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Improving Feed Efficiency Through Forage Strategies for Increasing Dairy Profitability and Sustainability

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IMPROVING FEED EFFICIENCY THROUGH FORAGE STRATEGIES FOR INCREASING DAIRY PROFITABILITY AND SUSTAINABILITY

BY

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

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ABBREVIATIONS

ADF	Acid detergent fiber
ADIN	Acid detergent insoluble nitrogen
AH	Alfalfa haylage
ATP	Adenosine triphosphate
BCS	Body condition score
BMR	Brown mid-rib
BW	Body weight
C	Control treatment diet
CH_4	Methane
Co	Cobalt
CO_2	Carbon dioxide
CP	Crude protein
CS	Corn silage
d	Day
DM	Dry matter
DMI	Dry matter intake
DIM	Days in milk
ECM	Energy corrected milk
EE	Ether extract
FCM	Fat corrected milk
H	Hydrogen
He	Helium
hr	Hour

iNDF	Indigestible neutral detergent fiber
NDF	Neutral detergent fiber
NDIP	Neutral detergent insoluble protein
kg	Kilogram
Mcal	Mega calories
ME	Metabolizable energy
MUN	Milk urea nitrogen
Na	Sodium
NDF	Neutral detergent fiber
NE_L	Net energy of lactation
NH_3	Ammonia
NRC	National research council
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
SC	*Saccharomyces cerevisiae*
SCFP	*Saccharomyces cerevisiae* fermentation product
SEM	Standard error of the mean
SCC	Somatic cell count
SNF	Solid non-fat
TCA	Tricarboxylic acid cycle
TMR	Total mixed ration
VFA	Volatile fatty acids
wk	Week

ABSTRACT

IMPROVING FEED EFFICIENCY THROUGH FORAGE STRATEGIES FOR INCREASING DAIRY PROFITABILITY AND SUSTAINABILITY

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Three studies were conducted to determine production parameters and study specific hypothesis in regard to improving feed efficiency through various forage utilization strategies with or without the inclusion of various supplemented products. The first study evaluated the supplementation of a cobalt-lactate product and its effects on fiber digestibility and milk production parameters when fed to cows consuming a 70% forage diet. Treatments included: 1) CONTROL diet containing 12.5 mg/cow/d of cobalt (carbonate carbonate) and 2) TEST diet being the same basal diet but including an additional 50 mg/cow/d of cobalt via a 1% Co-lactate product (Co-Max®). In a feeding trial with 24 late lactation cows, feeding the cobalt-lactate product had no effect on production parameters. However, cobalt-lactate supplementation decreased rumen ammonia concentrations, increased ruminal molar concentrations of acetate and numerically increased fiber digestion. The second study evaluated *Saccharomyces cerevisiae* fermentation products (SCFP); (Diamond V original XPC and two prototypes) on lactational performance and ruminal fermentation. Eight ruminally cannulated Holstein dairy cows were used in a replicated 4 x 4 Latin square. Treatments were: 1) Control (C): corn silage and haylage based ration; 2) XPC: C ration with 14 g/hd/d Original XPC; 3) Prototype 1 (P1): C ration with 5 g/hd/d P1; and 4) Prototype 2 (P2): C ration with 19 g/hd/d P2. Ruminal pH (6.06, 6.07, 6.02 and 6.13 for C, XPC, P1, and P2

respectively) was greater ($P < 0.05$) for cows fed P2 compared to cows fed other treatments. Rumen concentration and percentage of propionate and iso-butyrate were increased ($P < 0.05$) for cows fed P2 when compared to C with cows fed other treatments being intermediate and similar. The feeding of a dairy ration with P2 SCFP can improve ruminal pH while increasing propionate and iso-butyrate concentrations and percentages. The third study evaluated two forage production programs with subsequent feeding to evaluate the lactational performance of Holstein dairy cows. Thirty peak-lactation (58 DIM ± 2.9) Holstein dairy cows were used in a randomized complete block design. Treatments were: 1) CONTROL: normal forages (65% of diet) ration formulated using alfalfa haylage and corn silage produced with a standard university soil and agronomy program; 2) TEST: high forage level (65% of diet) ration formulated using alfalfa haylage and corn silage produced on an enhanced soil and agronomy program. Milk production was increased for cows fed TEST compared to cows fed control forage while DMI were similar. Energy corrected milk was increased for the TEST fed cows. There was an increasing trend in starch digestibility for cows fed TEST forage. Digestibility of NDF and ADF were increased for the TEST fed cows compared to cows fed CONTROL forages. Feeding higher quality forages obtained from enhanced agronomy procedures increased milk production, milk composition, and fiber digestibility when lactating dairy cows were fed a high forage ration. Based on these results, lactating dairy can greatly benefit from increases in forage quality and forage digestibility. Supplemental products such as SCFP can be utilized to aide in increases in propionate production which typically lead to increases in milk yield.

CHAPTER 1:

LITERATURE REVIEW

Introduction

Costs of grain and various feed ingredients have fluctuated greatly in recent years. In addition, the availability of certain commodities have become scarce in certain parts of the country. The result is that rations fed to livestock and in particular, lactating dairy cows, have risen dramatically in cost. Often times, the cost to produce a hundred kilogram of milk is below the milk price and therefore, the profitability of the dairy industry is negative, and producers are again losing equity. In the past, commodities and/or by-products have been used to reduce ration costs and improve profitability of the dairy operation. However, even these commodities are increasing in cost due to value and availability relative to corn and soybean meal. Therefore, new ways must be found to reduce feed costs to regain profitability and sustainability of the dairy industry to compete on a world market.

Dairy cattle are biologically designed to convert forages and other fibrous feeds into high quality products such as milk and meat. The predominant foundation behind rations for dairy cows is to provide a highly fermentable diet that supports high intakes and promotes consistent rumen fermentation. In an era of high priced concentrate feedstuffs, producers and nutritionists continue to seek ways to reduce feed costs. The utilization of high forage diets with lower starch concentrations are one option to reduce costs.

During periods of high corn prices, it has become increasing popular to feed at least 60% and potentially 70% of ration DM in the form of high quality forages. Typically, these diets are made up primarily of corn silage with the addition of alfalfa haylage with the goal of reducing ration costs. Through increased management practices, producers have improved their ability to grow and store larger quantities of consistent high-quality forages. The evaluation of NDF digestibility has helped nutritionists more effectively formulate high forage diets.

A common question when feeding high forage diets to high producing cows is whether productivity can be maintained when compared to more common, lower forage diets. Controlled research studies and field experiences have concluded it is possible to maintain production when utilizing high forage diets as long as consistent, high-quality forages are fed. Research has shown herds producing over 36 kg of milk fed rations containing more than 70% of the total ration DM as forage (Chase, 2011). High forage diets are beneficial in numerous ways including reduced feed costs, increased cow health, rumen homeostasis, and improved nutrient management. A couple of challenges with high forage diets include increased forage inventories and frequent monitoring of feedstuffs and rations. The quality and quantity of forages fed to the dairy herd are directly related to milk production, feed costs, nutrient balance, and farm profitability.

Feed efficiency is one way to improve the profitability and sustainability of the dairy operation. Feed efficiency is defined as the unit of milk produced per unit of dry matter intake. The energy content of the ration is the greatest factor affecting the feed efficiency of the lactating dairy cow (Casper and Mertens, 2007). The greatest factor

affecting the energy density of the diet are the nutrient digestibilities of the forages in the ration. Forages are the cheapest and most economical sources of nutrients on the farm when compared to grains, proteins, and various commodities sources. Therefore, increasing forage nutrient availability will increase their economic value relative to other commodities or by-products. The use of highly digestible forages may allow one to increase the amount used in the ration to meet the nutrient requirements of high producing dairy cows. In addition, meeting the nutrient requirements of dairy cows in later lactation may also be advantageous in order to reduce feed cost to improve profitability.

In addition to high forage diets, nutritionists continue to search for ways to increase producer profitability and dairy cow feed efficiency. Oftentimes, these increases are due to supplemental feed products that increase feed digestibility, shift VFA production, and/or increase milk production, while utilizing the same or reduced DMI levels. One area of focus was the inclusion of cobalt-lactate to increase nutrient digestibility. Supplemental Co has been proven to increase fiber digestion in the ruminant. Another area is the inclusion of *Saccharomyces cerevisiae* fermentation products (SCFP) which has commonly led to increases in feed efficiency and milk production.

This literature review will first describe the characteristics of forage metabolism and fermentation in dairy cows. It will then explore the role of high forage diets in today's dairy industry. Finally, it will discuss the impact of supplemental products such as cobalt-lactate and SCFP on dairy cow efficiency and production parameters.

Forage Fermentation in the Rumen

Diet fermentation is the result of physical and microbial activities which convert components of the diet into useful products for the animal, such as VFAs, microbial protein, and B-vitamins. Dairy cows moderate microbial populations in the rumen by supplying and masticating substrate regularly. Moreover, the addition of buffers, removal of acid products, the passing of microbial products, and the maintained conditions of the rumen all allow for microbial growth. The fermentation of forage is most commonly associated with intake, NDF, fiber digesting bacteria, and acetate production.

Intake

Total stomach volume (rumen) is very large in terms of capacity when compared to non-ruminant animals (Van Soest, 1994). This capacity is necessary in order to retain large levels of fibrous feedstuffs in the rumen for proper microbial fermentation. Ruminal volume is typically greater when diets contain high levels of roughage. The dry matter of rumen digesta can vary from 7% to over 14% of rumen wet weight, depending on forage roughage level. Additionally, rumen volume limits feed intake of high roughage diets (Della-Fera and Baile, 1984). Due to the various rates of solid and liquid rumen outflow, volume and rate of passage must be combined to determine true fractional outflow each day (Bell, 1959).

Particles in the rumen leave less rapidly than liquid due to the location of particles and higher proportions of roughage will also increase liquid flow (Van Soest, 1994).

This increase in liquid flow will help prevent inhibitory levels of fermentation end products. Lower quality roughages will also take longer to degrade in the rumen, thus decreasing intake.

Rumination is primarily stimulated by the intake of fibrous particles longer than 10 mm, such as chopped hay (NRC, 2001). These lightweight particles float and form a rumen mat, which is later regurgitated and chewed thoroughly.

The forage component most strongly related to rumination time of longer forage particles is NDF. Neutral detergent fiber levels are best described as negatively associated with digestibility and positively associated with the time spent ruminating (NRC, 2001). Consistent rumination will lead to consistent intakes, thus providing more dependable production from the cow.

Bacteria and Acetate Production

The rumen is a dominant feature of the digestive tract of dairy cattle. The rumen maintains a dense and varied population of microorganisms that ferment feed materials to primarily produce short-chain organic acids or VFAs along with methane and carbon dioxide. Additionally, this process provides substrate and energy for the production and growth of micro-organisms. Amount and type of micro-organisms are directly related to the type of diet being fed. The main organisms that breakdown feedstuffs in the rumen include bacteria, protozoa, and fungi (Van Soest, 1994).

Bacteria that digest structural carbohydrates (cellulose and hemicellulose), produce a great proportion of acetic acid, which is important for the production of milk

fat. These bacteria are sensitive to fats and lower pH in the rumen. If the rumen is too acidic or if too much fat is included in the diet, these bacteria can be eliminated or their growth rate can be greatly reduced (Van Soest, 1994). Furthermore, this reduction in cellulose digesting bacteria can reduce feed digestibility and can reduce DMI. This situation can be minimized with the use of high quality forages and by reducing the level of rapidly digestible carbohydrates.

Fibrous, structural carbohydrates are broken down by pectinolitic, hemi-cellulolytic, or cellulolytic bacteria in the rumen. During the breakdown of these components, several VFAs are produced with the production of acetate being higher in high-forage diets when compared to higher-concentrate diets. Highly fibrous feeds lead to microbial populations which produce high ratios of acetate to propionate. Acetate is necessary for the production of milk fat and low acetate levels can lead to milk fat depression. The production of propionate is a common end product of starch and sugar fermentation, less commonly associated with fibrous carbohydrates. However, most of the dairy cow's energy needed by the mammary system to produce lactose, the major osmotic constituent of milk, is obtained from propionate.

Structural Carbohydrate Nutrition

Structural Polysaccharides

Structural polysaccharides represent a large proportion of the cell wall material in plant cells. Typically, cellulose makes up the majority of structural carbohydrates present in nature. The cell wall of plants are initially made up of pectin. Although, pectin levels

are reduced significantly as the plant ages, while the levels of cellulose and lignin simultaneously increase (Van Soest, 1994). Cellulose and hemicellulose are known to be more digestible than lignin. The lignin portion of the cell wall is typically what is excreted and is found in higher levels in lower quality forages.

Methane Production

Dairy cows utilize their rumen microbes to metabolize carbohydrates by converting them to glucose which is then oxidized to pyruvate in the Embden-Meyerhof pathway and subsequently converted to acetate and various other VFA. Throughout this process, methane is created and should be viewed as an energy sink where H from all rumen microorganisms drains, allowing a greater yield of ATP production. The quantity of methane produced is often related to end-products of carbohydrate fermentation.

In comparison, high forage diets generally yield 2 to 3 times more methane as an end product than do high concentrate diets (Church, 1988). The reason for this is that CO_2 and H are byproducts from the conversion of glucose to acetate and butyrate. In contrast, higher concentrate diets generate a higher proportion of propionate through the succinate and acrylate pathways which accounts for all of the H produced (Church, 1988).

Methanogenic bacteria are sensitive to changes in dietary conditions. Instances such as increased passage and fermentation or decreased rumination or pH can reduce the amount of H available to methanogens. Animal performance can be increased with these ruminal changes as H is further retained and utilized in the creation of propionate, which

also increases the ME of the diet. Ionophores, such as monensin, function to shift the rumen population by selecting against gram positive bacteria which helps to increase propionate production and decrease CH_4. The supplementation of ionophores can significantly increase the animal performance and efficiency.

A recent review paper indicated that improving forage quality had a low to medium effectiveness on mitigating methane emissions (Gerber et. al., 2013). However, these same authors concluded that the effectiveness of change was variable when interactions of DMI and ration nutrients were considered.

Another study evaluated the effect of forage to concentrate ratio on milk production and methane emissions (Aquerre et. al., 2011). All treatments contained equal portions of alfalfa haylage and corn silage on a DM basis but varied by forage percent from 47 to 68%. Dry matter intake did not differ but there was a tendency of decreased milk yield in the higher forage diets. Researchers found significantly higher daily methane emissions from the high forage diet compared to the low forage diet. Daily methane emissions were 17% higher for the high forage diet as compared to the low forage diet.

Additional work is needed to better understand the interactions of DMI, forage quality, and forage intake on methane emissions. Ration nutrient balance and profitability will be important considerations needing attention in order to better evaluate the relationships between forages and methane emissions.

VFA Absorption and Metabolism

The importance of VFAs in the ruminant for a ME source is well understood. A majority of all VFAs produced are primarily absorbed in the rumen, reticulum, and omasum, with a small amount reaching the abomasum. Most of the acetate produced is carried by portal circulation to the liver unchanged while a small amount is absorbed through the rumen wall and converted to ketone bodies.

