.90	5.29
NDF	123	7.35	7.19	1.98	2.42	13.82
Cow variables						
DIM	121	92	84	44	1	193
DMI (kg/d)	114	22.31	22.75	3.76	5.25	29.10
Milk yield (kg/d)	114	33.23	31.75	6.22	19.80	51.60
Milk fat (%)	112	3.68	3.60	0.44	2.48	5.08
Milk fat yield (kg/d)	103	1.19	1.17	0.22	0.71	1.86
Milk protein (%)	116	3.09	3.08	0.23	2.63	3.84
Milk protein yield (kg/d)	107	1.04	1.01	0.20	0.62	1.58
Milk lactose (%)	82	4.76	4.81	0.16	4.34	5.11
Milk lactose yield (kg/d)	79	1.62	1.63	0.35	0.92	2.51
Rumen fermentation variables						
pH	52	6.23	6.16	0.27	5.73	7.04
NH ₃ (mg/dL)	51	12.98	12.60	6.23	5.10	26.70
Total VFA (mM)	79	111.39	112.10	20.13	65.80	153.40
Acetate (mol/100 mol)	83	63.43	62.60	6.18	52.40	87.10
Propionate (mol/100 mol)	83	21.60	20.40	4.07	13.90	38.60
Butyrate (mol/100 mol)	83	11.94	11.50	2.45	6.28	19.50
Acetate:propionate	55	3.03	3.09	0.49	2.06	3.90
Isobutyrate (mol/100 mol)	57	1.25	1.00	0.79	0.35	3.28
Isovalerate (mol/100 mol)	49	1.44	1.40	0.71	0.37	3.03
Valerate (mol/100 mol)	61	2.31	1.88	1.17	0.99	7.00
Total tract nutrient digestion variables (%)						
DMI	69	67.83	68.3	3.94	58.00	74.90
OM	60	69.57	69.93	4.22	59.90	80.00
Starch	45	93.48	94.4	3.91	81.90	98.70
CP	55	67.45	67.10	6.10	57.35	78.90
NDF	63	51.92	53.40	8.84	33.85	69.30

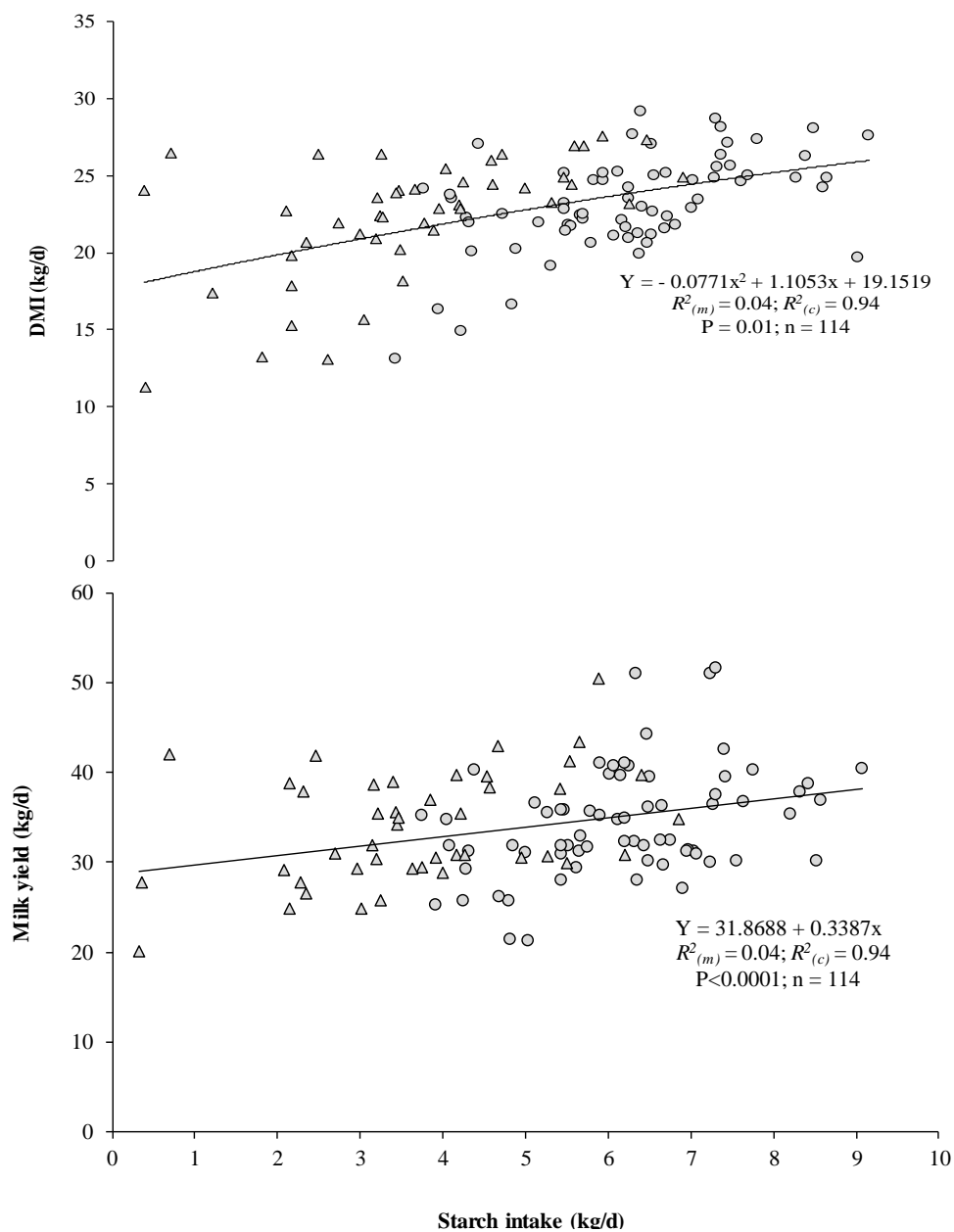
¹Number of treatments.

Table 10. Linear and quadratic regression equations used to measure response to different dietary starch intakes by partially replacing cereal grain with NFFS

Response variable	n ¹	Parameter	Estimate	SE	P-value	RMSE	R ² _(m)	R ² _(c)
DMI (kg/d)	114	Intercept	19.152	0.9034	<0.0001	0.0292	0.04	0.94
		Starch	1.105	0.3016	0.0005			
		Starch ²	-0.077	0.0297	0.01			
Milk yield (kg/d)	114	Intercept	31.869	1.1164	<0.0001	0.0261	0.009	0.97
		Starch	0.339	0.0935	<0.0001			
Milk fat (%)	112	Intercept	3.942	0.0827	<0.0001	0.0388	0.04	0.92
		Starch	-0.047	0.0106	<0.0001			
Milk fat yield (kg/d)	103	Intercept	1.074	0.0724	<0.0001	0.0224	0.003	0.97
		Starch	0.047	0.0213	0.03			
		Starch ²	-0.005	0.0020	0.03			
Milk protein (%)	116	Intercept	3.012	0.0437	<0.0001	0.0470	0.02	0.91
		Starch	0.017	0.0053	0.003			
Milk protein yield (kg/d)	107	Intercept	0.946	0.0368	<0.0001	0.0375	0.03	0.95
		Starch	0.019	0.0044	0.0003			
Milk lactose (%)	82	Intercept	4.755	0.0372	<0.0001	0.0571	0.00004	0.88
		Starch	-0.0006	0.0051	0.91			
Milk lactose yield (kg/d)	79	Intercept	1.459	0.1104	<0.0001	0.0236	0.003	0.98
		Starch	0.016	0.0069	0.02			

¹Number of observations.

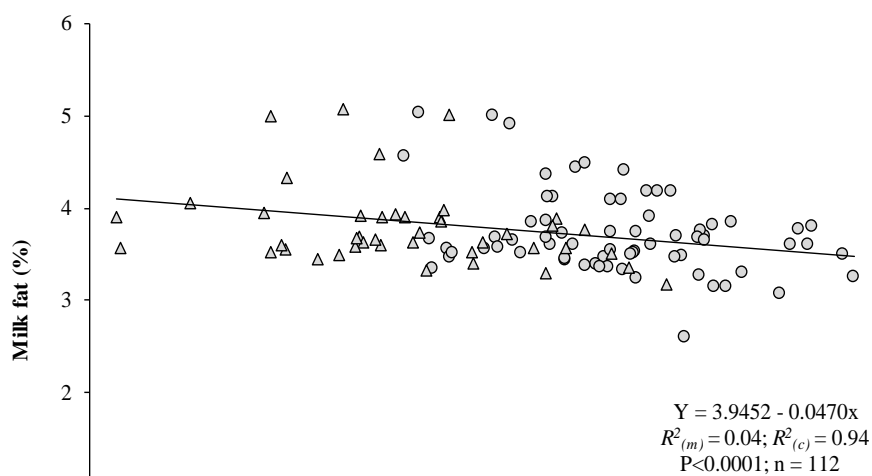
a



b

Figure 1. Response of DMI and milk yield to increased dietary starch in dairy cows. Observations were adjusted to the random effect of trial. Triangles indicated diets formulated with NFFS (25.94±11.52% as DM basis [reduced dietary starch]) and circles diets formulated with cereal grains (27.48±11.52% as fed [high dietary starch]).

a



b

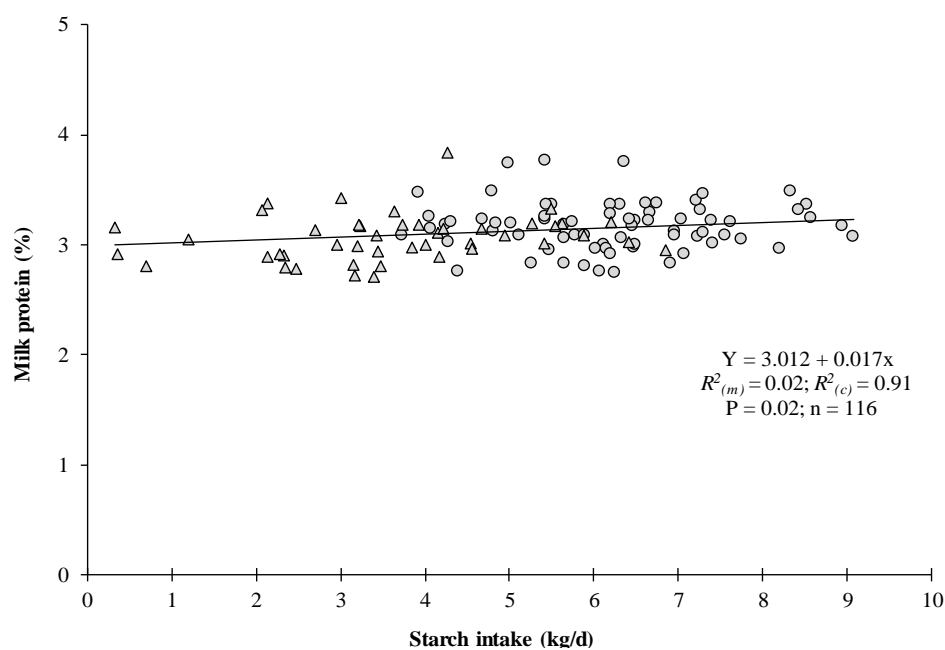


Figure 2. Response of milk fat and milk protein concentration to the increase of dietary starch in dairy cows. Observations are adjusted to the random effect of trial. Triangles indicated diets formulated with NFFS ($25.94 \pm 11.52\%$ as DM basis [reduced dietary starch]) and circles diets formulated with cereal grains ($27.48 \pm 11.52\%$ as fed [high dietary starch]).

Table 11. Best-fit regression equations of performance to different dietary starch and other nutrients intake by partially replacing cereal grains with NFFS

Response variable	n ¹	Parameter	Estimate	SE	P-value	VIF	RMSE	$R^2_{(m)}$	$R^2_{(c)}$
DMI (kg/d)	110	Intercept	4.379	0.7968	<0.0001	-	0.0128	0.81	0.98
		Starch	0.896	0.1253	<0.0001	9.4			
		CP	2.050	0.1730	<0.0001	1.5			
		NDF	0.935	0.0901	<0.0001	2.7			
		Starch×NDF	-0.046	0.0171	0.01	7.4			
Milk yield (kg/d)	110	Intercept	14.525	2.814	<0.0001	-	0.0174	0.25	0.98
		Starch	0.276	0.0926	0.004	1.2			
		DMI	0.462	0.1941	0.02	3.1			
		CP	1.795	0.5462	0.002	2.8			
Milk fat yield (kg/d)	103	Intercept	0.538	0.1105	<0.0001	-	0.0182	0.11	0.98
		Starch	0.015	0.0065	0.03	2.2			
		CP	0.068	0.0205	0.006	1.3			
		NDF	0.039	0.0108	0.0005	2.3			
Milk protein (%)	114	Intercept	3.013	0.0932	<0.0001	-	0.0495	0.19	0.90
		Starch	-0.020	0.0112	0.08	5.7			
		DIM	0.0003	0.0009	0.97	1.7			
		Starch×DIM	0.0004	0.0001	0.001	6.6			
Milk protein yield (kg/d)	107	Intercept	-0.008	0.0986	0.94	-	0.0319	0.58	0.96
		Starch	0.010	0.0039	0.01	1.1			
		DMI	0.044	0.0442	<0.0001	1.1			
Milk lactose (%)	82	Intercept	4.257	0.1333	<0.0001	-	0.0536	0.18	0.90
		DIM	0.022	0.0058	0.0004	-			
Milk lactose yield (kg/d)	75	Intercept	0.425	0.2110	0.05	-	0.0151	0.11	0.99
		Starch	0.012	0.0054	0.04	1.3			

DMI	0.027	0.0134	0.05	3.4
CP	0.109	0.0361	0.004	3

¹Number of observations.

Table 12. Equations for linear regression of rumen fermentation response to different dietary starch intake by partially replacing cereal grains with NFFS

Response variable	n ¹	Parameter	Estimate	SE	P-value	RMSE	$R^2_{(m)}$	$R^2_{(c)}$
pH	52	Intercept	6.299	0.0775	<0.0001	0.0373	0.003	0.95
		Starch	-0.009	0.0073	0.24			
NH ₃ (mg/dL)	51	Intercept	12.220	1.7748	<0.0001	0.0735	0.00007	0.89
		Starch	0.026	0.1943	0.89			
Total VFA (<i>Mm</i>)	79	Intercept	114.470	4.6885	<0.0001	0.0312	0.004	0.97
		Starch	-0.810	0.4629	0.09			
Acetate (mol/100 mol)	83	Intercept	66.153	1.3566	<0.0001	0.0416	0.03	0.92
		Starch	-0.584	0.1430	0.0004			
Propionate (mol/100 mol)	83	Intercept	20.171	0.8907	<0.0001	0.0361	0.02	0.93
		Starch	0.305	0.1187	0.01			
Butyrate (mol/100 mol)	83	Intercept	12.117	0.5307	<0.0001	0.0314	0.0003	0.96
		Starch	-0.024	0.0663	0.72			
Acetate:propionate	55	Intercept	3.296	0.1139	<0.0001	0.0631	0.02	0.93
		Starch	-0.049	0.0256	0.06			
Isobutyrate (mol/100 mol)	57	Intercept	0.950	0.1723	<0.0001	0.0233	0.009	0.98
		Starch	0.039	0.0178	0.03			
Isovalerate (mol/100 mol)	49	Intercept	1.059	0.1859	<0.0001	0.0335	0.01	0.97
		Starch	0.050	0.0151	0.002			
Valerate (mol/100 mol)	61	Intercept	1.875	0.2528	<0.0001	0.0352	0.02	0.95
		Starch	0.104	0.0498	0.04			

¹Number of observations.

