EFFECT OF SUGAR SUPPLEMENTATION IN LACTATING DAIRY COWS

by

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Abstract

During the past decades, the dairy industry has been challenged to find alternative approaches in order to feed cows without affecting their performance or increasing production costs. To accomplish these objectives, some options that have been implemented are the inclusion of short chain carbohydrates to replace starch and the addition of synthetic supplements to increase feed efficiency. In order to assess the impact of these strategies, an experiment was conducted to evaluate productive responses of lactating dairy cattle when they received sucrose and/or exogenous amylase in low starch diets. The results indicated that milk production, milk component profile, and feed efficiency were not significantly altered by the use of the enzyme, sucrose inclusion, or the combination of both. Comparing these results with the literature revealed apparent inconsistencies in responses to the inclusion of sugar in dairy rations. For that reason, a meta-analysis was performed to determine the impact of different sugar sources on milk production, and also to evaluate the impact of other dietary factors on response to dietary sugar. The results indicated that dry matter intake responses were significantly ($P < 0.05$) affected by an interaction between added sugar and dietary forage neutral detergent fiber content, but overall, dry matter intake tended to increase when sugar replaced corn grain in diets. Energy corrected milk was not affected by dietary sugar, but milk production showed a tendency to respond to treatment, dependent on an interaction between added sugar and rumen undegradable protein. In summary, sugar inclusion may promote small increases in dry matter intake, but the impact on milk production is inconsistent; both factors may be influenced by the diet to which sugar is added.

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Dedication

“*To dream is free*”

To **TIO MEMO,** with all the strength of my heart.

Chapter 1 - Literature Review

Carbohydrate nutrition in dairy cattle

The management of nutrition for high producing dairy cows is a challenge because it involves a series of dynamic interactions among dietary factors, the rumen microbial population, and the host animal in a complex ecosystem established in the ruminant digestive tract (Allen and Mertens, 1988). An animal needs to eat enough food to fulfill not only its needs but also to meet the requirements of the microbes in the rumen. When the diet provides timely availability of proper nutrients, the conditions are set up to generate the maximum levels of milk production.

In diets for dairy cattle, carbohydrates play an important role as energy sources; these nutrients represent about 60-70% of rations formulated to feed high producing cows (National Research Council, 2001; Hall et al., 2010). These compounds range from fiber to rapidly fermentable carbohydrates. Some of them come in the form of simple monomers (monosaccharides) while others are arranged in structures to form complex molecules (polysaccharides; Derevitskaya et al., 1978).

The carbohydrates have been broadly classified in two major groups: fiber and non-fiber components. The structural carbohydrates are quantified as neutral detergent fiber (NDF), which includes lignin, cellulose, and hemicellulose, and are considered the indigestible and slowly digested components of the ration. On the other hand, starch, sugars and pectin share the characteristic of being rapidly digested in the rumen. This group is known as the non-fiber carbohydrate (NFC) fraction (Grant et al., 1995).

Ruminants have the ability to use the carbohydrates of the cell wall by the action of the microbes present in their digestive tract. Bacteria, protozoa and fungi enzymatically break down the structure of cellulose and hemicellulose into less complex compounds that the host animal can uptake through the rumen epithelial wall (Allen, 1997; Aschenbach et al., 2011; Zebeli et al., 2012). But the ability of the microbial population to degrade fiber can be affected by numerous forage factors like type of forage, agronomic management, maturity at harvest, and fermentation or processing methods; as a consequence, the rate and extent of degradation can vary, and the delay in digestion following ruminal exposure varies (Galloway et al., 1991).

The NDF fraction is very important for ruminants, because it is critical to ensure the proper functionality of the rumen. The structural carbohydrates stimulate the appropriate motility of the rumen to promote rumination, secretion of saliva to regulate ruminal pH, and development of the ruminal mat that optimizes the fermentation processes (Tafaj et al., 2004; Clauss et al., 2011; Zebeli et al., 2012).

Non-fiber carbohydrates represent about 30 to 45% of the diet for cattle in milk production systems. They provide energy to the microbes and support microbial protein synthesis in the rumen (Aldrich et al., 1993; Berthiaume et al., 2010), which is the major source of high quality protein and amino acids to the lower digestive tract. Furthermore, the microbes produce volatile fatty acids (VFA), compounds that function as the major metabolic fuels for the host. Nevertheless, excessive amount of these products can generate negative responses like a reduction in ruminal fluid pH, the inhibition of cellulolytic activity in the rumen, and alterations in milk fatty acid profiles that lead to milk fat depression (Mullins and Bradford, 2010). Furthermore, microbial protein and VFA are not the only end products from carbohydrate fermentation; some gases like methane, hydrogen sulfide, and carbon dioxide are also generated and they must be eructated in order to ensure the proper function of the rumen and indeed the animal health (Ellis et al., 2012).

Because of the differences between fiber and non-fiber carbohydrates, the critical point in dairy nutrition is to find an optimal balance between these two fractions in order to maintain proper rumen metabolism (Zebeli et al., 2006; Plaizier et al., 2008) and a stable metabolic health status to optimize the productivity of dairy cattle (Zebeli et al., 2012).

Sources of dietary sugar in dairy diets and formulation approaches

Among all the non-fiber carbohydrates, starch contributes approximately 50 to 75% of the energy value of corn silage and corn grain, two of the main ingredients utilized in rations for dairy cattle (Hall et al., 2010). According to Patton et al, (2012), starch can be found in lactation diets at as low as 20% of diet dry matter (DM) and as high as 40%. However, the increasing demand and prices of these feedstuffs (Ranathunga et al., 2010; Bradford and Mullins, 2012) have forced farmers to opt for cheaper alternatives in order to maintain sustainable production.

To replace starch sources, one of the available options is the inclusion of sugar. In a common sense, the term sugar refers to carbohydrate chains with less than 20 units of saccharides (monosaccharides, disaccharides or oligosaccharides).

The benefits of using this type of supplement rely on their digestibility in the rumen. Compared to starch and structural carbohydrates, microbes invest less effort to reduce sugars to smaller units (Golder et al., 2012), and the microbes can use them faster as fuels and convert them to VFA that can be absorbed by the host animal in a short period of time (Nafikov and Beitz, 2007).

Sugars are available in different forms. For example, some forage contains high proportions of soluble carbohydrates; the perennial ryegrasses (*Lolium perenne*) are considered rich sources of this type of nutrient (Miller et al., 2001; Tas et al., 2006), and also some alfalfa cultivars (Berthiaume et al., 2010). Sugar cane is a crop that can be successfully produced under tropical and subtropical conditions (Pate, 1981), with high yields of biomass per unit area (Aranda et al., 2001). Sugar cane has a high sugar content (Peláez-Acero et al., 2008) and consequently relatively high energy levels (2.0 to 2.3 Mcal/kg; Correa et al., 2003). But these benefits are limited by the high content of NDF and low proportion of crude protein (CP) in sugar cane (Correa et al., 2003; Martin, 2005; Lascano et al., 2012).

The food industry generates alternative sugar sources for the animal industry. One such byproduct that is widely used in the dairy industry is citrus pulp. This ingredient has a high content of soluble carbohydrates and a digestible NDF fraction (Ben-Ghedalia et al., 1989; Ammerman and Henry, 1991; Miron et al., 2001). One study demonstrated that citrus pulp could replace corn grain in a total mixed ration (TMR) without affecting milk production (Solomon et al., 2000). Another cost-effective sugar source is molasses. This feedstuff can be extracted from different materials, including sugar cane, beet pulp, citrus, hemicellulose extract and starch extract (Curtin, 1983). The content of sugar in molasses can vary from ≥ 45% in the cases of the first three sources to ≥ 50% for hemicellulose and starch molasses (Association of American Feed Control Officials, 1982).

Molasses is a feed ingredient that is not only useful because of the energy it supplies, but also for the physical benefits it confers to the diets. Molasses can reduce dustiness and help to agglomerate small particles; it can also promote more uniformity in the diets consumed by individual animals by reducing sorting behavior in cows (Firkins, 2010; DeVries and Gill, 2012).

For research purposes, many authors have used pure forms of short chain carbohydrates in experimental rations for dairy cattle. These have included glucose, sucrose, and lactose, and in some studies fructose has been used, too (McCormick et al., 1999; Ordway et al., 2002; Vallimont et al., 2004; Broderick et al., 2008; Penner and Oba, 2009; Hall et al., 2010; Golder et al., 2012).

Endogenous sugar production

Sugars in the rumen are not only provided by external sources; microbes can produce them during the degradation of starch and structural carbohydrates. Hemicellulose is formed as polymers of xylose, arabinose, mannose, galactose and glucose, whereas cellulose and starch are glucose polymers, differing in the types of disaccharide bonds linking the monomers (Heinze and Koschella, 2005). The microbes first need to break down these polymers and convert them to monomers before metabolizing them to obtain energy. During these conversions, the microbes generate waste products that must be removed because they can affect the microbial population through mechanisms like feedback inhibition or toxicity, but these end products have high utility for the host animal (Ortega and Mendoza, 2003).

The primary organic acids produced during carbohydrate fermentation are acetate, propionate, butyrate, and lactate. All of them can be absorbed across the rumen wall by different mechanism in order to maintain a stable ruminal environment; some of these mechanisms are passive diffusion, facilitated diffusion, and active transport (Aschenbach et al., 2011). Also, some bacteria in the rumen can utilize organic acids as sources of energy for their own metabolism. The relative proportions of the individual organic acids vary according to fermentation patterns of sugars, starch, or soluble fiber, which are totally different due to their digestion characteristics (Marounek et al., 1985; Strobel and Russell, 1986; Heldt et al., 1999; DeFrain et al., 2004; Ellis et al., 2012). When sugar is included in rations, the molar proportions of acetate and butyrate are increased and the proportion of propionate is typically decreased (Heldt et al., 1999; Ribeiro et al., 2005; Guan et al., 2008; Martel et al., 2011).

The rate of absorption has been related to the extent of metabolism in the rumen epithelium and it increases with the chain length of the acid. Butyric acid has the highest rate of absorption, followed by propionic acid and acetic acid. Among these three VFA, propionate is transported to the liver where it is primarily utilized in the gluconeogenesis pathway to produce the glucose for the metabolism of the host animal (Pratti and de Resende, 2012), whereas acetate and butyrate are converted to acetyl-CoA and either enter the citric acid cycle to produce energy or are used for *de novo* fatty acid synthesis.

The population of microbes maintains a dynamic equilibrium depending on the substrate available, and a complex series of interactions between bacteria, protozoa and fungi. These interactions include competition, predation, interspecies hydrogen transfer, and mutualism, among others.

Microbial responses to dietary sugar

The addition of sugar to dairy rations impacts the microbial ecosystem in the rumen. Different microbial species, depending on the substrate they utilize (sugar or starch), generate different fermentation end products, and the speed of fermentation is positively related to the microbial mass production (Hall and Herejk, 2001; Golder et al., 2012).

Usually, when diets contain a large proportion of starch, some fiber-digesting bacteria (*Butyrivibrio fibrisolvens, Fibrobacter succinogenes* and *Provetella ruminicola*) begin to use non-fiber carbohydrates rather than fiber because they can degrade NFC faster and get energy rapidly (Miron et al., 2002). However, in order to get the energy from starch, fiber fermenting microbes must compete for the substrates against amylolytic bacteria (*Streptoccocus bovis, Butyrivibrio fibrisolvens, Bacteriodes ruminicola and Selenomonas ruminantium*). Which grow more aggressively in the presence of starch, and have advantage over fiber utilizers because they colonize the substrate first (Huntington, 1997).

In addition to the competition for energy, the end products from starch fermentation also impact microbial populations. The rate of organic acid production tends to increase when starch is present in the diet; this is attributed to the fact that starch can be fermented faster than fiber. This causes a decrease of ruminal pH, which alters the conditions for fiber digesters and reduces fiber degradation (Zebeli et al., 2012).

With the inclusion of sugars, the ruminal environment is less affected because starch-fermenting bacteria do not have affinity for sugars as energy sources; this substrate is rapidly degradable so fiber digesters can use it without the colonization disadvantage (Hall and Herejk, 2001; Miron et al., 2002; Firkins, 2010).

According to some authors, when sugars like sucrose are included in the diet, some of the sugar-utilizing bacteria store a portion of the carbohydrates in the form of glycogen that can be used to maintain the microbial population when the substrate is deficient (Hall and Herejk, 2001).

Acidosis, ruminal pH and milk fat depression

Another key factor is the ruminal pH. This parameter reflects the concentration of H^+ ions in the rumen contents. Variations in ruminal pH can alter daily feed intake, rumen motility, the microbial population, the fermentation products and absorption patterns (Storm et al., 2012).

In the rumen, the pH is associated with the concentration of organic acids, mainly VFA and lactic acid. This factor has a particular impact on the rumen because each microbial species has a different pH range in which it can survive, and in general, ruminal microbes can grow in a pH range between 5.5 and 7.5 (Febres and Vergara-López, 2007). The equilibrium in the ecosystem is driven not only by the production and absorption of the organic acids, but also by the input of saliva and feeds.