The first reaction in acetate metabolism is a conversion to acetyl-CoA in the cytoplasm which is mediated by acetyl-CoA synthetase. A large portion of liver acetate will escape oxidation and pass directly into peripheral circulation. Once absorbed, acetate will generally be oxidized in the TCA cycle or used for fatty acid synthesis (Church, 1988). In the absence of adequate levels of ATP-citrate lyase, glucose cannot supply enough acetyl-CoA for fatty acid synthesis. Therefore, acetate is the main precursor for lipogenesis in the ruminant. Production of adequate levels of acetate is essential in order to maintain sufficient quantities of milk fat. Acetate is the primary precursor of milk fatty acids up to and including palmitic acid. For these reasons, adequate levels of forage in the diet is necessary.

Utilization of High Forage Diets

The prices of grain and various feed ingredients have greatly fluctuated in recent years making it difficult to control the cost of dairy rations. Commodity prices are changing almost on a daily basis and the markets have been anything but calm (Alexander, 2008). The markets are in volatile times due to a number of reasons affecting

ingredient prices and uses (ethanol, bio-diesel, export, international value of dollar, etc.). In addition, the availability of some commodities has become scarce in certain parts of the country.

The result is that rations fed to livestock, and in particular, lactating dairy cows, have risen dramatically in cost. The cost to produce a hundred kg of milk is often below the milk price, therefore, the profitability for the dairy industry is negative, and producers are again losing equity. In order to keep dairy producers profitable and able to compete on the world market, methods must be found to reduce the cost to produce milk. However, one consolation to remember is that dairy cows require specific nutrients and not ingredients to optimize production. Therein lies an opportunity.

In the past, commodities have been used to reduce ration costs and improve profitability of the dairy operation. South Dakota State University is well known for its research on distillers grains and co-products (Schingoethe et al. 2009; Kalscheur. 2005) as an economical commodity to reduce ration costs. However, even these ingredients are increasing in cost and decreasing in availability due to their nutrient value relative to corn and soybean meal. Therefore, new ways must be found to reduce feed costs to regain profitability and sustainability of the dairy industry.

One area that has received little emphasis until recently is in the area of forage quality. Forages can represent from 40 to over 70% of the ration dry matter. Improving forage quality will improve the nutrient supply to the animal. It is one thing to talk about the importance of high quality forages and quite another to produce them. Dairy producers must have a passion for producing high quality forages, because forage quality

is determined by management. Managing the soil, purchasing the correct seed for the soil type, adjusting to weather conditions, harvesting, forage treatment aids, storage, and feed-out management are all part of the forage management program. When everything is done correctly, excellent quality forage can be obtained. Dairy producers must understand that forage quality cannot be too good if the goal is to lessen the reliance on commodities for feeding the cows.

A common question that arises when formulating high forage diets is "How much forage or forage-NDF can a dairy cow eat?" A ration formulated entirely on forage can be fed but applied knowledge suggests that this diet will not maximize production, efficiency, or profitability (Mertens, 2009). Two factors should be considered when maximizing forage use in high producing cows: 1) maximize the proportion of forage in the diet while allowing the cow to optimize production and 2) maximize the digestion and utilization of forage when it is included in the diet (Mertens, 2009). Nutrient uptake and digestibility drive lactation performance.

It is well known that the level of NDF can have a negative effect on the animal as intake and performance is reduced at high levels of NDF inclusion while low levels of NDF can reduce intake. This indicated that there is an optimal level of NDF that will maximize intake (Mertens, 2010). As the forage quality increases, the NDF level of the forage decreases encouraging increased intake. The gut fill effect is determined by forage-NDF content, forage particle size, fragility of forage-NDF, and NDF digestibility within a forage family (Allen, 2000).

Research indicates that dairy cows can consume higher quantities of NDF and forage than some of the previous guidelines recommend. In general, high producing cows should be fed diets that are less filling and highly fermentable in order to maximize DMI, whereas low producing cows should be fed diets that are more filling and less fermentable (Allen, 2011). The respective filling effect is determined by the concentration and digestion characteristics of the forage fiber (Allen, 2000). Oba and Allen (1999) reported that a 1 unit increase in NDF digestibility was associated with an increase in DMI of 0.17 kg and an increase in fat corrected milk of 0.25 kg. These higher digestibility forages would have a lower indigestible NDF fraction. It is important to remember that the intake and milk response to improved digestibility of NDF is greater for high producing cows when compared to lower producing cows.

Research in Sweden evaluated the effect of grass maturity on NDF intake (Rinne et. al., 2002). In situ data indicated significant decreases in rate of digestion and potential NDF digestion as forage maturity increased. Additionally, it was noted that early cut grass silages had a lower rumen fill and increased intake when compared to more mature forages.

Raffrenato and Van Amburgh (2010) evaluated the concept that higher digestible forages may have a greater portion of total NDF in the fast-digesting fraction of two proposed pools. In this study, they compared conventional and BMR corn silages. The BMR corn silage had 73.7% of the total NDF in the fast pool when compared to 60.7% in the conventional corn silage. The proportion in the slow NDF pool was 18.7% for

conventional while the BMR treatment was 13.1%. Furthermore, the iNDF was 20.6% of total NDF in the conventional and 13.1% for the BMR corn silage.

Another trial was conducted to evaluate the relationship between forage levels and forage digestibility on DMI, milk production, ruminal digesta, pool sizes, and fiber turnover (Grant and Cotanch, 2012). Conventional corn silage rations consisted of corn silage levels of 39 or 55% and had 52.6 or 68.3% total forage. Brown mid-rib treatments had 36 or 50% corn silage and 49.4 or 63.5% forage. Additionally, all treatments contained 13.3% haycrop silage.

Solids corrected milk was significantly increased by BMR corn silage when fed at a higher forage level. Total NDF intake was significantly higher for cows on the high BMR ration and the increased digestibility of the BMR diets allowed for greater intake and greater ruminal turnover. Rumen digesta mass was less for BMR fed cows indicating that cows more easily obtained the necessary nutrient supply from the small rumen NDF pool with a quicker turnover time. This study helps to provide a better understanding of this complex system through insight into the relationships of NDF, NDF digestibility, DMI, and rumen function.

Considerations for High Forage Diets

In order for high forage rations to work, the mindset of the producer and nutritionist should be consistent of the fact that this method can work. Management practices of consistent quality forages is a necessity as with the utilization of higher forage diets, there is less room for variability. Variations in quality will have immediate

ramifications on milk production as the level of forage in the ration increases. In order to eliminate variation, large inventories of high quality forage will need to be acquired through either a purchasing agreement or cropping program adjustments. More frequent analysis of forages are needed in order to reach the goals of the feeding program. With the increased analysis, ration formulation adjustments can be made more easily based on the results.

Additionally, feed management is increasingly important with the goal of having a constant supply of fresh feed available to the animals throughout the day. Due to the increased levels of silage in the diet, rations may heat up more quickly during hot times of the day which may lead to increased feed deliveries to cows. An increase in forage in the diet will typically lead to an increase in the bulkiness of the diet. The bulkier feed will require an increased number of feed pushups throughout the day. This less dense, bulkier feed may require additional mixes to feed the same number of cows and may lead to the decision of purchasing a larger mixer to increase efficiency.

In summary, feeding higher forage rations is an opportunity that should be evaluated in all dairy herds. Higher forage rations allow the cow to utilize a feedstuff, useless to man, to convert forage into milk. Forage quality and consistence defines the usefulness of this method in all on farm scenarios. These types of changes to the feeding program can take time as cropping programs are only gradually adjusted. There are numerous long-term advantages of high forage diets including higher milk component levels, improved cow health, and herd profitability.

Supplementation of Cobalt

Overview of Cobalt

Cobalt is a component of vitamin B_{12} (cobalamin). Provided that adequate cobalt is available in the diet, ruminal microbes can produce all of the vitamin B_{12} required by the cow. A very small percentage of diet Co will be incorporated into vitamin B_{12}. Deficient diets will utilize as much as 13 percent of the Co while satisfactory diets will utilize only 3 percent (NRC, 2001). Therefore, excess dietary cobalt that is not utilized as B_{12} or B_{12} analogs, should potentially be available for other uses.

Current recommendations for Co supplementation are estimated at 0.11 mg/kg of dietary DM which is based on supplying enough Co to keep tissue concentrations of B_{12} above 0.3 µg/L (NRC, 2001). Cobalt fed at 0.25 to 0.35 mg/kg of dietary DM, well above what is required for sufficient B_{12} synthesis, can enhance ruminal digestion of feedstuffs, especially lower quality forages (Lopez-Guisa and Satter, 1992). Addition of Co has been reported to increase total anaerobic bacteria in the rumen by 50 percent and increase lactic acid production in the rumen by 86 percent (Young, 1979). Cobalt toxicity causes reduced feed intake, weight loss, hyperchromemia, and eventually anemia (NRC, 2001).

The most recent research on Co supplementation in dairy cattle has focused on its effects on metabolism and production parameters in the cow. Kincaid and Socha (2007) focused on the effects of Co supplementation during late gestation and early lactation on milk and serum measures by utilizing 36 multiparous cows in a completely randomized block design at Washington State University. Concentrations of Co were included at one of three different levels from 55 days prior to calving to calving and were 0.15, 0.89, or 1.71 mg/kg on a DM basis. Lactating cows received diets containing 0.19, 0.57, or 0.93 mg/kg of Co from parturition through 120 days in milk. Samples collected included DMI (daily), BW (d -55, -20, 7 and 120), milk yield (daily), colostrum (at calving), individual

milk samples (monthly), blood (d -55, -20, 7, and 120), and liver biopsies (d -55, 7, and 120).

Serum vitamin B_{12} concentrations declined sharply in all cows between 55 and 20 d postpartum. Dietary Co supplementation tended to cause an increase in the concentration of vitamin B_{12} in colostrum and milk (0.089, 0.120, and 0.130 µg of Co/mL) at 120 days in milk. There was no effect of Co supplementation on DMI or yield of milk and milk components. Despite the liver having the highest Co concentration and being the main storage site, liver Co concentration was not affected by either Co intake or day of sampling. In conclusion, serum concentrations of vitamin B_{12} are reduced in the early dry period, and added dietary Co may increase ruminal synthesis of vitamin B_{12} as indicated by a tendency for increased vitamin B_{12} concentrations in colostrum and milk of cows supplemented with dietary Co.

Akins et al. (2013) examined the effects of Co supplementation and vitamin B_{12} injections on lactation performance and metabolism of dairy cows. For this study, forty-five cows at 60 d prepartum were blocked by expected calving date, and randomly assigned to 1 of 5 treatments in an RCB design with treatments starting at 60 d prepartum. The 5 treatments for this study were CON: no supplemental dietary Co, CoCarb: 25 mg/d of supplemental Co from Co carbonate, LCoGH: 25mg/d of supplemental Co from Co glucoheptonate, HCoGH: 75 mg/d of supplemental Co from Co glucoheptonante, and IB12: CON diet plus weekly 10 mg i.m. of vitamin B_{12} injections. Samples collected included BW (weekly), BCS (weekly), colostrum (at calving), milk yield (daily), blood (d -63, -57, -7, 1, 30, 90, and 150), and liver biopsies (d -60, 1, 30, 90, and 150).

Dry matter intake, BW, and BCS were not affected by treatment. The LCoGH treatment tended to have greater milk yield than CoCarb, and CON had similar milk

yields to the mean of LCoGH and HCoGH. Cobalt supplementation and vitamin B_{12} injections did not influence plasma or liver measures of energy metabolism. However, injections of vitamin B_{12} increased plasma, liver, and milk vitamin B_{12} contents. Dietary Co addition did not affect plasma vitamin B_{12} concentrations. Although, it increased milk vitamin B_{12} concentrations throughout lactation.

Kincaid et al. (2003) also evaluated the effect of dietary Co supplementation on Co metabolism and performance of dairy cattle. In this study, 36 cows were assigned to one of three treatments from 21 d prepartum to 120 d postpartum varying by Co per day of 0, 12, and 25 mg/d DM basis during prepartum. After parturition, dietary concentrations of Co were 0.37, 0.68, and 1.26 mg/kg.

Supplemental Co did not increase Co in serum, colostrum, milk, or liver. Primiparous cows secreted colostrum and milk with higher Co concentrations than multiparous cows. Additionally, serum B_{12} levels were higher in primiparous than multiparous cows and declined with increasing days in milk. Serum Co also decreased from 7 to 120 DIM.

Campbell et al. (1999) looked at the effect of Co on reproduction and milk yield on lactating cows receiving bovine somatotropin. They utilized 60 cows and blocked them by lactation number and incidence of retained fetal membranes. Two diets were utilized from calving to 154 DIM with the first being a control and the second being a control plus 26 mg of Cobalt as Co glucoheptonate. Days to first service, days open, days from first service to conception, services per conception, milk yield, milk components, and somatic cell counts were similar for control and supplemented cows.

Tiffany et al. (2006) looked at the influence of cobalt concentration on vitamin B_{12} production and fermentation of mixed ruminal microorganisms grown in continuous culture flow-through fermenters. For this study, four fermenters were fed 14 g of DM/d

with one of 4 levels of Co CO_3. Treatments were no 1) no Co, 2) 0.05 mg of Co/kg of DM, 3) 0.10 mg of Co/kg of DM, 4) 1.0 mg of Co/kg of DM. After a 3 day adjustment period, fermenters were sampled over a 3 day sampling period. Molar proportions of acetate, propionate, and isobutyrate, and acetate:propionate were not affected by the addition of supplemental Co. Cultures supplemented with 0.10 mg of Co/kg had greater vitamin B_{12} concentrations than those supplemented with 0.05 mg of Co/kg of DM, and increasing supplemental Co from 0.10 to 1.0 mg/kg of DM increased ruminal fluid vitamin B_{12} concentration.

In conclusion, research on Co supplementation is minimal and not completely understood. While we know Co is primarily stored in the liver, we do not know of many added benefits once sufficient vitamin B_{12} has been produced. Therefore, further research appears to be needed on the effects of Co supplementation on ruminal fermentation and metabolism of dairy cattle.

Supplementation of *Saccharomyces cerevisiae* Fermentation Products

Overview of Yeasts

Yeast is a unicellular fungi that does not reproduce via asexual spore production (Phaff, 1966). The most commonly fed yeast in the dairy industry is *Saccharomyces cerevisiae* (SC); a facultative anaerobic yeast often referred to as a bakers or brewers yeast. Most commonly, the yeast fed to ruminants are live cells or yeast culture mixes. A yeast culture is a fermented-yeast product that contains dead and live yeast, the culture media on which the yeast is grown on, and the metabolic by-products produced by the yeast during fermentation. This process generally involves inoculating the culture media

with live yeast cells, fermentation of the media, and drying of the fermented media. Live yeast products generally consist solely of live dried yeasts that are mixed with a carrier for easier distribution.

Yeast products are commonly supplemented around the world for inclusion in diets of production animals. Yeast is often supplemented in dairy cow diets with the goal of improving animal performance and is considered a "natural" alternative to using antibiotics. When fed to lactating dairy cows, several benefits have been reported including increased milk production, increased DMI, and increased milk fat production. How yeast directly improves animal health and performance is not yet known although a variety of mechanisms have been suggested and explored. These differences are often explained through changes in the rumen microbial population, rumen fermentation, intestinal nutrient flow, and diet digestibility. Most dietary compounds entering the rumen are broken down by various anaerobic microorganisms (primarily bacteria and protozoa) present in rumen fluid.

