University of São Paulo
"Luiz de Queiroz" College of Agriculture

Effect of length of storage on reconstituted sorghum grain silages treated with sodium benzoate on nutritive value and dairy cows performance

Willian Pereira dos Santos

Thesis presented to obtain the degree of Doctor in Science. Area: Animal Science and Pastures

Piracicaba
2019

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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1. Silagem de grãos 2. Proteolise 3. Produção de leite 4. Sorgo reconstituído I. Título

To my parents and brothers.

I DEDICATE

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I would like to thank God for life and be aware of that.

To my parents (Maria Hilda and Geraldo Pereira) and brothers (Gilson, Gildécio, Gilda, Gilvânio, Gilmacio and Gilvair) for their love, and all efforts they have been made to my growth as human being.

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RESUMO

Efeito do tempo de estocagem em silagens de grãos de sorgo reconstituído tratadas com benzoato de sódio no valor nutritivo e desempenho de vacas leiteiras

A ensilagem de grãos úmidos geralmente aumenta a digestão do amido e da proteína devido a proteólise durante o armazenamento. Porém, o tempo de armazenamento é fundamental para permitir que a matriz proteica seja degradada. O objetivo central desse trabalho foi avaliar o efeito do tempo de estocagem em silagens de sorgo grão reconstituído (SSGR) no desempenho de vacas leiteiras. De forma simultânea foi avaliado o efeito do benzoato de sódio no valor nutritivo de silagens de sorgo grão reconstituído. A hipótese foi que o uso de benzoato de sódio em SSGR reduz a atividade proteolítica devido suas propriedades antimicrobianas impactando no desempenho animal. Uma sequência de dois experimentos com vacas leiteiras da raça Holandesa foram formatados. O primeiro experimento avaliou o efeito de diferentes tempos de estocagem em SSGR tratadas ou não com benzoato de sódio (0.2 % base na matéria natural). Silagens não tratadas (Controle) e tratadas com aditivo (Benzoato) foram armazenadas por 30 ou 90 dias antes do fornecimento. Vinte vacas leiteiras (168 ± 87 dias em lactação) foram usadas em cinco quadrados latinos replicados 4 × 4 em arranjo fatorial 2 × 2. Sorgo grão foi reconstituído para 35 % de umidade em ambos os experimentos e ensilados em tambores plásticos com capacidade de 200-L. Os tratamentos foram: SSGR armazenados por 30 dias sem aditivo (30 CON), SSGR armazenados por 30 dias com benzoato de sódio (30 BEN), SSGR armazenados por 90 dias sem aditivo (90 BEN) ou SSGR armazenados por 90 dias com benzoato de sódio (90 BEN). Prolongando o tempo de armazenamento a concentração de 1,2-propanodiol e proteina solúvel aumentaram. A produção de leite foi maior em favor das vacas alimentadas com silagens armazenadas por 90 dias comparadas à 30 dias (31.2 vs. 30.0 kg/d). A digestibilidade do amido (89.3 vs. 86.9%) e da proteina (57.1 vs. 54.0%) foi maior para silagens armazenadas por 90 dias. O benzoato de sódio reduziu a concentração de ethanol (0.20 vs. 0.08% of DM), porém não alterou ao nível de significância estatística adotada nesse trabalho a proteína solúvel (CON = 18.9 vs. BEN = 15.6% of PB) e o N-amoniacal (CON = 4.38 vs. BEN = 3.94 % of N). O segundo experimento foi conduzido com 12 vacas leiteiras (170 ± 47 DEL) para avaliar o efeito do benzoato de sódio no valor nutritivo e desempenho de vacas leiteiras alimentadas com SSGR estocadas por 150 dias, tratadas (Benzoato) ou não (Controle) com benzoato de sódio. Uma dieta padrão contendo sorgo grão seco moído foi fornecida por 14 dias. No final do período de adaptação, os animais foram pareados dois a dois e distribuídos aleatoriamente a um de dois tratamentos (Controle ou Benzoato) fornecidos por 28 dias. Durante o período experimental as vacas receberam a mesma dieta sendo o sorgo seco totalmente substituído por SSGR. Silagens tradadas com benzoato de sódio tiveram menor concentração de etanol (0.84 vs. 0.18 % de MS) e etil lactato (388 vs. 157 mg/kg de MS) como consequência de uma menor população de leveduras (4.73 vs. 2.52 log ufc/g). A proteína solúvel foi reduzida nas silagens tratadas (26.2 vs. 20.6 % da PB). A estabilidade aeróbia foi mais alta nas silagens tratadas (51 vs. 146 h). Em conclusão, estender o tempo de estocagem em SSGR aumentou a eficiência alimentar e do uso do nitrogênio devido ao aumento na digestibilidade do amido e da proteína. O benzoato de sódio promoveu respostas típicas na fermentação de silagens, e não alterou o desempenho animal.

Palavras-chave: Silagem de grãos; Proteólise; Produção de leite; Sorgo reconstituído

ABSTRACT

Effect of length of storage on reconstituted sorghum grain silages treated with sodium benzoate on nutritive value and dairy cow performance

Ensiling high moisture grain often increases starch and protein degestibilities due proteolysis during storage. However, the length of storage is fundamental to allow great protein matrix break down. The central objective of this work was to evaluate the effect of length of storage of reconstituted sorghum grain silage (RSGS) on dairy cows performance. Simultaneously it was evaluated the effect of sodium benzoate on silage nutritive value and its impact on animal performance. The hypothesis was that sodium benzoate reduces proteolitic activity due its antimicrobial properties. Two sequential experiments with mid-lactation Holstein dairy cows were set. The first experiment evaluated the effect of different length of storages on RSGS treated or not with sodium benzoate (0.2% as fed). Silages treated with additive (Benzoate) and non-treated (Control) were stored for 30 or 90 days prior feeding. Twenty mid-lactation dairy cows with 168 ± 87 days in milk (DIM) were used in 5 replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement. Dry ground sorghum grain was reconstituted to 35% moisture and ensiled in 200-L plastic drums. Treatments were: RSGS stored for 30 days without additive (30 CON), RSGS stored for 30 days with sodium benzoate (30 BEN), RSGS stored for 90 days without additive (90 CON) and RSGS stored for 90 days with sodium benzoate (90 BEN). Lengthening silage storage increased 1,2-propanediol concentration and protein solubility of silages. Milk yield was greater in favor of cows fed silage stored for 90 days compared to 30 days (31.2 vs. 30.0 kg/d). Starch (89.3 vs. 86.9%) and protein (57.1 vs. 54.0%) digestibility was also greater for silages stored for 90 days compared to 30 days. Sodium benzoate reduced silage ethanol concentration (0.20 vs. 0.08% of DM), but did not altered statistically protein solubility (CON = 18.9 vs. BEN = 15.6% of CP) or ammonium-N (CON = 4.38 vs. BEN = 3.94 % of N). The second trial were conducted with 12 mid-lactation dairy cows (170 ± 47 DIM) to evaluated the effect of sodium benzoate on nutritive value and dairy cows performance fed RSGS stored for 150 days, treated (Benzoate) or not (Control) with sodium benzoate. Cows received a standard diet containing dry ground sorghum for 14 days. At the end of adaptation period, cows were paired blocked and randomly assigned to one of two treatments (Control or Benzoate) for 28 experimental days. During experimental period cows received the same diet with the exception of dry ground sorghum, which was totally replaced with RSGS. Silages treated with sodium benzoate had low ethanol (0.84 vs. 0.18 % of DM) and ethyl-lactate (388 vs. 157 mg/kg of DM) concentration as a consequence of low yeast population (4.73 vs. 2.52 log cfu/g). Soluble protein was reduced on treated silages (26.2 vs. 20.6 % of CP). Aerobic stability was higher for treated silages (51 vs. 146 h). Dairy cow performance was not altered by treating silages with sodium benzoate. In conclusion, extending the length of storage of RSGS increased dairy cows use feed efficient and nitrogen use efficiency due higher starch and protein digestibility. Sodium benzoate promoted typical response on silage fermentation and did not alter animal performance.

Keywords: Grain silage; Proteolysis; Milk yield; Reconstituted sorghum

1. INTRODUCTION

High moisture grain has been used to feed ruminants since the early 1900s (Matushima, 2006). However, the large adoptions of ensiling high moisture corn begin in the 1960s due to two main reasons. With the advent of new technologies as hybridization, chemical nitrogen fertilizer, herbicides and pesticide molecules, corn production in North America rapidly increased. Therefore, Canada and United States were not strategically prepared to store grain in bins due to increased production. On this way, the government invested big money to build structures to store grain as high moisture silage. However, there was limited knowledge about the concern. Interest in learning about ensiling high moisture corn peaked between the 1960s and 1980s and much information and doubts about the subject emerged during that time. Aspects involving proper moisture content, processing and animal performance were the science targets. Later, at the end of the 1980s and beginning of the 1990s, some scientists began to more thoroughly investigate the impact and reasons for proteolysis due to ensiling. They observed a positive correlation between proteolysis and starch digestibility suggesting a physicochemical barrier of proteins involving starch granules. Searching for a better understanding of the protein structure, they observed that prolamins was the main protein composing the protein matrix, which is divided in four groups based on molecular mass and AA composition (Phillippeau et al., 2000; Branlard and Bancel, 2006; Hoffman et al., 2011).

To the best of my knowledge, the influence of length of storage on proteolyses was first reported in the literature in 1971 by Neuhaus. In 2005, Benton et al. showed that starch and protein degradability in situ increases with length of storage when proper moisture conditions and processing were attempted. Although the findings of Benton et al. (2005) suggest increase in starch and protein digestibility in vivo, no animal trials evaluating performance of dairy or beef cattle fed silage stored for different times have yet been conducted.

Chemical additives are used in silage conservation with distinct applications. In high moisture corn silage, the first interest in treating silage with chemical additives was to reduce proteolysis (Baron et al., 1986). The high content of soluble nitrogen in high moisture grain silage was primarily associated with poor fermentation and low performance of cattle (Priggie et al., 1976). With the positive effect of proteolysis on the starch and protein digestibility established, the use of chemical additives in grain silage was would better fit as aerobic deterioration and dry matter losses controller (Morais et al., 2017).

Although studies have demonstrated the benefits of ensiling high moisture grain on starch and protein degradability in situ, there is less understanding of the performance of animals fed silages with different degree of proteolysis caused by different lengths of storage. On the other hand, treating silage with chemical additives might reduce proteolysis and decrease animal performance. The objective of this work was to evaluate the effect of different lengths of storage, and the use of chemical additive (sodium benzoate) in sorghum grain silages on proteolysis and dairy cow performance.

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2. LITERATURE REVIEW

2.1. GRAIN SILAGE BACKGROUND

There are reports in the literature since 1928 citing concern about high moisture corn for ruminants; however, the corn was not ensiled (Becker and Gallup, 1928). The highest interest in storing grains as silage began in the late 1950s (Van Fossen, 1964). The high cost to build bin structures, incentivize farms to develop new ways of keep grains safe and with great nutritional value was reported by Buchanan-Smith (1976). In the northern hemisphere, the ensiling of cereal grains increased concern over adoption due to the possibility of earliest harvest and saving energy to dry kernels at safe moisture conditions (< 13%). Another reason that stimulated the conservation of grains as silage was the increase in corn and sorghum productivity after the advent of single and double hybrids, inorganic nitrogen sources (urea and ammonium) and herbicides (Figure 1). From 1954 to 1964, the state of Iowa alone invested 12 million dollars in storage facilities for high moisture corn (Van Fossen, 1964). Despite all the infrastructure investments made by the government, knowledge about the process that occurs in the silo, environmental conditions and nutritive value of the crops was scarce at that point.

