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Physically Adjusted Neutral Detergent Fiber System: Effects of Dextrose and Non-Forage Fiber on Performance, Feeding Behavior, Digestibility, Rumen Fermentation and Rumen Bacterial Abundance in Dairy Cattle

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PHYSICALLY ADJUSTED NEUTRAL DETERGENT FIBER SYSTEM: EFFECTS OF DEXTROSE AND NON-FORAGE FIBER ON PERFORMANCE, FEEDING BEHAVIOR, DIGESTIBILITY, RUMEN FERMENTATION AND RUMEN BACTERIAL ABUNDANCE IN DAIRY CATTLE

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THESIS ACCEPTANCE PAGE

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

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LIST OF ABBREVIATIONS

DMI	Dry matter intake
BW	Body weight
CON	Control
d	Days
DIM	Days in milk
DM	Dry matter
ECM	Energy-corrected milk
NDF	Neutral Detergent Fiber
NFFS	Non-forage fiber source
NH_3	Ammonia
paNDF	Physically adjusted NDF
peNDF	Physically effective NDF
TMR	Total mixed ration
VFA	Volatile fatty acids
WSC	Water-soluble carbohydrates

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ABSTRACT

PHYSICALLY ADJUSTED NEUTRAL DETERGENT FIBER SYSTEM: EFFECTS OF DEXTROSE AND NON-FORAGE FIBER ON PERFORMANCE, FEEDING BEHAVIOR, DIGESTIBILITY, RUMEN FERMENTATION AND RUMEN BACTERIAL ABUNDANCE IN DAIRY CATTLE

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The newly proposed physically adjusted NDF system (paNDF) system aims to maintain a favorable rumen environment (e.g., pH) by taking into account both the physical and chemical composition of diet. Thus, the objective of this study was to determine the effects of water-soluble carbohydrates (WSC) and non-forage NDF inclusion in a paNDF system on production performance, rumen pH and fermentation profile, rumen bacterial abundance, feeding behavior and nutrient digestibility in late-lactating Holstein cows. Nine fistulated multiparous Holstein cows (2^{nd} and 3^{rd} parity) were used in a triplicated 3×3 Latin Square experiment. Each period lasted for 21 d with 14 d of adaptation and 7 d of sampling. All three diets including control (CON), dextrose (DEX) and non-forage NDF (NFFS) had similar crude protein (16.5%), net energy for lactation (1.74 Mcal/kg DM), and forage NDF (19.1%). On contrary, CON diet contained greater starch than DEX or NFFS diet due to more corn grain inclusion whereas DEX and NFFS diet contained greater concentrations of WSC (replacing corn grain) and non-forage NDF (replacing soybean meal and corn grain with soybean hulls), respectively. Milk yield (MY) and dry matter intake (DMI) were recorded daily. Milk samples from AM and PM milking over 3 d (6 samples/cow in each period) were analyzed for milk components. Rumen fluid was collected for bacterial abundance, volatile fatty acids (VFA), and ammonia (NH_3) whereas

fecal spot samples (8 timepoints per cow) were collected for total-tract nutrient digestibility using indigestible NDF as marker. Feeding behavior and rumination was measured using an ear attached accelerometer. Statistical model included the fixed effects of period, Latin Square, diet, and diet × period interaction and a random effect of cow within parity. Although MY, fat-and-protein corrected milk yield and milk component yields were not affected by the diet, cows fed DEX had 6 and 9% greater DMI than CON and NFFS cows, respectively. Diet did not affect rumen pH, NH_3-N and VFA, however, CON diet tended to have greater iso-butyrate and greater iso-valerate than DEX or NFFS. Inclusion of non-forage NDF increased DM and NDF digestibility of NFFS diet compared to CON or DEX diet whereas DEX had lower starch digestibility than CON or NFFS. Diet tended to affect the abundance of *F. succinogenes, P. ruminicola and S. bovis* being greater in abundance for DEX than NFFS whereas abundance did not vary between CON vs. DEX or CON vs. NFFS diet. Cows receiving the DEX diet tended to spend greater time eating than NFFS cows likely due to greater DMI. When expressed per unit of DM, NDF or forage NDF intake, DEX cows had lower rumination time than CON cows due to mainly increased DMI or NDF or forage NDF consumption. On contrary, eating and chewing time expressed per unit of NDF intake was greater in DEX than NFFS diet likely because DEX cows had greater DMI. Overall, our findings suggest that though replacement of corn grain either with dextrose or non-forage NDF in paNDF system affected DMI, nutrient digestibility, bacterial abundance and feeding behavior; however, implementing an adequate paNDF maintained a ruminal pH above 6.0 which most likely kept animals healthy without affecting lactation performance.

Key words: Feed efficiency, rumen environment, rumen pH, volatile fatty acids

CHAPTER 1. LITERATURE REVIEW

INTRODUCTION

The dairy cow is one of the many animals where humans have benefitted from its milk. Cattle milk comprises around 80% of total milk consumed across globe (FAO, 2020). Like other ruminant, dairy cow has an overly complex digestive system(Church, 1988).The stomach of dairy cattle is divided into four compartments: rumen, reticulum, omasum, and abomasum. Among four compartments, rumen can make up to 80% of the total abdominal cavity of a grown cow having a total capacity of around 80 L, this constitutes around 16% of the whole-body weight of the mammal (Niehaus, 2008).

The rumen environment can be considered as anaerobic and methanogenic fermentation chamber (Matthews et al., 2019). Rumen plays a key role in the degradation of the feedstuff fed to the cow. If we consider the cow as a machine, we must be sure to be able to make it as efficient as possible. The rumen microbiome composition is dependent not only on the host animal but also on diet composition (Monteiro et al., 2022). In a study, it was concluded that feed efficiency might be affected by the rumen microbes when residual feed intake was used as a measure of efficiency (Guan et al., 2008).

In previous years, the rumen microbiome had been a challenge to study and analyze due to to the difficulty to culture (Mullins et al., 2013). In ruminants, it was found that more than 67% of the abundance of rumen microbiome was made up of 7 families (*Prevotella, Butyrivibrio, Ruminococcus, Lachnospiraceae, Ruminococcaceae, Bacteroidales*, and *Clostridiales*), and can be considered as the core bacterial microbiome (Henderson, 2015).

Rumen pH is important in accordance with the health of a cow. The pH in the rumen of a cow can determine the biodiversity of the ruminal ecosystem and health of the animal because an alteration of the pH, normally associated with a decrease in pH can lead to subclinical acidosis or even clinical ruminal acidosis (Aschenbach et al., 2011). Subacute ruminal acidosis (SARA) occurs when the rumen pH is lower than 5.6 for a period greater than 3 hours over 24-h period (Plaizier et al., 2008). The rumen pH will decrease when volatile fatty acids (VFA) accumulate in the rumen with the rumen not being buffered enough by the animal (NRC, 2001). This happens when the feed has a higher concentration of grains than necessary, decreasing at the same time the proportion of forages. Rumination that can help maintain a healthy rumen pH through proper salivation containing buffers such as bicarbonate and phosphate, can be stimulated by the particle size of the of the diet fed to cows (Beauchemin, 2018).

Mertens (1997) emphasized not only the importance of neutral detergent fiber (NDF) content of the diet but also the importance of physically effective NDF(Mertens, 1997). Physically effective NDF (peNDF) is related to the particle size which influences the chewing activity of the animal, asserting that the welfare of the animal is maintained due to the buffering properties of the saliva of the animal. It is now recommended to use peNDF to be integrated with a physical adjusted NDF (paNDF). The paNDF system emphasizes the importance of particle size aiming to maintain the healthy rumen pH for better productivity, efficiency, and animal health (White et al., 2017b).

Non-forage fiber sources (NFFS) have been used with the purpose of having a diet with low forage due to several reasons such as price, availability, and quality (Swain, 1994). If a diet contains too much NFFS, rumination and saliva secretion are reduced due

to the fact that NFFS are fermented and passed through the rumen faster than forage fiber (Grant, 1997). Thus, it is extremely important to balance the usage of NFFS and forage fiber or NDF in dairy cattle diet in order to maintain the proper amount of rumination required for an animal to maintain a healthy pH in the rumen.

Dairy cattle diet contains diverse forms and types of sugars to some extent in most cases. According to (Oba, 2011), sugars are rapidly fermented in the rumen. Sugars may decrease in ammonia-N concentrations in rumen if the diet fed has not a high fermentation rate in the rumen (Oba, 2011). Thus, it is important to understand the influence of NFFS and sugars in a newly proposed paNDF system on rumen environment, fermentation, nutrient digestibility, and production performance of dairy cattle.

1. The ruminant

Ruminants belong to the Mammalia class and more specifically the Artiodactyla order. Cattle are part of this group, but they belong specifically to the category of true ruminants that have one stomach with four compartments, which include rumen, reticulum, omasum, and abomasum. Cattle, according to some archaeological findings, were domesticated around 8,500 years ago. With the process of selective breeding aiming to improve milk or meat and more completely formulated diets, cattle nowadays have been further categorized as dairy or beef cattle. Dairy cattle breeds are known to be able to convert feed (forages and grains) into high quality food, milk.