Research in the area of yeast supplementation to dairy cattle has shown inconsistent results across numerous peer-reviewed and non-peer-reviewed studies. One viable explanation for this inconsistency would be the wide variation in conditions across these studies. This would include differences in inclusion level, type of ration, DMI, and the use of additional feed additives along with other animal factors such as age, physiological stage, health, and stress status; all of which may affect yeast efficacy (Wagner et al., 1990).

Increased DMI has been observed in some studies (Dann et al., 2000) and decreased DMI in other studies (Schingoethe et al., 2004). Numerous studies have identified positive effects on milk production (Harrison et al., 1988; Abd El-Ghani, 2004; Hippen et al., 2007; Stella et al., 2007; Lehloenya et al., 2008; Ramsing et al., 2009); while others reported a positive trend in production (Williams et al., 1999; Dann et al., 2000; Wang et al., 2001) or found no significant differences (Robinson, 1997; Schingoethe et al., 2004).

Poppy et al. (2012) conducted a meta-analysis to include results from 36 separate studies to summarize the effects of supplementing SCFP to lactating dairy cows. These individual studies originated from both peer-reviewed and non-peer-reviewed sources and included research on both early lactation and later lactation cows. Across all studies, SCFP supplemented animals had a significantly increased milk yield (1.18 kg/d), 3.5% FCM (1.61 kg/d), ECM (1.65 kg/d), milk fat yield (60 g/d), and milk protein yield (30 g/d). Additionally, researchers found an increase in DMI for cows less than 70 DIM and a decrease in DMI for cows greater than 70 DIM. Therefore, cows at a greater DIM became more efficient in their milk production once passing 70 DIM.

Transition dairy cows

Kim et al. (2005) conducted a study feeding Holstein cows SCFP approximately 4 weeks prior to calving through 41 DIM. Treatments did not affect DMI prepartum but the day of calving and one day post-calving cows receiving SCFP had significantly higher DMI than control fed cows. Treatment had no effect on milk yield or components

in this trial. Additionally, a more sustained DMI response was reported when Jersey cows were supplemented with 60 g/d of SCFP 21 days prepartum through 140 days postpartum (Dann et al., 2000). Dry matter intake was significantly increased for animals receiving SCFP the week prior to calving (2.1 kg/d) and the first 21 DIM (1.8 kg/d). Cows receiving the SCFP treatment also peaked in milk 14 days earlier than control cows (43 vs. 57 DIM) but there was no difference in milk yield or components through 150 DIM.

Nocek et al. (2006) conducted a study utilizing 44 cows by assigning them to either a control or SCFP and *Enterococcus faecium* supplemented ration. This study was conducted 21 days prepartum through 70 DIM. Supplementation of the treatment significantly increased in situ corn silage (6.5%) and haylage (4.8%) DM digestibilities at 72 hours. Dry matter intake had a tendency (P=0.10) to increase prepartum (1.0 kg/d) and significantly increased after calving (2.7 kg/d) for test fed cows as compared to control fed cows. Milk yield was also significantly increased (2.3 kg/d) for cows receiving the test diet compared to the control diet. Researchers found no difference in milk component yield; however, the test diet significantly decreased milk fat percentage compared to control fed cows (0.32 %).

Block et al., (2000) utilized 64 cows in a similar type of study but differed by feeding either a control or a live SC with the addition of *Lactobacillus plantarum* and *E. faecium* diet from 21 days prior to calving through 70 days postpartum. Researchers found no difference in cow performance prepartum. However, postpartum DMI (1.9

kg/d), milk yield (1.0 kg/d), and milk protein concentration (0.1 %) were all significantly increased for the test fed cows as compared to the control fed cows.

Robinson and Garrett (1999) evaluated the effects of SC yeast culture when supplemented to Holstein cows from 23 days prior to calving through 56 days postpartum. No significant difference on DMI prior to calving was observed. After calving, there was a trend for increased DMI in multiparous cows and increased milk yield in primiparous cows. Vogel et al. (2005) also reported a similar response in a similar study except this time cows were fed from 21 days prior to calving through 75 DIM. Vogel et al. (2005) found no effect on DMI pre or postpartum but found a trend (P=0.08) for increased milk yield (4.3 kg/d) for cows receiving the SC culture vs. control fed cows.

Wohlt et al. (1998) researched varying levels of SC supplementation at 0 or 10 g/d beginning 30 days prior to calving through 28 days postpartum. On day 29, cows within each treatment group were reassigned to new SC treatment levels of 0, 10, or 20 g/d through 126 DIM. Various treatments had no effect on DMI prepartum. From parturition through 28 DIM, treatment had no effect on milk production or DMI. With the increase of treatment supplementation at DIM 29 from 10 to 20 g/d, these cows consumed more DMI (2.0 kg/d) from week 5 to 18 compared to cows decreased in amounts of SC from 10 to 0 g/d. Additionally, cows from week 5 to 11 fed increased levels of SC had increased 3.5% fat corrected milk when compared to cows with maintained or decreased treatment level supplemented cows (43.4, 39.0, 38.1 kg/d), respectively.

Ramsing et al. (2009) researched supplementation of SCFP at O, 57, or 227 g/d to 66 Holstein dairy cows. Treatments were fed from approximately 21 days prior to calving through 21 days after calving. Postpartum DMI were similar for all treatments. Milk yield was significantly greater for test cows when compared to control cows. Additionally, 3.5% FCM, ECM, and milk fat yield tended to be 10% greater for cows that were supplemented with yeast culture compared with nonsupplemented cows. Milk protein yield, milk protein percent, milk fat percent, and somatic cell score were not affected by treatment. Yeast culture supplementation improved prepartum DMI and postpartum performance and improved the ability of cows to transition during the periparturient period. Additionally, primiparous and multiparous cows responded similarly when supplemented with yeast culture.

Zaworski et al. (2014) evaluated different dosage levels of SCFP delivered to 42 Holstein cows. Treatments were 0 g/d, 56 g/d, or 112 g/d and were supplemented to transition cows starting approximately 28 days prior to calving through 28 days after calving. During the first day after calving, feeding SCFP decreased serum cortisol concentrations and at least tended to increase supplement intake and serum concentrations of calcium, glucose, urea N, and serum amyloid. During the first 4 weeks postpartum, supplementing SCFP versus no SCFP decreased milk SCC and increased milk production. Feeding the 112 g versus 56 g of SCFP had no additional benefits.

In comparison, research has shown no effect on cow performance when yeast is supplemented on either prepartum or postpartum diets. Wang et al. (2001) fed SCFP at a rate of 60 g/d to cows starting at 21 days prior to calving through 120 days postpartum.

Results show no effect of supplementation on milk production or DMI through 140 DIM. Robinson (1997) fed SC culture at 57 g/d from 23 days prior to calving through 56 days postpartum without any differences in cow performance pre or postpartum. Soder and Holden (1999) fed SC culture, alone and with enzymes, and found no significant differences on pre or postpartum DMI, milk yield, or composition from 28 days prior to calving through 92 DIM when supplemented at 15 to 20 g/d. Erasmus et al. (2005), in a similar study, fed cows SC culture from 21 days prior to calving through 56 DIM with the supplementation of approximately 51 g/d SC with no significant differences reported in postpartum DMI, milk production, or milk composition when supplemented to Holstein cows.

Lactating dairy cows

Kung et al. (1997) supplemented mid lactation dairy cows with SCFP for 77 days and early lactation cows for 28 days. Supplementation with SCFP did not affect cow performance for mid-lactation cows, but increased 3.5% FCM yield in early lactation cows when fed at 10 g/d compared to control cows (39.3 vs. 36.4 kg/d). Shaver and Garrett (1997) evaluated the effect of supplemental SCFP to mid-lactation cows in 11 high producing commercial dairy herds in Wisconsin. Feeding yeast significantly increased milk yield 0.9 kg/d and milk protein yield 0.03 kg/d. Milk fat was decreased 0.1 percent with no difference in milk fat yield across farms. Lehloenya et al. (2005) utilized treatments of control (C), yeast (Y), or yeast plus Propionibacteria (Y+P) supplemented diet to 31 cows from 2 weeks prepartum to 210 DIM. Yeast was fed at an

inclusion of 56.0 g/d and Propionibacteria at $6x10^{11}$ cfu/d. Milk fat percentage was significantly lower for the control fed cows when compared to the Y or Y+P treatment cows.

Acharya et al. (2015) utilized 80 mid-lactation Holstein cows to evaluate the effects of a common SCFP and a new SCFP prototype. Treatments were 0 SCFP (control), 14 g/d SCFP (XPC), 5 g/d SCFP (prototype 1), or 19 g/d SCFP (prototype 2). After 8 weeks of treatment supplementation, researchers found no difference in DMI, 3.5% FCM, or ECM. However, milk yield was increased for the prototype 2 treatment when compared to control with the other two treatments being intermediate. Additionally, researchers found an increase in propionate percentage for the prototype 2 when compared to control or XPC with prototype 1 being intermediate. Researchers felt this increase in propionate most likely lead to the increase in milk yield for the prototype 2 cows.

In comparison, research has also shown no effect with the addition of SC. Arambel and Kent (1990) utilized 20 Holstein dairy cows in early to mid-lactation which were allocated to either a control or test group (90 g/d of SCFP). Cows were fed treatment rations from approximately 65 DIM through 145 DIM. The addition of SCFP in the diet of early to mid-lactation Holstein cows had no effect on DM intake, milk yield, or apparent digestion of nutrients.

Health and metabolic effects

Yeast supplementation during the dry period has improved effects on feed intake during the transition period (Kim et al., 2005; Dann et al., 2000). This continues to be an important factor in decreasing the metabolic stress for cows during the transition period (Hayirli et al., 2002). Furthermore, it is likely that the supplementation of yeast can be used to decrease the incidence of metabolic diseases postpartum. Although, studies to date have not been conducted with enough animals to clearly assess this assumption.

The effects of yeast on rumen fermentation has been diffident. Enjalbert et al. (1999) evaluated the supplementation of SC culture to dry cows for 32 days prepartum. Supplementation of SC resulted in increased rumen total VFA concentrations prefeeding (83.7 vs. 68.8 mmol/l) and 1 hour after feeding (93.3 vs. 78.2 mmol/l). Prior to feeding, test cows had higher rumen propionate concentrations and tended ($P<0.10$) to have a lower acetate:propionate ratio (3.00 vs. 3.49) when compared to control fed cows. Rumen pH was not effected by treatment although ammonia-N was lower (103.1 vs. 148.5 mg/L) 3 hours after feeding for test cows as compared to control fed cows. In general, the use of yeast products prior to calving through early lactation has resulted in no differences in rumen pH, ammonia-N, or VFA concentrations (Robinson and Garrett, 1999; Varel et al., 1994). However, pre-calving increases in rumen pH (Nocek et al., 2003), a trend for increased propionate concentration and decreased acetate:propionate ratio has been reported (Erasmus et al., 2005).

Calf performance and health

Research in the area of yeast supplementation to calves has been far less explored than in lactating dairy cows. Dobicki et al. (2005) supplemented calves with SC at 20 or 40 g/kg feed. Calves receiving the test treatments increased average daily gain (0.03 kg/d) and feed efficiency (0.44 kg gain/kg feed) when compared to control fed calves. Calves supplemented with SC also exhibited an improvement in health and immune status due to decreased blood cholesterol, increased leukocyte and erythrocyte counts, and increased hemoglobin levels.

Galvào et al. (2005) fed live SC yeast calves exhibiting low IgG concentrations indicating a failure of passive transfer. Treatments of SC included: 0 (control), 0.5 g in grain/d for 84 days, 0.5 g in milk/d for 42 days, or 0.5 in grain/d for 84 days with the addition of 0.5 g in milk/d for 42 days. Pre-weaning, calves receiving only SC in milk or grain had decreased days with diarrhea. Post-weaning, calves receiving SC in grain of the combination of SC in milk and grain had decreased days with diarrhea.

Lianjiang et al., (2006) reported that the supplementation of SC culture significantly decreased plasma endotoxin concentrations and increased immune system function in calves with diarrhea. Lesmeister et al. (2004) supplemented calves with SC culture at 0, 10, or 20 g/kg calf starter for 42 days. At the conclusion of the study, claves receiving the high dose of SC starter were 5.1 kg heavier than control fed calves. No difference was found for feed efficiency between treatments along with no effect on calf health. Wagner et al. (1990) reported no effect of yeast culture on calf performance and

Seymour et al. (1995) reported no differences with the supplementation of live yeast on calf performance or health.

In conclusion, research on SCFP supplementation has shown benefit although it is not completely understood. While we know SCFP primarily increases milk yield and can increase feed efficiency in cows past peak lactation, we do not know all of the details as to how this supplement is utilized in the rumen. Therefore, further research appears to be needed on the effects of SCFP supplementation on ruminal fermentation and metabolism of dairy cattle.

Conclusions

In summary, feeding higher forage rations is an opportunity that should be evaluated in all dairy herds. Higher forage rations allow the cow to utilize a feedstuff, useless to man, to convert forage into milk. Forage quality and consistency defines the usefulness of this method in all on farm scenarios. These types of changes to the feeding program can take time as cropping programs are only gradually adjusted. There are numerous long-term advantages of high forage diets including higher milk component levels, improved cow health, and herd profitability.

Additionally, supplemental products to increase feed efficiency have been evaluated and can be beneficial to the dairymen. Research on Co supplementation is minimal and not completely understood. While we know Co is primarily stored in the liver, we do not know of many added benefits once sufficient vitamin B_{12} has been produced. Therefore, further research appears to be needed on the effects of Co

supplementation on ruminal fermentation and metabolism of dairy cattle. Research on SCFP supplementation has shown benefit although it is not completely understood. While we know SCFP primarily increases milk yield and can increase feed efficiency in cows past peak lactation, we do not know all of the details as to how this supplement is utilized in the rumen. Limited research has evaluated VFA profiles when supplementing SCFP. Therefore, further research appears to be needed on the effects of SCFP supplementation on ruminal fermentation and metabolism of dairy cattle.