Much progress was made during the ‘60s and ‘70s to better understand grain silage conservation and its use for ruminants. The first organized symposium to discuss knowledge of the subject was held in Oklahoma in 1976. Among the scientists were T.W. Perry (Purdue University) and Jimmy Clark (University of Illinois) which study HM for dairy cows. The conference scientific committee concluded that moisture is an important factor related to ensiling high moisture grain and should be above 30%. Neuhaus and Totusek (1971) reported that in vitro digestibility improvements were progressive with increments in moisture from 26 to 34%. Reconstitution of grains has been suggested as a promising tool to increase feed value by increasing starch and protein digestibility (Hinders, 1976). Priggie et al. (1976) reported that protein solubility in grain silage was slower and not quite as extensive in whole plant corn silage, although the mechanism has not been identified. With more protein utilization studies (Hinders, 1976), the increase in protein solubility of high moisture grain silage can potentially be an advantage instead of an occasional problem. Mechanical processing was recommended to increase digestibility compared to whole grain, but the results of research suggests that storage of whole and processed grain before feeding was more efficient (Fox, 1976). Although processing increases digestibility, when used in high concentrate diets (<15% of forage), ground or rolled grain was not recommended in function of digestive disorders (Dexheimer, 1973) cited by Fox (1976).

Over the next years, the scientific committee members resolved to dive deeper into the questions raised during that first symposium. These included elucidating mechanisms that increase protein solubility, different types of processing and better predictions of animal performance. In 2006, some 30 years after the 1976 symposium (and also in Oklahoma), the Cattle Grain Processing Symposium was organized, with a focus on a wider range of grain processing that were discovered in the intervening years.

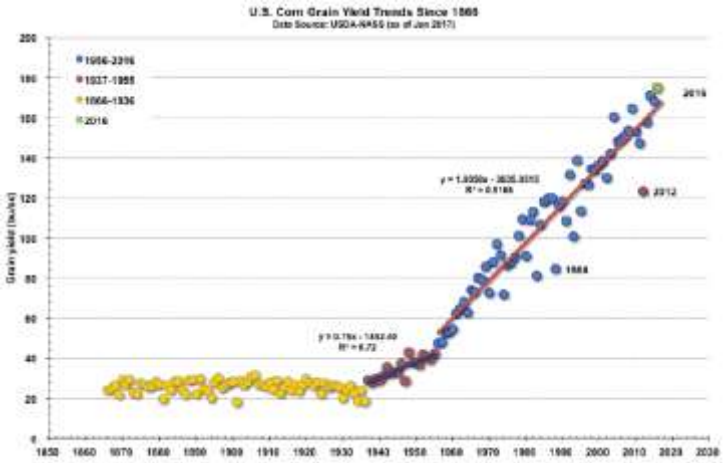

Figura 1. Evolution of productivity of corn in United States of America since 1866 (Nielson, R. L - Purdue University)

In Brazil, the first description of ensiling high moisture grain was in early 1980 in Castro-PR, to feed swine with posterior use in cattle nutrition (Kramer and Voorsluys, 1991). As in the United States, the reasons for utilizing the technology were basically economic. The first publication evaluating strategies to ensile high moisture corn grain in Brazil dates from 1996. Jobim et al. (1996) evaluated the effect of different proportions of cobs in the silage microbiology. The use of high moisture corn for cattle in Brazil has not historically been a big concern. Millen et al. (2009) report that only 12% of feed lots use high moisture grain as a second grain source, and none as the first grain source. The main processing methods for first grain source were finely ground (54.8%), and cracked (38.7%). In 2014, the same survey was applied and a modest increase from 12 to 18.8% was observed for adoption of high moisture corn (Millen et al., 2014). One of the reasons for low adoption of high moisture corn is that cattle farmers do not grow corn for self-utilization; they often go to the market to buy it. Also, the machinery needed to harvest and process the grain prior ensiling is quite expensive, necessitating large production to justify the investment.

Even knowing that ensiling high moisture grain increases starch digestibility, the technology received a boost in acceptance after the extensive reports that Brazilian hybrids have more vitreous endosperm and, consequently, low starch digestibility (Corrêa et al., 2002). After that, the use of reconstituted grain silage became more interesting. The first experiment in Brazil conducted to investigate the effect of ensiling reconstituted corn grain silage with flint endosperm was performed by Andrade et al. (2010). Dairy cows fed reconstituted grain silages for performance were evaluated two years later in 2012 by Bitencourt et al. Recently, Bernardes et al. (2018) performed a survey in 126 dairy farms to evaluate the main feed sources used in Brazilian conditions. Accounting for both corn and sorghum reconstituted silages, 25.2% of the farms are using reconstituted grain silages in the diets. These reports suggest an increased adoption of the technology by Brazilian cattle farmers.

2.2. GRAIN SILAGE FERMENTATION

Today we know that the same basic conditions, within different ranges, should be attempted for grain and whole plant silage fermentation. However, when ensiling high moisture corn became popular among North American farmers in the ’50s, information about grain silage was scarce. Although the concept of anaerobiosis was known to be important at the time for good silage fermentation, the scientific works were targeted to explain the better moisture content, the best way to store grain (whole or rolled) and how animals perform when fed high moisture compared to dry kernels. Two events in United States accelerated the adoption of high moisture corn grain. Purdue University in 1958 published a report where cattle fed high moisture corn had 5% better feed efficiency compared to dry corn, and Oklahoma University published another report in 1988 showing that cattle fed high moisture corn were more efficient than when fed steam-flaked corn (Matushima, 2006). Increased popularity of the subject resulted in greater interest in understanding the fermentation process.

Ensile the whole grain was the main form utilized in the beginnings (Fox 1976). Goodrich et al. (1975) evaluated the effect of different moisture content, processing and reconstitution on the fermentation profile of corn grain. They concluded that grains ensiled whole had higher dry matter losses compared to rolled grains (4.6 vs. 3.4%). Although there was some interaction between moisture content and processing method, ensiling whole grain independently of moisture content had higher pH (5.6 vs. 5.1%) as a consequence of low concentration of lactic, acetic and butyric acids ($P < 0.05$). Ethanol was also higher in unprocessed grain corn silage. Burmeister and Hartman (1966) observed high yeast activity in

high moisture corn and reported that this microorganism was primarily responsible for utilization of oxygen entering the silo. Increasing the moisture content from 21.5 to 33.1% drastically reduced the pH (5.90 vs. 4.82), increased the gas production/ kg of dry matter (1.44 vs. 4.96 litters), and all organic compounds measured (Lactic, acetic, propionic, and butyric acids and ethanol). These findings suggest an intense fermentation with increased moisture and processing method, which were maximized when rolled corn were ensiled with 33.1% moisture. Although the authors did not report density or package, it is presumed that low particle size and high moisture content allowed better packing and, consequently, low oxygen during ensiling, which resulted in better fermentation. Following the intensity of fermentation with high moisture content (>30%) and processing (ground or rolled), the ammonia nitrogen concentration also increases (Baron et al., 1986).

Comparing high moisture corn grain and reconstituted corn grain, Fernandes et al. (2014) observed low pH for high moisture compared to reconstituted (4.7 vs. 4.2) both with similar moisture content (33%, on average) and mean particle size (2.52 mm). The fermentation end products were different between treatments. Concentration of lactic acid was higher for high moisture corn (1.7 vs. 1.0% of DM), while ethanol concentration was higher for reconstituted corn grain silages (1.0 vs. 0.5% of DM). Acetic (0.30% of DM) and propionic (35mg/kg of DM) acids did not differ between treatments. Probably due to the high concentration of butyric acid (1700 mg/kg of DM) and 2,3-Butanediol (10.000 mg/kg of DM), the authors suggested a high activity of clostridium on reconstituted corn grain silage. Jobim et al. (1996) also observed an enhanced growth of clostridium, yeast and enterobacteria when cobs were added in high moisture corn grain silage. This result suggests that the presence of clostridium in silages is related with previous source of contamination and not with the reconstitution itself.

The fermentation of grain silages is much quicker than whole plant silage. Because of this, the concentration of total fermentation end products is low. The main end fermentation product in well-fermented non-inoculated (with hetero-fermentative bacteria) silage is lactic acid. Although reconstituted grain silage pH reaches values around 4.0 very rapidly, the aerobic stability is not quite long enough in function of low concentration of weak acids with properties to inhibit yeast growth. It could, however, be increased by inoculation with hetero-fermentative strain (Da Silva et al., 2018) or with chemical additives (Morais et al., 2017).

2.3. STORAGE LENGTH AND PROTEOLYSIS

Proteolysis has been reported in high moisture grain silages since the 1970s (McKnight et al., 1973). Priggie et al. (1976) ensiled high moisture corn grain with 28% moisture and kept silos stored for 56 days. Soluble nitrogen increased linearly from 15.8 to 38.2% of total N, when the length of storage ranged from 2 to 56 days. These results represent a 142% increase in soluble nitrogen. Baron et al. (1986) also report increase in nitrogen solubility when storage length was extended. However, the effect of time only increased nitrogen solubility when moisture content was higher than 33% moisture in this trial. Treatments were: high moisture corn ensiled for 0, 15, 30, and 90 days with 22, 26, 33 and 36% moisture. Baron et al (1986) also report the effect of organic acids (acetic:propionic:formic) and formaldehyde in partial inhibition of proteolysis. The interest of proteolysis inhibition increased when the negative performance of cattle fed high moisture corn with high soluble nitrogen content was reported by Sprage and Breniman (1969), cited by Priggie (1976). Controversially, some works indicated that nitrogen was more efficiently utilized when cattle were fed high moisture corn (Priggie et al., 1976).

For scientists, the mechanism involving proteolysis of high moisture corn was not as clear as it was for whole plant corn silage. Priggie (1976) suggested that organic acids and plant enzymes were the main contributor to solubilization of proteins. The contribution of bacterial enzymes was discarded because those scientists thought that with 21 days after ensiling, the lactic and acetic acids increased the high levels. Thus, they assumed that after the peak of acids, bacterial activity was reduced and, consequently, it could not contribute to proteolysis, which increased intensity after 45 days of ensiling (Baron et al., 1986). Neuhaus et al. (1971) observed no difference on in vitro digestibility of reconstituted sorghum grain silages stored for 10, 20 or 30 days. This result suggested that time is fundamental to allow high degrees of proteolysis. Priggie et al. (1976) report some findings of Florence et al. (1968) suggesting that during the storage of reconstituted sorghum grain, energy availability was increased due to disruption of protein matrix surrounding the starch granules.

Corn and sorghum have their starch granules involved by a protein matrix (Seckinger and Wolf, 1973). In general, the protein matrix is composed of prolamins, albumins, globulins, and glutelins (Branlard and Bancel, 2006). In sorghum, prolamins (called kafarins) represent between 50 to 70% of total proteins (Hamaker et al., 1995). The kafarins can be classified in α, β, γ based on molecular mass (Daltons) and A.A composition. The α, β, γ kafarins have approximately 23-28.000, 20.000 and 18.000 daltons, respectively, and α-kafarins represent 80-84% of the total fraction in vitreous endosperm (Belton et al., 2006).

Cereal grains can be separated in three major components; pericarp (6% of kernel), endosperm (85% of kernel), and germen (9% of kernel) for sorghum. Although the crude protein content on endosperm (10.5%) is lower than that found in germen (18.4%), the endosperm have the 80% of total crude protein in function of their high mass. This information helps support the impact of protein interaction with starch granules on the endosperm.