The types of carbohydrates from different feedstuffs and their fermentation in the rumen are the primary factors dictating the ruminal pH patterns. In the case of forage-based diets, the predominant carbohydrates are cellulose and hemicellulose. To degrade the fiber, the animal needs more mechanical activity (mastication and rumination) that increases the secretion of saliva and consequently the input of more buffers to the rumen (Maekawa et al., 2002). The predominant VFA produced in this type of diet is acetate, but propionate and butyrate also are produced in lower quantities (Kendall et al., 2009).

The inclusion of grains in diets alters the fermentation conditions and also the ruminal pH. The main carbohydrate present in grains is starch, which is rapidly degraded in the rumen. The fermentation of starch produces different proportions of VFA than fiber fermentation; as in fiber fermentation, acetate is produced in high amounts, but propionate production is increased to a greater extent, resulting in a decrease in the acetate: propionate ratio. Butyrate is also produced but rapidly absorbed in the rumen (Silveira et al., 2007). The buffering capacity in starch diets is less than in fiber diets because the animal masticates and ruminates less, and the stimulation for saliva production is diminished.

Diets with high proportions of starch and low fiber content offer good conditions for the development of acidosis (Enemark, 2008). This happens because the production of VFA and lactic acid is greater than the absorption rate and buffering capacity, and organic acids start to accumulate, causing ruminal pH to decline below 5.5 (Penner et al., 2007). The two possible levels of acidosis depend on the type of acid that is accumulated; acute acidosis occurs when lactic acid is accumulated, whereas sub-acute acidosis occurs in the absence of lactic acid accumulation.

Acidotic conditions severely impact animal performance. In the case of acute acidosis (pH < 5.0), there are more severe health problems and the clinical signs are evident (Reference Advisory Group on Fermentative Acidosis of Ruminants, 2007). On the other hand, in sub-acute acidosis (pH 5.0 to 5.5), the major effects are on milk production rather than health, caused by diminished dry matter intake and depressed milk fat content (Enemark, 2008; Aschenbach et al., 2011; Lechartier and Peyraud, 2011).

The fat is the most important and variable component in milk, and its concentration is easy to alter with changes of nutritional factors that modify the ruminal environment (Sutton, 1989). The interaction between grain and fiber fermentation is what primarily influences fermentation patterns, and subsequently changes in pH. This dynamic can generate changes in milk fat content (Bath, 1982). Many theories have been proposed to explain milk fat depression, but the most attractive is related to the effect of low pH on the biohydrogenation process that takes place in the rumen. Due to this alteration in biohydrogenation, the synthesis of milk fat in the mammary gland is inhibited by the increased proportions of unique rumen-derived fatty acids (eg. *trans*-10 $C_{18:1}$ and *trans*-10, *cis*-12 $C_{18:2}$; Bauman and Griinari, 2003).

When rumen pH drops, some of the steps in ruminal lipid metabolism are affected. Initially, hydrolysis is disturbed because the microbe population responsible for this step is sensitive to low pH. The other affected step is the isomerization of linoleic acid; instead of *cis*-9, *trans*-11 $C_{18:2}$, the intermediate product generated is *trans*-10, *cis*-12 $C_{18:2}$, which induces milk fat depression (Griinari et al., 1998). This happens because one of the two major lipolytic bacteria that is also a fiber-digesting microbe, *Butirivibrio fibrisolvens,* is affected by low pH. *Butirivibrio fibrisolvens* produces the isomer *cis*-9, *trans*-11 C18:2, and a reduction in this population likely contributes to a shift in the isomerization pathways. On the other hand, some strains of *Megasphaera elsdenii*, a lactate utilizing bacterium (Nagaraja and Titgemeyer, 2007), were identified as producers of *trans*-10, *cis*-12 $C_{18:2}$ (Kim et al., 2002). When this bioactive fatty acid reaches the mammary gland, the synthesis and secretion of short and medium chain fatty acids in milk declines (Pottier et al., 2006).

Despite the consequences of rapidly fermentable carbohydrate for ruminal pH, the inclusion of sugars in the diet seems to induce different effects. It is possible that short chain carbohydrates do not affect ruminal pH; indeed they could help to prevent drastic drops in pH and promote the digestibility of fiber (Penner et al., 2007; Penner et al., 2009; Firkins, 2010). This positive influence could minimize acidosis problems and also limit the risk of milk fat depression.

Part of the explanation behind this condition is related to lactic acid production and its metabolism. Lactate is a strong acid compared with VFA, which means that this compound can decrease rumen pH more than the VFA if it accumulates. However when the pH in the rumen is over 5.5, the rate of utilization of lactic acid is faster than production, and almost all the acid produced is used by lactate-utilizing bacteria and the impact on the ruminal pH is minimal.

The lactate-utilizing bacteria need to compete with starch fermenters for substrate, especially since starch provides the energy for both species. The key point here is that sugar inclusion could stimulate the growth of the microbes capable of metabolizing the lactic acid because they are able to use sugar instead of starch as primary energy source during periods when little lactate is available (Firkins, 2010).

The second key point is that sugar is important as fuel for fiber digesting bacteria. Sugars stimulate this population and more butyrate and acetate is produced in response to dietary sugar (Hristov and Ropp, 2003; DeFrain et al., 2006; Firkins et al., 2008). Butyrate is absorbed faster through the ruminal wall and it does not accumulate. It also impacts the function of ruminal epithelium to increase the blood flow, and in turn, uptake of all VFA, so the ruminal pH is regulate and acidosis risk is diminished (Firkins, 2010).

Another possible explanation about why sugar could positively impact the ruminal pH is that this carbohydrate is not fermented to produce acid directly. It could be stored in the form of glycogen; the bacteria use this compound as a reservoir for starvation situations. When the bacteria use the glycogen, the VFA production is regulated because they are produced slowly as energy is needed, so the impact on pH is less (Allen, 1997; Hall and Weimer, 2007; Penner and Oba, 2009).

Impact on digestion of other fractions

The usage of nitrogen in ruminants is highly related to the proportion of available energy in the diet (Dijkstra et al., 1998). Although some authors argue that rumen bacteria are not affected by asynchrony between energy and nitrogen supply because they have the capacity to adapt quickly to transient deficit situations (Newbold and Rust, 1992; Henning et al., 1993), others stated that synchronization between protein and energy supply could increase N efficiency and less of this nutrient could be lost in urine (Castillo et al., 2001; Miller et al., 2001).

Some references indicated that animals fed with fructose presented lower ruminal ammonia concentration compared with starch-fed animals (Golder et al., 2012), and the urinary nitrogen was decreased when sugars were included in dairy rations (Broderick and Radloff, 2004). In addition, the concentration of milk urea-nitrogen (MUN) was lower too (Delahoy et al., 2003). The explanation behind this is attributed to the fact that microbes could incorporate nitrogen from ammonia because sugars are metabolized faster than starch and they provide more energy immediately available for microbial protein synthesis (Hall and Herejk, 2001; Firkins et al., 2001; Miron et al., 2002)

Digestion of the NDF fraction can be impacted by sugar inclusion in rations for high producing cows. Fiber digesting bacteria, as mentioned previously, invest energy to produce adhesion molecules and cellulolytic enzymes to break down the cell wall, utilizing energy from rapidly degradable carbohydrates. Sugars provide this “fast energy”; first stimulate the colonization of the cellulose, and second they incite bacterial growth (Firkins, 2010). In support of this view, sucrose initiates microbial growth more rapidly than starch when evaluated *in vitro* (Hall and Herejk, 2001). Other studies indicated that inclusion of sugar in the ration up to 8% of DM improved NDF digestibility, possibly reflecting a change in microbial populations or an increment of fiber digesting bacteria present in the rumen (Vallimont et al., 2004).

The excessive addition of sugar can also cause detrimental effects on NDF digestibility (Oba, 2011), and some researchers suggest that this problem is a result of asynchrony between ruminal carbohydrate availability and rumen-degradable protein (RDP) supply, which can promote energy spilling by microbes, decrease rumen pH, and/or depress fiber digestibility (Oelker et al., 2009).

Production responses

Regardless of the impact on cow performance, some references indicated that the inclusion of sugar increases dry matter intake (Broderick et al., 2008; Penner and Oba, 2009; Penner et al., 2009), especially if it increases the digestibility of NDF. Increasing NDF digestibility typically decreases the time that a feed particle needs to be in the rumen to be degraded, so passage rate is increased. Other researchers found positive responses on intake when they combined sugar with fat to provide high-energy diets to cows instead of using starch (Garnsworthy et al., 2008). Nevertheless, this point is still controversial because other authors did not find such responses when they included these nutrients in diets for lactating cattle (Nombekela et al., 1994; DeFrain et al., 2006; Penner et al., 2009; Ranathunga et al., 2010; Hall et al., 2010).

In the case of milk production, most of the studies did not find significant differences between diets with starch and diets where sugar replaces some starch

(Ranathunga et al., 2010; Gencoglu et al., 2010; Hall et al., 2010), but the most significant differences between studies have been the effects of sugar on milk composition (Vallimont et al., 2004; DeVries and Gill, 2012).

The impact of meta-analysis in animal science

In the field of animal science, many years of investigation have provided a lot of information about different topics. However, when similar questions have been evaluated by different scientists, the results often did not coincide, even when the conditions of the trials were similar. Such inconsistencies make it difficult to elucidate the real impact of a given treatment or nutrient. In some cases, results are based on relatively small sample sizes and the variability of their outcomes is high (Erdreich et al., 2009).

Narrative reviews have been widely implemented to group the existing evidence on specific topics, with the intention of resolving the inconsistencies in the literature. Unfortunately, the lack of statistical or systematic support for conclusions in these reviews suggest that such approaches can be biased by the subjective opinions of the reviewer (Sargeant et al., 2006; Duffield et al., 2008b; Ceballos et al., 2009).

Meta-analytical techniques are considered more appropriate than narrative reviews for summarizing results of many studies, because they provide objectivity and statistically evaluated results. This approach is based on statistical scrutiny of a large collection of analytical results from individual, complex and sometimes apparently conflicting studies to quantitatively summarize effects, with appropriate weighting and identification of factors that explain any heterogeneity of the responses (Duffield et al., 2008a; Lean et al., 2009; Borenstein et al., 2009; Halasa et al., 2009). Another powerful application for meta-analysis to use existing data for the examination of the heterogeneity in responses to formulate novel hypothesis (Lean et al., 2009; Borenstein et al., 2009).

The success of a meta-analysis is dependent on a deep and sensitive literature search, because a failure to identify the majority of existing studies can lead to erroneous conclusions (Lean et al., 2009). At this point, many tools have been developed to reduce this type of risk and they provide strength to the results obtained from meta-analysis (Duval and Tweedie, 2000; Higgins and Thompson, 2002; Peters et al., 2007).

Meta-analyze have provided valuable information in the areas of rumen modifiers, milk fever, parasite control, mastitis, somatotropin, and reproductive manipulations (Oetzel, 1991; Rabiee et al., 2005; Carriquiry et al., 2008; Desnoyers et al., 2009; Rabiee et al., 2010; Chen et al., 2011; Reyher et al., 2012; Poppy et al., 2012; Rabiee et al., 2012).

Conclusion

Sugar seems to provide a good alternative for the dairy industry in order to reduce the proportion of high-cost sources of energy without negatively impacting the performance of the animal; however, there is still a lot controversy about its applicability, which needs to be clarified.

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Chapter 2 - Effects of dietary amylase and sucrose on productivity of cows fed low-starch diets

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Abstract

Recent studies have observed positive impacts of both sucrose and exogenous amylase on fiber digestion and productivity of dairy cattle. Our objective was to evaluate direct effects and interactions of amylase and sucrose on DMI, milk production, and milk components. Forty-eight multiparous Holstein cows between 70 and 130 DIM were randomly assigned to each of 4 pens (12 cows/pen). Pens were randomly assigned to treatment sequence in a 4 × 4 Latin square design balanced for carryover effects. The treatments were a control diet (36% NDF, 21% starch), the control diet with amylase (0.5 g/kg DM; Ruminstar, DSM), a diet with sucrose replacing corn grain at 2% of DM, and the sucrose diet with amylase (0.5 g/kg DM). All data were analyzed with mixed models including the fixed effect of sugar, amylase and their interaction and the random effects of period and pen. Milk data included the random effects of cow nested within pen and pen × period to provide the error term for the pen-level analysis. DMI was not affected by treatments. Milk yield and milk composition were not altered by the inclusion of sucrose or amylase; however, a tendency for an amylase by sucrose interaction was observed for milk protein content ($P = 0.06$), reflecting slightly lower milk protein concentrations for amylase and sucrose treatments (3.00 and 2.99 ± 0.03%) compared to control and amylase + sucrose treatments (3.02 and 3.03 ± 0.03%). Solids-corrected and fat-corrected milk yield variables were not significantly altered by treatment, although the direct effect of amylase approached significance for both variables (both $P = 0.13$), suggesting possible small increases with amylase supplementation (~0.5 kg/d). Feed efficiency (ECM/DMI) numerically increased with either amylase (1.57 ± 0.12) or sucrose (1.60 ± 0.12) treatment, but the combination of the two (interaction $P = 0.19$)

resulted in feed efficiency similar to the control treatment (both 1.50 ± 0.12). The inclusion of amylase or sucrose did not significantly affect DMI, productivity, or feed efficiency in mid-lactation cows fed low-starch, high-fiber diets.