CHAPTER 2:

COBALT-LACTATE INCLUSION IN A HIGH FORAGE TOTAL MIXED RATION FED TO LATE LACTATION DAIRY COWS

Abstract

Cobalt-lactate is a highly soluble source of Co in the rumen. Prior research evaluating higher Co feeding rates has been shown to increase ruminal fiber digestion. Feeding high forage rations to late lactation dairy cows to improve income over feed cost could potentially benefit from feeding higher ruminal soluble Co rates to enhance ruminal fiber and nutrient digestibility. Twenty-four late-lactation (238 ± 68.8 DIM and 36.4 ± 5.4 kg/d milk) Holstein dairy cows (10 primiparous and 14 multiparous), were blocked by milk yield, DIM, and parity and randomly assigned to 1 of 2 treatments. Treatments included: 1) CONTROL diet containing 12.5 mg/cow/d of cobalt (carbonate carbonate) and 2) TEST diet being the same basal diet but including an additional 50 mg/cow/d of cobalt, via a 1% Co-lactate product (Co-Max®, Ralco, Marshall, MN). Rations were 70% forage and 30% of the respective experimental grain mix on a DM basis with the forage blend consisting of 60% alfalfa baleage and 40% corn silage (DM basis). Cows were fed the CONTROL ration during the covariate period of 7 d followed by 4 weeks of data collection when CONTROL and TEST diets were fed. Milk production (26.2 and 25.8 kg/d for CONTROL and TEST, respectively throughout results) was similar (P = 0.72). Dry matter intakes (22.9 and 23.1 kg/d) were similar (P = 0.8). Yield of milk fat (1.02 and 1.09 kg/d), milk protein (0.87 and 0.91 kg/d) and lactose (1.17 and 1.26 kg/d) were

similar (P = 0.33, P=0.44, and P = 0.34 respectively). Body weights (684 and 674 kg) were not different (P = 0.11). Rumen ammonia concentrations were lower (P = 0.03) for cows fed TEST (12.3 mg/dL) as compared to cows fed CONTROL (15.8 mg/dL). Ruminal molar concentrations of acetate were higher (P = 0.04) for cows fed TEST (61.07%) as compared to cows fed CONTROL (59.47%). Feeding additional Co as cobalt-lactate did not influence milk production, milk composition, dry matter intake or body weight for lactating dairy cows fed a high forage ration, but did appear to alter ruminal fermentation.

Key words: dairy cattle, cobalt-lactate, high-forage diet

Introduction

Cobalt is an essential trace element in ruminant diets that is utilized by the rumen microbes for vitamin B_{12} production. Provided adequate dietary Co is available, ruminal microbes can produce the vitamin B_{12} required for both ruminal bacteria and the host animal (NRC, 2001). The dietary requirement of dairy cows for Co is 0.11 mg/kg DM, which is based on supplying enough Co to keep tissue concentrations of B_{12} above 0.3 µg/L (NRC, 2001). However, Mills (1981) found ruminal synthesis of B_{12} to increase 20-fold in sheep when levels of dietary Co was increased from 0.1 to 0.5 mg/kg. Additionally, Tiffany et al. (2006) found increased synthesis of vitamin B_{12} as dietary Co concentration increased from 0.1 to 1.0 mg/kg, using closed system fermenters. Furthermore, Allen (1986) reported increased cellulose digestibility of diets containing 10 mg/kg added Co, in vitro. Lopez-Guisa and Satter (1992) supplemented Co above NRC recommended levels to enhance diet utilization of corn crop residues in growing heifers.

In general, only 3 % of dietary Co is utilized for vitamin B_{12} production, though up to 13 % will be incorporated when insufficient Co is fed (Smith and Marston, 1970). A low forage to concentrate ratio diet has been shown to reduce ruminal synthesis of vitamin B_{12}, thus creating more analogs of vitamin B_{12}, which are not physically active (Walker and Elliot, 1972). In comparison, high forage diets tend to promote greater production of cobalamin, further increasing the ratio to other various analogues (Sutton and Elliot, 1972).

Limited research has determined the effect of Co lactate in dairy cattle diets on lactational performance. Beyond the utilization of Co for vitamin B_{12} production, very little is known about Co metabolism in the ruminant. Although research has shown the liver to retain concentrations of Co at varying levels dependent on animal age (Kincaid et al, 2003), the function of additional dietary Co has not yet been clearly defined. Thus, the objective of this study was to determine if Co supplementation in a high-forage diet during late lactation would affect rumen VFA and ammonia concentration, diet digestibility, and milk production parameters.

Materials and Methods

Ten primiparous and 14 multiparous cows averaging (mean ± SD) 36.4 ± 5.4 kg of milk/d and 238 ± 68 DIM were blocked by milk yield, DIM, and lactation number and randomly assigned to one of two treatment groups. The trial was 35 d in length with -7 d through 0 d being utilized for adaptation to the basal diet and Calan (American Calan, Inc., Northwood, NH.) door training. Days 1 – 7 were used for a covariate period and days 8 – 35 for data collection on respective treatments.

Cows were housed in a free-stall facility at the South Dakota State University dairy research and training facility (DRTF) with free access to water, milked 3 times daily (0600, 1400, and 2100 h), and fed once daily (0700 h) for *ad libitum* intake through individual mangers located in front of each Calan door. Total daily feed offerings were adjusted based on previous 24-h intake so refusals were approximately 5%. Amounts fed and refused were recorded daily. The experimental cows were cared for according to the

guidelines stipulated by South Dakota State University Institutional Animal Care and Use Committee. The health status of each animal was evaluated daily.

Treatments consisted of 2 diets (Table 1) fed as TMR, composed from a common basal mix that consisted primarily of 70% forage (40% corn silage and 60% alfalfa baleage), finely ground corn, dried distillers grains, and soybean hulls. Treatments were as follows; control (CONTROL) – Diet formulated to meet all nutrient requirements, including 12.5 mg/cow/d of cobalt (cobalt carbonate) and Test Diet (TEST) – the same basal diet as control diet plus the inclusion of cobalt lactate (Co-Max, Ralco Inc., Marshall, MN, USA) to provide 50 mg/cow/d of Co. Supplemental Co was incorporated into the test grain mix and all diets were prepared and delivered with a Calan Data Ranger (American Calan, Inc., Northwood, NH). All diets were formulated using AMTS (Agricultural Modeling and Training Systems, LLC, Groton, NY, USA), an applied mathematical nutritional model to predict lactating dairy cow performance.

Experimental Measures

Prior to the start of the experiment, samples of feedstuffs were analyzed and initial diets were formulated based on the feed analysis. Grain mixes for CONTROL and TEST diets were then formulated and tested for nutrient content prior to the start of the feeding study. The grain mix was mixed at the SDSU Feed Mill and delivered to the DRTF approximately every 2 wk. Individual treatment TMR were mixed for 5 min using a Super Data Ranger (American Calan, Inc., Northwood, NH) prior to dispensing the

TMR. Samples of the basal mix and TMR were collected and frozen (-20°C) weekly then composited by experimental period prior to analysis.

Daily intake was calculated from feed offered and refused and recorded daily. Total milk production was measured and recorded daily throughout the experiment via a recording system (DeLaval-ALPRO, Kansas City, MO) at each milking and saved to a Universal Serial Bus flash drive. Milk samples were collected (25 mL) 1 day weekly at each milking throughout the experiment, preserved using 2-bromo-2-nitropropane-1,3 diol, stored at 4°C after collection and analyzed for fat, true protein, lactose, MUN, SNF and somatic cells at Dairy Herd Improvement Association (Manhattan, KS) within 72 h.

Body weights and BCS (1 – 5 scale) were measured and recorded once each morning (1000 h) weekly. Two 10-mL blood samples were collected from the coccygeal artery into vacutainers tubes (containing K_2-EDTA (Becton Dickinson Vacutainer Systems, Rutherford, NJ)) on d 25 and 32 and immediately spun at 1,875 x g for 20 min to obtain plasma. Plasma samples were then frozen at –20°C until analysis of blood urea nitrogen was conducted. Rumen fluid samples were obtained via esophageal pump on d 25 and 32, analyzed for pH then two 10 mL samples were frozen at –20°C until analysis for VFA and ammonia concentration via gas chromatography. Fecal grab samples were collected during wk 3 and 4 every 8 hr for 3 d with forward advancement of 2 hr daily to account for diurnal variation.

Body condition scores were determined weekly by 3 individuals on a scale of 1 to 5, with 1 as emaciated and 5 as obese (Wildman et al., 1982), approximately 3 h after feeding. Body weights were electronically collected using a livestock scale (AWB-5K-

SYS, Triner Scale and Manufacturing Company, Inc., Olive Branch, MS) on Tuesday of each week, approximately 3 h after feeding.

Sample Analysis

Dry matter composition of forages was determined weekly by drying in a 105°C oven (Despatch LEBI-75, Despatch Industries, Minneapolis, MN) for 24 h and feed sheets adjusted accordingly. Composited samples of individual feeds and TMRs were shipped frozen in insulated shippers to Analab Laboratory (Fulton, IL) for analysis. Samples were analyzed using the following Association of Official Analytical Chemists International (1998) methods: DM (935.29), CP (990.03), ADF (973.18), NDF (2002.04), ADIN (973.18 and 976.06), NDIP (2002.04 without sulfite and 976.06), lignin (973.18), ash (942.05), Ca (985.01), P (985.01), Mg (985.01), Na (985.01), Cl (915.01), S (923.01), Fe (985.01), Cu (985.01), Zn (985.01), K (985.01), Mn (985.01), and pH (981.12). The remaining nutrient parameters were measured using the following methods: soluble protein (SP); (Krishnamoorthy et al., 1982), starch (Glucose Reagent Set, AMRESCO, Solon, OH and ALPKEM Corporation, 1990), oil (Damon, 1966), in vitro dry matter digestibility (IVDMD); (24 h ruminal and 24 h enzymatic digestion using the Kansas State Buffer (Marten and Barnes, 1980)), neutral detergent fiber digestibility (NDFD); (Van Soest et al., 1991, incubated for 30 h using the Kansas State Buffer (Marten and Barnes, 1980)), NH_3-N (United States Environmental Protection Agency, 1993, method 351.2 and International Organization for Standardization, 2013, method 11732), lactic acid (El Rassi, 1996), acetic acid (Cancalon, 1993), nonfiber carbohydrate

(NFC); (National Research Council, 2001), net energy for lactation (NEl); (National Research Council, 2001), relative forage quality (RFQ); (Rohweder et al., 1978), and sugar (Analab, Fulton, IL defined method, in process of entering a Single Laboratory Validation from the Association of American Feed Control Officials).

Milk samples were analyzed for concentrations of fat, true protein, SNF, and lactose via infrared absorbencies (B-2000 Infrared Analyzer; Bentley Instruments, Chaska, MN). Milk urea nitrogen was quantified using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments, Chaska, MN) and somatic cells were counted using dual laser flow cytometry (Somacount 500, Bentley Instruments, Chaska, MN). All milk analysis were completed using Association of Official Analytical Chemists International (2002) approved procedures. Energy-corrected milk yield was calculated as follows: 0.327 x milk yield + 12.95 x fat yield + 7.2 x protein yield. Solids-corrected milk production was calculated as: 12.3 x fat yield + 6.56 x SNF yield + 0.0752 x milk yield. Fat corrected milk was calculated as: 0.4 x milk yield + 15 x milk fat yield.

Rumen fluid samples were initially analyzed for pH immediately after collection, via esophageal tubing, using an electronic pH meter (Corning 350, Corning Inc., Corning, NY). The first 100 mL of rumen fluid was discarded to minimize saliva contamination. If the rumen fluid collected was at a pH > 7.0, rumen fluid was discarded and additional rumen fluid was collected to ensure minimal saliva contamination. Two 10-mL samples of rumen fluid were collected, where one 10-mL sample was added to a vial containing 200 µl of 50% (vol/vol) H_2SO_4 for later determination of NH_3-N and the other 10-mL

sample was added to a vial containing 2 mL of 25% (wt/vol) meta-phosphoric acid for later determination of VFA. After sample collection and preparation, rumen fluid samples were immediately stored at -20˚C. Rumen fluid samples were later thawed and centrifuged at 30,000 × g for 20 min at 20˚C (Eppendorf 5403, Eppendorf North America, Hauppauge, NY). Rumen fluid samples acidified with 50% (vol/vol) H_2SO_4 were analyzed for NH_3-N using procedures from Chaney and Marbach (1962). Ruminal fluid samples acidified with 25% (wt/vol) meta-phosphoric acid were prepared according to Erwin et al. (1961) and analyzed for VFA concentrations using an automated gas-liquid chromatograph (6890, Hewlett-Packard, Palo Alto, CA) with a flame-ionization detector. Once prepared, 1 µl of prepared sample was injected at a split ratio of 30:1 at the injection port (250°C). Volatile fatty acids were separated on a capillary column (15 m × 0.25 mm i.d.; Nukol, 17926–01C, Supelco Inc., Bellefonte, PA) with flow-rate of 30 mL/min of He using 2-ethylbutyrate as an internal standard. The column and detector temperature were maintained at 140°C and 250°C, respectively.

Blood plasma was analyzed for bloodurea nitrogen (BUN); (Point Scientific BUN UV Reagent Set; Point Scientific, Inc., Canton, MI).

Composited samples of individual fecal samples were shipped frozen in insulated shippers to Analab Laboratory (Fulton, IL) for analysis. Samples were analyzed using the following Association of Official Analytical Chemists International (1998) methods: DM (935.29), CP (990.03), ADF (973.18), NDF (2002.04), ADIN (973.18 and 976.06), NDIP (2002.04 without sulfite and 976.06), lignin (973.18), ash (942.05), Ca (985.01), P

(985.01), Mg (985.01), Na (985.01), Cl (915.01), S (923.01), Fe (985.01), Cu (985.01), Zn (985.01), K (985.01), and Mn (985.01).

Starch was measured using the following method: starch (Glucose Reagent Set, AMRESCO, Solon, OH and ALPKEM Corporation, 1990). An identical fecal sample was thawed and washed through a digestion analyzer (Nasco's Digestion Analyzer, Nasco, Fort Atkinson, WI) by cow. Residue from each screen was collected, dried, and dry sieved (grain sieves) to determine micron particle size and distribution.

Statistical Analysis

Data were analyzed as a randomized complete block design using PROC MIXED procedure of SAS (Version 9.4, SAS Institute, Inc., Cary, NC). Feed intake, milk production, milk composition, milk component yield, BW and BCS data were analyzed with week, treatment, parity, covariate and the interactions of treatment and week as fixed effects. Random effects included cow. Significance was declared at $P \leq 0.05$ and trends declared at $0.05 \leq P \leq 0.10$.

Results

Ingredient composition of diets offered are given in Table 2.1. Chemical composition of the diets are found in Table 2.2. CONTROL and TEST diets contained similar amounts of forages, but differed in source and amount of cobalt supplement. Post analysis of the TMR shows that diets were formulated and met formulation expectations.

Milk production, milk components, milk component production, FCM, ECM, and SCM did not differ ($P > 0.05$) between treatments (Table 2.3), which is in agreement with Akins et al. (2013), Kincaid and Socha (2007), and Campbell (1999). Milk production (26.2 and 25.8 kg/d for CONTROL and TEST, respectively throughout results) was similar ($P = 0.72$). Dry matter intakes (22.9 and 23.1 kg/d) were similar ($P = 0.81$) (Table 2). Yield of milk fat (1.02 and 1.09 kg/d), milk protein (0.87 and 0.91 kg/d) and lactose (1.17 and 1.26 kg/d) were similar ($P = 0.33$, P = 0.44, and $P = 0.34$), respectively for CONTROL and TEST. Body weights and body condition score were unaffected by treatment ($P > 0.05$).

Effects of treatments on rumen fluid samples can be found in Table 2.5. Rumen ammonia concentrations were lower ($P = 0.03$) for the TEST (12.3 mg/dL) as compared to the CONTROL (15.8 mg/dL) which could be explained by an increase in microbial protein synthesis. Ruminal percentage of acetate were significantly lower ($P = 0.04$) for the CONTROL (59.47%) as compared to the TEST (61.07%) which can explain the numerically higher fiber digestibility coefficients.

Effects of treatment on digestibility can be found in Table 2.6. When evaluating DM, CP, NDF, ADF, and starch digestibility percentage, there were no differences between the two treatments ($P > 0.05$). However, a numeric advantage in fiber digestion was observed, further describing the increase in acetate percentage.