Corn vitreousness of endosperm is negatively correlated with dry matter digestibility (Corrêa et al., 2002). There is a significant positive relationship between kernel vitreousness and content of prolamins in corn grains (Philippeau et al., 2000) and we suppose that the same characteristics are true for sorghum. When corn is ensiled as high moisture, the content of prolamins (zeins) is often low compared to dry corn (Larson and Hoffman, 2008). Hoffman et al. (2011) evaluated the effect of length of storage in high moisture corn and observed a significant reduction on the α-zeins after 240 days of ensiling compared to non-ensiled.

Published data about the concern show that extending length of storage increases starch and dry matter digestibility in function of proteolysis (Kung et al., 2018). Thus, the increase seems to be more pronounced in more vitreous endosperm, which is composed of more prolamins than globulins (Philippeau et al., 2000). Florence et al. (1968) already suggest the effect of ensiling on protein breakdown and increase in dry matter digestibility. However, the mechanism was unknown. Authors thought that plant enzymes and organic acids were the main contributors to proteolysis. Now we know that the main contributor to proteolysis in high moisture grain silages is bacteria (60% of total) and plant enzymes (30% of total) (Junges et al., 2017).

Although a considerable number of works evaluate the length of storage effect on starch digestibility of grain silages (in vitro and in situ), in vivo studies have not been performed to my knowledge. It is common to see in the literature affirmations that ensiling high moisture grains increase starch digestibility compared to dry kernels submitted to the same mechanical process. However, the length of storage is not considered in those trials.

2.4. SODIUM BENZOATE AS SILAGE ADDITIVE

Investigations of the use of benzoic acid as silage additive (as sodium salt, Sodium benzoate) began in Poland in 1971 with the intention to reduce proteolysis in whole crop cereal silages (Woolford, 1984). It was concluded that sodium benzoate was not effective in protein-rich silages; otherwise, when added to apples before ensiling, preservation was enhanced. Benzoic acid has been classified as silage fermentation inhibitor (Woolford, 1984;

McDonald, 1991). However, the salts of benzoic acid, which is the form found in commercial products, have little impact on microorganisms growth under high pH. Woolford (1975) observed that sodium benzoate was more effective in controlling yeast, and at low pH (< 5.0) the addition of sodium benzoate also inhibited hetero-fermentative lactic acid bacteria. The effectiveness of benzoic acid in inhibiting microbial growth is pH and dose dependent (Lambert and Stratford, 1998). Although the undissociated form is more efficient, the anion also contributes slightly with antimicrobial activity (Eklund, 1983).

Because benzoic acid could not be produced synthetically in large amounts until the 1900s, its use as a food preservative was limited. Benzoic acid is synthetically produced by hydrolysis of benzotrichloride, and then treated with mineral acids. Benzoic acid is also found in natural products such as yogurts and some foods, including cranberries, tomatoes, and mushrooms (Chipley et al., 1981). The mechanisms by which benzoic acid inhibits yeast growth were demonstrated by Lambert and Stratford (1999). They concluded that the concentration of benzoate within the cell, rather than the energy cost to pump hydrogen out of the cell, was the main inhibitory factor; however, ATP consumption was directly correlated with reduction in cell yield. Other mechanisms, such as inhibition of amino acids transport and inhibition of enzymatic systems within cells (Freese et al., 1973) have also been suggested.

Recently, the use of benzoic acid as a silage additive is often found in mixed rather than exclusive products. The reason is due to the different mechanism of actions involved when more than one compound is mixed. The frequently used form is in a mixed product that also contains sorbate, propionate, formates and nitrite. Da Silva et al. (2015) evaluated the effect of a commercial product containing 200 g/kg of sodium benzoate, 100 g/kg of potassium sorbate, and 50 g/kg of sodium nitrate (Safesil®, Ab Hanson and Möhring, Halmstad, Sweden) applied in high moisture corn in different dosages (0, 2, 3, and 4 L/ton). They observed a reduction in N-NH3 on treatments with high dose (4.0 L/ton) of Safesil®, suggesting inhibition of natural proteolysis after 90 days of storage. The concentration of water soluble carbohydrates (WSC) was also higher in treated silages in the function of yeast count reduction. As a consequence of low yeast activity, ethanol concentration and aerobic stability increased. The lactic acid bacteria (LAB), was also reduced in treated silages (5.19 vs. 4.82 log cfu/g). The reduction of LAB associated with low N-NH3 suggests that the reduced proteolysis might be a function of low activity of bacteria, which contributes significantly with proteolysis (Junges et al., 2017).

2.5. HYPOTESES AND OBJECTIVES

Ensiling of high moisture grain (sorghum and corn) increases its feed value. The reason is basically in the breakdown of a complex wire of proteins (mostly prolamins) surrounding the starch granules. When these protein matrices are soluble, the access of starch granules by rumen microbes is easier, increasing the rate and extent of ruminal starch digestion. However, two basic conditions must be attempted: proper mechanical process and moisture content. With these concepts established, the length of storage will be crucial to predict the increase in proteolysis. The literature frequently report increases in starch and protein digestibility when ruminants are fed with high moisture grain silage compared to dry kernel; however, those trials do not take into account the length of storage. Our first hypothesis is that feeding reconstituted grains silages stored for long periods increases feed efficiency of dairy cows.

As grain silage is prone to aerobic deterioration, the use of additives like sodium benzoate, which increases aerobic stability, is recommended. Although benzoic acid is very efficient in controlling yeast and bacillus, it could also inhibit lactic acid bacteria. Lactic acid bacteria plays an important function in proteolysis. Thus, the use of sodium benzoate could reduce proteolysis, which is interesting in high moisture grain silages. Our second hypothesis is that sodium benzoate reduces proteolysis in high moisture grain silages.

The objective of these trials was to evaluate the fermentation profile and nutritive value of reconstituted sorghum grain silages with different lengths of storage (short = 30 days, or long = 90 days), treated or not with sodium benzoate, on the performance of dairy cows.

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3. THE EFFECT OF LENGTH OF STORAGE ON THE NUTRITIVE VALUE OF RECONSTITUTED SORGHUM GRAIN SILAGE TREATED WITH SODIUM BENZOATE FOR DAIRY COWS PERFORMANCE

Abstract

Twenty Holstein cows with 168 ± 87 d in milk (mean ± SD) were assigned to a 4 × 4 Latin square design with a 2 × 2 factorial arrangement to evaluate the effects of two storage length (30 or 90 d) and sodium benzoate (control or 0.2% as fed) on the nutritive value of reconstituted sorghum grain silages (RSGS). Dry ground sorghum grain was rehydrated to 35% moisture and ensiled in 200-L plastic drums. Treatments were: RSGS stored for 30 d without sodium benzoate (30 CON), RSGS stored for 30 d with sodium benzoate (30 BEN), RSGS stored for 90 d without sodium benzoate (90 CON) and RSGS stored for 90 d with sodium benzoate (90 BEN). Diets contained 16.3% of RSGS. Silages stored for 90 d had higher concentration of 1,2-propanediol, soluble protein, and ammonia-N. Sodium benzoate reduced ethanol and ethyl-ester formation. Storing silages for 90 d instead of 30 d increased starch (89.3 vs. 86.9%) and protein (57.1 vs. 54.0%) digestibility compared to silages stored 30 d. Ruminal acetate to propionate ratio was lower in RSGS stored for 90 d than in RSGS stored for 30 d (3.8 vs. 3.3). Milk yield was increased from 30.0 to 31.2 kg/d in favor of cows fed RSGS stored for 90 d, without change DMI (23.5 kg/d on average). Hence, feed efficiency and nitrogen use efficiency were also increased in cows fed RSGS stored for 90 d. Sodium benzoate did not alter cows performance, but slightly increased plasma glucose (65.2 vs. 63.6 mg/dL). In conclusion, increasing storage period of RSGS from 30 to 90 d improve starch and protein digestibility, milk yield, and feed efficiency.

Keywords: Starch digestibility; Chemical additive; Milk yield; Proteolysis proteolysis

3.1. Introduction

Ensiling cereal grains as sorghum and corn has been associated with increased starch digestibility in ruminants (Owens et al., 1986). Replacing high moisture cereal grain silage for dry ground often increase feed efficiency, by increasing milk yield without affect DMI (Oba and Allen, 2003, Arcari et al., 2016) or by decreasing DMI without alter milk yield (Ferraretto et al., 2013).

Protein matrix break down is considered the main reason for increase starch digestibility in grain silages (McAllister et al., 1993). Plant and microbial enzymes are the main contributors to proteolysis during silage fermentation (Junges et al., 2017) and a minimum length of storage is required to improve starch digestibility (Benton et al., 2005).

It is well established in the literature that lengthening silage storage increases proteolysis and consequently, starch digestibility (Der Bedrosian et al., 2012, Kung et al., 2018). Meanwhile, to our knowledge, in vivo studies evaluating the performance of dairy cows fed high moisture grain silages with different lengths of storage have not been performed.

Sodium benzoate is a chemical additive, highly efficient to increase aerobic stability (Morais et al., 2017). However, sodium benzoate has been associated with a reduction in proteolysis during silage fermentation (Da Silva et al., 2015). Therefore, we hypothesized that

storing RSGS for a longer period increase starch digestibility and feed efficiency in dairy cows, whereas the addition of sodium benzoate may reduce proteolysis during silage fermentation and, consequently impair starch digestibility. The objective of this experiment was to evaluate silage conservation, rumen fermentation profile, starch digestibility and performance of dairy cows fed RSGS stored for thirty or ninety days, treated with or without sodium benzoate.

3.2. Material and Methods

All experimental procedures were approved by ethic committee animal use of University of São Paulo (protocol: 2017.5442.11.4).

3.2.1. Ensiling

A grain sorghum hybrid (BM 737 - Biomatrix®, Rio Claro, SP, Brazil) was sown as the second crop season in February of 2016. Grains were mechanically harvested (MF 3640, Massey Ferguson) at the end of July and stored in a metal bin with 12% moisture (KeplerWeber®, Campo Grande, MS, Brazil).

Ensiling was scheduled over time to allow two different lengths of storage (30 and 90 d) at silo opening. Silages stored for 90 d were prepared 60 d prior to silages stored for 30 d. Before silage making, sorghum dry matter was determined by oven drying at 105º C for 12 h and the result used to calculate the amount of water to achieve the target moisture (35%). Before ensiling, grains were ground with a hammer mill through a 2-mm sieve. Dry ground sorghum was mixed with water in a vertical feed wagon (VM4, Delaval®, Tumba, Sweden) for 15 min. Fifteen hundred kilograms of silage were prepared per treatment for each experimental period and ensiled in six 200-L plastic drums. Silage density was 1100 ± 50 kg/m3 as fed.

Sodium benzoate (0.2% as fed) was used as a second study factor combined in a factorial arrangement with the length of storage. Sodium benzoate was previously diluted in the water used for reconstitution. The ensiling was reproduced four times with 21 d intervals far from the before to ensure that the cows received silages stored for the same length of storage in all four experimental periods. Each ensiling period was considered as one block in the statistical analyses of silage outcomes.

3.2.2. Feeding trial

Twenty Holstein cows (16 multiparous and 4 primiparous) were housed in a free stall barn with sand beds and an individual feed monitoring system (Intergado Ltda., Contagem, Minas Gerais, Brazil) validated by Chizzotti et al. (2015). At the beginning of the trial, cows were 168 ± 87.4 DIM, milk yield 32.3 ± 5.3 kg/d, and BW 681 ± 47 kg (mean ± SD). Cows were grouped respectively by parity and milk yield. One group was composed by four multiparous rumen-cannulated cows in late lactation (288 ± 10 DIM) with a milk yield of 21.0 ± 2.0 kg/d. Cows were randomly assigned to a sequence of four treatments in a 2 × 2 factorial arrangement in a 4 × 4 Latin square design balanced for carry-over effect with 21-d periods (15 d of adaptation + 6 d of sampling). Treatments were; silage stored for 30 d without the addition of sodium benzoate (30 CON), silage stored for 30 d with sodium benzoate (30 BEN), silage stored for 90 d without sodium benzoate (90 CON), and silage stored for 90 d with sodium benzoate (90 BEN). Cows were fed (0600 and 1700 h) and milked (0600 and 1800 h) twice daily. Ration ingredients were mixed for 10 min in a pull type vertical mixer wagon, and delivered in amounts to allows 5 to 10 % of orts.

