VIVIANE BORBA FERRARI

Levels of concentrate and sources of non-fiber carbohydrates on productive performance and ruminal kinetics of finishing cattle

Orientador: Prof. Dr. Luis Felipe Prada e Silva

Pirassununga

2017

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Tese apresentada ao Programa de Pós-Graduação em Nutrição e Produção Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, para obtenção do título de Doutor em Ciências.

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Orientador:
Luis Felipe Prada e Silva

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CERTIFICADO

Certificamos que a proposta intitulada "Níveis de concentrado e fontes de carboidratos não-fibrosos sobre o desempenho produtivo e cinética ruminal de bovinos em terminação.", protocolada sob o CEUA nº 2784310715, sob a responsabilidade de **Luís Felipe Prada E Silva** *e equipe; Viviane Borba Ferrari* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 13/10/2015.

We certify that the proposal "Levels of concentrate and sources of non-fiber carbohydrates on productive performance and ruminal kinetics of finishing cattle.", utilizing 114 Bovines (114 males), protocol number CEUA 2784310715, under the responsibility of **Luís Felipe Prada E Silva** *and team; Viviane Borba Ferrari* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 10/13/2015.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 01/08/1 a 20/09/1 Área: Nutrição E Produção Animal

Origem:	Animais provenientes de estabelecimentos comerciais						
Espécie:	Bovinos	sexo:	Machos	idade:	a	N:	114
Linhagem:	Bos Indicus			Peso:	a		

Resumo: Dietas de terminação de gado de corte possuem níveis elevados de energia, o que representa um grande desafio para manutenção da estabilidade ruminal. Com o uso crescente da silagem de cana-de-açúcar na alimentação de ruminantes, se torna fundamental o entendimento dos efeitos de diferentes fontes energéticas sobre o metabolismo ruminal e desempenho animal. Objetiva-se com este estudo quantificar o efeito da substituição parcial do milho moído fino por milho floculado ou polpa cítrica, em dois níveis de concentrado na dieta, sobre o desempenho, características de carcaça, fermentação ruminal, cinética da degradação, passagem ruminal da fibra. O estudo consistirá de 6 rações experimentais a base de silagem de cana-de-açúcar, sendo dois níveis de concentrado na dieta (60% ou 80% na matéria seca) e três fontes de carboidratos não fibrosos (CNF): milho floculado ou a polpa cítrica, em substituição parcial ao milho moído. Para medição do desempenho e características de carcaça, serão utilizados 108 tourinhos Nelore com aproximadamente 350 kg, alojados em 36 baias, com três animais por baia, em delineamento experimental de blocos ao acaso com 6 repetições, o controle da alimentação será feito por baia, por meio da pesagem diária do alimento fornecido e sobras. Serão feitas pesagens após jejum de 16h, a fim de estimar o ganho médio diário e a eficiência alimentar. A deposição de gordura e crescimento muscular serão avaliados por ultrassonografia no início e fim do experimento. Amostras de sangue serão coletadas para dosagem de: glicose, nitrogênio ureico, proteínas totais e albumina. Para mensuração da cinética ruminal, seis novilhos Nelore canulados no rúmen serão utilizados em delineamento experimental de quadrado latino 6x6. A massa total do conteúdo ruminal e volume serão determinados por esvaziamento. Amostras serão retiradas de ambas as fases, sólida e líquida, para determinação do tamanho do compartimento ruminal de componentes da digesta. A dieta, sobras e digesta ruminal serão analisados para conteúdo de nutrientes. Fibra em detergente neutro indigestível será estimada como o conteúdo de FDN das amostras após fermentação in situ por 264 horas. A determinação do volume líquido e da taxa de passagem de líquidos pelo rúmen será realizada por polietilenoglicol de peso molecular 4.000.

Local do experimento:

São Paulo, 25 de junho de 2017

FICHA DE AVALIAÇÃO

Autor: FERRARI, Viviane Borba

Título: Levels of concentrate and sources of non-fiber carbohydrates on productive performance and ruminal kinetics of finishing cattle.

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Instituição: ____________________________ Julgamento: ____________

DEDICATION

I dedicate this thesis to my parents, Jesus and Lucí, and brothers Fábio and Gustavo who offered me unconditional love and support and have always been there for me. Thank you very much!

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I would like to thank God for life and strength to keep moving on and never give up on my studies.

To my family supporting me spiritually throughout my life. To my parents Jesus and Lucí, and brothers Fábio and Gustavo, for all the support given throughout this long journey.

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Hold the door, say "please", say “thank you"

Don't steal, don't cheat, and don't lie

I know you got mountains to climb

But always stay humble and kind

When the dreams you're dreamin' come to you

When the work you put in is realized

Let yourself feel the pride

But always stay humble and kind

(Lori McKenna)

ABSTRACT

FERRARI, V. B. **Levels of concentrate and sources of non-fiber carbohydrates on productive performance and ruminal kinetics of finishing cattle**. [Níveis de concentrado e fontes de carboidratos não-fibrosos sobre desempenho e cinética ruminal de bovinos em terminação]. 2017. 114 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2017.

The objective of this study was to quantify the effect of partial replacement of finely ground corn by steam-flaked corn or pelleted *citrus* pulp and two concentrate levels, either 60 or 80%, on rumen pH, *in vitro* digestibilities of tropical forages, performance and rumen fermentation of Nellore young bulls. Three rumen cannulated Nellore steers with average BW of 350 ± 15 kg were assigned in a 3×3 Latin Square design. The rumen pH was measured and rumen fluid used to evaluate *in vitro* DM and NDF digestibilities (IVDMD and IVNDFD) of tropical forages. Moreover, a total of 108 Nellore young bulls with initial BW of 365.3 ± 3.12 kg, were distributed in collective pens, with 3 animals per pen, in a randomized complete block design with 6 replicates in a 3×2 factorial arrangement of treatments. The performance, carcass traits, blood parameters, feeding behavior, and sorting index were evaluated. Additionally, six rumen-cannulated steers, weighing 345.10 ± 14 kg were arranged in 2 6×6 non-contemporary Latin squares, in a 3×2 factorial arrangement of treatments. The rumen was manually evacuated, an aliquot of the digesta was filtered to separate solid and liquid phases. Rumen fluid samples were taken at multiple times after feeding to determine pH, short chain fatty acids and ammonia nitrogen. The steam-flaked corn decreased *in vitro* digestibility of the forages evaluated, but increased rate of degradation. It also increased propionic acid, but decreased ammonia compared to the other diets. It decreased NDF total tract digestibility compared to *citrus* pulp. The steam-flaked corn increased fat deposition, with no impact on animal performance. The *citrus* pulp decreased lag time and increased rate of degradation. The pelleted *citrus* pulp caused the bulls to sort the diet against large particles, decreasing the DMI, performance, and rumen propionic acid concentration, but increased the acetic acid, disappearance rate of NDFd, and digestibilities of DM and OM. On the other hand, the 80% concentrate diets decreased passage rate of NDF, but increased the digestibilities of OM, DM, and performance, despite the lower intake. In conclusion, the inclusion of 80% concentrate in sugarcane silage based-diets lowers intake, and increases performance, the partial replacement of ground corn by steam-flaked corn improves carcass fat deposition. And *citrus* pulp increases rumen pH, fiber digestibility but decreases intake, and performance of finishing young Nellore bulls.

Keywords: Non-fiber carbohydrates. Productive performance. Ruminal kinetics.

RESUMO

FERRARI, V. B. **Níveis de concentrado e fontes de carboidratos não-fibrosos sobre desempenho e cinética ruminal de bovinos em terminação.** [Levels of concentrate and sources of non-fiber carbohydrates on productive performance and ruminal kinetics of finishing cattle]. 2017. 114 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2017.

O objetivo deste estudo foi quantificar o efeito da substituição parcial de milho finamente moído por milho floculado a vapor ou polpa cítrica peletizada e dois níveis de concentrado, 60 ou 80% sobre o pH, digestibilidade *in vitro* de forragens tropicais, desempenho e fermentação ruminal de tourinhos Nelore. Foram utilizados três novilhos Nelore canulados no rúmen PV médio de 350 ± 15 kg em um delineamento de quadrado latino 3×3. O pH do rúmen foi medido e o fluido do rúmen utilizado para avaliar as digestibilidades *in vitro* da MS e da FDN (DIVMS e DIVFDN) de forragens tropicais. Além disso, um total de 108 novilhos Nelore com PV inicial de 365,3 ± 3,12 kg, foram distribuídos em baias coletivas, com 3 animais por baia, em um delineamento de blocos ao acaso com 6 repetições em um arranjo fatorial 3×2 de tratamentos. As avaliações feitas foram desempenho, características de carcaça, parâmetros de sangue, comportamento alimentar e índice de seleção de partículas. Ademais, seis novilhos canulados no rúmen, pesando 345,10 ± 14 kg, foram organizados em 2 quadrados latinos não contemporâneos 6×6, em um arranjo fatorial 3×2 de tratamentos. O rúmen foi evacuado manualmente, uma alíquota da digesta foi filtrada para separar as fases sólida e líquida. As amostras de fluido de rúmen foram tomadas em várias horas após a alimentação para determinar o pH, ácidos graxos de cadeia curta, e nitrogênio amoniacal. O milho floculado diminuiu a digestibilidade *in vitro* de forragens avaliadas, mas aumentou a taxa de degradação, ácido propiônico, mas diminuiu a amônia em comparação com as outras dietas., e a digestibilidade do trato total do FDN em relação à polpa cítrica. O milho floculado aumentou a deposição de gordura, sem impacto no desempenho. A polpa cítrica diminuiu o Lag time e aumentou a taxa de degradação, e fez com que os touros selecionassem a dieta contra partículas grandes, diminuindo o CMS, desempenho e ácido propiônico do rúmen. Por outro, lado a polpa aumentou o ácido acético, a taxa de desaparecimento de FDNd e digestibilidades de MS e MO. As dietas com 80% de concentrado diminuíram a taxa de passagem do FDN, mas aumentaram as digestibilidades da MO, MS e desempenho, apesar da menor ingestão. Em conclusão, a inclusão de 80% de concentrado em dietas à base de silagem de cana-de-açúcar reduz a ingestão e aumenta o desempenho. A substituição parcial de milho moído por milho floculado melhora a deposição de gordura de carcaça. A polpa cítrica aumenta o pH do rúmen, e a digestibilidade da fibra, mas diminui o consumo e desempenho de tourinhos Nelore em terminação.

Palavras-chave: Carboidratos não-fibrosos. Cinética ruminal. Desempenho produtivo.

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ABBREVIATION LIST

A – Soluble fraction

ADF – Acid detergent fiber

ADG – Average daily gain

ADL – Acid detergent lignin

APS – Average particle size

ATP - Adenosine triphosphate

B – Potentially degradable insoluble fraction

BFT – Back-fat thickness

BW – Body weight

CD – Carcass dressing percentage

CH_4 – Methane

CO_2 – Carbon dioxide

CONC – Concentrate

CP – Crude protein

CS – Corn silage

DM – Dry matter

DMI – Dry matter intake

EE – Ether extract

G:F – Gain to feed ratio

GC – Ground corn

GIT – Gastro intestinal tract

HCO_3^- - Bicarbonate

iNDF – Indigestible neutral detergent fiber

IEDM – Intake efficiency of dry matter

IVDMD – *In vitro* dry matter digestibility

IVNDFD – *In vitro* Neutral detergent fiber digestibility

K_d – Degradation rate

K_p – Passage rate

LMA – *Longissimus lumborum* muscle area

MCP – Microbial crude protein

MM – Mineral matter

MP – metabolizable protein

N – Nitrogen

NDF – Neutral detergent fiber

NDFd – Digestible NDF

NE – Net energy

NE_g – Net energy of gain

NE_m – Net energy of maintenance

NFC – Non-fiber carbohydrate

N-NH_3 – Ammonia nitrogen

NSC – Non-structural carbohydrate

OM – Organic matter

PCP – Pelleted *citrus* pulp

pH – Power of hydrogen

RFT – Rump fat thickness

SC – Structural carbohydrate

SCFA – Short chain fatty acid

SFC – Steam-flaked corn

SI – Sorting index

SS – Sugarcane silage

TMR – Total mixed ration

TMT – Total mastication time

TDN – Total digestible nutrients

WSC – Water soluble carbohydrate

SUMÁRIO

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1 INTRODUCTION

The finishing phase of beef cattle in feedlots is an important part of the intensification process of the of Brazilian beef cattle industry, which allows for a greater stocking rate of the pastures during the winter period. However, during that phase, feed costs account for 70 - 80% of the total production costs (PACHECO et al., 2006), therefore the producer has the challenges to improve feed efficiency, to seek cheaper feedstuffs to include in the diet, such as by-products, to lower the cost of weight gain.

In ruminants, the carbohydrates comprehend from 70 to 80% of the diet and are fundamental for meeting the energy requirement of the animal, microbial crude protein and to maintain rumen health. Fiber represents the carbohydrate fraction of slower digestion rate, or indigestible, and depending on its inclusion, it can limit intake and performance (NUSSIO; CAMPOS; LIMA, 2011). On the other hand, the non-fiber carbohydrates have faster digestion rates and are comprised of sugars and fructans, starch, and pectins; all of which can differ in their effects on rumen pH or support of microbial growth (HALL; EASTRIDGE, 2014). The most used source of grain in feedlot diets in Brazil is the flint type corn, that is less digestible than dent corn, and the most commonly used processing methods of the kernel is cracking and grinding (OLIVEIRA; MILLEN, 2014).

Steam-flaking of grains, on the other hand, increases surface area for microbial attachment, disrupts the protein matrix that surrounds the grain, and also gelatinize the starch, breaking the intermolecular hydrogen bonds within the starch granule. Therefore, it increases the digestibility of starch, the energy value of corn, and performance relative to whole, dry-rolled, or ground corn (MCALLISTER et al., 2006). However, the increase of rumen starch digestibility can cause a sharp drop in rumen pH, and possibly reduce fiber digestibility and dry matter intake (VAN SOEST, 1994). Zinn, Owens and Ware (2002) summarized animal performance from 8 trials and reported an average decrease of 6.1% on DMI, but an increase of 5.4% in ADG and 12.2% in G:F ratio when either whole, ground, or dry-rolled corn was replaced by steam-flaked corn. However, the summarized trials were carried out in North America with *Bos taurus* cattle and dent type corn. Greater benefits with steam-flaking the grain should be expected when using flit corn with lower initial digestibility than dent corn.

Brazil is the main producer of orange and orange juice in the world, processing approximately 70% of the total fruit grown for orange juice production (SANTOS et al., 2013) thus, annually million tons of agro-industrial wastes are generated in Brazil. Therefore, an environmentally friendly and economically viable destination for agro-industrial byproducts is their use in animal feed, reducing feed costs of the farm (SILVA; REZENDE; INÁCIO, 2016). The pelleted *citrus* pulp is a byproduct, from *citrus* juice production and gained space in the national livestock market, mainly due to its price and nutritional qualities (MENDES NETO et al., 2007). Pelleted *citrus* pulp is considered an energetic concentrate feedstuff, with on average 85 to 90% of the energy value of corn (NRC, 2001). It is rich in pectin, a carbohydrate which is an amorphous substance that, despite being a component associated with the cell wall, it is almost completely digested in the rumen (90 to 100%). Therefore, compared to starch, pectin has a lower propensity to cause rumen pH drop, due to its fermentation being carried out by cellulolytic microorganisms, favoring the production of acetate and not lactate and propionate, as occurs in the starch fermentation (VAN SOEST; PETERSON; LEWIS, 1991).

Despite the lower nutritive value relative to corn and sorghum silages (ANDRADE; FERRARI JR; BRAUN, 2001), sugarcane presents high production per hectare - 73 ton/ha (CONAB, 2017), ease of cultivation, persistence, good acceptance by the animals, and lower production costs (LANDELL et al., 2002; FREITAS et al., 2006). In contrast to other forages, sugarcane does not reduce significantly its nutritive value in the dry season, and is notable for the increase in sucrose content at that time of year (BORGES; PEREIRA, 2003). The daily-harvested sugarcane freshly offered to the animals is a traditional practice however, it becomes problematic because of the difficulty of harvesting on rainy days, and the loss of its nutritional value during the summer. The recent development of conservation techniques of the whole sugarcane plant in the form of silage allows its use by a greater number of cattle ranchers in the most diverse production systems, throughout the year (SCHMIDT et al., 2007; QUEIROZ et al., 2008). However, there are few studies with sugarcane silage, and recommendations of inclusion in beef cattle diets.

Few studies evaluated sources of non-fiber carbohydrates in the beef cattle diets using sugarcane silage as a source of roughage (PEREIRA et al., 2007) being primordial the understanding of the digestion effects of different energy sources on ruminal metabolism and animal performance. Therefore, our hypothesis was that the partial replacement of the ground corn by steam-flaked corn would decrease intake, lower pH and fiber digestibility but improve or at least maintain average daily gain, improving feed efficiency. On the other hand, the *citrus* pulp would increase intake in diets with greater concentrate inclusion but maintain performance, increasing rumen pH, and acetic acid in the rumen. Thus, the objectives of this study were to quantify the effect of the different sources of non-fibrous carbohydrates – Ground corn, steam-flaked corn, and pelleted *citrus* pulp and two levels of concentrate (either 60 or 80% on a DM basis) in the sugarcane silage-based diets on:

- The *in vitro* digestibility of fiber and organic matter of forages, and ruminal pH;
- The dry matter intake, average daily gain, sorting index, feeding behavior, blood parameters, carcass traits;
- Ruminal passage rate, pH, short chain fatty acids, and ammonia nitrogen of Nellore young bulls in the finishing phase; and
- To describe the degradation curve of the non-fiber carbohydrates.

2 LITERATURE REVIEW

2.1 CURRENT SCENARIO OF BEEF CATTLE

According to the United States Department of Agriculture (USDA, 2017b), the world cattle herd, in 2016, was estimated in 989 million heads. The largest herd is in India, with 302.6 million heads, which represents 30.6% of the total number of animals. It is worth mentioning that for that country, the USDA considers cattle and buffaloes. Brazil is thus ranked second, with 219.2 million heads in 2016, equivalent to 22.2% of the world herd, but is the leader if considered only commercial bovine herds. After India and Brazil appears China, with 100.3 million heads, followed by the United States with 98.0 million and the European Union with 89.1 million heads. Therefore, Brazil and India together, account for more than half of the total herd in the world. According to the latest data from the Brazilian Institute of Geography and Statistics (IBGE, 2015), in 2014 the Brazilian herd was distributed mainly in Mato Grosso with 13.41% of the herd, in Minas Gerais with 11.43%, Goiás with 10.19%, Mato Grosso do Sul with 9.94% and Pará with 9.05%. These five states together, account for more than half of the national cattle herd.

According to the report released by the Brazilian Association of Meat Exporting Industries (ABIEC, 2017), the number of cattle slaughtered in the country in 2016 was 24.3 million heads, of which 19.3% was slaughtered in Mato Grosso, 14.9% in Mato Grosso do Sul, 11.3% in Goiás, 11.1% in Rondônia, 9.9% in São Paulo, 9.0% in Pará, and 7.6% in Minas Gerais. These seven states were responsible for more than 83% of the total cattle slaughtered in 2016. Even though Brazil is the greatest commercial bovine herd in the world, the global leader in beef and veal production are the United States of America with 11.5 million metric tons (carcass weight equivalent) in 2016, followed by Brazil with 9.3, European Union 7.8, China 7.0, India 4.2, and Argentina 2.7 million metric tons (USDA, 2017b). According to ABIEC (2017), 76.94% of the volume of beef exported was *in natura* meat (fresh beef). Additionally, the 6 largest importers of Brazilian fresh meat in 2016 were Hong Kong, responsible for the import of 181 thousand tons, followed by China with 165 thousand tons, Egypt with 165 thousand tons, Russia with 130.6 thousand tons, Iran with 95 thousand tons, and Chile with 70 thousand tons.

Millen et al. (2009) carried out an important survey with nutritionists who assist feedlots in several parts of Brazil and found that fresh sugarcane is the primary source of roughage, being used by 32.3% of nutritionists, followed by corn silage used by 25.8% of the nutritionists, sorghum silage by 22.6%, sugarcane bagasse by 9.7%, grass silage by 6.5% and sugarcane silage by 3.2% of the nutritionists interviewed. In that study, it was found corn and sorghum are the main source of energy used in Brazil (MILLEN et al., 2009). Furthermore, the results of that research indicate that most of the grains used in Brazilian feedlots were hard or flint corn (90.9%), being the primary processing method used as finely ground grain (54%), cracked (38.7%) and coarsely ground (6.5%) (MILLEN et al., 2009). Still, according to Millen et al. (2009), when questioned about the level of inclusion of concentrate on a DM basis in the diet, 41.9% nutritionists reported using 41 to 70% of concentrate in the diet, while 38.7% nutritionists used from 71 to 80%, 19.4% nutritionists used 81 to 90% and no nutritionist reported using more than 91% concentrate in the diet.

In a more recent same-type of study, Oliveira and Millen (2014) performed a survey with 33 nutritionists and reported that 87.9% used corn (predominantly flint type 96.5%), followed by 12.1% of sorghum as primary source of grain. Still, according to that survey, the primary grain processing method was cracked 57.6%, followed by finely ground 36.4%, steam-flaking 3.0% and high-moisture harvesting and storage 3.0%. The predominant level of concentrate inclusion is 81-90% on a DM basis being used by 42.4% of the nutritionists, 71-80% used by 39.4% of the consultants, 56-70% of concentrate used by 12.1%, and lees than 55% used by 6.1% of the nutritionists (OLIVEIRA; MILLEN, 2014). Vasconcelos and Galyean (2007) reported that in the USA, the corn is also the primary source of grain used (100% of nutritionists), however, the steam-flaking of corn is the main processing method, used by 65.5% of the feedlots, followed by dry-rolling used by 13.8%, and by high-moisture harvesting and storage used by 13.8% of the interviewed nutritionists. In the same study, the predominant level of concentrate range is reported to be 80-85%, used by 34.5%, 70-80%, adopted by 31.0% of the nutritionists, 60-70% of concentrate used by 20.7% of the interviewed consultants.

To achieve higher levels of production, animals need more energy-dense diets. Due to the lower digestibility and energy availability of forages compared to grains, it has been adopted to reduce the participation of roughage to minimum levels in the diet, since it does not compromise rumen health and optimize performance. In addition, forages tend to be more variable in their composition, which makes it difficult to compare results between studies (MERTENS, 2002). However, differently from USA, the Brazilian forages are cheaper than grains. Therefore, it is important to realize studies that evaluate levels of concentrate in the diet with different types of non-fiber carbohydrates feedlot diets.

2.2 LEVELS OF CONCENTRATE IN THE DIET

The microbial crude protein (MCP) can supply from 50 to almost 100% of all the metabolizable protein required by beef cattle, depending on the intake of undegraded protein in the rumen. The microbial growth in the rumen is influenced by the interaction of chemical, physiological and nutritional factors (HOOVER; STROKES, 1991). The energetic availability is indicated as a limiting factor for the microbial growth, so the manipulation of the diet by altering the forage to concentrate ratio, can increase the amount of fermented organic matter and, consequently, the protein synthesis, when there is more supply of energy (CLARK; KLUSMEYER; CAMERON, 1992). Burroughs, Trenkle and Vetter (1974) proposed that the synthesis of MCP corresponds to about 13.0% of the total digestible nutrients (TDN) consumption and the National Research Conuncil - (NRC, 1996), admits that this value is a good estimate. However, the production of MCP is lower with the use of low quality roughage. The low rate of passage leads to increased use of digested energy for microbial maintenance - including cell lysis (RUSSELL; WALLACE, 1988; RUSSELL et al., 1992), therefore, the efficiency of MCP synthesis from digestible energy is reduced.

Fiber is a complex material whose digestibility varies according to the forage species, variety, plant maturity and environmental conditions. In addition, greater weight gain is achieved with grain-rich diets, reducing the time required to reach slaughter weight. Additionally, from the operational point of view, the forages are more laborious in processing (cutting and grinding) and mixing, so animals tend to select the feed in a higher intensity than grain (MERTENS, 2002).

However, feeding high proportion of concentrate in the diet can cause changes in the microbial population, inhibiting fiber degradation (GRANT; MERTENS, 1992), increased production of butyrate and propionate instead of acetate, with concomitant lactate production causing a sharp decrease of pH (MERTENS, 1997; BEHARKA et al., 1998). This situation can lead to acidosis, consequently there is a decrease in ruminal motility (NOCEK, 1997; OWENS et al., 1998), there is alteration in the ruminal wall morphology (GAELBEL; BELL; MARTENS, 1989), keratinization of ruminal papillae (NOCEK; KESLER, 1980) and consequent decrease in the absorption of short chain fatty acids (HINDERS; OWEN, 1965). Other consequences such as tympanism, hepatic abscesses, ruminitis, laminitis (JENSEN et al., 1958), bronchopneumonia (KRAUSE; OETZEL, 2006) and even death of the animal have been reported because of ruminal acidosis. Thus, higher forage intake stimulates saliva secretion, increasing ruminal pH, avoiding such metabolic disturbances (MERTENS, 1997).