Conclusions

Feeding additional Co as cobalt-lactate did not influence milk production, milk composition, dry matter intake or body weight for lactating dairy cows fed a high forage ration. Feeding Co decreased ruminal ammonia concentrations which could indicate an increase in ruminal microbial protein synthesis and growth although we did not measure that specific characteristic. Feeding Co increased ruminal concentrations of acetate which would suggest increased fiber digestion.

The evaluation of Co in early lactation dairy cows is warranted to determine if enhancements in microbial protein synthesis (NH_3) and fiber digestion (acetate) are beneficial. Additional research in this area would include a titration study, *in vitro*, to determine the optimal Co levels for ruminal digestion. The lack of response of cows to supplemental Co was likely due to the elongated DIM of the study cows and a lower than expected quality of alfalfa forage.

Table 2.1 Ingredient and nutrient composition of diets[1,2]

	CONTROL[3]	TEST[4]
Ingredient, % of DM		
Alfalfa baleage	43.0	43.0
Corn silage	28.0	28.0
Corn, finely ground	22.0	22.0
Corn distillers dried grains	3.4	3.4
Soybean meal, 48%	0.08	0.08
Urea 281 CP	0.04	0.04
Magnesium oxide	0.006	0.006
Sodium bicarbonate	0.04	0.04
Calcium phosphate dical	0.04	0.04
Sodium phosphate mono H_20	0.004	0.004
Dynamate	0.04	0.04
Salt	0.006	0.006
Selenium yeast	0.002	0.002
Vitamin premix E, 44,000 IU/kg	0.01	0.01
Vitamin premix ADE[5]	0.03	0.03
Salt trace mineral	0.005	0.005
Cobalt lactate	0.0	0.02
Totals	52.34	52.34
Nutrient, % of DM		
DM, % as-fed	62.38	62.38
CP	17.32	17.32
ADF	19.66	19.66
NDF	28.14	28.14
Ether extract	3.40	3.40

[1]The TMR had a forage-to-concentrate ratio of 70:30 (dry matter basis) with the forage ratio containing 40% corn silage and 60% alfalfa haylage.
[2]The TMR ration was formulated using AMTS (Agricultural Modeling and Training Systems, Groton, NY).
[3]CONTROL=no additional supplementation of cobalt-lactate
[4]TEST= 50 mg/hd/d of cobalt(Cobalt-lactate)
[5]3,306,000 IU/kg vitamin A, 1,102,000 IU/kg vitamin D, and 1,100 IU/kg vitamin E.

Table 2.2 Nutrient chemical analysis by treatment[1]

	CONTROL	TEST
Nutrient, % of DM		
DM, % as-fed	61.64	62.97
CP	17.86	17.76
ADF	21.73	21.40
NDF	34.61	34.17
Cobalt, ppm in grain mix	<1.50	8.88
NE_L, Mcal/kg[2]	1.55	1.55

[1]CONTROL = no additional supplementation of cobalt-lactate; TEST = 50 mg/hd/d of cobalt.

[2]Estimated according to NRC (2001).

Table 2.3 Effects of treatment[1] on performance of lactating cows

	CONTROL	TEST	SEM	*P*
DMI, kg/d	22.9	23.1	0.87	0.81
Milk, kg/d	26.2	25.8	1.19	0.72
FCM[2]	25.2	27.1	1.75	0.27
ECM[3]	27.5	29.5	1.82	0.30
SCM[4]	29.1	31.2	2.00	0.30

[1]CONTROL = no additional supplementation of cobalt-lactate; TEST = 50 mg/HD/d of cobalt.
[2]Fat Corrected Milk = (0.4 x kg of milk) + (15 x kg of milk fat).
[3]Energy Corrected Milk = (0.327 x kg of milk) + (12.95 x kg of milk fat) + (7.2 x kg of milk protein).
[4]Solid Corrected Milk = (0.0752 x kg of milk) + (12.3 x kg of milk fat) + (6.56 x kg of SNF).

Table 2.4 Effects of treatment[1] on milk components

	CONTROL	TEST	SEM	*P*
kg/d				
Fat	1.02	1.09	0.08	0.33
Protein	0.87	0.91	0.05	0.44
Lactose	1.17	1.26	0.10	0.34
SNF[2]	2.25	2.40	0.16	0.36
%				
Fat	4.14	4.13	0.25	0.99
Protein	3.53	3.40	0.11	0.22
Lactose	4.68	4.71	0.07	0.66
SNF[2]	9.08	8.97	0.11	0.35
Other measures				
SCC[3] x 1,000, cells, mL	444	488	419	0.91
MUN[4], mg/dL	11.9	11.6	0.49	0.56
Body weight, kg	684	674	13.73	0.11
Body weight change, kg	11.23	-0.11	9.49	0.25
Body condition score	3.27	3.31	0.05	0.43

[1]CONTROL = no additional supplementation of cobalt-lactate; TEST = 50 mg/hd/d of cobalt.
[2]Solids Not Fat.
[3]Somatic cell count
[4]Milk urea nitrogen

Table 2.5 Effects of treatment[1] on rumen fluid

	CONTROL	TEST	SEM	*P*
pH	6.830	6.836	0.87	0.95
NH_3-N, mg/dL	15.8	12.3	1.51	0.03
Acetate, %	59.47	61.07	0.39	0.04
Propionate, %	22.15	21.13	0.48	0.22
Butyrate, %	13.27	12.86	0.25	0.16
Acetate:Propionate	2.73	2.92	0.13	0.17

[1]CONTROL = no additional supplementation of cobalt-lactate; TEST = 50 mg/HD/d of cobalt.

Table 2.6 Effects of treatments[1] on digestibility

	CONTROL	TEST	SEM	Diet
Dry matter, %	53.7	56.8	3.49	0.24
Crude protein, %	63.8	64.0	2.77	0.92
NDF[2], %	46.3	48.9	4.06	0.43
ADF[3], %	39.8	42.9	4.61	0.41
Starch, %	97.6	97.1	0.46	0.96

[1]CONTROL= no additional supplementation of cobalt-lactate; TEST = 50 mg/hd/d of cobalt.
[2]Neutral detergent fiber
[3]Acid detergent fiber

Table 2.7 Effects of treatment[1] on fecal particle size

	CONTROL	TEST	SEM	*P*
Sieve Number				
6, % on screen	19.9	20.6	0.02	0.72
18, % on screen	48.3	48.4	0.02	0.97
20, % on screen	10.2	10.3	0.00	0.79
25, % on screen	5.9	6.2	0.00	0.35
30, % on screen	5.9	5.9	0.00	0.88
40, % on screen	5.9	5.2	0.00	0.09
Bottom, % on screen	3.9	3.5	0.00	0.35
MPS[2], micron	1449	1481	33.1	0.35

[1]CONTROL = no additional supplementation of cobalt-lactate; TEST = 50 mg/hd/d of cobalt-lactate.
[2]Mean particle size

CHAPTER 3:

LACTATIONAL PERFORMANCE AND RUMINAL CHARACTERISTICS WHEN MID-LACTATION DAIRY COWS ARE FED SACCHAROMYCES CEREVISIAE FERMENTATION PRODUCTS

Abstract

This study evaluated *Saccharomyces cerevisiae* fermentation products (SCFP) (Diamond V original XPC and two prototypes) on lactational performance and ruminal fermentation. Eight ruminally cannulated (132 DIM and 34.4 kg/d milk) Holstein dairy cows (2 primiparous and 8 multiparous), were blocked by milk yield, DIM and parity and randomly assigned to treatments using a replicated 4 x 4 Latin square design. Treatments were: 1) CONTROL (CONTROL): corn silage and haylage based ration; 2) XPC: CONTROL ration with 14 g/hd/d Original XPC; 3) Prototype 1 (P1): CONTROL ration with 5 g/hd/d P1; and 4) Prototype 2 (P2): CONTROL ration with 19 g/hd/d P2. The SCFP were mixed with dried distillers grains and then mixed in the TMR at 454 g/hd/d. The experimental periods were 28 d with the first 21 d for dietary adjustment followed by 7 d of data collection. Milk yield (3x/d) was recorded daily and milk samples were collected at each milking (2 d) during wk 4. On d 25 or 27, rumens were evacuated, weighed, markers added (CoEDTA & valeric acid), mixed, the rumen-omasal orifice was blocked using a sponge, and rumen contents returned to the rumen. Ruminal samples were collected for 4 h at 20 min intervals to determine ruminal pH, ammonia, and VFA concentrations. After 4 h of sample collection, rumen contents were re-evacuated, re-

weighed, rumen-omasal sponge removed, and rumen contents returned. One cow died from causes unrelated to study objectives and her data was removed. Milk yield (30.7, 32.3, 32.0, 31.3 kg/d for CONTROL, XPC, P1, and P2, respectively) and intake of DM [(DMI); 24.5, 23.6, 23.6 and 25.3 kg/d, respectively] were similar ($P > 0.10$) for cows fed all treatments, but feed efficiency (1.26, 1.36, 1.36 and 1.24 kg/kg milk/DMI, respectively) and energy-corrected milk kg/DMI (1.42, 1.54, 1.52, and 1.38 kg/kg, respectively) were greater ($P < 0.01$) for cows fed XPC and P1 compared to cows fed CONTROL and P2. Milk composition was similar ($P > 0.10$) for cows fed all rations. Ruminal pH (6.06, 6.07, 6.02 and 6.13, respectively) was greater ($P < 0.05$) for cows fed P2 compared to cows fed other treatments. Rumen concentration and percentage of propionate and iso-butyrate were increased ($P < 0.05$) for cows fed P2 when compared to CONTROL with cows fed other treatments being intermediate and similar. The feeding of a dairy ration with P2 SCFP can improve ruminal pH while increasing propionate and iso-butyrate concentrations and percentages.

Key words: dairy cattle, volatile fatty acids, *Saccharomyces cerevisiae* fermentation products

Introduction

Feed efficiency (FE) is one of several ways to improve the profitability and sustainability of the dairy operation. Feed/production efficiency is defined as the unit of milk produced per unit of dry matter intake. The energy content of the ration is the greatest factor affecting the FE of the lactating dairy cow (Casper and Mertens, 2007). The greatest factor affecting the energy density of the diet is the digestibility of the ration (Casper and Mertens, 2007).

Saccharomyces cerevisiae fermentation products (SCFP) have been shown in previous studies to increase ration digestibility and FE of lactating dairy cows (Poppy et al., 2012). These increases in FE include increased milk yield, 3.5% FCM, ECM, milk fat yield and milk protein yield, while increasing DMI for early lactation cows (< 70 DIM) and decreasing DMI for post-peak lactation cows (> 70 DIM), while making them more efficient (Poppy et al., 2012).

Antioxidants have been shown to have some benefit in the ration of lactating dairy cows; however, the response has been small and inconsistent across studies (Poppy et al., 2012). Recently, a new prototype of SCFP with enhanced antioxidant activity has been created. Nutritionists, veterinarians, and dairy farmers need to identify the efficacy of these products in order to make sound decisions for their use in their production and management systems.

Past research has developed techniques to measure ruminal VFA absorption over time by dosing supraphysiological levels of valeric acid into the rumen and analyzing rumen VFA concentrations at numerous time points (Allen et al., 2000). Utilizing this

method along with dosing a metal chelate with EDTA can be beneficial for determining the fractional rate of absorption of VFA (acetate, propionate, and butyrate) across the rumen wall (Resende Júnior et al., 2006; Melo et al., 2013).

The purpose of this study was to evaluate a common SCFP in comparison to no supplementation and 2 novel SCFP on digesta volume, rumen pH, and VFA parameters in mid-lactation Holstein dairy cows. The hypothesis of this study was that SCPF supplementation in a TMR would affect rumen VFA production and their absorption.

Materials and Methods

The experimental cows were cared for according to the guidelines stipulated by South Dakota State University Institutional Animal Care and Use Committee and all procedures were approved by the committee prior to the start of the study. The health status of each animal was evaluated daily. Eight ruminally cannulated (132 DIM and 34.4 kg milk) Holstein dairy cows (2 primiparous and 6 multiparous), were blocked by milk yield, DIM and parity and randomly assigned to a replicated, 4 x 4 Latin square design. The trial included 4 periods with each period lasting 28 d. The first 21 d were for adjustment and adaptation to the experimental diet followed by 7 d of data collection.

Cows were housed in a free-stall facility at the South Dakota State University dairy research and training facility (DRTF) with free access to water, milked three times daily (0600, 1400, and 2100 h), and fed once daily (0700 h) for *ad libitum* intake using individual custom manufactured tubs located in front of each Calan (American Calan, Inc, Northwood, NH) door. Total daily feed offerings were adjusted based on previous

24-h intake so refusals were approximately 5%. Amounts fed and refused were recorded daily.

Treatments consisted of 4 diets (Table 3.1) fed as a TMR, composed from a common basal mix consisting of corn silage, alfalfa haylage, and finely ground corn. Treatments were as follows; 1) CONTROL (CONTORL) – Diet formulated to meet all nutrient requirements (NRC, 2001), with no inclusion of SCFP; 2) XPC (XPC) – the CONTROL diet plus the inclusion of 14 g/hd/d of SCFP (XPC, Diamond V, Cedar Rapids, IA, USA); 3) Prototype 1 (P1) – the CONTROL diet plus the inclusion of 5 g/hd/d of SCFP (Prototype 1, Diamond V, Cedar Rapids, IA, USA,); and 4) Prototype 2 (P2) – the CONTROL diet plus the inclusion of 19 g/hd/d of SCFP (Prototype 2, Diamond V, Cedar Rapids, IA, USA,). Supplemental SCFP were incorporated into dried distillers grains as a carrier and then mixed into individual treatment TMR at 454 g/hd/d. All diets were prepared and delivered with a Calan Data Ranger (American Calan, Inc, Northwood, NH). All diets were formulated using NDS Professional (Nutritional Dynamic System, Emilia, Italy), a Cornell net carbohydrate and protein system (CNCPS) based platform for ruminant diet formulation and evaluation to predict lactating dairy cow performance for a 628 kg Holstein cow producing 38.6 kg/d of milk with a 3.75 % fat and 3.36 % protein.

Data and Sample Collection and Analyses

Prior to the start of the experiment, samples of forages were analyzed and initial diets were formulated based on actual feed composition. Grain mixes for CONTROL

and test diets were then formulated and tested for nutrient content prior to the start of the feeding study. Samples of the basal mix and TMR were collected and frozen (-20°C), then composited by experimental period prior to analysis. Daily intake was calculated from feed offered and refused while recorded daily after being corrected for DM.

Total milk production was measured at each milking and recorded throughout the experiment (DeLaval-ALPRO, Kansas City, MO). Milk samples were collected (25 mL) 1 d during wk 3 and 4 at all milkings, throughout the experiment, preserved using 2-bromo-2-nitropropane-1,3 diol, stored at 4°C after collection and analyzed for fat, true protein, lactose, MUN, SNF and somatic cells within 72 h (Heart of America DHIA, Manhattan, KS 656502). Body condition scores (1 – 5 scale) were measured and recorded once each morning (1000 h) on 2 d during the final week of each period by 3 individuals(Wildman et al., 1982). Body weights were electronically collected using a livestock scale (AWB-5K-SYS, Triner Scale and Manufacturing Company, Inc., Olive Branch, MS) on 2 d during the final week of each period, approximately 3 h after feeding.