2. Ruminant stomach

The stomach of a ruminant is known as one with the highest evolutionary development in mammals (Church, 1988). Among the four compartments of ruminant stomach, rumen has an important function to cows and all ruminants, one of the reasons for the evolution of this pre-gastric fermentation compartment is that the ruminants needed a way to degrade phytotoxins and mycotoxins found in the feed. Rumen can contain a variety of microorganisms such as anaerobic bacteria, protozoa, archaea and fungi (Mackie et al., 2013). The rumen contains a ruminal fluid in which particles with distinct size float which is called rumen mat. In the rumen, the ruminal fluid can be retained approximately 8-12 hours, and the solids can be retained more than 48 hours depending on diet composition (Weimer, 2019). The retention of the ruminal fluid can be associated with salivation during chewing time, but not with salivation during resting time, given that the outflow rate of the ruminal fluid into the abomasum was positively correlated to the saliva secreted during chewing time (Maekawa, 2001). Decreasing particle size increases outflow rate from rumen (Huhtanen et al., 2007). The omasum is the third compartment in the stomach of the cow and its' function is to transition the content from the rumen into the abomasum. Given that the digestion from these two compartments varies, it also has the capacity to recycle water and some nutrients. The abomasum is the fourth compartment of the stomach, and many consider it the true stomach, given the strong acid and digestive enzymes secretion.

3. Rumen environment

The environment of rumen is anaerobic and methanogenic. It has its own environment in which specific microorganisms interact. The rumen microorganisms can utilize cellulolytic feeds and increase the productivity of them (Matthews et al., 2019). This rich and complex population starts since the birth of the calves and continues to change and develops throughout their early stages in a process in which the microbial populations succeed one another until it reaches a point of stability (Mackie et al., 2013). Abundance and diversity of microbial populations that inhabit specific regions in the rumen vary (Mackie et al., 2013) .

The ingesta in the rumen of a cow does not follow the normal pathways of non-ruminants. The feed in the rumen is in constant movement due to the continuous contractions of the reticulo-rumen. Some of the feed needs to be regurgitated, re-insalivated and re-swallowed all of these with the purpose of decreasing the particle size of the feed, enhancing the surface area of the feed that can later be used by the microorganisms in the rumen, buffering the pH of the rumen with saliva buffers such as bicarbonate, urea and phosphate, and to expel some of the excess of gases formed (Church, 1988). The mobility of the feed in the rumen plays a significant role, but this mobility can be affected as well, such as a distension in the rumen, bloating, or even if the osmotic pressure is too high in the rumen, stasis can occur. The mastication during the rumination tends to be slower and has a higher level of consistency than the mastication during eating (Beauchemin, 2018).

4. Rumen pH and efficiency

Cows have been through an intense process of selective breeding to the point that their productivity has reached new heights, due to this, the energy requirements have also changed. One of the solutions for this energy and nutrient requirements to be fulfilled is to introduce higher percentages of non-fiber carbohydrates (NFC) such as starch into the rations. The problem that arises due to the increase of NFC is that the forage amount is reduced in the ration which can cause a decrease in the ruminal pH due to an increase in the amount of fermentation end products (Bach et al., 2023). Rumen pH needs to be maintained above 6.0, in cases where the pH of the rumen is below 5.5,the cow is believed to have S.A.R.A. (Garrett et al., 1999). Also, trying to maintain the pH above SARA levels in the cows is difficult, this can be associated with some cows being more susceptible to experiencing SARA than others despite being fed the same diet.

In a study by Humer et al. (2015) in transition cows, some cows sorted their feed compared to the rest of the cows, in which they consume more of the rich fiber diets, enabling them to be more tolerant to SARA after parturition. Even so, one cannot confirm that this is the reason, but one can affirm that cows can differ in their ruminal pH despite being fed the same diet. Rumen acidity can cause lysis in gram-negative bacteria which causes an increase in some bacterial endotoxins lipopolysaccharides (LPS) which are one of the main agents to potentially affect the cows metabolism and immune response (Dong et al., 2011). According to Hoover (1986), a decrease in ruminal pH below 5.5 can cause a decrease in the production of fibrolytic microbes which can result in hindering fiber digestion. A low rumen pH has a negative effect in the productivity of dairy cows given the reasons that they affect energy intake and its absorption of VFAs (Allen, 1997).

According to Allen et al. (2006), having a high pH in the rumen can decrease energy intake and microbial protein production.

5. Rumen microbiome

Rumen is an anaerobic microbial ecosystem, and it hosts a variety of microbes such as bacteria, archaea, protozoa, and fungi; creating conditions in which all these microbes can function and grow (Wang et al., 2017). The microbial community in the rumen has the capacity of breaking down polysaccharides and producing VFAs, microbial biomass, and gases (Lengowski et al., 2016; Wallace et al., 2015; Wang et al., 2017). The microbes in the rumen can be differentiated and grouped by some of their main functions such as proteolytic, cellulolytic, and amylolytic (Henderson, 2015).

Rumen is not fully developed when a calf is born, this means that the rumen microbiome is not present as it is in an adult cow. Normally, a calf is fed by its mother and the rumen microbiomes are transmitted to the calf through the mother and its environment. In current farms, where productivity is essential a calf is separated from its mother, so the microbes need to be transmitted from the environment (airborne) and another form that can be transmitted is through the clothing or equipment of the workers that move from old and young groups of cattle. Once a microbe is retained by one calf the rest of the group will get it as well (Hobson et al., 1997). Although the rumen is an anaerobic habitat, and maintaining this state helps with the constancy of itself, the bacteria and protozoa in the rumen can survive to exposure to oxygen enabling them to be transmitted via airborne to calves (Hungate, 1960).

The rumen ecosystem can be considered to have three environments that interact such as the liquid phase, solid phase, and the rumen epithelial cells and protozoa (Ishler et al., 2016; Matthews et al., 2019). The solid phase comprises around 25% of the microbial mass, in this phase the slower-growing bacteria are attached given that the outflow rate of the solid phase is slower than that of the liquid phase; enabling the bacteria to maintain a stable population in the rumen. The liquid phase is the largest in the rumen with up to 70% of the microbial mass and its outflow rate is much faster than the solid phase. The rumen epithelial cells and protozoa contain around 5% of the microbial mass.

6. Rumen Bacteria

The Rumen ecosystem is very diverse and complete that it has the several domains of life; bacteria, protozoa, archaea, and fungi which all are present in the rumen. Most abundant microorganisms in the rumen are the bacteria. Bacteria population goes up to 10^{11} viable cells per gram, with up to 1000 phylotypes (Mackie et al., 2013). The rumen although it presents a core microbiome many factors affect its total population and diversity such as diet and host; some cows require more energy than others and also some cows are resistant to certain metabolic end-products (Henderson, 2015; Matthews et al., 2019). Some of the functional groups of bacteria can be grouped as proteolytic bacteria, cellulolytic bacteria, lipolytic bacteria, and lactate degrading bacteria.

Cellulolytic bacteria have a vital role in the function of the rumen. Cows have a based plant diet; cellulose is the main component of the cell wall of plants. Cellulose is digested in the rumen by these bacteria but their efficiency can also depend on the type of forage they are degrading as well as the maturity stage of the plant and what are the

cellulolytic bacteria present in the rumen digestive system (Castillo-Gonzalez et al., 2014). Cellulolytic bacteria in the rumen is susceptible to low pH according to Weimer (1996), where the three predominant cellulolytic bacteria genus such as Succinogenes, Ruminococcus, and Flavefaciens) do not grow in pH lower than 6.0.

Amylolytic bacteria are one of the core populations in the rumen microbiome. Some bacteria are present in all cattle being fed any ration such as *Prevotella ruminicola* (Hungate, 1966). According to Zhang et al. (2022), the ratio between cellulolytic and amylolytic bacteria must be maintained because when there are less cellulolytic bacteria and more amylolytic bacteria the ruminal pH will decrease. Another amylolytic bacteria species is the *Streptococcus bovis*. The population of these bacteria depends on the type of forage being fed to cows. When a high fiber diet is fed, the population of this bacteria will be low, but if a high grain-based diet is fed, population will increase in the rumen. The production of lactic acid derives from starch in the diet.

The pH in rumen needs to be maintained above 6.0 for cellulolytic bacteria to be able to reproduce. Lactate is the substrate needed for the growth of certain bacteria such as *Megasphaera elsdenii*, which in turns metabolize the lactate into VFAs (Castillo-Gonzalez et al., 2014). Rumen VFAs provide up to 60-80% of the metabolizable energy which cattle need on a daily basis (Mackie et al., 2013). The problem arises when more lactate is produced than the ones being metabolized causing acidosis in the rumen although lactate is associated with acute acidosis entirely; a high population of lactate utilizing bacteria is needed to prevent this.