3.2.3. Laboratorial analysis

From d-16 to d-20 of each experimental period, samples of each diet ingredient and orts were collected daily and frozen to form a composite sample per period. Samples were dried in a forced air oven for 72 h at 55ºC and ground through a 1 mm mesh screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Subsamples were analyzed for DM, ether extract, and ash according (AOAC, 1990; methods 934.01, 920.39 and 924.05, respectively). The NDF (expressed inclusive of residual ash, assayed with sodium sulfite and heat stable amylase) was analyzed using a TE-149 fiber analyzer (Tecnal Equipamentos, Piracicaba, Brazil). The CP was analyzed by Dumas method (Leco® FP-2000A nitrogen analyzer; Leco corp., St. Joseph, MI). Non-fiber carbohydrate was calculated as NFC = 100 – (CP + EE + ash + NDF). Starch content was analyzed according to Hall (2009). Indigestible NDF (iNDF) was measured by ruminal in situ incubation for 288 h (Huhtanen et al., 1994). The nutrient composition of consumed diets was calculated dividing daily nutrient intake by DMI (Table 1). The DMI was measured during d-16 to d-20 of each period as the difference between the amount of offered diet and orts (DM basis).

Subsamples (25 g) of RSGS were mixed with 225 g of deionized water and mixed 4 min in a stomacher. The extract was filtered in a 3 folder cheese-cloth and centrifuged at 10,000 × g for 15 min at -4 ºC. The supernatant was used to determine ammonia nitrogen

using the method of Chaney and Marbach (1962) adapted by Weatherburn (1967) and lactic acid (Price, 1969). The concentration of volatile fatty acids, alcohols and esters were analyzed using a gas chromatographer with a mass detector (GCMS QP 2010 plus, Shimadzu, Kyoto, Japan) using a capillary column (Stabilwax, Restek, Bellefonte, PA; 60 m, 0.25 mm, i.d., 0.25 m). The DM content was corrected for volatiles according to (Weissbach, 2009).

Ruminal gas production profile was measured using the methodology of Theodorou et al. (1994) as modified by Mauricio et al. (1999).In all assays, 1.0 g of dried sample was incubated in duplicate for 72 h in a water bath set at 39º C. Pressure measurements were made at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, 60, and 72 h post inoculation, using a pressure transducer and data logger (PDL200, LANA/CENA-USP, Piracicaba, SP, Brazil).. The rumen fluid was collected from two cannulated Holstein nonlactating cows fed corn silage and 3 kg of concentrate mixture (50 % dry ground sorghum, 43 % soybean meal, 3% urea, and 4% mineral and vitamin mix). Ruminal fluid was collected from the solid and liquid phases. The solid phase was collected from dorsal sac by hand and squeezed. The liquid phase was collected from ventral sac using a stainless steel probe. Fluids were placed separately in pre-warmed (~39ºC) flasks and transported immediately to the laboratory. Equal volumes of solid and liquid phases were mixed for approximately 10 s and then filtered through a 35 µm nylon filter and kept in a water bath (39ºC) with CO2 until inoculation. Gas volume production was expressed as mL/g of DM. A one-pool exponential equation Vt = Vf (1 - e-k(t-L)) with discrete lag period was fitted to data (Schofield et al., 1994) to estimate the following parameters: volume of gas at time t (Vt), fractional rate of gas production (k), and lag time (L).

The particle size distribution was measured using a Ro-Tap Shaker (Bertel® Ltda., Caieiras, SP, Brazil) with five sieves with nominal squares apertures of 4.75, 2.36, 1.70, 1.18, 0.6 mm, and bottom pan. Approximately 500 g of sample, dried at 55º C for 72 h in air forced oven, was used to perform the analyses.

Milk yield was measured during d 16 to 20 and samples for milk composition were collected on d 17 and 19 of each period in flasks with bronopol. Milk was analyzed for fat, protein, lactose, and MUN by mid-infrared spectroscopy using Fourier transform (Clínica do Leite, Piracicaba, SP, Brazil). Energy-corrected milk was calculated as milk energy secretion (NRC 2001) divided by 0.70 (assumes 0.70 Mcal/kg for milk with 3.7% fat, 3.2% protein, and 4.6% lactose). Three measures of efficiency were calculated: actual milk divided by DMI, energy-corrected milk divided by digestible organic matter intake, and milk nitrogen secretion divided by nitrogen intake.

Total-tract apparent digestibility of DM, OM, NDF, CP and starch were estimated using iNDF as a marker. Fecal grab samples were collected every 8 h during d-18 to 20 of each period, and composed per cow. Samples were dried and analyzed for DM, NDF, starch, CP, ash, and iNDF as described previously. Total tract apparent digestibility was calculated according to the follow equation {100 – (TMR iNDF/fecal iNDF) × (Fecal nutrient concentration/TMR nutrient concentration)}. Daily fecal starch excretion was calculated by multiplying daily fecal excretion by starch content in feces.

Chewing activity was evaluated on d-18 of each period by visual observation (eating and ruminating) every 10 min intervals for 24 h. Sorting behavior was measured on the same day according to the methodology of Leornardi and Armentano (2003). Particle size distribution of offered diets and orts were measured using two sieves 19 and 8-mm and a bottom pan of the Penn State Particle Size separator (Lammers et al., 1996).

Blood samples were obtained on d-21 of each period from coccygeal vessels before the morning feeding and 1, 2, 3, 6, and 12 h after feeding. Vacutainer tubes containing K-EDTA were used to collect samples for plasma urea nitrogen (PUN) analysis and tubes with K-EDTA and fluoride for glucose analyses. After sampling, blood was immediately centrifuged for 20 min at 2000 × g at room temperature (~21 ºC) and the plasma was frozen at –20ºC. The PUN and glucose were analyzed using a laboratory kit's Urea 500 and Glucose Enzimática Líquida (Doles Reagentes Para Laboratórios Ltda., Goiânia, Brazil).

Rumen samples were obtained on d-16 and 17 of each period from rumen-cannulated cows every 3 h across 24 h after the morning feeding. Samples of solid phase were taken by hand in four different portions of the ventral rumen and squeezed through a cheese-cloth in a Becker. The pH was immediately measured, and 100 mL of fluid were freeze in liquid nitrogen for 1 min and stored at -20ºC. Analyses of ammonia nitrogen and VFA were performed as described for silages.

Tabela 1. Ingredients and nutrient composition (mean ± SD) of the experimental diets with reconstituted sorghum grain silage (RSGS) stored for thirty (30) or ninety (90) days without (CON) or with (BEN) sodium benzoate

	30		90	
item	CON	BEN	CON	BEN
Ingredientes, % of diet DM				
Corn silage	49.4±0.73	49.3±0.75	49.3±0.69	49.3±0.79
RSGS	16.2±0.07	16.4±0.16	16.3±0.13	16.3±0.21
Dry ground sorghum	13.8±0.39	13.8±0.39	13.8±0.41	13.8±0.38
Soybean meal	14.8±0.26	14.8±0.25	14.8±0.27	14.8±0.24
Rumen protected soybean meal[1]	1.9±0.03	1.9±0.03	1.9±0.04	1.9±0.03
Urea	0.4±0.01	0.4±0.01	0.4±0.01	0.4±0.01
Calcium soap of palm oil	1.2±0.02	1.2±0.02	1.2±0.02	1.2±0.02
Mineral mix	2.2±0.04	2.2±0.04	2.2±0.04	2.2±0.04
DM, % as fed	51.1±0.75	51.1±0.73	51.1±0.78	51.1±0.68
Nutrients, % of DM				
CP	16.8±0.19	16.8±0.09	16.8±0.11	16.7±0.26
NDF	34.9±0.7	35.2±0.9	34.4±1.1	34.2±0.7
EE	3.3±0.06	3.3±0.04	3.3±0.04	3.3±0.03
Ash	6.1±0.47	6.1±0.41	6.1±0.41	6.1±0.42
NFC	38.9±1.3	38.6±1.6	39.5±1.6	39.6±1.1
Starch	31.8±1.16	32.6±0.85	32.1±1.14	32.9±1.20
Starch from, % of total				
Corn silage	37.1±1.1	37.0±1.2	37.0±1.1	37.0±1.3
RSGS	34.0±1.0	34.3±1.1	34.2±0.7	34.3±1.2
Dry ground sorghum	27.4±1.1	27.3±1.1	27.3±1.1	27.3±1.0
Particle size distribution, % as fed				
>19 mm	2.7±0.84	2.8±1.42	2.7±0.88	2.8±1.64
8-19 mm	22.8±2.87	22.9±3.80	23.3±2.07	23.4±2.07
<8 mm	74.5±3.64	74.0±5.08	73.9±2.94	73.8±3.57

[1] (Soypass®, Borregaard LignoTech, Fernandina Beach, FL, USA).

3.2.4. Statistical Analyses

Statistical analyses were performed using the PROC MIXED of SAS (version 9.3). Data from RSGS were analyzed as randomized block design using the following model: Yijk = μ + Bi + Tj + Ak + TAjk + eijk, where μ = overall mean, Bi = random effect of block (i = 1 to 4), Tj = fixed effect of length of storage (j = 30 or 90), Ak = fixed effect of additive (k = BEN or CON), TAjk = interaction between length of storage and additive, and eijk = residual error.

Cows outcomes were analyzed with following model: Yijklm = μ + Si + Cj(i) + Pk + Tl + Am + TAlm + eijklm, where μ = overall mean, Si = fixed effect of Latin square (I = 1 to 5), Cj(i) = random effect of cow within square (j = 1 to 20) , Pk = fixed effect of period (k = 1 to 4), Tl = fixed effect of length of storage (l = 30 or 90), Am = fixed effect of additive (m = BEN or CON), TAlm = interaction between length of storage and additive, and eijklm= residual error. Data collected over time (glucose and PUN) were analyzed as repeated measures using the same model including the effect of time and their interaction. The mean square of cow, period and treatment factors was used as the error to test the treatment effects. Data obtained from rumen cannulated cows were analyzed as a repeated measures using the previous model without the effect of Latin square. Degrees of freedom were adjusted using the Kenward-Roger method. The covariance structure was chosen based on the Akaike's information criterion among VC, CS, AR(1) and UN. Significant difference between main effects were considered when $P \leq 0.05$ and trends when $P > 0.05$ and ≤ 0.10. One cow was removed of the trial on the fourth period because of mastitis. All others 19 cows completed the experiment.

3.3. Results

3.3.1. Silages

Before ensiling, the dry matter content of ground sorghum was 87.8%. The moisture target for ensiling was 35%. Silage moisture ranged from 35.0 to 36.1% and there was no difference ($P > 0.10$) for dry matter content among treatments (Table 2). Ammonia nitrogen (30-d = 3.44 vs. 90-d = 4.89% of N) and soluble protein (30-d = 13.6 vs. 90-d = 20.9% of CP) increased 53.6 and 41.8% respectively ($P < 0.05$), in function of great length of storage. Before ensiling, ammonia nitrogen and soluble protein of sorghum grain was 0.58% of N and 8.5% of CP respectively. Sorghum grain silage stored for 30-d increased soluble protein in 62.5% in relation to non-fermented sorghum while silages stored for 90-d increased soluble protein by 146%. Sodium benzoate did not alter ($P > 0.10$) protein solubility (CON = 18.9 vs. BEN = 15.6% of CP) or ammonia concentration (CON = 4.38 vs. BEN = 3.95% of N).