On d 25 or 27, rumens were evacuated, digesta weighed, markers added (CoEDTA & valeric acid), mixed, reticulum-omasal orifice was mechanically blocked using a 25- by 12-cm, 7-cm high, 45 g/cm^3 density synthetic sponge during the period of rumen sampling, and rumen contents returned to the rumen using the procedure outlined by Melo et al. (2013). Ruminal samples were collected through the cannula by a perforated tube coupled to a suction device for 4 h at 20 minute intervals to determine ruminal pH, ammonia, and VFA concentrations. Sampling times were: 0, 20, 40, 60, 80,

100, 120, 140, 260, 180, 200, 220, and 240 min after returning the evacuated rumen content. After 4 h of sample collection, rumen contents were re-evacuated, re-weighed, reticulum-omasal sponge removed, and rumen contents returned. Cows did not have access to feed and water during the rumen sampling period. One cow died from conditions unrelated to study objectives and her data was removed.

Dry matter composition of forages was determined weekly by drying in a 105˚C oven (Despatch LEBI-75, Despatch Industries, Minneapolis, MN) for 24 h. Samples of the grain mix, individual forages, and TMR were collected weekly for further analysis and stored at -20˚C. Period composited samples of individual feeds and TMRs were shipped frozen in insulated shippers to Analab Laboratory (Fulton, IL) for nutrient analysis. Samples were analyzed using the following Association of Official Analytical Chemists International (1998) methods: DM (935.29), CP (990.03), neutral detergent fiber (NDF); (2002.04), acid detergent fiber (ADF); (973.18), acid detergent insoluble nitrogen (ADIN); (973.18 and 976.06), neutral detergent insoluble protein (NDIP); (2002.04 without sulfite and 976.06), lignin (973.18), ash (942.05), Ca (985.01), P (985.01), Mg (985.01), Na (985.01), Cl (915.01), S (923.01), Fe (985.01), Cu (985.01), Zn (985.01), K (985.01), Mn (985.01), and pH (981.12). The remaining nutrient parameters were measured using the following methods: soluble protein (SP); (Krishnamoorthy et al., 1982), starch (Glucose Reagent Set, AMRESCO, Solon, OH and ALPKEM Corporation, 1990), oil (Damon, 1966), *in vitro* dry matter digestibility (IVDMD) (24 h ruminal and 24 h enzymatic digestion using the Kansas State Buffer; (Marten and Barnes, 1980), neutral detergent fiber digestibility (NDFD); (Van Soest et

al., 1991, incubated for 30 h using the Kansas State Buffer (Marten and Barnes, 1980), NH_3-N (United States Environmental Protection Agency, 1993, method 351.2 and International Organization for Standardization, 2013, method 11732), lactic acid (El Rassi, 1996), acetic acid (Cancalon, 1993), nonfiber carbohydrate (NFC) (National Research Council, 2001), net energy for lactation (NE_L) (National Research Council, 2001), relative forage quality (RFQ); (Rohweder et al., 1978), and sugar (Analab, Fulton, IL, defined method, in process of entering a Single Laboratory Validation from the Association of American Feed Control Officials).

Milk samples were sent to Dairy Herd Improvement Association Heart of America (Manhattan, KS) for analysis of fat, protein, somatic cell count (SCC), lactose, and MUN using Association of Official Analytical Chemists International (2002) approved procedures. Milk fat, protein, and lactose were analyzed using near infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Milk urea nitrogen concentrations were determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments, Chaska, MN). Somatic cell counts were determined using a flow cytometer laser (Somacount 500, Bentley Instruments, Chaska, MN). Fat-corrected milk (3.5%) was determined using the following equation: (0.432 × kg milk) + (16.216 × kg fat) and ECM was determined using the following equation: (0.327 × kg milk) + (12.95 × kg fat) + (7.65 × kg protein) as described by Orth (1992).

Rumen pH were determined immediately (Corning 350, Corning Inc., Corning, NY). Two 10-mL aliquots of every sample were obtained: 1 sample was immediately

frozen at -20°C containing 2 mL of 25% (wt/vol) meta-phosphoric acid for later determination of VFA/Co content and the other 0.2 mL of a 50% H_2SO_4 solution was added before freezing for rumen ammonia determination.

Rumen fluid samples were thawed and centrifuged at 30,000 × g for 20 min at 20˚C (Eppendorf 5403, Eppendorf North America, Hauppauge, NY). Rumen fluid samples acidified with 50% (vol/vol) H_2SO_4 were analyzed for NH_3-N using procedures from Chaney and Marbach (1962). Ruminal fluid samples acidified with 25% (wt/vol) meta-phosphoric acid were prepared according to Erwin et al. (1961) and analyzed for VFA concentrations using an automated gas-liquid chromatograph (model 6890, Hewlett-Packard) with a flame-ionization detector. Once prepared, 1 µl of prepared sample was injected at a split ratio of 30:1 at the injection port (250°C). Volatile fatty acids were separated on a capillary column (15 m × 0.25 mm i.d.; Nukol, 17926–01C, Supelco Inc., Bellefonte, PA) with flow-rate of 30 mL/min of He using 2-ethylbutyrate as an internal standard. The column and detector temperature were maintained at 140°C and 250°C, respectively. The supernatant was analyzed for rumen ammonia and VFA content by plate reader and GC, respectively. The content of Co was determined by atomic absorption spectroscopy (AAnalyst 200, Perkin Elmer, Waltham, Massachusetts, USA) on supernatant samples diluted 1:12 with distilled water.

The fractional rate of rumen valeric acid absorption by the rumen wall was estimated by using a first-order kinetic model describing the exponential decay of the ratio of ruminal valeric acid to Co concentration over time (*k* val/Co): $C_t = Ae^{-kt}$, where: C_t = val/Co at time t, A = val/Co at time 0, and *k* = the fractional decay rate of val/Co,

procedures from. The fractional rate of Co concentration variation over time (*k* Co) was determined similarly, aiming at determining digesta dilution by water inflow to the rumen.

Statistical Analysis

All data were subject to least squares analysis of variance for a replicated 4 x 4 Latin square design using the PROC MIXED procedure of SAS (Version 9.4, SAS Institute, Inc., Cary, NC) as a repeated measures ANOVA. Week 4 data was utilized for analysis of feed intake, milk production, milk composition, milk component yield, BW, rumen pH, rumen ammonia, and VFA data, with square, period, cow(period), treatment, and all possible interactions as fixed effects. Random effect included cow(square). Repeated effect included time. Significance was declared at $P \leq 0.05$, tendency at $0.05 < P \leq 0.10$.

Results and Discussion

Ingredient and chemical composition of diets offered are given in Table 3.1. CONTROL and TEST diets contained similar amounts of forage and concentrate, but differed in source and amount of SCFP supplement. Post analysis of the total mixed ration shows that diets met formulation expectations and were consistent during the study (Table 3.2).

Milk production, milk component production, and DMI did not differ ($P > 0.05$) between treatments (Table 3.3) which was in agreement with others (Arambel and Kent,

1990). Feed efficiency was significantly increased ($P < 0.05$) for cows fed XPC and P1 treatments due to numerically lower DMI and numerically higher milk yield, when compared to cows fed C and P2, which is in agreement with other research supplementing SCFP (Poppy et al., 2012).

Ruminal Volume, pH, and Ammonia

The pre- and post-digesta volume (Table 3.4) for the 28 evacuations made during the experiment were 76.0 and 70.4 L, respectively, which is higher than other research published in this area (Melo et al., 2013). The pre- and post-digesta fresh weight was 91.5 and 82.3 kg, respectively. Rumen digesta variables for all treatments are presented in Table 3.4. While utilizing a synthetic sponge during the sampling procedure, we were able to effectively block the reticulum-omasum orifice to minimize the loss of ruminal contents to the lower tract. Lower changes in fresh weight and volume were found for cows fed P2 when compared to the large changes for cows fed the CONTROL. Rumen pH was increased ($P < 0.05$) for cows fed P2 compared to cows fed CONTROL, XPC, or P1, which may result in increased rumen homeostasis (Table 3.5). Decreases ($P < 0.05$) in rumen ammonia concentrations were found for cows fed P2 and P1 when compared to cows fed C and XPC (Table 3.5), which may indicate an increase in microbial protein synthesis, although this parameter was not measured.

Ruminal VFA Absorption and Production

There was only a 0.2%/h decrease ($P > 0.10$) in Co levels throughout the sampling period, with no difference between treatments (Table 3.6). There was a difference in the ratio of valeric acid to cobalt EDTA marker ratio with higher levels being absorbed for cows fed P1 compared to lower levels absorbed for cows fed XPC and P2 treatment, and intermediate levels being absorbed for cows fed CONTROL (Table 3.6).

Effects of treatments on rumen fluid VFA production can be found in Table 3.7. Ruminal concentrations of acetate were reduced ($P < 0.05$) for cows fed P2 when compared to cows fed XPC with cows fed CONTROL and P1 being intermediate. Ruminal propionate concentrations were significantly highest for the P1 treatment, lowest for CONTROL and XPC with P2 being intermediate. There was an increase in iso-butyrate concentration for the P2 treatment when compared to CONTROL, XPC, and P1. This increase may have an effect on increased milk production in a large scale production study due to iso-butyrate stimulating growth hormone release (Hultquist and Casper, 2015). Butyrate concentrations were reduced for cows fed P2 when compared to a higher level for cows fed XPC with cows fed CONTROL and P1 being intermediate. Lower levels of iso-valerate were found when cows were fed P2 compared to cows fed XPC and P1 with cows fed CONTROL being intermediate. There were no differences ($P > 0.10$) in total VFA concentration between the 4 treatments.

Effects of treatments on rumen fluid VFA molar percentage can be found in Table 3.7. Ruminal percentage of acetate were lower for cows fed P1 when compared to cows

fed CONTROL and XPC, with cows fed P2 being intermediate. An increase in the molar propionate percentage was found for cows fed P1 and P2 with CONTROL and XPC fed cows being reduced. Iso-butyrate percentage was increased for cow fed P2 in comparison to cows fed CONRTOL, XPC, and P1. There was a decrease in acetate to propionate ratio for cows fed P1 and P2 due to decrease in acetate with an increase in propionate when compared to cows fed CONTROL and XPC.

Conclusions

Feeding SCFP did not influence milk production, milk composition, dry matter intake, or body weight in this study. However, this study primarily focused on ruminal characteristics as this was a mechanism study rather than a production study. Feeding SCFP in the form of P1 or P2 decreased ruminal ammonia concentrations, which could indicate an increase in ruminal microbial protein synthesis. Feeding SCFP in the form of P1 or P2 increased ruminal molar percentages of propionate, while reducing ruminal acetate, resulting in a reduction in the acetate to propionate ratio. Feeding P2 resulted in an increase in iso-butyrate percentage when compared to cows fed C, XPC, and P1, which may lead to increased milk production in a large scale production study due to the stimulation of growth hormone release via iso-butyrate. However, this was not measured in this study. The evaluation of SCFP in a large, mid lactation dairy cow production study is warranted to determine if enhancements in VFA concentration and percentage are beneficial on production parameters.

Table 3.1 Ingredient composition of diets[1]

	CONTROL	XPC	P1	P2
Ingredient, % of DM				
Corn silage	23.0	23.0	23.0	23.0
Alfalfa haylage	10.0	10.0	10.0	10.0
Corn, finely ground	7.95	7.95	7.95	7.95
Soybean meal, 47.5%	3.39	3.39	3.39	3.39
Corn distillers dried grains	2.98	2.95	2.97	2.94
Whole cottonseed, fuzzy	2.31	2.31	2.31	2.31
Soy Best PEARL	1.50	1.50	1.50	1.50
Soybean hulls, ground	0.587	0.587	0.587	0.587
Calcium carbonate	0.427	0.427	0.427	0.427
Energy booster	0.251	0.251	0.251	0.251
Sodium bicarbonate	0.251	0.251	0.251	0.251
Salt, white	0.210	0.210	0.210	0.210
Fat animal veg blend	0.210	0.210	0.210	0.210
Blood meal	0.168	0.168	0.168	0.168
Magnesium oxide	0.084	0.084	0.084	0.084
Trace mineral premix	0.084	0.084	0.084	0.084
Urea 281 CP	0.042	0.042	0.042	0.042
Vitamin premix E	0.025	0.025	0.025	0.025
Vitamin premix ADE	0.008	0.008	0.008	0.008
XPC, Diamond V	-	0.03	-	-
P1, Diamond V	-	-	0.011	-
P2, Diamond V	-	-	-	0.042

[1]CONTROL = no supplementation of SCFP; XPC = supplementation of 14 g/hd/d of original XPC SCFP; P1 = supplementation of 5 g/hd/d of prototype 1 SCFP; P2 = supplementation of 19 g/hd/d of prototype 2 SCFP.

Table 3.2 Nutrient composition (% of diet dry matter (DM) unless otherwise noted) of grain mix (**GM**), corn silage (**CS**), alfalfa haylage (**AH**), and total mixed ration (TMR).

	Feed ingredient			
Nutrient	GM	CS	AH	TMR[1]
DM, %	87.9	41.3	47.2	52.5
CP	23.6	7.36	26.9	18.0
SP[2], % CP	24.3	54.5	67.9	44.3
NDF	15.9	38.7	33.4	29.4
ADF	9.7	23.4	25.9	19.0
ADIN	----	0.27	1.14	0.55
NDIP[3]	2.6	0.52	1.86	1.34
NFC	48.3	48.0	30.5	43.6
Starch	30.9	34.3	----	26.0
NE_L, Mcal/kg	----	1.65	1.61	1.77
Oil	7.16	2.69	2.47	4.07
IVDMD[4]	----	70.5	78.2	82.6
NDFD[5], % NDF	----	46.3	63.0	58.6
Lignin	----	2.29	5.68	3.48
Ash	7.58	3.82	8.62	6.23
NH_3-N, ppm	----	1,080	4,162	----
Ca	1.15	0.18	1.64	0.79
P	0.44	0.20	0.38	0.33
Mg	0.45	0.17	0.36	0.29
K	1.07	0.77	2.82	1.45
Na	0.80	0.03	0.08	0.31
Cl	0.61	0.16	0.68	0.53
S	0.25	0.05	0.27	0.21
Fe, ppm	234	67	258	205
Cu, ppm	40	2.75	8.25	20.5
Zn, ppm	203	25	37.8	99
Mn, ppm	189	32	48.0	100
pH, 0-14	----	3.88	4.95	----
Lactic Acid	----	5.36	4.44	----
Acetic Acid	----	1.70	0.66	----

[1]The nutrient composition of the TMR was an average of the TMR for each treatment.
[2]SP = Soluble protein.
[3]DIP = Neutral detergent insoluble protein.
[4]IVDMD = *In vitro* dry matter digestibility.
[5]NDFD = Neutral detergent fiber digestibility, 30 h.