7. Rumen Archaea

The archaea group is also present in the rumen, specifically the phylum Euryarchaeota, which consists of methanogens. Methanogens have a wide variety of twenty-eight genera and 113 species. So far, only a few species have been isolated from the rumen: *Methanobacterium fomicicum, Methanobacterium bryantii, Methanobrevibacter ruminantium, Methanobrevibacter millerae, Methanobrevibacter olleyae, Methanomicrobium mobile,* and *Methanoculleus olentangyi (Matthews et al., 2019).* The methanogens in the rumen constitute of around 0.3 to 3.3% of the microbial mass in the rumen (Janssen & Kirs, 2008).

Methane production in the cattle accounts for a loss of energy of 6% according to Johnson and Johnson (1995), this data is within the range that Mohammed et al. (2004) mentioned which was between 6 to 10% of loss of energy due to methane production. The range mentioned by (Wallace et al., 2014) is less conservative than the ones mentioned before, and the loss of energy goes from 2 to 12% of energy. These variations may be possible due to several reasons such as cattle breeds, size of cattle, types of feed being fed and many others. Despite variation in the loss of energy from different studies we may as well consider that a loss of energy can be impactful in the cattle given that it might impact in other areas such as reproduction, milk production, and growth. Function of methanogen is not just to produce methane but also to remove H_2 from forming in the rumen which may impact the rate of fermentation or the formation of VFAs (Ishler et al., 2016; Janssen & Kirs, 2008).

The pH in the rumen of a dairy cow is constantly changing. This variable depends on many factors, such as feed, breed of the cow, size, and many others. Usually, SARA

can be defined thanks to the measurement of pH values. The measurement have to be make taken into consideration the low pH value and the amount of time it was maintained with this value (Allen et al., 2006). In a study made by Duffield et al. (2004), they affirmed the difference in pH levels in the same timepoint due to the location where the ruminal fluid was taken from, stating that the central part of the rumen tended to have lower levels than the rest.

Diet plays a significant role in the ruminal pH of cattle. High starch diets decrease the rumen pH and increase the variation of it. With an increase in the fluctuations of ruminal pH a cow can start to increase the insulin secretion as well (Oba & Allen, 2003). Also, this can cause an increase in the population of amylolytic bacteria; affecting the growth of cellulolytic bacteria and NDF digestibility (Aschenbach et al., 2011). SARA can be caused by VFAs elevation in the rumen due to the reason that lactate is not present in high abundance when cows have SARA. Lactate is more related with acute acidosis due that lactic acid producing bacteria such as Streptococcus bovis which thrive in pH below 5.2 (Stone, 2004).

8. Volatile fatty acids

The VFAs produced in the rumen are mainly absorbed into the blood streams from the rumen (Hungate, 1960). As pointed above that the rumen is the main provider of energy supply to cattle, providing up to 70% of the total energy mainly from short chain fatty acids (Khiaosa-ard & Zebeli, 2014). The VFA is mainly absorbed in the dissociated form by the rumen wall (Allen et al., 2006). The capacity of absorption is affected by various factors such as the pH of the rumen and papillae surface area (Dirksen et al., 1985). Other factors

that affect the absorption rate are rumen size and degree of fill (Dijkstra et al., 1993). Supplying energy to the host is not the only action done by the VFAs absorption; it also helps to stabilize the ruminal pH by removing the protons in the rumen (Beauchemin, 2018).

According to Allen (1997) hydrogen ions can be removed from the rumen through the rumen wall as the absorption of VFAs if the pH is close to 6.0. The absorption of VFA is affected due to a low ruminal pH, and low pH increased the VFA absorption but motility wasn't taken into consideration (Allen et al., 2006). It was found that motility is needed since absorption occurs in the ruminal epithelium and motility was needed for a better absorption of VFA.

The VFAs included as end products of microbial fermentation are acetic acid, propionic acid, and butyric acid. Their total population is strongly associated with the consumption of organic matter (OM) and ruminally degraded organic matter (ROM).

9. Rumination

Adult cattle in normal conditions will tear the vegetation and swallow with little mastication, it is partially digested in the rumen by its microbial population. The addition of saliva helps the feed to be swallowed easier and increases the specific gravity which helps the forage to pass from the rumen (Church, 1988). Contractions in the reticulum and rumen help to mix the ingesta, saliva and microbiome population. A contraction of the reticulo-rumen also serves to the process of rumination in which a bolus from the mat fiber in the rumen will go through the esophagus back into the mouth of the cattle and be further chewed decreasing its particle size and returned to the rumen (Hobson et al., 1997).

Saliva in cattle contains buffers that act upon reaching the ruminal fluid to regulate the pH in cattle such as urea, bicarbonate, and phosphate. Half of the bicarbonate in the rumen comes from the saliva secretion (Maekawa, 2001; Owens et al., 1998). According to Allen et al. (2006), the buffering capacity of the saliva is attributed during rumination and not during eating or resting. During eating rumen pH decreases in the rumen but the saliva secretion helps with the outflow rate into the omasum. Salivation during eating is 2-3 times higher than the salivation rate during resting and similar during rumination (Maekawa, 2001).

10. Particle size of diet

Particle size and particle size distribution of diet plays a key role in the efficiency and health of the cattle. Cows that eat diets with larger particle size tend to decrease DMI. In a study by Kononoff and Heinrichs (2003), when there was a decrease in the particle size of the alfalfa haylage being fed to the cattle there was an increase in DMI. They associated this with having a larger particle size in the feed will increase the rate of gut fill in cattle. The fiber particle size affects the chewing and rumination, with a proper particle size rumination is promoted (Stone, 2004).

Feeding activity in cattle vary not only due to particle size but also from moisture, as such some particles tend to be chewed more by the cow and despite having a larger particle size in the feed when the bolus is produced the size can be considerable smaller (Schadt et al., 2012). The particle size in the rumen also impacts on rumination with larger particles which form the rumen fiber mat induce rumination and smaller particles induce

outflow rate but particles with a size larger than 1.18 mm tend to show resistance to leave the rumen (Poppi et al., 1980).

10.1 Physically effective neutral detergent fiber (peNDF)

Neutral detergent fiber (NDF) that plays an important role, measures the amount of fiber in the feed such as cellulose, hemicellulose, and lignin (National Academies of Sciences et al., 2021). Feed NDF provides the chemical characteristics of fiber, but it cannot provide particle size and density information (Mertens, 1997). Physically effective NDF (peNDF) and effective NDF (eNDF) that was clarified by Mertens (1997) measure the ability of fiber to maintain the milk fat production and refer it as "effective". Physically effective NDF (peNDF) is the percentage of feed particle that stimulates the cattle's chewing activity and helps maintain the floating fiber mat in the rumen, which comprises of large particles.

Several models have been developed aiming to quantify the peNDF in a diet. Mertens (1997) proposed that the particle size that is greater than 1.18 mm in size for Penn State Particle Separator (PSPS) are considered as physically effective. Zebeli et al. (2012) proposed that particle size that is greater than 8 mm in size of PSPS sieve is considered as physically effective NDF. Zebeli et al. (2012) proposed that the range of proper peNDF retained on an 8 mm sieve was between 14.9% and 18.5% of the feed taken into consideration the goal intended, either prevention of SARA or maximizing intake.

10.2 Physically adjusted neutral detergent fiber (paNDF)

Physically effective NDF (peNDF) has come to play a significant role in diets, and it is a tool that many nutritionists use to form a proper diet. White et al. (2017a) re-evaluated the peNDF and came up with a new term called physically adjusted NDF (paNDF) as the dietary NDF multiplied by the particle size of individual NDF also with particle size descriptors using chemical and physical properties. These diet descriptors are the ones responsible for predicting DMI, rumination time, and rumen pH as well.

The paNDF system by White et al. (2017b) was created to serve as a guide to know the proper requirements of particle size on the diet to optimize the ruminal pH interacting with other components of the diet. The system was created to observe the effects of the physical and chemical composition of the diet in the ruminal pH of lactating cows. One of the few problems that raised with this study is that 1) this study was taken from individually fed cows, 2) it also didn't take into consideration of ruminal buffers, 3) it considered only high forage diet with CP concentrations of greater than 17.0% and 4) cows included weigh close to 630 kg.

11. Non-forage fiber in the dairy cattle diet

Dietary starch has become more expensive over the years and one solution nutritionists have come to accept is reducing dietary starch while including highly digestible forages or non-forage fiber sources (Dann et al., 2015). Fiber has a vital role in normal rumen function. The fiber has to be of good quality and a proper particle size so cows have an optimal chewing activity, maximum DMI, normal ruminal fermentation, and milk fat percentage (Grant, 1997).

Non-forage fiber sources (NFFS) come from high-fiber by-products by several industries. NFFS bring more benefits than just price reduction, in some cases it can even increase the price of the diet, like inclusion of protein as a nutrient (Bradford & Mullins, 2012), decreasing the forage bulkiness in the rumen which can decrease DMI due to the gut fill. Still forage NDF (fNDF) is used as the main factor for a diet and the recommended level of fNDF ranges from 15 to 19% of DM (National Academies of Sciences et al., 2021). Non-forage sources have 50% of the effective NDF (eNDF) value as the forage eNDF. If a diet needs to have a total NDF of 25% then 19% at a minimum will come from fNDF, and starch concentration cannot exceed 30%. If the fNDF decreases by 1% then total NDF has to increase by 2% and starch will have to be decreased by 2% as well (National Academies of Sciences et al., 2021). NFFS are high in fiber but unlike forages these are highly passed from the rumen (Bradford & Mullins, 2012). We can relate this rapidly passage rate of the NFFS with a need of NDF increase in the diet because without sufficient fiber in the rumen chewing and saliva secretion will decrease affecting the health of the cattle (Grant, 1997).

