University of São Paulo
Luiz de Queiroz College of Agriculture

Propionic acid based additive on aerobic stability of total mixed rations and performance of lactating dairy cows

Ariádna Patricia Ribeiro

Dissertation presented to obtain the title of Master in Science. Area: Animal Science and Pastures

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To my parents, brothers and friends
I DEDICATE

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CONTENTS

RESUMO 6
ABSTRACT 7
LIST OF TABLES 8
1. INTRODUCTION 9
REFERENCES 9
2. LITERATURE REVIEW 11
2.1. TMR AS A NUTRITIONAL STRATEGY FOR DAIRY COWS 11
2.2. AEROBIC STABILITY OF TMR 13
2.3. FEED SAFETY IN TMR 14
2.4. PROPIONIC ACID-BASED ADDITIVES ON TMR: MODE OF ACTION 17
2.5. PROPIONIC ACID ON ANIMAL PERFORMANCE AND METABOLISM 19
REFERENCES 21
3. PROPIONIC ACID BASED ADDITIVE ON TOTAL MIXED RATIONS FOR LACTATING DAIRY COWS PERFORMANCE 27
3.1. INTRODUCTION 27
3.2. MATERIAL AND METHODS 28
3.2.1. Experimental diets 28
3.2.2. Feeding trial 30
3.2.3. Animal performance: nutrient intake, milk yield and composition 32
3.2.4. Feeding behavior and particle sorting 34
3.2.5. Statistical analyses 35
3.3. RESULTS AND DISCUSSION 35
3.4. CONCLUSIONS 43
REFERENCES 44

RESUMO

Aditivo a base de ácido propiônico na estabilidade aeróbica de rações completas e desempenho de vacas lactantes

Doze vacas Holandesas com peso corporal médio de 612 kg, 178 ± 60 dias de lactação (média ± DP) e produção média diária de leite de 27 ± 6,5 kg (média ± DP) foram alocadas a um delineamento em switch-back para avaliar o efeito da aplicação de um aditivo à base de ácido propiônico na ração mista total (TMR) durante a mistura dos ingredientes, imediatamente antes da oferta no cocho, sobre o desempenho animal. O conjunto de tratamentos foi desenhado para realizar uma comparação entre duas estratégias de alimentação: vacas recebendo duas ofertas diárias de TMR sem tratamento (Controle); e vacas recebendo uma única oferta diária de TMR tratada com o aditivo a 2 L/t de matéria natural (Aditivo). A composição da dieta foi a mesma para ambos os tratamentos, com 44,59% de silagem de milho, 6,40% de pré-secado de Tifton, 21,11% de silagem de milho grão reidratado, 9,25% de polpa cítrica, 16,33% de farelo de soja e 2,32% de mistura mineral-vitamínica. Vacas alimentadas com a TMR tratada com aditivo aumentaram significativamente a produção diária de leite em 2,4 kg sem alterações significativas no consumo de matéria seca (CMS), embora apenas um ligeiro aumento não significativo tenha sido observado para a eficiência alimentar. Os teores de sólidos totais, gordura e lactose foram diluídos no leite das vacas alimentadas com a TMR tratada, embora a produção diária desses componentes tenha sido maior devido ao aumento da produção de leite. O teor de proteína bruta e o nitrogênio ureico do leite (NUL) não foram alterados pela estratégia de alimentação. A densidade energética do leite foi semelhante entre os tratamentos, embora a excreção de energia através do leite tenha sido maior para as vacas alimentadas com a TMR tratada com aditivo. A contagem de células somáticas (CCS) foi significativamente maior para as vacas quando alimentadas com a TMR tratada. O aditivo alterou o comportamento de seleção em favor de partículas mais longas da ração em relação ao tratamento controle. Vacas que receberam uma única oferta diária de TMR tratada com aditivo passaram menos tempo ingerindo e mais tempo ruminando, com maior tamanho de refeição, menor duração da refeição e redução do número de refeições diárias. A frequência de oferta de TMR pode ser reduzida, com aumento na produção de leite em 10,2%, na presença de aditivo a base de ácido propiônico.

Palavras-chave: Frequência de alimentação, Estabilidade aeróbica, Ração completa, Estratégia nutricional

ABSTRACT

Propionic acid based additive on aerobic stability of total mixed rations and performance of lactating dairy cows

Twelve Holstein cows with average body weight of 612 kg, 178 ± 60 days in milk (mean ± SD) and average daily milk yield of 27 ± 6.5 kg (mean ± SD) were assigned to a switch-back design to evaluate the effect of applying a propionic acid-based additive on total mixed ration (TMR) during the mixture of ingredients, immediately before feeding, on the animal performance. The set of treatments was designed to perform a comparison between two feeding strategies: cows receiving two daily offers of TMR without treatment (Control); and cows receiving a single daily offer of TMR treated with the additive at 2 L/t of as fed matter (Additive). The ration composition was the same for both treatments, with 44.59% corn silage, 6.40% Tifton haylage, 21.11% rehydrated corn grain silage, 9.25% citrus pulp, 16.33% soybean meal and 2.32% mineral-vitamin mix. Cows fed the additive-treated TMR significantly increased daily milk yield in 2.4 kg without significant changes in dry matter intake (DMI), although only a slight non-significant increase was observed for feed efficiency. The contents of total solids, fat and lactose were decreased in the milk of cows fed the treated TMR, although daily yield of these components was greater due to increased milk yield. Milk crude protein content and milk urea nitrogen (MUN) were not altered by feeding strategy. Energy density of milk was similar across treatments, although energy excretion through milk was greater for the cows fed the additive-treated TMR. Somatic cell count (SCC) was significantly higher for the cows when fed the treated TMR. The additive shifted sorting behavior in favor of longer particles of the ration, in relation to the control treatment. Cows receiving a single daily offer of additive-treated TMR spent less time ingesting and more time ruminating, with greater meal size, shorter meal length and decreased daily meal frequency. TMR offering frequency might be reduced, with a 10.2% increase in milk yield, by using propionic acid based additive.

Keywords: Feeding frequency, Aerobic stability, Total mixed ration, Feeding strategy

LIST OF TABLES

Table 1. Survey on studies with ruminal infusions of propionic acid..20

Table 2. Inclusion level of ingredients and composition of formulated diets...29

Table 3. Chemical composition of offered diets...30

Table 4. Chemical composition of the ingredients 30

Table 5. Nutrient intake by dairy cows fed the TMR treatments...36

Table 6. Performance of dairy cows fed the TMR treatments...38

Table 7. Additive and propionic acid intake by cows fed the treated TMR 40

Table 8. Feeding behavior of dairy cows fed the TMR treatments..42

1. INTRODUCTION

The diets for production ruminant animals are formulated with the aim of providing the animal with raw material – the nutrients – so it can both generate the product of interest (meat, milk, wool) and maintain its basic biological functions (maintenance), in addition to compensate for possible energy and nutrient drains (pregnancy, locomotion activity). In this context, the total mixed ration (TMR) is a nutritional strategy that aims to guarantee the supply of all the necessary nutrients to the animal in a uniform and simultaneous manner (Coppock et al., 1981), allowing adequate intake by the animal. The simultaneous supply of all ingredients that compose the diet reduces the risk of sorting and the unbalanced intake of nutrients (NRC, 2001).

As it is a mixture of ingredients, including forages and silages that have a high moisture content and their own microflora, TMR is a mixed diet very prone to spoilage while served in the bunk. Silages are also the ingredients that most contribute to aerobic deterioration, as they initiate this process after being removed from the silo and exposed to atmospheric oxygen. The use of additives based on organic acids, mainly propionic acid, is a strategy commonly used to enhance the conservation of feeds, mainly silages and hay. Its antimicrobial properties allow the control of yeasts, fungi and spoilage bacteria, increasing the feed's resistance to aerobic spoilage (Morais et al., 2017). Thus, TMR can also benefit from the use of such chemical additives, extending the maintenance of its nutritional quality in the feed bunk after being offered to the animals.

The aim of this study was to evaluate the effects of the inclusion of a propionic acid-based additive in the TMR immediately before feeding on the performance and selective behavior of dairy cows. For the animal performance trial, the treatments were designed in order to provide a feeding management strategy to challenge the preservative potential of the additive, so the additive-treated TMR was offered only once a day, while the control TMR was split in two daily offers. With that, feeding frequency and additive are offered as a joint strategy to reach better efficiency.