Key words: sugar, enzyme, lactation.

Introduction

In addition to the requirements that an animal has for maintenance and growth, dairy cows face increased demands for energy during lactation. In order to ensure an adequate supply of nutrients, carbohydrates are used as the main source of energy; this type of nutrient can provide over one half of the needed energy in farm animals' diets (Nafikov and Beitz, 2007). Most diets for dairy cattle contain between 25 and 30% starch (Weiss et al., 2011; Ferraretto et al., 2011; Ellis et al., 2011; Lechartier and Peyraud, 2011; Lascano et al., 2012; Golder et al., 2012; DeVries and Gill, 2012). The high ruminal degradability of starch provides substrate to support VFA production and microbial growth and therefore enzyme production and microbial crude protein production. This enzymatic degradation is very important for the whole body metabolism because through it, glucose precursors are generated and fuel supply for the whole metabolism is ensured (Lemosquet et al., 2009). Another source of energy utilized for ruminant rations in the past years has been sugars, short chain carbohydrates that are rapidly degradable in the rumen, which could increase fiber degradation (Miller et al., 1969; Masuda et al., 1999).

The inclusion of exogenous amylase in diets for high producing cows is designed to enhance the utilization of carbohydrates present in feeds. In non-ruminant animals, the salivary glands can secrete saliva with amylase to begin breaking down starch as soon as food enters the mouth. On the other hand, ruminants do not have salivary amylase (McDougall, 1948); the microbial population in the rumen is largely responsible for the degradation of starch.

The addition of exogenous amylase has been evaluated primarily as a method to increase starch degradability. However, the literature suggests that amylase may improve productivity of lactating cows independent of effects on total-tract starch digestion (DeFrain et al., 2005; Cabrita et al., 2007; Ferraretto et al., 2011). In fact, one of the most consistent responses to exogenous amylase is an increase in NDF digestibility (Bowman et al., 2002; Gencoglu et al., 2010). Likewise, recent studies have suggested that inclusion of sugar sources to reach dietary sugar concentrations of 5 – 8% may also promote fiber digestion (Broderick et al., 2008; Firkins, 2010). These findings suggest that sugars, whether provided in the diet or produced by the activity of exogenous amylase, may promote the growth of fiber-digesting bacteria and thereby increase fiber digestibility.

The purpose of the present study was to evaluate dry matter intake, milk production, and milk components in lactating dairy cows fed exogenous amylase, sucrose, or both in a low-starch diet.

Materials and methods

The experimental procedures for this experiment were approved by the Institutional Animal Care and Use Committee at Kansas State University.

Design and treatments

Forty-eight Holstein cows between 70 and 130 days in milk (101 ± 22 DIM, mean ± SD) from Kansas State University Dairy Cattle Teaching and Research Unit were blocked by parity (1.70 ± 0.88 lactations) and stage of lactation. Within block, cows were randomly assigned to 1 of 4 pens (12 cows/pen). Pens were then randomly assigned to treatment sequence in a 4 x 4 Latin square design and balanced for carryover effects.

Treatments were: (1) control diet, (2) the control diet with amylase added at 0.5 g/kg DM, (3) a diet with sucrose replacing corn grain at 2% of DM, (4) and the sucrose diet with amylase added at 0.5 g/kg DM. The amylase used was Ronozyme RumiStar (lot # AUN01024 and AUN01028, DSM Nutritional Products, Basel, Switzerland; Novozymes, Bagsvaerd, Denmark) with an expected amylase activity of 240 KNU[1]/g (FAO, 2004). Table 1 shows the complete description of the diets. The forages used in the diets were analyzed approximately 1 month prior to the start of the study, and formulation was adjusted at that time. Dry components of the diet (not including cottonseed) were blended into a premix approximately 2 wk prior to the start of the study, and samples were submitted to DSM Nutritional Products Analytical Services Center (Basel, Switzerland) for determination of amylase activity (Jung and Vogel, 2008). Each ration was delivered as a total mixed ration (TMR), and corn silage dry matter was determined twice weekly to adjust its inclusion rate. Cows were fed once daily for *ad libitum* intake and milked 3 times daily throughout the experiment. Treatment periods were 28 d, with 24 d for diet adaptation and 4 d for sample and data collection.

Sample and data collection

During the experiment, the amount of feed offered and refused were measured for each pen daily to determine dry matter intake (DMI). During the final 4 days of each period, samples of orts and each feed ingredient were collected daily and composited by period for analysis. Total mixed ration samples were collected daily and analyzed for particle size on 2 days by using the Penn State Particle Separator (Lammers et al., 1996;

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[1] KNU = Kilo novo units; amount of α-amylase which dextrinizes 5.26 g of starch dry substance per hour under standard conditions of pH 7.1 and 37°C (FAO, 2004).

Kononoff et al., 2003). Milk yield was recorded for each cow daily. Milk samples were collected at each milking during the 4–d collection periods. Body condition scores were determined by 2 trained investigators at the beginning of period 1 and at the end of each period using a 1 to 5 scale (Wildman et al., 1982).

Sample analyses

Milk samples were analyzed for concentrations of fat, lactose, true protein (B-2000 Infrared Analyzer; Bentley Instruments Inc., Chaska, MN), urea nitrogen (MUN spectrophotometer; Bentley Instruments Inc.), and somatic cells (S=CC 500, Bentley Instruments Inc.; Heart of America DHIA, Manhattan, KS).. Energy-corrected milk was calculated by using the formula 0.327 × kg milk yield + 12.86 × kg fat + 7.65 × kg protein (Dairy Record Management System, 2011).

Feed ingredient, TMR, and orts samples were dried in a 55°C forced-air oven for 72 h to determine their DM concentration. To prepare the samples for the subsequent analyses, samples were ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA) through a 1-mm screen, and were then composited by period. To express all the nutrients on a common DM basis, the samples were dried at 105°C in a forced-air oven for more than 8 h. Ash concentration was determined after 5 h of oxidation at 500°C in a muffle furnace. Concentration of NDF was determined in the presence of sodium sulfite and amylase with an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY; Van Soest et al., 1991). Crude protein was determined by oxidation and detection of N_2 (Leco Analyzer; Leco Corp, St. Joseph, MI). Crude fat was determined by ether extraction (AOAC, 2000). Total sugars were quantified in a commercial laboratory by incubation with invertase followed by measurement of reducing sugars (Martel et al. 2011). Starch was determined by alpha-amylase and glucoamylase digestion, followed by colorimetric glucose quantification with a commercial kit (Autokit Glucose; Wako Chemicals USA, Richmond, VA). All analyses were performed in duplicate.

Statistical analysis

Two cows were removed from the study for health reasons unrelated to treatments; one was removed in period 1, and the other in period 4. Feed intake data for each pen was divided by the number of cows present in that pen on that day. All data were analyzed with mixed models including the fixed effect of sugar, amylase, and their interaction and the random effects of period and pen. Milk data included the random effects of cow nested within pen and pen × period to provide the error term for the pen-level analysis. Denominator degrees of freedom were checked to verify that pen was treated as the experimental unit for all variables. Data were analyzed by using the REML procedure of JMP (version 8.0, SAS Institute, Cary, NC). Significance of the treatments was declared at $P < 0.05$ and tendencies at $P < 0.10$.

Results and discussion

The amylase activity in this study was evaluated at the beginning of the trial in accordance with Firkins et al. (2001) who indicated that the enzymes need to be periodically checked. The values obtained were 405 KNU/kg for the treatment that only included amylase and 351 KNU/kg for the diet that combined amylase and sucrose. These values are in the desired range and are similar to those determined in other experiments conducted with this enzyme (Weiss et al., 2010; Gencoglu et al., 2010; Ferraretto et al., 2011).

Nutrient analyses for the experimental diets are provided in Table 3. The concentrations of NDF were relatively high for mid-lactation cows across these diets, but this was by design. The experiment was intended to assess responses to added sucrose and/or amylase in low-starch diets. The lack of effect on intake could be attributed to the high proportion of this nutrient in accordance with some authors, who suggested that high NDF content in diets for lactating cattle causes a physical fill that limits the dry matter intake (Qiu et al., 2003; Kendall et al., 2009; Weiss et al., 2011). Crude protein concentrations were approximately 16.5%, which can be considered excessive amount of the nutrient in the diet, and that is reflexed in MUN concentrations (Table 2).

The body condition score was not different across studies, animals in control diet had on average 2.81±0.296, 2.81±0.266 under sucrose treatment and enzyme diet, and 2.78±0.296 with the diet that had sucrose + enzyme mixture. The results obtained from the milk component analysis and production of cows fed the treatment diets are detailed in Table 2. Dry matter intake was not impacted significantly by the diets. Previous studies have generated inconsistent responses when amylase was included as a supplement in dairy rations; some studies demonstrated increases in DMI (Klingerman et al., 2009), whereas others failed to detect any change in DMI (DeFrain et al., 2005; Gencoglu et al., 2010; Weiss et al., 2011). In spite of the differences in results, most of the studies coincided in two things; the first is that the enzyme altered the site of starch digestion and that could impact the feed consumption (DeFrain et al., 2005; Klingerman et al., 2009; Gencoglu et al., 2010; Weiss et al., 2010); also the inclusion of amylase might increase other fractions digestibility would affect intake.

A tendency for an amylase by sucrose interaction was observed for milk protein content (*P* = 0.06), reflecting slightly lower milk protein concentrations for amylase and sucrose treatments compared to control and amylase + sucrose treatments. This interaction was not observed for milk protein yield; the direct amylase effect approached significance for this variable (P = 0.11), reflecting marginally greater protein yield (20 g/d) when amylase was included in the diets. Likewise, solids-corrected and fat-corrected milk yield variables were not significantly altered by treatment, although the direct effect of amylase approached significance in both cases (both P = 0.13), suggesting possible small increases with amylase supplementation (~0.5 kg/d).

One possible justification for the lack of improvement in milk production could be attributed to the storage of sugars by some bacteria and protozoa in the form of glycogen. In this case, microbes store glycogen from different dietary sources (glucose, fructose, sucrose or fructan) in order to have energy reservoirs that can be utilized later, thereby temporarily reducing fermentation acid production (Lou et al., 1997; Hall and Weimer, 2007; Penner and Oba, 2009). Some bacterial species (i.e *Provetlla bryantii*) are known to ferment glycogen to acetate and succinate during starvation situations (Lou et al., 1997). The stored resources in the form of glycogen are slowly metabolized to generate VFA. This form of VFA production is more stable and starch fermentation is slowed down through this process which could increase the ruminal pH, favoring the environment for fiber fermenting bacteria (Nagaraja and Titgemeyer, 2007).

One key outcome in this study was feed efficiency. Some researchers indicated that dietary amylase positively impacted feed efficiency of lactating cows (Ferraretto et al., 2011), but others did not find differences in feed efficiency when amylase was included in low-starch diets (Weiss et al., 2011). In the current study, efficiency for the control diet (ECM/DMI) was 1.50; either amylase (1.57) or sucrose (1.60) treatment alone numerically increased efficiency, but the combination of the two resulted in feed efficiency identical to the control treatment. Although this interaction was not significant (P = 0.19), these results certainly do not suggest that amylase would produce synergistic benefits with high sugar content in lactation diets.

The total dietary concentrations of sugar in this study were 6.3 and 6.8% for the diets without sucrose, and 8.4 and 8.6% on DM basis for the treatments that were supplement with this nutrient. In all the cases, the added sugar values do not exceeded the 5% of DM basis; this is lower than the 8% identified by some researchers as the threshold to have negative responses on cows' performance (Broderick et al., 2008; Firkins et al., 2008). Furthermore, the diets that included amylase appeared to have higher sugar content (0.2 – 0.5%) than the treatments that lacked the enzyme, suggesting possible enzyme activity during feed storage. This outcome did not match with the results of Weiss et al. (2011) who indicated that amylase in their study was not able to provide more sugar at the feed level. On the other hand, our results coincide with Ferraretto et al. (2011), who obtained higher values of sugars when they added amylase.

Table 3 also shows particle size distribution in the TMRs as determined using the Penn State Particle Separator. According to the guidelines for this system, the top sieve should retain between 6 and 10% of the TMR (Kononoff et al., 2003), whereas in the present study the top sieve retained around 20% of DM for all the treatments, which demonstrated that the diets had high concentrations of effective fiber.