Another important approach is the manipulation of the forage to concentrate ratio on the voluntary feed intake. Some theories have been postulated to explain the mechanism by which excess fermentation and subclinical acidosis leads to a decrease in DM intake. Recent research with beef cattle (FALEIRO et al., 2011) indicate that the pH drop *per se* is not responsible for the DMI depression. One theory is that the accumulation of short chain fatty acids (SCFAs) would promote increased rumen osmolarity, which in turn would be directly responsible for the drop in feed intake (OWENS et al., 1998). The high osmolarity, both in rumen and blood, has been related to the control feed intake (CARTER; GROVUM, 1990; LANGHANS; ROSSI; SCHARRER, 1995), however this hypothesis is difficult to prove in *in vivo* experiments.

Another hypothesis, more accepted nowadays, is that ruminal fermentation products of animals fed high-concentrate diets promote a decrease in dry matter intake, is called the Hepatic Oxidation Theory (ALLEN; BRADFORD; OBA, 2009). In this theory, the products of ruminal fermentation, more precisely propionate is accepted as the primary sign of satiety, as its flow to the liver increases expressively during meals. The propionate that reaches the liver, can be used for gluconeogenesis or oxidized through acetyl CoA, producing ATP. This excess ATP would lead to negative feedback in the brain by activating the satiety center in the hypothalamus (ALLEN; BRADFORD; HARVATINE, 2005). Although propionate is extensively metabolized by the ruminant liver, there is a small net metabolism of acetate or glucose, which may explain why these fuels do not actually induce hypophagia. Lactate is metabolized in the liver but has less effect on the satiety stimulus, probably due to the greater latency to reach the liver during meals (ALLEN; BRADFORD; OBA, 2009).

Research involving metabolic inhibitors demonstrated a cause and effect relationship between energy status and eating behavior. Stimulation of intake by inhibition of fatty acid oxidation was associated with reduced hepatic energy status by measuring ATP concentration, ATP:ADP ratio, and potential phosphorylation in the liver. The hepatic nerve is the mean of communication that allows the Central Nervous System (CNS) to receive information about the hepatic energy status controlling appetite (ALLEN; BRADFORD; OBA, 2009). Langhans, Egli and Scharrer (1985), through hepatic vagotomy reported a block in the satiety stimulus. In this same rationale, another study by Anil and Forbes (1988) has shown that hepatic vagotomy and total damage of the hepatic nerves in lambs eliminate the hypophagic effects in animals receiving propionate infusion in the portal vein.

Another mechanism regulating the DMI is rumen fill, where the voluntary intake of ruminants is limited by the consumption of diets with high proportion of roughages, because of the restricted flow of the digesta through the gastro intestinal tract (GIT), resulting in the decrease of the feed intake. Balch and Campling (1962) reported that the consumption varies inversely proportional to the forage filling capacity, which is represented by the fibrous mass. Similarly, Van Soest (1965) reported that voluntary intake of forage by sheep is more highly correlated with NDF content than other dietary bromatological characteristics. However, dietary fiber content is not the only variable of feedstuffs responsible for the rumen fill (MERTENS, 1987). The filling effect also varies according to particle size, particle fragility, rate and extent of digestion.

The reticulum-rumen is considered local in the GIT in which the distension limits the DMI in forage-based diets. Tension receptors located mainly in the reticulum and cranial part of the rumen are responsive to distension (LEEK, 1969). With decreasing particle size of the diet, either by grinding or pelletization, there is usually an increase in voluntary intake (MINSON, 1963), due to the decrease in the volume of the feed inside the rumen and increase in the rate of passage of this feed, being retained for less time. The relationship between digestibility and forage intake is well established, however this relationship is not linear (BLAXTER; WAINMAN; WILSON, 1961). The DMI of feedstuffs with low digestibility is limited by the physical distention in the GIT, which decreases as the forage digestibility increases (ALLEN; BRADFORD; OBA, 2009). Conrad, Pratt and Hibbs (1964) suggested that there is a limit to where voluntary consumption is physically limited and is replaced by metabolic satiety as the forage digestibility increases. The increase in the proportion of concentrate feedstuffs results in greater DMI and greater feed efficiency (G:F), to a certain extent, from which the DMI is reduced while maintaining the same energy consumption due to the greater energy concentration in the diet (GALYEAN; DEFOOR, 2003). The relationship between increased dietary energy and G:F is also not linear, showing that with the increase in dietary ME, G:F increases in a quadratic fashion (KREHBIEL et al., 2006).

Dung, Shang and Yao (2014) worked with rice straw-based diets, evaluating forage to concentrate ratios (80:20, 60:40, 40:60 and 20:80) on the *in vitro* fermentation of the diet and observed increased digestibility of DM, OM, in the total production of SCFA, MCP, and also a decrease in pH with the increase of the concentrate and decrease of rice straw inclusion in the diet. In the study of Lee et al. (2006) using the same forage to concentrate ratios from the previous study, on ruminal parameters of rumen cannulated Hereford x Holstein steers, observed a significant reduction in the concentration of ammonia nitrogen ($N\text{-}NH_3$), from 156.8 to 101.0 mg N / L, increase on DMI and production efficiency of MCP from 51.6 to 72.4 g / d when forage inclusion decreased from 80 to 20%. Costa et al. (2013) observed greater intramuscular fat deposition in the *longissimus lumborum* and *semitendinosus* muscles in

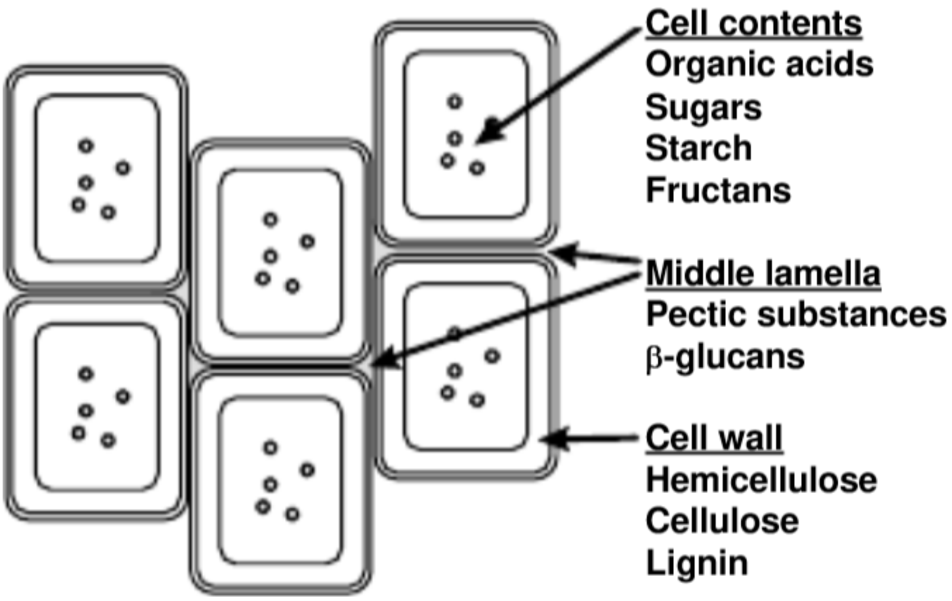
Barrosã animals fed diets with 30% corn silage compared to animals fed diets with 70% of the same forage.

2.3 CARBOHYDRATE DIGESTION

Carbohydrates are the most abundant biomolecules on Earth, they have the formula $(CH_2O)_n$ in plants, their synthesis occurs through photosynthesis where, in the presence of light energy, the carbon present in the atmosphere in the form of CO_2 is combined with water and transformed into carbohydrates. Its main functions in plant cells are: source and reserve energy and structure (LEHNINGER, 2000).

In ruminants, unlike non-ruminant, there is no salivary amylase for starch digestion, and carbohydrates are fermented in the rumen with the action of the enzymes of microorganisms to SCFA (mainly acetate, propionate and butyrate) and are absorbed by the rumen wall and used as energy. For ruminants, carbohydrates comprise between 70 and 80% of the ration composition and are essential for meeting energy requirements, microbial protein synthesis and animal health (MERTENS, 1996). Carbohydrates are divided into two major groups: those of the cell wall (structural carbohydrates - SC) and those of cellular content (non-structural carbohydrate - NSC). Fiber is a term used to establish a nutritional concept being defined by nutritionists as the indigestible or slow digestion fraction of the food that occupies space in the GIT (BERCHIELLI; PIRES; OLIVEIRA, 2011). Therefore, the cell wall cannot be taken into account as an exact measure of fiber, since it includes pectic substances that are of high digestibility (MERTENS, 1996). Non-fibrous carbohydrates (NFC) are a fraction easily and almost completely digested by most animals, and comprises organic acids, mono- and oligosaccharides, fructose, starch, pectin and other carbohydrates except hemicellulose and cellulose found in the NDF fraction (VAN SOEST, 1994; HALL, 2003). The figure 1 represents the location of the carbohydrates and lignin within the plant cell structure. The organic acids, sugars, starch, fructans are located in the cell content, while pectic substances, and β-glucans in the middle lamella, which is considered the “cement” of the cell wall of two plant cells. And lastly, hemicellulose, cellulose and lignin are found in the cell wall of the plant.

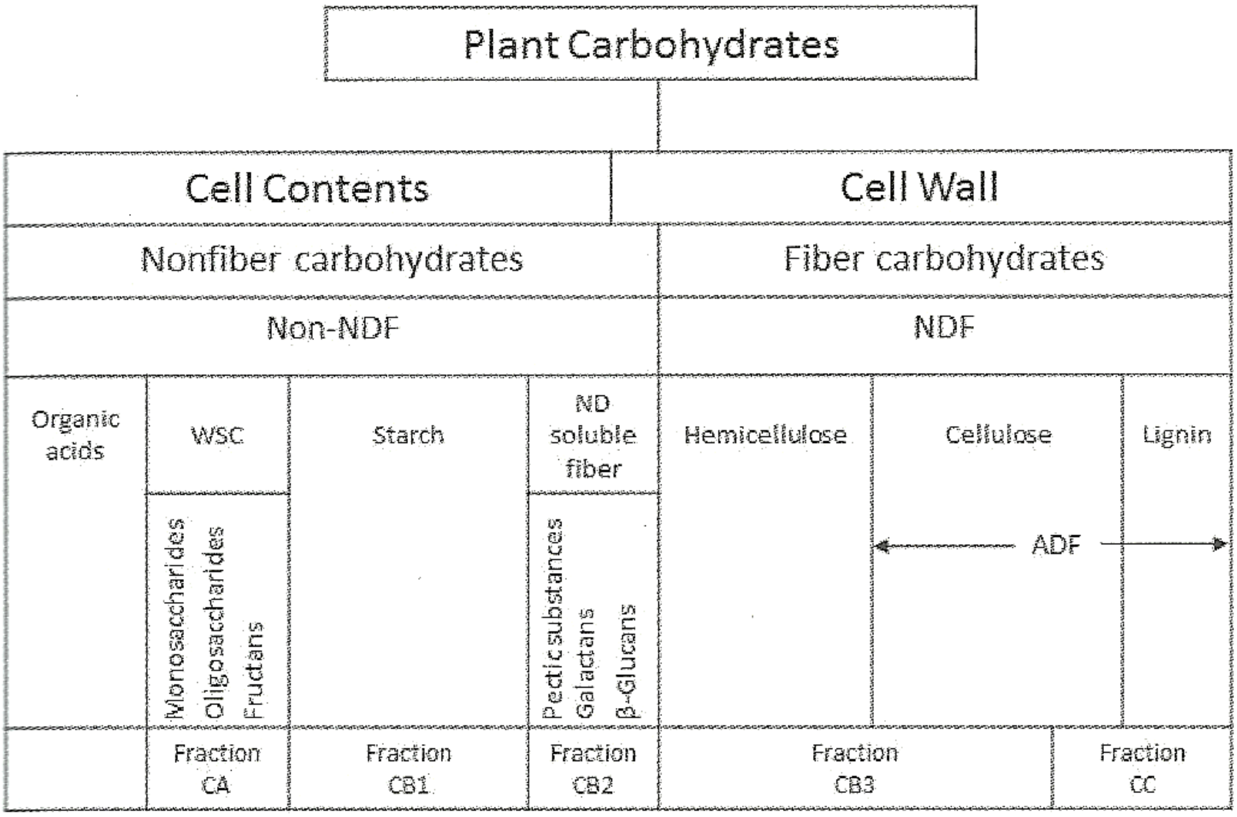
Figure 1 - Location of carbohydrate and lignin within the plant cells



Source: (HALL, 1998)

The NRC (2016) has adopted a simplified carbohydrate fractionation approach based on analytical methodology, similarly of digestion rates among fraction in the rumen that separates the carbohydrates in six fractions according to the Figure 2: Organic acids (OA); Water-soluble carbohydrates (WSC) including sugars and fructans (Fraction CA), Starch (Fraction CB1), Neutral detergent soluble fiber (Fraction CB2), Available NDF (Fraction CB3), and Unavailable NDF (Fraction CC). The OA and WSC are completely available in the rumen, however, unlike WSC, the OA from fermented feeds do not provide substrate for microbial growth in the rumen. Fructans are part of the CB2 fraction, but are classified as part of the WSC because they are soluble in water and rapidly degraded. The CB1 and CB2 fractions are moderately available in the rumen, and CB3 represents slowly digestible fiber and CC the indigestible portion in the rumen.

Figure 2 - Simplified fractionation of plant carbohydrates



Source: (NRC, 2016).

Legend: ADF = acid detergent fiber, ND = neutral detergent, NDF = neutral detergent fiber, WSC = water-soluble carbohydrates. Fiber carbohydrates represent call wall recovered as NDF (fiber that is soluble in neutral detergent is not included). Non-fiber carbohydrates represent the carbohydrate fraction that is soluble in neutral detergent. Although fructans are actually part of the soluble fiber fraction, in this classification they are considered part of the WSC fraction because they are soluble in water and are very rapidly available in the rumen. Although not carbohydrates, organic acids are considered part of the non-NDF fraction. Organic acids are utilized as energy subtracts by the animal, but they do not support appreciable microbial growth in the rumen and therefore are not included in the fraction CA. Lignin is considered part of the fiber carbohydrate fraction because it is included in the ADF and NDF and affects their digestibility. Fractions CA, CB1, CB2, CB3 and CC are described in the text.

Below is a partial list of sugars found in the feedstuffs according to Emanuele and Sniffen (2014):

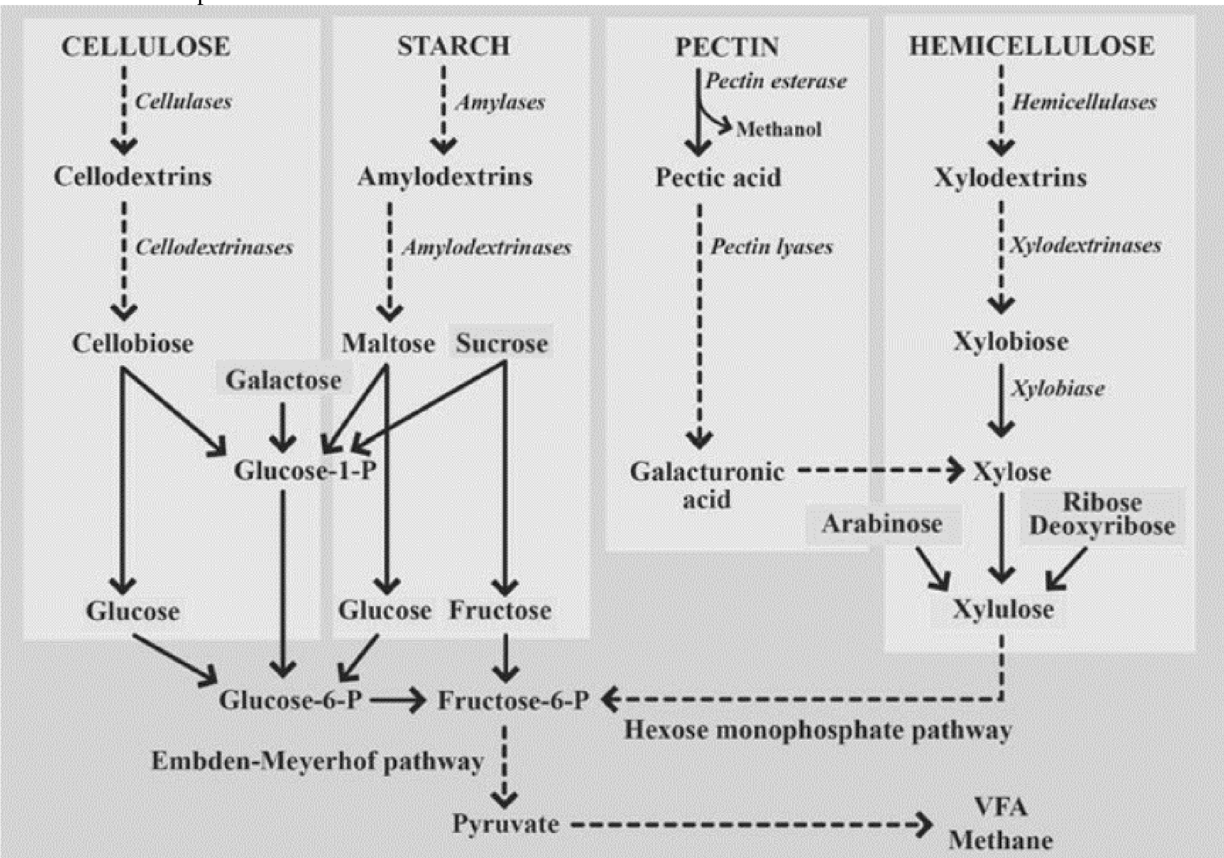
- Monosaccharides: 5-carbon sugars – part of hemicellulose, RNA and DNA: Arabinose, Ribose and Xylose; 6-carbon sugars – diverse across all plants: Glucose, Fructose, Galactose, and Mannose.

- Disaccharides: Sucrose – found in all plants and consists of Glucose + Fructose; Maltose – found in all plants and consists of Glucose + Glucose alpha-linkage as starch; Lactose – found in milk and consists of Glucose + Galactose.
- Trisaccharides: Raffinose – found in cottonseeds and sugar beet pulp and consists of Galactose + Fructose + Glucose; Maltotriose – found in corn distiller's grains and consists of Glucose + Glucose + Glucose.
- Tetrasaccharides: Stachyose – found in soybeans and soybean solubles and consists of Galactose + Galactose + Glucose + Fructose.
- Polysaccharides: Fructans – mainly in grasses and consist of fructose; Galactans – mainly in alfalfa and soybeans and consist of galactose; Pectins – mainly in *citrus* pulp, alfalfa and soybeans and contain arabinose + galactose; Cellulose – all plants and contains long chains of glucose; Hemicellulose – all plants, higher concentration in grasses and contains arabinose, xylose, mannose, galactose, and glucuronic acids; Starch – diverse among plants and contains long chains of glucose.

Again, according to Kozloski (2011), carbohydrates that are not degraded in the rumen pass to the small intestines, and if starch is included in this fraction, it can be hydrolyzed by the pancreatic and the intestinal enzymes, yielding glucose, that will be absorbed. Part of the residual carbohydrates that reach the larges intestines can be fermented as well as in the rumen, but most of it is excreted in the feces. The substrate fermented and the rumen environment determine the species of rumen microorganisms that, are going to proliferate and determine the end products of fermentation (ANTUNES; RODRIGUEZ; SALIBA, 2011). The figure 3, represents the rumen digestion of the carbohydrates and the enzymes acting in each of the pathways.

Bacteria, ciliated protozoa, and fungi produce a variety of enzymes that breakdown the glycosidic bonds producing oligo-, di-, and monosaccharides. Several species of those bacteria and protozoa and all species of ruminal fungi possess cellulolytic activities, and the major cellulolytic bacteria can also digest hemicelluloses and pectin. However, there are some non-cellulolytic bacteria that can digest hemicelluloses too. In the rumen, pectin is completely digested, and starch is rapidly digested, but its extent depends on the grain type and processing. Additionally, rumen has active sugar fermenting bacteria that are important because of their propensity for fast growth and production of lactic acid in situations where rumen has excessive fermentable carbohydrate (NAGARAJA, 2016).

Figure 3 - Carbohydrate fermentation in the rumen, intermediate and end products and the main enzymes involved in each process



Source: (NAGARAJA, 2016)

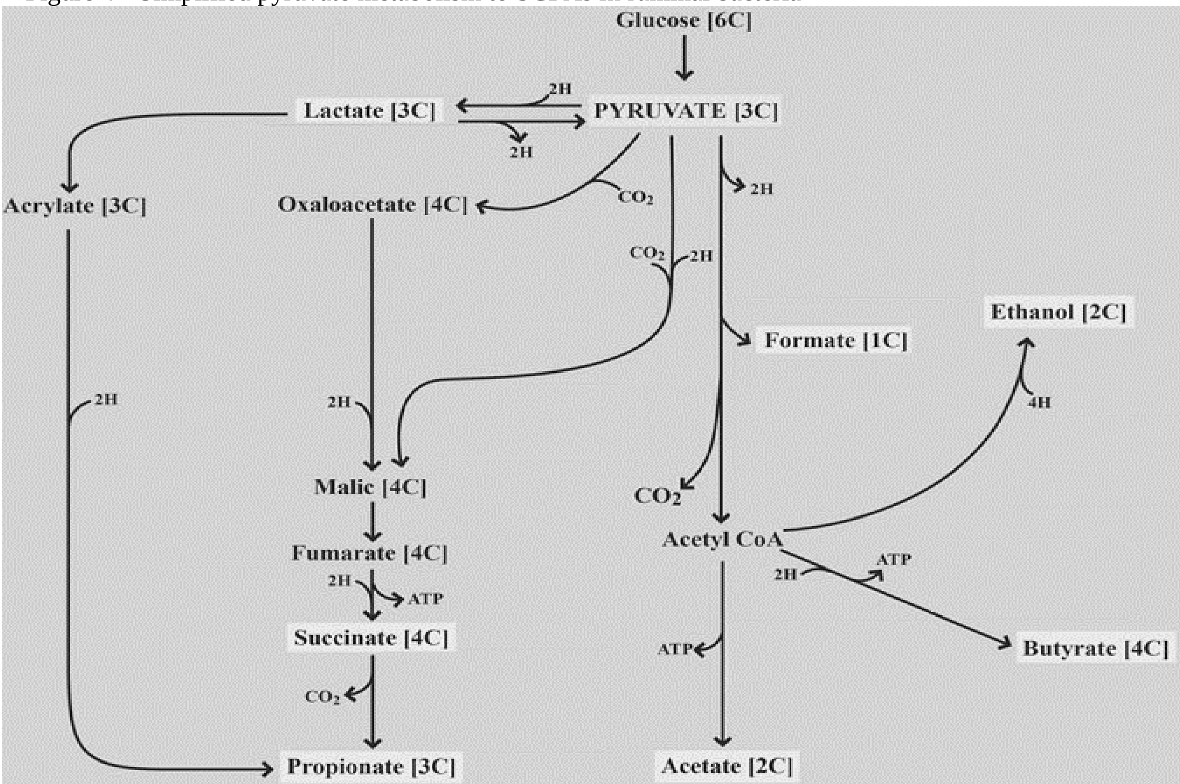
The common product from carbohydrate digestion in produced in the rumen is pyruvate. The fermentation products produced from pyruvate yield gases and short chain fatty acids (SCFAs): acetate, propionate, and butyrate, and their proportion depend on the type of feed, microorganism and ruminal conditions like pH and dilution rates. The acetate and butyrate are interconvertible, and their formation is given from acetyl-CoA produced from pyruvate (Figure 4). The acetate concentrations are reduced as the concentrate inclusion increases, and can be explained due to the inhibition of the cellulolytic microorganisms, and protozoa, which are the main producers of acetate. The increase of carbohydrate in the diet is also associated with the fast degradation of non-structural carbohydrate and rumen pH drop (ANTUNES; RODRIGUEZ; SALIBA, 2011). The propionate formation involves two main routes: one involving the succinate and oxaloacetate, and the second involving acrylate from lactate (BERGMAN, 1990). The fist pathway is the most occurring in the rumen in the propionate formation, however the propionate production from acrylate is more important for animals consuming high concentrate diets (LENG, 1970). Formate is utilized to produce methane. Lactate can be metabolized to either acetate, propionate, or butyrate. However, if lactate production exceeds the rate of fermentation, it accumulates in the rumen (NAGARAJA, 2016).

The butyrate formation may occur through two pathways: the inverse of β-oxidation, from 2 molecules of acetate, or from malonyl-CoA that combines with acytel-CoA, and reduced to butyrate (LENG, 1970). The amount of each SCFA produced in the rumen vary according to the type of diet due to metabolic pathways used by different populations of microorganisms that digest each dietary carbohydrate. In forage-based diets, the cellulolytic and *saccharolytic* bacteria will be the most active, therefore cellulose digestion and soluble sugar fermentation by these microbes will increase acetate formation (OWENS; GOETSCH, 1988). However, in grain rich-diets, amylolytic bacteria is the microbial population predominant and, its digestion results in an increased in propionate production (NAGARAJA, 2016).