The addition of sodium benzoate reduced ($P < 0.05$) the ethanol concentration by 46% (CON = 0.15 vs. BEN = 0.08% of DM), ethyl lactate by 56% (CON = 73 vs. BEN = 32 mg/kg of DM) and ethyl acetate by 63% (CON = 29 vs. BEN = 10.5 mg/kg of DM) compared to non-treated silages. When silages were stored for 90-d, the concentration of 1,2-propanediol was increased ($P = 0.04$) and propionic acid concentration tended to be twice higher ($P = 0.10$) compared with silages stored for 30-d (Table 2). The concentration of lactic

and acetic acid was not altered by treatments and averaged 2.27 ± 0.04 and 0.23 ± 0.03% of DM (mean ± SD), respectively.

Before ensiling, dry ground sorghum had the geometric mean particle size of 890 ± 41.6 μm and fractional rate of gas production of 3.6%/h. The particle size was not altered by treatments after ensiling and averaged 874 ± 7.2 μm ($P = 0.75$). The rate of gas production was increased 21.1% (3.75 vs. 4.75% / h) in favor of silages stored for 90-d compared to 30-d ($P = 0.03$). There was no effect of treatment ($P > 0.10$) on lag time and cumulative gas production which averaged 3.2 ± 0.12 h and 260 ± 3.0 mL/g of DM, respectively. Sodium benzoate did not alter the kinetics of gas production.

Tabela 2. Composition, fermentation profile, and in vitro gas production of reconstituted sorghum grain, ensiled for thirty (30) or ninety (90) days, without (CON) or with sodium benzoate (BEN)

	30		90			*P*-value[1]		
Item	CON	BEN	CON	BEN	SEM	S	A	S ×A
Nutrient								
DM, % as fed	63.4	64.2	64.0	64.1	0.89	0.47	0.34	0.44
$DM_{corr,}$ % as fed	63.9	65.0	64.2	64.5	0.90	0.93	0.14	0.35
Soluble protein, % of CP	14.3	12.9	23.5	18.3	2.61	0.03	0.25	0.50
NH_3-N, % of N	3.68	3.20	5.08	4.69	0.473	<0.01	0.21	0.88
Starch, % of DM	71.6	70.3	69.6	69.3	1.42	0.29	0.55	0.72
Fermentative profile								
pH	4.01	3.98	4.02	4.06	0.04	0.41	0.75	0.42
Lactic acid, % of DM	2.33	2.22	2.30	2.24	0.260	0.98	0.52	0.85
Acetic acid, % of DM	0.20	0.21	0.29	0.22	0.067	0.41	0.58	0.47
Ethanol, % of DM	0.24	0.10	0.16	0.07	0.039	0.20	0.01	0.57
1,2-Propanediol, mg/kg of DM	39	51	202	106	51.9	0.04	0.37	0.26
2,3-Butanediol, mg/kg of DM	104	65	97	55	23.4	0.70	0.08	0.94
Propionic acid, mg/kg of DM	14	20	28	39	9.6	0.10	0.37	0.75
Ethyl lactate, mg/kg of DM	65	32	33	81	9.4	0.37	<0.01	0.42
Ethyl acetate, mg/kg of DM	35	13	23	8	7.3	0.21	0.02	0.62
Butyric acid, mg/kg of DM	3.2	4.7	3.5	3.3	1.25	0.66	0.62	0.53
1-propanol, mg/kg of DM	4.3	1.2	16.3	10.0	5.15	0.10	0.41	0.79
In vitro gas production								
Lag time, h	3.4	3.1	3.0	3.2	0.14	0.45	0.95	0.12
Fractional rate of gas production, %/ h	3.9	3.6	4.6	4.9	0.37	0.03	0.85	0.35
Cumulative gas production, mL/g of DM	260	263	255	264	5.21	0.68	0.22	0.50

[1]Probabilities for effect of storage length (S), additive (A) and interaction between storage length and additive (S × A).

3.3.2. Animal Performance

Dry matter and organic matter intake were not altered by treatments (P > 0.10) and averaged 23.4 ± 0.32 and 13.5 ± 0.18 kg/d, respectively (Table 3). There was a tendency to increase milk yield (P = 0.10) and ECM (P = 0.07) in 1.2 kg/d when cows received diets containing silage stored for 90-d compared to silages stored for 30-d. Treatment with silages stored for 90-d tended (P = 0.10) to increase feed efficiency, calculated as milk yield / DMI by 4.7% (30-d = 1.27 vs. 90-d = 1.33) and ECM / DOMI by 4.8% (30-d = 2.16 vs. 90-d = 2.27). Although milk components were not different among treatments, longer storage

increased ($P \leq 0.05$) daily excretion of fat, protein, and lactose due high milk production on treatment 90-d of storage. The efficiency of conversion of nitrogen from ration into milk nitrogen tended to increase by 5.4% (30-d = 24.1 vs. 90-d = 25.4; P = 0.07). The MUN concentration (30-d = 14.7 vs. 90-d = 13.8 mg/dL; P = 0.10) was lower in cows fed diets containing silages stored for 90-d compared to silages stored for 30-d. Nevertheless, daily excretion of MUN was not altered by treatments (Table 3). Sodium benzoate did not alter milk yield or milk composition.

The NDF intake (8.2 ± 0.25 kg/d) and digestibility of NDF (29.2 ± 0.66%) did not differ across treatments (Table 4). When cows were fed diets with silages stored for 90-d there was an increase in the total tract starch digestibility (30-d = 86.9 vs. 90-d = 89.3%; P = 0.04) and a tendency (P = 0.06) to reduce fecal starch (30-d = 9.3 vs. 90-d = 7.9 %). Digestibility of crude protein and dry matter also tended (P = 0.10) to be higher when cows received diets containing silages stored for 90-d (Table 4). Sorting index and chewing behavior were not affected by treatments (Table 4). Cows fed silages stored for 90-d tended (P = 0.10) to have lower acetate to propionate ratio compared to cows fed silages stored for 30-d (30-d = 3.8 vs. 90-d = 3.3). Sodium benzoate increased (P = 0.01) plasma glucose from 63.6 on control treatment to 65.2 mg/dL on benzoate treatment.

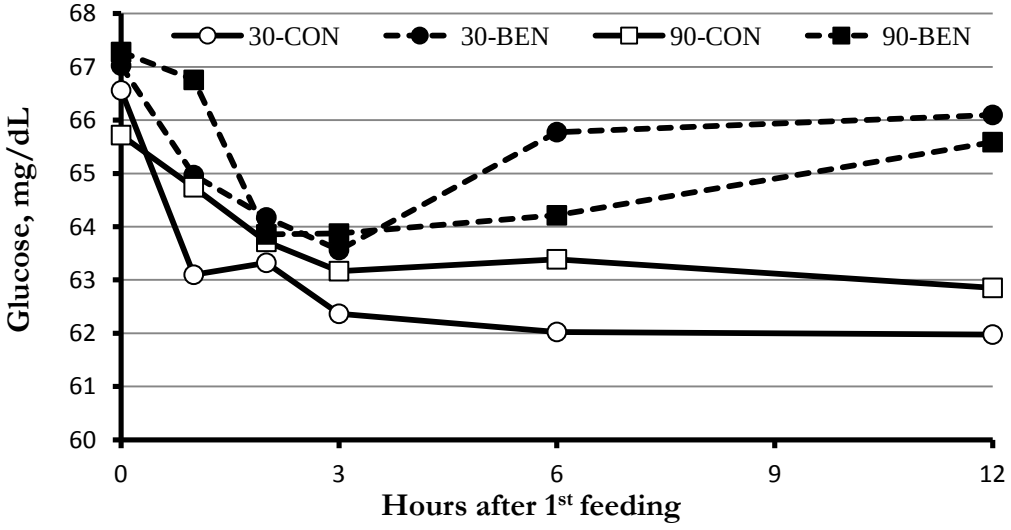

Figura 2. Plasma glucose concentration along the day of dairy cows fed reconstituted sorghum grain silages stored for thirty (30) or ninety (90) days without (CON) or with (BEN) sodium benzoate. SEM=0.847.

Tabela 3. Performance of dairy cows fed reconstituted sorghum grain silage stored for thirty (30) or ninety (90) days without (CON) or with (BEN) sodium benzoate

	30		90			*P*-value[1]		
Item	CON	BEN	CON	BEN	SEM	S	A	S ×A
DMI, kg/d	23.4	23.6	23.9	22.8	0.58	0.84	0.31	0.40
DOMI, kg/d	13.5	13.5	13.9	13.2	0.34	0.75	0.22	0.23
N-Intake, kg/d	0.647	0.634	0.647	0.606	0.0172	0.45	0.44	0.76
Milk, kg/d	29.4	30.6	31.4	31.0	1.25	0.10	0.55	0.27
ECM, kg/d	28.9	29.5	30.8	30.0	1.02	0.07	0.99	0.27
Fat, %	3.59	3.59	3.56	3.56	0.141	0.63	0.57	0.55
Fat, kg/d	1.012	1.057	1.128	1.096	0.0407	0.01	0.81	0.21
Protein, %	3.23	3.23	3.22	3.20	0.017	0.71	0.68	0.60
Protein, kg/d	0.956	0.960	1.029	0.973	0.0730	0.04	0.22	0.16
Lactose, %	4.63	4.59	4.61	4.61	0.045	0.70	0.20	0.28
Lactose, kg/d	1.399	1.389	1.495	1.426	0.0644	0.05	0.40	0.26
MUN, mg/dL	14.6	14.7	13.9	13.7	0.563	0.07	0.91	0.83
MUN, g/d	43.0	43.7	43.3	42.1	2.47	0.86	0.70	0.86
Milk/DMI	1.26	1.28	1.30	1.35	0.456	0.10	0.19	0.67
ECM/DOMI	2.13	2.19	2.23	2.30	0.094	0.10	0.29	0.77
Milk N/N intake, %	23.9	24.2	25.8	25.1	0.89	0.07	0.77	0.49

[1]Probabilities for effect of storage length (S), additive (A) and interaction between storage length and additive (S × A)

Tabela 4. Total tract apparent digestibility of nutrients, fecal starch, chewing and sorting behavior of dairy cows fed reconstituted sorghum grain silage stored for thirty (30) or ninety (90) days without (CON) or with (BEN) sodium benzoate

	30		90			*P*-value[1]		
Item	CON	BEN	CON	BEN	SEM	S	A	S × A
NDF intake, kg/d	8.3	8.3	8.3	7.8	0.21	0.13	0.14	0.17
NDF intake, % of BW	1.25	1.25	1.25	1.19	0.03	0.17	0.17	0.18
Digestibility, %								
DM	57.9	57.8	58.8	59.2	0.72	0.10	0.86	0.69
OM	60.7	60.6	61.7	61.8	0.78	0.16	0.98	0.87
NDF	28.2	28.9	30.5	29.1	1.74	0.46	0.84	0.53
CP	53.6	54.4	57.1	57.0	2.12	0.10	0.83	0.81
Starch	87.0	86.8	89.2	89.3	0.12	0.04	0.95	0.89
Fecal starch, %	8.9	9.7	8.0	7.9	0.72	0.06	0.62	0.50
Chewing behavior, min/d								
Ingestion	225	218	213	229	13.8	0.96	0.65	0.26
Rumination	516	516	502	499	19.6	0.47	0.76	0.90
Chewing	742	727	714	728	28.7	0.53	0.98	0.47
Particle sorting, % as fed								
>19 mm	100	96	99	102	3.5	0.49	0.93	0.29
8-19 mm	98	98	99	99	0.76	0.27	0.94	0.44
<8 mm	101	101	101	100	0.32	0.20	0.30	0.23

1Probabilities for effect of storage length (S), additive (A) and interaction between storage length and additive (S × A)

Tabela 5. Probabilities of the effect of storage length, additive hour and their interactions on blood metabolites and rumen parameters of dairy cows.