In addition to determining production responses to these diets, we also attempted to model the economic impacts of the treatment diets. Using local milk component values and estimated feed costs for Kansas in March and April 2011, we calculated both gross milk income and cost of feed for each treatment (Table 4). The two diets that contained sucrose were more expensive than the other treatments because of the very high cost of this experimental ingredient, making these comparisons somewhat unrealistic. On the other hand, by adding amylase to the control ration, solids-corrected milk production was slightly higher despite a decrease in DMI, resulting in an estimated increase in income over feed cost of $0.37/cow per day (if no cost is attributed to the amylase treatment). Therefore, based on these results, dairy nutritionists would theoretically be justified to incorporate amylase into diets if the added cost is less than $0.37/cow daily.

Conclusions

In contrast with previous studies in which exogenous amylase significantly improved feed efficiency of cows fed low-starch diets, we did not observe any significant effects of amylase, sucrose, or their interaction on intake, productivity, body condition, or feed efficiency in mid-lactation cows fed low-starch, high-fiber diets. Nevertheless, the small but economically meaningful numeric increases in feed efficiency with amylase and sucrose treatments were consistent with previously observed improvements in fiber digestibility in response to similar treatments. Based on feed efficiency responses, our results hint at the conclusion that amylase may not be as advantageous in diets that are already high in sugar content.

The inconsistencies between our findings and those of some previous studies highlight the fact that there remain some unexplained interactions of amylase with animal or dietary factors. The mechanistic effects and potential interactions of amylase and sucrose may be worth evaluating at the ruminal level. Examination of the effects of such treatments on the rumen microbial ecosystem, ruminal pH, and the kinetics of fiber degradation may provide a more precise understanding of the modes of action for these supplements.

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Figures and tables

Table 2.1 Ingredient composition of diets

Ingredient[1]	Treatment[2]	
	Control	Sucrose
Corn silage	38	38
Alfalfa hay	28	28
Wet corn gluten feed[3]	10	10
Ground corn	8	6
Sucrose	-	2
Whole cottonseed	5	5
Expeller soybean meal[4]	6	6
Soybean meal	2	2
Micronutrient premix[5]	4	4

[1] Values are expressed as a percentage of diet dry matter.

[2] Each diet was tested with and without amylase (Rumistar, DSM, Basel, Switzerland) added.

[3] SweetBran, Cargill Inc., Blair, NE

[4] Soy Best, Grain States Soya, West Point, NE

[5] Premix consist of 57% limestone, 8% magnesium oxide, 4% selenium premix (600 ppm Se), 2% trace mineral salt, 2% vitamin A premix (30 kIU/g), 0.4% vitamin D premix (30 kIU/g), 7% vitamin E premix (20 kIU/g), 4% 4-Plex (Zinpro Corp, Eden Prairie, MN) and 15% Menhaden fish meal.

Table 2.2 Sucrose and amylase effects on intake, productivity, and milk composition of lactating dairy cows fed low-starch diets

	Control		Amylase			*P*-value		
	Control	Sucrose	Control	Sucrose	SEM	Amylase	Sugar	Interaction
Dry matter intake, kg/d	23.5	22.2	22.9	23.9	1.3	0.42	0.89	0.11
Milk yield, kg/d	34.3	34.0	34.9	34.5	1.2	0.21	0.35	0.93
Milk fat, %	3.67	3.69	3.66	3.72	0.092	0.70	0.22	0.56
Milk protein, %	3.02	2.99	3.00	3.03	0.026	0.42	0.88	0.06
Milk lactose, %	4.78	4.77	4.78	4.77	0.028	0.90	0.19	0.95
MUN, mg/dl	16.9	16.7	16.4	16.6	0.48	0.45	0.93	0.67
SCC linear score	2.08	2.01	2.34	1.97	0.27	0.53	0.21	0.38
Fat yield, kg/d	1.25	1.24	1.26	1.27	0.060	0.16	0.88	0.49
Protein yield, kg/d	1.03	1.01	1.04	1.04	0.030	0.11	0.28	0.35
Lactose yield, kg/d	1.64	1.62	1.67	1.64	0.070	0.21	0.27	0.91
SCM[1], kg/d	32.3	32.0	32.7	32.7	1.30	0.13	0.49	0.64
ECM[2], kg/d	35.2	34.8	35.6	35.6	1.30	0.13	0.51	0.59
BCS change / 28 d	0.013	-0.012	-0.010	-0.116	0.045	0.17	0.18	0.37
ECM/DMI	1.50	1.60	1.57	1.50	0.12	0.82	0.77	0.19

[1] SCM = (12.3 × fat yield) + (6.56 × SNF yield) - (.0752 × milk yield); (Tyrell and Reid, 1965)

[2] ECM = (.327 × milk yield) + (12.86 × fat yield) + (7.65 × protein yield); (Dairy Record Management Systems, 2010)

Table 2.3 Nutritional composition and particle size characterization of the diets

	Control		Amylase	
% of DM	Control	Sucrose	Control	Sucrose
DM, % as-fed	57.0	55.6	54.7	56.8
OM	91.5	91.6	91.3	91.4
CP	16.5	16.5	16.5	16.3
NDF	35.6	35.2	35.4	34.9
Starch	21.4	20.6	21.4	20.9
Sugars	6.3	8.4	6.8	8.6
EE	3.2	3.0	3.2	3.0
Particle size[1]				
Top, %	20.3	20.2	21.4	21.1
Middle, %	36.3	35.3	33.5	33.9
Bottom, %	27.0	27.8	28.1	27.6
Pan, %	16.4	16.7	17.0	17.4

[1] Top sieve >1.9 cm, middle sieve 0.31 to 1.9 cm, bottom sieve 0.18 to 0.31 cm and pan <0.18 cm (Kononoff et al. 2003).

Table 2.4 Estimated profitability of the treatments

	Control		Amylase[1]	
$/cow per day	**Control**	**Sucrose**	**Control**	**Sucrose**
Gross milk income	15.63	15.46	15.84	15.82
Feed cost	6.17	6.38	6.01	6.87
Income over feed cost	9.46	9.08	9.83	8.95

[1] Feed costs for Amylase diets do not include any cost for the enzyme.

Chapter 3 - Meta-analysis of the effects of dietary sugar on intake and productivity of dairy cattle

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Abstract

A meta-analysis was performed to determine the effects of dietary sugar on feed intake and milk production in lactating dairy cattle. The database used in this analysis included 18-treatment comparisons from 10 studies reported between 1985 and 2012. Treatment comparisons were used only if 1) either sucrose (n = 9) or molasses (n = 9) replaced corn grain without adding fat, and 2) sugar added by treatment ranged from 2 to 5% of DM. In addition, 1 study was excluded because the SD for the DMI response was three times the mean SD across studies and was identified as outlier by the box plot because it was outside of the 95% confidence interval for the mean. The meta-analysis included studies analyzed by both fixed effects and mixed effects statistical models. To account for the differences in SEM reported for treatment means by these approaches, studies with repeated measures that used a mixed effects model were re-analyzed to estimate fixed effects model SEM; this approach allowed for consistent weighting across studies. The meta-analysis was conducted using a random effects model. First, responses to sucrose and molasses were compared using Cochran's *Q* statistic, and no evidence for heterogeneity across sugar sources was found in either DMI ($P = 0.24$) or ECM ($P = 0.60$) responses. Therefore, different sugar sources were pooled for the remaining analyses. In the final data set, ECM and DMI responses to added sugar were moderately correlated ($r = 0.68$, $P < 0.001$). No evidence of publication bias was observed for DMI, milk production, milk fat content or milk protein content, although for ECM, the trim and fill method suggested that 2 additional studies with negative responses would be required to generate a normal response distribution. The combined data included in this analysis showed that the DMI response was influenced by an interaction of the amount of sugar added with the content of forage NDF (fNDF) in the diet ($P < 0.05$) and the interaction of added sugar with RUP tended to impact milk production ($P = 0.09$).

The addition of sugar tended (*P* = 0.07) to increase DMI by 0.38 kg/d (95% confidence interval: -0.04 to +0.80 kg/d) and milk fat content by 0.085% (95% confidence interval: -0.008 to +0.178). On the other hand, no effect was detected for milk yield, ECM and milk protein content. In summary, results of this analysis suggest that the addition of 2 to 5% dietary sugar may promote small increases in DMI and milk fat, but do not consistently increase ECM in lactating dairy cattle. Interactions of added sugar and dietary fNDF content are worthy of further investigation.

Key words: molasses, sucrose, lactation, dry matter intake

Introduction

High demands for energy in lactating dairy cows, especially to maintain profitable production levels, are mostly covered by the dietary inclusion of highly fermentable carbohydrates. Traditionally, the predominant nutrient used to meet this requirement has been starch (Weiss et al., 2011). Fermentable carbohydrates provide the energy supply for microbial activity in the rumen and ensure the appropriate supply of glycogenic nutrients for milk production (van Knegsel et al., 2007). Most recommendations suggest starch levels between 25 to 35% on DM basis (Gencoglu et al., 2010); however, factors like variability across types of grain, processing, differences in particle size, and mixtures of different sources could alter the responses by the cows (Firkins et al., 2001).

In the rumen, the end-products of starch fermentation could generate responses like pH reduction, which negatively affects the growth of fibrolytic bacteria. Cellulotic and hemicellulotic microorganisms are sensitive to an acidic rumen environment, especially if pH drops below 5.5, resulting in a reduction of fiber digestibility (Sung et al., 2007). Dietary starch also alters the profile of VFA produced, by increasing the amount of propionic acid and reducing the acetic acid. In addition, a reduction in DMI has been reported when the quantity of grain added to the diet exceeds the recommended concentration of starch (Calsamiglia et al., 2007).

To lessen the negative effects of high starch concentrations and to reduce the cost of the diets, alternative energy supplements have been included in dairy rations (Carver, 2007). Molasses, whey and citrus pulp are some examples of feedstuffs used to replace corn starch, and these ingredients share a high content of sugar (Heldt et al., 1999), which is water soluble and rapidly fermented in the rumen (Chamberlain et al., 1993).

Sugars in the rumen can undergo hydrolysis and fermentation in less than one hour, providing large amounts of rapidly available energy for microbial protein production (Penner and Oba, 2009). Based on their features, it is possible that sugars could have similar effects as starch. However, sugar inclusion could provide substrate for lactic acid-utilizing bacteria, and supporting a stable population of these microbes may provide a buffer against lactic acid accumulation and ruminal pH depression. Subsequently, this effect may result in a better environment for cellulolytic bacteria and increased fiber digestibility (Firkins, 2010).

In addition to the potential metabolic effects of sugars, another rationale for including them in diets for lactating cows is the effect on diet palatability, given that cows tend to prefer sweet flavors (Nombekela et al., 1994). Also, sugars supplied in a liquid form can help to bind large and small particles in the rations, with the advantage of reducing sorting behavior and improving diet uniformity.

Despite the fact that sugar has been added to diets for lactating cattle for many years, the impact of this strategy remains controversial. To answer this question with confidence, responses to dietary sugar need to be evaluated across large numbers of animals with a variety of diet types. Meta-analytical techniques provide the statistical framework to assess results of numerous studies simultaneously, providing more statistical power and a broader inference space than any individual study. Based on the guidelines developed in other fields (Zwahlen et al., 2008; Greenwald et al., 2009; Crowther et al., 2010), we performed a meta-analysis to assess the effects of sugar inclusion in dairy diets.

Materials and methods

Literature review

The information used for this meta-analysis included treatments from studies reported between 1985 and 2012. To find as many studies as possible, an extensive review was performed during 2012. Multiple databases, including CAB abstracts, Web of Science, Agricola, Agris, CRIS, PubMed, Science Direct, S-PAC and Google Scholar were searched to identify relevant papers, books, abstracts, or conference proceedings that could match the selection criteria (Lean et al., 2009; Ceballos et al., 2009). Some of the terms used to find relevant information were "sugar", "sucrose", "molasses", "dairy", "starch replacement", "liquid feed", "dry matter intake", "milk production", "sugar and intake" and "dietary sugar". When one paper cited another paper with similar characteristics, the referenced paper was reviewed to see if it followed the defined criteria (Duffield et al., 2008a).

Inclusion and exclusion criteria

Treatments were included in the analysis only if the added sugar ranged between 2% and 5% of the diet DM. Studies with less than 2% added sugar were excluded because the physical effect of such treatment was likely to have greater impact than the change in the nutrient profile (Bradford and Mullins, 2012). The upper limit was established based on the results presented in previous studies suggesting that responses to dietary molasses are beneficial up to that point, after which the responses are negative (Broderick and Radloff, 2004). Furthermore, very few treatments were found in the literature with sugar added at more than 5% of DM. In addition, only studies that directly tested responses to molasses or sucrose in lactating cows were evaluated; other sources of sugar were excluded because of too many confounding nutrients or because of poor representation in the literature (i.e. lactose). If the sugar sources contained any fat, they were excluded from the meta-analysis to avoid possible confounded effects unless fat source and amount were balanced in the control diet.

A total of 10 published studies were found with at least 1 treatment comparison meeting these criteria, and 2 studies published in abstract form were included in the database, providing 18 total treatment comparisons. Data published in abstract form only were included to reduce publication bias as much as possible (Duffield et al., 2008a).