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2. LITERATURE REVIEW

2.1. TMR as a nutritional strategy for dairy cows

Since the 1950s, the supply of roughage, consisting of forages, in addition to protein and high energy concentrates, mineral and vitamin sources as a single feed for dairy cows has gained notoriety. Since then, productive and reproductive efficiency, technological advances and profitability in the dairy industry in the last 70 years have been highly associated with the physical integrity and nutritional/microbial quality of the feed, as well as the way it is offered to these animals. (Schingoethe, 2017).

The first studies attributed to this feeding strategy were developed by Harshbarger (1952), and later by McCoy et al. (1966), which was the first complete study to be published. Both sought to evaluate the offer of feed in a single mixture format to lactating cows in comparison to the offer of roughage and concentrate separately, and observed that the animals submitted to the single mixture responded with an increase in DM intake, milk yield, feed efficiency and milk fat content. Less competition between cows during feeding (Coppock et al., 1981), and lower frequency of digestive disorders, especially in the peripartum stage (Hernandez-Urdaneta et al., 1976), were also reported.

The results of these and other trials published in that period corroborated the observations and outcomes obtained in the field, consolidating the unique blending of feeds as the main diet and nutrition strategy for confined high-yield dairy cows in the United States (Schingoethe, 2017). Currently, this is the main feeding method adopted for different categories across the ruminant animal industry, especially high production cows in confinement in most countries with emphasis on milk production (Snowdon, 1991), arriving in Brazil at the end of the 1970s.

The term TMR - Total Mixed Ration was defined by Coppock et. al. (1981) as a proportional mixture of all the ingredients that make up the diet, and when correctly homogenized, a single ration is formed to be offered to the animals ad libitum, making it difficult for them to sort the ingredients in the bunk. Achievement of basic guarantees of consistency between the formulated diet and the ration actually offered, regarding the chemical and physical composition, is an essential part of maximizing the performance of the cows and obtaining the best nutritional and economic value from the feed. The term TMR is used synonymously with single mix, total diet, complete diet, complete feed and mixed total feed.

According to NRC (2001), the objective of offering a total diet is to provide the opportunity for cows to consume the amount of feed determined in the diet formulation, approaching precision nutrition, where the nutritional requirements of the animals are ensured, without deficiencies or excesses, maximizing the use of existing foods and consequently increasing efficiency in their use, in addition to decreasing their excretion into the environment (Sova et al., 2014).

In metabolic terms, it is expected that the use of TMR provides the construction of a homogeneous flow of nutrients to the rumen throughout the day, promoting a favorable environment for the rumen microbiota. Consequently, it contributes to a more uniform fermentation, maximizing the use of nutrients present in ingested feeds, synchronization between energy and protein availability in the rumen, ruminal pH stability and animal longevity (Devries et al., 2005).

In practical terms, the use of the total diet optimizes the operational sector of dairy farms and reduces labor costs (Schingoethe, 2017). When associated with animal data processing, it allows the organization of the herd in groups of animals of similar production conditions, days in milk and reproductive status, which favors the formulation of diets to meet specific nutritional demands for each group without compromising the routine of the production unit. (Lammers et al., 2003).

Among other advantages, it becomes unnecessary to supply a free choice of mineral and vitamin supplements (Coppock, 1977), which allows greater safety in the use of non-protein nitrogen compounds, especially urea (Schingoethe, 2017). In addition, it promotes the incorporation of less palatable ingredients into the diet, as the high levels of forage contribute to dilute and mask the taste of unpleasant ingredients (Schingoethe, 2017). This can be an important strategy because it increases the range of ingredients available to bovine nutrition, which can reduce costs in the feed industry and guarantees a sustainable destination for resources with potential, such as co-products from different industries.

James Cox (2008) emphasizes that the inclusion of any ingredient must be endorsed in an effective nutritional management. This means that we must first know the nutritional specifications of the ingredients in order to achieve nutritional accuracy in the formulated TMR.

Among the disadvantages of using a total diet, the need to invest in infrastructure for storing ingredients and equipment, such as tractors and mixing wagon with load cell, may become unfeasible for small properties. According to USDA (2014), nearly 90% of large herds (>500 cows/herd) in the US were fed TMR compared to <20% of small herds (30-99 cows/herd).

In properties that adopt the TMR as strategy for feed management, perhaps the main obstacle is to transform the formulated diet into a consumed diet. It is admitted that on a dairy farm there are three diets:

I. Formulated diet, which results from formulation programs, supported by the chemical composition of the ingredients, laboratory analysis, herd data and goals and objectives designed to be achieved (Rossow and Aly, 2013);

II. Prepared and offered diet, highly dependent on operator care when mixing the ingredients, which is sensitive to factors such as loading order, mixing time, amount loaded, type of mixing car (Allen, 2000; Rossow and Aly, 2013) and method of delivering in the feed bunk;

III. Ingested diet, determined by the behavior of ingestion, mainly the sorting of particles with different sizes (Rossow and Aly, 2013).

This happens because, despite the fact that the TMR preparation process is relatively simple, it is highly dependent on the human factor, therefore subject to error. The preparation of the diet can directly interfere with the physical characteristics of the diet since it is closely linked to the health integrity of the ingredients in the preparation of the TMR and the time of exposure to the environmental characteristics.

2.2. Aerobic stability of TMR

As with silages, exposure of TMR to air favors deterioration and promotes a faster "heating rate", leading to the disruption of aerobic stability (AS). As a result, there is loss of nutrients and negative effects on voluntary intake of dry matter and, consequently, on the performance of the animals (Kung Jr, 2009). This results in lower shelf life of the TMR in the bunk, promoting an increase in the amount of food rejected by the cows and labor costs due to frequent delivery of feed or removal of excess leftovers in the bunk (Seppälä et al., 2016).

The term aerobic stability is defined as the time in hours required for measurable changes in temperature to occur (Siqueira et al., 2005), which is when the feed temperature exceeds the ambient temperature by 2ºC (Moran et al., 1996; Ranjit and Kung, 2000; Driehuis et al., 2001; Kung Jr. et al., 2003). In practice, aerobic stability represents the resistance of the evaluated material to heating, and can be characterized as the “lag” phase of growth of aerobic microorganisms (Ranjit and Kung, 2000).

There is enough data (O'Kiely et al., 2021) reporting that, as in silages, TMR deteriorates due to exposure to air, and its nutritional value is decreased due to the loss of fermentation products, such as lactic and acetic acid, which become substrates for microbial growth (Honig et al., 1999; Whitlock et al., 2000; Pahlow et al., 2003). According to Jobim et al. (2007), the ability of feeds to maintain stability when exposed to oxygen is a very important factor in determining their quality.

It is possible that the maintenance of aerobic stability of TMR is closely linked to the quality of the forage used in its manufacture. Therefore, careful maintenance of the AS of forage and grain silages is essential, especially in the warmer periods of the year, when combined factors such as temperature and environmental humidity reach greater impact on the stability of the substrate (Ashbell et al., 2002).

Aerobic spoilage is inevitable and invariably accompanied by losses of fermentation volatile products, proteins and carbohydrates (Woolford, 1984). Therefore, it becomes a strong indicator of nutrient and dry matter losses in the form of carbon dioxide and water (Woolford et al., 1977).

2.3. Feed safety in TMR

The total diet is a ration composed of different ingredients based on forages, grains and cereals and their derivatives, vitamins and minerals. The "shelf life" of a TMR – its durability in the bunk – occurs due to intrinsic factors such as the type of ingredients, nutritional composition, water activity, pH, epiphytic microflora, aeration, degree of processing and particle size at the time of preparation of the TMR, in addition to extrinsic factors such as time of exposure to environmental conditions, temperature, humidity and type of bunk or structure in which the ration is delivered.

The process of homogenization of ingredients, mainly in diets for dairy cows with high forage inclusions between 35 and 50%, creates a microenvironment (nutrient x temperature x humidity) favorable to the development of beneficial and non-beneficial microorganisms, including fungi, wild yeasts and bacilli present in fresh forages and mainly in forages and grains preserved by anaerobic fermentation. The latter are possibly the most unstable ingredients present in traditional diets offered in most Brazilian dairy farms.