Table 13. Best-fit regression equations for rumen fluid parameters in cows different DMI, NDF, and dietary starch intakes by partially replacing cereal grains with NFFS

Response variable	n ¹	Parameter	Estimate	SE	P-value	VIF	RMSE	$R^2_{(m)}$	$R^2_{(c)}$
NH ₃ (mg/dL)	51	Intercept	2.178	3.2201	0.50	-	0.0649	0.16	0.91
		CP	2.612	0.7585	0.002	-			
Total VFA (<i>Mm</i>)	79	Intercept	97.264	9.9174	<0.0001	-	0.0250	0.03	0.98
		Starch	-3.389	0.8430	0.0002	3.32			
		DMI	2.732	0.6993	0.0003	2.82			
		NDF	-4.007	1.2744	0.003	3.67			
Acetate (mol/100 mol)	83	Intercept	57.911	3.7676	<0.0001	-	0.0389	0.05	0.93
		Starch	-0.742	0.1578	<0.0001	1.2			
		DMI	0.416	0.1769	0.02	1.2			
Isobutyrate (mol/100 mol)	57	Intercept	0.244	0.3208	0.45	-	0.0231	0.05	0.99
		Starch	0.029	0.0150	0.06	1.1			
		DMI	0.035	0.0132	0.01	1.1			

¹Number of observations.

Table 14. Equations for linear and quadratic regression of total tract nutrient digestion response to different dietary starch intake by partially replacing cereal grains with NFFS

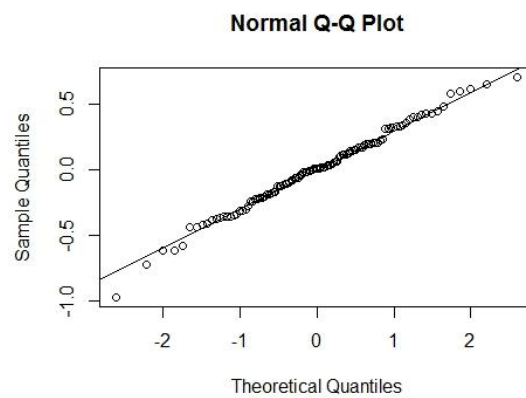
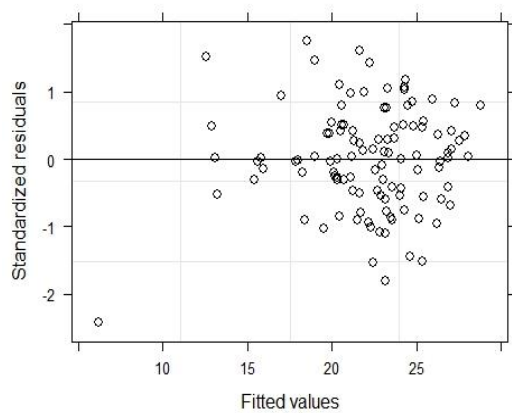
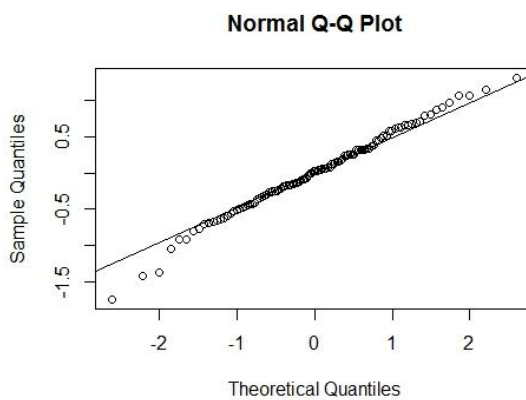
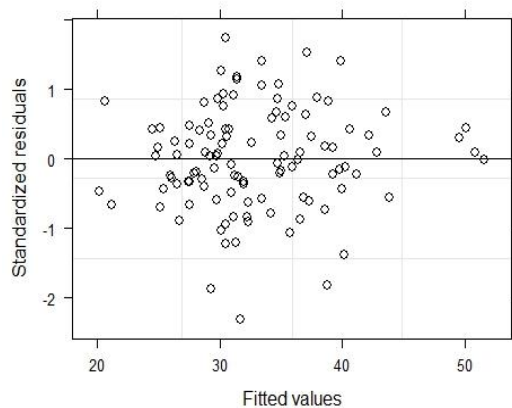
Response variable (%)	n ¹	Parameter	Estimate	SE	P-value	RMSE	$R^2_{(m)}$	$R^2_{(c)}$
DM	69	Intercept	63.059	1.8300	<0.0001	0.0779	0.06	0.84
		Starch	1.648	0.6879	0.02			
		Starch ²	-0.139	0.0651	0.04			
OM	60	Intercept	69.331	1.2163	<0.0001	0.0559	0.00009	0.90
		Starch	0.002	0.1636	0.98			
Starch	45	Intercept	94.485	1.5167	<0.0001	0.0843	0.005	0.80
		Starch	-0.173	0.2157	0.43			
CP	55	Intercept	61.657	2.3380	<0.0001	0.0368	0.03	0.94
		Starch	2.286	0.8360	0.01			
		Starch ²	-0.229	0.0770	0.005			
NDF	63	Intercept	64.390	1.8187	<0.0001	0.0692	0.24	0.88
		Starch	-2.351	0.3737	<0.0001			

¹Number of observations.

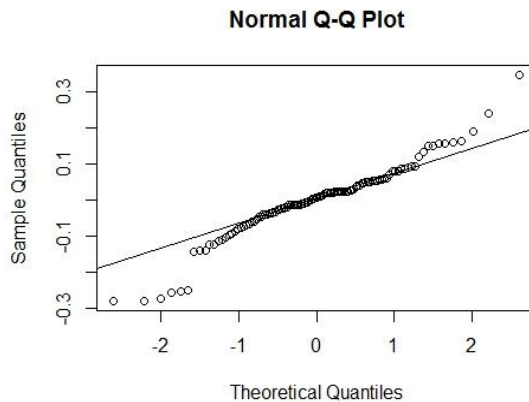
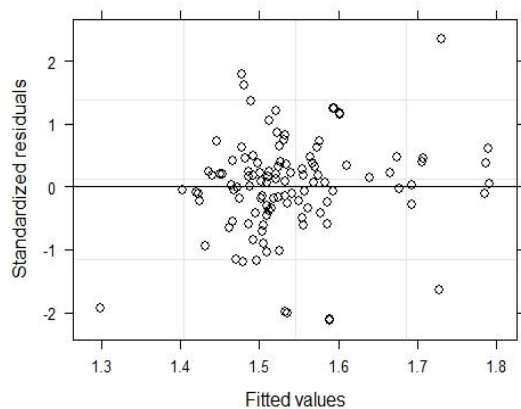
Table 15. Best-fit regression equations of total tract nutrient digestion to different DMI, CP, NDF, and starch intakes by partially replacing cereal grains with NFFS

Response variable (%)	n ¹	Parameter	Estimate	SE	P-value	VIF	RMSE	$R^2_{(m)}$	$R^2_{(c)}$
DM	69	Intercept	70.843	1.8496	<0.0001	-	0.0839	0.04	0.81
		NDF	-0.457	0.2162	0.04	-			
OM	60	Intercept	79.686	5.2774	<0.0001	-	0.0506	0.20	0.94
		DMI	-1.118	0.3875	0.007	2.8			
		CP	4.809	1.9033	0.02	2.9			
		NDF	-0.642	0.2207	0.006	1.1			
NDF	63	Intercept	76.298	6.4010	<0.0001	-	0.0610	0.23	0.90
		Starch	-4.946	0.9704	<0.0001	7.4			
		NDF	-1.559	0.6675	0.03	2.8			
		Starch×NDF	0.347	0.1224	0.007	4.6			

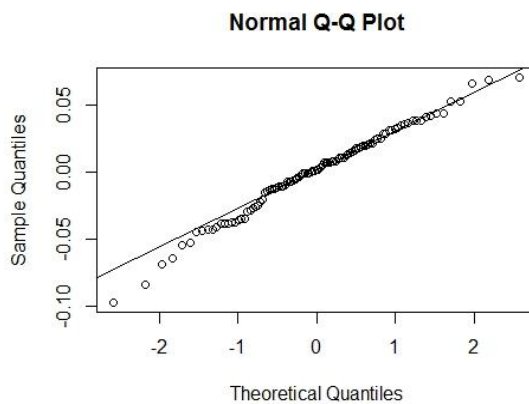
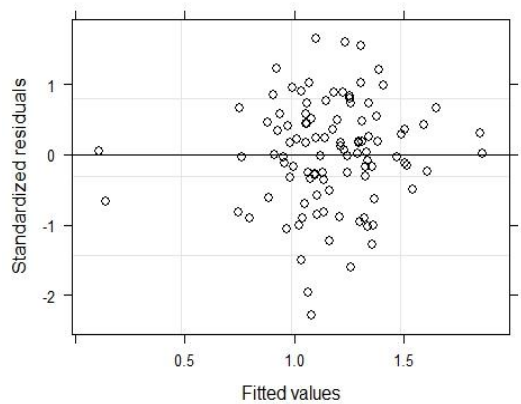
¹Number of observations.

Figure 3. Residuals vs. fitted and Q-Q plots for the evaluated variables*Cow performance***DMI****Milk yield**

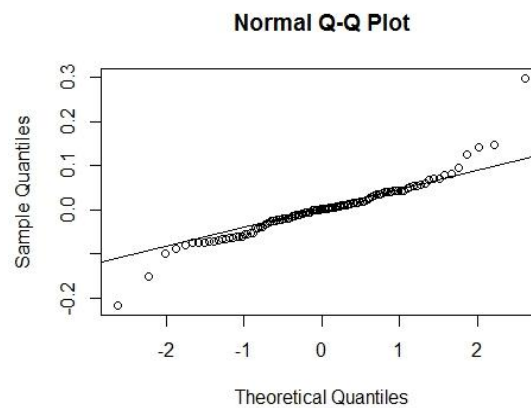
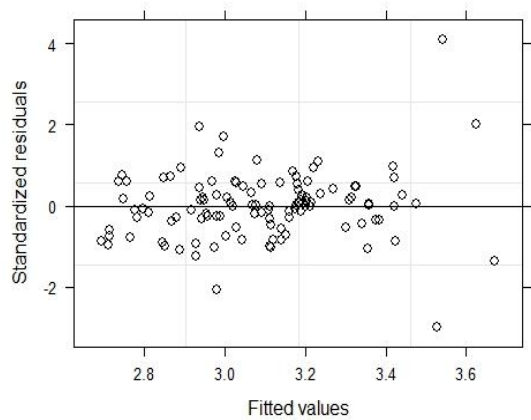
Milk fat percentage



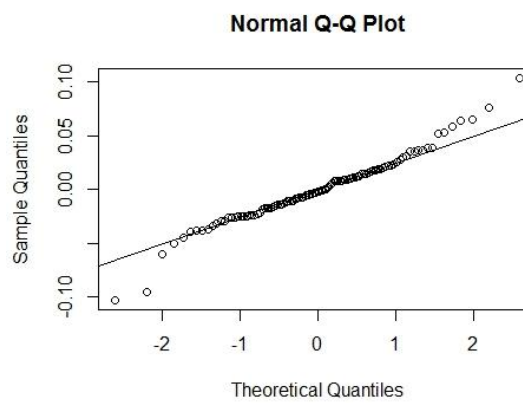
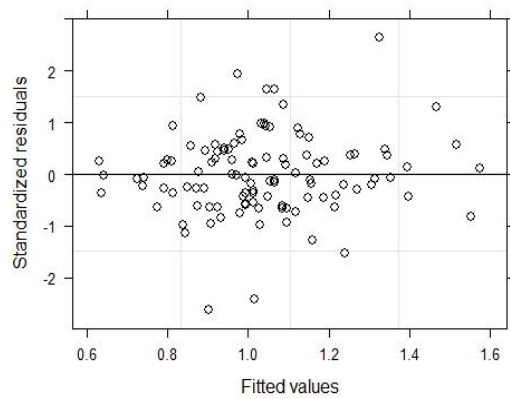
Milk fat yield



Milk protein percentage

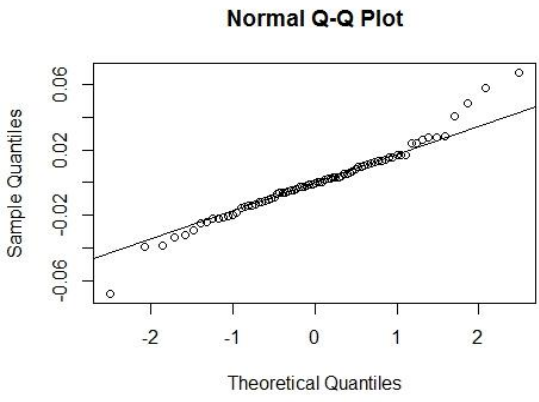
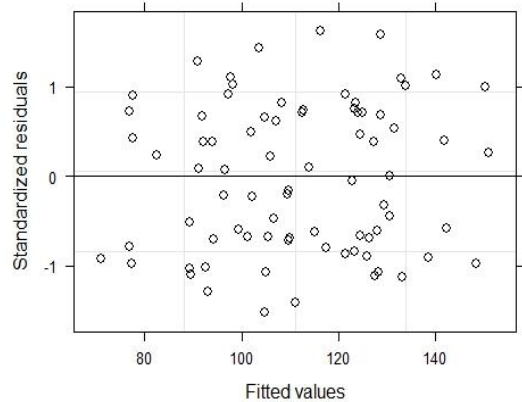


Milk protein yield

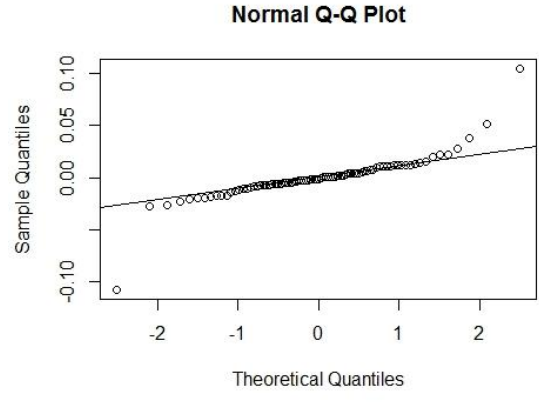
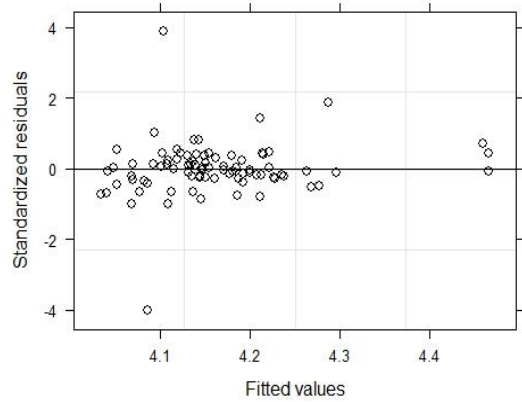