Table 3.3 Effects of treatment[1] on production performance

	CONTROL	XPC	P1	P2
DMI, kg/d	24.5	23.6	23.6	25.3
Milk, kg/d	30.7	32.3	32.0	31.3
Feed efficiency, kg	1.26[b]	1.36[a]	1.36[a]	1.24[b]

[ab]Means in same row with different superscripts differ significantly for treatment effect.
[1]CONTROL = no supplementation of SCFP; XPC = supplementation of 14 g/hd/d of original XPC SCFP; P1 = supplementation of 5 g/hd/d of prototype 1 SCFP; P2 = supplementation of 19 g/hd/d of prototype 2 SCFP.

Table 3.4 Effects of treatment[1] on rumen digesta parameters

Item	CONTROL	XPC	P1	P2	SEM
Returned					
Fresh weight, kg	91.5	90.2	92.9	91.2	4.07
Dry matter, % of fresh	17.0	16.9	17.3	15.9	0.71
Dry matter, kg	15.5	15.3	16.0	14.4	0.96
Volume, L	75.5	74.9	76.9	76.8	3.43
After sampling					
Fresh weight, kg	79.4	79.2	82.8	87.8	4.63
Dry matter, % of fresh	14.8	14.2	14.8	13.9	0.67
Dry matter, kg	11.7	11.4	12.4	12.0	0.91
Volume, L	67.7[b]	67.8[b]	70.4[ab]	75.8[a]	5.01
Fresh weight change, kg	11.6[a]	11.0[ab]	9.8[ab]	3.0[b]	3.10
Volume change, L	7.8[a]	7.1[a]	6.1[ab]	0.5[b]	0.95

[ab] Means in same row with different superscripts differ significantly for treatment effect.

[1]CONTROL = no supplementation of SCFP; XPC = supplementation of 14 g/hd/d of original XPC SCFP; P1 = supplementation of 5 g/hd/d of prototype 1 SCFP; P2 = supplementation of 19 g/hd/d of prototype 2 SCFP.

Table 3.5 Effects of treatment[1] on rumen fluid pH and ammonia concentration

	CONTROL	XPC	P1	P2	SEM
pH	6.06[b]	6.07[b]	6.02[b]	6.13[a]	0.09
NH_3-N, mg/dL	15.24[a]	15.23[a]	13.07[b]	12.34[b]	0.56

[ab] Means in same row with different superscripts differ significantly for treatment effect.
[1]CONTROL = no supplementation of SCFP; XPC = supplementation of 14 g/hd/d of original XPC SCFP; P1 = supplementation of 5 g/hd/d of prototype 1 SCFP; P2 = supplementation of 19 g/hd/d of prototype 2 SCFP.

Table 3.6 Absorption coefficients of rumen marker by treatment[1]

	CONTROL	XPC	P1	P2	SEM
k Co, % h^{-1}	-0.20	-0.26	-0.22	-0.22	0.09
k Val/Co, % h^{-1}	14.41[ab]	13.74[b]	16.06[a]	13.87[b]	0.95

[ab] Means in same row with different superscripts differ significantly for treatment effect.
[1]CONTROL = no supplementation of SCFP; XPC = supplementation of 14 g/hd/d of original XPC SCFP; P1 = supplementation of 5 g/hd/d of prototype 1 SCFP; P2 = supplementation of 19 g/hd/d of prototype 2 SCFP.

Table 3.7 Effects of treatments[1] on VFA concentration and percentage

	CONTROL	XPC	P1	P2	SEM
Acetate, mM	54.59[ab]	56.70[a]	55.27[ab]	52.73[b]	2.64
Propionate, mM	23.80[b]	23.60[b]	27.67[a]	25.32[ab]	2.78
Iso-butyrate, mM	1.39[b]	1.40[b]	1.45[b]	1.56[a]	0.09
Butyrate, mM	9.81[b]	10.40[a]	9.47[b]	8.96[c]	0.39
Iso-valerate, mM	2.56[b]	2.81[a]	2.78[a]	2.30[c]	0.13
Total VFA[2], mM	92.13	94.91	96.61	90.85	7.00
Acetate, molar %	59.01[a]	59.46[a]	57.52[c]	58.61[b]	0.89
Propionate, molar %	25.71[c]	24.63[d]	27.87[a]	27.10[b]	1.13
Iso-butyrate, molar %	1.55[b]	1.54[b]	1.55[b]	1.71[a]	0.03
Butyrate, molar %	10.84[b]	11.26[a]	10.11[c]	10.00[c]	0.28
Iso-valerate, molar %	2.90[b]	3.12[a]	2.97[b]	2.60[c]	0.12
Acetate:propionate	2.30[b]	2.41[a]	2.06[c]	2.16[c]	1.41

[ab] Means in same row with different superscripts differ significantly for treatment effect.
[1]CONTROL = no supplementation of SCFP; XPC = supplementation of 14 g/hd/d of original XPC SCFP; P1 = supplementation of 5 g/hd/d of prototype 1 SCFP; P2 = supplementation of 19 g/hd/d of prototype 2 SCFP.
[2]Does not include valerate.

CHAPTER 4:

PRODUCTION OF HIGH QUALITY AND DIGESTIBLE FORAGES TO INCREASE MILK PRODUCTION AND NUTRIENT SUPPLY WHILE REDUCING FEED COSTS FOR LACTATING DAIRY COWS

Abstract

This study evaluated 2 forage production programs with subsequent feeding to evaluate the lactational performance of Holstein dairy cows. Thirty peak-lactation (58 DIM ± 2.9 and 38.9 kg/d milk ± 7.6) Holstein dairy cows (8 primiparous and 22 multiparous), were blocked by milk yield, DIM, and parity and randomly assigned to 1 of 2 treatments using a randomized complete block design. Treatments were: 1) CONTROL: normal forages (65%) ration formulated using alfalfa haylage and corn silage produced via standard soil and agronomy programs; 2) TEST: high forage level (65%) ration formulated using alfalfa haylage and corn silage produced on an enhanced soil (base saturtions) and agronomy program (foliar applications). Cows were fed the CONTROL ration during the covariate period of 7 d followed by 12 weeks of data collection when CONTROL and TEST diets were fed. Milk production was increased (P = 0.04) for cows fed TEST compared to cows fed control forage (32.6 and 36.9 kg/d for CONTROL and TEST, respectively throughout results). Dry matter intakes (23.9 and 22.8 kg/d) were similar (P = 0.46). Milk fat yields (1.18 and 1.27 kg/d) were similar for cows fed both forage programs (P = 0.21) but milk protein (0.98 and 1.09 kg/d; P = 0.037), lactose (1.62 and 1.88 kg/d; P = 0.032), and total solids (3.77 and 4.25 kg/d; P = 0.045) yields were

increased for cows fed TEST forages compared to cows fed CONTROL forages. Milk urea nitrogen (14.42 and 14.93 %; P = 0.37) and somatic cell score (3.98 and 3.71; P = 0.63), were similar between treatments. Fat-corrected milk (4%) tended (P = 0.09) to be higher (33.6 and 39.0 kg/d) for cows fed the TEST forages compared to cows fed the CONTROL forage. Energy corrected milk was increased (P = 0.05) for the TEST fed cows (33.0 and 36.8 kg/d). Body weights (630 and 664 kg) were similar (P = 0.14). Rumen ammonia concentrations (18.4 and 19.1 mg/dL) were similar (P = 0.63). A decrease (P = 0.004) in ruminal butyrate percentage was found for cows fed the TEST diet. Ruminal propionate concentration (P = 0.10) and percentage (P = 0.10) tended to increase when cows were fed TEST forages compared to cows fed CONTROL forages. There was a trend (P = 0.06) for an increase in starch digestibility for cows fed TEST forage compared to CONTROL fed cows (97.9 and 98.4 % digestible). Digestibility of NDF (48.5 and 54.7 % digestible, P = 0.03) and ADF (48.3 and 54.4 % digestible, P = 0.02) were increased for the TEST fed cows compared to cows fed CONTROL forages. Feeding higher quality forages obtained from enhanced agronomy procedures increased milk production, milk composition, and fiber digestibility when lactating dairy cows are fed a high-forage ration.

Key words: high-forage diet, forage quality, dairy cattle

Introduction

Costs of grain and various feed ingredients have fluctuated greatly in recent years. In addition, the availability of certain commodities are scarce in certain parts of the country. The result is that rations fed to livestock and in particular, lactating dairy cows, have risen dramatically in cost. Often times, the cost to produce a hundred kilograms of milk is below the milk price and therefore, the profitability of the dairy industry is negative and producers are again losing equity. In the past, commodities and/or by-products have been used to reduce ration costs and improve profitability of the dairy operation. However, even these commodities are increasing in cost due to value and availability relative to corn and soybean meal. New ways must be found to reduce feed costs to regain profitability and sustainability of the dairy industry to compete on a world market.

Dairy cattle are biologically designed to convert forages and other fibrous feeds into high quality products such as milk and meat. The predominant foundation behind rations for dairy cows are to provide a highly fermentable diet that supports high intakes and promotes consistent rumen fermentation. In an era of high priced concentrate feedstuffs, producers and nutritionists continue to seek ways to reduce feed costs. The utilization of high-forage and lower-starch diets is one option to reduce costs.

During periods of high corn prices, it has become increasing popular to feed at least 60%, and potentially 70%, of ration DM in the form of highly digestible forages. Typically, these diets are made up primarily of corn silage with the addition of alfalfa haylage. Through increased management practices, producers have improved their ability to grow and store larger quantities of consistent high-quality, highly digestible

forages. The evaluation of NDF digestibility has helped nutritionists more effectively formulate high forage diets. A common question when feeding high-forage diets to high producing cows is whether productivity can be maintained when compared to the more common lower forage diets. Controlled research studies and field experiences have concluded it is possible to maintain production when utilizing high forage diets as long as consistent, high-quality, highly digestible forages are fed (Chase, 2011). Research has shown herds producing over 36 kg of milk fed rations containing more than 70% of the total ration DM as forage (Chase, 2011). High forage diets are beneficial in numerous ways including reduced feed costs, increased cow health, rumen homeostasis, and improved nutrient management (Chase, 2011). A couple of challenges with high forage diets include increased forage inventories and frequent monitoring of feedstuffs and rations. The quality and quantity of forages fed to the dairy herd are directly related to milk production, feed costs, nutrient balance, and farm profitability.

Feed efficiency is one way to improve the profitability and sustainability of the dairy operation. Feed efficiency is defined as the unit of milk produced per unit of dry matter intake. The energy content of the ration is the greatest factor affecting the feed efficiency of the lactating dairy cow (Casper and Mertens, 2007). The greatest factor affecting the energy density of the diet are the digestibilities of the forages in the ration (Casper and Mertens, 2007). Forages are the cheapest source of nutrients on the farm when compared to grains, proteins, and various commodities sources. Therefore, increasing forage nutrient availability will increase their economic value relative to other commodities or by-products. The use of highly digestible forages may allow one to increase the amount used in the ration to meet the nutrient requirements of high

producing dairy cows. In addition, meeting the nutrient requirements of dairy cows in later lactation may also be advantageous in order to reduce feed cost to improve profitability.

The purpose of this study was to evaluate the lactational performance of dairy cows when fed forages produced via standard soil and agronomy program compared to an enhanced soil (base saturations) and agronomy management (foliar applications) program in the productions of forages for formulating rations and feeding lactating dairy cows.

Materials and Methods

This research trial was conducted at the South Dakota State University Dairy Research and Training Facility (DRTF); (Brookings, SD) and all procedures were approved by the SDSU Institutional Animal Care and Use Committee prior to the start of the study. Thirty peak-lactation (58 DIM ± 2.9 and 38.9 kg milk ± 7.6) Holstein dairy cows (8 primiparous and 22 multiparous), were blocked by milk yield, DIM, and parity and randomly assigned to 1 of 2 treatments in a randomized complete block design. The trial was 13 wk long with the first 7 d for diet adaptation and adjustment followed by 84 d of data collection.

Cows were housed in a free-stall facility at the DRTF with free access to water, milked 3 times daily (0600, 1400, and 2100 h), and fed once daily (0700 h) for *ad libitum* intake through individual mangers located in front of each Calan door. Total daily feed offerings were adjusted based on previous 24-h intake so refusals were approximately 5%. Amounts fed and refused were recorded daily. The health status of each animal

was evaluated daily and all other bedding, cow monitoring, and manure scraping followed normal DRTF procedures

Treatments were: 1) CONTROL: normal forages (65%) ration formulated using alfalfa haylage and corn silage produced via standard university soil and agronomy programs; 2) TEST: high forage level (65%) ration formulated using alfalfa haylage and corn silage produced on an enhanced soil (base saturations) and agronomy program (foliar applications) (Ag Spectrum, De Witt, IA). The grain mix was similar among both treatments and was mixed at the SDSU Feed Mill and delivered to the DRTF approximately every 2 wk. Cows were fed the CONTROL ration during the 7 d covariate period followed by 12 weeks of data collection when CONTROL and TEST diets were fed. All diets were prepared and delivered with a Calan Data Ranger (American Calan, Inc., Northwood, NH). All diets were formulated using NDS Professional (Nutritional Dynamic System, Emilia, Italy), a CNCPS based platform for ruminant diet formulation and evaluation to predict lactating dairy cow performance for a 616-kg Holstein cow producing 38.6 kg/d of milk, 3.75 % fat, and 3.36 % protein.

Data and Sample Collection

Prior to the start of the experiment, samples of forages were analyzed and initial diets were formulated based on actual feed composition. Dry matter composition of forages was determined weekly by drying in a 105˚C oven (Despatch LEBI-75, Despatch Industries, Minneapolis, MN) for 24 h and feed sheets adjusted accordingly. Samples of the grain mix, individual forages, and TMR were collected and frozen (-20°C) weekly for

future analysis. Daily intake was calculated from feed offered and refused and recorded daily after being corrected for DM.

Milk production was recorded electronically (DeLaval-ALPRO, Kansas City, MO) at each individual milking and saved daily to a Universal Serial Bus flash drive. Two milk samples were collected at all milkings each wk for each individual cow. One set of milk samples were composited by day on a weighted basis proportional to milk production and frozen for potential future analysis at -20˚C. The other set of individual milk samples were sent to Dairy Herd Improvement Association Heart of America (Manhattan, KS) for analysis of fat, protein, somatic cell count (SCC), lactose, and MUN using Association of Official Analytical Chemists International (2002) approved procedures. Milk fat, protein, and lactose were analyzed using near infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Milk urea nitrogen concentrations were determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments, Chaska, MN). Somatic cell counts were determined using a flow cytometer laser (Somacount 500, Bentley Instruments, Chaska, MN). Somatic cell counts were converted to a linear somatic cell score (SCS) using the following equation: [(ln(SCC/100))/0.693147] + 3, as described by Schroeder (2012). Fat-corrected milk (3.5%) was determined using the following equation: (0.432 × kg milk) + (16.216 × kg fat) and ECM was determined using the following equation: (0.327 × kg milk) + (12.95 × kg fat) + (7.65 × kg protein) as described by Orth (1992).