Authors such as McDonald (1981), Woolford (1990) and Amaral et al. (2008) suggest that the anaerobic state is one of the factors responsible for the conservation of ensiled forages.

The exposure of this type of material to aerobic conditions allows organisms that were sporulated resume metabolic activity, proliferating rapidly, generating heat and triggering chemical activities when using nutrients as an energy substrate. The presence of oxygen in the silage determines the multiplication of some groups of aerobic microorganisms that consume the energetic compounds present, increasing the losses of dry matter (DM) and decreasing the nutritional value of the food (Pahlow et al., 2003).

Ranjit and Kung (2000), when studying the effects of anaerobiosis in corn silage, reported losses of 3.4% of DM and 1.4% of soluble carbohydrates at the time of silo opening. Until the third day of exposure to air, losses increased to 5.3% and 60%, respectively. In that study, the pH values increased from 3.9 to 5.0 and the lactic and acetic acid contents were reduced from 7.52% to 1.35% and from 1.88% to 0.08% of DM, respectively.

Tropical climates such as in Brazil favor the heating of feed during the processes of storage and delivery to the animals, which is intensified in the summer months, when high temperatures promote the growth of fungi, yeasts and some bacilli. The increase in feed temperature may be associated with microbial multiplication on it, which can intensify the deterioration process of the feed supplied to cows. The greatest intensity of deterioration occurs when ambient temperature exceeds 30ºC, favoring the proliferation of microorganisms, greater yield of CO_2 and greater pH increase (Ashbell et al., 2002).

In silages, the first group of microorganisms to develop in the presence of oxygen are yeasts, which are probably the first TMR colonizers. This is because these organisms can multiply over a wide range of pH 3 to 8 (McDonald, 1981). Yeasts consume lactic acid and sugars, and release carbon dioxide, water, and generate heat (Woolford, 1990), and its metabolic activity triggers the spoilage process in feeds that were preserved at anaerobic conditions. Ranjit and Kung (2000) observed an increase in yeast numbers from approximately 10^6 CFU/g at the time of silo opening to more than 10^8 CFU/g within 36 hours of exposure to air. In corn silage, these organisms cause an increase in pH, which can reach values in the range of 5 and 6, allowing the development of other undesirable microorganisms (McDonald et al., 1991). Like acetic acid bacteria, which also act in this phase oxidizing ethanol to acetic acid, the genus Acetobacter can oxidize acetate and lactate to carbon dioxide and water.

The bacilli start to develop at the beginning of aerobic deterioration. Their metabolism possibly creates conditions for some strict anaerobic organisms, such as clostridia, to develop during aerobic deterioration due to the coexistence of aerobic and anaerobic niches in the silages. The anaerobic niches originate from the oxygen consumption by aerobic microorganisms active in the oxidation processes, which result in anaerobic points in the aerated mass, favoring the growth of clostridia (Pahlow et al. 2003). Filamentous fungi also develop during the advanced stages of aerobic deterioration in silages (Woolford, 1990).

The intake of silage or any other feed with signs of deterioration by dairy cows is undesirable, as it poses a risk to animal health (Cai et al., 1999). Feeds under these conditions have high activity of potentially pathogenic or undesirable microorganisms (Lindgren et al., 2002) and mycotoxin synthesis (Richard et al., 2009). In addition to lower nutritional value, they may result in lower voluntary intake, complete refuse of feed and negative effects on animal performance (McDonald et al., 1991; Kung Jr. et al., 1998).

Deoka et al. (1982) reported that voluntary intake of TMR by sheep was negatively affected when corn silage exposed to aerobiosis for 5 days was added, compared to animals that received fresh corn silage. The pH of this material was increased from 3.97 to 6.35 on the fifth day of exposure. Hoffman and Ocker (1997) evaluated the performance of 18 lactating cows fed TMR containing fresh high moisture grain silages “taken daily from the silo” vs. deteriorated high moisture grain silages “taken from the silo 14 days before feeding and stored on a concrete floor”. The animals that consumed the deteriorated silage responded with a decrease of 3.2 kg of milk/day.

In a study conducted by Wichert et al. (1998), dairy cows expressed refusal of whole-plant corn silages with low hygienic quality, decreasing intake by 10 to 20%, compared to fresh silage. Gerlach et al. (2013) noted similar responses in goats fed corn silage exposed to aerobic conditions for 4 days, suggesting that the low integrity of this component in the TMR had a negative impact on voluntary intake and feed preference in goats.

Whitlock et al. (2000) observed that the supply of deteriorated corn silages had a negative impact on the nutritional value of TMR, on DM intake and on the digestibility of cannulated steers. These animals were conditioned to a TMR composed of 90% corn silage and 10% concentrate, where the roughage portion originated four treatments: I. 100% normal silage; II. 75% normal and 25% deteriorated; III. 50% normal and 50% deteriorated; and IV. 25% normal and 75% deteriorated. The animals fed with 75% deteriorated silage decreased their intake by 17%, and the digestibility of neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) and organic matter (OM) in 7.2, 9.9, 4.1 and 5.0 percentage units, respectively, when compared to the control group. These authors also reported that the supply of 25% deteriorated silage partially or totally disrupted the solid phase of the rumen.

The response of the animals in that study demonstrates that spoiled feeds cause significant changes in the quality of the diet, which may reduce weight gain or milk production. The decrease in DM intake observed in that study seems to be motivated by the low acceptability and digestibility of the animals, which conditioned the decrease of ruminal passage rate and compromise of the synthesis of microbial protein generated by the imbalance in the supply of nitrogen and energy in the rumen environment. In a study conducted with heifers, Windle and Kung (2013) also associated the intake of deteriorated silage with lower DM intake, loss of digestive efficiency, increased number of yeasts in the rumen fluid and poorer intestinal health.

Heller et al. (2021) reported that already aerobically deteriorated TMR may have higher pH and lower concentrations of lactic acid, acetic acid and ethanol than fresh TMR. In this context, the search for additives capable of promoting greater stability of the total mixture and reducing microbial activity becomes desirable.

2.4. Propionic acid-based additives on TMR: mode of action

Short-chain organic acids (SCOA) are widely used in food production and conservation due to their high effectiveness in maintaining organoleptic characteristics, preserving hygienic integrity and, consequently, increasing their shelf life. In dairy cattle nutrition, use of SCOA is mainly linked to grain conservation and preservation of fermented foods, such as plant and grain silages and haylages, reducing microbial activity when exposed to aerobiosis (Haque et al., 2009). When used in the silage process, they can inhibit the growth of undesirable microorganisms present in the ensiled material and, thus, improve silage fermentation, preventing the deterioration process in the silo and subsequently prolonging aerobic stability (Kleinschmit and Kung, 2006).

In silages, during the feed-out phase, high concentrations of lactic acid become a substrate for aerobic organisms and the low concentrations of short-chain fatty acids, such as propionic and acetic, might be insufficient to protect the feed against yeasts and fungi (Moon, 1983). SCOA have strong antimicrobial and antifungal properties, in which propionic acid expresses greater efficiency, followed by acetic, lactic and citric acids. Among the other organic acids, benzoic and sorbic acid stand out. Most organic acids with antimicrobial activity have a chain length between one and four carbons, and a pKa between 3 and 5.

Propionic acid (PA) and its derivative salts, such as sodium and ammonium propionate, can be used as pH buffers, preservatives and flavoring agents (Smith and Hong-Shum, 2003). Approximately 66% of the annual world production of PA is for use in animal nutrition (Sauer et al., 2008; Xu et al., 2011; Quitmann et al., 2014). Propionic acid has antimicrobial properties that have been extensively reported in the literature, usually resulting in improved aerobic stability of both forages and grain silages. The inhibition of yeasts, fungi and some bacteria are promoted by the undissociated form of the acid, whose concentration is higher at low pH values (Lambert and Stratford, 1999). At pH 6.5, the undissociated form of the acid accounts for only 1%, while it increases to more than 50% when pH drops below 4.8.