	P-values[1]						
item	S	A	S×A	H	S×H	A×H	S×A×H
Blood metabolites, mg/dL							
Glucose	0.56	0.01	0.51	<0.01	0.83	0.58	0.90
Urea nitrogen	0.83	0.16	0.49	<0.01	0.12	0.68	0.54
Rumen parameters							
pH	0.12	0.32	0.46	<0.01	0.71	0.72	0.16
Ammonia N, mg/dL	0.04	0.22	0.88	0.83	0.55	0.91	0.94
Proportion, mol/100 mol							
Acetate	0.73	0.16	0.54	0.01	0.92	0.58	0.88
Propionate	0.14	0.87	0.49	<0.01	0.85	0.67	0.40
Butyrate	0.27	0.36	0.27	0.17	0.12	0.47	0.81
Iso-Butyrate	0.59	0.58	0.33	0.01	0.29	0.19	0.81
Valerate	0.25	0.22	0.92	0.07	0.17	0.97	0.44
Iso-Valerate	0.34	0.81	0.89	<0.01	0.56	0.13	0.97
Acetate to propionate ratio	0.10	0.58	0.40	0.01	0.95	0.63	0.67
Concentration, mM							
Acetate	0.69	0.67	0.77	<0.01	0.12	0.50	0.63
Propionate	0.61	0.96	0.56	<0.01	0.84	0.63	0.34
Butyrate	0.40	0.34	0.14	<0.01	0.82	0.45	0.79
Iso-Butyrate	0.75	0.36	0.13	0.03	0.94	0.74	0.56
Valerate	0.16	0.37	0.32	<0.01	0.63	0.18	0.61
Iso-Valerate	0.33	0.92	0.44	0.49	0.99	0.41	0.85
Total concentration	0.81	0.88	0.28	0.01	0.21	0.87	0.42

[1]Probabilities for effect of storage length (S)**,** additive (A), interaction between storage length and additive (S×A), hour (H), interactions between storage length and hour (S×H), additive and hour (A×H), and among storage length, additive, and hour (S×A×H).

3.4. Discussion

Grain silage conservation requires adequate moisture content to allows microbial growth and activity (Phalow, 2003). Despite the wide range of moisture capable to result in well fermented silages, literature data suggest that 35% moisture is the optimal target to enhance fermentation, increase proteolysis and starch degradability (Neuhaus and Totusek, 1971; Barol et al., 1986; Gomes et al., 2018). Based on this founds we reconstituted sorghum grains with the aim to reach 35% moisture. Although there was no difference between treatments, the average values of pH, lactic acid and acetic acid are in agreement with literature data for high-quality moisture grain silages (Morais et al., 2017).

Ethanol concentration was low in treated silages due to a straight effect of benzoic acid on yeast metabolism, which is the main microorganism responsible for ethanol production in silages (Pahlow et al., 2003). Although other weak-acids as acetic, propionic, and butyric be able to control yeasts in acidic conditions (Moon, 1983), those compounds were not altered when silages were treated with sodium benzoate. These founds support that benzoic acid was the compound responsible for the reduction on ethanol concentration. The formation of ethyl esters of lactate and acetate, which depend on the ethanol concentration, was also reduced because of the lower concentration of ethanol on treated silages (Weiss, 2012). The trend of sodium benzoate reducing 2,3-butanediol in treated silages might be related to the inhibition of enterobacteria (Nishino and Shinde, 2007). Additionally, enterobacteria have been associated with proteolysis (McDonald, 1991). It has been suggested that sodium benzoate can mitigate proteolysis in grain silages (Da Silva et al., 2015). Controversially, protein solubility and ammonia nitrogen content, which indicate proteolysis, were not altered by sodium benzoate in this trial ($P > 0.10$).

Plant and bacterial enzymes contribute with around 90% of proteolysis in corn grain silages (Junges et al., 2017). When proteolysis is desired, conditions that allow plant and bacterial proteolytic enzymes must be attempted. Moisture, processing and length of storage have been shown the most important factors which increase protein-matrix breakdown, increasing starch and protein digestibility in grain silages (Benton et al., 2005, Hoffman et al., 2011). The greater degree of proteolysis in grain silages is highly correlated with in vitro starch degradability (Ferraretto et al., 2014). In the current trial, the greater proteolytic activity of silages stored for 90-d compared with 30-d certainly allowed easier access to the starch granules by rumen microbes, resulting in faster degradation of starch as indicated by greater rate of gas production in vitro. We expected that sodium benzoate would slower the rate of gas production on treated silages, due to a possible inhibition of proteolysis, but it did not happen.

In acidic conditions, there are species of lactobacilli that can use lactate enabling the formation of acetate and 1,2-propanediol (Oude Elferink et al., 2001). Therefore, only 1,2-propanediol was increased due length of storage on this trial. If it is considered that each mole of lactate is metabolized to equimolar amounts of acetate and 1,2-propanediol, the 109 mg difference of 1,2-propanediol should result in similar production of acetic acid, which was very small to make any difference in acetic acid final concentration. Moreover, 1,2-propanediol can be metabolized into 1-propanol and propionic acid by L. diolivorans

(Krooneman et al., 2002) supporting biologically the tendency of silages stored 90-d have high concentration of these end fermentation products.

Ensiling high moisture grains has been shown to increase starch and protein digestibility (Wilkerson et al., 1997), as a result of the breakdown of protein matrix which is an important physiochemical barrier to starch digestion in ruminants (Owens et al., 1986). In sorghum, proteins are distributed in the endosperm (75%), germ (22%) and pericarp (3%) (Bean et al., 2016). It was mentioned above the importance of main conditions that enable proteolysis throughout ensiling, however, it's important to emphasize that when proteolysis is wished, physical barriers can not limit endosperm access by bacteria. Compiled data from experiments published between 2012 and 2016 showed an increase of 5 to 10% in vitro starch degradability of corn silage within 45 days of ensiling followed by similar increments between 45 and 120 days (Kung et al., 2018). In reconstituted grain silages, the increments in starch degradability seem to be slower (Gomes et al., 2018) and length of storage higher than two months might be adopted to optimize starch digestibility (Da Silva et al., 2019).

When dairy cows are fed high moisture grain silage compared to dry ground feed efficiency is frequently increased. The increase in milk yield with similar DMI (Oba and Allen, 2003, Arcari et al., 2016) or reduced DMI with similar milk yield (McCaffree et al., 1968, Ferrareto et al., 2013) are typical behaviors. Although ensiling of cereal grains often increase dairy cows feed efficiency, the silage length of storage has to be considered. In this trial all treatment diets were composed with grain silages, nevertheless, the feed efficiency was also different.

In the current trial, the sixty days difference on storage period allowed cows to produce 1.2 kg/d more milk without change DMI. The higher milk production of cows fed silages stored for 90-d is partially justified for an increase in starch and protein digestibility. The energy content of 1.2 kg of milk considering the average percentage of fat (3.57%), protein (3.22%), and lactose (4.61%) was calculated (0.826 Mcal). The difference in starch (2.35%) and protein (3.05%) digestibility contributes with an additional of 178 and 120 g of digested starch and protein respectively. Applying the coefficient efficiency of 0.82 from DE to ME (NRC, 2001) and 0.64 From ME to milk energy production (Moe and Tyrrel, 1972), we estimated that increases in starch and protein digestibility would explain 47.5 and 41.1% of the difference in milk yield based on energy partition. The left 11.3% might be a consequence of microbial protein synthesis, although we did not measure any variable to estimate the contribution of this source.

The MUN tended to differ between treatments, even though diet CP was similar. Nousiainen et al. (2004) reported that diet CP was a more accurate predictor of MUN than CP/ME ratio. Conversely, Fadul-Pacheco et al. (2015) found a low correlation between CP and MUN, suggesting a better understanding of other herd characteristics to explain differences in MUN. Using Nousiainen equation, MUN was estimated in 14.4 mg/dL, which was underestimated for diets with silages stored for 30-d, and overestimated for diets with silages stored for 90-d. It suggests greater ruminal use of ammonia nitrogen when grain silage with faster rate of starch degradation was fed. As rumen ammonia-N concentration was higher in silages for 90-d, due to the increase of protein solubility and deamination, a faster supply of energy to the ruminal microbes might increase AA utilization without pass-through ammonia-N pool (Hirstov et al., 2005). Even though rumen ammonia-N was higher on cows fed silages stored for 90-d, the plasma urea nitrogen, which is highly correlated with MUN (Baker et al., 1995), did not differ between treatments.

The higher digestibility of diets containing grain silage stored for 90-d reduced MUN concentration. However, daily excretion of MUN was not altered (43.02 g/d). Hence, the reduction in MUN can be explained mathematically as a consequence of dilution. On the other hand, daily excretion of protein was 43 g higher when cows fed silage stored for longer period. The supply of AA to support this difference in protein yield probably came from rumen microbes, and sorghum proteins, which had greater digestibility in function of extensive proteolysis. In the current study, cows that received diets with higher digestible RSGS were more efficiently in convert nitrogen from diet to milk-N. Within the same N-intake, as in this trial, the only way to increase milk-N efficiency is retaining more nitrogen as milk components. True protein usually represents more than 90% of total-N excreted in milk (Baker et al., 1995, Ahvenjarvi and Huhtanen 2018). Broderick (2003) observed a highly significant linear decrease in N-efficiency use as daily N-intake was increased; however, he also observed a linear increase in N-efficiency use as diet NFC/NDF ratio increases. These results support that to improve dairy cows N-efficiency use, strategies to increase milk true protein yield without extra needs of nitrogen intake, are encouraged. Ruminal fermentation profile was not changed by lengthening silage storage. Oba and Allen (2003) fed high producing dairy cows with dry or HMC and detected an increase in total VFA production in favor of HMC without any shift in VFA proportion. Changes in rumen fermentation pattern were observed in situations when NDF/NFC ratio was drastically altered by replacing forage for concentrate (Russel , 1998 and Sutton et al., 2003). Although starch digestibility was increased with length of storage, the forage concentrate ratio were similar in this trial. The

tendency of higher ECM/DOMI followed by the reduction of acetate to propionate ratio in favor of silages stored for 90-d indicate low rumen methane production. Russel (1998) suggested that propionate production and methanogenesis competing for reducing equivalents. In this way, the faster starch digestibility in the present trial, probably favored propionate formation instead of methane.

Unexpectedly, sodium benzoate increased plasma glucose concentration, with a pronounced difference 6 h after post first feeding. Intravenous injection of sodium benzoate in sheep has been associated with the pancreatic endocrine system, inducing insulin and glucagon secretion (Mineo et al., 1995). Phillips et al. (1969) infused short-chain fatty acids and glucagon in normal and depancreatized sheep and found acid-induced hyperglycemia. Mineo et al. (1995) suggested similar mechanism involved in plasma glucose following benzoic acid administration. Benzoic acid has been also described as a stimulatory of pancreatic amylase secretion in sheep (Katoh and Yajima, 1989). This founds bring us an idea that sodium benzoate can increase plasma glucose in dairy cows by promoting higher amylase secretion in the hindgut or mechanism involving glucagon and insulin regulation. Thus, further studies are warranted to confirm the impact of sodium benzoate on glucose concentration in dairy cows.