12. Water soluble carbohydrates diet

Dairy cattle diets may contain up to 70% carbohydrates including NDF, starch and water-soluble carbohydrates (WSC). Sugars, such as glucose, galactose, fructose, sucrose, lactose, and maltose are WSC. WSC are considered as carbohydrates that are rapidly fermented in the rumen (Oba, 2011). Though starch and NDF comprise a bulk amount of carbohydrates in a dairy cattle diet, sugars can also play a vital role in the diet.

Oba (2011) concluded that when starch is being partially replaced by sugar, the rumen pH decreases. In this study, the percentage of forage used was around 10%.

Another *in-vitro* study by Piwonka and Firkins (1996) found that the inclusion of glucose in the ruminal fluid decreased the digestion of NDF. Lactic acid was also produced due to glucose fermentation. The lactic acid was only fermented into butyrate and propionate. This study also found that glucose fermentation produced a proteinaceous inhibitor which inhibits fibrolytic microorganisms.

Sugar has also an increasing effect on DMI in lactating dairy cows (Oba, 2011). The main reason for the increase in DMI is due to flavor in a study made by Nombekela et al. (1994), in early lactating cows they found that adding sugars to the cows increase the DMI and found that cows tended to prefer sweetened diets. WSC has a high energy density without implicating the pH giving faster accessible energy for the dairy cow (Oba, 2011). According to the National Academies of Sciences et al. (2021), there is still a need to recollect more data if the WSC in a diet might affect the NDF requirements.

CONCLUSION

Proper usage of NDF in dairy cattle diets is crucial not only to improve production efficiency but also to maintain rumen and animal health and related functions such as reproduction. The effectiveness of the particle size of NDF is essential to maintain the proper ruminal pH of cattle. Rumen is a complex environment where microorganisms thrive depending on temperature, pH, motility, and rumen outflow rate. Some microorganisms grow and thrive in a specific range of ruminal pH such as cellulolytic

bacteria. Cellulolytic bacteria are the main fiber digesters in the rumen and if the pH is lower than the 6.0, the growth of the cellulolytic bacteria is affected negatively, and other bacteria start to thrive in this environment such as the amylolytic bacteria which are lactate producers. Lactate is associated with further decreasing the ruminal pH affecting the environment of the rumen. If the pH falls below 5.6 in the rumen, dairy cattle are more susceptible to develop SARA resulting in decreased milk production, laminitis and reduced VFA absorption by the rumen wall. VFAs are better absorbed in a slightly acidic ruminal pH and if the ruminal pH is higher than 7.0 the productivity can also be affected in dairy cattle due to the decrease in DMI. Particle size of the feed also plays a significant role in the rumen pH of cattle. Salivation is one of the mechanisms that cattle can buffer the ruminal pH given that saliva contains high alkaline substances such as urea, phosphate, and bicarbonate. Salivation occurs in three distinct stages in cattle which are rumination, eating, and resting.

Rumination and eating produce the highest amount of saliva but rumination has a higher impact in the buffering of the rumen given that while eating usually cows also ingest high degradable sources of starch that are related to decrease in the pH of the rumen. Rumination is associated with the contractions of the reticulo-rumen, but it is propelled by the fiber mat which is floating in the rumen. The fiber mat is composed of large particles. The particle size of the feed also plays a significant role in formulating diets, and it has been associated with the NDF to be able to maintain a proper rumen function. Physically effective NDF stimulates a cow in their chewing activities. The paNDF system is not used to predict ruminal pH but to maintain it between 6.0 and 6.1 (National Academies of Sciences et al., 2021). This system helps to maintain the correct rumen environment in

which rumen bacteria thrive as efficiently as possible (White et al., 2017a, 2017b). This is one of the main importance of using this system. WSC can cause an effect on the pH in the rumen as well increasing it (Oba, 2011). Literature yet warrants to understand if the effect of the paNDF system would change even if there was a change in starch vs sugar inclusion in the diet. NFFS also can be used as an alternative to feed fiber from other sources but the inclusion of this source in the diet toned to made ensuring that the eNDF is maintained in certain ranges to maintain proper pH in the rumen (National Academies of Sciences et al., 2021).

PHYSICALLY ADJUSTED NEUTRAL DETERGENT FIBER SYSTEM: EFFECTS OF DEXTROSE AND NON-FORAGE FIBER ON PERFORMANCE, FEEDING BEHAVIOR, DIGESTIBILITY, RUMEN FERMENTATION AND RUMEN BACTERIAL ABUNDANCE IN DAIRY CATTLE

ABSTRACT

The newly proposed physically adjusted NDF system (paNDF) system aims to maintain a favorable rumen environment (e.g., pH) by taking into account both the physical and chemical composition of diet. Thus, the objective of this study was to determine the effects of water-soluble carbohydrates (WSC) and non-forage NDF inclusion in a paNDF system on production performance, rumen pH and fermentation profile, rumen bacterial abundance, feeding behavior and nutrient digestibility in late-lactating Holstein cows. Nine fistulated multiparous Holstein cows (2^{nd} and 3^{rd} parity) were used in a triplicated 3×3 Latin Square experiment. Each period lasted for 21 d with 14 d of adaptation and 7 d of sampling. All three diets including control (CON), dextrose (DEX) and non-forage NDF (NFFS) had similar crude protein (16.5%), net energy for lactation (1.74 Mcal/kg DM), and forage NDF (19.1%). On contrary, CON diet contained greater starch than DEX or NFFS diet due to more corn grain inclusion whereas DEX and NFFS diet contained greater concentrations of WSC (replacing corn grain) and non-forage NDF (replacing soybean meal and corn grain with soybean hulls), respectively. Milk yield (MY) and dry matter intake (DMI) were recorded daily. Milk samples from AM and PM milking over 3 d (6 samples/cow in each period) were analyzed for milk components. Rumen fluid was collected for bacterial abundance, volatile fatty acids (VFA), and ammonia (NH_3) whereas

fecal spot samples (8 timepoints per cow) were collected for total-tract nutrient digestibility using indigestible NDF as marker. Feeding behavior and rumination was measured using an ear attached accelerometer. Statistical model included the fixed effects of period, Latin Square, diet, and diet × period interaction and a random effect of cow within parity. Although MY, fat-and-protein corrected milk yield and milk component yields were not affected by the diet, cows fed DEX had 6 and 9% greater DMI than CON and NFFS cows, respectively. Diet did not affect rumen pH, NH_3-N and VFA, however, CON diet tended to have greater iso-butyrate and greater iso-valerate than DEX or NFFS. Inclusion of non-forage NDF increased DM and NDF digestibility of NFFS diet compared to CON or DEX diet whereas DEX had lower starch digestibility than CON or NFFS. Diet tended to affect the abundance of *F. succinogenes, P. ruminicola and S. bovis* being greater in abundance for DEX than NFFS whereas abundance did not vary between CON vs. DEX or CON vs. NFFS diet. Cows receiving the DEX diet tended to spend greater time eating than NFFS cows likely due to greater DMI. When expressed per unit of DM, NDF or forage NDF intake, DEX cows had lower rumination time than CON cows due to mainly increased DMI or NDF or forage NDF consumption. On contrary, eating and chewing time expressed per unit of NDF intake was greater in DEX than NFFS diet likely because DEX cows had greater DMI. Overall, our findings suggest that though replacement of corn grain either with dextrose or non-forage NDF in paNDF system affected DMI, nutrient digestibility, bacterial abundance and feeding behavior; however, implementing an adequate paNDF maintained a ruminal pH above 6.0 which most likely kept animals healthy without affecting lactation performance.

Key words: Feed efficiency, rumen environment, rumen pH, volatile fatty acids

INTRODUCTION

The inclusion of forage in a diet is important to maintain healthy rumen. This helps the rumen to have a healthy pH and helps to reduce the outflow rate from the rumen by giving the microorganisms in the rumen to better digest the substrate with which they thrive. NDF was used as a measure to propose the correct ratio between forage and concentrate (Mertens, 1997). Although NDF measures the chemical composition of fiber it does not measure the physical composition of fiber. Physically effective NDF (peNDF) is the fragment of the diet that induces a cow to perform the chewing activity and mainly forms the fiber mat in the rumen. Chewing activity can be affected by the particle size of the roughage in diets (White et al., 2017b). One of the biggest concern involving peNDF as a guideline for diet formulation is that it lacks a validated method to measure the effectiveness of fiber and its requirements (National Academies of Sciences et al., 2021). The value of peNDF can be calculated by multiplying the content of NDF in a feed by the proportion contain in a 1.18mm sieve (White et al., 2017a). Although this can also cause concerns in the usage of the peNDF seeing that it assumes that NDF is distributed equally across all sieves. The National Academies of Sciences et al. (2021) recommended to use forage NDF (fNDF) instead of total NDF to implement on the diets formulations.