The antimicrobial activity of propionic acid is reported mainly against fungi and bacteria (Barbosa-Cánovas, 2003). Salts, such as sodium ammonium propionate, promote a similar effect against yeasts and filamentous fungi at low pH (Schnürer and Magnusson, 2005), although they are less aggressive than the acid itself to animal health, which is why they are commonly used in most commercial additives. In its undissociated form, propionic acid is lipophilic, which allows it to penetrate the cellular lipid membrane more easily. Once in the cytoplasm, it starts to acidify the medium by dissociating into anions and protons (Cherrington et al., 1990; Davidson, 2001; Burt, 2004). Cytoplasmic acidification leads to the interruption of enzymatic reactions and nutrient transport systems, compromising their macromolecular functions (Cherrington et al., 1991). Russell (1992) also mentions that the accumulation of anions within the cytoplasm of bacterial cells is the main toxic effect of organic acids. According to Cherrington et al. (1991), they can also cause changes in the permeability and activity of the bacterial cell membrane, in addition to altering the activity in calcium channels, leading to ionic imbalance and loss of ions. Damage to the enzyme system compromises energy production and synthesis of structural components, making it difficult to conduct and transport intracellular adenosine triphosphate (ATP) (Cherrington et al., 1990). Exporting excess protons requires consumption of cellular ATP, which can result in the depression of all energy in the cell, decreasing energy for cell proliferation and, in more severe cases, leading to cell death and resulting in some degree of bacteriostasis (Davidson, 2001).

Goeser et al. (2015), in a meta-analysis, report consolidated effects of PA on the decrease of DM losses during storage and on aerobic stability due to the inhibition of microbial growth. When incorporated into TMR, they act in a similar way as in silages, controlling the increase in the temperature of the mixed feed offered and decreasing undesirable microbial activity and succession capable of altering the physical, chemical and sensory characteristics of the feed. This increases the exposure time that the feed can tolerate, which allows to reduce the number of daily offers when based on a consistent feeding strategy.

2.5. Propionic acid on animal performance and metabolism

From 55 to 75% of the energy digested by ruminants is in the form of short-chain volatile fatty acid (SCVFA), which is the main energy input for these neoglycogenic animals. When produced by the ruminal microbial fermentation of digesta, they are released into the rumen in the form of propionic, acetic and butyric acids. Of these, PA stands out for its hypophagic activity (limiting intake) in ruminants (Anil and Forbes, 1980; Allen, 2014). Therefore, its absorption results in hepatic oxidation control of these animals, which is well documented (Allen, 2000).

According to Bauman et al. (1971), PA production rates in the rumen of lactating cows fed normal or low forage diets, with an average daily intake of 15 kg of DM, range from 13 to 31 mol/d. In cows with an average daily intake of 12 kg DM of a similar diet, as described by Sutton et al. (2003), PA production rates ranged from 17 to 36 mol/d. Allen (1997) reported that the total amount of SCVFA production ranges from 42 to 115 mol/d in lactating cows. The concentration of these acids in the rumen can increase from 15 to 45% of the total fermentation, depending on the digestibility of the diet, so that the production of PA can reach 52 mol/d.

Studies with higher intakes of propionic acid through diet or rumen infusion suggested that the feed intake of animals does not seem to be linked to the fulfillment of their requirements, as it would be natural to expect, but to satiety effects linked to specific fuels, which is currently known as Hepatic Oxidation Theory (Allen, 2000; Allen et al., 2009). At first, this process was detected in rats (Langhans and Scharrer, 1992; Friedman 1995), in which it was observed that satiety was triggered by a signal originating in the liver and transmitted to the brain by the vagus nerve, being affected by the hepatic oxidation of fuels and ATP generation. Koch et al. (1998) describe a temporal link between voluntary intake and hepatic energy status, based on the premise that the regulation of feed intake passes through oxidative metabolism in the liver.

Allen (2000) suggests that the same mechanism probably also applies to ruminants. Therefore, propionate is classified as an obligate anaplerotic metabolite, stimulating hepatic oxidation of acetyl-CoA (Gualdrón-Duarte and Allen, 2017), which explains its hypophagic characteristic (Anil and Forbes, 1980; Allen 2014). Thus, the greater supply of PA in the rumen during the meal favors a greater arrival of propionate into the liver, promoting the stimulus for satiety and changes in feed intake patterns. Studies with rapid rumen infusions performed within 5 minutes increased meal size and reduced meal frequency compared with longer infusions of 12 to 15 minutes, as shown in Table 1.

Table 1. Survey on studies with ruminal infusions of propionic acid

Study		DIM	IT	PAD	MF	DMI	FIT
		d	min	mol		kg	min
Maldini e Allen, (2018)a	controle	13,8 ± 2,9	0	0	12	18.6	300
Oba e Allen, (2003)a	trat. 1	113 ± 26	0	0	13.8	28.9	316
Oba e Allen, (2003)a	trat. 2	159 ± 26	0	0	14.8	26.6	322
Maldini e Allen, (2019)a	trat. 1	PPi	5	0.5	13	21.6	248
Maldini e Allen, (2019)a	trat. 2	PPi	5	0.2	10	16.96	185.8
Bradford e Allen (2007)a	trat. 1	51 ± 19	5	0.54	12.9	18.6	291.54
Maldini e Allen, (2018)a	trat. 1	13,8 ± 2,9	5	1.25	8.5	10.4	195.5
Oba e Allen, (2003)a	trat. 1	113 ± 26	14	0.14	13.8	28.6	326
Oba e Allen, (2003)a	trat. 1	113 ± 26	14	0.29	16.2	30.4	346
Oba e Allen, (2003)a	trat. 1	113 ± 26	14	0.43	15.5	27	320
Oba e Allen, (2003)a	trat. 1	113 ± 26	14	0.57	14.4	26	318
Oba e Allen, (2003)a	trat. 1	113 ± 26	14	0.71	15	27.2	298
Oba e Allen, (2003)a	trat. 1	113 ± 26	14	0.86	14.2	26.4	298
Oba e Allen, (2003)a	trat. 2	159 ± 26	14	0.33	12	23	306
Oba e Allen, (2003)a	trat. 2	159 ± 26	14	0.67	12.2	16.6	220
Maldini e Allen, (2019)a	trat. 1	PPi	15	0.5	16	24.6	260
Maldini e Allen, (2019)a	trat. 2	PPi	15	0.2	12	16.52	226
Bradford e Allen (2007)a	trat. 1	52 ± 19	15	0.54	12.8	19.1	311.04
Maldini e Allen, (2018)a	trat. 2	13,8 ± 2,9	15	1.25	11.2	10	224

DIM – days in milk; IT – infusion time; PAD – propionic acid dosage; MF – meal frequency; DMI – dry mater intake; FIT – feed intake time.

It is likely that excess propionate in the rumen can lead to saturation of the hepatic uptake pathway, which results in lower initial extraction by the liver, despite the liver being highly efficient in extracting propionate from the blood due to the high activity of propionyl-CoA synthetase in ruminant hepatocytes (Ricks and Cook, 1981; Demigné et al., 1986). Higher levels of propionate may remain in the bloodstream, bypassing the liver, which likely extends anaplerosis of the Krebs cycle and hepatic acetyl-CoA oxidation over time, increasing the interval between meals and depressing their frequency (Maldini and Allen, 2019).

Differences in feeding intake behavior are closely linked to the origin (ingestion or infusion), levels and exposure time of propionate in the liver (Maldini and Allen, 2018), the liver's ability to metabolize propionate and its metabolites (Gualdrón-Duarte and Allen, 2018) and the availability of acetyl-CoA (Piantoni et al., 2015).