Besides carbohydrates, SCFAs are also formed from protein and lipid digestion. The SCFAs normal concentration in the rumen is 60-160 mmol/L, and are considered the waste of fermentation for the microbes, but are the main source of energy for the ruminants – 50 to 70% of the digestible energy of the feedstuffs (KOZLOSKI, 2011). According to Coelho da Silva and Leão (1979) the SCFA concentration in the rumen depends on factors such as: Total production, absorption by the rumen wall epithelium, passage to the omasum or abomasum, dilution with the saliva, utilization by the microorganisms and conversion to other compounds. The branched-chain fatty acids, are extremely important for the growth of bacteria that degrade structural carbohydrates, but they are exclusively formed from peptides and amino acids (resulting from protein digestion). These compounds are readily captured by ruminal bacteria, and peptides are hydrolyzed when they get into the bacterial cell and most of the amino acids are deaminated. The deamination of valine, leucine and isoleucine results in isobutyrate, isovalerate, and 2-methylbutyrate, respectively (MEMBRIVE, 2016).

Lactate is a strong acid with pKa (pH value which an acid is found 50 % in its protonated form - HSCFA and 50 % in its non-protonated form - $SCFA^-$) equal to 3.8, while the SCFA are weak acids with pKa equal or lower than 4.8. Most part of the SCFA are found in the anionic form, in other words non-protonated, or non-acid (as acetate, propionate, butyrate) forms (BERCHIELLI, 2011). It happens because SCFA tend to donate protons to the ruminal environment (when rumen pH is above 6.2). Therefore, the lower the ruminal pH gets, the greater SCFA amount in the rumen is in its protonated form (HSCFA). Thus, SCFA production is the main way of reducing the rumen pH (OWENS; BASALAN, 2016). Therefore, these SCFA have to be removed from the rumen to avoid sharp pH drop and decrease in the fiber digesting microbes.

Figure 4 - Simplified pyruvate metabolism to SCFAs in ruminal bacteria



Source: (NAGARAJA, 2016)

Rumen buffering is realized by two major mechanisms, one trough saliva that contains phosphate and bicarbonate (HCO_3^-) that is released during chewing, and the other is the HCO_3^- input in the rumen through the rumen epithelium. When rumen pH is over 4.8, there will be a greater amount of $SCFA^-$ that do not carry H^+ to bloodstream during its absorption, not reducing acidification. In the dissociated form, $SCFA^-$ are electrically charged molecules and are more unstable in the rumen (MILLEN et al., 2016). The permeability of ruminal epithelium for charged molecules is extremely low, and because of that passive diffusion has been attributed only to molecules in their protonated forms – HSCFA (GABEL; ASCHENBACH; MULLER, 2002). Therefore, $SCFA^-$ are bicarbonate dependent for absorption, which means that when the $SCFA^-$ is absorbed, a molecule of HCO_3^- is released into the rumen fluid (ASCHENBACH et al., 2009). On the other hand, when pH is lower than 4.8, the HSCFA is absorbed by the rumen wall (papilla and epithelium) by lipophilic diffusion, taking H^+ proton with them, reducing acidification without exchange with HCO_3^- (MILLEN et al., 2016).

Only a small amount of the absorbed acetate is utilized by the liver due to the low activity of the acetil-CoA synthetase compared to adipose tissue and muscle. Therefore, the lipogenesis mostly occurs in adipose tissue utilizing the acetate. The carbon of the acetate is incorporated to fatty acids either by the adipose tissue or by the mammary gland by lactating cows most of the time, faster than the carbon from the glucose. The propionate and butyrate are metabolized by the liver, and by the ruminal and intestinal epitheliums. Butyrate is mostly absorbed by the intestinal and ruminal epitheliums, and propionate is mostly absorbed by the liver (BERGMAN, 1990).

The common route for the metabolism of acetate, propionate and butyrate is the Krebs cycle. Butyrate and acetate enter as acetyl CoA and propionate as succinyl CoA in this cycle, regenerating oxaloacetate, producing CO_2, ATP. The propionate is the main precursor of glucose in ruminants, as acetate, and butyrate have an even number of carbons so they can not contribute for a net synthesis of glucose (BERGMAN, 1990; BERCHIELLI; PIRES; OLIVEIRA, 2011).

2.4 PELLETED *CITRUS* PULP

Annually, million tons of agro-industrial wastes are generated in Brazil. An environmentally friendly and economically viable destination for agro-industrial byproducts is their use in animal feed, reducing feed costs of the farm (SILVA; REZENDE; INÁCIO, 2016). The pelletized *citrus* pulp is a byproduct, of *citrus* juice production and has been gaining more space in the national livestock market, mainly due to its price and nutritional qualities (MENDES NETO et al., 2007). *Citrus* pulp is from mainly orange juice extraction, but can be made up from by-products of other *citrus* fruits such as lemon, tangerine and pineapple (FRAPE, 2008). In this context, Brazil is the main producer of orange and orange juice in the world. The global orange production for season 2016/17 is forecast up 2.4 million metric tons from the previous year to 49.6 million, as a larger Brazilian crop more than offsets smaller crops in China and the United States. Fruit for processing is up 2.8 million tons on higher production in Brazil. Exports, however, are up only slightly as most of the expanded supplies will be used for processing. Global orange juice production for 2016/17 is forecast up sharply to 2.0 million metric tons as Brazil rebounds from the lowest production in nearly 3 decades (USDA, 2017a).

After the juice extraction, remains the pulp, hulls and seeds. The process of obtaining the *citrus* pulp consists of three stages: Initially, the mass of *citrus* residue is dehydrated, molasses is added and finally, pelletized. In the beginning of the process, calcium hydroxide is added to facilitate the dehydration of the mass and promote a pH adjustment therefore, it is a feedstuff rich in calcium and low in phosphorus (YAMANAKA, 2005). The period of production of *citrus* pulp begins in May and ends in January, coinciding with the inter-harvesting of grains such as corn and sorghum and the ending season of animals in the feedlot. Thus, producers can account on an important energy supplement in the months when corn reaches high prices.

When analyzed in the nutrition approach, agro-industrial by-products can be presented as excellent quality nutritional sources. Another advantage in the use of these foods is the possibility of greater flexibility in the diet formulation, depending on the variety of ingredients. In addition, some by-products may contain special or complementary nutrients to those already in traditional diets (MENEGHETTI; DOMINGUES, 2008). The pelleted *citrus* pulp is considered an energetic concentrate food, but due to its fiber content, pectin and rumen fermentation aspect is considered as an intermediate product between roughage and concentrate (FEGEROS et al., 1995). Therefore, the inclusion of *citrus* pulp allows the elevation of dietary fiber content without affecting the energy content of finishing diets.

Regarding to the nutritional aspect, the main carbohydrate of the pelleted *citrus* pulp is pectin, which is a polysaccharide formed by a linear chain of galacturonic acid, interrupted by units of rhamnose. The galacturonic acid chain is bound by α1-4 glycosidic bonds, similar to what occurs in starch, but differs in the axial bond of carbon 4. Pectin is an amorphous substance, partially soluble in water, totally soluble in NDF and of rapid and extensive degradation by ruminal microorganisms (VAN SOEST, 1994). It is located on the middle lamella of the cell wall and serves as cell adhesion (SALISBURY; ROSS, 1991). Moreover, pectin has no direct bonding with lignin, evidencing that the extent of pectin degradation by rumen microbes does not appear to be negatively affected by plant lignification (JUNG et al., 1992). And despite being a component associated with the cell wall, is almost completely digested in the rumen (90 to 100%), in addition, has a lower acidogenic rumen effect than corn (NOCEK; TAMMINGA, 1991), and it is fermented to more acetic acid than propionic acid.

Pectin is invariably the fastest degradation carbohydrate, while the starch and cellulose have very variable degradation according to the source, so the quality of these compounds reflects the rate of digestion. Compared to starch, pectin has a lower propensity to cause ruminal pH drop, due to its fermentation being carried out by cellulolytic microorganisms, favoring the production of acetate and not of lactate and propionate, as occurs in the fermentation of the starch. Another important factor is that the galacturonic acid structure promotes potential buffering through the exchange of cations. Therefore, pectin becomes an important carbohydrate to be included in high starch diets (VAN SOEST; ROBERTSON; LEWIS, 1991).

Due to its rapid ruminal degradability, the pelleted *citrus* pulp is an interesting food to be used in diets with high concentrations of soluble protein, which contributes to the better utilization of the ammonia produced, reducing its toxic effects on the rumen. This is because when the rate of ammonia synthesis by microorganisms exceeds its use, there is a rise in ammonia concentrations in the rumen with consequent increase in urea excretion, resulting in loss of protein (MORRISON; MACKIE, 1996). This residue of juice production usually consists of 60-65% of peels, 30-35% of bagasse and about 10% of seeds (CRAWSHAW, 2004), and its nutritional value can be modified depending on the variety, proportion of peel, seeds or bagasse of the fruits used and the type of processing. This food presents around 85 to 90% of the energy value of corn (NRC, 2001), Table 1).

Citrus pulp can be consumed by cattle in both wet and dry forms, but dry *citrus* pulp is more easily hauled and managed, and it is usually chosen by farmers when has to be transported to long distances because it becomes lighter when water is absent, and cheaper per kg purchased. Non-pelleted, dry *citrus* pulp is a bulky feedstuff, having a low density of approximately 303 kg/m^3. For this reason, and because of economic limitations on transportation, the pelleting of *citrus* pulp is favorable. Pelleting increases handling efficiency and decreases dustiness. Upon pelleting of the *citrus* pulp, the bulk density was increased up to 674 kg/m^3 (ARTHINGTON; KUNKLE; MARTIN, 2002).

Table 1 - Chemical composition of pelleted *citrus* pulp compared to corn

Nutrient	*Citrus* Pulp	Corn
Dry matter	80-90%	90%
Crude protein	7.0%	9%
NDF	25%	11.61%
ADF	24%	4.13%
Lignin	1.0%	1.10%
TDN	77%	85.65%
Ca	1.59%	0.03%
P	0.08%	0.25%
Starch	0.2%	66.25%
Pectin	25%	-

Source: Harris Jr. (1993)

However, some palatability issues have been related to the inclusion of *citrus* pulp in the diet. Assis et al. (2004) reported that many factors can alter the palatability of the *citrus* pulp such as: physical form, fruit variety used, and the proportion of seeds. Most of the papers found in the literature regarding acceptability and palatability of this by-product are studies with horses. In a study with Moreira et al. (2015) evaluated levels of *citrus* pulp in the diet (0, 7, 14, 21 and 28%) and observed that horses preferred concentrates with the addition of 0, 7 or 14% of *citrus* pulp, and the control concentrate was consumed the most. They also concluded that animals can identify the presence of *citrus* pulp in concentrates, and prefer concentrates without added *citrus* pulp. Ott, Feaster and Leib (1979) investigated the acceptability of diets with increasing levels of *citrus* pulp (0, 15 and 30%) and observed that diets with the inclusion of 30% of *citrus* pulp have been rejected by horses.

The results of researches evaluating the *citrus* pulp inclusion on beef cattle performance and metabolism are diverse. In the study of Prado et al. (2000), it can be concluded that none of the replacement levels of corn (40, 60, 80 and 100% in DM) by pelleted *citrus* pulp changed the weight gain, DM, CP, NDF intakes, G:F ratio, carcass dressing, fat deposition or rib-eye area of uncastrated bovines (F1-Nelore x Angus). However, in an experiment replacing finely ground corn by pelleted *citrus* pulp in diets of finishing bulls receiving 30% of sugarcane silage and 70% of concentrate, Pereira et al. (2007) observed a decrease in DM intake and weight gain when the animals received diets with 75 and 100% replacement of *citrus* pulp per corn in the concentrate, in relation to the 50% replacement diet. The authors expected a positive effect of *citrus* pulp on DMI, due to the lower starch content and, therefore, better ruminal pH maintenance. These results suggest that 30% of sugarcane silage in the diet was sufficient to maintain ruminal pH stability, even in the diet without *citrus* pulp (55.9% corn). Martínez-Pascual and Fernández-Carmona (1980) evaluated diets contained 10% alfalfa hay and 90% concentrate with 0, 15, 30, 45 and 60% of *citrus* pulp and found no differences on digestibility coefficients of the various feed fractions except that for ADF which increased as *citrus* pulp increased. In the same trial authors found no effect on hyperkeratosis incidence and daily gains, feed efficiency, and dressing percentage up to 30% of *citrus* pulp, but in greater quantities the animal response was poorer.

2.5 STARCH DIGESTION AND CORN PROCESSING

Cereal grains are comprised of three main morphological parts. The pericarp, which is the external layer that protects the grain, rich in fiber, highly lignified, and with low digestibility. The germ, rich in oil, and the endosperm rich in starch. The starch is a non-structural polysaccharide synthesized by the plants, found mainly in the endosperm of cereal grains, roots, tubers and stems, and its main function is energy reserve for dormancy, grain germination, growth, and regrowth (WANG et al., 1998). Starch is consisted entirely of glucose, and is arranged in two types of polymers: amylose and amylopectin, that differentiate from each other regarding the length of the molecule and chemical properties. Hydrogen bonds keep together these two polymers, resulting in a highly-organized structure (MCALLISTER et al. 2006). The amylose, is a linear molecule with α-(1→4) linkages, which means glucose being linked between carbon atom 1 of one molecule and carbon atom 4 of the adjacent molecule. A small proportion of α-(1→6) linkages may also be present in amylose molecules. The other polymer is the amylopectin, an α-(1→4)-linked glucose polymer with a great number of α-(1→6)-linked branches, and is longer than amylose (HALL, 1998).

Starch granules are formed by the deposition of rings that consist of alternating crystalline layers, represented by the more compact structure of amylopectin, and amorphous layers, represented mainly by amylose. Starch granules can be classified as high-amylose when amylose to amylopectin ratio is greater than 36% of the granule, normal when is between 16-35%, and waxy when this value is lower than 16%. Starch rich in amylose is less digested than amylopectin-rich starch (MCALLISTER, 2006). The proportion of the two vary among species and varieties, and amylose:amylopectin ratio increases with corn grain maturity. Waxy starches, when heated in water, swells much more than non-waxy starches, indicating that amylose restricts granule swelling. It is thought that amylose increases the intermolecular hydrogen bonding, limiting both swelling and enzymatic hydrolysis (ROONEY; PFLUGFELDER, 1986).

Starch granules within cells are embedded in a protein matrix, and the property of this protein matrix may also be related to the type of proteins present in the kernel. The corn kernel contains two main types of protein: zein, occurring in the endosperm, poorly digested, and glutelin, occurs in lesser amounts in the endosperm, and germ, and has greater digestibility than zein. Some corn hybrids have been selected for low zein concentration, therefore, for greater digestibility (MCDONALD et al., 2002). The density of this protein matrix, and its interaction with the starch varies with cell location in the grain, and may reduce its susceptibility to enzymatic hydrolysis, and digestibility. The protein matrix is sparse, fragmented, and loosely associated with the starch granules, and starch is more susceptible to enzymatic attack in the floury endosperm. However, in the vitreous region, the protein matrix is densely compacted with starch granules, and well developed (HARMON; TAYLOR, 2005; SANTOS; MARQUES; DÓREA, 2016). The difference in the digestibility between slowly fermentable grains and the more rapidly fermentable grains, is in the protein matrix property of these grains (MCALLISTER et al., 1990). The protein matrix of the sorghum and corn is denser thus, limiting the access of amylolytic microorganisms to the starch granule. On the other hand, the protein matrix of wheat and barley is more diffuse, not preventing the access of microorganisms (MCALLISTER et al., 2006).

In North America, almost all cultivated corn is dent (*Zea mays* ssp. Indentata), with soft porous starch, and in South America, the *flint* corn, harder grain, is predominant (*Zea mays* ssp. Indentura). Dent yellow corn, typically used in ruminant nutrition in the USA, was originated from the breeding of *flint* with farinaceous corn genotypes. *Flint* corn contains high concentrations of vitreous endosperm and is more slowly digested in the rumen than corn with higher concentrations of farinaceous endosperm (PHILIPPEAU; MICHALET-DOREAU, 1997). Floury corn endosperm types have lower zein content compared to the *flint* and dent corn endosperm (GIUBERTI, 2014). Vitreousness is defined as the proportion of vitreous endosperm in relation to total endosperm (SANTOS; MARQUES; DÓREA, 2016). The greater the vitreousness of corn grain is, the harder the grain, and the lower the ruminal starch degradability is. Vitreousness of Brazilian hybrids in mature stage averaged 73.1%, and the North-American hybrids, vitreousness averaged of 48.2%. Additionally, they measured density of Brazilian *flint* hybrids, and it averaged 1.268 g/cm^3, while the US dent hybrids averaged 1.201 g/cm^3. They also reported, a negative and significant correlation between both vitreousness and density of the corn grain with digestibility of starch (CORRÊA et al., 2002).

Starch hydrolysis in the rumen starts with the action of enzymes such as: α-amylase, β-amylase, amyloglucosidase, isoamylase, phosphorylase, pullulanase that can break alpha 1-4 and alpha 1-6 linkages (TESTER; KARKALAS; QI, 2004; MCALLISTER et al., 2006). These enzymes are secreted by microorganisms, mainly amylolytic bacteria, and are predominant in animals fed starch-rich diets (YOKOYAMA; JOHNSON, 1988). They hydrolyze the starch granules towards the interior of the endosperm cells leaving, the protein matrix intact (MCALLISTER et al., 2006). Then, the degraded starch molecule, results mainly in maltose and glucose, and saccharolytic bacteria will ferment these substrates through glycolytic route producing pyruvic acid. This is the intermediate step way where the carbohydrates have to go through before the conversion to SCFA, CH_4, and CO_2 in the rumen (YOKOYAMA; JOHNSON, 1988).

The starch digestion in the small intestine is not complete. It occurs in two different ways: digestion in the intestinal lumen by the pancreatic α-amylase enzyme, and digestion in brush borders by intestinal enzymes produced by the intestinal mucosa, such as maltase and isomaltase, similarly as occurs in non-ruminants (HARMON, 2009). The α-amylase reaches the intestinal lumen via pancreatic duct and hydrolyzes α-(1-4) glycosidic bonds of amylopectin producing maltose, maltotriose and α-(1-6) glycosidic linkages of amylopectin, producing several limit-dextrins (HARMON, 2009). The optimal pH for α-amylase activity in the intestinal lumen is 6.9, and a decrease of 0.5 in the pH reduced the amylase activity in 20% (RUSSEL et al., 1981). The starch digestion is completed in the intestinal villi by maltase and isomaltase activities, producing glucose (HARMON, 2009). These two enzymes are disaccharidases that present greater activity in the jejune and ileum compared to duodenum (HARMON, 1992). Maltase digests maltose molecules, and the isomaltase hydrolyzes the α-(1-6) glycosidic linkages of amylopectin branch points producing free glucose (ANTUNES; RODRIGUEZ; SALIBA, 2011).

The presence of starch in the small intestine is not the main factor to stimulate α-amylase secretion, but the energy intake, which determines the enzyme secretion, because it stimulates the production and microbial protein flow (RUSSELL; YOUNG; JORGENSEN, 1981; HARMON, 2009). Additionally, Owens, Zinn and Kim (1986) suggested that the size of particles containing starch may be a more limiting factor to the starch digestion in the small intestine, than the starch supply itself. Glucose absorption does not seem to be a limiting factor to starch digestion in the small intestine (HUNTINGTON; HARMON; RICHARDS, 2006), and this mechanism is mediated by the transporter GLUT2 without energy consumption to absorb glucose (HARMON, 2009). Glucose absorption contributes 33% of the total glucose supply of a beef steer; 44% of that glucose supply comes from organic acids from starch fermentation in the rumen and 23% from other carbon sources, such as amino acids (HUNTINGTON et al., 1996). Starch that is not digested in the small intestine is again submitted to fermentation in the large intestine by microbial enzymes yielding SCFA, microbial protein, methane and heat (SANTOS; MARQUES; DÓREA, 2016).

There are several cereal grains available for feeding ruminants, which can vary in size, texture, shape, maturity, moisture, and digestibility, thus justifying the processing of these foods because it allows to significantly improve the physical form and the nutritive value of beef cattle. The main purpose of grain processing is to increase energy availability, in order to increase animal performance (OWENS et al., 1997).

Traditional processing methods reduce particle size with or without the addition of water or steam (BEAUCHEMIN et al., 1994). Processing the grain by either milling, rolling, pelleting or flaking, breaks down digestion barriers such as hull, pericarp and protein matrix and allows microbial access to starch sheltered within endosperm cells. In addition, the ability of the ruminal microbiota to digest grain depends, among other factors, on particle size (GALYEAN; WAGNER; OWENS, 1981; BEAUCHEMIN et al., 1994), so finer processed particles are digested faster than coarsely ground particles, due to the greater contact area for colonization.

The milling or lamination processes are the most common methods of processing the grain. The 'fracture' of the grain by milling generally generates a large variety of particle size; on the other hand, the grain lamination results in more uniform grains. To further increase digestion, the whole grain, rolled or milled can be fermented with the addition of moisture (24 to 35%). Grain fermentation may occur in the presence of moisture inherent to the grain due to early harvest (wet grain) or when water is added to the dry grain before fermentation (reconstituted grain). However, to obtain the steam-rolled or steam-flaked grain, the whole dried grain is moistened with steam and crushed by roll. The steam-rolled grain receives moisture for a shorter period of time, its grains are thicker and a smaller portion of the starch is gelatinized compared to the steam-flaked grain (OWENS; SODERLUND, 2006).

The steam-flaking is a multi-step process, that promotes starch gelatinization of the grain. In this process, approximately 5 to 7% water is applied, then the grain absorbs water reaching 19–24 % of moisture (ARMBRUSTER, 2006). The grain is also exposed to steam from 30 to 60 min in a stainless-steel chamber. Then, the corn passes between pre-heated rollers, and the distance between them is adjusted to obtain the desired density (THEURER et al., 1999a; ZINN; OWENS; WARE, 2002). Bulk density, is typically measured as lb/bushel or g/L, and is an indirect measurement that reflects degree of processing. Gelatinization refers to the irreversible 'swelling' of the starch granule, which is the loss of its native structure in function of some applied energy that will be responsible for breaking intermolecular hydrogen bonds. This process can be caused by several factors, such as: thermal, mechanical, chemical agents or a combination of them. During gelatinization, the granules absorb water, increase in volume, and expose part of their amylose thus becoming more susceptible to enzymatic degradation (ROONEY; PFLUGFELDER, 1986).

After gelatinization, however, the gelatinized starch can undergo to a process known as retrogradation by which the starch molecules can reassociate and form tight bound structures and stabilized by hydrogen bonds, in which starch resists digestion by the amylases. This phenomenon is mainly associated with the cooling rate of the flake after addition of temperature, in which grains maintained at high temperatures for a long period of time, can have this process dramatically accelerated. In addition, the retrogradation is associated with the storage condition (high temperature and humidity) (JOUPPILA; ROOS, 1997) and the amylose content (LII; LAI; SHEN, 2004). Thus, grains with starch granules with higher amylose content are more susceptible to this retrogradation process than those with low amylose content.

In studies with processing, digestibility of corn increased by about 25% when ground (CLARK; CROOM; HARSHBARGER, 1975) or cracked (MOE; TYELL; HOOVEN, 1973) compared to the whole grain. Much of the difference in the digestibility of ground or cracked corn is caused by the 7 to 10% increase in starch digestion, but some of this increase is impaired by the reduction in dietary fiber digestibility. Studies have shown that flocculation increases about 10 to 20% of starch digestibility, but the digestibility of the fiber decreases in the same proportion (PLASCENCIA; ZINN, 1996). However, the starch digestion site is more affected by grinding than total starch digestibility. Based on *in situ* assays, approximately 44% of coarsely cracked corn starch is digested in the rumen, compared to 60-65% of finely ground corn (CERNEAU; MICHALET-DOREAU, 1991; LYKOS; VARGA; CASPER, 1997).

In a study with diets containing 50% of corn and 20% of whole cottonseed, Zinn (1987) concluded that steam-flaked corn contained 13.4 and 14.2% more NE_m and NE_g, respectively, than dry-rolled corn. These results show the benefit of the level of processing in the energy of the grain, which is consequently, expected improvements in the performance of the animals fed with them. Working with corn grain processing, Owens et al. (1997) reported lower DMI when the animals received diets with steam-flaked corn (8.34 $kg.d^{-1}$) when compared to animals fed dry-rolled corn (9.43 $kg.d^{-1}$). The authors attributed these results to the increase of metabolizable energy present in corn after steam-flaking, reducing consumption, and maintaining high weight gain. Theurer et al. (1999a) evaluated steam flaked-sorghum and observed an increased ruminal digestibility of dry matter and starch, and increased total tract digestibility of starch, compared to dry-rolled sorghum. However, the digestibility of small and large intestines as % of the intake was decreased, but as % of the entry, the starch digestion was greater in the small with no differences for the large intestine.