The physically adjusted NDF is a newly proposed system in which not only is the physical properties of the diet included but also the chemical composition of forage, starch, NDF and fNDF. The paNDF model was not designed to predict the ruminal pH but to maintain the mean in between 6.0 and 6.1 considering the amount of feed needed in the 8mm and 19mm sieve on a Penn State Particle Separator (PSPS). The paNDF system was designed with the purpose of demonstrating the interaction between chemical composition

and physical form can affect rumen pH. As a recommendation, the National Academies of Sciences et al. (2021) suggests including the difference in moisture of the diet as well as analyzing NDF concentrations. NDF concentrations vary among diets and even from source to source. As such it is recommended that the diets should be analyzed for NDF as well as for DMI.

Still, it is unexplored how water-soluble carbohydrates (WSC), or non-forage fiber sources (NFFS) might affect rumen environment and the fermentation profile under a paNDF system. WSC are known to be able to degrade rapidly in the rumen as such their inclusion in a diet using the paNDF system might be hard to determine due to the reason that NDF and starch has a slower degradability rate. Another difference is that WSC does not vary that much due to the source compared to NDF and starch. NFFS also has a high variability, but this is mainly due to the amount of peNDF in the diet given that NFFS needs to be retained in the rumen much longer to be fully degraded. As such, the need to include these two diet sources in a paNDF system to observe the response they have given their availability as different sources to formulate diets.

Therefore, the objective of this study was to determine the effects of dextrose and non-forage fiber source with a similar particle size distribution of diets on performance (DMI, milk yield, milk components, and efficiency), rumen pH, VFA and ammonia-N, total-tract nutrient digestibility, bacterial abundance, and rumination and feeding behavior. We hypothesized that replacing corn grain with pure dextrose or non-forage NDF in a paNDF model would maintain a similar pH across diets while improving production performances without hindering rumen fermentation.

MATERIAL AND METHODS

Experimental Design and Dietary Treatments

All animals procedures were conducted in accordance of the South Dakota State University Institutional Animal Care and Use Committee protocol (2109-059A). A total of nine rumen fistulated multiparous (2^{nd} and 3^{rd} lactation) late-lactating (191 ± 22 DIM) Holstein cows were enrolled in a triplicated 3 x 3 Latin Square design study. Each study period consisted of 21 d with 14 d of adaptation to the diet and 7 d of sampling. Within Latin Square, each cow was assigned to either a control (CON) or dextrose (DEX) or non-forage fiber source (NFFS) based diet (Table 2.1), and then diets were switched accordingly at the end of each period. Diets were formulated to meet the NASEM (2021) nutrient requirements of late-lactating Holstein cows aiming to contain 16.5% CP, 17.4 Mcal.kg DM of NE_L. In DEX diet, corn grain and supplemented fat was replaced with pure dextrose to decrease starch and increase WSC as compared to the CON diet. In NFFS diet, corn grain and soybean meals were replaced with soybean hulls to decrease starch and increase non-forage NDF as compared to the CON diet. Diets were fed *ad libitum* as a total mixed ration (TMR) with a 5-10% refusal. Cows had *ad libitum* access to drinking water and were housed in a free-stall barn with a 60% stocking density.

Table 2.1. Ingredients and chemical composition of control and experimental diets

Items	Diets[1]			
	CON	DEX	NFFS	
Ingredients, % of DM[2]				
Corn silage	32.10	32.10	32.10	
Alfalfa hay	13.89	13.89	13.89	
Alfalfa haylage	7.22	7.22	7.22	
Cottonseed Fuzzy	2.25	2.25	2.25	
Soybean meal	4.52	4.38	2.08	
Corn grain	22.46	17.52	17.09	
Soybean hulls	8.51	9.12	16.91	
Energy Booster 100	0.45	0.00	0.46	
Urea	0.03	0.08	0.09	
Salt	0.31	0.31	0.31	
Sodium bicarbonate	1.20	0.77	0.16	
Calcium phosphate	0.11	0.15	0.19	
Magnesium oxide	0.21	0.21	0.21	
Amino Plus	6.28	6.56	6.58	
Vitamin mix	0.26	0.26	0.26	
Trace mineral	0.18	0.18	0.18	
Dextrose	0.00	5.00	0.00	
Chemical composition, % of DM				
DM, g/100g fresh matter	58.6	58.8	58.8	
Forage DM	53.2	53.2	53.2	
Concentrate DM	46.8	46.8	46.8	
Crude protein	16.5	16.5	16.5	
Rumen ungradable protein	9.9	9.9	9.9	
Rumen degradable protein	6.6	6.6	6.6	
Total NDF[3]	28.4	28.4	32.4	
Forage NDF	19.1	19.1	19.1	
Non-forage NDF	9.3	9.3	13.3	
peNDF, % of NDF				
Starch	28.0	24.5	24.3	
WSC[4]	5.4	10.1	5.0	
Starch + WSC	33.4	34.6	29.3	
NE_L, Mcal/kg	1.74	1.75	1.74	
Ash	7.9	7.5	7.4	
Calcium	0.63	0.63	0.68	
Phosphorus	0.37	0.36	0.36	

[1]CON = control diet, NFFS = non-forage fiber diet, and DEX = dextrose diet; [2]DM=Dry matter; [3]NDF=Neutral detergent fiber; [4]WSC=Water-soluble carbohydrates.

Feed Intake

Cows were fed individually once at (0900 h) using the Calan gate system (American Calan, Inc.; Northwood, NH). Individual feed intake was recorded daily, and feed was moved closer to the cows at 1900 h. Cows were milked twice daily at 0700 h and at 1830 h.

Feed and Milk Samples

The DM content of individual forages (corn silage, alfalfa hay and alfalfa haylage) was determined weekly (100ºC for 24 h) and the TMR was adjusted accordingly. Individual samples of forages and grain-mix for each diet were sampled weekly and stored at -20ºC to be analyzed later. Before analysis, each ingredient or grain-mix sample was thawed and composited by Latin Square period and then dried at 55ºC for 48 h and ground to pass 1-mm screen in a Wiley Mill (Arthur H. Thomas, Philadelphia, PA). Every ingredient was then analyzed for DM (method 934.01, AOAC International, 2016), ash (method 942.05, AOAC International, 2016), NDF (method 2002.04, AOAC International, 2016), ADF and ADL (method 973.18, AOAC International, 2016), ether extract (method 920.39, AOAC International, 2016), CP (method 954.01, AOAC International, 2016), and Starch (Hall, 2009) content.

The PM and AM milk samples for each individual cow (6 milkings per cow for over 3 d) were taken in milk bottle containing bronopol as preservative from d 15-17. Milk samples from each period were stored in refrigerator until the last day of sampling and then samples were analyzed for milk components (total solids, fat, protein, lactose, fatty acids, somatic cell count and MUN) with infrared analyzer using a Foss FT6000 (Foss Electric, Hillerød,

Denmark; DHIA Laboratory, Sauk Centre, MN, USA). Daily milk component yields for were calculated weighting for each milking (AM and PM). The fat-and-protein corrected milk (FPCM) was calculated as: [FPCM (kg/d) = milk production (kg/d) × (0.1226 × milk fat% + 0.0776 × true protein% + 0.253)] (IDF, 2015). We calculated feed efficiency by dividing milk yield or FPCM with DMI.

Collection and Analyses of Rumen Fluid

Rumen fluid was collected via the rumen cannula at 8 different time points over 24-h period with 3-h intervals from d 15 through d 18 of each period. About 100ml of rumen fluid was collected from the ventral sac of the rumen at each time point and then the sample were strained using a commercial cheese cloth. The pH of each sample was measured in duplicate immediately after collection. Rumen fluid samples (10 mL) were stored in an aliquot containing 200 μL of 50% sulfuric acid for rumen NH_3, another 10 mL were stored in an aliquot containing 2 mL of 25% metaphosphoric acid for rumen VFA. Ammonia N for each rumen sample was analyzed as per method of Chaney and Marbach (1962). The VFA samples were analyzed for total VFA, acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate and lactate (DairyOne Laboratory). A 2-mL of the rumen fluid was stored at -80 °C to determine bacterial abundance. Bacterial abundance was measured at 1200h (3-h after feeding) for each period.