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3. PROPIONIC ACID BASED ADDITIVE ON TOTAL MIXED RATIONS FOR LACTATING DAIRY COWS PERFORMANCE

ABSTRACT

Twelve Holstein cows with average body weight of 612 kg, 178 ± 60 days in milk (mean ± SD) and average daily milk yield of 27 ± 6.5 kg (mean ± SD) were assigned to a switch-back design to evaluate the effect of applying a propionic acid-based additive on total mixed ration (TMR) during the mixture of ingredients, immediately before feeding, on the animal performance. The set of treatments was designed to perform a comparison between two feeding strategies: cows receiving two daily offers of TMR without treatment (Control); and cows receiving a single daily offer of TMR treated with the additive at 2 L/t of as fed matter (Additive). The ration composition was the same for both treatments, with 44.59% corn silage, 6.40% Tifton haylage, 21.11% rehydrated corn grain silage, 9.25% citrus pulp, 16.33% soybean meal and 2.32% mineral-vitamin mix. Cows fed the additive-treated TMR significantly increased daily milk yield in 2.4 kg without changes in dry matter intake (DMI), although only a slight non-significant increase was observed for feed efficiency. The contents of total solids, fat and lactose were decreased in the milk of cows fed the treated TMR, although daily yield of these components was greater due to increased milk yield. Milk crude protein content and milk urea nitrogen (MUN) were not altered by feeding strategy. Energy density of milk was similar across treatments, although energy excretion through milk was greater for the cows fed the additive-treated TMR. Somatic cell count (SCC) was significantly higher for the cows when fed the treated TMR. The additive shifted sorting behavior in favor of longer particles of the ration, in relation to the control treatment. Cows receiving a single daily offer of additive-treated TMR spent less time ingesting and more time ruminating, with greater meal size, shorter meal length and decreased daily meal frequency. TMR offering frequency might be reduced, with a 10.2% increase in milk yield, by using propionic acid based additive.

Keywords: Propionic acid, Total mixed ration, Feeding frequency, Dairy cows

3.1. Introduction

The total mixed ration (TMR) is a nutritional strategy that aims to guarantee the supply of all the necessary nutrients to the animal in a uniform and simultaneous manner (Coppock et al., 1981), allowing adequate intake by the animal. However, the presence of high moisture ingredients such as silages and forages, which have their own microbial populations, makes the TMR a mixed feed probably quite susceptible to aerobic deterioration.

The use of additives based on organic acids, mainly propionic acid, is a common strategy to enhance the conservation of foods, mainly silages and hay. Its antimicrobial properties allow the control of yeasts, fungi and spoilage bacteria, increasing the food's resistance to aerobic spoilage (Morais et al., 2017). Thus, TMR can also benefit from the use of such chemical additives, extending the maintenance of its nutritional quality in the feed bunk.

The aim of this trial was to evaluate the effects of the inclusion of a propionic acid-based additive in the TMR immediately before feeding on the performance and selective behavior of dairy cows. The treatments were designed in order to provide a nutritional strategy to challenge the preservative potential of the additive, in which the TMR treated with the product was offered only once a day, while the control TMR was split in two daily offers.

3.2. Material and methods

All experimental procedures were approved by the ethics committee for animal use of the University of São Paulo – Luiz de Queiroz College of Agriculture (protocol nº 1690070722).

3.2.1. Experimental diets

The basal diet of the feeding trial was formulated according to the NRC (2001) software (version 1.1.9, December 2012). The level of inclusion of ingredients and the nutritional composition of the formulated diet are shown in Table 2. The nutritional composition of the offered diets is described in Table 3. The proposal of this trial was based on the use of two feeding strategies employing diets of identical physical and chemical compositions. The first strategy consisted of two daily offers without the presence of the additive, and the second strategy was a daily single offer with the use of the additive.

Table 2. Inclusion level of ingredients and composition of formulated diets

Ingredients, % DM of TMR	Trat. Additive	Trat. Control
Corn silage	44.59	44.59
Tifton 85 haylage	6.40	6.40
Rehydrated corn grain	21.11	21.11
Citrus pulp	9.25	9.25
Soybean meal	16.33	16.33
Mineral-vitamin mix	2.32	2.32
Additive, mL/kg as fed	2.00	-
Chemical composition (formulated)		
DM, % as fed	48.9	48.9
CP, % DM	15.6	15.6
NDF, % DM	34.5	34.5
Forage NDF, % DM	27.9	27.9
EE, % DM	3.0	3.0
Starch, % DM	26.6	26.6
Diet NE_L, Mcal/kg	1.56	1.56

DM – dry matter; CP – crude protein; NDF – neutral detergent insoluble fiber; EE – ether extract; NE_L – net energy for lactation.

Table 3. Chemical composition of offered diets

Item	Trat. Additive		Trat. Control	
	Mean	SD	Mean	SD
DM, % as fed	42.55	2.37	43.34	1.67
CP, % DM	17.03	2.47	17.37	2.80
NDF, % DM	37.18	5.61	38.08	5.08
Forage NDF, % DM	23.84	0.50	23.84	0.49
Ash, % DM	7.30	1.18	7.87	3.14
Particle size, % as fed				
> 19 mm	12.06	1.10	11.72	1.49
8-19 mm	31.32	1.25	33.15	2.65
4-8 mm	15.09	0.82	14.31	1.20
<4 mm	41.53	1.20	40.81	2.19

DM – dry matter; CP – crude protein; NDF – neutral detergent insoluble fiber.

Table 4. Chemical composition of the ingredients

Item	Corn silage		Tifton haylage		Rehydrated corn grain		Citrus pulp		Soybean meal	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, % as fed	31.66	1.01	49.91	1.82	59.31	1.29	84.60	0.84	86.98	0.17
CP, % DM	10.33	0.41	13.53	2.10	14.27	0.06	7.48	0.29	48.45	0.48
NDF, % DM	43.33	1.34	70.57	3.73	11.97	0.40	25.25	0.37	17.90	0.77
Ash, % DM	8.42	0.23	0.88	0.01	1.50	0.11	7.37	0.25	7.16	0.24

DM – dry matter; CP – crude protein; NDF – neutral detergent insoluble fiber.

3.2.2. Feeding trial

The animal performance trial was carried out at the Experimental Facility of Free Stall Barn for Dairy Cattle “Prof. Vidal Pedroso de Faria" at the Animal Science Department of the Luiz de Queiroz College of Agriculture (ESALQ) – University of São Paulo (USP), located in Piracicaba-SP. Twelve Holstein cows were allocated to the trial, which presented an average body weight of 612 kg, mean DIM of 178 ± 60 d and average daily milk yield of 27 ± 6.5 kg. Cows were blocked based on calving order (primiparous vs. multiparous), milk yield and body condition score. Two groups of six animals each were formed, which were randomly allocated to a sequence of two treatments in a Switch-back design, conducted simultaneously, with three periods of 28 days and response to treatments measured in the fourth week starting at d-21.

The following treatments were applied: Trat. Additive – a daily single offer of TMR with the additive (2.0 mL/kg TMR as fed) at 6:30 am; and Trat. Control – two daily offers of TMR without additive at 6:30 am and at 6:00 pm. The application of the additive was carried out via spraying over the TMR during mixing in a vertical forage wagon model VM4 (DeLaval® , Tumba, Sweden), equipped with a side discharge, without dilution in water, with the aid of a pressure pump equipped with a Teejet Extended Range 110° fan spray nozzle, Orange - 0.23 to 0.45 L/min. To ensure that both diets were physically similar, the ingredients were added to the forage wagon starting with the lowest density (Tifton 85 haylage, whole-plant corn silage, soybean meal, citrus pulp, rehydrated corn and mineral-vitamin mix), and the mixing time was of 12 minutes for both diets. To avoid contamination of the additive on the control treatment, the additive-treated TMR was prepared first, and the wagon was then sanitized with the help of a semi-industrial high pressure washer before the control TMR was made. After the second feeding in the afternoon (only for the control treatment), both treatments had the feed manually turned over in the bunk for three minutes, immediately before the access of the animals in the return of the milking parlor, in order to guarantee the same stimulus to intake. The amount of feed offered per cow was calculated based on the weight of the orts from the previous day, aiming to guarantee a minimum of 10% orts in the bunk the following morning.

The additive used (Fresh Cut™ Plus; Kemin South America, Indaiatuba, SP, Brazil) is formed by a blend containing acetic acid, propionic acid (65.66% wt/wt), ammonium hydroxide, polysorbate 80, water, benzoic acid, butylated hydroxitoluene, tartrazine yellow artificial dye and bright blue artificial dye, with a density of 1.059 g/mL and pH 5.15. Recent studies developed by Muck et al. (2018), Santos et al. (2019), Dias et al. (2021) and Gheller et al. (2021) used similar proportions of propionic acid directly in the total diet, which was the reference for this study.