Rumen fluid samples were collected on Thursday of wk 4, 8, and 12 at 3 h after feeding via esophageal tube attached to a hand-operated pump. The first 100 mL of

rumen fluid was discarded to minimize saliva contamination. After collection, rumen fluid was mixed thoroughly and pH was measured immediately using an electronic pH meter (Corning 350, Corning Inc., Corning, NY). If the rumen fluid collected was at a pH > 7.0, rumen fluid was discarded and additional rumen fluid was collected to ensure minimal saliva contamination. Two 10-mL samples of rumen fluid were collected, where one 10-mL sample was added to a vial containing 200 µl of 50% (vol/vol) H_2SO_4 for later determination of NH_3-N and the other 10-mL sample was added to a vial containing 2 mL of 25% (wt/vol) meta-phosphoric acid for later determination of VFA. After sample collection and preparation, rumen fluid samples were immediately stored at -20˚C.

Two 10-mL coccygeal artery and two 10-mL mammary vein blood samples were collected using Vacutainer tubes containing K_2-EDTA (Becton Dickinson Vacutainer Systems, Rutherford, NJ) on Thursday of wk 4, 8, and 12 at approximately 3 h after feeding for later analysis. One 6-mL coccygeal artery blood sample using a Vacutainer tube containing sodium fluoride (Beckton Dickinson Vacutainer Systems, Rutherford, NJ) was also collected on Thursday of wk 4, 8, and 12 at 3 h after feeding for later analysis of glucose. Fecal grab samples were collected during wk 4, 8, and 12 every 8 hr for 3 d with forward advancement of 2 hours daily to account for diurnal variation.

Body condition scores were determined weekly by 3 individuals on a scale of 1 to 5, with 1 as emaciated and 5 as obese (Wildman et al., 1982), approximately 3 h after feeding. Body weights were electronically collected using a livestock scale (AWB-5K-SYS, Triner Scale and Manufacturing Company, Inc., Olive Branch, MS) on Thursday of wk 4, 8 and 12, approximately 3 h after feeding.

Laboratory Analysis

At the end of the trial, feed samples were thawed and composited by period before being sent to Analab (Fulton, IL) for DM and nutrient analysis. Samples were analyzed using the following Association of Official Analytical Chemists International (1998) methods: DM (935.29), CP (990.03), neutral detergent fiber (NDF) (2002.04), acid detergent fiber (ADF) (973.18), acid detergent insoluble nitrogen (ADIN) (973.18 and 976.06), neutral detergent insoluble protein (NDIP) (2002.04 without sulfite and 976.06), lignin (973.18), ash (942.05), Ca (985.01), P (985.01), Mg (985.01), Na (985.01), Cl (915.01), S (923.01), Fe (985.01), Cu (985.01), Zn (985.01), K (985.01), Mn (985.01), and pH (981.12). The remaining nutrient parameters were measured using the following methods: soluble protein (SP); (Krishnamoorthy et al., 1982), starch (Glucose Reagent Set, AMRESCO, Solon, OH and ALPKEM Corporation, 1990), oil (Damon, 1966), *in vitro* dry matter digestibility (IVDMD) (24 h ruminal and 24 h enzymatic digestion using the Kansas State Buffer (Marten and Barnes, 1980), neutral detergent fiber digestibility (NDFD); (Van Soest et al., 1991, incubated for 30 h using the Kansas State Buffer (Marten and Barnes, 1980), NH_3-N (United States Environmental Protection Agency, 1993, method 351.2 and International Organization for Standardization, 2013, method 11732), lactic acid (El Rassi, 1996), acetic acid (Cancalon, 1993), nonfiber carbohydrate (NFC); (National Research Council, 2001), net energy for lactation (NE_L); (National Research Council, 2001), relative forage quality (RFQ); (Rohweder et al., 1978), and sugar (Analab, Fulton, IL defined method, in process of entering a Single Laboratory Validation from the Association of American Feed Control Officials).

Rumen fluid samples were thawed and centrifuged at 30,000 × g for 20 min at 20˚C (Eppendorf 5403, Eppendorf North America, Hauppauge, NY). Rumen fluid samples acidified with 50% (vol/vol) H_2SO_4 were analyzed for NH_3-N using procedures from Chaney and Marbach (1962). Ruminal fluid samples acidified with 25% (wt/vol) meta-phosphoric acid were prepared according to Erwin et al. (1961) and analyzed for VFA concentrations using an automated gas-liquid chromatograph (model 6890, Hewlett-Packard) with a flame-ionization detector. Once prepared, 1 µl of prepared sample was injected at a split ratio of 30:1 at the injection port (250°C). Volatile fatty acids were separated on a capillary column (15 m × 0.25 mm i.d.; Nukol, 17926–01C, Supelco Inc., Bellefonte, PA) with flow-rate of 30 mL/min of He using 2-ethylbutyrate as an internal standard. The column and detector temperature were maintained at 140°C and 250°C, respectively. Blood plasma taken 3 h after feeding was analyzed for glucose (Liquid Glucose (Oxidase) Reagent Set; Pointe Scientific, Inc., Canton, MI).

Statistical Analysis

Data were analyzed as a randomized complete block design using PROC MIXED procedure of SAS (Version 9.4, SAS Institute, Inc., Cary, NC). Feed intake, milk production, milk composition, milk component yield, plasma glucose, rumen pH, rumen ammonia, VFA, BW, and BCS data were analyzed with week, treatment, parity, and the interactions of treatment and week as fixed effects. Random effects included cow. Significance was declared at $P \leq 0.05$ and trends declared at $0.05 \leq P \leq 0.10$.

Results and Discussion

Ingredient composition of diets offered are given in Table 4.1. Chemical composition of the diets are found in Table 4.2. CONTROL and TEST diets contained similar amounts of forages, but differed in source due to pre-determined agronomy program. Post-experiment analysis of the total mixed ration shows that diets were formulated and met formulation expectations.

Milk production (Table 4.3) was increased ($P = 0.04$) when cows were fed the TEST ration which agreed with other researchers (Chase, 2011). Dry matter intakes were similar between treatments ($P = 0.46$). Yield of milk fat was similar for CONTROL and TEST fed cows ($P = 0.21$), while yields of milk protein ($P = 0.037$), lactose ($P = 0.032$), and total solids ($P = 0.045$) were increased ($P < 0.05$) for cows fed the TEST treatment compared to CONTROL fed cows. Milk urea nitrogen ($P = 0.37$) and SCS ($P = 0.63$) were similar between treatments. Fat corrected milk (4%) tended ($P = 0.09$) to be higher for cows fed TEST forages compared to CONTROL fed cows. Energy corrected milk was increased ($P = 0.05$) for cows fed TEST compared to CONTROL fed cows. Body weights were similar ($P = 0.14$). Rumen ammonia concentrations (Table 4.4) were similar ($P = 0.63$) for cows fed TEST as compared to cows fed CONTROL.

A decrease ($P = 0.004$) in ruminal butyrate percentage was found for cows fed the TEST diet. Propionate concentration ($P = 0.10$) and percentage ($P = 0.10$) tended to increase when cows were fed the TEST diet. No differences were found in total VFA production.

There was a trend for an increase ($P = 0.06$) in starch digestibility (Table 4.5) for cows fed TEST when compared to CONTROL fed cows. Digestibility of NDF ($P =$

0.03) and ADF (P = 0.02) were increased for the TEST fed cows compared to cows fed CONTROL forages. Feeding higher quality forages obtained from enhanced agronomy procedures did increase milk production, milk composition, and fiber digestibility for lactating dairy cows fed a high forage ration.

Conclusions

Feeding higher quality forage positively influenced milk production, milk composition, and fiber digestibility for lactating dairy cows fed a high forage ration. Increases in milk production can partially be explained by increases in NDF and ADF digestibility for the TEST fed forages. Additionally, increases in the rate of digestion for the alfalfa haylage NDF is assumed to be a contributing factor in the increase in animal productivity. There is limited, published research in this area. Further research is warranted to aid in the clarification of how forages produced via enhanced agronomy/forage programs can be utilized in rations to increase lactating cow production parameters and health.

Table 4.1 Ingredient composition of diets based on dry matter (DM)

Item	DM, kg.	% of diet DM
Corn silage	20.0	37.51
Alfalfa haylage	12.5	23.44
Ground corn, fine	8.4	15.75
Distillers grain	3.0	5.63
Whole cotton seed	3.0	5.63
Soybean meal, 47.5% CP solvent	2.7	5.06
Soy Best pearl	1.851	3.47
Limestone, ground 38% Ca	0.44	0.83
Salt, white	0.25	0.47
Sodium bicarbonate	0.22	0.41
Energy booster	0.2	0.38
Dicalcium phosphate dihy	0.15	0.28
Diamond V XP	0.12	0.23
Dynamate	0.111	0.21
Vitamin ADE premix	0.1	0.19
LysiPEARL	0.07	0.13
Potassium chloride, Red	0.06	0.11
Mepron	0.055	0.10
Urea, 281 CP	0.05	0.09
Magnesium Oxide	0.04	0.08
Rumensin 90	0.004	0.01
Total	53.321	100.0

Table 4.2 Nutrient composition of CONTROL and TEST treatments (%DM)

Nutrient, %	CONTROL[1]	TEST[2]
DM, % as-fed	56.6	56.8
CP	18.5	19.2
SP[3], % of CP	44.0	42.0
ADF	18.1	17.1
NDF	27.9	26.6
Starch	25.9	26.3
NDFD[4]	58.5	60.4
IVDMD[5]	82.7	84.1

[1]CONTROL=rations utilizing forages produced through standard soil and agronomy programs.
[2]TEST=rations utilizing forages produced through enhanced soil and agronomy programs.
[3]Soluble protein.
[4] NDFD = Neutral detergent fiber digestibility, 30 h.
[5] IVDMD = *In vitro* dry matter digestibility.

Table 4.3 Effects of treatment on performance of lactating cows

	CONTROL[1]	TEST[2]	SEM	*P*
DMI, kg/d	23.9	22.8	1.56	0.46
Milk, kg/d	32.6	36.9	1.99	0.04
FCM[3]	33.6	39.0	3.04	0.09
ECM[4]	33.0	36.8	1.84	0.05
Fat, kg/d	1.18	1.27	0.08	0.21
Protein, kg/d	0.98	1.09	0.05	0.04
Lactose, kg/d	1.62	1.88	0.12	0.03
Fat, %	3.61	3.39	0.16	0.20
Protein, %	2.98	3.90	0.08	0.30
Lactose, %	4.93	4.97	0.09	0.67
SNF[5], %	8.79	8.78	0.13	0.90
SCC[6] x 1,000, cells, ml	230	250	273	0.94
MUN[7], mg/dL	14.4	14.9	0.55	0.37
BW, kg	630	664	22.5	0.14
BCS	3.01	3.00	0.06	0.86

[1]CONTROL=rations utilizing forages produced through standard soil and agronomy programs.
[2]TEST=rations utilizing forages produced through enhanced soil and agronomy programs.
[3]Fat Corrected Milk = (0.4 x kg of milk) + (15 x kg of milk fat).
[4]Energy Corrected Milk = (0.327 x kg of milk) + (12.95 x kg of milk fat) + (7.2 x kg of milk protein).
[5]Solids not fat
[6]Somatic cell count
[7]Milk urea nitrogen

Table 4.4 Effects of treatment on rumen fluid

Measurement	CONTROL[1]	TEST[2]	SEM	*P*
pH	6.796	6.720	0.06	0.22
NH_3-N, mg/dL	18.36	19.12	1.57	0.63
Acetate, %	60.22	59.94	0.93	0.09
Propionate, %	21.48	22.80	0.76	0.10
Butyrate, %	12.99	12.01	0.31	0.01
Acetate:propionate	2.84	2.69	0.13	0.24

[1]CONTROL=rations utilizing forages produced through standard soil and agronomy programs.
[2]TEST=rations utilizing forages produced through enhanced soil and agronomy programs.

Table 4.5 Nutrient digestibility by cows fed CONTROL or TEST forages

Measurement	CONTROL	TEST	SEM	*P*
Dry matter, %	75.5	75.3	0.59	0.69
Crude protein, %	74.0	75.8	1.42	0.20
NDF, %	48.5	54.7	2.78	0.03
ADF, %	48.3	54.4	2.54	0.02
Starch, %	97.9	98.6	0.27	0.06

[1]CONTROL=rations utilizing forages produced through standard soil and agronomy programs.
[2]TEST=rations utilizing forages produced through enhanced soil and agronomy programs.

OVERALL SUMMERY AND CONCLUSIONS

This research fulfilled our initial overall objective to expand the understanding of how forage feeding strategies, at a high dietary inclusion rate, affects lactating cow performance. The practice of feeding cobalt-lactate to late-lactation dairy cows was evaluated in Chapter 2. In Chapter 3 it was determined how the inclusion of *Saccharomyces cerevisiae* fermentation products (SCFP) in mid-lactation cows affects animal performance. In Chapter 4 we examined the inclusion of highly digestible forages in peak lactation diets and its effect on increasing milk yield.

Supplementing cobalt resulted in similar production parameters between treatments which is in agreement with results found in other research on cobalt supplementation (Akins, 2013; Kincaid and Socha, 2007; Campbell, 1999). Feeding additional Co as cobalt-lactate did not influence milk production, milk composition, dry matter intake or body weight for lactating dairy cows fed a high forage ration. Feeding Co decreased ruminal ammonia concentrations which could indicate an increase in ruminal microbial protein synthesis and growth although we did not measure that specific characteristic. Feeding Co increased ruminal concentrations of acetate which would suggest increased fiber digestion. The evaluation of Co in early lactation dairy cows is warranted to determine if enhancements in microbial protein synthesis (NH_3) and fiber digestion (acetate) are beneficial. Additional research in this area would include a titration study, in vitro, to determine the optimal Co levels for ruminal digestion. The lack of response of cows to supplemental Co was likely due to the elongated DIM of the study cows and a lower than expected quality of alfalfa forage.

From the results in Chapter 3, feeding SCFP did not influence milk production, milk composition, dry matter intake, or body weight in this study. However, this study primarily focused on ruminal characteristics as this was a mechanism study rather than a production study. Feeding SCFP in the form of Prototype 1 (P1) or Prototype 2 (P2) decreased ruminal ammonia concentrations, which could indicate an increase in ruminal microbial protein synthesis. Feeding SCFP in the form of P1 or P2 increased ruminal molar percentages of propionate, while reducing ruminal acetate, resulting in a reduction in the acetate to propionate ratio which is in agreement with Acharya et al. (2015). Feeding P2 resulted in an increase in iso-butyrate percentage when compared to cows fed C, XPC, and P1, which may lead to increased milk production in a large scale production study due to the stimulation of growth hormone release via iso-butyrate. However, this was not measured in this study. The evaluation of SCFP in a large, mid lactation dairy cow production study is warranted to determine if enhancements in VFA concentration and percentage are beneficial on production parameters.

From the results in Chapter 4, feeding higher quality, more digestible forage increased milk production, milk composition, and fiber digestibility for lactating dairy cows fed a high forage ration. High quality forage, supplemented at a high level, can increase feed efficiency through increased levels of ECM which is explained by increases in milk production with maintained DMI. Increases in NDF and ADF digestibility in the Test forage aids the largest explanation for this increase in milk yield and animal performance.

In conclusion, these results demonstrated that high-forage diets can be fed to lactating dairy cows, with or without the supplementation of cobalt or SCFP, to maintain

or increase lactational performance without the added cost of higher concentrate diets. High-forage diets made up of locally produced, highly-digestible forage can be used to reduce input feed costs and ultimately improve animal performance through increases in animal productivity.

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