Not only can the method of processing but also the density of the flake affect the digestion of the grain. Theurer et al. (1999b) evaluated the effect of grain density on ruminal metabolism and concluded that by decreasing the flake density of corn from 437 to 283 $g.L^{-1}$, the proportion of starch digested in the rumen and total tract is increased by 11 and 2%, respectively. In the same work, the authors reported a linear increase of starch and dry matter digestibilities either in the rumen and total tract when evaluated decreasing sorghum densities (437, 360 to 283 $g.L^{-1}$). Hales et al. (2010) worked with non-castrated bovine males fed steam-flaked corn at two densities with alfalfa as roughage and observed a 4% increase in feed efficiency when the flake density decreased from 386 to 335g L^{-1}.

Zinn, Owens and Ware (2002) summarized performance 8 trials and reported an average decrease of 6.1% on DMI, but an increase of 5.4, and 12.2, for ADG and G:F ratio when dry-processed corn grain, either whole, ground, or dry-rolled was replaced by steam-flaked corn. Additionally, Santos, Marques and Dórea (2016) compiled 11 more recent papers that replaced dry-rolled corn by steam-flaked corn in the diets of finishing cattle performance, and found a 3.4% decrease in DMI, a 8.3% increase in ADG and, consequently, a 12.3% improvement on the G:F ratio. Both summaries evaluated trials carried out in the North America with *Bos taurus* cattle and dent type corn, so greater benefits are expected with the steam-flaking of flint corn, which has much lower digestibility than dent corn (SANTOS; MARQUES; DÓREA, 2016). The same author, evaluated 5 trials performed in Brazil with flint corn type, which compared the effects of replacing finely-ground, dry-rolled or whole corn by steam-flaked corn in the diets of finishing Nellore cattle on performance. This summary concluded that the steam-flaking of corn reduced 7.5% the DM intake, and increased 15.7, and 24.8% the ADG and G:F ratio, respectively. Those differences found in Brazilian trial with flint corn and Zebu animals are twice greater than those found in North America.

2.6 SUGARCANE AS ROUGHAGE

According to CONAB (2017) the sugarcane production for 2016/17 is estimated in 657.18 million tons, 1.3% lower than the previous year. The harvested area was 9.05 million hectares, 4.6% greater when compared to 2015/16, being 52% located in the São Paulo State. The sugar production reached 38.7 million tons, 15.5% greater than the previous year, due to the better prices. The ethanol production was 27.8 billion liters, 8.7% lower due to the preference for producing sugar (CONAB, 2017). However, no information is available on the amount of sugarcane produced for ruminant feed.

Due to the ease in the cultivation, sugarcane is adopted as supplementary roughage for cattle in the dry season of the year and is considered as one of the most interesting options for minimizing feed costs for ruminants and maximizing the net revenue in the activity (NUSSIO; ROMANELLI; ZOPOLLATTO, 2003). The sugarcane, among all the grasses, has the greatest potential of DM production. Its adequate nutritional value coincides with the season of forage shortage (OLIVEIRA, 2001), from May to September, as well as the ease of establishment and management of the crop and the possibility of harvesting during drought periods (FREITAS et al., 2006).

However, the nutritional limitations of this forage, such as low ingestion due to the high NDF content, the need for strategic manpower for daily cutting and chopping, low protein content (approximately 3.5%), low ruminal degradation of fiber (OLIVEIRA, 2001). Low levels of minerals such as calcium (0.39%), phosphorus (0.3%), magnesium (0.24%), sulfur (0.14%), and manganese, not meeting the requirements of the animals NRC (2016), so they must be supplemented correctly. In addition to urea, traditionally used as a low-cost non-protein ingredient, sources of true protein, such as soybean and cottonseed meal, soybeans or corn gluten feed must be present in sugarcane diets for high animal performance (NUSSIO et al., 2009).

Sucrose is the most abundant disaccharide in plants It is found in high concentrations in sugarcane, approximately 20%, and in sugar beet, 15-20%, and it is also present in other roots such as carrots, and it occurs in many fruits. Sucrose is formed from one molecule of glucose and one molecule of fructose joined together through an oxygen bridge between their respective carbon atoms 1 and 2 (MCDONALD et al., 2002). Ruminant animals do not secrete sucarase, and sucrose is digested by the enzyme sucrose phosphorylase produced by multiple rumen microorganisms (NIWIŃSKA, 2012). On forage-based diets, supplementation of 1 kg/day of sucrose decreased ruminal NDF digestion, and increased lag time for NDF digestion (KHALILI; HUHTANEN, 1991). The decrease in NDF digestibility with the sugar supplementation may be a function of drop in ruminal pH and or a competition between fiber and NFC fermenting bacteria for nitrogen and other nutrients (JONES; HOOVER; MILLER WEBSTER, 1998).

Traditionally, the use of sugarcane is based on daily cutting and immediate supply of fresh forage in the feed bunk, which increases the costs with manpower. In this context, the conservation of sugarcane in the form of silage can be an interesting strategy, since it allows the concentration of cutting and cultural treatments of the sugarcane, allowing an increase in the longevity of the field, favoring a more uniform regrowth and greater efficiency in weed control (NUSSIO et al., 2009). These advantages of silage production to the detriment of the fresh sugarcane supply can favor the decision by silage, despite the lower digestibility and consumption of sugarcane silage compared to fresh sugarcane.

Montañez-valdez et al. (2013) observed greater DM, NDF, ADF and pH when bromatological composition of sugarcane silage was compared to fresh material with no differences for protein nor rumen pH, and lower *in situ* DM digestibility. Roman et al. (2011) worked with sugarcane silage or corn silage and found greater DM intake for the animals that received corn silage (10.5 kg) compared to those that received sugarcane silage (10.2 kg), however, with no differences for average daily gain (1.3 kg/d) between the two silages.

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3 EFFECT OF DIFFERENT SOURCES OF NON-FIBER CARBOHYDRATE ON RUMINAL PH AND *IN VITRO* DIGESTIBILITY OF TROPICAL FORAGES

ABSTRACT. The present study was conducted to determine the effect of non-fiber carbohydrates (NFC) sources on rumen pH and *in vitro* dry matter (IVDMD) and neutral detergent fiber (IVNDFD) digestibility of forages. The NFC sources used were ground corn (GC), steam-flaked corn (SFC), pelleted *citrus* pulp (PCP) and were fed with sugarcane silage as roughage. The rumen pH was measured and rumen fluid used to evaluate IVDMD and IVNDFD of bermudagrass hay (Hay); corn silage (CS), and sugarcane silage (SS). Three rumen cannulated Nellore steers with average BW of 350 ± 15 kg were assigned in a 3×3 Latin Square design. A separate trial was conducted to describe the *in vitro* DM degradation curves of GC, SFC and PCP at 0, 6, 12, 18, 24, 30, 36, 42, and 48 h of incubation. The statistical model for the first trial included fixed effects of treatments (Diet), analyzed feedstuff (Feed), and their interaction (Feed × Diet), and the random effects of incubation time, square, and period. The significance was considered at ≤ 5% probability. The degradation curves of the concentrates were adjusted using the PROC NLIN procedure from SAS, and the equation parameters compared using confidence intervals. Feeding SFC decreased rumen pH compared to GC and PCP, at 6 h after feeding ($P = 0.04$). The rumen fluid from animals fed diets with SFC decreased IVDMD ($P = 0.03$) and IVNDFD ($P = 0.02$) of forages, compared to GC and PCP when measured after 48 h, with no effects for 30 h of incubation. The PCP had lower insoluble potentially degradable fraction (B), lower Lag Time, and greater B-fraction degradation rate. Moreover, SFC had greater degradation rate than GC. In conclusion, sugarcane silage has lower fiber digestibility among the evaluated roughages. Steam-flaked corn decreased rumen pH, fiber and dry matter digestibility, but increased the rate of degradation compared to ground corn. The *citrus* pulp decreased the lag time and increased the rate of degradation compared to the corn feedstuffs, either ground or steam-flaked.

Keywords: Corn processing. *Citrus* Pulp. Fiber digestibility.

3.1 INTRODUCTION

Animal performance is directly related to nutrient intake, which, in turn, depends on physical and chemical nature of the feeds and diet digestibility. Highly digestible carbohydrates are widely used to achieve greater digestibility and performance levels. Dry processing of cereal grains provides greater surface area for microbial attachment and disruption of the protein matrix surrounding starch, and wet processing can also gelatinize the starch, breaking the intermolecular hydrogen bonds within the starch granule (NOCEK; TAMMINGA, 1991). With greater digestibility, the ruminal fermentation capacity is maximized, resulting in increased microbial protein synthesis and short chain fatty acids (SCFA), particularly propionic acid, resulting in greater energy flow to the portal vein (THEURER et al., 1999). However, the increase of rumen starch digestibility can cause sudden drop in rumen pH, with possible reduction in fiber digestibility (VAN SOEST, 1994) and in dry matter intake (BENGOCHEA et al., 2005).

Pelleted *citrus* pulp (PCP), similar to corn, is a non-fiber carbohydrate (NFC) feedstuff used as prompt energy for ruminant fermentation, with fast and extensive rumen degradability (SNIFFEN; ROBINSON, 1987; SUNVOLD et al., 1995), but with high pectin concentration (22-40% DM - ARTHINGTON; KUNKLE; MARTIN, 2002; BAMPIDIS; ROBINSON, 2006). However, unlike cereal grains, it does not contain significant amounts of starch, thus preventing rumen acidosis because it tends to yield more acetate and little lactate. Therefore, PCP is usually included in replacement of rapid fermentable starchy feedstuffs (HALL; EASTRIDGE, 2014).

Sugarcane silage is a roughage source with poor fiber digestibility and high levels of soluble carbohydrate (sucrose 12.5% – OLIVEIRA et al., 2012). However, there are very few information regarding the effect of different NFC sources in sugarcane silage diets. In this context, the present study aimed to evaluate the effect of diets containing three NFC sources (ground, or steam-flaked corn, and *citrus* pulp) in sugarcane silage-based diets on rumen pH and *in vitro* DM and NDF digestibility of different forage sources.

3.2 MATERIAL AND METHODS

All experimental procedures were approved by the University of São Paulo Animal Bioethics Committee (protocol number 2784310715).

3.2.1 Animals and Diets

The trial was conducted to measure the effect of three NFC sources: steam-flaked corn (SFC - density of 415 g.L-1) and pelleted *citrus* pulp (PCP), partially replacing ground corn (GC) on rumen pH and cellulolytic activity of rumen fluid. Samples of Bermuda-grass (*Cynodon dactylon* cv. Coast-cross) hay (Hay), corn silage (CS), and sugarcane silage (SS), were incubated *in vitro* for either 24 or 48 h. Three rumen cannulated Nellore steers with average BW of 350 ± 15 kg were used in this study. The animals were housed in individual pens with concrete floor and covered feed bunks. Each pen contained individual automatic drinking troughs. The animals were assigned to treatments in a 3×3 Latin Square design, with three periods and three diets. Periods lasted 14 d each, with the first 12 d for diet adaptation and the last two days for serial measurement of pH and collection of rumen fluid for *in vitro* digestibility.

The three different diets were formulated to be iso-nitrogenous and promote a body gain of 1.4 kg.day-1, according to the NRC (2000) recommendations (Table 2). Food was provided ad libitum once daily at 0800 h, and the offered amount was adjusted daily allowing for 5% of orts.

Table 2 - Composition of the experimental diets with three sources of non-fiber carbohydrate on a DM basis

Item[1]	GC	SFC	PCP
Sugarcane silage	40.0	40.0	39.9
Ground corn	51.8	15.6	15.5
Steam-flaked corn	-	36.3	-
Pelleted *citrus* pulp	-	-	36.3
Soybean meal	5.0	5.0	5.0
Urea	1.1	1.1	1.6
Limestone	0.8	0.8	-
Mineral mixture[2]	1.1	1.1	1.1
Salt	0.2	0.2	0.2
Dicalcium phosphate	-	-	0.4

[1]GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
[2]Guaranty levels per kg: Calcium 210 g; Cobalt 24 mg; Copper 720 mg; Sulfur 74 g; Fluorine 240 mg; Phosphorus 24 g; Iodine 40 mg; Magnesium 30 g; Manganese 1500 mg; Selenium 8 mg; Sodium 60 g; Zinc 2080 mg; Monensin 1830 mg.
Source: (FERRARI, 2017).

3.2.2 Chemical procedures

The samples were dried for 72 h in a forced-ventilation oven at 55 °C and ground to pass a 1-mm screen at a Willey mill (Tecnal, Piracicaba, Brazil). The ash and DM were analyzed according to AOAC (2000) methods 942.05 and 930.15, respectively. The N was determined by combustion (Leco protein/N analyzer, model FP-528; Leco Corp., St. Joseph, MI) and CP determined multiplying N content by 6.25, and concentration of NDF was determined according to Van Soest, Robertson and Lewis (1991) using heat stable α-amylase (Sigma A3306; Sigma Chemical Co., St. Louis, MO) using the ANKOM A200 Fiber Analyzer (ANKOM Technology Corp.). The ADF and ADL were analyzed according to (VAN SOEST; ROBERTSON, 1985). The chemical composition of the feedstuffs is presented in (Table 3).

Table 3 - Chemical composition of feedstuffs

Item	Chemical composition, DM%[1]							
	DM	MM	CP	EE	NDF	ADF	ADL	NFC
Roughage samples,								
Corn silage	32.4	4.1	7.2	2.23	59.0	33.5	7.5	27.5
Sugarcane silage	33.3	5.5	3.2	1.23	75.4	48.0	10.6	14.7
Coast-cross hay	91.5	4.7	6.4	1.06	83.6	39.4	7.2	4.2
Concentrate samples,								
Ground corn	88.3	1.4	9.9	2.03	13.7	3.5	5.8	73.0
Steam-flaked corn	93.9	0.9	8.4	2.1	15.5	4.2	2.3	73.1
Pelleted *citrus* pulp	94.1	7.2	7.7	1.79	21.4	14.8	10.9	61.9

[1]DM = dry matter, MM = mineral matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, NFC = non-fiber carbohydrate.
Source: (FERRARI, 2017).

3.2.3 Degradation curve of concentrates

To better describe the three NFC sources used in this study, a degradation curve trial was performed. Ground corn, SFC and PCP were fermented in a DaisyII incubator (ANKOM Technology Corp., Fairpoint, NY) device for 0, 6, 12, 18, 24, 30, 36, 42, and 48 hours of incubation. The rumen fluid was collected from a rumen-cannulated young bull fed a diet with 60% corn silage and 40% concentrate consisted of ground corn, soybean meal, urea and mineral mixture. Approximately 0.75 g of each NFC source was weighted in F-57 bags in triplicate per time of incubation. As the DaisyII incubator consists of four fermenter bottles, two rounds were made. In the first round, samples were fermented during 6, 12, 18 and 24 hours, while in the second-round samples were fermented at 30, 36, 42 and 48 hours. Thus, each fermenter was intended for each fermentation time. The IVDMD analysis was performed following the methodology proposed by Holden (1999). Rumen fluid was added to each fermenter, and then CO_2 was added to remove O_2 and accelerate the anaerobic process. Time 0 hour of incubation was done by immersing the bags in distilled water at 37 °C for 10 minutes. Then, the bags were dried in oven at 105 ºC for 12 hours and weighted again. The results were used to adjust the DM degradation curve according to the model proposed McDonald (1981) described as follows:

$Deg = a + [b \times (l - e^{-Kd \times t})]$, where:

Deg = degradability in time t (%);

a = parameter equation; intersection of the exponential model when time t = 0 hour, corresponding to immediately soluble fraction if there was no lag-time;

b = parameter equation; would be potentially degradable fraction if there was no lag-time;

A = soluble fraction (%);

B = potentially degradable insoluble fraction = [(a + b) - A];

K_d = rate of degradation of the fraction B (%.h-1);

t = incubation time (h);

e = represents the base of the natural logarithms;

Lag = "Lag-time" or colonization time = $[\ln (b/B)] / K_d$.

3.2.4 Ruminal pH and digestibility of roughages

On the 13th and 14th d of each experimental period, approximately 4 kg of rumen content of each animal were collected from caudal, ventral, and cranial areas of the rumen via cannula, mixed and filtered using a 1-mm nylon mesh (Albercan Group, Itajubá, Brazil). Approximately 1.5 L of the collected liquid was transported in insulated bottles preheated with water at 37 °C for *in vitro* analysis of the roughage digestibility to be incubated for 30 and 48 hours. Approximately 0.5 g of roughage samples were weighted inside F-57 bags and placed in a DaisyII incubator (ANKOM Technology Corp., Fairpoint, NY). The *in vitro* DM and NDF digestibility of roughages were determined after 30 and 48 h of incubation, performed following the same methodology described previously. After 30 or 48 h of fermentation, the bags were analyzed for NDF as described earlier and dried in an oven at 105 ºC for 12 h for NDF and DM *in vitro* digestibility determination, respectively. Moreover, on the 14th d, the pH of rumen liquid was measured with portable pH-meter (Tec-3MP, Tecnal, Brazil) immediately before the first meal, considered time 0, and after 1, 3, 6, 9, and 12 hours post feeding.

3.2.5 Statistical analysis

The degradation curve of the concentrates was adjusted using the procedure PROC NLIN of the statistical package of SAS, being chosen the secant method (DUD), and the equation parameters compared using confidence interval. Rumen pH was analyzed using fixed effects of diet, random effects of period and repeated measures over time. The data of the roughage digestibility trial were analyzed in a 3 × 3 Latin Square design using the MIXED procedure of the SAS version 9.2 for Windows (SAS Institute Cary, NC, USA). The model included fixed effects of feedstuff (Feed), diet, and their interaction (Feed × Diet), and the random effects of animal and period. The degrees of freedom and the tests were adjusted using the Kenward-Roger method. The means were compared with the least significant difference and significance was considered at ≤ 5% probability level.

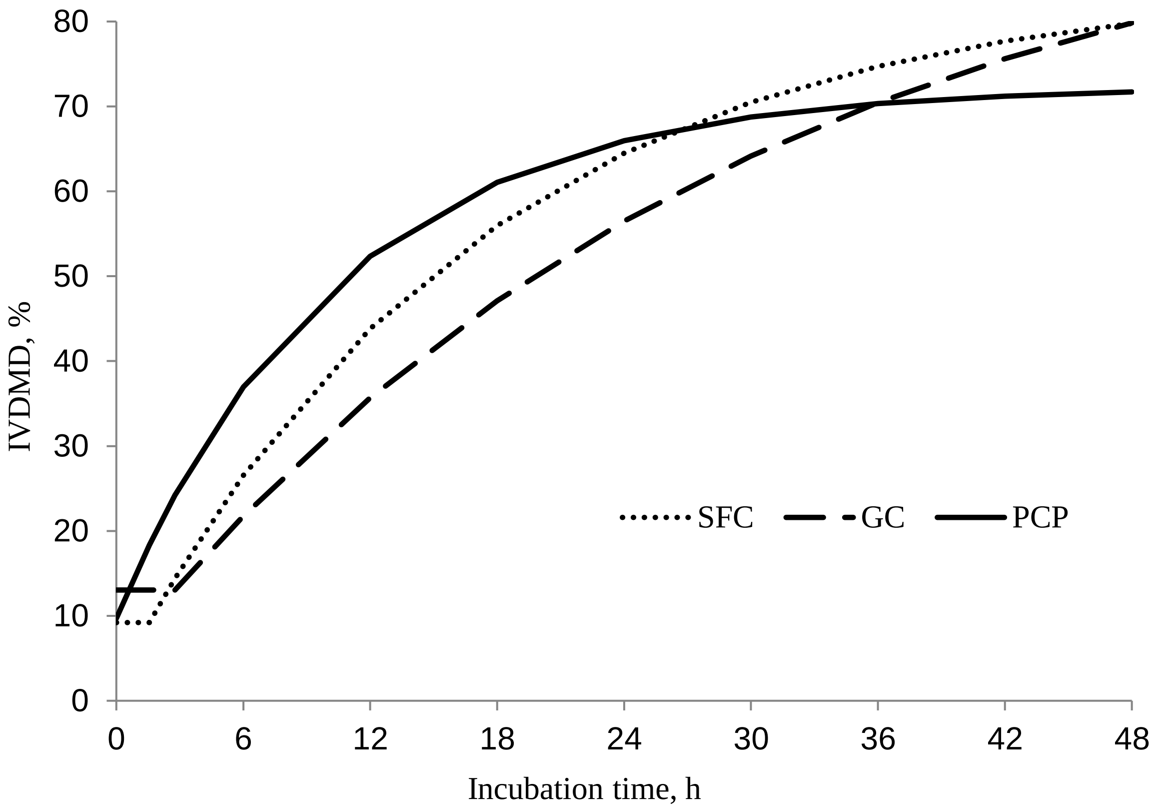
3.3 RESULTS AND DISCUSSION

3.3.1 Degradation curve

The degradation curve of the three NFC sources is presented in Figure 5.

To better characterize the three NFC sources used in this study, the feedstuffs were incubated at several time points and the parameters of the degradation curve were generated. Pelleted *citrus* pulp had lower lag-time, lower potentially degradable fraction and faster rate of degradation than the other two corn sources. The lower lag-time and faster rate of digestion of PCP is likely related to the accessibility of particles for microbe degradation. Pectin has high cation exchange capacity that can cause the plant to attract and bind hydrogen ions (MCBURNEY; VAN SOEST; CHASE, 1983). When these ions are bound to the feed rather than free in the liquid phase, the less acid is the rumen, and pectin also increases the rate at which the rumen microbes digest the feed (MCBURNEY; VAN SOEST; CHASE, 1983). Although PCP had faster degradation, the total degradation was lower than for corn sources, likely reflecting the greater total NDF and ADL content in the feedstuff.

Figure 5 - Potential degradation curve of DM for steam-flaked corn (SFC), ground corn (GC) and pelleted *citrus* pulp (PCP) at 0, 6, 12, 18, 24, 30, 36, 42 and 48 hours after feeding with the Lag time



Source: (FERRARI, 2017).

Analyzing the curve parameters, PCP had shorter Lag Time, smaller B fraction and greater K_d compared to both corn sources (Table 4). Also, SFC had greater K_d than GC (Table 4).

Table 4 - Feed fractions of the concentrate feedstuff, degradation rate and Lag time

Item[1]	Treatment			SEM
	GC	SFC	PCP	
A	0.13	0.09	0.10	0.043
B	0.86[a]	0.76[a]	0.63[b]	0.038
K_d	0.033[c]	0.059[b]	0.095[a]	0.007
Lag	2.77[a]	1.55[a]	0.00[b]	0.728

[1]Within a row, means without a common superscript differ ($P \leq 0.05$).
A=Soluble fraction; B=Potentially degradable insoluble fraction; K_d=rate of degradation of the B fraction; Lag=Lag-time; GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
Source: (FERRARI, 2017).

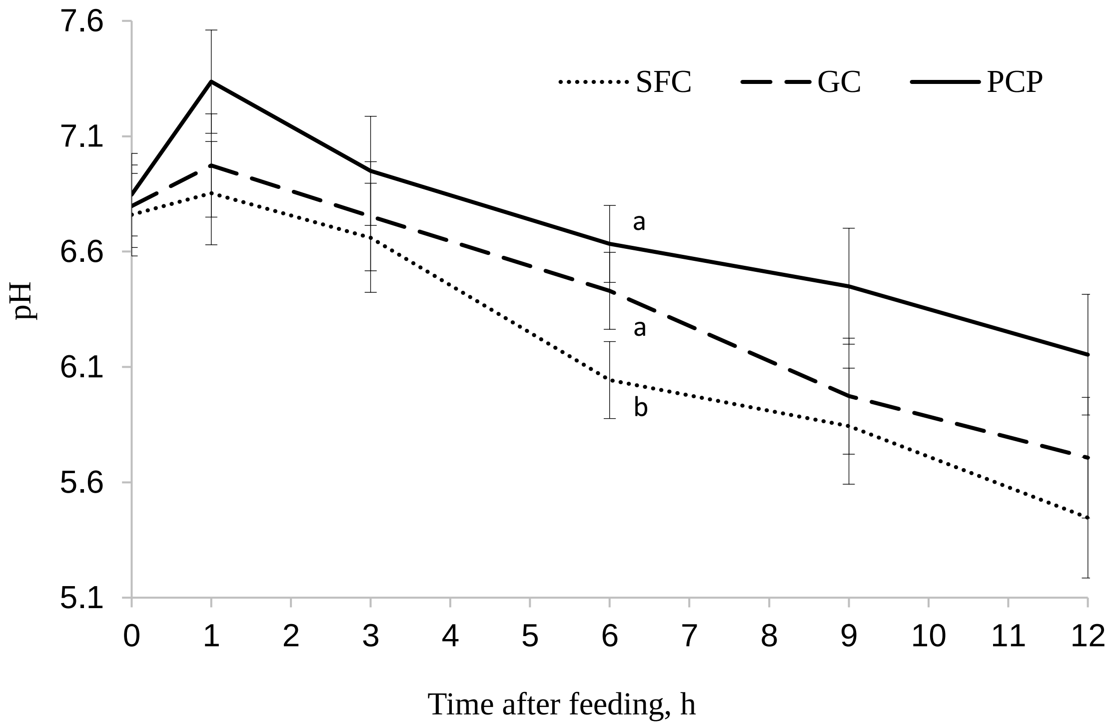
The processing effect of the corn sources was demonstrated in the degradation curve, with faster degradation rate for SFC than GC. Steam-flaking improves starch accessibility to rumen microbes accelerating the rate of DM degradation (NOCEK; TAMMINGA, 1991).