The free-stall barn had a slotted concrete floor and sand beds, with an open ridge at the roof to ensure satisfactory environmental conditions and animal welfare. The animals had free access to water, and the barn was equipped with individual feed bunks of 100 cm of linear space each, capable of individual access control (Intergado Ltda., Contagem, Minas Gerais, Brazil), which allows continuous measurement of feeding behavior for all experimental animals individually. The identification of the animal occurs through an exclusive passive transponder (High-Performance ISO Half Duplex Electronic ID Tag, Allflex, St-Hyacinthe) in the shape of an earring, placed in the right ear of the animals.

The animals were distributed in two lots in the barn, reaching a density of 1 cow/21.6 m^2 (15.8 m^2 of floor + 5.8 m^2 of bedding), which complied with the protocols for confined animals (Arave et al al., 1974; Telezhenko et al., 2012). Cows were grouped into the lots 14 days before the start of the experiment to promote the social adaptation of the animals to each other and identify possible dominance relationships, during which the cows were allocated to their respective bunks to promote the usual adaptation of feed intake.

Cows were milked twice daily, at 5:00 am and at 5:00 pm, using a double four herringbone parlor. The waiting and milking rooms were located 100 m from the free-stall barn, distance covered four times/day, which was the only locomotion activity outside the barn to which the animals were submitted during the experimental period. Other aside facilities like the footbath alley and body weighing scale were available right next to the free-stall barn. The animals were passed through the footbath three times a week, upon returning from the milking parlor in the morning.

3.2.3. Animal performance: nutrient intake, milk yield and composition

The animal performance trial was structured in 28 experimental days each, being 21 days of adaptation and 7 days of sampling and measurements, totalizing an experimental period of 84 days. In each period, each group of six animals was fed with the TMR of one of the treatments, following a switch-back design, with the change of treatment between periods. The ensiling of the rehydrated corn took place 14 days before the beginning of each experimental period in order to standardize the storage time in 35 days at the beginning of each sampling week.

The milk yield of each cow was measured between d-21 and d-27 of each period, with the aid of a continuous flow meter model Fi7 (DeLaval®, Tumba, Sweden) coupled to the milking system. Sampling of milk from each animal took place between d-22 and d-24 of each period, using cup-type collectors (DeLaval®, Tumba, Sweden) coupled to the meters, with two daily samples being taken (one for each milking), totalizing six samples for each animal per experimental period. These samples were immediately frozen and sent for near infrared (NIRS) analysis (Clínica do Leite, Piracicaba, São Paulo, Brazil) of fat, crude protein, lactose, milk urea nitrogen content and somatic cell count (SCC). The equation of Tyrrell and Reid (1965) was used to calculate the energy corrected milk (ECM):

$$\text{ECM (kg/d)} = (0.327 \times \text{milk yield}) + (12.95 \times \text{fat yield}) + (7.2 \times \text{protein yield}) \quad \text{(Eq. 1)}$$

The energy content and milk energy yield were calculated based on the equations below described in NASEM (2021):

$$\text{Milk } NE_L \text{ (Mcal/kg)} = (0.0929 \times \%\text{ fat}) + (0.055 \times \%\text{ protein}) + (0.0395 \times \%\text{ lactose}) \quad \text{(Eq. 2)}$$

$$NE_L \text{ yield (Mcal/d)} = \text{Milk yield (kg/d)} \times \text{Milk } NE_L \text{ (Mcal/kg)} \quad \text{(Eq. 3)}$$

Samples of ingredients used in the composition of the diets were collected daily between d-21 and d-27 of each period, producing daily subsamples immediately frozen at -20 ºC. At the end of each period, these subsamples were thawed and homogenized to obtain a single composite sample. Samples were then dried in a forced air circulation oven at 55ºC for 72 hours and ground in a Willey mill with a 1 mm sieve for later laboratory determination of dry matter (AOAC, 1990, method 934.01), ash (AOAC, 1990, method 924.05), crude protein (Dumas method – AOAC, 1990, method 992.23) and ash-corrected neutral detergent fiber with thermostable amylase and sodium sulfite (Mertens, 2002).

The TMR offered to each animal was weighed and sampled between d-21 and d-27 of each experimental period, forming daily subsamples immediately frozen at -20 ºC. At the end of each period, these subsamples were thawed and homogenized to obtain a single composite sample. Orts from each animal were measured and sampled between d-22 and d-28, before the first feed offer in the morning. Daily subsamples were immediately frozen at -20 ºC, thawed and homogenized at the end of each period to form a single composite sample.

A 400 g fraction of each daily subsample of TMR and orts was kept to determine the particle size distribution using the Penn State Particle Separator according to the method described by Lammers et al. (1996) and Heinrichs and Jones (2013), using the set of 19 mm, 8 mm and 4 mm sieves, plus the bottom box. A fraction of 500 g of each composite sample was dried in a forced air circulation oven at 55°C for 72 hours and ground in a Willey mill with a 1 mm sieve to determine the contents of dry matter (AOAC, 1990, method 934.01), ash (AOAC, 1990, method 924.05), crude protein (Dumas method – AOAC, 1990, method 992.23) and ash-corrected neutral detergent fiber with thermostable amylase and sodium sulfite (Mertens, 2002).

A fraction of 100 g of TMR samples was kept to prepare aqueous extracts at the proportion of 25 g of sample to 225 g of deionized water, which were homogenized in a stomacher for 4 minutes and then filtered through 3 folder cheesecloths. After immediately measurement of pH, extract samples were centrifuged at 10,000 rpm for 10 minutes, and the supernatant was placed in 2 mL micro tubes and frozen at -40 ºC. These samples were analyzed for lactic acid (Pryce, 1969), volatile fatty acids, esters and ethanol by gas chromatography (GCMS QP2010 Plus; Shimadzu®, Kyoto, Japan) using a capillary column (Stabilwax; Restek®, Bellefonte, PA; 60 m long, 0.25 mm outer diameter, 0.25 μm film thickness) and correction of dry matter content for volatile compounds according to Weissbach (2009).

The individual feed intake of the cows was measured daily between d-21 and d-27 of each period through the feed monitoring system installed in the feed bunks (Intergado Ltda., Contagem, Minas Gerais, Brazil).

3.2.4. Feeding behavior and particle sorting

Feeding behavior was measured for 48 hours, between d-22 and d-24 of each period, recording every 10 minutes the activities of feed and water ingestion, rumination and idleness. The recording started at 08:00 am, when the animals had access to the morning feed offer right after milking, on d-22 and ended at the same time on d-24 of each period. During the time in which they remained in the milking and waiting room, the animals did not have access to the diet, only water, so the assessment of feeding behavior included four hours of feed restriction between 5:00 am-6:00 am and 5:00 pm-6:00 pm on both days of evaluation in each period, recording only the other activities. Average meal size, length and frequency were calculated using the feed monitoring system (Intergado Ltda., Contagem, Minas Gerais, Brazil). The first meal of the first and second offers corresponded to the first feed intake activity after the morning and afternoon offers, respectively, being measured between d-21 and d-27 of each period.

To determine the particle sorting behavior and the granulometric distribution of the particles present in the offered TMR to each animal and their respective orts, the Penn State Particle Separator was used as described by Lammers et al. (1996) and Heinrichs and Jones

(2013), as previously described. The input for each particle size retained on the y-mm sieve was determined based on the following equation:

[Offered x (%offer(y)/100)] – [Orts x (%orts(y)/100)] = Intake(y)

(Eq. 4)

With the actual input in each sieve, the particle size distribution for intake was estimated. The particle sorting index for each sieve was then calculated [(%intake(y) / %offer(y)) x 100]. According to Leonardi and Armentano (2003), indexes <100% were interpreted as refusal, =100% as no selection and >100% as preferential intake.

3.2.5. Statistical analyses

The parameters of animal performance including feed intake, milk yield and composition, ingestive behavior and particle sorting were analyzed using the MIXED procedure from SAS (SAS Institute Inc., Cary, NC), as a switch-back design according to the following model:

$$\gamma_{ijk} = \mu + T_i + P_J + A_k + \varepsilon_{ijk}$$

(Eq. 5)

Where:

μ = mean;

T_i = fixed effect of treatment (i= A, B)

P_J = random effect of period (j= 1, 2, 3)

A_k = random effect of animal (k= 1,2,3...12)

ε_{ijk} = experimental error

Differences were declared significant when $P \leq 0.05$ and trends when $0.05 < P \leq 0.10$.