Isolation and Amplification of Ruminal Fluid Bacterial DNA using qPCR

The rumen fluid previously collected and stored at -80°C was thawed in a warm bath at 45°C until a consistent liquid form was formed. After that, the rumen fluid was shaken to mix the sample. In a 2 mL microtube, 0.4 g of zirconia beads (0.3 g 0.1 mm + 0.1 g 0.5 mm) weights and 1000 µL of lysis buffer were added together with 250 µL of homogenized rumen fluid. Following, the microtubes were placed in a bead beater for 3 min to be homogenized. Once taken out, the samples were placed in a warm bath to be incubated at 70°C for 15 mins having to be inversed every 5 min. Following the warm bath, the samples were later centrifuged for 5 min at 16×10^3 g at 4°C. A total of 1000 µL of cell lysate solution was removed and placed in a fresh 2 ml microtube and placed on ice. Another 300 µL of lysis buffer was added to the microtube with the cell debris and the process was repeated from the bead beater and forward. Later, we removed 300 µL of cell lysate solution and placed it with the other 1000 µL previously stored in ice. To the total amount of cell lysate solution, we added 20% of ammonium acetate 10M (1.3 mL lysate solution + 0.26 mL ammonium acetate). The solution was mixed by vortexing for 5s. After making sure the solution was homogenized, the microtube was incubated in ice for 5 min to be later centrifuged at 16×10^3 g for 10 min at 4 °C. A total of 1000 µL of supernatant was transferred to fresh microtubes adding 1000 µL of isopropanol and both were mixed by vortexing. The samples were incubated for 30 min on ice and then were centrifuged at 16×10^3 g for 15 min at 4 °C. The supernatant was removed and 300 µL of 70% ethanol was added to the pellet remaining in the microtube. The solution was centrifuged at 16×10^3 g at 20 °C and following that, ethanol was removed and the microtubes were aired to dry the pellet. The pellet was then resuspended with inhibitex solution 1000 µL (Applied

Biosystems, Waltham, MA) and vortexed until the pellet dissolved completely. The solution was left at room temperature for 10 min. In a centrifuge tube 600 µL of the DNA pellet in inhibitex was added with 25 µL of proteinase K (Applied Biosystems) and mixed by vortex. Another 600 µL of AL buffer (Applied Biosystems) was added and mixed by vortex. The solution was applied in a mixed column and centrifuged at $16x10^3$ g at room temperature for 1 min. The column was then rinsed with 500 µL of wash solution AW1 (Applied Biosystems) centrifuge at $16x10^3$ g for 1 min at room temperature followed by a second wash solution AW2 (Applied Biosystems) centrifuge at $16x10^3$ g for 3 min at room temperature. The centrifuge tube was centrifuged at $16x10^3$ g for 3 min at room temperature to dispose of traces of wash buffer. The sample was eluted using 50 µL of AE buffer (Applied Biosystems) and left to incubate at room temperature for 1 min and finally the sample was centrifuged at $16x10^3$ g for 1 min at room temperature. Later the sample was stored at -20 ºC to be used later for qPCR. All samples were diluted to 10 ng/ul and tested in triplicates using 1 negative control in triplicate in each plate. The program MxPro was used using a SYBR green with dissociation curve to know the cycle threshold (ct) values for each primer used (*Megasphaera elsdenii, Fibrobacter succinogenes, Prevotella ruminicola, and Streptococcus bovis*). Relative abundance (RA) for each species was calculated using the formula,

$$RA\ (\%) = \frac{1}{2^{(Avg\ ct\ value\ test\ primer - Avg\ ct\ value\ universal\ primer)}}$$

Table 2.0 - Species-specific primers used in real-time qPCR assay for the quantification of bacterial abundance

Target bacterial species		Primer sequence (5'- 3')	Reference
Fibrobacter succinogenes	F	GCGGGTAGCAAACAGGATTAGA	(Abdelmegeid et al., 2018)
	R	CCCCCGGACACCCAGTAT	
Megaspheara elsdenii	F	AGATGGGGACAACAGCTGGA	(Abdelmegeid et al., 2018)
	R	CGAAAGCTCCGAAGAGCCT	
Prevotella ruminicola	F	GAAAGTCGGATTAATGCTCTATGTTG	(Stevenson & Weimer, 2007)
	R	CATCCTATAGCGGTAAACCTTTGG	
Streptococcus bovis	F	TTCCTAGAGATAGGAAGTTTCTTCGG	(Abdelmegeid et al., 2018)
	R	ATGATGGCAACTAACAATAGGGGT	
Total Bacteria	F	CCTACGGGAGGCAGCAG	(Muyzer et al., 1993; Mosoni et al., 2007)
	R	ATTACCGCGGCTGCTGG	

[a]F: forward primer
[b]R: reverse primer

Feeding Behavior and Particle Size Distribution

During sampling week, we recorded rumination, eating, chewing (rumination + eating), lying, active and high active time for each cow in each period (EarTag, CowManager Sensor™, Harmelen, The Netherlands). We then calculated the intake adjusted rumination, eating, and chewing time by dividing those values with DM, NDF and fNDF intake.

We collected each TMR sample daily (1000g) during the sampling period to determine particle size distribution and physically effective particles (PEP). The daily TMR sample was composited by diet and period and then particle size was determined using PSPS. The PEP was calculated by summing particle weights retained in sieves that were >1.18 mm in screen size (Kononoff et al., 2003).

Total-Tract Nutrient Digestibility

To determine total tract digestibility of nutrients, we collected fecal spot samples from each cow (8 collections per cow) in each period from d 15-18. Fecal samples were composited by cow and period. The composited fecal samples were dried at 55ºC for 72 h and ground to pass 1-mm screen in a Wiley Mill (Arthur H. Thomas, Philadelphia, PA). The fecal samples were analyzed for DM, OM, NDF, starch and CP as per the method described above for feed samples. We also analyzed fecal and TMR samples for 240-h indigestible NDF (iNDF) which were used as an internal marker for estimation of fecal output and nutrient digestibility. The fecal output was calculated following the method of Cochran et al. (1986). Then, nutrient digested was calculated by subtracting fecal nutrient output from nutrient consumed. Digested nutrients were divided by nutrient consumed to determine nutrient digestibility which was expressed as percentage. During this sampling, we also collected orts samples for each individual cow to determine nutrient intake properly accounting for sorting (if any). Orts samples were composited by cow and period, and analyzed for DM, OM, NDF, starch and CP as per method described for feed ingredients.

Statistical Analyses

Aside from DMI, MY and milk components, all other variables were averaged to a single mean value for each cow in each period. Dry matter intake, MY, and milk components were analyzed as a repeated measure whereas all other variables were analyzed as non-repeated measure using the following mixed model (lme4 R package):

$$Yijkl = \mu + P_i + Sq_j + C_k + D_l + PD_{il} + Eijkl$$

Where Yijkl is the response variable; μ is the overall mean; P_i is the fixed effect of i^{th} period, i = 1, 2, 3; Sq_j is the fixed effect of j^{th} square, j = 1, 2, 3; C_k is the random effect of k^{th} cow within parity, k = 1, 2, … 9; D_l is the fixed effect of l^{th} diets, l = 1, 2, 3; PD_{il} is the interaction term of i^{th} period and l^{th}diet, and Eijkl is the error term. Significance and tendences were declared at $P \leq 0.05$ and $0.05 > P \leq 0.10$, respectively. A Tukey's post hoc test was performed when the *P*-value for diet was ≤ 0.10.

RESULTS AND DISCUSSION

Diets and Particle Size Distribution

Though all diets contained similar concentrations of forage NDF, CP and NE_L, the CON and DEX diet contained 4% less total NDF than NFFS diet due to increased inclusion of soyhull in the NFFS diet (Table 2.1). On the contrary, CON diet had 3.6 percentage unit more starch than DEX or NFFS diet due to greater inclusion of corn grain. The DEX diet contained more WSC than CON or NFFS diet (10.1 vs. 5.2% of diet DM) due to inclusion of pure dextrose.

Regarding particle size distribution as shown in Table 2.2, all three diets contained statistically similar proportion of long (> 19 mm), medium (8.0-19 mm) and fine (< 1.18 mm) particles whereas CON diet had lower proportion of short (1.18-7.9 mm) particle than DEX or NFFS diet due to disproportionate inclusion of corn grain, dextrose and soybean meals across diets. All three diets also contained similar physically effective particle (PEP) which is defined as the proportion of particle size that is greater than 1.18 mm in-size.

Table 2.2. Particle size distribution of experimental diets

Items	Diet[1]			SEM	*P* value		
	CON	DEX	NFFS		Diets	Period	Diets×Period
Particle consumption, as-fed, kg/d							
Long, >19 mm	2.41[c]	8.11[a]	4.54[b]	0.36	<0.001	<0.001	<0.001
Medium, 8.0–19 mm	11.0[b]	12.5[a]	11.1[b]	0.83	<0.001	<0.001	0.209
Short, 1.18–7.9 mm	6.00[b]	6.89[a]	7.20[a]	0.53	<0.001	<0.001	0.023
Fine, <1.18 mm	27.9	27.5	27.0	2.28	0.358	0.005	<0.001
Sorting index[3], %							
Long, >19 mm	93.0[b]	110[a]	110[a]	2.99	<0.001	0.030	<0.001
Medium, 8.0–19 mm	92.8	94.5	93.6	2.11	0.313	<0.001	0.089
Short, 1.18–7.9 mm	101[a]	101[a]	103[b]	0.79	0.137	<0.001	<0.001
Fine, <1.18 mm	95.8[b]	95.8[b]	99.2[a]	0.75	<0.001	<0.001	<0.001

[2]Geometric mean particle size, and [3]geometric standard deviation of particle size, calculated using Particle Size Analysis Spreadsheet (Jud Heinrichs, 2022).