3.3.2 Rumen pH and *in vitro* digestibility

There was a NFC × Time interaction on rumen pH (P = 0.04, Figure 6) in which the diet with SFC decreased rumen pH compared to PCP and GC diets at the time 6h. *Citrus* pulp is composed of approximately 25% of pectin, which is a carbohydrate with rapid fermentation (CAMPBELL; WILLIAMS; EISEMANN, 2002) but lower potential for rumen acidification. Strobel e Russell (1986) found greater concentrations of acetic acid, a weak acid, and less lactic acid and in the rumen when pectin was fermented compared to starch. Marino et al. (2011) evaluated diets containing approximately 70 % concentrate and observed that animals fed *citrus* pulp presented pH values above 6.0 throughout the 12-h period after feeding, similarly to the present study. However, in that study, when animals were fed finely-ground corn or high-moisture corn silage, those authors reported pH values around 5.6 from 2 to 6 h after feeding. In the current study, despite the ground corn being a processed starchy feedstuff, the effect on rumen pH was similar to PCP.

The only observed difference in the present study was a lower rumen pH after 6h of feeding when GC was partially replaced with SFC. Starch digestion can be improved by processing the grain, which includes decreasing the particle size by grinding or breaking the kernel, crushing using rolls, adding or not moisture or heat (OWENS; BASALAN, 2016). The steam-flaking method breaks the protein matrix associated with starch granules, causes starch gelatinization, increases the surface area of the kernel, and facilitates the digestion of starch granules by amylolytic enzymes (NOCEK; TAMMINGA, 1991). In this regard, Zinn, Adams and Tamayo (1995) observed lower rumen pH 4 h after feeding for SFC compared to dry rolled corn, and this effect was associated with increased SCFA concentration in the rumen.

Figure 6 - Rumen pH from animals fed Steam flaked corn (SFC), Ground Corn (GC), Pelleted *citrus* pulp (PCP). a,b Means within the same time period with different lowercase letters differ ($P \leq 0.05$)



Source: (FERRARI, 2017).

There was no Feed × Diet interaction effect ($P > 0.05$) for any parameter of digestibility (Table 5). There was a Feed effect on both DM and NDF digestibility either after 30 or 48 h of incubation ($P < 0.01$, Table 5). The CS had the greatest value, followed by SS and lastly by Hay for IVDMD, both after 30 and 48 h of incubation. For the IVNDFD after 30 h, the CS had the greatest value compared to SS and Hay, with no differences between them. However, when IVNDFD was measured after 48 h of incubation, CS presented the greatest value, followed by SS and Hay (Table 5).

The rumen fluid from animals fed with SFC decreased both DM ($P = 0.03$) and NDF ($P = 0.02$) digestibility of the roughage sources, when measured after 48 h of incubation (Table 5). There was no effect of treatments on either DM or NDF *in vitro* digestibility when measured after 30 h of incubation (Table 5).

Table 5 - Effect of treatment on IVDMD and IVNDFD of roughage feedstuffs after 30 and 48 hours of incubation

Item[1]	Feedstuff[2]			SEM	Diet[3]			SEM	*P*-Value[4]		
	SS	CS	Hay		GC	SFC	PCP		Feed	Diet	Feed × Diet
IVDMD 30 h	35.6[b]	55.3[a]	27.4[c]	0.95	39.6	39.3	39.4	0.95	<0.01	0.84	0.82
IVDMD 48 h	42.0[b]	66.4[a]	38.2[c]	1.56	50.0[a]	46.7[b]	49.9[a]	1.59	<0.01	0.03	0.81
IVNDFD 30 h	14.7[b]	24.4[a]	13.1[b]	1.29	17.7	17.3	17.2	1.29	<0.01	0.78	0.74
IVNDFD 48 h	23.1[c]	43.1[a]	26.1[b]	2.08	32.3[a]	27.8[b]	32.2[a]	2.12	<0.01	0.02	0.89

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). *In vitro* Dry matter (DM) and neutral detergent fiber (NDF) digestibility after 30 and 48 h of incubation.
[2]Feedstuff: sugarcane silage (SS); corn silage (CS) and Coast-cross hay (Hay).
[3]Diet: ruminal fluid of animals fed ground corn (GC), steam-flaked corn (SFC) and pelleted *citrus* pulp (PCP).
[4] Feed = effect of feedstuff; Diet = effect of Diet; Feed × Diet = Feed and Diet interaction effect.
Source: (FERRARI, 2017).

The cellulolytic activity of the rumen fluid of animals fed with SFC was lower than the other two NFC sources, when measured after 48h of incubation. When rumen pH values fall below 6.0, the growth of the cellulolytic organisms can be reduced, allowing for an increase in the amylolytic flora which are propionate-producing microbes (KAUFMAN; HAGEMEISTER; DURKSEN, 1980), therefore impairing fiber digestion. The results of the present study agree with those of Owens and Soderlund (2012) who found that rumen and total tract digestibility of fiber decreased 42.4 and 12.7%, respectively, when animals were fed steam-flaked corn compared to animals fed dry-rolled corn. Similarly, Manríquez et al. (2016) observed a 25.1% decrease in the rumen fiber digestibility when animals were fed SFC compared to dry rolled corn.

Comparing the three roughage sources analyzed in the current experiment, the *in vitro* DM digestibility was associated with the fiber content of the feedstuffs. Corn silage and sugarcane silage with greater NFC content than Bermuda-grass hay. Oliveira et al. (2011) reported DM digestibility of corn silage to range from 72.2 to 74.8% and NDF digestibility from 44.8 to 46.3% after 48 h of incubation, values slightly greater than the present study (66.4 and 43.1% for DM and NDF digestibility, respectively). Reis et al. (2003) reported DM digestibility of 43.7% for Bermuda-grass hay, and Oliveira et al. (2007) evaluated sugarcane silage and reported *in vitro* digestibility of 60.8 and 40.1% for DM and NDF, respectively. The lower fiber digestibility of sugarcane was again demonstrated in this experiment. Sugarcane had a higher proportion of lignin in the cell-wall, which likely explains the lower *in vitro* NDF digestibility (YOU et al., 2017).

3.4 CONCLUSION

In conclusion, sugarcane silage had the lowest fiber digestibility among the evaluated roughages. Replacing ground corn with steam-flaked corn increased rate of rumen degradation, decreased rumen pH and therefore, reduced *in vitro* DM and NDF digestibility of the forages evaluated. Pelleted *citrus* pulp had lower lag time and faster rate of degradation compared to the corn regardless the processing method.

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4 CONCENTRATE LEVELS AND NON-FIBROUS CARBOHYDRATE SOURCES ON PERFORMANCE, CARCASS TRAITS, FEEDING BEHAVIOR, AND SORTING INDEX OF FINISHING NELLORE YOUNG BULLS

ABSTRACT - The objectives of this study were to quantify the effect of the partial replacement of ground corn (GC) by stem-flaked corn (SFC) or pelleted *citrus* pulp (PCP) in two concentrate levels (CONC, either 60 or 80% on a DM basis) in sugarcane-based diets on intake, performance, carcass traits, blood parameters, feeding behavior, and sorting index of finishing Nellore young bulls. To increase the power of test, the trial was repeated in two years: 2012 and 2013, with a total of 108 Nellore young bulls with initial body weight of 365.3 ± 3.12 kg. In each year, 54 Nellore young bulls were distributed in 18 pens, with 3 animals per pen, in a randomized complete block design with 6 replicates in a 3×2 factorial arrangement of treatments, with three non-fiber carbohydrates (NFC) and two concentrate levels (CONC). The animals were divided in three blocks by initial body weight. There was a significant NFC × CONC interaction on DMI and NDF intake (kg/d). Bulls fed GC with 60% concentrate had greater DMI (P = 0.04) compared to the other treatments. The NDF intake was decreased by SFC and PCP when fed at 60% concentrate, and increased by PCP when fed at 80% concentrate, compared to GC (P = 0.05). There was a significant NFC × CONC interaction on FBW and ADG (P < 0.05). The FBW was decreased (P = 0.03) when bulls were fed SFC and PCP with 60% concentrate compared to GC diets, with no differences on 80% concentrate diets. The SFC and the PCP decreased the ADG (P < 0.01) when fed with 60% concentrate, and the PCP decreased this parameter compared to GC and SFC corn with 80% concentrate included in the diet. Increasing concentrate from 60 to 80% of the diet increased G:F ratio (P < 0.01), HCW (P < 0.01), and CD (P = 0.03). Substituting GC by PCP decreased G:F ratio (P = 0.05) and HCW (P = 0.01). The SFC increased fat deposition (P = 0.03) and blood urea concentration (P = 0.02). Feeding the 80% concentrate decreased ingestion (P = 0.05) and rumination times (P < 0.01). The PCP increased Ingestion time (P = 0.05), decreased rumination (P < 0.01) and IEDM (P < 0.01). The inclusion of 80% concentrate in the diet caused the animals to sort for particles greater than 8 mm (P < 0.04) in a greater intensity. Also, PCP promoted a greater rejection of >19 mm particles compared to diets with GC and SFC. The replacement of ground corn with *citrus* pulp decreased the animal performance, carcass weight and dressing, and caused the animals to sort the diet against larger particles. The 80% concentrate inclusion in the diet increased animal performance, carcass weight and dressing compared to 60% concentrate. The steam-flaked corn increased fat deposition, with no impact on animal performance.

Keywords: Pelleted *citrus* pulp. Steam-flaked corn. Sugarcane silage.

4.1 INTRODUCTION

Improving performance of beef cattle is generally associated with the maximization of nutrient intake and providing a high-quality diet. There are, however, basically two mechanisms of intake regulation. One is the regulation based on hepatic oxidation that occurs mainly in finishing diets that are rich in non-fibrous carbohydrates. The rapid digestion of these carbohydrates results in the accumulation of propionate and the hepatic oxidation of this compound, promotes a signaling of metabolic satiety (ALLEN; BRADFORD; OBA, 2009). The other mechanism is rumen fill, which usually occurs in diets with high inclusion of forage and of low digestibility (ALLEN, 1996). However, an optimum level of forage in the diet is necessary, because it stimulates the selective retention of particles, increasing the ruminal digestibility of the diet, helping to avoid the sharp decline, or at least maintain the rumen pH (MERTENS, 2002).

Sugarcane is a roughage source and has a high production per hectare in the dry season and lower production costs compared to other forages (NUSSIO; ROMANELLI; ZOPOLLATTO, 2003). However, due to its low fiber digestibility (PRESTON; LENG, 1980; CARVALHO et al., 2010) it is necessary to include a source of high-quality carbohydrate in order to increase the energy intake of sugarcane-based diets for finishing beef cattle. In corn grain, starch granules are involved in a protein matrix that acts as a primary barrier to starch digestion. The steam-flaking of corn is a process that adds moisture and temperature to the whole grain, while mechanically causing a disruption of the protein matrix, increasing starch digestion and animal performance (OWENS, 2005). However, the increased starch digestibility can depress DMI, and fiber digestion in the rumen (OWENS; SODERLUND, 2006). *Citrus* pulp, is a high pectin by-product highly digested in the rumen, and has 85 to 90% of the energy value of dent corn (NRC, 2001). But, pectin fermentation, produces more acetic acid, it is less likely to decrease fiber digestion than corn sources. It is usually included in replacement of rapid fermentable starchy feedstuffs (HALL; EASTRIDGE, 2014).

Thus, studies evaluating different sources of non-fibrous carbohydrates in the concentrate and inclusion levels of sugarcane silage as roughage are important to determine the performance of cattle in the finishing phase. Our hypothesis is that partial substitution of GC with PCP would increase intake and maintain average daily gain in diets with 80% of concentrate, and that partial substitution of GC with SFC would decrease intake and increase or at least maintain daily gain, increasing feed to gain ratio in sugarcane silage-based diets.

Therefore, the objective of the present study was to quantify the effect of partial replacement of ground corn by steam-flaked corn or *citrus* pulp at two levels of concentrate in sugarcane silage based diets on intake, performance, carcass traits, sorting index and feeding behavior of finishing Nellore young bulls.

4.2 MATERIAL AND METHODS

All experimental procedures were approved by the Animal Bioethics Committee of the University of São Paulo (protocol number 2784310715).

4.2.1 Experimental site

The experiment was carried out at the Department of Nutrition and Animal Production, School of Veterinary Medicine and Animal Science, University of São Paulo, in the facilities of the Beef Cattle Research Laboratory, campus Pirassununga (Southeast of Brazil, 21º59'46" S, 47º25'33"). According to Köppen classification, Pirassununga has the Cwa climate type, characterized by hot and wet summer (mean temperature of the hottest month greater than 22 ºC), and dry winter (mean temperature of the coldest month below 18 ºC) and approximately 1100mm of rainfall annually. The rainy season is from end of October to beginning of March.

4.2.2 Animals and diets

To increase the power of test, the feedlot performance experiment was conducted in subsequent years (2012 and 2013). In each year, 54 Nellore young bulls in good sanitary and nutritional status, age of 20 to 24 mo old, and average initial BW of 365.3 ± 32.12 kg were used. Prior to the beginning of the experiment the animals were treated against ectoparasites with 40 mL/animal of flumethrin (Bayticol® Pour-on 1%, Bayer) and injectable broad-spectrum anti-helminth with 10 mL/animal of albendazole oxide (Ricobendazole®, Ourofino Animal Health). Then, the animals were weighed after 16-hour of food fasting and randomly distributed within 3 blocks according to initial body weight. The young bulls were then, allocated to 18 pens: 3 collective pens (with 3 animals) per treatment per year. Pen dimensions were 9 × 3 m with concrete floor, 3 meters of covered bunk, and shade. Automatic water troughs were located in each pen. During the first 30 days, the animals received a step-up adaptation with a gradual increase of the final content of concentrate in the diet (30, 40, 50, 60, 70, and 80% with 5 day-long each of the six steps). There was another 14-day of initial adaptation before the beginning of the trial, and the trial itself was composed by four periods of 21 days each.

Six diets were randomly assigned to the pens, in a 2 × 3 factorial arrangement of treatments. The factors consisted of two levels of concentrate in the diet (CONC) either 60 or 80% on a dry-matter basis, and three NFC sources: pelleted *citrus* pulp (PCP) and steam-flaked corn (SFC - density 415 $g.L^{-1}$), partially replacing ground corn (GC). The diets, formulated on a dry matter basis according to the Nutrient Requirement of Beef Cattle suggested by the NRC software (2000) for DOS® to be isoproteic (Table 6), and promote an ADG of 1.4 kg/d. Sugarcane silage was used as the roughage source.

Table 6 - Composition and analyzed nutrient content of experimental diets with three sources of non-fibrous carbohydrate and either 60 or 80% concentrate on DM basis

	60% concentrate			80% concentrate		
Item[1]	GC	SFC	PCP	GC	SFC	PCP
	---------------------------- % of diet DM ----------------------------					
Sugarcane silage[2]	40.0	40.0	39.9	20.0	20.1	20.0
Ground corn	51.8	15.6	15.5	72.1	21.5	21.4
Steam-flaked corn	-	36.3	-	-	50.5	-
Pelleted *citrus* pulp[3]	-	-	36.3	-	-	50.4
Soybean meal	5.0	5.0	5.0	5.0	5.0	5.0
Urea	1.1	1.1	1.6	0.8	0.8	1.4
Limestone	0.8	0.8	-	0.8	0.8	-
Mineral mixture[4]	1.1	1.1	1.1	1.1	1.1	1.1
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Dicalcium phosphate	-	-	0.4	-	-	0.5
Analyzed composition	---------------------------- % of diet DM ----------------------------					
DM	64.5	64.9	64.8	76.1	76.1	76.5
CP	12.00	11.8	12.5	12.3	12.1	12.1
NDF	31.6	32.0	33.5	21.0	21.3	23.6
Forage NDF	25.7	25.7	25.6	12.8	12.9	12.8
ADF	23.0	22.6	27.8	15.4	14.4	21.6
ADL	3.8	4.1	3.8	2.7	3.0	2.6
Starch	35.4	35.73	12.22	48.3	47.89	15.62
TDN	69.93	71.84	67.71	77.80	80.29	74.51
APS[5]	5.91	7.73	7.83	4.68	6.75	6.83
	---------------Expected dietary NE values, Mcal/kg of DM[6]--------------					
NE_m	1.63	1.69	1.56	1.88	1.95	1.77
Ne_g	1.03	1.08	0.96	1.24	1.30	1.15

[1]CP = crude protein, ADF = acid detergent fiber, ADL = acid detergent lignin, TDN = total digestible nutrients.
[2]DM = 30.9%; CP = 4.67%; NDF = 64.2%; ADF = 43.2%; ADL = 7.33%, Starch = 1.62%.
[3]DM = 88.1%; CP = 6.79%; NDF = 16.2%; ADF = 16.9%; ADL = 1.39%, Starch = 2.58%.
[4]Guaranty levels per kg: Calcium 210 g; Cobalt 24 mg; Copper 720 mg; Sulfur 74 g; Fluorine 240 mg; Phosphorus 24 g; Iodine 40 mg; Magnesium 30 g; Manganese 1500 mg; Selenium 8 mg; Sodium 60 g; Zinc 2080 mg; Monensin 1830 mg. [5]Average Particle Size of the diets according to Jones and Heinrichs (2002).
[6]Calculated based on the net energy (NE) requirements or maintenance and gain equations of NRC (2000).

Source: (FERRARI, 2017)

Before the harvest, the brix of sugarcane to be ensiled was evaluated to measure its maturity. The assessment was made from the plant broth by portable refractometer (model RT 30ATC, Instrutherm, São Paulo Brazil) after making small cuts in the top, middle and bottom portions of 10 plants (Figure 7). The mean Brix was 20.14%, with values above 18% recommended for animal feeding or harvesting for silage (BRIEGER, 1968).

Figure 7 - Measurement of the brix content on the sugarcane harvested for silage confection

Source: (FERRARI, 2017)

The silage was made from the sugarcane field belonging to FMVZ – USP Pirassununga of approximately 20 ha of the variety IACSP 93-3046, using a silage crop harvester (Premium Flex FM2-90, Menta Mit, Cajuru, SP), regulated for particle size of approximately 8 mm (Figure 8). It was also used a microbiological inoculant composed of the heterolactous bacterium *Lactobacillus buchneri* (strain NCIMB 40788 LALSIL Cana; Lallemand Animal Nutrition, Aparecida de Goiânia, GO, Brazil) in order to improve forage conservation in the silage process, besides limiting the alcoholic formation of and dry matter losses. The inoculant was pre-diluted 1 sachet (100 g) in 50 liters of water and sprinkled with a very fine beak at the time of chopping in the proportion of 1L/ton of chopped sugarcane. The chopped sugarcane was discharged with a tipper truck at the Beef Cattle Research Laboratory and packed 3 minutes/ton of sugarcane (Figure 9) to avoid colonization of aerobic microorganisms and to maintain the quality of the feedstuff. The silo was the surface type, covered with double-faced canvas of 200 microns with 8 m of width and was sealed for 30 days until opening.

Figure 8 - Sugarcane harvest in tipper truck

Source: (FERRARI, 2017)

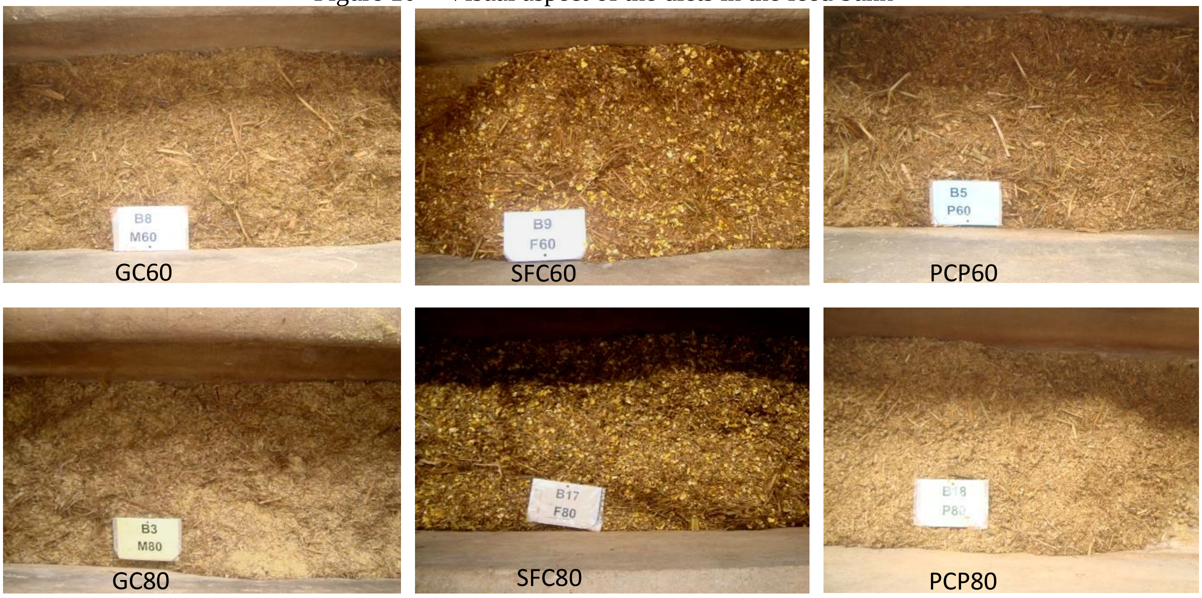
Figure 9 - Sugarcane packing for silage confection

Source: (FERRARI, 2017)

The steam-flaked corn used (SL Alimentos, Londrina, PR) remained at 100 ^{o}C at 17% humidity for 30 minutes and then rolled in regulated cylinders for a final thickness of 1.0 mm. After flocculation, the corn flakes were dried at temperature between 100-200 °C and cooled, and the final moisture was reduced to 13% and density of 415 g.L^{-1}.

The diets were offered as a Total Mixed Ration (TMR, Figure 10), the mixture of the roughage with the concentrate were made in a vertical screw mixer. The concentrates of each treatment were prepared at the Ration Plant through the mixing of the ground ingredients. In diets containing pelleted *citrus* pulp and steam-flaked corn, those ingredients were weighed daily separately and integrated into mixer at the time of delivery.

Figure 10 - Visual aspect of the diets in the feed bunk



Legend: where GC = ground corn, SFC = Steam-flaked corn, and PCP = Pelleted *citrus* pulp, and either 60 or 80% of concentrate in the diet

Source: (FERRARI, 2017)

Animals were fed the experimental diets *ad libtum*, twice daily, in two equal feedings at 0700 and 1500 h. Feed control was made by pen daily, by weighing the food provided and orts. The orts were kept within a range of 3-5% of the total feed provided on the previous day, making daily adjustments of food supply.

4.2.3 Performance and carcass traits

Samples of dietary ingredients and orts from each pen were collected weekly, dried at 55 ^{o}C for 72 h and ground to pass a 1-mm screen in a Wiley mill (Wiley Mill, Model 4; Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for DM and ash following the AOAC, 2000 methods 930.15 and 942.05, respectively. Concentration of N was measured by combustion method (Leco model FP-528; LECO Corporation, St. Joseph, MI) and CP content was calculated as the percentage of N in the sample multiplied by 6.25. The NDF content was determined using the method described by Van Soest, Robertson and Lewis (1991) using sodium sulfide and heat stable α-amylase (Sigma A3306; Sigma Chemical Co., St. Louis, MO) in an ANKOM A200 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY). Acid detergent lignin and ADF were analyzed according to Van Soest and Robertson (1985).

For calculation of average daily gain (ADG) animals were weighted at the beginning of the experiment and at the end of each 21-d period after 16-h solid fasting. The gain to feed ratio (G:F) was calculated by ADG:DMI ratio. The DMI was also evaluated as percentage of the BW (DMI% BW).

Fat deposition and muscular growth were measured at the beginning and at the end of the experiment using an Aloka 500V system equipped with a 3.5-MHz, 17.8-cm long transducer (Aloka USA, Inc., Wallingford, CT) coupled to an acoustic guide for better adaptation to the anatomy of the animal in order to analyze *Longissimus dorsi* muscle area (LMA), 12^{th} rib fat thickness on *Longissimus dorsi* muscle (BFT), and fat thickness on *Biceps femoris* muscle (rump fat thickness - RFT).