Rumen fermentation

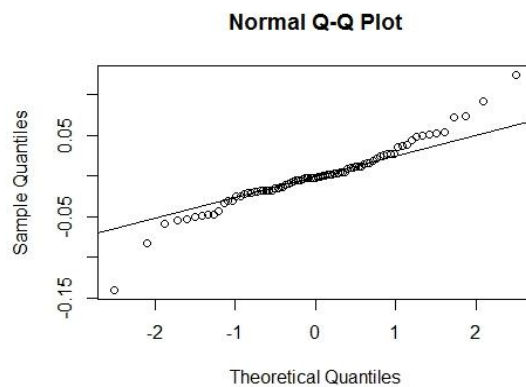
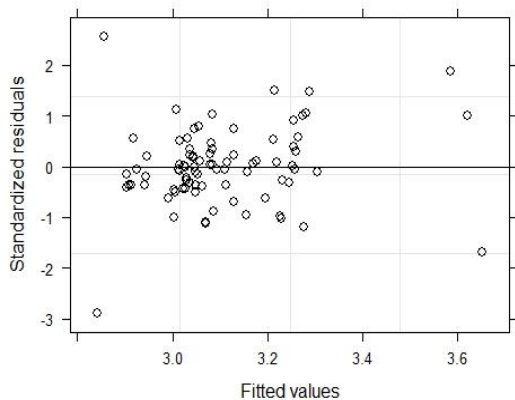
Total VFA



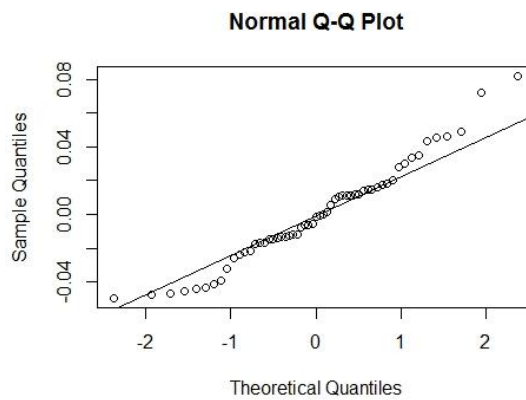
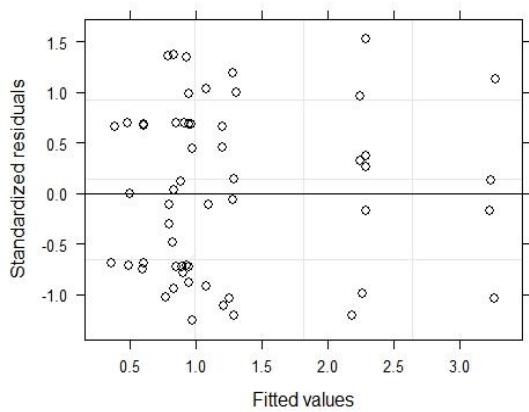
Acetate



Propionate

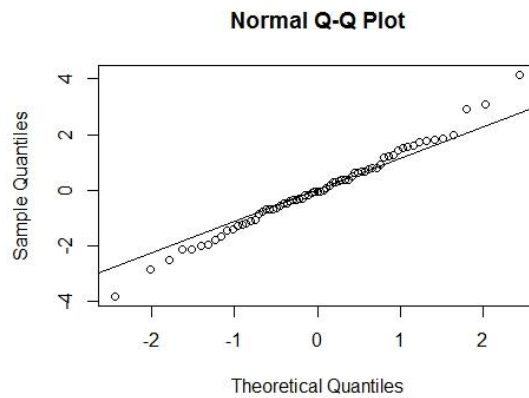
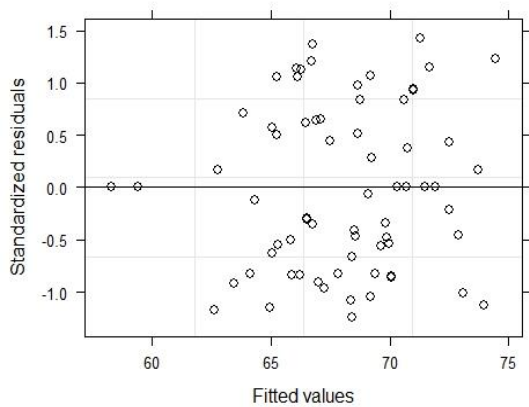


Isobutyrate

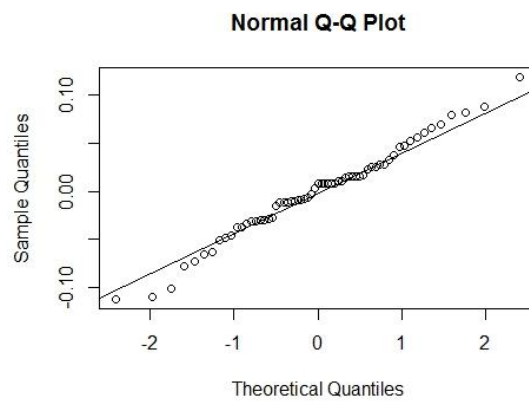
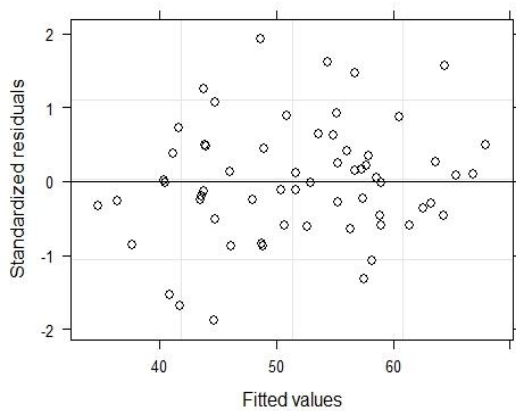


Total tract nutrient digestibility

DM digestibility



NDF digestibility



CHAPTER 4:
IMPACT OF DIETARY STARCH CONCENTRATION WITH TWO TYPES OF
CORN SILAGE ON THE PERFORMANCE OF LACTATING DAIRY COWS

Abstract

This study was designed to evaluate the effects of feeding different corn silage types and dietary starch concentrations on the performance and nutrient digestion of lactating dairy cows. Forty-eight Holstein cows were assigned to 1 of 4 diets using a randomized complete block design with a 2-week covariate period followed by 8-week experimental period. Experimental diets were arranged as a 2×2 factorial with 2 types of corn silages (CONV and BMR) and 2 dietary starch concentrations (19% and 25% of DM). Diets were formulated to contain 60.7% forage and 39.3% concentrate on DM basis. A portion of dried ground corn grain was replaced with soybean hulls and beet pulp to decrease dietary starch concentration. Silage \times starch interaction significantly affected yields of milk ($P=0.03$), ECM ($P=0.05$), lactose ($P=0.03$), and FE ($P=0.05$), and tended to affect milk protein ($P=0.08$) and SNF ($P=0.07$). Milk yield was similar between cows fed BMR-25% starch (45.73 kg/d) and CONV-19% starch (44.0 kg/d), but was greater than for cows fed the other diets (43.40 kg/d). Cows fed BMR-25% starch produced 2.1 kg/d more ECM than with the other diets. Cows fed BMR-19% starch yielded the lowest milk lactose. There was no effect of diet on DMI, milk fat concentration and yield, total solids concentration, MUN, SCS, BW, BCS, and plasma blood glucose, BHB, and PUN ($P>0.05$), however, over time cows fed BMR silage diets tended ($P=0.06$) to have greater DMI than cows fed CONV silage diets. Milk protein and SNF concentrations were affected by dietary starch, resulting in greater protein concentration in cows fed 25%

starch compared to those fed 19% starch. Feed efficiency was the least for cows fed BMR-19% starch. Apparent total tract nutrient digestibility was affected by dietary starch concentration. Digestibility of DM ($P=0.02$), OM ($P=0.01$), and CP ($P=0.04$) was greater in cows fed 25% than those fed 19% starch diets. The performance response observed in dairy cows can be explained by the positive effects of dietary starch concentration on nutrient digestibility and the associated increase in DMI in response to BMR corn silage diets. The inclusion of BMR corn silage had a positive effect on dairy cow performance and contributed considerably to the energy needed by the cow to maintain an optimal milk production in reduced starch diets.

Keywords: BMR corn silage, milk yield, starch concentration.

Introduction

Corn silage represents an excellent source of energy for lactating dairy cows because of its fiber and starch content. In general, CONV corn silage yields more dry matter than its isogenic BMR silage (Lauer, 2015), but CONV silage has higher lignin and lower digestibility than BMR silage (Sattler et al., 2010). In fact, Sattler et al. (2010) demonstrated that BMR corn silage had 15 g/kg NDF and 22 g/kg less lignin than CONV silage, which contributed to the higher *in vitro* digestibility of DM (51 g/kg) and NDF (94 g/kg) observed in BMR silage. Therefore, the high NDF digestibility of BMR silage may contribute to improved dairy cow performance in reduced starch diets.

The positive contribution of higher NDF digestibility in BMR silage diets to milk yield has been reported in some studies. Taylor and Allen (2005a) observed increases of 0.50 kg/d in total tract NDF digestibility in dairy cows fed BMR corn silage diets with floury endosperm compared to cows fed CONV corn silage diets, which resulted in 2.1 kg/d more milk (Taylor and Allen, 2005b). Similarly, Ferrareto et al. (2015) reported 4.6% units higher NDF digestibility in week 13 of the experiment for BMR corn silage diets compared to CONV silage diets. This effect contributed to increase milk yield by 2.2 kg/d in BMR corn silage diets; ECM, however, was identical between treatments. Those effects were later confirmed through a meta-analysis by Ferrareto and Shaver (2015) where 162 treatments means from 54 feeding trials indicated an increase in milk production of 1 kg/d in cows fed BMR corn silage diets compared to cows fed CONV silage diets. Part of the increased milk production was attributed to higher total tract NDF digestibility in the BMR corn silage diets (2.5%) compared to CONV silage diets.

Although NDF digestibility increases in diets with BMR corn silage, starch digestion kinetics vary with this silage variety. Oba and Allen (2000b) reported that rumen starch digestion decreased 0.4 kg/d in diets with BMR corn silage when compared to diets with CONV silage. The starch flow to the duodenum however increased 0.6 kg/d in cows fed BMR corn silage diets. These effects were confirmed by Greenfield et al. (2001) who indicated that starch digested in the rumen was lower (0.6 kg/d), and the starch flowed to the duodenum was greater (1.2 kg/d) in cows fed diets with BMR corn silage than cows fed CONV silage diets. Increased passage rate of starch could be explained by the higher DMI in cows fed BMR silage, though increases of DMI was only observed by Oba and Allen (2000b). Even though fiber and starch digestion are clearly understood in cows fed BMR corn silage diets, no study has examined modifying the starch concentration in the diet. Previous research indicated that DMI, milk yield, and ECM decreased when soy hulls and beet pulp were used to reduce dietary corn starch (Sanchez-Duarte et al., 2016). Even when using NFFS with highly digestible fiber in reduced starch diets, the low fiber digestion of the CONV corn silage probably contributed to rumen fill, which could have decreased DMI and consequently the energy available to the cow. Therefore, the objective of this study was to include more digestible NDF through BMR corn silage in reduced starch diets. It was hypothesized that the high fiber digestion from BMR corn silage would not limit DMI in reduced starch diets. As a result, production response should improve in cows fed BMR reduced starch diets compared to those fed CONV corn silage reduced starch diets, but similar to cows fed CONV corn silage diets with increased starch concentration.

Materials and Methods

Cows, Treatments, and Diets

The experiment was carried out at the USDA-ARS Dairy Forage Research Center farm in Prairie du Sac, Wisconsin, USA. All procedures regarding care and handling cows in this experiment were approved by the University of Wisconsin Institutional Animal Care and Use Committee. Forty-eight lactating [24 primiparous (138.5±28.1 DIM and 602.0±24.5 kg of BW) Holstein cows and 24 multiparous (138.9±21.0 DIM and 668.9±36.2 kg of BW)] were blocked by parity, DIM, and milk production then randomly assigned to a 2-wk covariate period in a randomized complete block design. Following the adjusted covariate period, cows were fed one of four diets in a 2 × 2 factorial arrangement of treatments during eight weeks. Experimental diets were formulated with two different corn silages (CONV corn silage and BMR corn silage) and two dietary starch concentrations (19% and 25%). The diet formulated with BMR corn silage containing 25% starch was fed to all cows during the covariate period. The proportion of corn silages along with alfalfa haylage was maintained constant in all four experimental diets. The reduced starch concentration in the diet with 19% starch was accomplished by replacing 8.2% ground shelled corn with soybean hulls and beet pulp (Table 16). All other ingredients were maintained in the same proportion across diets. Experimental diets were formulated to meet nutrient requirements of a mature Holstein cow with 750 kg of BW, BCS 3.0, and 90 DIM, producing 45 kg/d of milk with 3.5% fat and 3.0 protein (NRC, 2001). Cows were fed the ration in tie-stalls as a TMR once daily at 0900 h adjusting their daily feeding rate based on 5 to 10% orts yield.

Measurements and Sampling

Feed intake was measured daily in all cows throughout the experiment using the amount of feed offered and orts. Forage samples from three days were collected three times a week to adjust for DM using NIRS. Samples of TMR, orts, forages, and individual feedstuffs were collected daily, stored at -20°C, while a weekly composited sample of each was used for DM analysis. The DM analysis of all samples was performed weekly. From the weekly TMR composited samples, 500 g of fresh material was used to determine particle size distribution in each diet (Heinrichs, 2013).

Individual rumination monitoring was measured visually in all cows during week 7 and 8 by 5 individuals for 26 consecutive hours. Direct visual observation consisted on walking in front of the cow stalls every 5 min. The following feeding behavior variables were measured: standing up or lying down either 1) eating: defined as when the cow was consuming feed from the bunk or masticating feed particles, 2) rumination: defined as when the cow was chewing her cud, 3) doing nothing: defined as when the cow was neither eating nor ruminating, and 4) drinking: defined as when the cow was drinking water. Cow behavior was evaluated according to Cook et al. (2016). A meal event was defined when the cow was observed eating for 1 or more times preceded by an observation that was not defined as eating. Standing, laying, and rumination events were defined as when the cow was observed for at least two consecutive times standing, laying or rumination, but which were preceded by a different observation. Total time for each behavior was calculated by the sum of observations multiplied by the 5-min interval between each. Rumination plus eating time was used to calculate chewing. Time spent

ruminating, eating or chewing per kg of DMI or NDF intake were calculated from week 7 and 8 using the average of those variables.