Data extraction

To assess dietary factors that may influence responses to sugar, the dietary concentrations of fat, NDF, forage NDF (fNDF), starch, crude protein (CP), rumen undegradable protein (RUP), and rumen degradable protein (RDP) were included for each treatment comparison. When these values were not reported, typical compositions of feedstuffs were assumed to derive estimated nutrient concentrations (NRC, 2001). In all the studies included, sugar sources replaced corn grain, but few studies reported basal concentrations of either sugar or starch, limiting the ability to evaluate these important factors. However, to allow for comparisons across sucrose and molasses studies, total sugar added was estimated for each treatment. Molasses was assumed to be 55% sugar on a DM basis unless actual sugar content was reported. Finally, responses for each comparison were calculated as the mean for the sugar treatment minus the mean for the appropriate control treatment. The corresponding standard error of each mean was recorded. One of the selected studies was removed from the meta-analysis as an outlier because the variance calculated for DMI was more than 8 times greater than the mean variance across studies (Z-score: 3.3).

Statistics

The analysis included original studies with either fixed effects or mixed effect statistical models. In the cases where repeated measures designs (i.e. Latin squares) were originally analyzed with mixed effect models, they were re-analyzed by the authors to estimate fixed effect model SEM in order to weight all the studies on similar bases.

The meta-analysis was conducted by using a random effects model, with the purpose of determining the variation or heterogeneity among studies by following the assumption that the true effects follow a normal distribution. By accounting for within-study variation and variation in the true effect across studies, the random effects model assigns weight properly to model both sources of variation (Sutton et al., 2000; Borenstein et al., 2009; Ceballos et al., 2009).

The model used to estimate the random effects was:

$$Y_i = \mu + \theta_i + \varepsilon_i$$

Where:

Y_i = is the effect of the i^{th} study

μ = is the true effect

θ_i = is the random effect of the study

ε_i = is the residual error

To evaluate the influences of dietary factors in the responses, we included them in a separate model with a two-step process. First, we tested each dietary factor, squared terms, and all 2-way interactions in a backward regression model using the Stepwise Procedure of SAS 9.3 (SAS Institute Inc., 2011). Data were weighted using the weight statement as recommended by St-Pierre (2001). The second step was done to test all the factors that were retained at a significant level ($P<0.05$) in the backward regression were then evaluated in the Mixed Procedure of SAS 9.3 with a random intercept for each study and weighting as described for the Stepwise Procedure.

Heterogeneity

In order to draw proper overall conclusions, and to determine if the observed effects were related to the type of sugar, we tested the between-study variation or heterogeneity, which is identified when the variation between the results of the studies is more than the differences that would be expected due to random error alone (Higgins and Thompson, 2002; Wallace et al., 2009). The heterogeneity test used was Cochran's Q statistic and it describes the extent of variability in effect across studies (Lean et al., 2009).

Publication bias/ trim and fill method

A common problem with the literature is the tendency of investigators to publish only studies with positive responses to treatments. Since the main objective of this research is to provide an unbiased assessment of the effects of sugar on production outcomes, we statistically evaluated the available evidence to estimate the risk of publication bias in this literature (Duffield et al., 2008b; Lean et al., 2009).

The funnel plot was the tool used to obtain a visual perspective of possible publication bias. This type of graphs plots the standard error of the difference (SED) for each treatment comparison on the Y axis, and on the X-axis the difference in means (Halasa et al., 2009). To indicate the absence of publication bias, the funnel must have symmetrical shape (Duffield et al., 2008a), where studies with more weight (lower SED) are closer to the overall mean, whereas less weighted studies have more scatter, but remain symmetrical on either side of the overall mean (Halasa et al., 2009).

To corroborate the information obtained from the funnel plots, we performed the Trim and Fill Method (Duval and Tweedie, 2000; Rothstein et al., 2005; Erdreich et al., 2009). This procedure estimates any possible missing studies and re-estimates the mean effect size by including the predicted missing values (Duval and Tweedie, 2000; Sterne et al., 2001; Ceballos et al., 2009).

Results and discussion

The analysis for variation between studies (heterogeneity) is presented in Table 1. Cochran's *Q*-test was performed to test the null hypothesis that all studies shared a common effect size for DMI, milk yield, ECM yield, milk fat content and milk protein content, across both sucrose and molasses sugar sources. With the results obtained, we failed to reject the null hypothesis and concluded that there was no evidence for a differential response between sucrose and molasses.

The visual evaluation of publication bias in the form of funnel plots is shown in Figures 1 to 5. For DMI (Figure 1), milk yield (Figure 2), and milk composition (fat and protein in Figures 4 and 5), the distribution of the studies revealed symmetry about the mean effect size, and that indicated that there was no evidence for publication bias. Statistical analysis by the Fill and Trim method also failed to find evidence of missing studies for these variables.

In the case of ECM, the responses were not dramatically different; Figure 3 does not provide strong evidence for publication bias, at least visually. However, the Trim and Fill method suggested that two possible additional studies with negative response were required to generate a normal response distribution (Duval and Tweedie, 2000). Overall, however, there was little evidence to suggest that publication bias is a serious problem in this data set.

The result of the meta-analysis for each outcome variable is presented in the form of a forest plot (Figures 6 to 10). The result for DMI for this study suggested that replacing corn grain with sugar in a range between 2 and 5% of DM in dairy rations tended (P = 0.08) to increase DMI by 0.38 kg/d (Figure 6). One possible explanation for this tendency is related to the palatable effect of sugar sources in rations (Broderick and Radloff, 2004; Kitessa et al., 2004). Alternatively, such an effect could also be explained by better attachment of bacteria to structural carbohydrates, more rapid digestion of fiber, decreasing the ruminal residence time of fiber and increasing DMI. A third possible mechanism is by reducing the net propionate absorption. Some authors indicate that when propionate exceeds the liver's requirements to produce glucose it sends a negative feedback that reduces intake (Allen et al., 2009; Firkins, 2010). By providing sugar, the proportions of VFA produced in the rumen are altered; more acetate, butyrate, valerate and branched chain VFA are produced but not typically propionate (Heldt et al., 1999; Broderick and Radloff, 2004; Hall and Weimer, 2007; Oelker et al., 2009; Lechartier and Peyraud, 2011), so this feedback could be prevented. However, we did not conduct a meta-analysis of sugar effects on ruminal VFA. Regardless of the mechanism, the putative impact on DMI was not reflected in milk production; the results presented in Figures 7 and 8 did not reveal evidence that sugar inclusion also affected milk or ECM yield, which could be expected with the increment in DMI.

Sugar inclusion showed a tendency to increase milk fat percentage (mean +0.0850 ± 0.0474%, $P = 0.07$). This may be attributed to the fact that sugars provide a better ruminal environment for microbes responsible for biohydrogenation, in part by increasing ruminal pH (Broderick and Radloff, 2004; Firkins, 2010; Martel et al., 2011). Enhanced ruminal biohydrogenation decreases the absorption of fatty acid intermediates that suppress milk fat synthesis in the mammary gland.

Dietary factors

The analysis with dietary factors revealed that DMI responses were significantly altered by the interaction of fNDF content and the amount of added sugar (Figure 11). The plot demonstrates that greater sugar inclusion negatively impacted the DMI responses when dietary fNDF was low (Figure 12); conversely, when dietary fNDF was high, DMI responses increased as more sugar was added (Figure 13).

We hypothesize that in rations with high fNDF, the inclusion of sugar provided more available energy to the cellulolytic bacteria, which didn't have to compete with starch digesting bacteria for the same energy source, allowing them to colonize the fiber substrate faster, which could increase the digestibility of the diet, allowing the animal to consume more (Mouriño et al., 2001; Firkins, 2010). In low fNDF diets, on the other hand, rumen fill is unlikely to be the factor limiting feed intake, and improving fiber digestibility is therefore unlikely to increase DMI (Allen et al. 2009).

The analysis of milk production (Figure 14) indicated that the interaction between amount of added sugar and dietary RUP tended to influence the milk yield response ($P = 0.09$). The addition of progressively more sugar with the lowest concentration of RUP tended to diminish milk production (Figure 15); on the other hand, with more RUP in the diets, greater sugar inclusion tended to increase milk production (Figure 16). These findings are inconsistent with typical advice that sugar supplementation requires additional RDP to generate production responses. It is possible that this association reflect interactions of sugar with forage types that differ in protein availability (i.e. alfalfa silage vs. corn silage) rather than RUP *per se*. This association was statistically marginal, and for both dietary interactions, it is important to note that the 18 treatment comparisons were inadequate to fully represent the interaction space shown in the graphs. The associations should be viewed as suggestions for forming testable hypotheses, rather than as establishing a definite cause-and-effect relationship.

No dietary factors or interactions were found to significantly affect the ECM yield, milk fat content, or milk protein content responses.

Conclusions

In summary, an assessment of responses across 18 treatment comparisons failed to demonstrate clear production effects when sugar was added at 2 to 5% of diet DM. However, interactions between fNDF content of diets and the amount of sugar added by treatments suggest that one reason for variable responses to dietary sugar may be the wide range of diets in which these treatments were tested. Further research to directly test the differential effects of sugar supplementation in high fNDF vs. low fNDF diets may help to clarify the importance of considering which diets benefit the most from the addition of sugar sources.

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Figures and tables

Table 3.1 Heterogeneity analysis for sucrose vs. molasses differences in means for dry matter intake, milk production, and milk components when corn grain was replaced with ingredients providing 2 to 5% (DM basis) sugar.

Parameter	*Q* - Value	*P* - Value
Dry matter intake, kg/d	1.35	0.24
Milk yield, kg/d	0.29	0.59
Energy-corrected milk, kg/d	0.29	0.60
Fat content, %	0.05	0.81
Protein content, %	0.02	0.90

Figure 3.1 Funnel plot of standardized mean differences of dry matter intake when 2 to 5% dietary sugar replaced corn grain in diets for dairy cattle. The X-axis represents the difference in means. The Y-axis indicates the standard error. The open circles are the observed studies. Open diamond represents the observed point estimate. The solid diamond represents the imputed point estimate including predicted missing studies. Coincidental diamonds indicate the absence of publication bias.

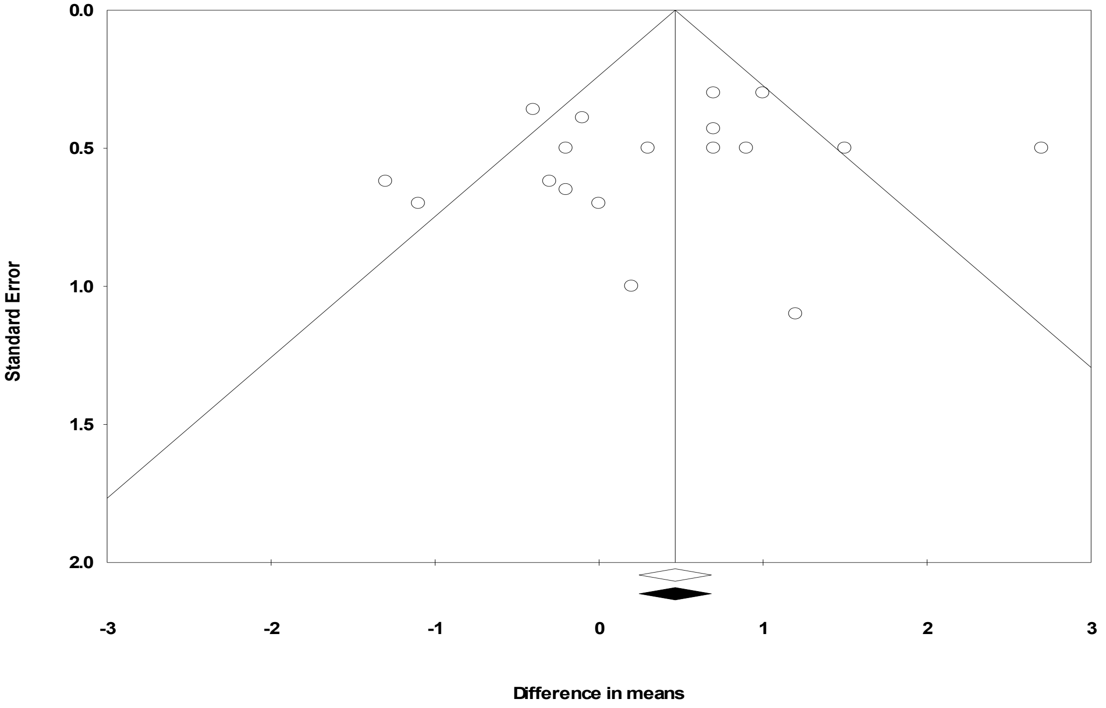


Figure 3.2 Funnel plot of standardized mean differences of milk yield when 2 to 5% dietary sugar replaced corn grain in diets for dairy cattle. The X-axis represents the difference in means. The Y-axis indicates the standard error. The open circles are the observed studies. Open diamond represents the observed point estimate. The solid diamond represents the imputed point estimate including predicted missing studies. Coincidental diamonds indicate the absence of publication bias.