At the end of the experiment the bulls were transported and slaughtered at a commercial packing plant (JBS Friboi - Lins/SP) in accordance with current guidelines after 16-h fast from solids and water. The bulls were stunned by cerebral concussion, suspended, and exsanguinated through the jugular vein. Then, the weights of each half carcasses were obtained, and the hot carcass weight (HCW) obtained by summing the two half-carcasses of the same animal. The final BW (FBW) was adjusted for HCW by dividing carcass weight by the decimal fraction of the average dressing percentage of all steers (HCW/average CD). Therefore, in making this correction the ADG and G:F ratio were recalculated.

4.2.4 Blood metabolites

At the end of each period, before the first meal, blood samples were collected into two vacuum tubes (BD Vacutainer, Becton and Dickinson, NJ, USA) by jugular venipuncture from each bull from all treatments. One tube contained fluoride oxalate for glucose determination the other without anticoagulant for total protein, albumin, and urea determination in plasma samples. Plasma from the tubes without anticoagulant was obtained by centrifugation of samples at 700 × g for 20 min at 4°C, collected, and stored at –20°C until analysis. All blood samples were analyzed colorimetrically according to standard procedures using commercial kit (Randox Laboratories, São Paulo, SP, Brazil) in an ABS-200 automatic biochemistry analyzer (CELM, São Caetano do Sul, SP, Brazil).

4.2.5 Feeding behavior

The animals were observed on the 18th day of each experimental period, with visual monitoring with observations every 5 minute for 24 hours starting at 0800 h, to determine the time animals spent with rumination, ingestion, idle and other activities according to Bürger et al. (2000). The total mastication time (TMT) was calculated by adding total feeding time plus total rumination time, the intake efficiency of DM (IEDM, kg/h) was calculated by dividing the DMI (kg/d) by time spent on feed ingestion (h/d).

4.2.6 Sorting index

The TMR and orts were weekly sampled, consisting of 5 points of the feed bunk from each pen, totaling about 700 g to determine the particle size distribution using stratifying sieves (Penn State Particle Separator - PSPS, Nasco, Fort Atkinson, WI), as described by Heinrichs and Kononoff (2002). The PSPS contains 3 overlapping sieves with apertures of 19; 8; 1.18 mm, and the bottom pan. To determine the intensity of sorting of large particles, the sorting index (SI) was calculated as the actual intake/expected intake for particles retained in each sieve. Actual intake was determined as the amount of feed offered × particle size distribution in the TMR (percentage of fresh material) − the amount of orts × particle size distribution in orts (percentage of fresh material). Expected intake was determined as the particle size distribution of the TMR (percentage of fresh material) × actual fresh feed intake. A SI of 1 indicates no sorting, a SI < 1 indicates sorting against and >1 sorting for particles retained in each screen (LEONARDI; ARMENTANO, 2003).

4.2.7 Statistical analysis

The statistical analyses were conducted using the MIXED procedure of the SAS version 9.1.2 for Windows (SAS Institute Cary, NC, USA). Data were analyzed as a randomized complete block design, with a 3 × 2 factorial arrangement of treatments. The model included the fixed effects of the three NFC sources (NFC: SFC, PCP, and GC) and two concentrate levels (CONC: 60 or 80%), and their interaction (NFC×CONC). Year was included in the model as a random effect, and pen was considered as the experimental unit, with 3 pens per treatment per year.

Denominator degrees of freedom were calculated using the Kenward–Roger approximation. Different error covariance structures were investigated and the one that best fit, according to the Bayesian information criterion, was selected. When there was a significant interaction, the effects of treatments were compared using the SLICE option of the MIXED procedure. Treatment effects were considered significant at $P \leq 0.05$. Tendency was considered when probability values were ≤ 0.10.

4.3 RESULTS AND DISCUSSION

4.3.1 Intake, performance and carcass traits

The means for all treatments are presented in Table 7. There was a significant NFC × CONC interaction on DMI and NDF intake (kg/d). Bulls fed GC concentrate increased DMI (P = 0.04, Table 7) in the 60% concentrate diets compared to SFC and PCP. The NDF intake was decreased by SFC and PCP when fed at 60% concentrate, and increased by PCP when fed at 80% concentrate, compared to GC (P = 0.05, Table 7). There was a significant NFC × CONC interaction on FBW and ADG (P < 0.05). The FBW was decreased (P = 0.03, Table 7) when bulls were fed SFC and PCP with 60% concentrate compared to GC diets, with no differences on 80% concentrate diets. The SFC and the PCP decreased the ADG (P < 0.01) when fed with 60% concentrate, and the PCP decreased this parameter compared to GC and SFC corn with 80% concentrate included in the diet.

The main effects of NFC on performance are presented in Table 8. The PCP increased NDF intake as percentage of BW (NDF% BW, P = 0.01, Table 8) compared to GC and SFC, decreased DMI% BW (P = 0.05), and G:F (P = 0.05) compared to GC. The PCP also decreased HCW (P = 0.01) compared to SFC and GC diets. On the other hand, SFC increased both BFT and RFT (P = 0.03) compared to the other two carbohydrates.

The main effects of CONC on performance are presented in Table 9. Increasing concentrate from 60 to 80% of the diet decreased DMI% BW (P = 0.01), NDF intake %BW (P < 0.01) and tended to decrease CP intake %BW (P = 0.09, Table 9). Increasing concentrate from 60 to 80% of the diet increased G:F ratio (P < 0.01), HCW (P < 0.01), and CD (P = 0.03, Table 9), the NE_m (P = 0.02) and the NE_g (P = 0.02).

Table 7 - Nutrient intake, and performance of Nellore young bulls fed diets containing three sources of non-fibrous carbohydrate and either 60 or 80% concentrate

Item[1]	60% CONC			80% CONC			SEM	*P*-value[2]		
	GC	SFC	PCP	GC	SFC	PCP		CONC	NFC	CONC×NFC
DMI, kg/d	10.4[a]	9.5[b]	9.0[b]	9.3[b]	9.3[b]	9.0[b]	0.4	0.02	0.01	0.04
NDFI, kg/d	3.20[a]	2.97[b]	3.09[ab]	1.97[d]	1.96[d]	2.23[c]	0.10	<0.01	0.05	0.05
CPI, kg/d	1.21	1.12	1.10	1.12	1.09	1.09	0.05	0.08	0.11	0.30
DMI, %BW	2.37	2.25	2.14	2.16	2.10	2.09	0.12	0.01	0.05	0.38
NDFI, %BW	0.73	0.71	0.74	0.46	0.44	0.52	0.04	<0.01	0.01	0.27
CPI, %BW	0.28	0.27	0.26	0.26	0.25	0.25	0.26	0.09	0.24	0.71
IBW, kg	372	361	365	364	367	363	14	0.73	0.52	0.25
FBW, kg	492[a]	472[bc]	466[c]	484[abc]	489[ab]	475[abc]	14	0.29	0.04	0.03
ADG, kg/d	1.49[a]	1.25[b]	1.18[c]	1.43[a]	1.48[a]	1.28[b]	0.07	<0.01	<0.01	<0.01
G:F, kg/kg	0.141	0.131	0.128	0.152	0.158	0.135	0.016	0.02	0.05	0.42
HCW, kg	261	250	246	261	265	251	7	0.04	0.01	0.15
CD, %	53.0	53.0	52.8	53.9	54.2	52.9	0.5	0.03	0.14	0.42
LMA, mm	79.8	75.3	75.3	76.4	80.1	77.8	1.7	0.40	0.70	0.20
BFT, mm	3.7	4.1	3.0	3.7	4.5	3.5	0.4	0.30	0.03	0.83
RFT, mm	5.6	5.9	4.7	4.1	6.3	5.2	0.4	0.64	0.03	0.18
Dietary NE values, Mcal/kg of DM[3]										
NEm	1.83	1.77	1.78	1.95	1.99	1.83	0.13	0.02	0.37	0.39
NEg	1.19	1.14	1.15	1.30	1.34	1.20	0.29	0.02	0.37	0.39

[1]Within a row, means without a common superscript differ ($P \leq 0.05$).
NDFI: neutral detergent fiber intake, CPI: crude protein intake, %BW: intakes expressed as percentage of body weight, IBW: initial body weight, FBW: final body weight, ADG: average daily gain, G:F: gain to feed ratio, HCW: hot carcass weight, CD: carcass dressing, LMA: *Longissimus dorsi* muscle area, BFT: Back-fat thickness, RFT: rump fat thickness (fat thickness on *Biceps femoris* muscle). GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
[2]CONC: concentrate inclusion effect, NFC: non-fibrous carbohydrate effect, CONC×NFC: CONC and NFC interaction effect.
[3]Calculated from intake and performance data based on the net energy (NE) requirements of maintenance and gain equations of NRC (2000).
Source: (FERRARI, 2017)

It was our overall hypothesis that partial substitution of GC by PCP would increase intake and maintain average daily gain, and that partial substitution of GC by SFC would decrease intake and increase performance in sugarcane silage based diets. However, the greater concentrate inclusion in the diet and partial replacement of GC by PCP or SFC decreased DMI. Regulation of intake in ruminants fed high-forage diets is primarily a function of physical fill or rumen distention for diets that are energetically dilute and less digestible (WALDO, 1986). When DMI is limited by distention, the limitation results from the low rate of removal of digesta from the rumen by digestion, absorption, and passage (ALLEN, 2000). Our results, however, indicate that intake was not regulated by NDF content, because diets with greater roughage inclusion promoted greater NDF intake with no reduction in DMI.

Table 8 - Nutrient intake, and performance of Nellore young bulls fed diets containing three sources of non-fibrous carbohydrate.

Item[1]	GC	SFC	PCP	SEM	*P*-value
DMI, %BW	2.26^{a}	2.18^{ab}	2.11^{b}	0.12	0.05
NDFI, %BW	0.60^{b}	0.58^{b}	0.63^{a}	0.04	0.01
G:F, kg/kg	0.146^{a}	0.144^{ab}	0.132^{b}	0.015	0.05
HCW, kg	261^{a}	258^{a}	249^{b}	7	0.01
BFT, mm	3.69^{b}	4.29^{a}	3.23^{b}	0.36	0.03
RFT, mm	4.85^{b}	6.11^{a}	4.92^{b}	0.35	0.03

[1]Within a row, means without a common superscript differ ($P \leq 0.05$).
NDFI: neutral detergent fiber intake, %BW: intakes expressed as percentage of body weight, G:F: gain to feed ratio, HCW: hot carcass weight, BFT: Back-fat thickness, RFT: rump fat thickness (fat thickness on *Biceps femoris* muscle). GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
Source: (FERRARI, 2017)

Bhattacharya and Harb (1973) in a trial with lambs, tested increasing levels of PCP replacing corn in the ration and reported when PCP is beyond 40% the palatability tends to decline. However, Cribbs et al. (2015) partially replaced steam-flaked corn with PCP in an inclusion lower than 40%, at 0, 10 and 20% in starter (d 0 to 28), transition (d 28 to 42) and finishing (d 42 to 56) diets fed to receiving cattle and found a linear decrease in DMI, ADG and G:F ratio as PCP increased regardless the type of diet offered. On the other hand, in another trial evaluating levels of replacement of either GC or SFC by PCP (0, 25, 50, and 75%), Gouvêa et al. (2016) observed a linear increase in DMI but a linear decrease in FBW, ADG, G:F ratio, with no effects on HCW as PCP increased in the diet replacing SFC. However, when PCP replaced GC diets, there were quadratic responses for FBW, DMI, ADG, and HCW with the 50% replacement being the optimum level of inclusion. Differently, from the negative effect of increasing *citrus* pulp in steam-flaked corn diets on performance, the authors attributed this quadratic effect in ground corn diets to a more favorable rumen environment for digestion when *citrus* pulp replaced half of the corn.

Table 9 - Nutrient intake, and performance of Nellore young bulls fed diets either 60 or 80% concentrate

Item[1]	60%	80%	SEM	*P*-value
DMI, %BW	2.25[a]	2.12[b]	0.12	0.01
NDFI, %BW	0.73[a]	0.47[b]	0.04	<0.01
G:F, kg/kg	0.133[b]	0.148[a]	0.015	0.02
HCW, kg	252[b]	259[a]	6.8	0.04
CD, %	52.9[b]	53.7[a]	0.4	0.03
Dietary NE values, Mcal/kg of DM[2]				
NEm	1.79[b]	1.92[a]	0.13	0.02
NEg	1.16[b]	1.28[a]	0.2	0.02

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). NDFI: neutral detergent fiber intake, %BW: intakes expressed as percentage of body weight, G:F: gain to feed ratio, HCW: hot carcass weight, CD: carcass dressing,
[2]Calculated from intake and performance data based on the net energy (NE) requirements of maintenance and gain equations of NRC (2000).
Source: (FERRARI, 2017)

Possible factors affecting intake of PCP include density, texture, physical size of the pellet (approximately 1.8 cm length in this study) or taste differences between *citrus* sources (CRIBBS et al., 2015). Karadeniz (2004) reported dramatic differences in main organic acid in fruits which can compromise *citrus* pulp palatability. Lemon has the highest concentration of citric acid and lowest pH among species and varieties of *citrus* analyzed in that study, so the proportion of each fruit present in the *citrus* pulp may influence intake. However, in the present study there is no information on the proportion of lemon or other fruits that composed this by-product.

On the other hand, the DMI depression when bulls were fed SFC and higher concentrate inclusion compared to GC, can be explained by greater propionate being produced in the rumen. Propionate is a primary end product of starch fermentation, and its production rate varies depending on starch concentration and fermentability (ALLEN, 2000). The high inclusion of steam-flaked corn increases extent of starch digestion, with a consequent increase in the propionate produced in the rumen (ZINN; OWENS; WARE, 2002), which is the primary fuel stimulating hepatic oxidation, decreasing intake (ALLEN; BRADFORD; OBA, 2009). Some of the animal performance from the present study can be explained by the effects of treatments on DMI.

Both ADG and FBW were decreased by PCP and by SFC at 60% concentrate diets, and by PCP at 80% concentrate diets. And, contrary to our hypothesis, partially substituting GC by PCP did not maintain performance, regardless of the level of concentrate inclusion, decreasing

G:F ratio and HCW, which can be attributed to lower intake. Feeding the 80% concentrate-diet increased G:F ratio, HCW, CD, and, and energy value of the diets even with lower DMI compared to 60% concentrate-diets. For Nkrumah et al. (2006), feed efficiency may be influenced by several potential metabolic and physiological pathways such as efficiency of conversion of gross energy into metabolizable energy (because of differences in digestibility, generation of gases during ruminal fermentation, absorption of nutrients, waste excretion, and heat production) and the subsequent efficiency of metabolizable energy conversion to retained energy for maintenance and growth.

Caetano et al. (2015) evaluated either high-moisture or finely-ground flint corns and levels of roughage NDF (rNDF) in sugarcane silage-based diets with 8% of whole lint cottonseed, and concluded that the high-moisture corn (HMC) decreased the DMI but increased the G:F ratio, with no effects on the ADG. They also estimated the optimal concentration of rNDF using the first derivative of second order polynomials to be 13.3% for FBW, 13.0% for ADG, 12.8% for HCW, 13.7 and 11.3% for DMI when fed HMC and ground corn, respectively. In their study, the conclusion is that a greater overall performance is achieved when 12.8% of rNDF is fed with whole cottonseed and 14.7% rNDF without the cottonseed. Another study evaluated levels of *in natura* sugarcane bagasse in diets that contained ground corn and *citrus* pulp – a great source of fiber, in the concentrate, and concluded that the animal performance was increased when 15% of bagasse was increased in the diet (BULLE et al. 2002). If the bagasse is considered to have 85% of NDF the rNDF of the 15% - bagasse diet would be 12.75%, similar to the value reported by Caetano et al. (2015). In the present study, the rNDF of the 80% concentrate diets were 12.8% and the 60% diets were 25.7%.

Opposed to our initial hypothesis, substituting GC by SFC did not improve G:F ratio. Owens and Gardner (2000) observed an increase on feed efficiency, carcass weight, dressing, and fat thickness when animals were fed steam-flaked corn rather than dry-rolled corn diets. The authors attributed these results to a shift on site of digestion, with increased fat deposition being related to greater starch digestibility and less ruminal escape of dietary starch. According to Pearce, Pethick and Masters (2008), increased propionate production stimulates insulin concentration, which is the hormone regulating the fat deposition in the carcass. However, in the present study, the SFC only increased fat thickness, with no effects on ADG or G:F ratio. According to McLeod et al. (2007), the increase of glucose supply in the small intestines stimulates the fat tissue deposition, and visceral and omental fat. One explanation for the lack of effect of SFC on performance is the increase in the fat deposition which requires more energy compared to the same amount of muscle deposited. The fat deposition process is more efficient than protein, but requires more energy per unit of weight gain because of the high caloric density of fat, and because the muscle is approximately 75% water (LAWRENCE; FOWLER, 2002).

In a Brazilian trial Marques et al. (2016) evaluated four diets containing either steam-flaked flint corn with 6% sugarcane bagasse, or diets with whole flint corn with increasing levels of sugarcane bagasse (0, 3, and 6%). In that trial, there was a quadratic increase on FBW, ADG and DMI, and an increasing linear tendency for HCW and Fat thickness as bagasse increased in the diets. Moreover, the steam-flaked corn diets increased NEm and NEg of the diets by 17 and 23%, increased 20% the G:F ratio due to a decreased 17% the DMI compared to the whole corn diets, with no effects on fat thickness. Santos, Marques and Dórea (2016) evaluated 5 trials performed in Brazil with flint corn, comparing the replacement of finely-ground, dry-rolled or whole corn by steam-flaked corn on performance of finishing Nellore cattle. They concluded that the steam-flaking of corn reduced on average 7.5% the DM intake, and increased 15.7% the ADG, and 24.8% the G:F ratio, respectively.

One probable explanation for the lack of improvement on performance, except for greater fat thickness, of the SFC diets on the present study, may have been the flake density and frequency of grain flaking and delivery to the experimental site. In the present study, the flake density was 415 $g.L^{-1}$ and the whole amount of corn for the trial was delivered in the feedlot cattle facilities once, at the beginning of the trial, while most of the studies reported flake density of approximately 310 $g.L^{-1}$ and the corn was delivered twice monthly. Moreover, after gelatinization with steam-flaking, gelatinized starch can undergo to retrogradation, which is the reassociation of starch molecules e and formation of tight bound structures stabilized by hydrogen bonds, in which starch resists to amylase digestion. This process is associated with the delay in the cooling rate of the flake after addition of temperature, to high temperature and humidity (JOUPPILA; ROOS, 1997) and to greater amylose content (LII; LAI; SHEN, 2004) which is the case of Brazilian corn types.

4.3.2 Blood metabolites

The means of blood parameters for all treatments are presented in Table 10. The main effects of NFC on blood parameters are presented in Table 11. The only effect on blood parameter, was of carbohydrate source on urea. The SFC increased blood urea concentration ($P = 0.02$, Table 11). There was no effect of treatments on blood glucose, albumin or total protein concentration ($P \geq 0.11$).

Table 10 - Blood parameters of Nellore young bulls fed diets containing three sources of non-fibrous carbohydrate and either 60 or 80% concentrate

Item[1]	60% CONC			80% CONC			SEM	*P*-value[2]		
	GC	SFC	PCP	GC	SFC	PCP		CONC	NFC	CONC×NFC
Glucose, mg/dL	65.6	74.2	67.7	68.7	67.6	67.6	8.3	0.59	0.37	0.24
Urea, mg/dL	28.3	34.8	31.4	32.9	34.8	29.0	2.2	0.60	0.02	0.14
Album, g/dL	3.47	3.60	3.58	3.50	3.58	3.58	0.07	0.92	0.21	0.93
Total prot, g/dL	6.86	6.94	7.00	7.13	7.15	7.05	0.16	0.11	0.85	0.92

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
[2]CONC: concentrate inclusion effect, NFC: non-fibrous carbohydrate effect, CONC×NFC: CONC and NFC interaction effect.
Source: (FERRARI, 2017)

Table 11 - Blood parameters of Nellore young bulls fed diets containing three sources of non-fibrous carbohydrate and either 60 or 80% concentrate

Item[1]	GC	SFC	PCP	SEM	*P*-value
Glucose, mg/dL	67.15	70.89	67.64	2.8	0.37
Urea, mg/dL	30.58[b]	34.78[a]	30.18[b]	2.2	0.02
Album, g/dL	3.48	3.59	3.58	0.05	0.21
Total prot, g/dL	7.09	7.15	7.18	0.12	0.84

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
Source: (FERRARI, 2017)

Theurer et al. (1999) stated that steam-flaking of corn or sorghum compared to steam-rolling of corn or dry-rolling of sorghum improves cycling of urea to the gastro-intestinal tract, because of greater ruminal and total tract starch digestion by dairy cows. The greater the processing level of the grain, the greater is the rumen degradability of the carbohydrates, therefore, more energy is available to utilize the rumen ammonia for microbial crude protein production (OWENS; ZINN, 1988). In this sense, more protein is being enzymatically broken down in the small intestines, and more amino acids are being absorbed into the bloodstream (SANTOS; PEDROSO, 2011). Consequently, the greater rumen starch digestibility of SFC could explain the increased blood urea nitrogen in bulls receiving SFC compared to GC in the present study. All the blood parameters, however are within the reference values considered as normal for bovine animals according to Kaneko, Harvey, and Bruss (1997).

4.3.3 Feeding behavior

The means of feeding behavior for all treatments are presented in Table 12.

Table 12- Feeding behavior of Nellore young bulls fed diets containing three sources of non-fibrous carbohydrate and either 60 or 80% concentrate

Item[1]	60% CONC			80% CONC			SEM	P-value[2]		
	GC	SFC	PCP	GC	SFC	PCP		CONC	NFC	CONC×NFC
Rum, h/d	7.7	6.8	6.1	5.7	5.2	4.8	0.7	<0.01	<0.01	0.91
Ing, h/d	3.3	3.2	4.4	3.1	2.8	3.4	0.3	0.05	0.05	0.53
IEDM, kg/h	3.23	3.10	2.09	3.25	3.91	2.48	0.29	0.09	<0.01	0.38
TMT, h/d	11.0	10.0	10.5	8.8	8.0	8.2	0.9	<0.01	0.11	0.99
Idle, h/d	12.8	13.7	13.3	15.0	15.9	15.6	0.4	<0.01	0.14	0.98
Others, h/d	0.22	0.16	0.27	0.17	0.20	0.18	0.02	0.11	0.31	0.16

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). Rum: rumination, Ing: ingestion, IEDM: intake efficiency of dry matter, TMT: total mastication time (Rum + Ing), Others: 24 h – (Rum + Ing + Idle), GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
[2]CONC: concentrate inclusion effect, NFC: non-fibrous carbohydrate effect, CONC×NFC: CONC and NFC interaction effect.
Source: (FERRARI, 2017)

The main effects of NFC on feeding behavior are presented in Table 13. The PCP increased Ingestion time ($P = 0.05$) compared to GC and SFC, decreased rumination time ($P < 0.01$) compared to GC and IEDM ($P < 0.01$) compared to GC and SFC, indicating that the bulls consumed less feed per hour spent at the feed bunk.

Table 13 - Feeding behavior of Nellore young bulls fed diets containing three sources of non-fibrous carbohydrate

Item[1]	GC	SFC	PCP	SEM	P-value
Rumination, h/d	6.70^{a}	6.00^{ab}	5.45^{b}	0.70	<0.01
Ingestion, h/d	3.05^{b}	3.24^{b}	3.9^{a}	0.23	0.05
IEDM, kg/h	3.24^{a}	3.51^{a}	2.29^{b}	0.23	<0.01

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). IEDM: intake efficiency of dry matter, GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
Source: (FERRARI, 2017)

Feeding the 80% concentrate diet tended to increase IEDM ($P = 0.09$, Table 12), increased idle time ($P < 0.01$, Table 14), decreased ingestion time ($P = 0.05$), and rumination time ($P < 0.01$), and consequently the TMT ($P <0.01$) compared to 60% concentrate diet (Table 14).

Among the factors that may influence the rumination time of the animals are: the particle size of the diet (BERCHIELLI; PIRES; OLIVEIRA, 2011), fiber content (BALCH, 1971), forage quality (WELCH; SMITH; GODSON, 1970) and insalivation (KICK; PAUL GERLAUGH; SCHALK, 1937). However, in the present study, the rumination was decreased when greater concentrate and PCP were included in the diet, probably as a consequence of a greater digestibility of the diets with less roughage, and *citrus* pulp, despite the greater fiber content. Furthermore, animals fed PCP increased time spent eating per day but reduced the amount consumed per time ingesting, but did not increase feed intake compared to other treatments. One possibility is the selective behavior due to palatability traits of PCP as described earlier in this chapter. When dry ingredients are mixed in a total mixed ratio, they generally tend to separate into fine, high-density particles at the bottom of the bunk and long, lower density particles on top (LEONARDI; ARMENTANO, 2003). Therefore, ruminants are able to sort the diets utilizing their noses to push away particles and tongues to selectively eat (BEAUCHEMIN, 1991). The total mastication time was decreased by the higher inclusion of concentrate in the diets as a consequence of the decreased rumination and ingestion times. Consequently, the idle time increased compared to 60% concentrate diets.