3.5. Conclusions

Increasing the storage length of reconstituted sorghum grain silage from 30 to 90 d increased starch digestibility, milk yield and feed efficient and nitrogen use efficiency. Sodium benzoate promoted typical benefits on silage fermentation profile although did not alter the performance of dairy cows. Further studies are warranted to prospect sodium benzoate effect on glucose metabolism of dairy cows.

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4. SHORT COMMUNICATION: THE EFFECT OF SODIUM BENZOATE ON THE NUTRITIVE VALUE OF RECONSTITUTED SORGHUM GRAIN SILAGE FOR DAIRY COWS

Abstract

The objective of this trial was to evaluate the nutritive value of reconstituted sorghum grain silage treated with sodium benzoate for dairy cows. Fifteen-hundred kilograms of dry ground sorghum were reconstituted to 350 g/kg moisture and ensiled in 200-L plastic drums. At ensiling, grain was either treated with sodium benzoate at 2g/kg as fed (Benzoate) or nothing (Control). Twelve mid-lactating Holstein cows with 170 ± 47 days in milk received a standard diet for 14 days and then were paired blocked and assigned to one of two dietary treatments for 28 d. Diets contained (dry matter basis): 360 g/kg corn silage, 48 g/kg Tifton haylage, 122 g/kg whole cottonseed, 184 g/kg soybean meal, 27 g/kg mineral-vitamin premix , and 258 g/kg reconstituted sorghum grain silage (Control or Benzoate). The dry matter intake, milk yield and composition were measured weekly, whereas rumen fermentation profile and feeding behavior were measured on the last week of comparison. Silages treated with sodium benzoate had lower concentrations of ethanol ($P = 0.05$), ethyl-esters ($P < 0.05$) and soluble protein ($P = 0.02$), and less yeast counts ($P < 0.01$). Therefore, silage treated with benzoate had a higher aerobic stability ($P < 0.01$). Ruminal acetate to propionate ratio was lower in cows fed the Control diet (2.66 vs. 2.96), due to the greater proportion of propionate in the rumen fluid (19.0 vs. 17.8 mol/100 mol of volatile fatty acids). However, dry matter intake, milk yield and composition did not differ among treatments. Sodium benzoate changed silo and rumen fermentation profile without altering the performance of mid-lactation dairy cows.

Keywords: Aerobic stability; Chemical additive; Grain silage; Milk yield; Rumen fermentation

4.1. Introduction

Storing dry kernels requires facilities with high investment in construction and maintenance. In this way, ensiling reconstituted cereal grains has advantages considering storage management and nutritive value. The low cost of storage and the improvement in starch digestibility during the fermentation (Benton et al., 2005) are some benefits that justify ensiling reconstituted grains on the farm.

Due to the high monetary value of the ensiled material, strategies capable of reducing fermentative losses and increase aerobically stability are welcome. In properly made grain silages, dry matter (DM) loss during fermentation are often low, but those silages are typically prone to aerobic deterioration, especially in warm weather. Therefore, the use of silage additives with antifungal capacity has been recommended to increase the aerobic stability of grain silages (Morais et al., 2017). Nonetheless, to our knowledge the performance of animals fed reconstituted grain silage treated with sodium benzoate has not been studied in tropical zones.

Sodium benzoate is a microbial growth controller and its efficiency is both dose- and pH-dependent (Russel, 1991). Despite the benefits of sodium benzoate in inhibit the development of undesirable microorganism, it could also reduce intensity of proteolysis (Da Silava et al., 2015). In grain silages, proteolysis is mainly promoted by bacteria (Junges et al.,

2017) and has been positively associated with starch digestibility (Hoffman et al, 2011; Der Bedrosian et al, 2012).

The objective of this study was to evaluate the aerobic stability and the nutritive value of reconstituted sorghum grain silages treated with sodium benzoate (2g/kg as fed basis) for mid-lactating dairy cows.

4.2. Materials and methods

The experimental protocol was approved by the Bioethics Committee of Animal Use of the Luiz de Queiroz College of Agriculture, University of São Paulo (protocol number 2016/25).

4.2.1. Ensiling

The sorghum hybrid (BM 737, Biomatrix®) was sown in March 2015 and the grain harvested at the end of June 2015. Dried grains were stored in metal bins (KeplerWeber®, Campo Grande, MS, Brazil) at 130 g/kg of moisture until silage making.

Before ensiling, sorghum grain was ground through a hammer mill with a 2-mm screen. The rehydration of the ground sorghum before ensiling was performed in a feed mixer wagon (VM4, Delaval®, Tumba, Sweden) up to 350 g/kg of moisture. Fifteen-hundred kilograms of reconstituted sorghum grain silage was prepared for each treatment and ensiled in six 200-L plastic drums (twelve drums in the total). During the water reconstitution, treatments were settled as: Control (no additive) and Benzoate (2 g/kg of sodium benzoate, as fed basis). The sodium benzoate was previously diluted in the water used for reconstitution of the grains.

After packed and closed, silos were weighted to calculate the wet density (1050 ± 55 kg /m3). After 150 d of storage (at least), silos were weighted to determine gas loss and opened for feeding the cows.

4.2.2. Silage sampling and analyses

A non-centrifuged silage extract (25 g of sample + 225 g of deionized water) was homogenized for 4 min in a stomacher and further diluted (10-2 to 10-6) in Ringer's solution for microbial counts. Malt extract agar (Himedia, Mumbai, India), was used for enumeration of yeast after 48 h of incutation at 30ºC. Lactic acid bacteria (LAB) were enumerated using de

Man Rogosa, and Sharpe agar (Acumedia, Lansing, USA) after 48 h of incubation at 30ºC. Plates containing 30 to 300 colony-forming units (CFU) were enumerated.

An aerobic stability test was performed in a controlled room temperature at 21 ± 0.8 ºC. On the last week of the trial samples of 3 kg of each silage treatment were allocated in plastic buckets (five for each treatment) and a temperature sensor (Novus tagtemp®, FL, USA) was placed in the center for record temperature every hour. Aerobic stability was defined as the time to silage temperature rise 2ºC above the ambient temperature.

During silage feed out, samples were collected daily and kept frozen at –20º C to form a composite sample per silo (200-L plastic drum). Aqueous extracts were prepared from the composite sample (25 g + 225 g of deionized water) and homogenized for 4 min in a stomacher. The extract was filtered through 3 folder cheese-cloth and an aliquot of 10 mL was centrifuged for 15 min at 10,000 × g. The supernatant was analyzed for fermentation end products. Lactic acid (Pryce, 1969) and ammonia (Weatherburn, 1967) were determined by colorimetric methods and volatile fatty acids, alcohols and esters were analyzed using a gas chromatograph with a mass detector (GCMS QP 2010 plus, Shimadzu, Kyoto, Japan), using a capillary column (Stabilwax, Restek, Bellefonte, PA; 60 m, 0.25 mm, i.d., 0.25 m). The dry matter content corrected for volatiles was calculated as DMcorr (g/kg as fed) = DMoven (g/kg as fed) + n-alcohols (g/kg as fed) + 2,3-butanediol (g/kg as fed) + 0.95 × volatile fatty acids (g/kg as fed) + 0.77 × 1,2-propanediol (g/kg as fed) + 0.08 × lactic acid (g/kg as fed) (Weissbach, 2009).

4.2.3. Cows, experimental design and diets

For the feeding trial, twelve multiparous Holstein cows (616 ± 50 kg BW, 170 ± 47 DIM) yielding 30.1 ± 4.2 kg/d of milk at the beginning of the trial were housed in a free stall barn with sand beds and individual feedbunks (Intergado Ltda., Contagem, Minas Gerais, Brazil; Chizzotti et al., 2015). The temperature inside the barn was daily recorded by two digital temperature recorders and averaged 25.4ºC with minimal at 23.6ºC and maximum 27.8ºC (Novus tagtemp®, FL, USA). Cows were fed once a day at 0700 h a standard diet for 14 d. Diets in the adaptation period was composed by 360 g/kg corn silage, 48 g/kg Tifton haylage, 184 g/kg soybean meal, 122 g/kg cottonseed, 258 g/kg dry ground sorghum, and 27 g/kg mineral and vitamin premix. At the end of the adaptation period, cows were paired blocked based on milk yield and randomly assigned to one of the two treatments (Control or Benzoate) for 28 d. During the experimental period, cows received the same diet with the exception of dry ground sorghum, which was totally replaced with reconstituted sorghum

grain silages. Nutrient composition of experimental diets was: 163 g/kg crude protein (CP), 350 g/kg neutral detergent fiber (aNDF), 48 g/kg ether extract, 382 g/kg non fiber carbohydrates (NFC), and 55 g/kg ash.

4.2.4. Sample collection and analyses

Samples of feed ingredients and refusals were collected daily and kept at -20ºC to form a composite sample per week. Composite samples were dried in an air-forced oven at 55ºC for 72 h and ground through a 1-mm diameter mesh screen with a Willey Mill (Thomas Scientific, PA, USA). Sub-samples were analyzed for DM, ether extract, and ash (AOAC, 1990; methods 934.01, 920.39 and 924.05, respectively), aNDF (assayed with sodium sulfite and heat stable amylase using a TE-149 fiber analyzer; Tecnal Equipamentos, Piracicaba, Brazil), and CP by the Dumas method (Leco® FP-2000A nitrogen analyzer; Leco corp., St. Joseph, MI). Soluble protein of reconstituted sorghum silages was determined by Krishnamoorthy et al. (1982). The content of non-fiber carbohydrates was calculated as NFC = 100 – (CP + EE + ash + aNDF).

Cows were milked twice daily at 0600 and 1700 h and milk production recorded daily. Average milk yield by week was used to compare treatments. Milk samples were collected once a week for analysis of fat, protein, lactose, and MUN by mid-infrared spectroscopy using Fourier transform (Clínica do Leite, Piracicaba, SP, Brazil). Daily excretion of fat, protein and lactose was computed as milk yield multiplied by the content of solids. Feeding behavior was evaluated on d 23 by visual observation every 10 min intervals for 24 h. Meal duration was defined as the ratio between eating time (min) and number of meals. An inter-meal interval of 20 min was considered to define a meal. Total chewing (min/d) was computed as the sum of eating and ruminating. Rumen fluid was collected on d 28 with a flexible orogastric tube 12 ± 0.5 h after the morning feeding. One sub-sample was used to measure the pH and another one was immediately frozen in liquid nitrogen. Samples were kept frozen at -20ºC until analyses of volatile fatty acids by GC-MS as described for silage.

4.2.5. Statistical Analyses

Cows outcomes were analyzed using the PROC MIXED of SAS (version 9.3) with the following model $Yij = \mu + Bi + Tj + eij$, where μ = overall mean, Bi = random effect of block (I = 1 to 6), Tj = fixed effect of treatment (j = Control or Benzoate), and eij= residual

error. Data from silages were analyzed with the previous model excluding the effect of block. Differences were declared when $P \leq 0.05$ and trends when $0.05 < P \leq 0.10$.