Blood samples were collected in all cows approximately 3 hours after feeding through venipuncture of the tail (coccygeal) vein on two consecutive days during wk 4 and 8. Blood was drawn into 10 mL vacutainer tubes containing lithium heparine for plasma urea N and BHB analysis, and in 7 mL vacutainer tubes containing sodium flouride-potassium oxalate for glucose analysis (Becton, Dickinson, and Company, Franklin Lakes, NJ). Blood samples were centrifuged at $2,400 \times g$ for 20 min at 5°C and then the plasma stored at -20°C for later analysis.

Fecal samples were collected in all cows on 3 consecutive days during week 8 to estimate total tract nutrient digestion. In total, 6 fecal samples (from 8 to 12 h intervals) per cow with approximately 20 g each were obtained directly from the rectum or spontaneous release in all animals and then dried immediately as described under laboratory analysis.

Cows were milked thrice daily at 0600, 1200, and 1900 h, and milk yield was recorded per day. Individual milk samples from each milking were collected 2 consecutive days in each week and send to a commercial laboratory for milk composition analysis. Body weight of each cow was recorded three consecutive days approximately 3 h after feeding on weeks 2, 4, 6, and 8. On weighing day, BCS was evaluated by 3 individuals according to Wildman et al. (1982).

Laboratory Analysis

Samples of TMR, orts, forages, and individual feedstuffs were dried by triplicate at 55°C for 48 h in a Thermo Scientific Heratherm oven (OMH 750L units, Thermo

Fisher Scientific Inc., Waltham, MA). Then, only two from those samples were further dried at 105°C for 24 h in the same oven. Fecal samples were dried in a Precision Elect oven (460/230, Precision Quincy, Woodstock, IL). All dried samples were ground to 4-mm particle size (Wiley mill, model 4, Arthur H. Thomas Co., Philadelphia, PA). Ground samples were further analyzed for DM, CP, NDF, ADF, starch, and ash. Ether extract was estimated using the EE contents in feeds from NRC (2001) and diet composition. Crude protein percentages were determined by analyzing total N using a combustion assay (Leco FP-2000 N Analyzer, Leco Instruments Inc., St. Joseph, MI). The analysis of NDF was determined by gravimetric determination of amylase-treated NDF using beakers or crucibles (Mertens, 2002), and ADF by the refluxing method (973.18; AOAC international, 1990). Starch was analyzed on sub-samples that were ground through a 1-mm screen of an abrasion mill (Udy Corp., Fort Collins, CO) using the methodology proposed by Hall (2015). Samples were incubated in screw cap tubes with thermostable α -amylase in 30 mL sodium acetate (pH 5.0) for 1 h at 100°C with periodic mixing (initial vortex, and then vortex at 10, 30, and 50 min) to gelatinize and partially hydrolyze α -glucan. Then, amyloglucosidase was added, and the reaction mixture was incubated in a water bath at 50°C for 2 h hand mixed once (vortex after the first hour). After incubation, 20 mL of distilled water was added and tubes were inverted $\pm 10 \times$ to mix completely. Approximately 2 mL of the solution were centrifuged at $1000 \times g$ for 10 min and then 0.1 mL working test solution and standards (in duplicate) were added into 16×100 mm glass tubes. Finally, 3.0 mL of glucose oxidase–peroxidase was added to each glass tube, tubes were vortexed, covered with plastic film to seal and incubated in a 50°C

water bath for 20 min. Absorbance was read at 505 nm. Samples of TMR, forages, and individual feeds were corrected for free glucose.

Ash concentration was analyzed by incinerating 1 g of sample for 8 h at 450°C in a muffle furnace (942.05; AOAC International, 1998). Organic matter was then calculated as 100 – % ash. Based on the nutrient analysis, NFC was calculated as 100 – (% CP + % NDF + % EE + % ash) according to NRC (2001). Individual forage and feedstuff analysis along with the proportion of each ingredient in the ration were used to calculate the chemical composition of the experimental diets. In addition, TMR samples were also analyzed to validate the calculated chemical composition of the diets. The analysis of minerals (Ca, P, Mg, K, and S) in TMR samples were analyzed using wet chemistry by Dairyland laboratories, Inc. (Arcadia, WI). Particle size distribution was determined in TMR samples by using the 4-5screen Penn State Particle Separator according to (Heinrichs, 2013).

Plasma metabolites were composited by cow and week before the analysis and then analyzed with commercial enzymatic or colorimetric kits using a micro-plate spectrophotometer (SpectraMax 190, Molecular Devices, Sunnyvale, CA). Serum glucose was analyzed by the glucose oxidase reagent (Cat. No G7521. Pointe Scientific Inc., Canton, MI) as described by (Trinder, 1969). Plasma urea N was analyzed with the methodology diacetylmonoxime (DAM; Stanbio Laboratory, Boerne, TX). Serum BHBA was determined by the β -Hydroxybutyrate reagent (Cat. No H7587-58. Pointe Scientific Inc., Canton, MI) according to Williamson et al. (1962).

Total tract nutrient digestion was determined *in situ* in the rumen of two cows using iADF by incubating bags (pore size of 25 μ m) during 288-h (Huhtanen et al.,

1994). Analysis of DM, OM, CP, NDF, ADF, and starch in TMR and feces samples along with the internal marker were utilized to estimate the total tract nutrient digestibilities following the equation used by Ferrareto et al. (2015): apparent total tract nutrient digestibility (%) = $100 - [100 \times (\text{TMR marker concentration/fecal marker concentration}) \times (\text{fecal nutrient concentration/TMR nutrient concentration})]$.

Milk samples were sent to AgSource Milk Analysis Laboratory (Menomonie, WI) for the analysis of fat, protein, SNF, MUN, and SCC. Milk components were analyzed using a Foss FT6000 spectrum analyzer (method 972.16; AOAC International, 1998; Foss Electric A/S, Hillerod, Denmark). Total solids were calculated adding the content of milk fat percentage to the amount of SNF. Somatic cell counts were converted to linear SCS.

Statistical Analysis

All data were analyzed by the MIXED procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC) as a randomized complete block design. The statistical analysis considered week as repeated measured for all production variables and silage particle size, while every two week was the repeated measure for BW, BCS, BW change, and BCS change. Data collected during the 2-wk covariate period were used as covaroiates in the statistical model for all measurements, except for behavior, digestibility, and blood variables. Data of behavior (wk 7 and 8), digestibility (wk 8), and blood (week 4 and 8) variables were evaluated using information of a single time point using the same model except week and the interaction week \times treatment were not included in the model. The model included fixed effects of treatments, week, and the interaction treatment \times week. Bloock was considered as a random in the data analysis using the following model:

$$Y_{ijkl} = Cov + CS_i + St_j + (CS \times St)_{ij} + W_k + B_l + (CS \times W)_{ik} + (St \times W)_{jk} + e_{ijkl}$$

Where: Y_{ijkl} = dependent variable, Cov = effect of covariate, CS_i = effect of corn silage i ($i = 1$ to 2), St_j = effect of starch concentration j ($j = 1$ to 2), $(CS \times St)_{ij}$ = interaction between corn silage i and starch concentration j , W_k = effect of week k ($k = 1$ to 8), B_l = effect of block l ($l = 1$ to 6), $(CS \times W)_{ik}$ = interaction between corn silage i and week k , $(St \times W)_{jk}$ = interaction between starch concentration j and week k , and e_{ij} = random residual error.

For each variable, the covariance structure corresponded to the lowest AIC and Bayesian information criterion (BIC) was selected. Interactions with $P > 0.05$ were sequentially removed from the model. Data were reported as least square means and the Tukey's test was used for separation of treatment means. Statistical significance in all variables was declared at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

Results and Discussion

Nutrient Composition of Diets, Feeds, and Particle size of Diets

Table 16 indicates the nutrient composition of experimental diets. The concentration of CP averaged 16.70%, but diets with 19% starch had on average 3.2% more NDF than diets with 25% starch. The fiber differences between diets with 19 and 25% starch concentrations resulted from the addition of soybean hulls and beet pulp as a replacement for corn grain. The actual starch concentrations of the diets were close to the formulated starch of the diets (20.03 and 24.97%, for 19 and 25% starch, respectively).

Nutrient composition of feedstuffs and forages used to formulate the experimental diets is in Table 17. There was higher NDF concentration (1.84%) in BMR corn silage

compared to CONV silage. However, the NDFD-30h and uNDF-30h were 8% and 2.4% lower, respectively, in BMR silage than CONV silage. Those differences have been clearly associated with the higher lignin content in CONV corn silage respect to BMR corn silages (Sattler et al., 2010). Starch concentration was 1.8% higher in CONV silage than BMR silage.

Particle size distribution of the diets was different by corn silage type (Table 18). A higher percentage of particles was observed in the upper screen (0.16%) and bottom pan (2.21%; $P=0.04$) in diets formulated with CONV silage than those with BMR silage. The proportion of particles retained in the middle screen however, was 2.11% greater in BMR corn silage diets ($P=0.004$). It is likely that the proportion of soybean hulls and beet pulp, as well as the fragility of the BMR silage contributed to those differences, as particle size distribution was similar between silages (Table 19). Similar particle size distribution has been observed in diets formulated with CONV and BMR corn silages (Akins and Shaver, 2014; Ferraretto et al., 2015).

Performance, Plasma Metabolites, Cow Behavior, and Nutrient Digestibility

The productive response of dairy cows to feeding different corn silage and starch concentration is shown in Table 20. Dry matter intake was similar among treatments (Table 28; $P>0.05$). However, DMI tended to be different by the silage \times week interaction ($P=0.06$), indicating a higher intake in cows fed BMR corn silage diets compared to cows fed CONV silage diets (Figure 4). Similar to these results, Akins and Shaver (2014) reported during an 11 week experiment that cows fed BMR corn silage diets tended to have a greater DMI compared to cows fed CONV corn silage diets. However, a meta-analysis of 48 articles concluded that DMI was 0.9 kg/d higher in cows

fed BMR corn silage diets than cows in CONV silage diets. This was explained by the low lignin (2.1% NDF; Ferraretto and Shaver, 2015) and high total tract NDF digestibility (8%; Table 26) in the BMR silage used to formulate the diets respective to CONV silage. The interaction silage \times starch also tended to affect milk protein ($P=0.08$), total solids ($P=0.07$), and SNF ($P=0.07$) yields.

There were silage \times starch interaction effects for milk yield and ECM (Table 20; $P\leq 0.05$). Cows fed BMR-25% starch yielded the most milk (45.73 kg/d) and ECM (46.83 kg/d) across all treatments. Milk yield and ECM were similar between cows fed BMR-19% starch and cows fed CONV corn silage diets regardless of the starch concentration. Zhao et al. (2016) observed a higher milk yield and ECM in dairy cows fed high starch diets (28 and 34%) with low NDF content (30 and 34%) than cows in low starch diets (18 and 23%) with high NDF content (38 and 41%). However, they combined different proportions of CONV corn silage, oat hay, and corn grain to formulate those starch concentration and in the present study, soybean hulls and beet pulp were used to achieve the dietary starch concentrations. Ferraretto and Shaver (2015) indicated an increase of 1.5 kg/d milk yield and 1 kg/d FCM in cows fed BMR corn silage diets respect to CONV corn silage diets. The increases in milk yield found by Ferraretto and Shaver (2015) were associated to the increase of 0.9 kg/d DMI in cows fed BMR corn silage diets, which was confirmed in the present study.

The interaction silage \times starch also affected FE ($P=0.03$) and milk lactose yield ($P=0.05$; Table 20). Cows fed BMR-19% starch had the lowest FE (1.66) of all treatments (averaging 1.74). VandeHaar et al. (2016) indicated that dietary starch content has little effect on FE, which was observed in cows fed CONV corn silage with either, 19

and 25% starch concentration in the present study. Edwards (2008) found that feed efficiency was affected more by the concentration of corn silage inclusion in the diet than the corn silage type. This author indicated higher FE in diets with 50% BMR corn silage than diets with 35% of either, BMR or CONV corn silage. Thus, the low feed efficiency of cows in BMR-19% starch can be partially explained by the slightly increased DMI and similar or lesser ECM of these cows respect to the cows in the other treatments. Milk lactose yield was the greatest for cows fed BMR-25% starch and the least for cows in BMR-19% starch, which can be related to the response of milk yield by those treatments.

Dietary starch concentration affected milk protein ($P=0.05$) and SNF concentrations ($P=0.001$) and tended to increase milk lactose concentration ($P=0.06$; Table 20). Cows fed 25% starch regardless of the silage type produced on average more milk protein (2.99%) and SNF (8.82%) than cows fed diets with 19% starch (milk protein = 2.91% and SNF = 8.68%). Increased milk protein concentration might have been the result of more microbial AA flow to the small intestine in cows fed diets with high starch concentration, increasing the AA available to the mammary gland for milk protein synthesis (Cantalapiedra-Hijar et al., 2014). These results are similar to those obtained by Fanchone et al. (2013) who associated increased milk protein concentrations with increased flow of EAA (948.5 g/d) and NEAA (1457.5 g/d) to the duodenum. In their experiment, cows fed starch-based diets (30.7%) were compared to cows fed fiber-based diets (15.2% starch; EAA = 847.5 g/d and NEAA = 1300 g/d) in either low (11%) or high (14.75%) CP diets. There is not much information reporting the effect of dietary starch on the concentration of SNF in milk. It is likely the increased milk protein ($P=0.05$) and the

trend ($P=0.06$) for higher lactose in response to dietary starch contributed to the increase in the concentration of SNF in the milk of cows fed diets with 25% starch concentration.

Dietary treatments did not affect the concentrations of milk fat and total solids concentrations, milk fat yield, MUN, and SCS (Table 20; $P>0.05$). Batajoo and Shaver (1994) found a linear decrease in milk fat concentration in response to changes in rumen pH and total VFA concentrations when the contents of dietary starch increased from 17.6 to 32.9%. A recent meta-analysis of the literature (Ferrareto and Shaver, 2015) reported lower milk fat and MUN concentrations in cows fed BMR corn silage diets than those fed CONV silage diets. In this meta-analysis, the proportion of NDF in the diets varied from 24 to 41% and the proportion of dietary starch also varied from 20 to 36%, which might have contributed to the milk fat and MUN differences. However, the dietary NDF and starch concentrations in the present study were not as high as in those studies, therefore milk fat concentration was not significantly affected

There was no effect of dietary treatments on BW, BCS, and their changes, indicating that cows did not mobilize body fat in support of milk production ($P>0.05$; Table 20). The concentration of plasma BHB as indicator of fat mobilization confirmed cows did not mobilize fat to support milk production ($P>0.05$; Table 21). In addition, concentration of glucose and PUN concentrations in blood plasma were not significantly affected by the treatments ($P>0.05$; Table 21). Therefore, neither, BW, BCS, or plasma metabolites explained the cow's response to different dietary treatments in this study.