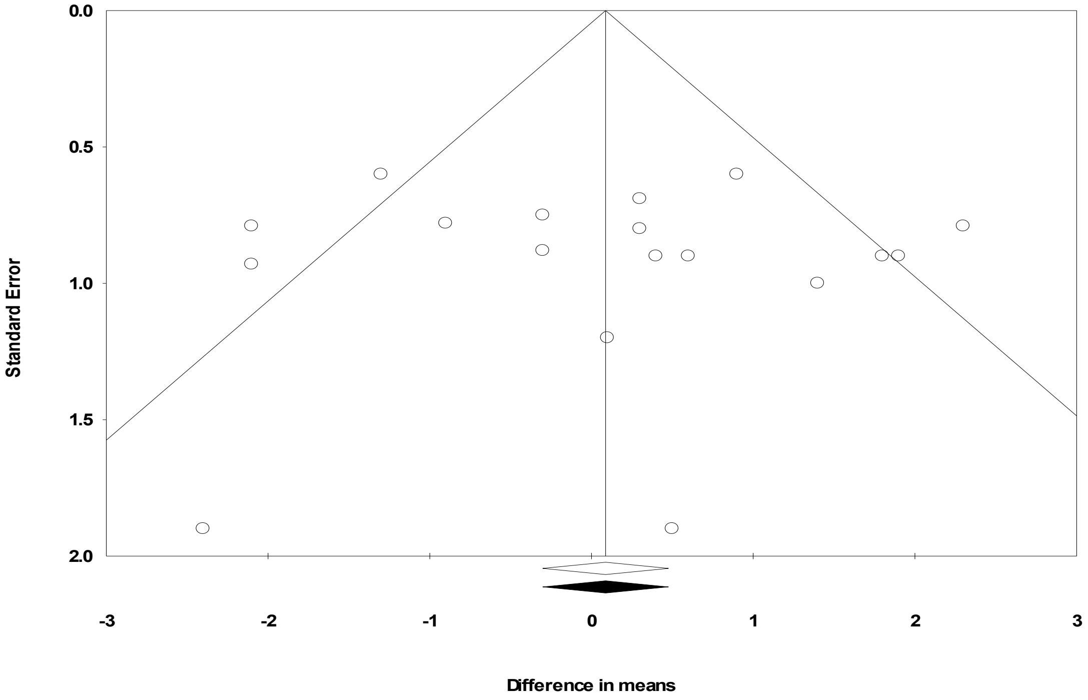


Figure 3.3 Funnel plot of standardized mean differences of energy-corrected milk when 2 to 5% dietary sugar replaced corn grain in diets for dairy cattle. The X-axis represents the difference in means. The Y-axis indicates the standard error. The open circles are the observed studies. The solid circles are possible missing studies after the Trim and Fill method. Open diamond represents the observed point estimate. The solid diamond represents the imputed point estimate including predicted missing studies.

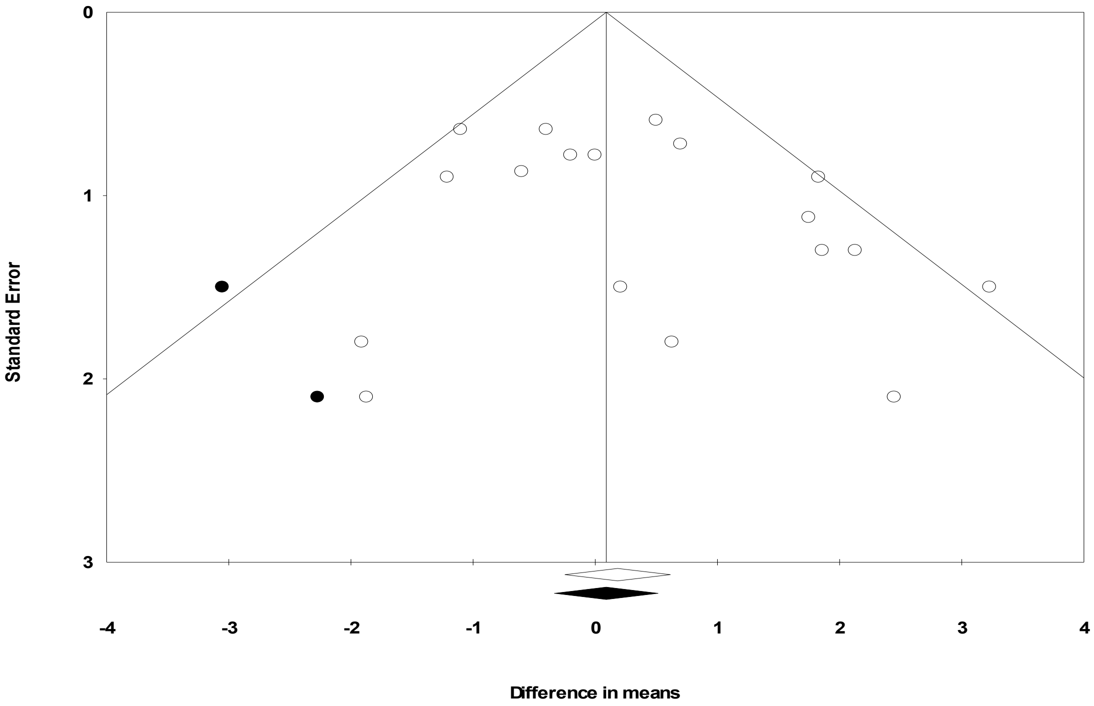


Figure 3.4 Funnel plot of standardized mean differences of milk fat content when 2 to 5% dietary sugar replaced corn grain in diets for dairy cattle. The X-axis represents the difference in means. The Y-axis indicates the standard error. Open diamond represents the observed point estimate. Solid diamond represents the imputed point estimate including predicted missing studies. Coincidental diamonds indicate the absence of publication bias.

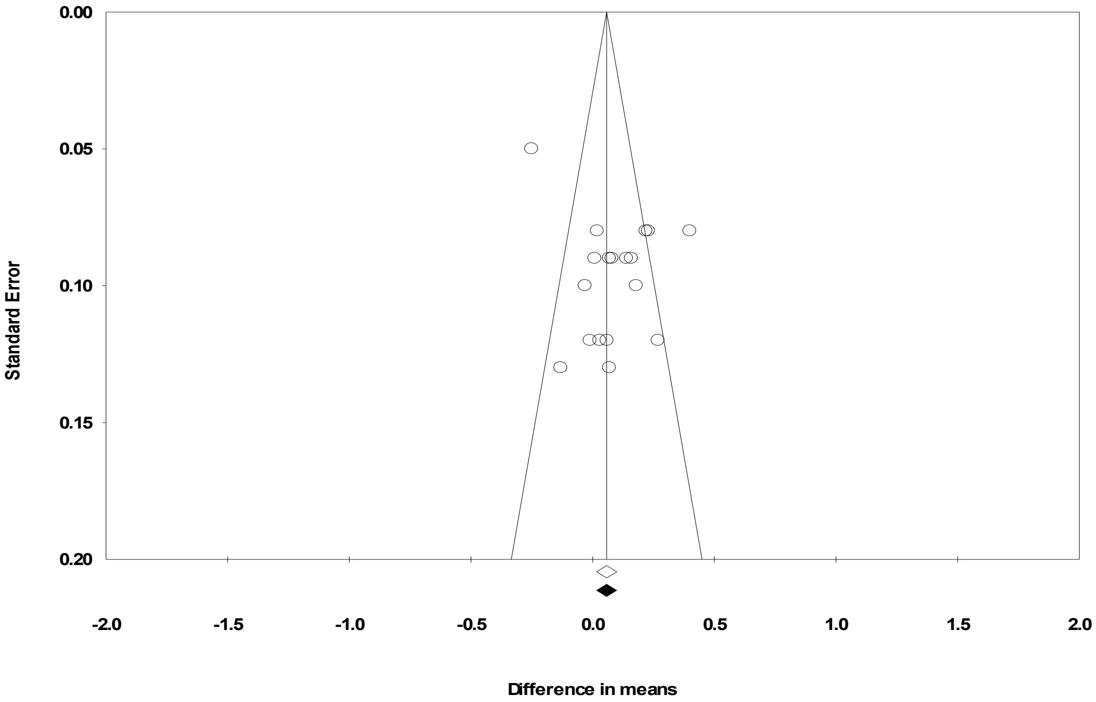


Figure 3.5 Funnel plot of standardized mean differences of milk protein content when 2 to 5% dietary sugar replaced corn grain in diets for dairy cattle. The X-axis represents the difference in means. The Y-axis indicates the standard error. The open circles are the observed studies. Open diamond represents the observed point estimate. The solid diamond represents the imputed point estimate including predicted missing studies. Coincidental diamonds indicate the absence of publication bias.

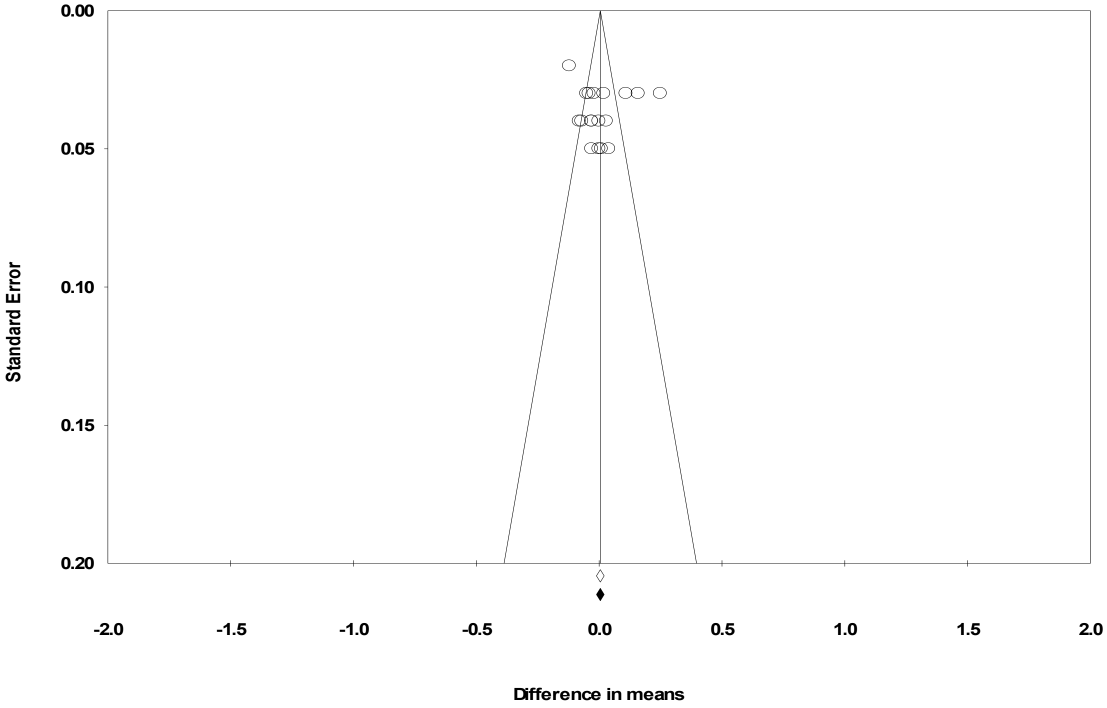
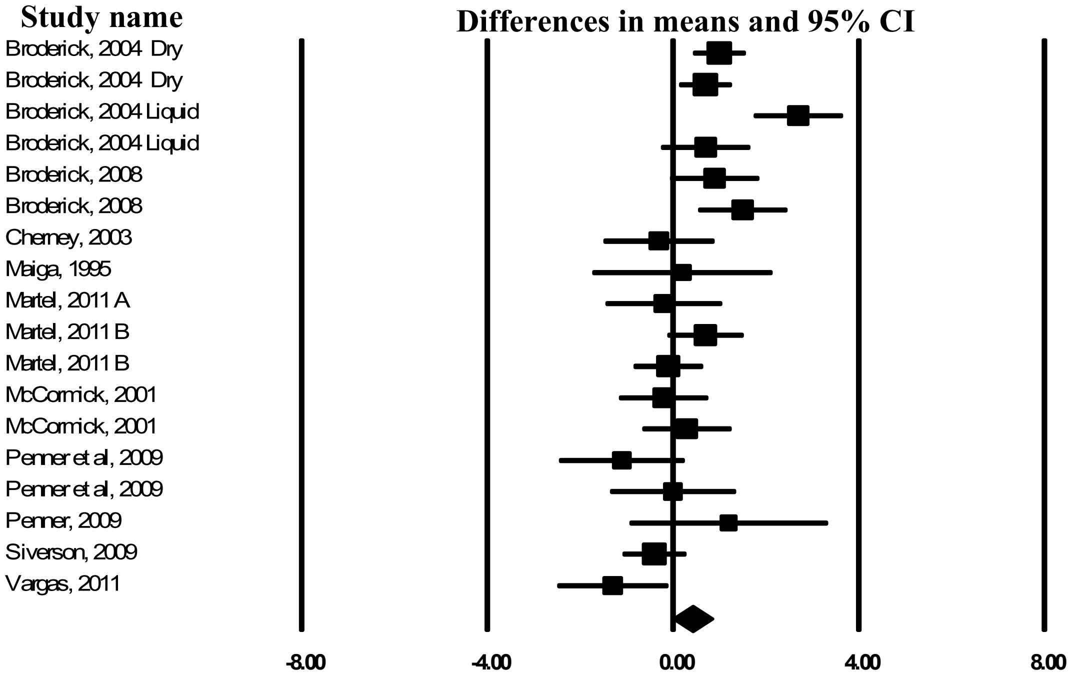
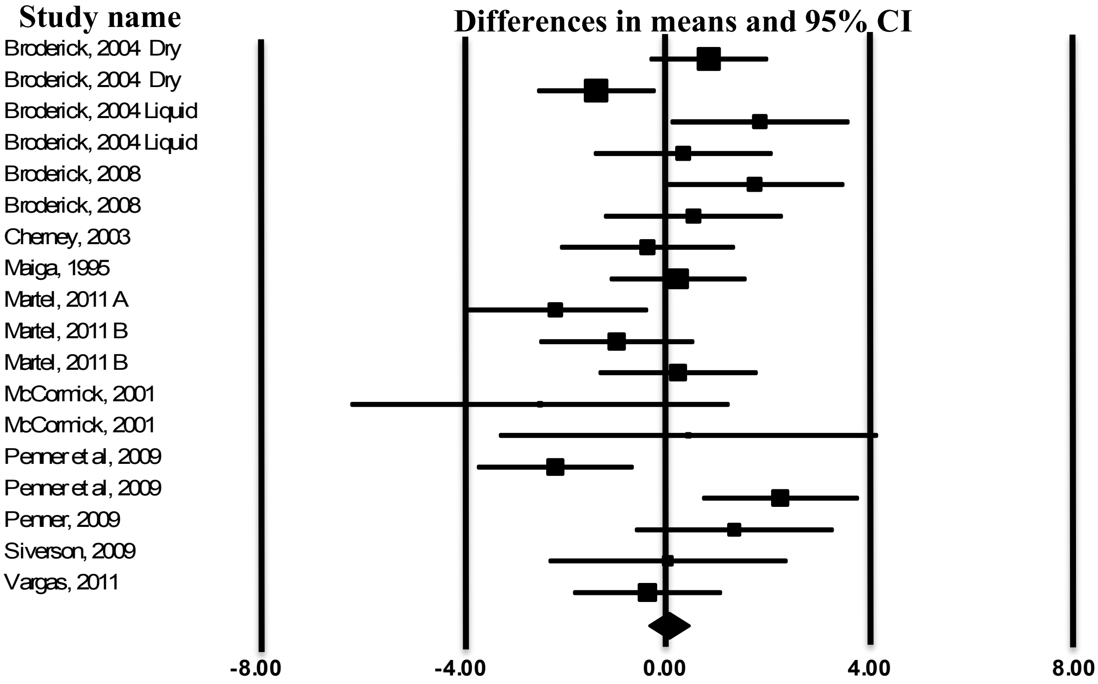