Table 14 - Feeding behavior of Nellore young bulls fed diets containing either 60 or 80% concentrate

Item[1]	60%	80%	SEM	*P*-value
Rumination, h/d	6.87	5.23	0.7	<0.01
Ingestion, h/d	3.68	3.11	0.18	0.05
TMT, h/d	10.52	8.36	0.24	<0.01
Idle, h/d	13.26	15.47	0.25	<0.01

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). TMT: total mastication time (Rumination + Ingestion), GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
Source: (FERRARI, 2017)

4.3.4 Sorting index

The means of all treatments on sorting index are presented in Table 15. There was no effect of diet on 1.18-8 mm particles ($P > 0.05$) nor CONC×NFC effect on any of the sorting indexes ($P > 0.05$). The averages of the main effects NFC on sorting index are presented in Table 16. The PCP promoted a greater rejection of >19 mm particles compared to diets with GC and SFC ($P = 0.05$, Table 16). Animals fed SFC sorted against <1.18 mm particles ($P = 0.05$) compared to GC and PCP diets, which almost did not sort this particle size (sorting index close to 1). There was a tendency of bulls fed GC to sort for the 1.18-8 mm particle size ($P = 0.07$) compared to SFC and PCP.

Table 15 - Sorting index of Nellore young bulls fed diets containing three sources of non-fibrous carbohydrate and either 60 or 80% concentrate

Item[1]	60% CONC			80% CONC			SEM	P-value[2]		
	GC	SFC	PCP	GC	SFC	PCP		CONC	NFC	CONC×NFC
>19 mm	0.940	0.937	0.925	1.011	1.022	0.957	0.032	0.02	0.05	0.26
8-19 mm	1.001	1.017	1.007	1.044	1.046	1.009	0.013	0.04	0.22	0.26
1.18-8 mm	1.022	1.013	1.011	1.031	1.015	0.994	0.010	0.79	0.07	0.43
<1.18 mm	0.984	0.936	0.974	0.976	0.900	1.030	0.022	0.84	<0.01	0.15

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). A SI of 1 indicates no sorting, a SI < 1 indicates sorting against and >1 sorting for particles retained in each screen (LEONARDI; ARMENTANO, 2003), GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
[2]CONC: concentrate inclusion effect, NFC: non-fibrous carbohydrate effect, CONC×NFC: CONC and NFC interaction effect.
Source: (FERRARI, 2017)

Table 16 - Sorting index of Nellore young bulls fed diets containing three sources of non-fibrous carbohydrate

Item[1]	GC	SFC	PCP	SEM	P-value
>19 mm	0.975[a]	0.980[a]	0.941[b]	0.032	0.05
1.18-8 mm	1.027	1.014	1.003	0.01	0.07
<1.18 mm	0.980[a]	0.918[b]	1.002[a]	0.022	<0.01

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). A SI of 1 indicates no sorting, a SI < 1 indicates sorting against and >1 sorting for particles retained in each screen (LEONARDI; ARMENTANO, 2003), GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
Source: (FERRARI, 2017)

The inclusion of 80% concentrate in the diet caused the animals to sort for particles >19 mm ($P = 0.02$, Table 17) and to sort for particles from 8 to 19 mm ($P = 0.04$) in a greater intensity compared to 60% concentrate diets.

Table 17 - Sorting index of Nellore young bulls fed diets containing either 60 or 80% concentrate

Item[1]	60%	80%	SEM	P-value
>19 mm	0.934[b]	0.997[a]	0.032	0.02
8-19 mm	1.008[b]	1.033[a]	0.013	0.04

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). A SI of 1 indicates no sorting, a SI < 1 indicates sorting against and >1 sorting for particles retained in each screen (LEONARDI; ARMENTANO, 2003).
Source: (FERRARI, 2017)

Feeding different diets to the animals may change fermentation in the rumen and the greater acid production can influence eating behavior and motivate ruminants to sort for longer particles to stimulate motility and balance rumen pH. In the present study, diets with greater inclusion of concentrate caused the animals to sort for larger particles (>8 mm) in a higher intensity. According to Leonardi and Armentano (2003), decreasing the forage level and particle size of the diet motivated cows to sort for coarser particles. These results are supported by Beauchemin and Yang (2005) who evaluated different particle sizes of the corn silage fed to dairy cows and related the preference of the cows for coarser particles to a greater fermentation and short chain fatty acid accumulation of the diets in the rumen. On the other hand, diets with SFC did not promote preference of the bulls for the coarser particles, but promoted rejection of the <1.18 mm particle size, in a greater intensity compared to PCP and GC diets.

The PCP caused the bulls to sort against the >19 mm particles, which can be explained by the palatability, and particle physical form and size, since *citrus* pellets averaged 19 mm of length, in the present study. Moreover, visually, the majority of the feedstuff retained in the two upper screens of the PSPS of the orts was PCP, demonstrating selectivity of the bulls against the pellets. Zebeli et al. (2009) demonstrated that lowering the particle size of the diet modulated the selective consumption of feedstuffs and improved intake of energy and nutrients of dairy cows. If that is the case, grinding of PCP would be expected to increase intake.

4.4 CONCLUSION

The partial replacement of ground corn with pelleted *citrus* pulp decreased the animal performance, carcass weight and dressing, and caused the animals to sort the diet against larger particles. The 80% concentrate inclusion in the diet increased animal performance, carcass weight and dressing compared to 60% concentrate. The steam-flaked corn increased fat deposition, with no impact on animal performance.

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5 LEVELS OF CONCENTRATE AND SOURCES OF NON-FIBROUS CARBOHYDRATES ON RUMEN KINETICS AND PARAMETERS OF NELLORE CATTLE FED FINISHING DIETS

ABSTRACT – The objective of this study was to evaluate the effect of partial replacement of finely ground corn (GC) by steam-flaked corn (SFC) and pelleted *citrus* pulp (PCP) and two concentrate levels (CONC) on dry matter intake, total apparent digestibility of nutrients, rumen kinetics and parameters of Nellore cattle fed finishing diets. Six rumen-cannulated steers, weighing 345.10 ± 14 kg were arranged in 2 non-contemporary Latin squares 6x6 in a 3x2 factorial arrangement of treatments with 3 non-fiber carbohydrates – NFC (SFC and PCP partially replacing GC) and 2 concentrate levels in the diet (60 and 80% DM). The sugarcane silage was used as roughage. The feed intake was measured per animal. The rumen was manually evacuated, the mass and rumen fluid volume were determined. An aliquot of the digesta was removed to determine chemical composition. Another sample was filtered to separate solid and liquid phases. Samples were taken from both phases to determine the size of the rumen compartment (pool) digesta components. Rumen fluid was sampled to determine pH, SCFA and N-NH_3. Feeding 80% concentrate diets decreased NDF intake (P < 0.01), NDF disappearance rate (P < 0.01), NDFd disappearance rate (P < 0.01) and kp of NDF (P < 0.01) compared to 60% concentrate. Substituting GC by PCP decreased DMI (P = 0.05) with no differences to SFC, and increased disappearance rate of NDFd (P < 0.01) compared to the other two carbohydrate sources. There was a NFC × CONC interaction on total tract apparent digestibility of starch (P < 0.01), the GC and the PCP decreased the starch digestibility when fed with 60% concentrate in the diet compared to the other treatments. Feeding 80% concentrate increased total tract digestibility of OM (P < 0.01), and DM (P < 0.01). Feeding PCP increased rumen pH (P <0.01), acetic acid (P < 0.01), and total tract digestibility of OM (P < 0.01), DM (P < 0.01), and total tract digestibility of NDF (P < 0.01) compared to SFC. The PCP also decreased propionic acid (P < 0.01). Partial replacement of GC by SFC increased propionic acid (P < 0,01), decreased acetic acid (P < 0.01) and rumen ammonia concentration (P = 0.03). In conclusion, the 80% concentrate diets decreased intake, and passage rate of NDF, but increased the digestibilities of OM and DM. The *citrus* pulp, decreased the DMI and propionate concentration in the rumen, but increased the acetic acid due to increased disappearance rate of digestible NDF, and digestibilities of DM and OM. The SFC, in turn increased propionate, but decreased ammonia and NDF digestibility.

Keywords: Ammonia nitrogen. Fatty acids. Rumen pH.

5.1 INTRODUCTION

High performance of beef cattle depends on animal ability to consume and extract energy from food. The short chain fatty acids are considered the main source of energy for the ruminants – 50 to 70% of the digestible energy of the feedstuffs, and formed not only from carbohydrate, but also from protein and lipid digestion (KOZLOSKI, 2011). Decision-making at the moment of the formulation of beef cattle diets depends, much more on the degradation profile of the food taking into account the ruminal dynamics, than on its bromatological characteristics alone. Among the factors that affect animal performance are dietary forage, energy levels, ruminal digestibility of food, production of short chain fatty acids.

Despite the lower nutritive value in relation to corn and sorghum silages (ANDRADE et al., 2004), sugarcane presents characteristics of great interest such as high dry matter production per area, ease of cultivation and persistence of the crop, good acceptance by animals; high soluble carbohydrate content and lower production cost (LANDELL et al., 2002; FREITAS et al., 2006). In contrast to other forages, sugarcane does not reduce its nutritive value significantly in the dry season, and is notable for the increase in sucrose content at that time of the year (BORGES; PEREIRA, 2003).

The most common type of corn used in Brazil is the flint corn, differently from the North America that uses predominantly the dent corn. Flint corn contains high concentrations of vitreous endosperm and is more slowly digested in the rumen than corn with higher concentrations of farinaceous endosperm (PHILIPPEAU; MICHALET-DOREAU, 1997). The steam-flaking of corn promotes starch gelatinization, which is the loss of the grain native structure, and the exposure of part of their amylose thus becoming more susceptible to enzymatic degradation (ROONEY; PFLUGFELDER, 1986). However, the increase of rumen starch digestibility can cause a sharp drop in rumen pH, and possibly reduce fiber digestibility, and dry matter intake (VAN SOEST, 1994).

Pelleted *citrus* pulp is a by-product rich in pectin and sugars which are invariably the fastest degradation carbohydrates, while starch and cellulose have very variable degradation depending on the source. Compared to starch, pectin has lower propensity to cause rumen pH drop, due to its fermentation occurring by cellulolytic microorganisms, favoring the production of acetate and not of lactate and propionate. Therefore, pectin becomes an important carbohydrate to be included in high starch diets (VAN SOEST; ROBERTSON; LEWIS 1991). Moreover, Brazil is the greatest orange juice producer in the world therefore, million tons of agro-industrial wastes are generated. An environmentally friendly and economically viable destination for agro-industrial byproducts is their use in animal feed, reducing feed costs of the farm (SILVA; REZENDE; INÁCIO, 2016).

In this sense, it is important to study different sources of non-fibrous carbohydrates and inclusion levels of sugarcane silage on the rumen kinetics of finishing cattle. Our hypothesis is that the steam-flaked corn was going to increase total tract digestibility of starch, and propionate concentration. The pelleted *citrus* pulp, in turn, would improve the rumen environment increasing acetate and fiber digestibility, mainly in diets with greater concentrate inclusion. Therefore, the objective of this study was to quantify the effect of the partial replacement of finely ground corn by steam-flaked corn, and pelleted *citrus* pulp, and two levels of concentrate in the diet of finishing Nellore bulls, on the total tract digestibility of nutrients, rumen pH, ammonia, short chain fatty acids, kinetics of degradation and rumen passage of the fiber.

5.2 MATERIAL AND METHODS

All experimental procedures of this experiment were approved by the Animal Bioethics Committee of the University of São Paulo under the same protocol number (2784310715) of the previous chapter.

5.2.1 Animals and diets

The experiment was realized in 2013 at the same site of the experiment described in the previous chapter. In this experiment, 6 Nellore rumen-cannulated steers, with average initial BW of 345.10 ± 14 kg, 20 months old, were assigned into two balanced non-contemporary 6 × 6 Latin squares. The steers were kept in tie stalls in pens with concrete floor, individual feed trough and water bunk.

The experiment consisted of 6 periods of 14 days each, with 10 days for diet adaptation and the last 4 days for sample collections. The step-up protocol to adaptation of the animals to the diets and experimental diets studied were the same as for the performance experiment already described. Diets were fed at 0800 h, and during the last four days of each period, samples of ingredients and orts were collected and analyzed for DM and nutrient intake calculations. The DM and nutrient intake calculations were made by subtracting the DM and nutrients of orts in the feed bunk from the DM and nutrients of the diet offered.

Samples of dietary ingredients and orts from each pen were dried at 55 ºC for 72 h and ground to pass a 1-mm screen in a Wiley mill (Wiley Mill, Model 4; Arthur H. Thomas, Philadelphia, PA). Samples were then analyzed for DM and ash following the AOAC, 2000 methods 930.15 and 942.05, respectively. Concentration of N was measured by combustion method (Leco model FP-528; LECO Corporation, St. Joseph, MI) and CP content was calculated as the percentage of N in the sample multiplied by 6.25. The NDF content was determined using the method described by Van Soest, Robertson and Lewis (1991) using sodium sulfide and heat stable α-amylase (Sigma A3306; Sigma Chemical Co., St. Louis, MO) in an ANKOM A200 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY). Acid detergent lignin and ADF were analyzed according to Van Soest and Robertson (1985). Starch content was determined by spectrophotometry after enzymatic degradation (Amyloglucosidase AMG 300L, Novozymes) according to Bach Knudsen (1997).

5.2.2 Total tract digestibility

Total tract apparent digestibility of DM, OM, NDF, and starch was determined by spot fecal sample collections. A total of 8 fecal spot samples were collected from each animal with 9 h intervals on d 11, 12, and 13 of each experimental period. In each collection time, approximately 250 g of feces were manually sampled, directly from the rectum, pooled by bull and dried in a forced air oven at 60°C for 48 hours and ground through a 2-mm screen in a Wiley mill. Feces and diets were then analyzed for indigestible NDF (iNDF) as internal marker, to estimate total fecal production. Samples were weighed into nylon Dacron bags (50 μm pore size), placed into a mesh bag and the iNDF was determined as the residual NDF after 288-h *in situ* incubation in a rumen-cannulated steer (HUHTANEN; KAUSTELL; JAAKKOLA, 1994) with four replicates each sample. The steer was fed a diet with forage:concentrate ratio of 60:40, composed by corn silage and the concentrate consisted of ground corn, soybean meal, urea and mineral mixture. After the incubation period samples were removed from rumen and washed with running water and analyzed for NDF using a ANKOM A200 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY). The iNDF was expressed as a percentage of the original NDF content. Additionally, diets and feces were analyzed for DM, OM, NDF and starch as described earlier to calculate the digestibility from nutrient ingestion, fecal excretion and concentration in the feces.

5.2.3 Rumen fiber kinetics

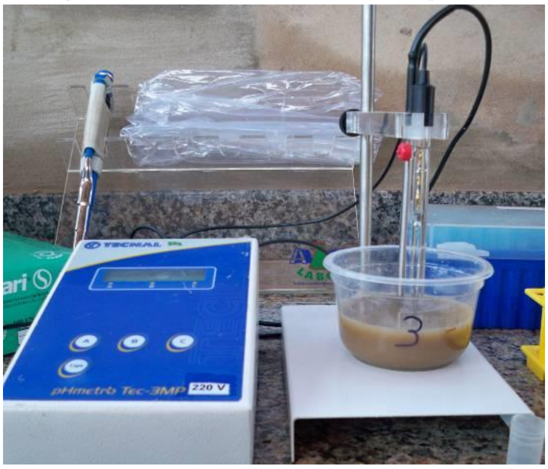
Entire ruminal contents of the steers were manually evacuated through ruminal cannula on d 12 at 1000 h (2 h post-feeding) and on d 13 at 0600 h (2 h before feeding) of each period. During rumen content removal, an aliquot of 10% of the digesta was separated to allow a more accurate and representative sample. The aliquots were filtered through 4 layers of a 1-mm nylon mesh (Albercan Group, Itajubá, Brazil) to determine the solid and fluid digesta weights according to Dado and Allen (1995). Ruminal contents from both phases (solid and liquid) were mixed and sampled for DM and nutrient pool size. The remaining digesta was then returned to the rumen of the same steer. The ruminal fiber kinetics was calculated based on iNDF rumen pool size (DADO; ALLEN, 1995). The NDF disappearance rate was calculated as NDF intake divided by ruminal mass of NDF, and the NDF k_p was calculated dividing iNDF intake per hour, by total mass of ruminal iNDF.

5.2.4 Ruminal fermentation

Ruminal fluid samples were collected at 0 (before feeding), 1, 3, 6, 9, and 12 h after feeding on d 14 of each period. Samples were taken from cranial, ventral, and caudal areas of the rumen via cannula, mixed, and filtered through 4 layers of a 1-mm nylon mesh (Albercan Group, Itajubá, Brazil). Approximately 100 mL of ruminal fluid was divided into two aliquots: one aliquot for measuring rumen pH and another for short chain fatty acids (SCFA) and ruminal ammonia nitrogen ($N\text{-}NH_3$) analysis.

Rumen fluid pH was measured immediately with a portable pH-meter (Tec-3MP, Tecnal, Brazil) as illustrated in Figure 11. After centrifuging the remaining aliquot of rumen fluid at 6,500 × *g* for 15 min, one milliliter of 1 *N* sulfuric acid was added to another 2 mL of the supernatant and frozen at –20°C for ammonia-N determination according to the phenol-hypochlorite method (BRODERICK; KANG, 1980). Another 2-mL subsample of the supernatant was taken, mixed with 0.4 mL of formic acid and frozen at –20°C for SCFA determination.

Figure 11 - Measurement of rumen pH



Source: (FERRARI, 2017)

Rumen SCFA from fluid was measured in the Rumen Fermentability Laboratory of the School of Animal Science and Food Engineering – FZEA, USP Pirassununga by gas chromatography with a capillary column (Stabilwax; Restek, Bellefonte, PA) using the method described by Erwin, Marco and Emery (1961) and adapted by Getachew, Makkar and Becker (2002). Acidified rumen fluid samples were centrifuged at 14,500 × *g* for 10 min. One milliliter of supernatant was transferred into a vial with 100 µL of internal standard (2-ethyl-butyric acid 100 m*M*; Chem Service, West Chester, PA). Concentrations of SCFA were determined using a gas chromatography (GC-2014; Shimadzu, Kyoto, Japan), with split injector and dual flame ionization detector temperatures at 250 °C and column temperature at 145 °C. External standards were prepared with the acetic, propionic, and butyric acids (Chem Service). For calculations of SCFA, the software GCSolution (Shimadzu) was used for separation and integration of chromatographic peaks.

5.2.5 Statistical analysis

The statistical analyses were conducted using the MIXED procedure of the SAS version 9.1.2 for Windows (SAS Institute Cary, NC, USA). Data were analyzed as two non-contemporary 6 × 6 Latin squares design with 6 experimental periods and 6 treatments. Each bull within an experimental period was considered an experimental unit. The model included the fixed effects of concentrate level (CONC: 60 or 80%), of the three NFC sources (NFC: SFC, PCP, and GC), and their interaction (CONC×NFC), as well as the random effect of square, steer within square, and periods. For rumen pH, SCFA, and ammonia, data were analyzed using repeated measures and time × treatments interactions were included as fixed effect in the model.

Denominator degrees of freedom were calculated using the Kenward–Roger approximation. Different error covariance structures were investigated and the one that best fit, according to Bayesian information criterion, was selected. When there was a significant interaction, the effects of treatments were compared using the SLICE option of the MIXED procedure. Treatment effects were considered significant at $P \leq 0.05$. Tendency was considered when probability values were ≤ 0.10.

5.3 RESULTS AND DISCUSSION

5.3.1 Rumen pool size and kinetics

The averages of intake, rumen pool size and fiber kinetics for all treatments are presented in Table 18.

Table 18 - Intake, pool size, nutrient content and rumen kinetics of Nellore steers fed diets containing three sources of non-fibrous carbohydrate and either 60 or 80% concentrate

Item[1]	60% CONC			80% CONC			SEM	*P*-value[2]		
	GC	SFC	PCP	GC	SFC	PCP		CONC	NFC	CONC×NFC
			--------------- Intake, kg/d ---------------							
DMI	10.75	10.25	9.71	10.40	9.38	9.00	0.9	0.09	0.05	0.84
CPI	1.15^{ab}	1.17^{a}	1.16^{a}	1.31^{a}	1.09^{b}	1.08^{b}	0.10	0.80	0.13	0.04
NDFI	3.48	3.34	3.48	2.24	2.03	2.30	0.24	<0.01	0.22	0.84
			------------- Rumen mass, kg -------------							
OM	43.0a	42.8a	39.9b	41.8a	43.7a	44.4a	2.1	0.68	0.49	0.03
DM	5.00	4.95	4.61	4.78	5.10	5.09	0.31	0.73	0.50	0.10
NDF	3.50^{a}	3.37^{ab}	3.04^{b}	3.10^{ab}	3.48^{ab}	3.32^{ab}	0.21	0.99	0.19	0.05
iNDF	2.04	2.06	1.96	1.96	2.11	2.10	0.14	0.85	0.67	0.55
			-------------Fiber kinetics, %/h-------------							
Disap. rate NDF	4.30	4.15	4.90	3.01	2.46	2.91	0.31	<0.01	0.02	0.34
Disap. rate NDFd	4.69	4.67	6.97	3.45	2.47	4.12	0.42	<0.01	<0.01	0.10
Kp of NDF	4.26	4.00	4.00	2.67	2.43	2.26	0.40	<0.01	0.50	0.92

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). CPI: crude protein intake, NDFI: neutral detergent fiber intake, iNDF: indigestible neutral detergent fiber, NDFd: digestible neutral detergent fiber, Kp: passage rate, Disap.: disappearance rate of NDF or NDFd. GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
[2]CONC: concentrate inclusion effect, NFC: non-fibrous carbohydrate effect, CONC×NFC: CONC and NFC interaction effect.
Source: (FERRARI, 2017)

There was a NFC × CONC interaction ($P < 0.05$) on CPI and rumen mass of OM and NDF. The SFC and PCP decreased CPI when fed 80% concentrate, and PCP with 60% concentrate decreased the rumen mass of OM and NDF (Table 18). The means for the parameters affected by main effect of NFC are presented in Table 19. Substituting GC by PCP decreased ($P = 0.05$) DMI with no differences to SFC, and increased ($P < 0.01$) disappearance rate of NDFd compared to the other two carbohydrates sources (Table 19). The PCP also tended to increase disappearance rate of NDF compared to SFC with no differences to GC ($P = 0.07$).

Table 19 - Intake and rumen kinetics of Nellore steers fed diets containing three sources of non-fibrous carbohydrate

Item[1]	GC	SFC	PCP	SEM	*P*-value
DMI, kg/d	10.58^{a}	9.82^{ab}	9.36^{b}	0.81	0.05
Disap. rate NDF, %/h	3.66	3.34	3.91	0.2	0.07
Disap. rate NDFd, %/h	4.07^{b}	3.57^{b}	5.54^{a}	0.3	<0.01

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). Disap.: disappearance rate of NDF or NDFd. GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
Source: (FERRARI, 2017)

The main effects of concentrate inclusion on intake and rumen kinetics are presented in Table 20. Feeding 80% concentrate diets decreased NDF intake ($P < 0.01$, Table 20), NDF disappearance rate ($P < 0.01$), NDFd disappearance rate ($P < 0.01$) and kp of NDF ($P < 0.01$), and tended to decrease DMI ($P = 0.09$), compared to 60% concentrate.

Table 20 - Intake and rumen kinetics of Nellore steers fed diets containing either 60 or 80% concentrate

Item[1]	60%	80%	SEM	*P*-value
DMI	10.24	9.59	0.79	0.09
NDFI	3.43^{a}	2.19^{b}	0.22	<0.01
-------------Fiber kinetics, %/h-------------				
Disap. rate NDF	4.46^{a}	2.81^{b}	0.21	<0.01
Disap. rate NDFd	5.44^{a}	3.35^{b}	0.31	<0.01
Kp of NDF	4.04^{a}	2.57^{b}	0.25	<0.01

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). NDFI: neutral detergent fiber intake, Disap.: disappearance rate of NDF or NDFd. GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
Source: (FERRARI, 2017)

Similarly to the performance trial, the PCP decreased, and the 80% concentrate diets tended to decrease the DM intake for reasons already described in the previous chapter. Moreover, greater concentrate inclusion in the diet decreased NDF intake, which consequently decreased NDF and NDFd disappearance rates and kp of NDF. However, the greater concentrate diets decreased the passage rate, but it did not increase total tract digestibility of fiber, only the OM and DM digestibilities (Table 12). Poore, Moore and Swingle (1990), conducted a trial evaluating the effects of altering dietary concentrates levels, either 30, 60 or 90% of a diet based on flaked sorghum, wheat straw and hay on fiber digestion. Similarly to the present study, the total tract digestibility of NDF was not altered by the concentrate levels. In their trial however, rumen passage rate of fiber was not altered when concentrate was increased from 30 to 60%, but it decreased by 20% when concentrates were increased to 90%. Probably, in the present study, the passage rate of animals fed 80% concentrate was decreased due to metabolic satiety decreasing appetite and intake, once the lower concentrate diets increased intake demonstrating no rumen fill. Despite the PCP did not alter the NDF intake, it increased the disappearance rate of NDFd and tended to increase the disappearance rate of NDF compared to the other energy sources. This fact can be explained by the greater digestibility of fiber when animals were fed *citrus* pulp. The bacteria that degrade low-digestibility fiber, usually increases the lag time, which negatively reflects on the disappearance rate (VAN SOEST, 1994). Therefore, in the present study, the highly-digestible fiber present in the *citrus* pulp increased the disappearance rates, and also probably due to shorter lag time.