The effects of dietary treatments on cow behavior are shown in Table 22. No differences were observed between treatments for standing, lying, rumination, eating, and chewing parameters ($P>0.05$). Although the inclusion of different corn silage affected

particle size of the diets (Table 18), it did not have a significant effect on cow behavior. Oba and Allen (2000a) observed greater meal sizes and longer intervals between meals in cows fed BMR corn silage diets compared to those in CONV silage diets, with high NDF content when the proportion of the silages were increased up to 50-55%. However, both parameters were lesser and shorter respectively, for cows in BMR corn silage diets compared to those in CONV silage diets at low NDF content, obtained by decreasing the proportions of silage to 32-36%. Regarding NFFS, Marchesini et al. (2011) did not report significant effects on rumination time, meals per day, and meal duration with the inclusion of NFFS in diets of dairy cows.

Apparent total tract nutrient digestibility is reported in Table 23. Dietary treatments did not affect intake of DM, OM, CP, and NDF ($P>0.05$); starch concentration however affected ADF and starch intakes ($P<0.05$). Cows fed diets with 25% starch consumed 0.5 kg/d less ADF regardless of silage type, although 1.52 kg/d more starch than cows fed 19% starch. Despite the minimal effect of diets on nutrient intake, digestibility of DM, OM, and CP were significantly affected by starch concentration. Cows fed 25% starch in either, CONV or BMR silages digested on average more DM (2.42%), OM (2.46%), and CP (2.30%) compared to those on 19% starch diets. Zhao et al. (2016) formulated diets with four dietary starch concentrations (17.6, 23.2, 28.8, and 34.4%) by replacing corn grain with CONV corn silage and oat hay. The authors reported a linear increase on DM, OM, and CP digestibility as dietary starch concentration increased. Similar to the present study, Zhao et al. (2016) also reported increases of DMI, milk yield, and ECM in response to increased starch. Therefore, Zhao's experiment and

the present study demonstrated the importance of dietary starch concentration on nutrient digestion and milk production regardless of corn silage type.

Digestibilities of NDF and ADF were not affected by treatments in the present study (Table 23; $P>0.05$), but the digestion of starch tended to be different by the interaction silage \times starch (Table 23; $P=0.08$). Cows fed CONV-25% starch and BMR-19% starch diets tended to have higher starch digestibility. An evaluation of the importance of NDF digestibility on milk yield demonstrated that for every unit increase in in vitro or *in situ* NDF digestibility, milk yield increased 0.23 kg/d and ECM 0.25 kg/d (Oba and Allen, 1999). In the present study however it was not possible to detect the effect of corn silage type on fiber digestion, although cows fed BMR-25% starch had the highest milk yield and ECM, and cows fed BMR-19% starch performed similar to cows in CONV silage regardless of starch concentration. It was therefore speculated that there must be an important contribution of the BMR silage to improve milk yield in cows fed reduced starch diets.

Conclusions

Cow fed diets with BMR corn silage tended to increase DMI over time compared to CONV silage diets. The highest milk yield, ECM and yield of milk protein, lactose, total solids, and SNF was observed in cows fed BMR-25 starch, but cows in BMR-19 starch produced the same amount of milk and ECM than cows fed CONV silage diets regardless of the starch concentration. However, FE was the lowest in cows fed BMR-19 starch. Cows fed 25% starch regardless of silage type improved the concentrations of milk protein, lactose, and SNF compared to cows with 19% starch. All these effects are explained by the increased digestibility of DM, OM, and CP in response to diets with

25% starch, and the potential increase in DMI with BMR corn silage. It can therefore be concluded that the inclusion of BMR corn silage has an important effect on dairy cow performance, and contributes considerably to the energy needed by the cow to maintain an optimal milk production in reduced starch diets.

Table 16. Ingredient and nutrient composition of experimental diets containing CONV and BMR corn silage with different starch concentrations

Item	CONV		BMR	
	19%	25%	19%	25%
Ingredients, % of DM				
Corn silage	40.00	40.00	40.00	40.00
Alfalfa haylage	20.69	20.69	20.69	20.69
Ground shelled corn	5.40	13.67	8.67	16.95
Beet pulp	5.69	1.98	3.79	0.34
Soybean hulls	6.55	1.98	5.17	0.34
Soybean whole roasted	5.17	5.17	5.17	5.17
Canola meal	10.57	10.57	10.57	10.57
Dried distillers grains with solubles	3.10	3.10	3.10	3.10
Rumen-inert fat ¹	0.34	0.34	0.34	0.34
Mineral and vit. premix ²	2.48	2.48	2.48	2.48
Nutrients, % of DM				
DM, % of diet	45.51	45.67	44.75	45.67
CP	16.73	16.60	16.80	16.62
aNDFom	35.04	31.46	33.99	30.74
NDF from forages	23.10	23.10	23.84	23.84
ADF	25.47	22.26	24.79	21.56
Starch	19.50	25.22	21.20	24.72
NFC ³	35.75	39.47	36.66	39.88
Ether extract	4.63	4.88	4.67	5.20
Ash	7.85	7.59	7.88	7.56
Ca	1.43	1.15	1.41	1.14
P	0.41	0.46	0.43	0.42
Mg	0.43	0.40	0.42	0.41
K	1.48	1.46	1.46	1.40
S	0.29	0.29	0.29	0.28

CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

¹Energy Booster 100 (Milk specialties Global, Co., Dundee, IL).

²Contained: Vitamin A, 290 IU/Kg; vitamin D, 58 IU/Kg; vitamin E, 1,365 IU/Kg; Calcium, 15.69%; Magnesium, 4.35%; Potassium, 0.54%; Sulfur, 0.95%; Sodium, 14.81%; Chloride, 6.67%; Salt, 11.03%; Iron, 778.21 Mg/kg; Zinc, 2,807.97 Mg/kg; Manganese, 2,601.300 Mg/kg; Copper, 518.81 Mg/kg; Iodine, 60.19 Mg/kg; Cobalt, 42.55 Mg/kg; Selenium, 14.64 Mg/kg (Vita Plus, Madison, WI); Rumensin®, 444.43 g/ton (Elanco, Greenfield, IN).

³NFC = 100 – (% NDF + % CP + % EE + % ash).

Table 17. Nutrient composition of forages and feeds used in diets containing CONV and BMR corn silage with different starch concentrations

Nutrient, % of DM (unless noted)	CONV	BMR	Alfalfa silage	Ground	Beet pulp	Roasted soybean	Soybean hulls	DDGS	Canola meal
	corn silage	corn silage		shelled corn					
DM, %	35.88	34.95	33.55	86.32	89.57	95.08	90.77	89.10	89.23
CP	6.60	6.91	25.18	7.89	8.97	37.08	10.81	31.51	41.08
aNDFom	38.10	39.94	37.97	8.85	35.00	18.15	67.53	29.03	23.78
NDFD-30h	56.75	64.79	43.43	-	-	-	-	-	-
uNDFom-30h	16.48	14.06	21.47	-	-	-	-	-	-
Starch	36.57	34.77	0.18	60.83	1.00	0.69	0.18	2.33	0.72
Ether extract	3.31	4.14	4.10	3.63	0.95	20.43	1.23	7.57	3.91
NFC	49.54	46.73	26.23	79.56	46.91	24.92	17.10	26.21	29.72
Ash	3.50	3.38	10.55	1.33	8.17	5.35	5.06	5.69	8.14

NFC = 100 – (% NDF + % CP + % EE + % ash).

Table 18. Particle size distribution of diets containing CONV and BMR corn silage with different starch concentrations

Screen ² , % as fed retained in each sieve	CONV ¹		BMR		SEM	Effect ³ (<i>P</i> > <i>F</i>)		
	19%	25%	19%	25%		CS	St	CS × St
Upper (>19.0 mm)	1.44	1.42	1.38	1.17	0.06	0.04	0.11	0.17
Middle (19.0-8.0 mm)	41.43	42.04	43.63	44.06	0.49	0.004	0.32	0.86
Lower (8.0-4.0 mm)	19.51	19.57	19.62	20.06	0.25	0.28	0.35	0.48
Bottom pan (<4.0 mm)	37.62	36.97	35.37	34.71	0.51	0.003	0.24	0.99

¹CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

²Particle size distribution measured according to Heinrichs (2013).

³CS = corn silage effect (CONV vs. BMR); St = dietary starch effect (19% vs. 25%); CS × St = interaction silage by starch concentration.

Table 19. Particle size distribution of CONV and BMR corn silage

Screen ² , % retained in each sieve	CONV ¹	BMR	SEM	<i>P</i> > <i>F</i>
Upper (>19.0 mm)	3.65	3.82	0.29	0.69
Middle (19.0-8.0 mm)	69.06	70.05	0.82	0.42
Lower (8.0-4.0 mm)	18.36	18.14	0.52	0.77
Bottom pan (<4.0 mm)	8.93	7.99	0.43	0.16

¹CONV = conventional corn silage; BMR = brown midrib corn silage

²Particle size distribution measured according to Heinrichs (2013).

Table 20. Lactation performance of cows fed diets containing CONV and BMR corn silage with different starch concentrations

Item	CONV ¹		BMR		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	19%	25%	19%	25%		CS	St	CS × St
DMI, ³ kg/d	25.85	26.77	26.95	26.82	0.54	0.29	0.47	0.33
Milk, ⁴ kg/d	44.09 ^{ab}	43.40 ^b	43.40 ^b	45.73 ^a	0.69	0.25	0.24	0.03
Fat, ⁴ %	3.83	3.89	3.87	3.88	0.09	0.91	0.66	0.74
Fat, kg/d	1.66	1.67	1.63	1.73	0.05	0.77	0.28	0.34
Protein, ⁴ %	2.91	3.00	2.90	2.98	0.04	0.65	0.05	0.99
Protein, ⁴ kg/d	1.27	1.27	1.23	1.32	0.02	0.92	0.12	0.08
Lactose, %	4.91	4.93	4.89	4.97	0.02	0.67	0.06	0.20
Lactose, kg/d	2.16 ^{ab}	2.11 ^{bc}	2.08 ^c	2.20 ^a	0.04	0.89	0.32	0.03
Total solids, ⁴ %	12.52	12.68	12.51	12.67	0.11	0.92	0.17	1.00
Total solids, kg/d	5.40	5.30	5.20	5.53	0.11	0.88	0.32	0.07
SNF, ⁴ %	8.69	8.81	8.67	8.83	0.04	0.98	0.001	0.55
SNF, kg/d	3.89	3.88	3.79	4.06	0.08	0.58	0.10	0.07
MUN, ⁴ mg/dL	11.06	10.52	11.37	10.96	0.35	0.29	0.18	0.85
SCS ⁵	2.19	2.37	2.32	2.23	0.09	0.92	0.61	0.12
ECM, ⁶ kg/d	45.09 ^b	44.90 ^b	44.20 ^b	46.83 ^a	0.71	0.47	0.09	0.05
ECM/DMI	1.76 ^a	1.69 ^{ab}	1.66 ^b	1.76 ^a	0.04	0.72	0.68	0.05
BW, ⁴ kg	646.24	644.83	649.77	640.78	5.08	0.96	0.31	0.46
BW change, ⁴ kg/d	1.01	0.96	1.81	-0.84	6.20	0.90	0.73	0.74
BCS ⁷	2.82	2.80	2.83	2.75	0.03	0.56	0.10	0.33
BCS change	-0.02	-0.02	-0.006	-0.02	0.02	0.86	0.76	0.87

^{abc}Means in rows with different superscripts differ (*P* ≤ 0.05).

¹CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

²CS = corn silage effect (CONV vs. BMR); St = dietary starch effect (19% vs. 25%); CS × St = interaction silage by starch concentration.

³CS × S interaction (*P* = 0.06)

⁴Effect of week (*P* < 0.05)

⁵SCS = log (SCC).

⁶ECM = [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein yield (kg)].

⁷Body condition score: 1 = emaciated to 5 = obese (Wildman et al., 1982).

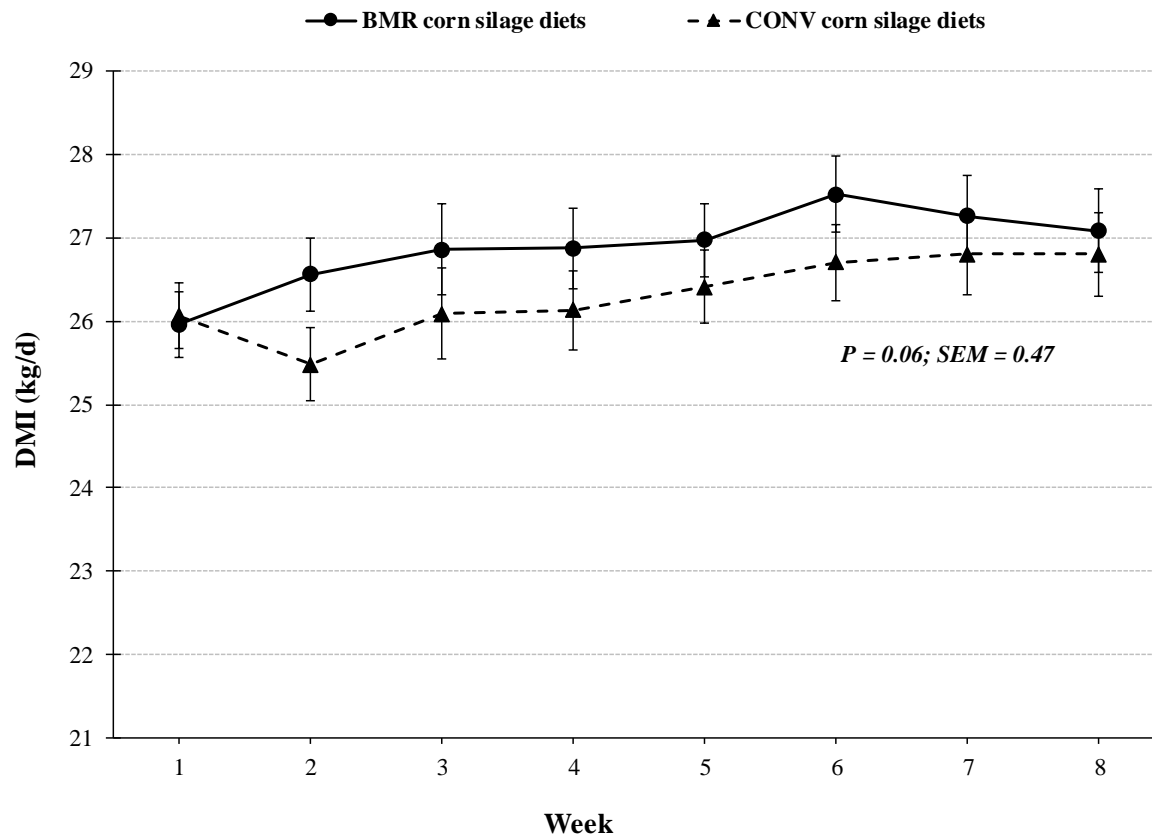


Figure 4. Effect of corn silage \times week on DMI.