Figure 3.6 Meta-analysis to determine dry matter intake responses when 2 to 5% dietary sugar replaced corn grain in dairy rations. The overall mean and SEM is included below the plot.



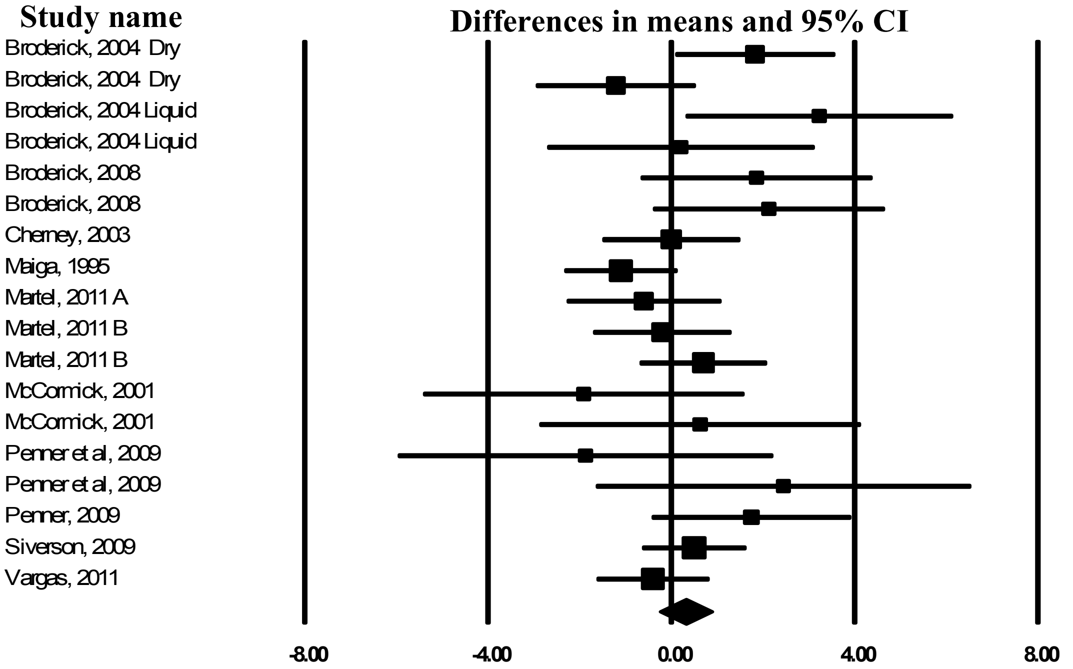
Mean + 0.38 ± 0.21 kg/d *P* = 0.08

Figure 3.7 Meta-analysis to determine responses on milk yield when 2 to 5% dietary sugar replaced corn grain in dairy rations. The overall mean and SEM is included below the plot.



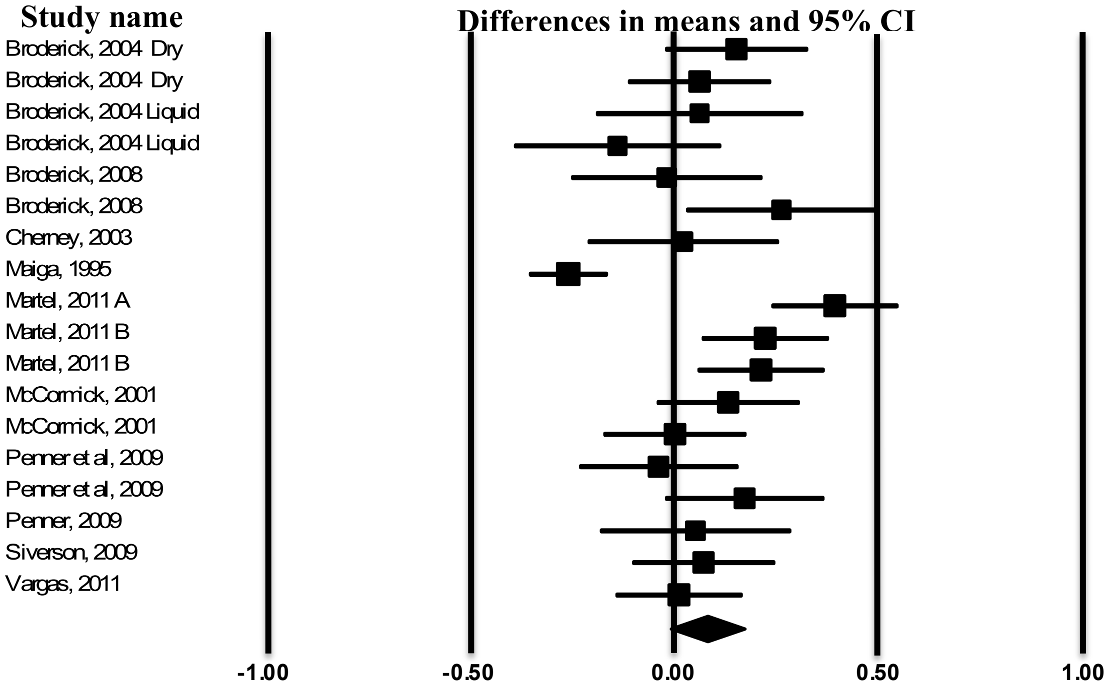
Mean + 0.11 ± 0.33 kg/d ***P*** **= 0.74**

Figure 3.8 Meta-analysis to determine responses on energy-corrected milk yield when 2 to 5% dietary sugar replaced corn grain in dairy rations. The overall mean and SEM is included below the plot.



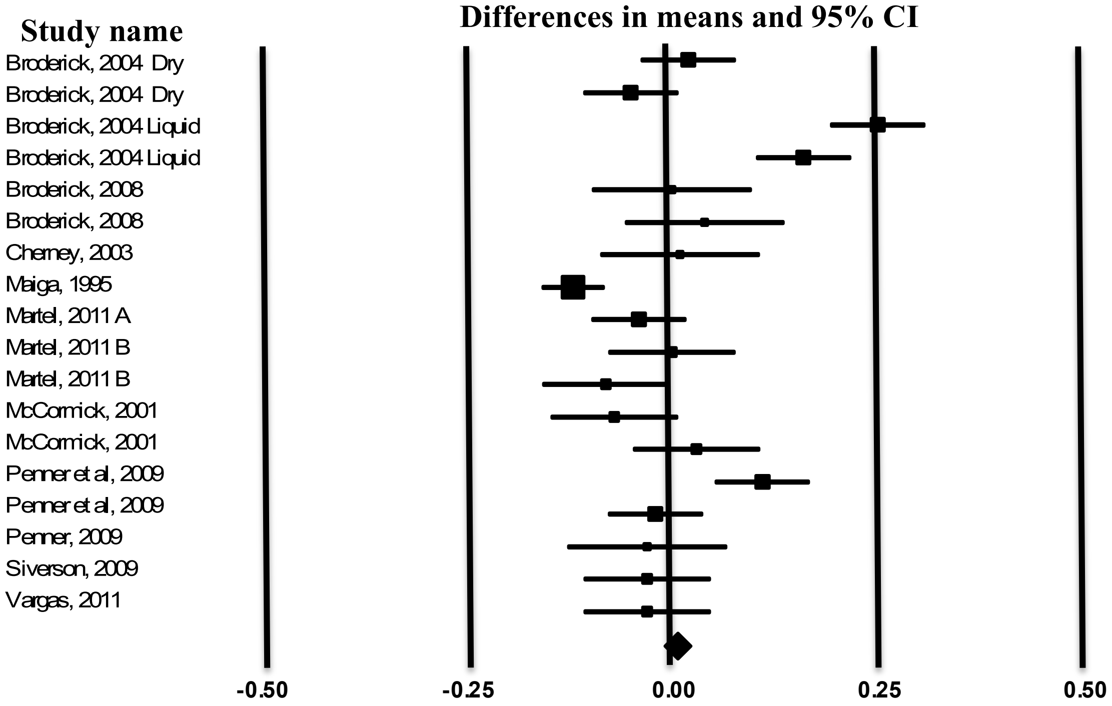
Mean + 0.29 ± 0.30 kg/d ***P*** **= 0.33**

Figure 3.9 Meta-analysis to determine responses on milk fat content when 2 to 5% dietary sugar replaced corn grain in dairy rations. The overall mean and SEM is included below the plot.



Mean + 0.085 ± 0.05%   *P* = 0.07

Figure 3.10 Meta-analysis to determine responses on milk protein content when 2 to 5% dietary sugar replaced corn grain in dairy rations. The overall mean and SEM is included below the plot.



Mean + 0.008 ± 0.03% ***P*** **= 0.74**

Figure 3.11 Plot of the interaction ($P < 0.05$) between added sugar (X) and forage NDF (Z) affecting dry matter intake (Y). $Y = 13.1567 – 4.4028 X – 0.5419 Z + 0.1690 X*Z$.