5.3.2 Rumen parameters and digestibility

The averages of all treatments for rumen parameters and digestibility are presented in Table 21. There was a NFC × CONC interaction on total tract apparent digestibility of starch ($P < 0.01$, Table 21), the GC and the PCP decreased the starch digestibility when fed with 60% concentrate in the diet compared to the other treatments. Feeding PCP increased rumen pH ($P < 0.01$), acetic acid ($P < 0.01$), and total tract digestibility of OM ($P <0.01$), and DM ($P <0.01$), and increased total tract digestibility of NDF ($P < 0.01$; Table 22) compared to GC and SFC diets. Feeding PCP also decreased propionic acid ($P < 0.01$) and, consequently, increased acetate to propionate (A:P) ratio ($P < 0.01$) compared to GC and SFC diets (Table 22). The by SFC increased propionic acid ($P < 0.01$), decreased acetic acid ($P < 0.01$) and consequently decreased A:P ratio ($P < 0.01$), and rumen ammonia concentration ($P = 0.03$; Table 22) compared to GC diets. Total SCFA concentration and indigestible NDF mass of rumen were not influenced by any treatment ($P > 0.05$).

Table 21- Rumen parameters and total tract digestibility of Nellore steers fed diets containing three sources of non-fibrous carbohydrate and either 60 or 80% concentrate

Item[1]	60% CONC			80% CONC			SEM	P-value[2]		
	GC	SFC	PCP	GC	SFC	PCP		CONC	NFC	CONC×NFC
Ruminal pH	6.43	6.32	6.74	6.25	6.41	6.62	0.08	0.45	<0.01	0.15
Ammonia, mg/dL	13.4	12.0	13.0	17.4	10.1	15.9	2.2	0.35	0.03	0.16
Total SCFA, m*M*	92	99	97	101	99	103	5	0.43	0.85	0.73
--------- Percent of total SCFA --------										
Acetic acid	60.9	59.1	64.9	61.1	58.2	66.3	2.1	0.82	<0.01	0.55
Propionic acid	22.4	23.3	18.8	21.9	25.1	18.1	1.1	0.80	<0.01	0.38
Butyric acid	12.4	12.2	12.8	11.5	11.0	12.6	1.0	0.19	0.26	0.76
A:P ratio	2.76	2.61	3.52	2.84	2.32	3.79	0.21	0.69	<0.01	0.31
------ Total tract digestibility, % ------										
OM	60.5	62.6	68.4	71.9	62.9	75.7	4.0	<0.01	<0.01	0.13
DM	57.9	59.9	66.9	70.0	60.4	74.6	4.1	<0.01	<0.01	0.13
NDF	32.3	31.8	45.7	38.9	16.7	48.3	6.3	0.66	<0.01	0.14
Starch	82.5[c]	95.3[a]	91.0[b]	92.4[a]	95.5[a]	94.0[ab]	1.8	<0.01	<0.01	<0.01

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). SCFA: short chain fatty acid, A:P ratio: acetate:propionate ratio, NDF: neutral detergent fiber. GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
[2]CONC: concentrate inclusion effect, NFC: non-fibrous carbohydrate effect, CONC×NFC: CONC and NFC interaction effect.
Source: (FERRARI, 2017).

Table 22 - Rumen parameters and total tract digestibility of Nellore steers fed diets containing three sources of non-fibrous carbohydrate

Item[1]	GC	SFC	PCP	SEM	P-value
Ruminal pH	6.34[b]	6.36[b]	6.68[a]	0.06	<0.01
Ammonia, mg/dL	15.37[a]	11.08[b]	14.44[a]	1.62	0.03
--------- Percent of total SCFA --------					
Acetic acid	60.99[b]	58.66[c]	65.60[a]	1.79	<0.01
Propionic acid	22.14[b]	24.19[a]	18.42[c]	0.83	<0.01
A:P ratio	2.80[b]	2.51[b]	3.65[a]	0.19	<0.01
------ Total tract digestibility, % ------					
OM	66.18[b]	62.81[b]	72.04[a]	3.53	<0.01
DM	63.94[b]	60.22[b]	70.79[a]	3.77	<0.01
NDF	35.61[b]	24.22[c]	47.00[a]	4.78	<0.01

[1]Within a row, means without a common superscript differ ($P \leq 0.05$). A:P ratio: acetate:propionate ratio, NDF: neutral detergent fiber. GC: ground corn, SFC: steam-flaked corn, PCP: pelleted *citrus* pulp.
Source: (FERRARI, 2017).

Feeding 80% concentrate increased total tract digestibility of OM ($P < 0.01$), and DM ($P < 0.01$) compared to 60% concentrate (Table 23).

Table 23 - Total tract digestibility of Nellore steers fed diets containing either 60 or 80% concentrate

Item[1]	60%	80%	SEM	*P*-value
OM	63.86[b]	70.15[a]	3.36	<0.01
DM	61.62[b]	68.36[a]	3.59	<0.01

[1]Within a row, means without a common superscript differ ($P \leq 0.05$).
Source: (FERRARI, 2017).

The average rumen pH of cattle fed high-grain diets is usually between 5.6 and 6.2 (SCHWARTZKOPF-GENSWEIN et al., 2003). However, multiple feed factors can influence the pH and its variation along the day such as rate of ruminal feed digestion, forage-to-concentrate ratio, source of grain, and extent of grain processing as well as size, number, and frequency of meals. In this sense, comparing the mean pH values of the present trial it is possible to conclude that the sugarcane silage fed was very effective in buffering the rapidly-fermenting carbohydrates and maintaining pH values above the ones reported in the literature.

In our trial, PCP increased rumen pH, acetic acid, and the A:P ratio, regardless of the concentrate level. *Citrus* pulp is a high pectin source, hence, when included in substitution of rapidly fermentable starchy feeds, the negative effect of high-energy diets on rumen pH and fiber digestibility can be, at least partially, avoided (BAMPIDIS; ROBINSON, 2006). Moreover, the pectin has high cation exchange capacity, causing the plant to attract and bind hydrogen ions. Therefore, when these ions are bound rather than free in the liquid phase, the less acidic is the rumen (MCBURNEY; VAN SOEST; CHASE, 1983). Likewise, Santos et al. (2001) reported increased rumen acetate when lactating cows were fed with PCP, and decreased acetate when fed with SFC. Moreover, PCP increased Disappearance rate of NDFd, total tract digestibilities of DM, and OM compared to the other treatments, and also increased digestibility of NDF compared to SFC, probably due to pectin traits. Pectin is an amorphous substance, which is a neutral detergent soluble fiber (NRC, 2016), and of rapid and extensive degradation by ruminal microorganisms (VAN SOEST, 1994). These results are supported by previous research (BAMPIDIS; ROBINSON, 2006) that showed a linear increase in apparent digestibility of NDF as levels of PCP increased in the diet. The greater NDF digestibility may be explained by the different pattern of pectin fermentation which, although rapidly fermented in the rumen, unlike starch, yields little lactate and more acetic acid, causing less of a decline in rumen pH (STROBEL; RUSSELL, 1986) and better conditions for fiber fermentation (BUENO et al., 2002). Also, PCP contains a readily digestible NDF fraction, contrary to the slowly degrading sugarcane NDF (MIRON; YOSEF; BEN-GHEDALIA, 2001). Additionally, the lower DMI when steers were fed PCP may have contributed to a higher NDF total tract digestibility.

As seen in the present study, steam-flaking of corn increased propionate concentration and decreased acetate. The same result was obtained by Simas et al. (2008) in a dairy cattle study comparing SFC with finely- or coarsely-ground corn. However, greater processing of grains can negatively impact on fiber digestion, as seen in the present study compared to PCP diets. According to Russell and Wilson (1996), the efficiency of microbial protein synthesis is negatively affected when ruminal pH is below 6.2, which can decrease fiber digestibility. Our findings, however, shows no negative effect of 80% concentrate inclusion, nor an effect of SFC on rumen pH, but a decrease on total tract NDF digestibility when SFC was included in the diet compared to PCP.

The steam-flaking of corn can also influence protein metabolism. In the present study, replacing GC with SFC decreased rumen ammonia concentration. Ruminal microorganisms can utilize ammonia and, when the utilization rate exceeds the production rate, ammonia concentration in rumen decreases (RUSSELL et al., 1992). Crocker et al. (1998) evaluated levels of replacement of dry-rolled corn with SFC and reported decreased concentrations of ruminal ammonia N and a linear increase in total tract digestibility of starch as inclusion of steam-flaked corn increased. Therefore, the rate of starch digestion may influence the ammonia utilization by the microbes, which likely explains the lower ammonia concentration in the rumen when SFC replaced GC in the present study, and suggests that there was greater microbial protein synthesis when GC was replaced with SFC. The greater concentrate inclusion in the diets increased not only the OM, but also the DM digestibilities compared to 60% concentrate diets. This fact can be explained by the lower passage rate, which increases the mean retention time of the digesta in the rumen allowing the rumen content to be fermented for longer period of time, thus increasing the digestibility.

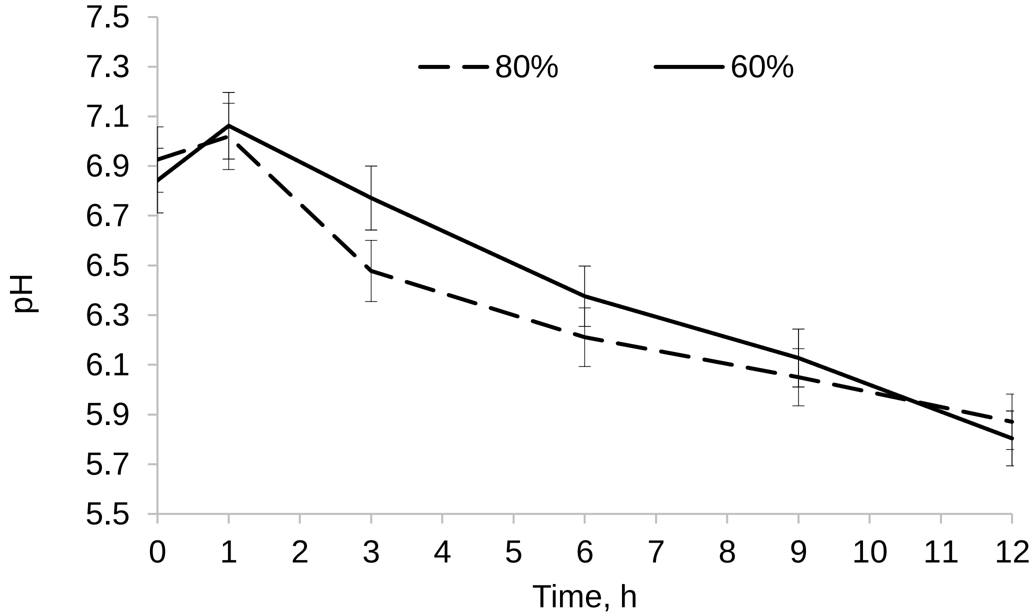
Substituting GC by SFC increased total tract digestibility of starch when animals were fed diets containing 60% concentrate compared to the other carbohydrate sources, in the present study. Starch granules are encapsulated in a protein matrix that acts as a primary barrier to starch digestibility. Steam-flaking process consists of the moisture uptake by starch granule and the disruption of the protein matrix (by shear forces on hot grain during flaking), optimizing starch digestion and increasing the feeding value of corn above that of whole or dry-rolled corn (ZINN; OWENS; WARE, 2002). In the study of Santos et al. (2001), when steam-flaked corn diets were fed, the total tract digestibility of starch were increased by 30.3%, respectively, when compared to diets containing coarsely ground corn. In the present study however, GC and PCP offered with 60% concentrate in the diet, decreased the starch digestion compared to SFC and the 80% concentrate diets. This fact can probably be explained by the greater intake of the lower concentrate inclusion, processing method and availability of starch granules compared to SFC.

For the measures taken over time, there was no interaction Time × NFC (P = 0.32), Time × NFC × CONC (P = 0.59), on rumen pH, but there was an interaction of Time × CONC (P = 0.01, Figure 12), where 60% concentrate in the diets increased rumen pH on time 3 h post feeding. There was no interaction of Time × NFC (P = 0.96), Time × CONC (P = 0.27), nor Time × NFC × CONC (P = 0.92), on Total SCFA. There was no interaction Time × CONC (P = 0.18), nor Time × NFC × CONC (P = 0.17), but there was an interaction of Time × NFC (P < 0.01, Figure 13), on Acetic acid in the rumen, when PCP increased this parameter on times 0, 1, 6, 9 and 12 relative to SFC and GC. There was no interaction of Time × CONC (P = 0.98), nor Time × NFC × CONC (P = 0.82), but there was a significant interaction of Time × NFC (P = 0.02, Figure 14) on Propionic acid in the rumen, where PCP decreased this parameter compared to GC and SFC in all times measured. There was no interaction of Time × NFC (P = 0.25), Time × NFC × CONC (P = 0.13), but an effect of Time × CONC (P = 0.01, Figure 15) on Butyric acid. There was no interaction Time × CONC (P = 0.18), Time × NFC × CONC (P = 0.29) on Acetic : Propionic ratio in the rumen, but there was an interaction of Time × NFC (P < 0.01, Figure 16), where PCP increased this parameter in all times measured compared to GC and SFC. There was no interaction of Time × NFC (P = 0.19), Time × CONC (P = 0.24), Time × NFC × CONC (P = 0.94), on ammonia concentration in the rumen.

Despite the total SCFA did not differ among treatments, and propionic acid only increased for SFC diets regardless the concentrate inclusion, the rumen pH differed only on time 3 h post-feeding when 80% concentrate was included in the diets. The explanation for that is probably due to the peak of fermentation of diets with 80% of concentrate to lactate, decreasing the pH shaper than diets with greater forage inclusion in that time point. Lactate is a product of fermentation that can be metabolized to either acetate, propionate, or butyrate. However, if the production of lactate exceeds the rate of fermentation, it can accumulate in the rumen (NAGARAJA, 2016).

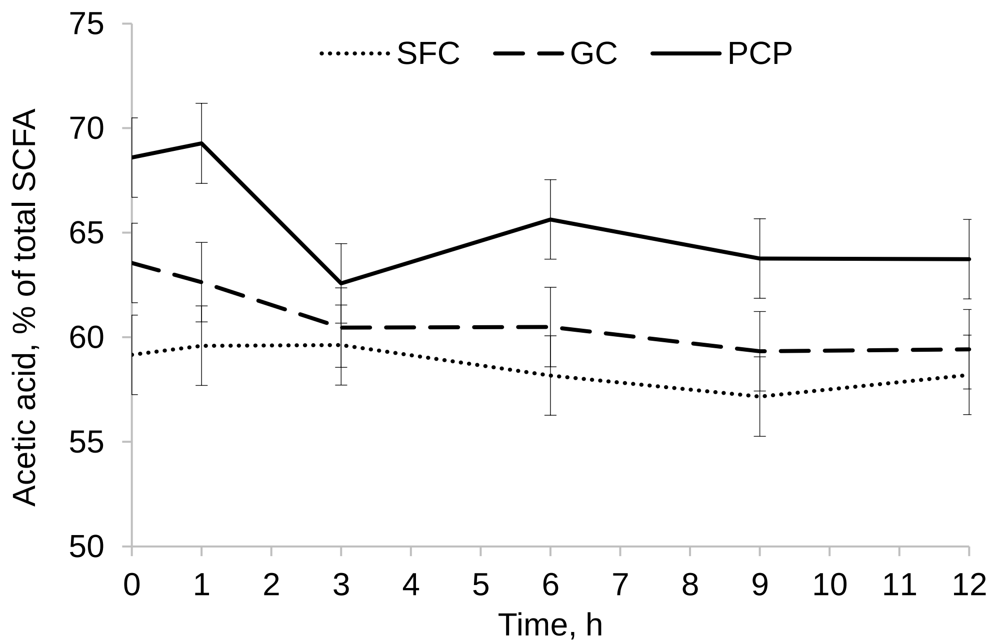
Interestingly, the 80% concentrate decreased the butyric acid concentration also after 3 h post-feeding. One possible explanation for that is the greater production of butyrate from acetate by the bacteria *Rosenburia Faecalis*. This bacteria prosses the enzymes acetate CoA transferase and acetate kinase activities, that are capable of producing butyrate from acetate (DUNCAN et al., 2002). The lower concentrate inclusion (greater forage level), may not have increased the acetate concentration in the rumen probably due to the increased conversion of this substrate to butyrate.

Figure 12 - Rumen pH relative to time of diets containing Steam flaked corn (SFC), Ground Corn (GC), Pelleted *citrus* pulp (PCP). The interaction of non-fiber carbohydrate × time of sampling was significant (P = 0.01)



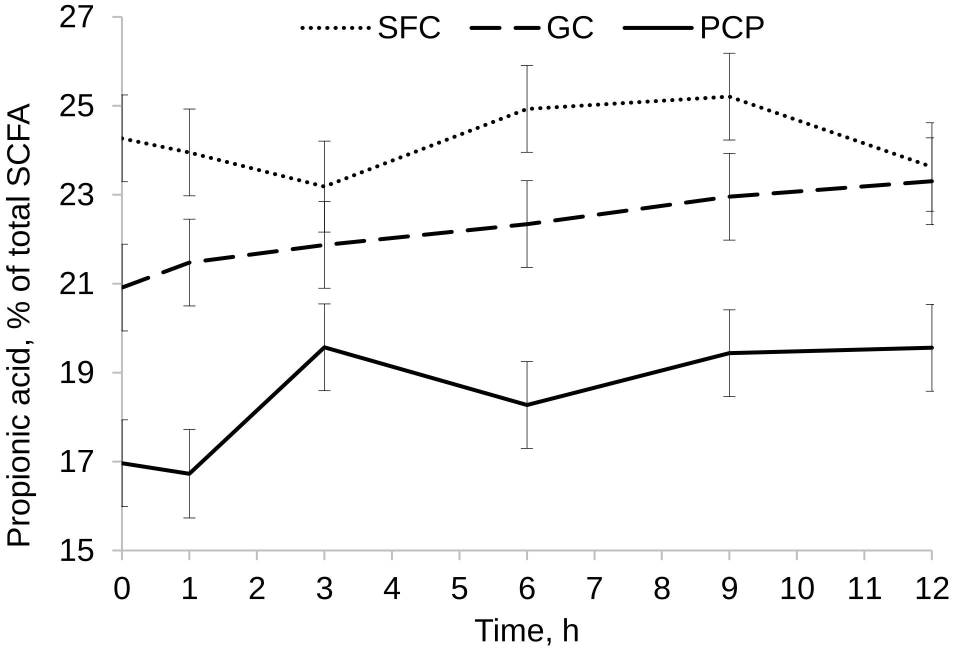
Source: (FERRARI, 2017).

Figure 13 - Proportion of Acetic acid relative to time post-feeding of diets containing Steam-flaked corn (SFC), Ground Corn (GC), Pelleted *citrus* pulp (PCP). Time × NFC interaction, P < 0.01



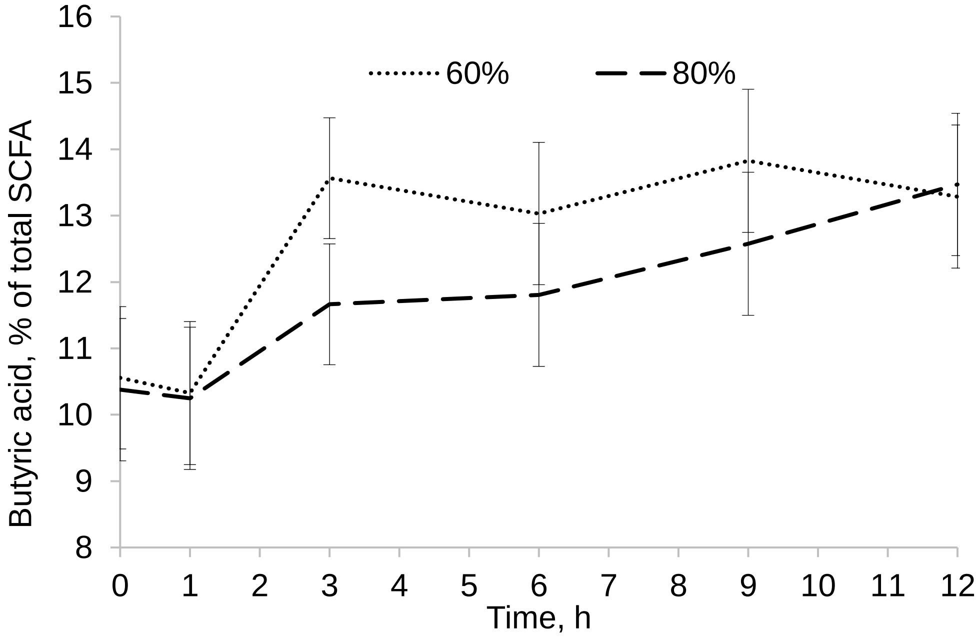
Source: (FERRARI, 2017).

Figure 14 - Proportion of Propionic acid relative to time post-feeding of diets containing Steam-flaked corn (SFC), Ground Corn (GC), Pelleted *citrus* pulp (PCP). Time × NFC interaction, P = 0.02



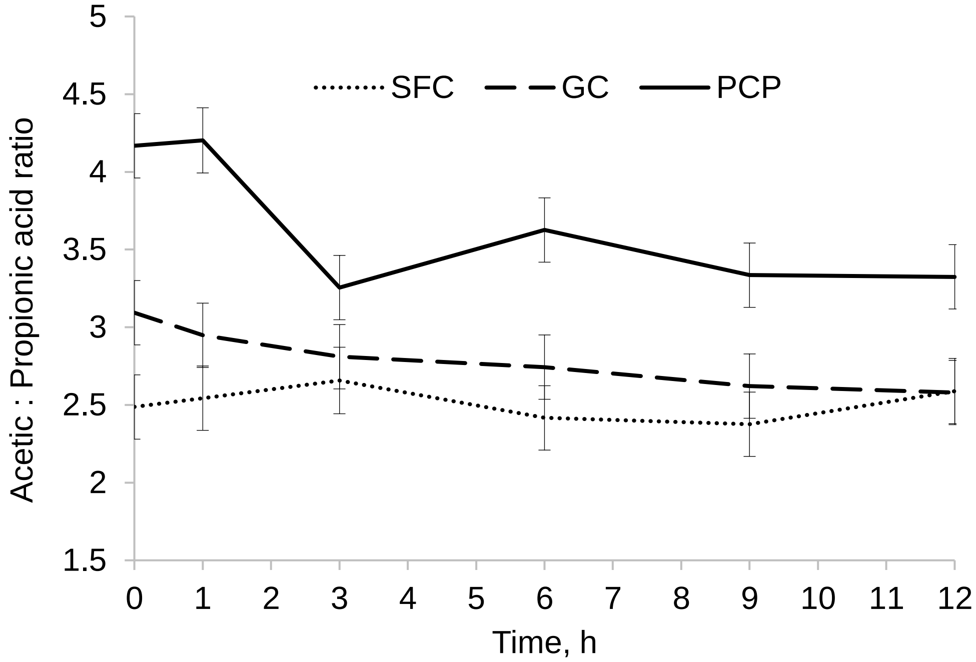
Source: (FERRARI, 2017).

Figure 15 - Proportion of Butyric acid relative to time post-feeding of diets containing Steam-flaked corn (SFC), Ground Corn (GC), Pelleted *citrus* pulp (PCP). Time × NFC interaction, P = 0.01



Source: (FERRARI, 2017).

Figure 16 - Acetic : Propionic acid ratio to time post-feeding of diets containing Steam-flaked corn (SFC), Ground Corn (GC), Pelleted *citrus* pulp (PCP). Time × NFC interaction, $P < 0.01$



Source: (FERRARI, 2017).

5.4 CONCLUSION

In conclusion, the 80% concentrate diets decreased intake, and passage rate of NDF, but increased the total tract digestibilities of OM and DM. The *citrus* pulp also decreased intake, but increased the rumen pH and acetic acid because of greater disappearance rate of fiber. The steam-flaked corn in turn, increased propionate, but decreased ammonia compared to the other diets and fiber digestibility compared to the pelleted *citrus* pulp.

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