Table 21. Plasma metabolites of cows fed diets containing CONV and BMR corn silage with different starch concentrations

Item	CONV ¹		BMR		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	19%	25%	19%	25%		CS	St	CS × St
Glucose, mg/dL	55.07	56.07	56.99	55.71	0.89	0.39	0.88	0.21
BHB, mmol/L	0.48	0.45	0.44	0.46	0.03	0.55	0.80	0.41
PUN, mg/dL	13.29	12.59	12.32	13.56	0.51	0.99	0.60	0.07

¹CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

²CS = corn silage effect (CONV vs. BMR); St = dietary starch effect (19% vs. 25%); CS × St = interaction silage by starch concentration.

Table 22. Behavior of dairy cows fed diets containing CONV and BMR corn silage with different starch concentrations

Item	CONV ¹		BMR		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	19%	25%	19%	25%		CS	St	CS × St
Standing								
Time, min/d	417.71	469.17	413.75	438.13	28.25	0.51	0.16	0.61
Events/d	10.88	11.38	11.17	11.54	0.61	0.67	0.42	0.91
Laying								
Time, min/d	796.25	744.58	802.21	773.75	33.96	0.50	0.13	0.67
Events/d	9.71	10.21	10.13	11.00	0.70	0.39	0.33	0.79
Rumination								
Time, min/d	475.00	455.46	458.13	467.08	15.95	0.86	0.72	0.34
Time, min/kg of DMI	18.14	16.81	17.73	17.45	0.89	0.90	0.37	0.56
Time, min/kg NDF intake	51.08	52.77	52.04	55.45	2.61	0.49	0.33	0.74
Periods/d	11.71	12.58	12.67	13.25	0.50	0.09	0.13	0.76
Duration of period, min	41.46	37.17	37.00	35.85	2.16	0.13	0.17	0.40
Eating								
Time, min/d	206.46	218.96	214.58	220.00	8.85	0.61	0.32	0.69
Time, min/kg of DMI	7.90	8.05	8.28	8.24	0.44	0.52	0.90	0.84
Time, min/kg NDF intake	22.25	25.26	24.31	26.15	1.29	0.26	0.07	0.66
Meals/d	16.83	15.96	15.67	16.58	0.84	0.75	0.98	0.29
Duration of meal, min	12.64	14.54	14.20	14.07	0.62	0.73	0.46	0.17
Chewing								
Time, min/d	681.46	674.42	672.71	687.08	18.15	0.91	0.84	0.55
Time, min/kg of DMI	26.05	24.86	26.01	25.70	1.18	0.74	0.53	0.71
Time, min/kg NDF intake	73.33	78.03	76.34	81.60	3.43	0.34	0.15	0.93

¹CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

²CS = corn silage effect (CONV vs. BMR); St = dietary starch effect (19% vs. 25%); CS × St = interaction silage by starch concentration.

Table 23. Apparent total tract nutrient digestibility of cows fed diets containing CONV and BMR corn silage with different starch concentrations

Item	CONV ¹		BMR		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	19%	25%	19%	25%		CS	St	CS × St
Intake, kg/d								
DM	26.25	27.47	25.99	27.22	0.92	0.78	0.19	0.99
OM	25.95	27.28	25.73	27.02	0.91	0.79	0.16	0.99
CP	4.33	4.54	4.33	4.48	0.15	0.82	0.23	0.85
NDF	9.0	8.58	8.57	8.21	0.30	0.19	0.20	0.91
ADF	6.34	5.87	6.10	5.54	0.22	0.19	0.02	0.83
Starch	5.12	6.93	5.51	6.73	0.22	0.66	<0.001	0.18
Digestibility, %								
DM	68.87	71.89	70.59	72.41	1.80	0.25	0.02	0.53
OM	69.33	72.48	71.15	72.92	1.74	0.23	0.01	0.46
CP	67.23	70.50	69.14	70.45	7.83	0.38	0.04	0.36
NDF	55.04	56.43	56.04	56.86	2.59	0.65	0.49	0.86
ADF	52.00	53.61	55.09	54.27	3.07	0.26	0.80	0.47
Starch	97.06	97.41	97.21	96.71	0.32	0.32	0.68	0.08

¹CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

²CS = corn silage effect (CONV vs. BMR); St = dietary starch effect (19% vs. 25%); CS × St = interaction silage by starch concentration.

CHAPTER 5:
IMPACT OF DIETARY STARCH CONCENTRATION WITH TWO TYPES OF
CORN SILAGE ON THE PERFORMANCE AND GAS EMISSIONS IN
LACTATING DAIRY COWS

Abstract

The objective of this study was to evaluate the effects of different corn silages and starch concentrations on the performance, nutrient digestion, and gas emissions (CH₄, NH₃, and CO₂) in lactating dairy cows. After the completion of an 8-week production study, 48 Holstein cows were allocated to 1 of 4 air-flow controlled chambers (2 cows/chamber) for 6 d, in a randomized complete block design. Chamber was the experimental unit. Cows were fed 1 of 4 diets arranged as a 2 × 2 factorial with 2 corn silage hybrids (CONV and BMR corn silage) and 2 dietary starch concentrations (19 and 25%). Performance data from the last 6 d and emission measurements from the last 3 d were recorded and used for analysis. To decrease starch concentration soybean hulls and beet pulp replaced a portion of corn grain in the diet. There was no effect of corn silage and dietary starch concentration on DMI, milk yield, ECM, and FE. While concentrations and yields of milk protein, lactose, total solids, and SNF were also not affected, milk fat concentration was greater ($P<0.03$) in cows fed diets formulated for 25% starch rather than 19% starch. A silage × starch interaction was observed for MUN ($P=0.03$), but values from all treatments were considered normal. Total tract nutrient digestibility was not affected by corn silage or starch concentration ($P>0.05$), but starch digestibility was lower in BMR-25% starch and CONV-19% starch diets ($P=0.05$). Diets did not affect CH₄, NH₃, and CO₂ emissions or those gases expressed per kg of milk yield and ECM

($P>0.05$). Nevertheless, the emissions of CH_4 and CO_2 expressed per kg of DM, OM, and starch digested was different by corn silage type and starch concentration ($P<0.05$). Cows fed BMR corn silage and 25% starch produced less CH_4 and CO_2 per kg of DM, OM, and starch digested, than cows in CONV silage. However, cows fed 25% starch produced more NH_3 and CO_2 per kg of NDF digested in any silage type. In general, diets formulated with BMR silage and 25% starch concentration represent a practical opportunity to reduce gas emissions per unit of nutrient digested in dairy farms.

Keywords: BMR corn silage, starch, milk, gas emissions

Introduction

One of the main problems associated with dairy cattle nutrition is the production of CH₄, NH₃, and CO₂, which contribute to the accumulation of greenhouse gases in the atmosphere. During enteric fermentation cows produce CH₄ (Knapp et al., 2014), and also CO₂ to some extent (Amon et al., 2001). Emissions of NH₃ and CO₂ on the other hand, are associated with urine and manure management (Amon et al., 2001; Zhu et al., 2012). Of these gases, CH₄ is the one that has received the most attention in dairy cattle nutrition. Enteric CH₄ is mainly produced by Archaea species of microorganisms by using metabolic H₂ formed during the fermentation of carbohydrates (Knapp et al., 2014), and protein in the rumen (Wallace et al., 2015). Opportunities such as feeding and nutrient management, utilization of rumen modifiers in the diet, and improvements on genetics to increase animal production have been proposed as key strategies to mitigate CH₄ emissions in dairy cows (Knapp et al., 2014). However, the manipulation of dietary carbohydrates seems to be one of the most practical and less costly strategies to be applied in commercial dairy farms.

Carbohydrates are the main contributors to CH₄ production. Of these, fibrous carbohydrates increase CH₄ production significantly more when compared to soluble carbohydrates (Moe and Tyrrel, 1979). Of the soluble carbohydrates, sugars have been demonstrated to have a higher potential for CH₄ production than starch (Czerkawski and Breckenridge, 1969). However, modelling comparison between starchy and fibrous concentrates has demonstrated that CH₄ production is 22% higher with fibrous-based concentrates (Benchaar et al., 2001). Forages with high NDF and greater lignification have been demonstrated to produce high enteric CH₄ per unit of DMI (Archimède et al.,

2011). Hence, forages with less lignin content may contribute to reduced enteric CH₄ production.

Corn silage BMR contains less lignin and increased NDF digestibility than CONV corn silage. As a result, BMR corn silage may potentially reduce CH₄ production in dairy cows, though little information has been generated about it. Tine et al. (2001) reported a trend of 0.3 Mcal/d less CH₄ energy emitted in lactating dairy cows fed BMR corn silage compared to those fed CONV corn silage; no effects however were found when treatments were applied to dry cows. More recently, Schwarm et al. (2015) did not find an effect on enteric CH₄ emission in dairy heifers fed BMR or CONV corn silage. The authors reported however a trend in daily CH₄ production of 16 l/kg of NDF digested in heifers with BMR corn silage diets compared to those with CONV silage.

There is some information generated comparing enteric CH₄ emissions in dairy cattle fed BMR corn silage versus CONV corn silage. There is no information however on the effects of BMR corn silage and CONV corn silage on enteric CH₄ emissions in lactating dairy cow fed different starch concentrations. The objective of this study was to evaluate the effects of CONV and BMR corn silages in combination with different starch concentrations on the performance, nutrient digestion, and gas emissions of lactating dairy cows. The hypothesis was that cows fed BMR corn silage would produce less CH₄ than those fed CONV corn silage regardless of the dietary starch concentration.

Materials and Methods

Cows, Treatments, and Diets

The experiment was conducted at the USDA-ARS Dairy Forage Research Center farm in Prairie du Sac, Wisconsin, under the protocols approved by the University of

Wisconsin Institutional Animal Care and Use Committee. Forty-eight lactating Holstein cows [24 primiparous (209.54±23.13 DIM and 629.03±33.63 kg of BW) and 24 multiparous (209.92±20.92 DIM and 691.81±38.69 kg of BW)] were blocked in pairs by parity, then randomly assigned to the measurements chamber using a randomized complete block design. Cows were fed four diets using a 2 × 2 factorial arrangement for 8 weeks (Chapter 4) and week 9 was used for measurements in this study. Experimental diets were formulated with two different corn silages (CONV and BMR) and two starch concentrations (19% and 25%). The proportions of corn silages and alfalfa haylage were maintained similar across diets and the reduction of dietary starch was accomplished by replacing 8.2% ground corn with soybean hulls and beet pulp (Table 24). All other ingredients were maintained similarly between the diets. Experimental diets were formulated to meet the nutrient requirements for a mature Holstein cow with 750 kg BW, BCS of 3.0, and 90 DIM producing 45 kg/d with 3.5% fat and 3.0% protein (NRC, 2001). Cows were fed diets as TMR once daily at 1000 h in tie-stall chambers adjusting feed intake to 5 to 10% orts.

Measurements and Sampling

Daily feed intake was measured during the whole week by difference between feed offered and orts. Three days average forage DM was analyzed by NIRS from a three-day composite sample with data from the previous week used to adjust dietary DM. Samples of TMR, orts, forages, and individual feedstuffs were collected daily, stored at -20°C, and then a weekly composite sample of each was used for DM analysis. The analysis of DM in all samples was performed at the end of the week. At the end of each week 500 g of sample were used to determine particle size distribution in each diet.

Total tract nutrient digestion was estimated through fecal samples collected in all cows on 3 consecutive days during the week. In total, 6 fecal samples (from 8 to 12 h intervals) per cow of approximately 20 g each were obtained directly from the rectum or spontaneous release in all animals and then dried immediately

Cows were milked thrice daily at 0600, 1200, and 1900 h, and milk production recorded daily. Individual milk samples from each milking were collected 2 consecutive days during the week, then sent to a commercial laboratory for milk composition analysis.

Gas emissions were measured during the last three days of the week in a modified tie-stall dairy barn at the US Dairy Forage Research Center in Prairie du Sac, Wisconsin. The barn contained 4 chambers designed to measure gas emission rates from cows by using a mass balance of the system, according to Drewry et al (2015). Each chamber was approximately 4.73 m deep by 5.36 m wide by 2.87 m high and could hold up to 3 cows using tie-stalls. An inlet duct equipped with a blower and electric heater provided ventilation to the chambers, while outlet ducts with exhaust fans were used to expel air out of each chamber. The inlet and outlet ducts were equipped with pitot tubes (Ultratech Industries, Inc., Ultraprobe AMPS, Garner, NC), temperature and humidity sensors (Campbell Scientific, HC2S3, Logan, UT), and a custom cross-sectional air sampler to monitor the air flow and gas concentrations. The air samplers were connected to a multi-point sample switching system (Air Quality Analytical, MSS, Wilmington, NC) and Fourier Transform Infrared Spectroscopy gas analyzer (Gasmeter, Model DX4015, Helsinki, Finland). Prior to measurements of gas emissions, chambers were calibrated according to Drewry et al. (2015).

Laboratory Analysis

Samples of TMR, Orts, forages, and individual feedstuffs were dried by triplicate at 55°C for 48 h in a Thermo Scientific Heratherm oven (OMH 750L units, Thermo Fisher Scientific Inc., Waltham, MA). Only two of those samples were further dried at 105°C for 24 h in the same oven. Fecal samples were dried in a Precision Elect oven (460/230, Precision Quincy, Woodstock, IL). All dried samples were ground to 4-mm particle size (Wiley mill, model 4, Arthur H. Thomas Co., Philadelphia, PA). Ground samples were further analyzed for DM, CP, NDF, ADF, starch, and ash. Ether extract was estimated using the EE contents in feeds from NRC (2001) and diet composition. Crude protein percentages were determined by analyzing total N using a combustion assay (Leco FP-2000 N Analyzer, Leco Instruments Inc., St. Joseph, MI). The analysis of NDF was determined by gravimetric determination of amylase-treated NDF using beakers or crucibles (Mertens, 2002), and ADF by the refluxing method (973.18; AOAC international, 1990). Starch was analyzed on sub-samples ground through a 1-mm screen of an abrasion mill (Udy Corp., Fort Collins, CO) using the methodology proposed by Hall (2015). Samples were incubated in screw cap tubes with thermostable α -amylase in 30 mL sodium acetate (pH 5.0) for 1 h at 100°C with periodic mixing (initial vortex, and then vortex at 10, 30, and 50 min) to gelatinize and partially hydrolyze α -glucan. Then, amyloglucosidase was added, and the reaction mixture was incubated in a water bath at 50°C for 2, then hand mixed once (vortex after the first hour). After incubation, 20 mL of distilled water was added and tubes were inverted $\pm 10 \times$ to mix completely. Approximately 2 mL of the solution were centrifuged at $1000 \times g$ for 10 min, then 0.1 mL working test solution and standards (in duplicate) were transferred to 16×100 mm

glass tubes. Finally, 3.0 mL of glucose oxidase–peroxidase were added to each glass tube, vortex them, covered with a plastic film to seal them, and incubated at 50°C for 20 min. Absorbance was read at 505 nm. Samples of TMR, forages, and individual feeds were corrected for free glucose.