3.3. Results and discussion

The inclusion of the additive in the TMR for lactating cows, at the concentration used (2 L/t as fed) in this study and associated with the frequency of diet offering, did not affect the intake of DM (DMI), NDF and OM (Table 5). However, the intake of crude protein (CPI) increased significantly ($P \leq 0.01$) compared to the control treatment. Dias et al. (2021) reported similar results to those observed in this study when using a similar dietary strategy for the intake of DM and OM. Kung et al. (1998) did not observe significant effects on DMI when adding propionic acid to the total diet, suggesting that this response was due to the high nutritional and hygienic quality of the corn silage that composed the diets, and to the advanced stage of lactation of the animals.

Such results contrast with those described by Gheller et al. (2021), who describe an increase in the intake of DM, OM, NDF, ether extract (EE), non-fibrous carbohydrates (NFC) and total digestible nutrients (TDN). However, they also reported an increase in CPI by animals fed TMR treated with products based on organic acids (acetic, formic and propionic). Such results were associated with a decrease in the concentration of hypophagic compounds present in the total diet (Van Os et al., 1995; Allen, 2000) and the maintenance of aerobic stability of the TMR over 24 hours (Kung et al., 1998). On the other hand, Krizsan et al. (2012) describe a depression in DMI when organic acids were added to the total diet.

Pazdiora et al. (2011) evaluated the effects of TMR splitting in 1 to 3 daily offers for dairy cows and heifers, and did not find significant differences in intake and performance, claiming that a reduction in the frequency of supplying TMR to animals can be made in order to decrease operational costs. Some of this variation across studies may be attributed to differences in experimental procedures, additive dosage, cows breeds, days of lactation of the animals, diet composition, feed level, and chemical and hygienic quality of the diet components.

Table 5. Nutrient intake by dairy cows fed the TMR treatments

Item	Treatment		SEM	*P*-value
	Additive	Control		
DMI, kg/d	22.3	21.6	0.89	0.59
NDF intake, kg/d	8.12	7.92	0.38	0.75
CP intake, kg/d	5.13	3.82	0.45	<0.01
OM intake, kg/d	4.52	3.97	0.22	0.09

Although no differences were observed in DMI, the total diet with the addition of propionic acid improved the productive performance of the animals (Table 6), significantly increasing milk yield by 2.4 kg/d, an increase of 10.2% compared to the control treatment. This effect was maintained in the milk yield corrected for 3.5% of fat and for energy, both with 1.9 kg/d more than the control ($P \leq 0.05$), a relative increase of 8.3% and 8.2%, respectively. Despite the increased milk yield, there was no significant difference in feed efficiency ($P = 0.42$). However, the use of the additive seems to have allowed for a better energy and nutritional use of the food ingested, since there was no difference in the total intake of dry matter and NDF.

Consequently, although there was a trend towards a decrease in the lactose content, the milk lactose yield in cows fed with the additive was significantly higher ($P \leq 0.01$) compared to the control. For Asimov and Krouze (1936), the higher milk yield may be explained by the higher concentration of readily available propionate compared to the control treatment (Table 7), which can be absorbed through the intestinal mucosa by passive diffusion and used for ATP production through the Krebs cycle (Sahoo and Jena, 2014) necessary for the synthesis processes in the mammary gland. In addition, propionate can be carried to the liver, where it is used for the synthesis of glucose, a precursor of lactose in the mammary gland (Wang et al., 2016).

According to Akers (2017), the increase in lactose content is closely related to the increase in total milk productivity. Lactose is an important precursor in milk synthesis, as it is the main osmotic component of milk, being responsible for extracting water into the milk, increasing the volume produced. Due to the close relationship between the synthesis of lactose and the amount of water drained into the milk, the concentration of lactose is the least variable among milk components, representing 4.4 to 5.2% of the total milk composition.

On the other hand, the concentration of fat showed a significant decrease ($P \leq 0.01$) in the milk of the animals that ingested the additive, probably due to a dilution of this component. As these animals showed a significant increase in the volume of milk produced, the daily yield of fat excreted in milk was not affected ($P \leq 0.16$).

Table 6. Performance of dairy cows fed the TMR treatments

Item	Treatment		SEM	*P*-value
	Additive	Control		
DMI, kg/d	22.3	21.6	0.89	0.59
Milk, kg/d	25.9	23.5	1.34	0.01
Milk/DMI	1.22	1.15	0.08	0.42
FCM 3.5%, kg/d	24.7	22.8	1.18	0.05
ECM, kg/d	25.1	23.2	1.16	0.03
Fat, %	3.28	3.41	0.07	0.01
Fat, kg/d	0.83	0.78	0.04	0.16
Crude protein, %	3.26	3.26	0.06	0.92
Crude protein, kg/d	0.82	0.75	0.03	0.01
Lactose, %	4.44	4.48	0.03	0.06
Lactose, kg/d	1.16	1.06	0.06	0.01
Total solids, %	11.86	12.02	0.12	0.02
Total solids, kg/d	3.05	2.80	0.14	0.02
DDE, %	8.64	8.65	0.06	0.74
Milk NE_L, Mcal/kg	0.66	0.67	0.01	0.12
NE_L, Mcal/d	16.8	15.6	0.77	0.04
Milk NE_L/DMI	0.79	0.76	0.05	0.56
SCC, mil/mL	99.3	41.8	8.10	<0.01
MUN, mg/dL	12.5	13.1	0.27	0.27
Casein, %	2.56	2.56	0.05	0.92
CASP, % Prot.	78.3	78.4	0.15	0.96

FCM – fat-corrected milk; ECM – energy-corrected milk (Tyrrel and Reid, 1965); DDE – defatted dry extract; NE_L – net energy for lactation; SCC – somatic cell count; MUN – milk urea nitrogen; CASP – percentage of casein in milk crude protein.

Daily milk protein yield showed a significant increase in cows fed with the additive (P ≤ 0.01). This was probably due to the increased milk yield and the higher intake of CP of the animals in this treatment. Despite not showing significance (P = 0.27), milk urea nitrogen content (MUN) was numerically lower (12.5 vs. 13.1) for cows receiving the additive-treated TMR, which may indicate greater efficiency in the synchrony of degradation between carbohydrates and protein in the rumen. It is likely that the maintenance of aerobic stability, associated with the antimicrobial effect of propionic acid and other components of the additive over 24 hours, provided the animals with a higher quality feed than the control treatment, when submitted to extended exposure time in the bunk, with reduced proteolysis and less deamination and decarboxylation of amino acids (Daniel et al., 2016; Oliveira et al., 2017; Santos et al., 2019). Similar results have been reported in silages treated in the during ensiling with blends of organic acids that present in their composition more than 50% of AP, in dosages between 0.8 and 3.0% of the DM consumed by the animals (Nagel and Broderick, 1992; Oliveira et al., 2017; Muck et al., 2018). The significant differences ($P = 0.02$) in content and yield of milk total solids can also be explained by the increase in milk yield.

We observed a significant increase ($P \leq 0.01$) in the somatic cells count (SCC) in the milk of cows when fed the additive-treated TMR. However, the values were below 100,000 cells/mL, which is described as normal by Dohoo and Leslie (1991), so that only values above these are associated with mastitis (Bradley and Green, 2005).

Tyler et al. (1997), DeVries and von Keyserlingk (2005) and DeVries et al. (2010) suggest in their results a strong correlation between the feeding beahvior of the herd immediately after milking and the risk of intramammary infection, which can be potentiated by the lowest feeding stimulus at that moment. This is because the teat canal remains dilated after milking for approximately 120 minutes, allowing penetration by microorganisms (Mc Donald, 1975). It is possible that the cows fed the additive were more susceptible to teat contamination, as they ended the first meal activity after milking about 10 minutes earlier throughout the period (Table 8). Tyler et al. (1997), DeVries et al. (2010) and Watters et al. (2013) reported similar results when testing different frequencies of fresh feed delivery throughout the day. According to these authors, there was a significant increase in the SCC of milk from animals submitted to lower rates of feed offering, which presented less time dedicated to feed intake in the post-milking periods and, consequently, a longer time spent lying down.