[4]CS = corn silage; AH = alfalfa hay; AHL = alfalfa haylage.

[5]CON = control diet; NFFS = non-forage fiber diet; and DEX = dextrose diet.

Production Performances and Feed Efficiency

The main effects and interactions for production performance and feed efficiency variables are presented in Table 2.3. Though CON and NFFS diet had similar DMI, the cows fed DEX diet consumed 6 and 9% greater DMI than NFFS and CON diet, respectively (P = 0.05). In this study, MY, FPCM and feed efficiency (MY/DMI or FPCM/DMI) did not vary among diets.

The increase in DMI in the DEX diet can be mainly attributed to the palatability of the dextrose included in DEX det. In an experiment conducted by Nombekela *et al*. (1994) concluded that greater proportion of sucrose inclusion in lactating cows diet increases both DMI and MY. On contrary, in our study, we did not find milk production response in

agreement with Gao and Oba (2016), who also did not observe an increment in MY due to increased DMI. Moreover, a few other factors might also explain reason for not observing an increment in MY in our study due to increased DMI. First, the cows in Nombekela et al. (1994) were in early stage of lactation while the ones in the present study were in late lactation. The energy requirements in these two stages in lactations vary, as the energy requirement for a late-lactation cow tends to be directed more into fat deposition and fetal growth. Also, body weight is difficult to measure in a Latin square design. Additionally, the period effect was profound and significant mainly because of decreasing milk production over time as cows were progressing toward end of the lactation period in 2nd and 3rd period ($P < 0.01$).

Table 2.3. Production performances and feed efficiency of cows fed control and experimental diets

Variables	Diets[1]			SEM	*P* value		
	CON	DEX	NFFS		Diets	Period	Diets×Period
DMI[2]	27.5[b]	30.0[a]	28.3[b]	2.06	0.05	0.001	0.333
MY[3], kg/d	31.3	29.4	30.2	2.51	0.190	<0.001	0.432
FPCM[4], kg/d	37.5	37.0	37.6	4.86	0.978	<0.001	0.791
MY/DMI, kg/kg	1.30	1.12	1.27	0.14	0.142	0.009	0.453
FPCM/DMI, kg/kg	1.40	1.25	1.42	0.24	0.465	0.003	0.893

[1]CON = control diet, NFFS = non-forage fiber diet, and DEX = dextrose diet; [2]DMI=Dry matter intake, a common superscript within a row does not differ between diets (P < 0.05); [3]MY=Milk yield, [4]FPCM=Fat-and-protein corrected milk.

Percentage and Yield of Milk Components

The percentages and yields of milk components including fatty acid profile and MUN are presented in Table 2.4. None of these variables were affected by the diets (*P* > 0.10). However, most variables except milk fat, fatty acids including *de novo* and mixed fatty acids and SNF were affected by the experimental period. Like production performance, milk component yields were affected by the period being lower yield of milk component in 2nd and 3rd period than first period mainly because of decreased milk yield while cows were progressing toward the end of lactation.

Table 2.4. Percentage and yield of milk components of cows fed experimental diets

Variables	Diets[1]			SEM	*P* value		
	CON	DEX	NFFS		Diets	Period	Diets×Period
Milk component, %							
Protein	3.69	3.92	3.94	0.52	0.601	<0.001	0.884
Fat	4.10	4.44	4.43	0.85	0.470	0.442	0.689
Fatty acid (FA)	3.87	4.19	4.18	0.79	0.472	0.609	0.693
Denovo FA	1.11	1.22	1.21	0.26	0.426	0.257	0.667
Preformed FA	1.24	1.31	1.34	0.24	0.549	0.001	0.624
Mixed FA	1.58	1.72	1.70	0.30	0.485	0.808	0.615
Lactose	4.09	4.16	4.17	0.16	0.631	<0.001	0.938
SNF[2]	8.98	9.17	9.25	0.60	0.380	0.419	0.942
MUN[3]	9.83	9.84	9.59	0.60	0.787	<0.001	0.218
Milk component yield, kg/d							
Protein	1.33	1.32	1.35	0.22	0.976	<0.001	0.789
Fat	1.47	1.49	1.51	0.28	0.953	<0.001	0.797
Fatty acid	1.39	1.41	1.43	0.26	0.955	<0.001	0.799
Lactose	1.44	1.36	1.37	0.13	0.537	<0.001	0.463
SNF	3.19	3.03	3.09	0.27	0.619	<0.001	0.495
Total Solids	4.67	4.53	4.61	0.44	0.862	<0.001	0.666

[1]CON = control diet, NFFS = non-forage fiber diet, and DEX = dextrose diet; [2]SNF=Solids-not-fat; [3]MUN=Milk urea nitrogen

Rumen Fermentation

The main effects and interactions of variables related to rumen fermentation are presented in Table 2.5. None of the variables were affected by the diet except iso-butyrate and iso-valerate. Cows fed CON diet had greater iso-valerate and tended to have greater iso-butyrate than NFFS or DEX diets. The rumen pH measured in 3-h intervals is shown in Figure 2.1. The rumen pH did not differ across diets. However, rumen pH was affected by the period. Rumen fluid sampling time had a significant effect on rumen pH when we included sampling time in the model of rumen pH data analysis. As shown in Figure 2.1, rumen pH followed a typical trend where pH was higher in the morning before feeding time (0830 h), then pH dropped ~3 h after feeding (1200 h) and again ~219 h after feeding time (1800 h)

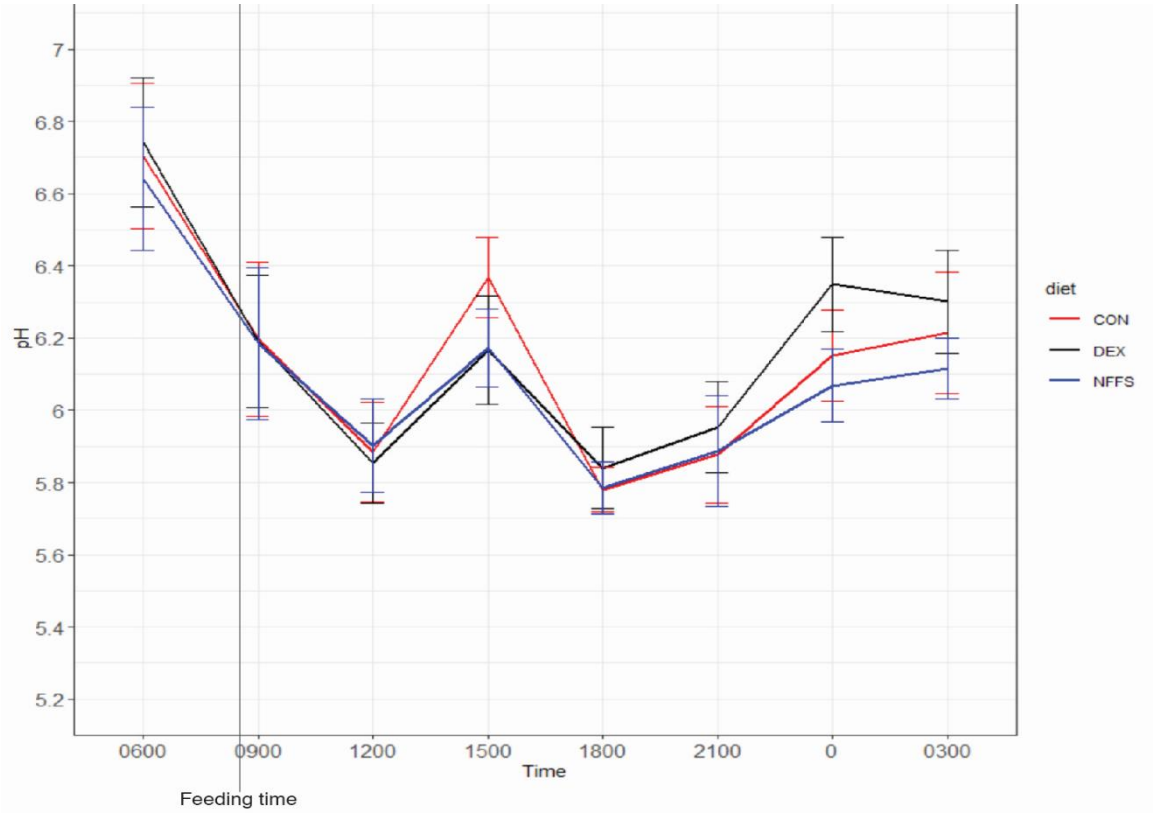
Storm et al. (2012) concluded that iso-butyrate shows highest concentration gradient compared to acetate, propionate and butyrate while it also has the lowest absorption rate constant provided that absorption rate constant is inversely proportional to concentration gradient. Liu et al. (2009) reported that iso-butyrate supplementation did not influence DMI but did increase milk yield. In the present study, CON cows had greater Iso-butyrate concentrations in rumen fluid, but milk yield was not different between CON and DEX or between CON and NFFS.