Ash concentration was analyzed by heating 1 g of sample for 8 h at 450°C in a muffle furnace (942.05; AOAC International, 1998); OM was calculated as $100 - \% \text{ ash}$. Based on the nutrient analysis, NFC was calculated as $100 - (\% \text{ CP} + \% \text{ NDF} + \% \text{ EE} + \% \text{ ash})$ according to the NRC (2001). Individual forage and feedstuff analysis along with the proportion of each ingredient in the ration were used to calculate the chemical composition of the experimental diets. In addition, TMR samples were also analyzed to validate the calculated chemical composition of the diets. Mineral analysis (Ca, P, Mg, K, and S) in TMR samples was performed by Dairyland laboratories, Inc. (Arcadia, WI) using wet chemistry.

Total tract nutrient digestion was determined *in situ* using iADF by incubating bags (25 μm pore size) in the rumen of two cows during 288-h (Huhtanen et al., 1994). Analysis of DM, OM, CP, NDF, ADF, and starch in TMR and feces samples along with the internal marker were utilized to estimate total tract nutrient digestibility according to Ferrareto et al. (2015): $\text{apparent total tract nutrient digestibility (\%)} = 100 - [100 \times (\text{TMR marker concentration/fecal marker concentration}) \times (\text{fecal nutrient concentration/TMR nutrient concentration})]$.

Milk samples were sent to AgSource Milk Analysis Laboratory (Menomonie, WI) to analyze fat, protein, SNF, MUN, and SCC. According to AgSource laboratories, milk components were analyzed using a Foss FT6000 instrument (method 972.16; AOAC

International, 1998; Foss Electric A/S, Hillerod, Denmark). Total solids were calculated adding milk fat percentage to the concentration of SNF; somatic cell counts were converted to a linear SCS.

Gas concentrations were measured every 20 seconds by the FTIR gas analyzer. The analyzer would measure three samples from a single source before moving on to the next one in the order as follows: Chamber 1, Chamber 2, Chamber 3, Chamber 4, and then from the inlet. With a 40 second flush period between sources, the analyzer could successfully analyze three samples from all of the five sources in 7 minutes and 40 seconds, and would immediately begin again. The ambient pressure, airflow, and temperature and relative humidity were measured at 30 second time intervals. The flux of each gas into and out of the chambers is numerically integrated using Simpson's Method over the course of each day. This information is then used to determine how much of each gas is being generated in each chamber. Time periods where cows were removed for milking were excluded from the data set. In fact, only data after 1 h after cows returned to the chambers were used. In average, gases were measured for 11.6 h per day with two cows in a chamber. The emissions for the emitted gas per each sampling interval was calculated as the product of gas concentration in the outlet (g/m^3) and the volumetric air flow (m^3), but corrected for gas concentration in the inlet air (g/m^3).

Statistical Analysis

All data were analyzed by the MIXED procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC) as a randomized complete block design. Statistical analysis considered weekly averages of TMR particle size and daily DMI, milk production, and milk components concentration and yield. Averages for cow total tract nutrient

digestibility from their respective time of sampling were considered in the analysis. Three days gas emissions average from the week were used in the analysis. Model selection was based on the smallest AIC and BIC.

The effect of dietary treatments was evaluated with the following model:

$$Y_{ijk} = \mu + CS_i + St_j + (CS \times St)_{ij} + B_k + e_{ijk}$$

Where: Y_{ij} = dependent variable, μ = overall mean, CS_i = effects of corn silage i ($i = 1$ to 2), St_j = effects of starch concentration j ($j = 1$ to 2), $(CS \times St)_{ij}$ = interaction between corn silage i and starch concentration j , B_k = effect of block l ($l = 1$ to 6), and e_{ij} = random residual error. Data were reported as least square means and the Tukey's test was used for separation of treatment means. Statistical significance in all variables was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

Results and Discussion

Nutrient Composition of Diets, Feeds, and Particle Size of Diets

Nutrient composition of diets is reported in Table 24. Concentration of CP was similar across diets averaging 16.79%; diets with 25% starch had on average 3.4% less NDF than those with 19% starch. This is a reflection of adding soybean hulls and beet pulp to decrease a portion of corn grain in reduced starch diets. Actual starch concentration in formulated diets averaged 25.2% and 20.36%.

Nutrient composition of individual feeds used to formulate experimental diets is shown in Table 25. The main difference between silages was NDF content and its digestibility. The content of NDF in CONV corn silage was 3.6% higher than BMR silage. However, the NDFD-30h and uNDF-30h were 8.44 and 1.93% higher, respectively in CONV corn silage compared to BMR corn silage.

Performance and Nutrient Digestibility

The effect of diets on the performance of lactating dairy cows is reported in Table 26. There was a silage \times starch interaction for MUN ($P=0.03$); cows fed CONV-25% starch had the lowest MUN (10.81 mg/dL) and similar to cows fed BMR-19% starch (11.20 mg/dL), but different from CONV-19% and BMR-25% starch. This effect might be the result of a better nitrogen use by cows associated with the amount of starch and rate of carbohydrate digestion of the diets. Ranges of MUN for all treatments in the present study however are considered normal (10-16 mg/dL; Powell et al., 2014).

Dietary treatments did not influence DMI, milk yield, ECM, and FE (Table 26). Similar results of diets formulated with BMR and CONV corn silages on DMI have been reported in dairy heifers (Schwarm et al., 2015) and lactating dairy cows (Tine et al., 2001; Akins and Shaver, 2014). However, a meta-analysis of 48 published papers (Ferraretto and Shaver, 2015) reported greater DMI in cows fed BMR corn silage diets (24.9 kg/d) compared to those fed CONV silage diets (24 kg/d). As a result there was more milk production (1.5 kg/d) and FCM (1.1 kg/d) in cows with BMR corn silage diets. Regarding starch, no effects have been observed of the concentration of dietary starch on DMI (Akins et al., 2014; Dann et al., 2014; Dann et al., 2015). Dann et al. (2015) however, indicated that cows fed 26% starch produced more milk than those with 21% starch. Although BMR corn silage and dietary starch concentration have demonstrated to affect significantly DMI and milk yield, it was not possible to find differences between diets on DMI and milk yield related to these factors in the present study. Therefore, FE was also similar between cows fed BMR and CONV corn silage with any starch concentration in our study.

Considering all milk components and yields, there was only a significant and a trend effect of dietary starch concentration on milk fat concentration and milk fat yield, respectively (Table 26). Cows fed 25% starch produce on average 0.25% more milk fat and tended to have 0.13 kg/d milk fat than cows fed 19% starch concentration. Milk fat yield tended to increase as a response of the increased milk fat concentration, but is challenging to explain the increase of milk fat concentration in diets with the highest starch content as milk fat concentration decrease in response of diets high in starch (Batajoo and Shaver, 1994; Dann et al., 2015).

Apparent total tract nutrient digestibility is shown in Table 27. There was no significant effects of diet on intake and digestion of DM, OM, and CP ($P>0.05$). However, the intake of NDF, ADF, and starch was significantly affected by starch concentration. Intakes of NDF and ADF were 0.78 kg/d and 1.19 kg/d higher, respectively in cows fed 19% starch compared to those in diets with 25% starch. Cows fed 25% starch had 1.33 kg/d higher starch intake than those in diets with 19% starch. Those differences were directly related to the addition of feed with higher fiber content (soybean hulls and beet pulp) to reduce starch in the experimental diets. Although starch affected fiber intake, the digestibility of the fiber fractions was similar between treatments ($P>0.05$). There was a silage \times starch interaction for starch digestibility; cows fed BMR-25% starch had the lowest starch digestibility, and similar to those fed CONV-19% starch. Digestibility results paralleled the results of cow performance, which indicated no effect of dietary treatment.

Gas emissions

The effect of dietary treatments on gas emissions and emissions expressed per kg of milk yield and ECM is reported in Table 28. The actual emissions of CH₄, NH₃, and CO₂ and their expression per kg of milk and ECM were similar between treatments ($P>0.05$). Hassanat et al. (2017) did not find significant effects between CONV and BMR corn silage on CH₄ emissions in lactating dairy cows. However, decreasing dietary starch concentration from 19 to 11% by increasing the proportion of DDGS in the diet, decreased CH₄ emissions 20 g/d (Benchaar et al., 2013). Although actual gas emissions were not significantly affected by diets in the present study, gas emissions per kg of nutrient intake differed between treatments (Table 28). The silage × starch interaction had an effect on the emissions of CH₄ and CO₂ ($P<0.05$) per kg of DM and OM intake, and tended to affect the emissions of NH₃ per kg of DM and OM intake (Table 29; $P=0.06$). Cows fed BMR-25% starch produced 1.22 and 1.25 less g of CH₄/kg of DM and OM intake, respectively. Similarly, cows fed BMR-25% starch produced lower CO₂/kg of DM and OM intake than those fed CONV-25% starch, but CO₂ emissions/kg of DM and OM intake were similar between cows in BMR-25% starch and those in BMR-19% starch and CONV-19% starch. Emissions of NH₃/kg of DM and OM intake tended to be greatest in cows fed CONV-25% starch. Findings from the present study are in line with those reported by Hassanat et al. (2017) who reported less CH₄ emissions per kg of DMI in cows fed BMR corn silage, but are in disagreement with results indicated by Benchaar et al. (2013) who reported less CH₄ emission in cows fed reduced starch diets.

Starch concentration affected CH₄, NH₃, and CO₂ emissions per kg of NDF intake (Table 29; $P<0.05$). Cows fed 25% starch produced on average 7.4 g CH₄, 0.62 g NH₃,

and 245.67 g CO₂ more per kg of NDF intake. Nevertheless, CH₄ and CO₂ emissions per kg of starch intake were 19.3 and 617.71 g lower in cows fed 25% starch than those fed 21%. Moreover, along with dietary starch intake, CH₄ and CO₂ emissions was affected by silage type, indicating 6.78 and 198.36 g of CH₄ and CO₂ less, respectively, in cows fed BMR silage than cows in CONV silage.

Gas emissions expressed per kg of nutrient digested are reported in Table 30. There were no silage × starch interactions between treatments; silage however affected significantly CH₄ and CO₂ emissions per kg of DM and OM digested ($P < 0.05$). Emissions of CH₄ were 2.36 g lesser per kg of digested DM and tended to be 2.29 g lesser per kg of OM digested ($P = 0.06$) in cows fed BMR silage compared to those in CONV silage. In addition, those cows produced 72.50 g CO₂/kg DM digested and 70 g CO₂/kg OM digested less than cows fed CONV silage. Dietary starch concentration significantly affected emissions of NH₃ and CO₂ per kg of NDF digested ($P < 0.05$) and tended to affect CH₄ emission per kg of NDF digested ($P = 0.06$). Cows fed 25% starch produced on average 1.49 g NH₃ and 955.8 g CO₂ more per kg of NDF digested, and tended to produce also 18.5 g CH₄ more per kg of NDF digested. On the other hand, both silage and starch affected the CH₄ and CO₂ emissions per kg of starch digested ($P < 0.05$). Cows fed BMR silage produced 6.69 g CH₄ and 195 g CO₂ less per kg of starch digested than those in CONV silage. In addition, cows fed 25% starch diets had 19.64 g CH₄ and 625.91 g CO₂ lower per kg of starch digested than cows fed 19% starch diets. All these results may be linked to an optimum fiber digestion with BMR silage (less retention time; Huhtanen et al., 2016). This could be explained by optimal rumen fermentation due to higher starch concentrations (more rumen propionate; Johnson and Johnson, 1995). This

would in turn result in reduced enteric CH₄ and CO₂, as well as N excretion, with decreased NH₃ formation via urine in the cows. Therefore, less gas emissions per unit of nutrient digested was obtained in the present study.

Conclusions

Dry matter intake, milk yield, ECM, FE, concentrations and yields of milk protein, lactose, total solids, SNF, and total tract nutrient digestibility were similar between treatments, with milk fat concentration higher in diets with 25% starch. Milk urea nitrogen was lowest in cows fed CONV-25% starch, but similar to cows fed BMR regardless of starch concentration. Total tract nutrient digestibility was unaffected by diets, but starch digestibility was lower in BMR-25% starch and CONV-19% starch diets. Total emissions of CH₄, NH₃, and CO₂ and their emissions expressed per kg of milk yield and ECM were similar between treatments. However, cows fed BMR corn silage along with feeding 25% starch produced less CH₄ and CO₂ per kg of DM, OM, and starch digested compared to those fed CONV corn silage; although cows fed 25% starch produced more NH₃ and CO₂ per kg of NDF digested. Therefore, BMR corn silage and high starch diets represent a practical opportunity to reduce gas emissions per unit of nutrient digestion in dairy farms.

Table 24. Ingredient and nutrient composition of experimental diets containing CONV and BMR corn silage with different starch concentrations

Item	CONV		BMR	
	19%	25%	19%	25%
Ingredients, % of DM				
Corn silage	40.00	40.00	40.00	40.00
Alfalfa haylage	20.69	20.69	20.69	20.69
Ground shelled corn	5.40	13.67	8.67	16.95
Beet pulp	5.69	1.98	3.79	0.34
Soybean hulls	6.55	1.98	5.17	0.34
Soybean whole roasted	5.17	5.17	5.17	5.17
Canola meal	10.57	10.57	10.57	10.57
Distillers dried grains with solubles	3.10	3.10	3.10	3.10
Rumen-inert fat ¹	0.34	0.34	0.34	0.34
Mineral and vitamin premix ²	2.48	2.48	2.48	2.48
Nutrients, % of DM				
DM, % of diet	46.81	46.70	46.60	46.62
CP	16.87	16.70	16.90	16.72
aNDFom	35.61	32.28	34.14	30.58
NDF from forages	22.86	22.87	24.31	24.31
ADF	25.13	22.33	24.68	21.11
Starch	19.45	25.68	21.26	24.72
NFC ³	35.05	38.53	36.57	39.61
Ether extract	4.58	4.97	4.56	5.49
Ash	7.89	7.52	7.83	7.60
Ca	1.55	1.13	1.43	1.11
P	0.45	0.47	0.46	0.42
Mg	0.44	0.42	0.45	0.42
K	1.43	1.48	1.44	1.35
S	0.29	0.28	0.29	0.27

CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

¹Energy Booster 100 (Milk specialties Global, Co., Dundee, IL).

²Contained: Vitamin A, 290 IU/Kg; vitamin D, 58 IU/Kg; vitamin E, 1,365 IU/Kg; Calcium, 15.69%; Magnesium, 4.35%; Potassium, 0.54%; Sulfur, 0.95%; Sodium, 14.81%; Chloride, 6.67%; Salt, 11.03%; Iron, 778.21 Mg/kg; Zinc, 2,807.97 Mg/kg; Manganese, 2,601.300 Mg/kg; Copper, 518.81 Mg/kg; Iodine, 60.19 Mg/kg; Cobalt, 42.55 Mg/kg; Selenium, 14.64 Mg/kg (Vita Plus, Madison, WI); Rumensin®, 444.43 g/ton (Elanco, Greenfield, IN).