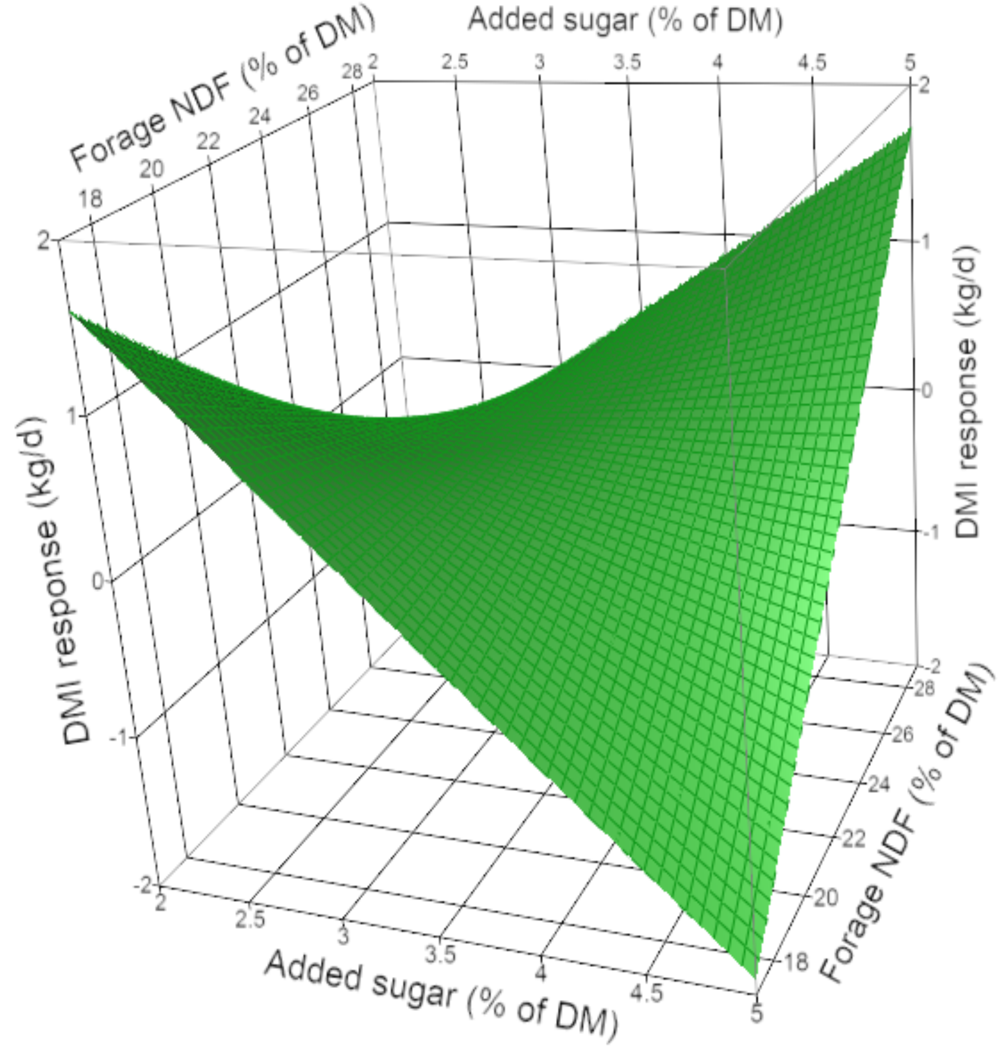


Figure 3.12 Predicted dry matter intake response to amount of added sugar when forage NDF is fixed at 18% of DM.

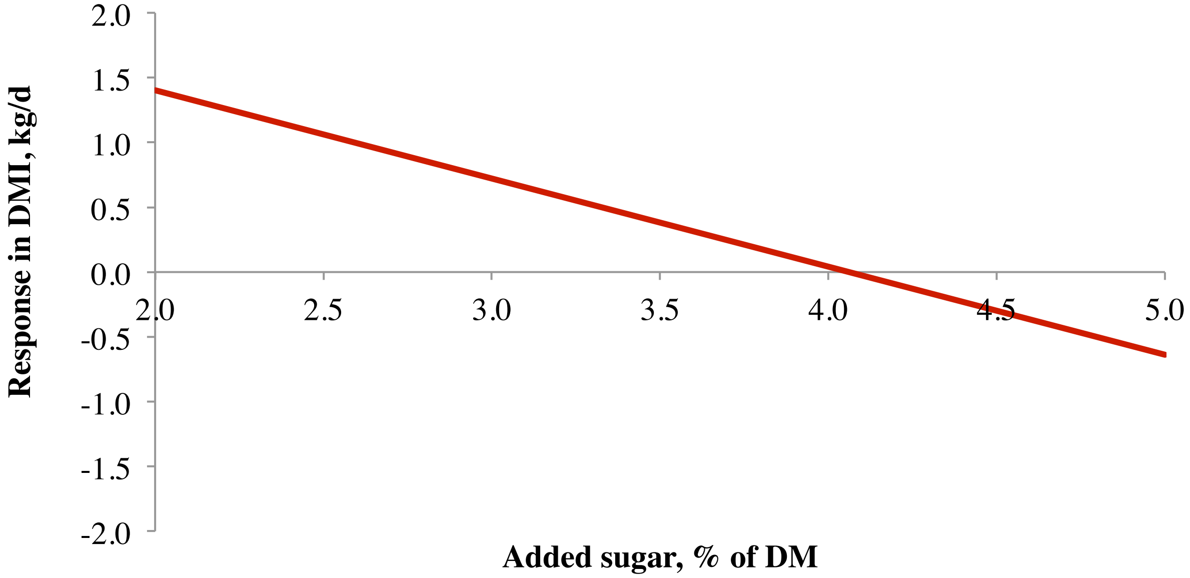


Figure 3.13 Predicted dry matter intake response to amount of added sugar when forage NDF is fixed at 28% of DM.

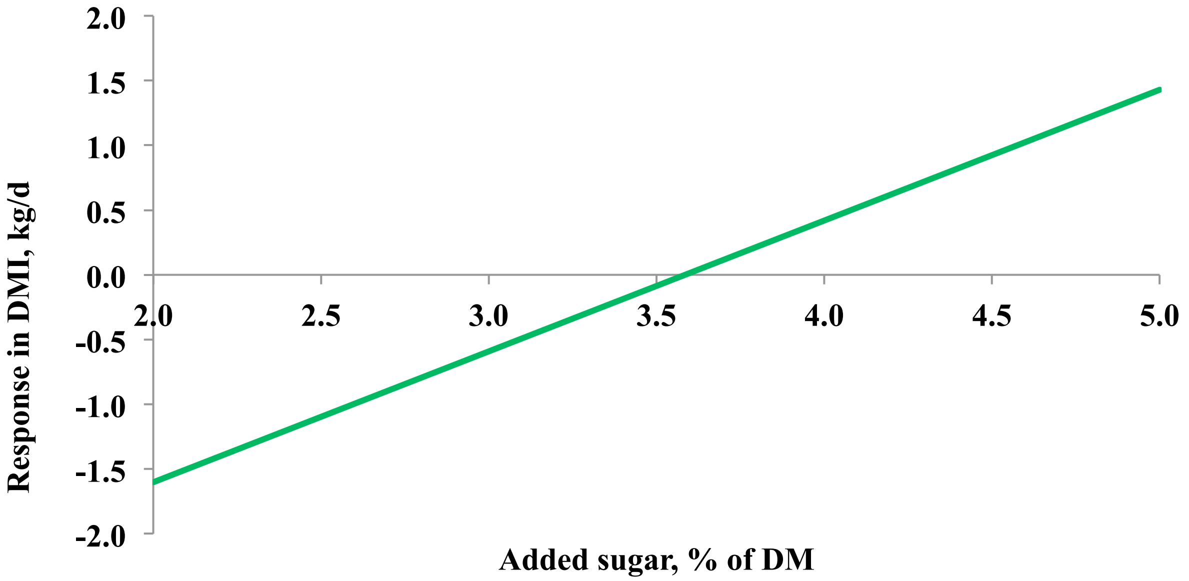


Figure 3.14 Plot of the interaction ($P = 0.09$) between added sugar (X) and RUP (X) affecting milk production (Y). $Y = 15.1597 - 3.7657\ X - 1.8612\ Z + 0.4705\ X*Z$.

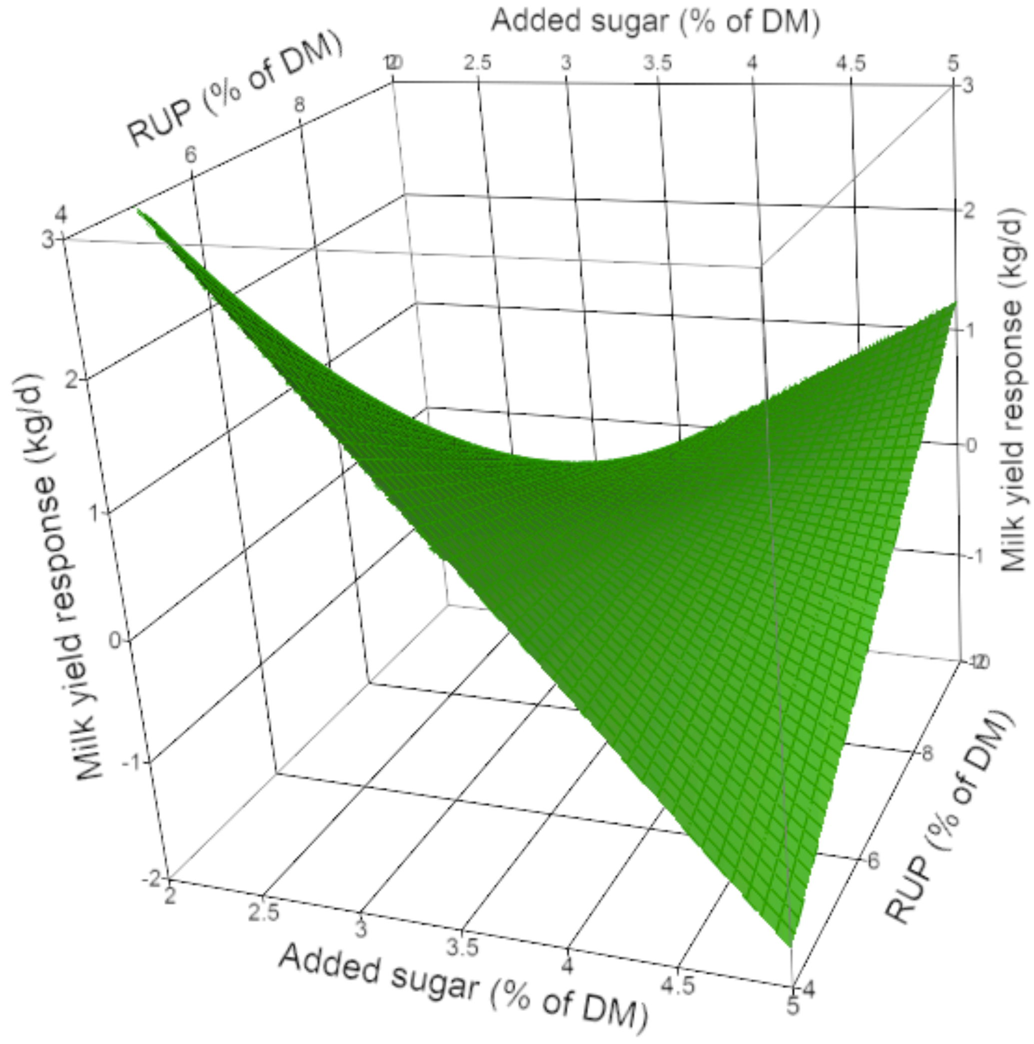


Figure 3.15 Predicted milk production response to amount of added sugar when RUP is fixed at 4% of DM.

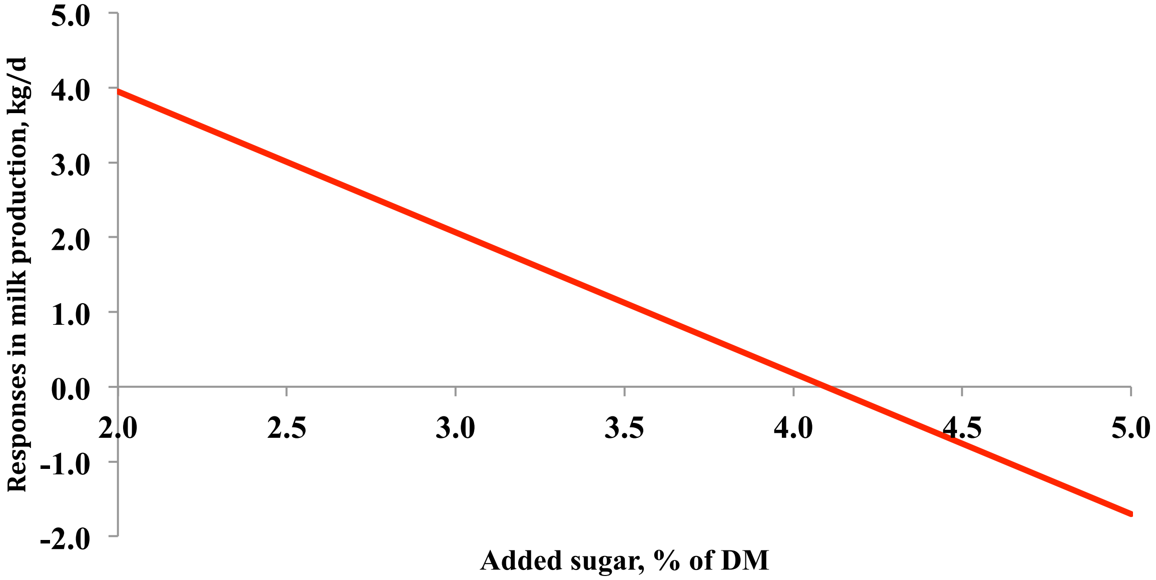


Figure 3.16 Predicted milk production response to amount of added sugar when RUP is fixed at 10% of DM.