Table 2.5. Rumen pH, volatile fatty acids and ammonia of cows fed experimental diets

Variables	Diets[1]			SEM	*P* Value[3]		
	CON	DEX	NFFS		Diets	Period	Diets×Period
Total VFA[2], mM	152	142	153	9.38	0.285	0.286	0.905
pH	6.14	6.17	6.09	0.16	0.462	<0.001	0.568
VFA, mol/100 mol							
Acetate (A)	64.0	62.3	64.1	2.24	0.506	0.219	0.258
Propionate (B)	23.2	23.4	22.3	2.13	0.669	0.298	0.184
Butyrate	9.82	11.06	10.54	0.77	0.145	0.154	0.971
Iso-butyrate	0.79[a]	0.70[b]	0.71[b]	0.03	0.100	0.056	0.121
Valerate	1.63	2.06	1.94	0.45	0.474	0.218	0.458
Iso-valerate	0.55[a]	0.48[b]	0.47[b]	0.04	0.032	0.043	0.174
Lactate	0.26	0.28	0.29	0.04	0.685	0.128	0.918
A:P	2.87	2.72	2.95	0.35	0.639	0.260	0.226
NH_3-N, mg/dL	4.97	7.10	7.43	2.56	0.451	0.704	0.779

[1]CON = control diet, NFFS = non-forage fiber diet, and DEX = dextrose diet; [2]VFA=Volatile fatty acids; [3]A common superscript within a row does not differ between diets ($P < 0.05$).

Figure 2.1. 3-hours interval rumen pH of cows fed different diets



Digestibility of Nutrients

Digestibility of nutrients are shown in Table 2.6. In this study, both DM (P <0.05) and NDF (P < 0.01) digestibility were greater for NFFS than CON or DEX diet. On the contrary, starch digestibility was lower for DEX than CON or NFFS diet (P < 0.01).

Forage NDF can be replaced with inclusion of non-forage NDF in a diet if diet contains minimum level of physically effective fiber so to stimulate rumination sufficiently. Replacement of grains with non-forage NDF affect productivity as well as the digestibility of nutrients due to the changes of outflow rate (Bradford & Mullins, 2012). In this study, DM and NDF digestibility were greater in NFFS diet than CON or DEX diet.

Inclusion of non-forage fiber using soyhulls in the NFFS diet might have increased both DM and NDF digestibility since NDF from soyhulls is highly digestible (National Academies of Sciences et al., 2021).

Table 2.6. Apparent nutrient digestibility of cows fed experimental diets (OM and CP digestibility will be added)

Variables	Diets[1]			SEM	*P* Value		
	CON	DEX	NFFS		Diets	Period	Diets×Period
DM (%)	63.6[b]	62.4[b]	69.6[a]	1.75	< 0.05	0.14	<0.01
OM (%)							
NDF (%)	38.0[b]	40.0[b]	55.0[a]	2.81	<0.01	0.39	<0.01
Starch (%)	98.0[a]	97.2[b]	98.0[a]	0.32	<0.01	0.12	<0.01
CP (%)							

[1]CON = control diet, NFFS = non-forage fiber diet, and DEX = dextrose diet; [2]DM=Dry matter; [2]OM=Organic matter; [3]A common superscript within a row does not differ between diets (P < 0.05).

Abundance of Ruminal Bacteria

The main effects and interactions for ruminal bacterial abundance are shown in Table 2.7. The abundance of *M. elsdenii* was not affected by the diet although there was a period effect (*P* <0.05). On the contrary, diet tended to affect the abundance of *F. succinogenes, P. ruminicola* and *S. bovis* (*P* < 0.10) being greater in abundance of DEX than NFFS whereas abundance did not vary between CON vs. DEX or CON vs. NFFS diets.

Prevotella is one of the most abundant families in the rumen, as such their functions vary. *P. ruminicola* has different functions in the rumen such as being one of the major ammonia producing species, proteolytic species, ureolytic species, amylolytic species, pectinolytic species and hemicellulolytic species. As such, it is hard to determine what the driver is for increasing abundance for this species due to its' multiple functionalities and without analyzing more bacterial species. The increase in this species was assumed to be

related to the decreased starch content and increased WSC or dextrose in DEX than CON diet. Increased WSC in the DEX diet might have influenced the abundance of *Streptococcus bovis and Prevotella ruminicola* due to increased proteolytic activity provided the similar concentrations of RDP in all three diets.

Table 2.7. Rumen bacterial abundance of cows fed experimental diets

Variables	Diets[1]			SEM	*P* Value[2]		
	CON	DEX	NFFS		Diets	Period	Diets×Period
M. elsdenii[3](%)	$7.5x10^{-3}$	$7.7x10^{-3}$	$8.5x10^{-3}$	$1.0x10^{-3}$	0.53	0.004	0.16
F. succinogenes[4](%)	0.173	0.205	0.294	0.05	0.22	0.020	0.91
P. ruminicola[5](%)	0.313^{ab}	0.205^{b}	0.577^{a}	0.20	0.10	0.24	0.92
Str. bovis[6](%)	$0.80x10^{-3bc}$	$1.3x10^{-3ab}$	$2.5x10^{-3a}$	$0.40x10^{-3}$	0.08	0.34	0.76

[1]CON = control diet, NFFS = non-forage fiber diet, and DEX = dextrose diet; [2]A common superscript within a row does not differ between diets ($P < 0.05$); [3]*Megasphaera elsdenii;* [4]*Fibrobacter succinogenes;* [5]*Prevotella ruminicola;* [6]*Streptococcus bovis*

Feeding and Rumination Behavior

The feeding and rumination behavior variables are shown in Table 2.8. Daily, DEX cows tended to spend greater time eating than NFFS cows which is aligned with increased DMI in DEX cows though DEX cows had less active time than NFFS cows. When these behaviors are expressed per unit of DM, NDF or forage NDF intake, DEX cows had lower rumination time per kg of DM, NDF or fNDF than CON cows. On contrary, eating and chewing time expressed per unit of NDF intake was greater in DEX than NFFS diet due to more time spent by the DEX cows to consume greater amount of DM.

Table 2. 8. Feeding and rumination behaviors of cows fed experimental diets.

Variables	Diets[1]			SEM	*P* value[4]		
	CON	DEX	NFFS		Diets	Period	Diets×Period
Activity, min/d							
Rumination	493	501	514	41.2	0.403	0.106	0.552
Eating	174^{ab}	186^{a}	168^{bc}	85.2	0.076	<0.001	0.325
Chewing	669	689	684	112.7	0.599	<0.001	0.490
Lying	463	451	446	46.1	0.688	<0.001	0.674
Active	116^{ab}	105^{bc}	119^{a}	18.1	0.028	0.106	0.004
High active	196	200	195	20.1	0.653	0.005	0.212
Intake-adjusted rumination time							
min/kg of DM[2]	19.1^{a}	17.2^{b}	18.9^{ab}	2.29	0.095	<0.001	0.589
min/kg of NDF[3]	67^{a}	61^{b}	59^{b}	7.92	0.009	<0.001	0.605
min/kg of fNDF[4]	100^{ab}	90^{c}	99^{bc}	11.9	0.095	<0.001	0.589
Intake-adjusted eating time							
min/kg of DM	6.46	6.34	6.11	3.37	0.576	0.017	0.959
min/kg of NDF	22.6^{ab}	22.2^{b}	18.9^{c}	11.1	0.003	0.022	0.935
min/kg of fNDF	33.8	33.2	31.9	17.7	0.568	0.017	0.962
Intake-adjusted chewing time							
min/kg of DM	25.7	23.7	25.2	5.38	0.229	0.008	0.814
min/kg of NDF	90.4^{ab}	83.3^{b}	77.8^{c}	18.1	0.003	0.011	0.829
min/kg of fNDF	135	124	132	28.2	0.229	0.008	0.817

[1]CON = control diet, NFFS = non-forage fiber diet, and DEX = dextrose diet; [2]DM=dry matter; [3]NDF=neutral detergent fiber; [4]fNDF = forage neutral detergent fiber; A common superscript within a row does not differ between diets ($P < 0.05$).

CONCLUSIONS AND FUTURE RESEARCH

In conclusion, we observed an increment in DMI when corn grain was replaced with dextrose. However, intake was not affected when corn grain and soybean meal was replaced with soyhulls. Milk yield, FPCM, percentage and component of milk, and feed efficiency did not differ between diets. Diet did not affect rumen pH and fermentation profile except iso-butyrate and iso-valerate had greater concentrations in CON than DEX or NFFS diet. Inclusion of soybean hulls (non-forage fiber or NFFS diet) by replacing corn grain increased DM and NDF digestibility whereas starch digestibility decreased when corn grain was replaced with pure dextrose. The bacterial abundance tended to increase in DEX diet than CON or NFFS diet. Despite changing microbial populations, the rumen pH was not affected confirming our hypothesis in which a proper paNDF system will maintain a rumen pH above SARA thresholds even though rumen bacterial populations differ as well as different digestibility rate in the rumen.

Future research should focus on understanding the interactions between the rumen microbiome, pH and paNDF system, expanding the number of microbial species measured. Finally additional studies are needed to assess a wider range of NFFS and WSC concentrations on dairy cattle performance and rumen fermentation to determine recommendations for the dairy industry.

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