Table 7. Additive and propionic acid intake by cows fed the treated TMR

Item	Mean	SD
Additive intake, g/d	109.8	28.67
Additive intake, g/kg DMI	4.9	0.20
PA intake, g/d	72.1	18.82
PA intake, g/kg DMI	3.2	0.13
PA intake, mol/d	0.97	0.25
PA intake, mol/kg DMI	0.04	0.00

PA – propionic acid

The association between the frequency of feed delivery and the addition of the additive directly to the TMR, at the dose used in this study, affected the particle sorting indexes (Table 8). When receiving the additive, the cows selected in favor of the fraction retained on the sieve >19 mm ($P \leq 0.01$), refused the fraction between 8-19 mm ($P \leq 0.01$), did not present significant selection in the intake of the 4-8 mm fraction ($P = 0.76$), and refused particles <4 mm ($P \leq 0.01$), demonstrating an atypical behavior for lactating dairy cows. It is possible that the use of propionic acid and its salts made the fibrous-rich fraction of the ration more attractive, increasing its acceptance by animals, since these compounds may also be used in the feed industry as flavoring agents (Samel et al., 2018). Huber and Soejono (1977) and Stallings et al. (1979) reported similar behaviors for forage silages treated with propionic acid, with an increased intake of the treated forages by dairy cows.

The greater selection for particles retained on the 19-mm sieve by the animals when fed the additive may explain the higher intake of crude protein, since the particles retained on the top sieve were basically composed of Tifton 85 haylage (13.53% CP), in addition to straw, leaves and stems of corn plants. These plant parts are usually also concentrated in fiber components. However, despite sorting in favor of such particles by cows when fed with the additive, there was no difference in NDF intake between treatments. As the animals when fed the control TMR selected in favor of particles between 8-19 mm ($P \leq 0.01$), which also have a considerable portion of fibrous material, it is likely that there was a numerical compensatory effect for this variable, with only a possible change in the ingested NDF profile. Mature portions of forages, such as stems and old leaves, may present greater deposition of lignin (Van Soest, 1994), requiring greater chewing activity by the animal. However, the time spent with ingestion per kg of NDF consumed was significantly ($P \leq 0.01$) higher for animals that received the control diet, and there was no significant difference in rumination time per kg of NDF ingested (P = 0.16).

It is important to emphasize that the TMR reported for both treatments had similar chemical and physical composition and moisture content, as observed in Table 3. Using diets with chemical and physical composition close to that of the present study, Lahr et al. (1983) and Leonardi and Armentano (2003) reported sorting indexes similar to those observed in the present trial for the control TMR. Recent studies conducted in Brazil with TMR treated with propionic acid-based additives immediately before offering reported no changes in selection, and the sorting indexes were similar to those observed for the control treatment in our study (Dias et al., 2021; Gheller, et al., 2021).

Benchaar et al. (2020), when evaluating feeding frequency, found no differences in intake, total tract digestibility, performance and ruminal parameters of dairy cows. However, they reported a preference for ingesting long particles larger than 8 mm. Phillips and Rind (2001) and DeVries and Von Keyserlingk (2005) reported that cows receiving two or more daily offers tend to spend less time feeding in the morning and more time feeding in the late afternoon, in addition to having a greater preference for NDF intake. Sova et al. (2013) reported similar results in which cows fed twice a day sought to ingest long particles (>19 mm) compared to those fed once a day.

Animals submitted to a lower frequency of daily feed offers receive less stimulus to visit the feed bunk throughout the day, which can promote high intake levels during meals. The demand for concentrate during this scenario may be associated with the preference for fresh ingredients in a diet with longer exposure time in the bunk. This is particularly important as the provision of fresh feed is more effective to stimulate feeding activity of group-housed dairy cattle (DeVries and von Keyserlingk, 2005).

Table 8. Feeding behavior of dairy cows fed the TMR treatments

Item	Treatment		SEM	*P*-value
	Additive	Control		
Particle sorting index, % as fed				
>19 mm	114.3	84.2	3.53	<0.01
8-19 mm	97.2	101.4	0.66	<0.01
4-8 mm	98.3	98.8	0.72	0.76
<4 mm	98.6	102.4	0.73	<0.01
Ingesting, min/d	188	258	9.2	<0.01
Ruminating, min/d	599	546	10.1	<0.01
Chewing, min/d	788	803	11.8	0.46
Idleness, min/d	625	621	12.6	0.82
Water intake, min/d	27	21	2.1	0.05
Ingesting/NDF intake, min/kg	25.4	36.3	1.85	<0.01
Ruminating/NDF intake, min/kg	79.6	71.8	3.65	0.16
Meal frequency, meals/d	6.1	8.9	0.27	<0.01
Meal size, kg	3.4	2.8	0.19	0.03
Meal length, min	20.0	20.8	1.00	0.49
1st meal/1st offer size, kg	4.4	4.4	0.32	0.84
1st meal/1st offer length, min	21.7	32.3	2.21	<0.01
1st meal/2nd offer size, kg	3.7	3.4	0.29	0.42
1st meal/2nd offer length, min	20.3	28.0	2.02	<0.01

The combination of results suggests that the dosage of the additive used in this study possibly affected the intake, sensory (smell and taste) and/or chemotactic (liver oxidation) regulatory systems (Allen et al., 2009). It is possible that the greater supply of propionic acid in the rumen during the meal promoted a greater arrival of propionate to the liver, generating the stimulus for satiety and interruption of feed intake, and promoting changes in dietary patterns which were not observed for the control treatment. Studies with ruminal infusions conducted by Oba and Allen (2003) and Maldini and Allen (2019) showed a decrease in the number of daily meals associated with a shorter ingesting time when faster ruminal infusions (5 minutes) were applied, suggesting an interaction between the greater arrival of propionic acid in the rumen and the accumulation of propionate in the liver within a short time. Pazdiora et al. (2011) describe a decrease in the number of daily meals in animals treated once daily compared to two and three times a day. Ferreira (2006) found no difference ($P > 0.05$) in ingesting time, intake rate and DMI, evaluating one, two, three or four daily offers. According to Thiago et al. (1992), the amount of feed consumed by the ruminant in a given period is dependent on the number of meals in that interval, ingesting time and feeding rate of each meal. Each of these processes is the result of the interaction of the animal's metabolism and the physical and chemical properties of the diet by stimulating satiety receptors.

There were no significant differences for the time spent on idleness ($P = 0.46$) and chewing ($P = 0.82$). However, cows fed with additive had longer rumination time ($P \leq 0.01$), since these animals sought to ingest a particle size greater than 19 mm, which is normally associated with a higher content of physically effective NDF and a higher content of structural carbohydrates. The efficiency of the rumination process interacts with the nature of the ingested fraction. Several authors cited by Mertens (1997) demonstrated that rumination activity is a characteristic that reflects the physical and chemical properties of foods, such as NDF concentration, particle size and moisture.

The water intake activity had a significant effect, following the higher milk yield of the additive-treated TMR. The production of milk is one of the main water drains for the dairy cow (Duque et al., 2012), so that peaks in water intake may occur shortly after milking (Andrigueto et al., 1988). Water intake is directly correlated with milk yield (Castle and Thomas, 1975; Little and Shaw, 1978; Appuhamy et al., 2016).

3.4. Conclusions

TMR treated with a propionic acid-based additive immediately before feeding, offered only once daily, increased milk yield by 10.2% in dairy cows in comparison to untreated TMR offered twice daily. The presence of the additive in the TMR promoted a shift in the sorting behavior in favor of longer particles in the ration, increasing the time spent on rumination to the detriment of the time spent on feed intake. The strategy combining a single daily offer with the additive-treated TMR promoted larger meals in a shorter time, decreasing the frequency of daily meals without changing total dry matter intake of dairy cows. TMR offering frequency might be reduced, with a 10.2% increase in milk yield, by using a propionic acid based additive. To the best of our knowledge this is the first report on combining Fresh Cut™ Plus and reduced offering frequency as strategy.

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