³NFC = 100 – (% NDF + % CP + % EE + % ash).

Table 25. Nutrient composition of forages and feeds used in diets containing CONV and BMR corn silage with different starch concentrations

Nutrient, % of DM (unless noted)	CONV	BMR	Alfalfa silage	Ground					
	corn silage	corn silage		shelled corn	Beet pulp	Roasted soybean	Soybean hulls	DDGS	Canola meal
DM, %	35.74	35.75	35.27	85.57	88.73	95.87	90.32	88.50	88.38
CP	6.56	6.77	25.62	7.85	8.58	37.84	10.90	32.01	41.30
aNDFom	37.47	41.08	38.06	9.53	35.51	17.45	67.63	28.44	23.00
NDFD-30h	57.33	65.77	43.59	-	-	-	-	-	-
uNDFom	15.99	14.06	21.47	-	-	-	-	-	-
Starch	33.89	35.44	0.19	61.27	1.20	0.88	0.28	2.23	0.49
EE	3.00	4.38	4.10	3.90	0.96	21.65	1.35	7.46	3.87
NFC	50.67	45.21	26.06	78.53	47.06	23.74	16.71	26.88	30.29
Ash	3.34	3.64	10.29	1.44	7.89	5.37	5.15	5.21	8.14

NFC = 100 – (% NDF + % CP + % EE + % ash).

Table 26. Lactation performance of cows fed diets containing CONV and BMR corn silage with different starch concentrations

Item	CONV ¹		BMR		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	19%	25%	19%	25%		CS	St	CS × St
DMI, kg/d	25.38	25.09	24.89	25.94	0.64	0.77	0.56	0.31
Milk, kg/d	41.97	41.81	39.87	42.61	1.34	0.64	0.35	0.29
Fat, %	3.91	4.07	3.68	4.02	0.11	0.18	0.03	0.42
Fat, kg/d	1.62	1.71	1.47	1.65	0.07	0.15	0.08	0.49
Protein, %	3.04	3.10	3.05	3.08	0.07	0.89	0.45	0.78
Protein, kg/d	1.29	1.25	1.21	1.29	0.05	0.74	0.72	0.19
Lactose, %	4.91	4.89	4.87	4.82	0.07	0.29	0.48	0.69
Lactose, kg/d	2.09	1.98	1.95	2.03	0.09	0.64	0.84	0.26
Total solids, %	12.66	12.92	12.47	12.68	0.20	0.23	0.19	0.33
Total solids, kg/d	5.35	5.32	5.06	5.43	0.18	0.57	0.32	0.27
Solids-not-fat, %	8.78	8.85	8.78	8.75	0.11	0.60	0.87	0.58
Solids-not-fat, kg/d	3.74	3.57	3.51	3.69	0.14	0.72	0.98	0.24
MUN, mg/dL	11.95 ^a	10.81 ^b	11.20 ^{ab}	11.81 ^a	0.37	0.74	0.49	0.03
SCS ³	2.13	2.57	2.46	2.18	0.22	0.91	0.72	0.13
ECM ⁴ , kg/d	44.05	44.71	40.78	44.57	1.42	0.25	0.13	0.28
ECM/DMI	1.74	1.79	1.65	1.74	0.06	0.25	0.26	0.81
BW, kg	624.15 ^b	653.22 ^a	658.96 ^a	619.87 ^b	20.52	0.96	0.72	0.02

^{ab}Means in rows with different superscripts differ (*P* < 0.05).

¹CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

²CS = corn silage effect (CONV vs. BMR); St = dietary starch effect (19% vs. 25%); CS × St = interaction silage by starch concentration.

³SCS = log (SCC).

⁴ECM = [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein yield (kg)].

Table 27. Apparent total tract nutrient digestibility of cows fed diets containing CONV and BMR corn silage with different starch concentrations

Item	CONV ¹		BMR		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	19%	25%	19%	25%		CS	St	CS × St
Intake, kg/d								
DM	25.12	24.58	24.71	26.07	0.59	0.37	0.49	0.13
OM	24.90	24.44	24.52	25.91	0.60	0.38	0.45	0.14
CP	4.18	4.12	4.15	4.37	0.11	0.27	0.39	0.15
NDF	8.65	7.72	8.40	7.79	0.24	0.70	0.003	0.49
ADF	6.03	5.24	6.82	5.25	0.16	0.51	0.001	0.47
Starch	4.89	6.30	5.25	6.49	0.15	0.07	<0.001	0.57
Digestibility, %								
DM	66.44	66.42	70.23	70.36	2.37	0.31	0.50	0.53
OM	67.16	70.00	70.83	70.89	2.30	0.31	0.51	0.53
CP	65.06	67.00	69.34	69.26	2.39	0.19	0.70	0.68
NDF	51.17	52.80	56.98	52.32	3.44	0.41	0.64	0.34
ADF	49.49	50.64	54.77	49.44	3.68	0.55	0.54	0.35
Starch	97.27 ^{ab}	97.61 ^a	97.57 ^a	96.66 ^b	0.47	0.28	0.35	0.05

^{ab}Means in rows with different superscripts differ (*P* < 0.05).

¹CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

²CS = corn silage effect (CONV vs. BMR); St = dietary starch effect (19% vs. 25%); CS × St = interaction silage by starch concentration.

Table 28. Gas emissions of cows fed diets containing CONV and BMR corn silage with different starch concentrations

Item	CONV ¹		BMR		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	19%	25%	19%	25%		CS	St	CS × St
CH ₄ , g/d	502.95	515.92	493.95	497.42	12.95	0.20	0.43	0.65
CH ₄ , g/kg of Milk	12.00	12.36	12.40	11.68	0.36	0.71	0.64	0.17
CH ₄ , g/kg of ECM	11.45	11.56	12.11	11.17	0.34	0.69	0.24	0.14
NH ₃ , g/d	22.27	27.56	23.44	23.07	2.65	0.42	0.24	0.18
NH ₃ , g/kg of Milk	0.53	0.66	0.59	0.55	0.06	0.59	0.42	0.10
NH ₃ , g/kg of ECM	0.51	0.62	0.58	0.52	0.06	0.78	0.61	0.12
CO ₂ , g/d	16,588	17,067	16,358	16,719	872.96	0.32	0.16	0.84
CO ₂ , g/kg of Milk	395.68	407.30	411.49	391.68	20.05	0.99	0.76	0.24
CO ₂ , g/kg of ECM	377.15	380.79	401.74	374.23	18.10	0.47	0.35	0.22

¹CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

²CS = corn silage effect (CONV vs. BMR); St = dietary starch effect (19% vs. 25%); CS × St = interaction silage by starch concentration.

Table 29. Gas emissions per kilogram of nutrient intake of cows fed diets CONV and BMR corn silage with different starch concentrations

Item	CONV ¹		BMR		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	19%	25%	19%	25%		CS	St	CS × St
CH ₄ , g/kg of DM intake	20.01 ^b	20.94 ^a	20.05 ^b	19.11 ^c	0.46	0.003	0.98	0.002
CH ₄ , g/kg of OM intake	20.17 ^a	21.07 ^a	20.21 ^a	19.23 ^b	0.45	0.003	0.86	0.002
CH ₄ , g/kg of NDF intake	58.04	66.82	58.05	64.13	1.92	0.57	0.001	0.23
CH ₄ , g/kg of starch intake	102.88	81.76	94.31	76.76	2.08	<0.001	<0.001	0.15
NH ₃ , g/kg of DM intake	0.88	1.13	0.95	0.90	0.10	0.28	0.22	0.06
NH ₃ , g/kg of OM intake	0.90	1.14	0.96	0.90	0.10	0.26	0.25	0.06
NH ₃ , g/kg of NDF intake	2.57	3.60	2.81	3.02	0.33	0.49	0.02	0.11
NH ₃ , g/kg of starch intake	4.56	4.41	4.48	3.60	0.45	0.17	0.12	0.26
CO ₂ , g/kg of DM intake	659.36 ^{ab}	692.87 ^a	664.15 ^{ab}	640.60 ^b	33.08	0.05	0.66	0.02
CO ₂ , g/kg of OM intake	664.75 ^{ab}	696.80 ^a	669.33 ^{ab}	644.51 ^b	32.77	0.05	0.76	0.03
CO ₂ , g/kg of NDF intake	1913.74	2209.49	1958.16	2153.75	115.14	0.92	0.004	0.37
CO ₂ , g/kg of starch intake	3390.00	2704.59	3123.94	2573.94	152.34	0.005	<0.001	0.28

^{abc}Means in rows with different superscripts differ (*P* < 0.05).

¹CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

²CS = corn silage effect (CONV vs. BMR); St = dietary starch effect (19% vs. 25%); CS × St = interaction silage by starch concentration.

Table 30. Gas emissions per kilogram of nutrient digested of cows fed diets containing CONV and BMR corn silages with different starch concentrations

Item	CONV ¹		BMR		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	19%	25%	19%	25%		CS	St	CS × St
CH ₄ , g/kg of DM digested	30.34	30.45	28.71	27.37	1.49	0.05	0.58	0.52
CH ₄ , g/kg of OM digested	30.23	30.34	28.68	27.32	1.46	0.06	0.59	0.53
CH ₄ , g/kg of NDF digested	116.39	131.12	104.39	126.64	11.08	0.39	0.06	0.69
CH ₄ , g/kg of starch digested	105.81	83.78	96.69	79.53	2.47	0.002	<0.001	0.09
NH ₃ , g/kg of DM digested	1.33	1.64	1.35	1.29	0.16	0.17	0.32	0.13
NH ₃ , g/kg of OM digested	1.33	1.64	1.35	1.29	0.16	0.18	0.32	0.18
NH ₃ , g/kg of NDF digested	5.10	6.98	4.93	6.01	0.80	0.36	0.03	0.52
NH ₃ , g/kg of starch digested	4.70	4.52	4.59	3.73	8.70	0.18	0.13	0.31
CO ₂ , g/kg of DM digested	1002.09	1008.86	948.53	917.41	69.18	0.04	0.72	0.58
CO ₂ , g/kg of OM digested	998.15	1005.10	947.58	915.65	5.79	0.05	0.70	0.55
CO ₂ , g/kg of NDF digested	3856.17	4344.44	3446.19	4257.79	429.61	0.40	0.04	0.58
CO ₂ , g/kg of starch digested	3488.41	2771.62	3202.21	2667.18	5.04	0.007	<0.001	0.17

¹CONV = conventional corn silage; BMR = brown midrib corn silage; 19% and 25% = starch concentrations in diets.

²CS = corn silage effect (CONV vs. BMR); St = dietary starch effect (19% vs. 25%); CS × St = interaction silage by starch concentration.

OVERALL SUMMARY AND CONCLUSIONS

Findings of this research allowed us to elucidate the effects of reducing dietary starch concentrations with NFFS in diets with different protein sources and corn silage types on DMI, milk production, milk composition, total tract nutrient digestion, and gas emissions in lactating dairy cows. Chapter 2 evaluated the effects of reducing dietary starch concentrations in combination with 2 different protein sources supplements (SBM and CM). Chapter 3 explored the effects of reducing dietary starch with NFFS through a meta-analysis. Chapters 4 and 5 determined how corn silage type (CONV vs. BMR) may improve the performance and nutrient digestion of dairy cows fed reduced starch diets and the potential effect of combining BMR corn silage and high starch diets on CH₄, NH₃, and CO₂ emissions, respectively.

Decreasing dietary starch in diets with SBM and CM had a negative effect on performance and nutrient digestion in dairy cows. Cows fed 27% starch diets had higher DMI, milk yield, and ECM compared to cows fed 21% starch diets. Milk fat concentration was reduced in CM-27% starch, but those cows had the lowest MUN. Milk protein concentration and yield were the least for CM-21% starch diets, however FE was greater in these cows compared to other treatments. The milk fat concentration response was explained by a higher rumen isobutyrate concentration in cows with CM-21% starch and lower milk yield and ECM was explained by a lower DM and OM digestibility in cows fed diet with 21% starch concentration.

Data from the meta-analysis confirmed the negative impact of reduced dietary starch with NFFS on dairy cow performance. As starch intake increase from 1 to 9 kg/d in the cows, DMI responded quadratically, and milk yield, milk protein concentration,

and milk lactose yield increased positively. However, milk fat concentration decreased linearly as starch intake increased. Milk fat depression was explained by the reduced concentration of total VFA and acetate in the rumen and by the decreased NDF digestibility in response to increasing dietary starch intake. The positive impact of increased starch intake on milk yield was explained by the linear increase of propionate, acetate to propionate ratio, isobutyrate, isovalerate, and valerate in the rumen along with the quadratic DM digestibility and linear CP digestibility in response to increased starch intake.

Findings from Chapter 4 revealed the importance of including BMR corn silage in reduced starch diets formulated with NFFS. Dry matter intake tended to be higher over time in cows fed BMR corn silage compared to cows with CONV corn silage. Cows fed BMR-25% starch diets produced higher milk yield and ECM than cows in CONV-25% starch, but cows fed BMR-19% starch produced the same amount of milk than cows with CONV corn silage with any starch concentration. Feed efficiency was lower in cows fed BMR-19% starch than the other treatments. Milk protein concentration was higher in cows fed 27% starch diets than cows in 19% starch diets. Positive effects of milk yield, ECM, and milk protein concentration was mainly explained by the increased DM and OM digestibility in response to feeding diets with 25% starch and by the potential increase of DMI in cows fed BMR corn silage.

Chapter 5 indicated that BMR corn silage and dietary starch concentration only have small effects on dairy cow performance and nutrient digestion, but they affected significantly gas emissions per kilogram of nutrient digested. There was no effect of corn silage and dietary starch concentration on DMI, milk yield, ECM, FE, concentrations and

yields of milk protein, lactose, total solids, and SNF, but milk fat concentration was greater for cows fed diets with 25% starch than diets with 19% starch. Total tract nutrient digestibility was not statistically affected by corn silage type or starch concentration. Starch digestibility however was the lowest with BMR-25 and CONV-19 diets. Diets did not effect on the emissions of CH₄, NH₃, and CO₂ or the emissions of those gases expressed per kilogram of milk yield and ECM, but cows fed BMR corn silage and 25% starch produced less CH₄ and CO₂ per kilogram of DM, OM, and starch digested than cows in CONV corn silage and 19% starch. Nevertheless, cows fed 25% starch in any silage type produced more NH₃ and CO₂ per every kilogram of NDF digested.

In conclusion, formulate diets with 19-21% starch by reducing a portion of corn grain with NFFS may affect negatively the DMI and milk production in dairy cows by decreasing DM and OM digestibility. The inclusion of BMR corn silage to diets with 19-21% starch diets could maintain similar DMI and milk production as cows fed diets with 27% starch in response of increasing nutrient digestion with the advantage of reducing the emissions of CH₄ and CO₂ per kilogram of nutrient digested.

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