4.3. Results and discussion

4.3.1. Silage fermentation

Silages treated with sodium benzoate had lower concentrations of ethanol, ethyl lactate, and ethyl acetate compared with untreated silages (Table 1). The low gas losses on treated silages are in agreement with low ethanol concentration. Each mol of glucose fermented to ethanol produce 2 mols of CO2 which increases the gas losses (McDonald, 1991). The lower concentration of ethanol and esters were a result of low yeasts growth which is the main microorganism responsible for ethanol production during silage fermentation (Pahlow et al., 2003). The yeast growth is inhibited by mechanisms involving amino acid uptake (Freese et al., 1973), enzymatic systems in the citric acid cycle (Bosund, 1962), and phosphofructokinase (Francois et al., 1986). The formation of the esters such ethyl-lactate and ethyl-acetate depends on ethanol, lactate and acetate concentration (Weiss, 2012). As lactate and acetate concentration were not different between treatments, lower concentrations of ethyl-lactate and ethyl-acetate in silages treated with benzoate was certainly a result of lower ethanol concentration. Although some fermentation products in silages, such as acetic acid, has been correlated with lower DM intake (Kriszan et al., 2007, Daniel te al., 2013) or change on the daily pattern (Hutchinson and Wilkins, 1971; Santos et al., 2017), ethanol and ethyl-esters have not been associated with changes on intake by ruminants (Gerlach et al., 2013; Daniel et al., 2013).

In addition to the benefits of sodium benzoate in reduce ethanol concentration and increase aerobic stability (Morais et al., 2017), the antimicrobial capacity of sodium benzoate might decrease proteolysis during silage fermentation (Da Silva et al., 2015). In the current trial, sodium benzoate decreased the proteolysis, which is mainly performed by silage bacteria (Junges et al., 2017). The concentration of soluble protein was reduced from 263 g/kg to 206 g/kg of CP and ammonia nitrogen from 43.4 to 33.0 g/kg of N when silages were treated with sodium benzoate (Table 1). The lower concentration of 2,3-butanediol on treated silages also indicate that benzoate restricted the development of enterobacteria (Nishino and Shinde, 2007), which have proteolytic activity (McDonald et al., 1991).

Tabela 6. Fermentation profile of reconstituted sorghum grain silages with or without sodium benzoate

	Treatment[1]			
Item	Control	Benzoate	SEM	*P*-value
DM_{corr}, g/kg	656	657	38	0.94
pH	3.94	3.78	0.05	0.11
Soluble protein, g/kg of CP	262	206	10.5	0.02
$N\text{-}NH_3$, g/kg of N	43.4	33.0	1.5	0.01
Lactic acid, g/kg of DM_{corr}	17.33	15.45	2.62	0.68
Acetic acid, g/kg of DM_{corr}	3.46	3.85	0.15	0.19
Propionic acid, mg/kg of DM_{corr}	52	57	11.2	0.77
Butyric acid, mg/kg of DM_{corr}	11	10	1.45	0.74
Ethanol, g/kg of DM_{corr}	8.40	1.85	1.21	0.05
1,2-Propanediol, g/kg of DM_{corr}	0.96	0.91	0.30	0.91
2,3-Butanediol, mg/kg of DM_{corr}	171	40.5	7.50	<0.01
Ethyl acetate, mg/kg of DM_{corr}	24	12	2.08	0.03
Ethyl lactate, mg/kg of DM_{corr}	388	157	21.6	<0.01
BAL, log cfu/g	4.66	4.16	0.09	0.01
Yeast, log cfu/g	4.73	2.52	0.45	0.02
Aerobic stability, h	51.7	146.1	0.76	<0.01
Gas losses, g/kg of DM	1.21	0.67	0.071	0.01

[1]Control: reconstituted sorghum grain silage without any additive; Benzoate: reconstituted sorghum grain silage treated with sodium benzoate at 2 g/kg as fed.

4.3.2. Dairy cows performance and rumen fermentation profile

Although treated silages had high aerobic stability, cow's performance was similar between treatments (Table 2). Rumen pH and proportions of acetate, butyrate and valerate were not changed by treatments. However, animals fed reconstituted sorghum grain silages treated with sodium benzoate had lower proportion of propionate and higher acetate to propionate ratio (Table 3). Ensiling grains with high moisture content increases the degradation of the protein matrix surrounding the starch granule, which results in faster rate of starch degradation in the rumen (Hoffman et al., 2011). Nevertheless, the antimicrobial capacity of sodium benzoate and its negative impact on silage proteolyses may have slightly constrained starch degradation in the rumen. In this trial, silages treated with sodium benzoate had lower soluble protein and N-NH3 concentration. Ammonia and soluble protein has been used as predictors of proteolysis and has a positive correlation with starch degradation in high moisture corn (Ferraretto et al., 2014). This result suggests a lower rate of starch degradation in cows fed the benzoate treatment, as indicated by the lower proportion of propionate in the rumen fluid.

Tabela 7. Dry matter intake and lactation performance of dairy cows fed reconstituted sorghum grain silages with or without sodium benzoate

	Treatment[1]			*P*-value[2]		
Item	Control	Benzoate	SEM	T	W	T × W
DMI, kg/d	19.5	19.6	0.75	0.91	0.39	0.68
Milk, kg/d	27.2	27.5	1.95	0.93	0.02	0.30
Fat, g/100g	3.18	3.22	0.201	0.90	0.07	0.87
Fat, kg/d	0.893	0.860	0.048	0.65	<0.01	0.68
Protein, g/100g	3.23	3.26	0.065	0.77	0.01	0.55
Protein, kg/d	0.900	0.873	0.040	0.63	0.26	0.58
Lactose, g/100g	4.65	4.63	0.291	0.66	0.06	0.42
Lactose, kg/d	1.29	1.24	0.066	0.59	0.02	0.69
Urea N, mg/dL	15.1	15.5	0.36	0.44	<0.01	0.18
Milk/DMI	1.44	1.40	0.035	0.42	0.13	0.18

[1]Control: reconstituted sorghum grain silage without additive; Benzoate: reconstituted sorghum grain silage treated with sodium benzoate at 2 g/kg as fed.
[2]T: fixed effect of treatment, W: fixed effect of week, T × W: interaction between treatment and week.

Diet treatments did not alter eating and ruminating times. Nevertheless, cows fed the Control treatment had approximately two more meals per day than the Benzoate treatment. Changes in ruminal fermentability of starch capable of altering the flux of propionate to the liver can alter the daily pattern of feed intake (Allen et al., 2009). The hepatic oxidation theory suggests that satiety and hunger are controlled by the energetic status of the hepatocytes in ruminants (Allen et al., 2009), and propionate is the VFA most extensively used by liver as a fuel (Reynolds, 1995). Oba and Allen 2003 observed an increase in meal bouts with lower meal size and attributed that behavior of a greater production of propionate in the rumen, when highly fermentable diets were fed to dairy cows. Similar results were observed on this trial suggesting the same mechanism controlling the feeding behavior. Due to the capacity of grain silages treated with sodium benzoate changing the profile of rumen VFA, further studies enrolling fresh cows are warranted. Fresh cows are more prone to hypophagic effects of propionate than mid lactation (Oba and Allen, 2003b) because greater propionate in the liver stimulates acetyl CoA oxidation, causing satiety (Allen, 2009). On this way, grain silages when treated with sodium benzoate might reduce the negative impact of high fermentable diet on dry matter intake in fresh cows.

Tabela 8. Chewing, ingestion behavior, and rumen fermentation profile of dairy cows fed reconstituted sorghum grain silages with or without sodium benzoate

	Treatment[1]			
Item	Control	Benzoate	SEM	*P*-value
Feeding behavior				
Eating, min/d	217	200	23.3	0.46
Ruminating, min/d	656	598	25.0	0.12
Chewing, min/d	875	798	28.2	0.11
Meal bouts, /d	8.74	6.80	0.623	0.04
Meal duration, min/meal	26.1	29.4	2.56	0.36
Rumen parameters				
pH	5.86	6.06	0.15	0.37
Acetate, mol/100 mol of VFA	50.60	52.62	1.70	0.42
Propionate, mol/100 mol of VFA	18.99	17.80	0.45	0.04
i-Butyrate, mol/100 mol of VFA	5.83	4.85	0.41	0.12
Butyrate, mol/100 mol of VFA	17.17	17.80	1.24	0.73
i-Valerate, mol/100 mol of VFA	1.50	1.80	0.22	0.35
Valerate, mol/100 mol of VFA	6.00	5.20	0.29	0.11
A:P ratio	2.66	2.96	0.09	0.03

[1]Control: reconstituted sorghum grain silage without additive; Benzoate: reconstituted sorghum grain silage treated with sodium benzoate at 2 g/kg as fed.

4.4. Conclusion

Sodium benzoate increased the aerobic stability, decreased the concentrations of volatile organic compounds and reduced proteolysis in sorghum grain silages. Although the silage treated with sodium benzoate increased the acetate to propionate ratio in the rumen fluid, dietary treatments did not alter the performance of mid-lactating dairy cows.

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5 GENERAL CONCLUSIONS AND CONSIDERATIONS

It is already known that ensiling increase nutritive value of grain silages. Previous trials have been demonstrated increments on dry matter (+15%) and starch (+10%) degradability when grains are ensiled at properly conditions (Benton, 2005; Der Bedrosian et al., 2012; Kung et al., 2018; Da Silva et al., 2019). On the best of my search, it was not common to find data reporting in situ protein degradability, however it is also increased. Literature data suggest at least two months of storing to improve dry matter degradability in two thirds of the total in reconstituted corn grain silages (Fernandes, 2014; Da Silva et al., 2019). When total tract dry matter and starch digestibility were evaluated, the increments were not as high compared to in situ studies.

Arcari et al. replaced dry ground corn from reconstituted ground corn grain silage stored for 90 days and observed an increase on dry matter (+ 5.7%), starch (+ 6.2%), and crude protein (+ 4.3%) digestibilities. Schonel et al. replaced dry ground sorghum for reconstituted ground sorghum grain silage stored for 80 days and observed increased digestibilities of dry matter (+ 3.1%), starch (+ 9.9 %) and crude protein (+ 5.2%). In this present thesis was evaluated the effect lengthening storage from 30 to 90 days of reconstituted sorghum grain silages. Increasing storage length increased dry matter (+ 2.1%), starch (+ 2.7%) and protein (+ 5.6 %) digestibilities.

Although there is a positive linear correlation between starch digestibility and milk yield (Firkins et al., 2001), it is not a rule, and the increase in milk production is not totally explained by the increase of starch digestibility. The system is more complex, and takes into account microbial protein synthesis, site of starch digestion, and in silages due proteolysis, high protein digestibility. Considering all the benefits of feeding dairy cows with reconstituted grain silages compared to dry ground or ensiled for short time, we estimated the increment of milk per ton of corn/sorghum grain silage based on three experiments (Arcari, 2016; Schonel, 2016 and Santos, 2019). Independently of dairy cows performance (Increase milk with similar dry matter intake or similar milk yield with lower dry matter intake), grain silages (stored for > 80 days) promoted increase on productivity of 450 kg of milk / DM ton of corn or sorghum compared to dry ground. When comparison was made from grain silages stored for 90 or 30 days the difference was 400 kg of milk / DM ton of sorghum.

The use of chemical additives is recommended to reduce losses, increase aerobic stability, and control undesirable microorganisms which can produce toxins and mycotoxins. In grain silages gas losses is typically low (average 1.5%; raging from 0.5 to 5%) (Morais et al., 2017). Considering only the benefit in control losses, the use of sodium benzoate at 0.2%

in grain silages is not profitable at current prices of sorghum (R$ 600,00/ton) and sodium benzoate (R$ 16,00 kg). However, considering the increase in aerobic stability and reducing probability of mycotoxins occurrence it became interesting. It is not easy to apply monetary value for aerobic stability response or guarantee that using additives, mycotoxins will not happen. On this way, the use of sodium benzoate on reconstituted grain silages is recommended when the price spent with the additive is equal the price saved due losses reduction. Thus, the increase of aerobic stability and low probability of mycotoxins occurrence comes as an additional benefit.

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