SÃO PAULO STATE UNIVERSITY "JÚLIO DE MESQUITA FILHO"
SCHOOL OF AGRICULTURAL AND VETERINARY SCIENCES
JABOTICABAL CAMPUS

UTILIZATION OF RECONSTITUTED CORN GRAIN SILAGE IN SUPPLEMENTS FOR FINISHING BEEF CATTLE ON PASTURE: EVALUATION OF SUPPLEMENT AEROBIC STABILITY AND CATTLE PERFORMANCE

Saulo Teixeira Rodrigues de Almeida
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2024

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AUTHOR'S CURRICULAR DATA

Saulo Teixeira Rodrigues de Almeida was born on June 8, 1996, in Belo Horizonte, MG, Brazil. He is the son of Laura Stella Teixeira and José Carlos Rodrigues de Almeida. He graduated in Animal Science from the Federal University of Lavras (UFLA, 2015 – 2021). During his undergrad, he was a member of the Brazilian Forage Team (NEFOR) (2017 – 2021), teaching assistant of "Analytical Chemistry" and "Biostatistics" courses and received a scientific initiation scholarship (FAPEMIG). He spent one year (2019 -2020) at The University of Maine, USA, completing an internship in feed conservation and ruminant nutrition. Saulo was awarded as the best student in the Animal Science Program. In 2022, he began his master's at Unesp Jaboticabal and APTA Colina and received a FAPESP scholarship. In 2023, he spent 5 months at The University of Adelaide, Australia, as a visiting researcher, conducting research on barley processing and degradability.

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SUMARY

CERTIFICADO Nº 005/2022 – CEUA

Certificamos que o projeto apresentado dia 18 de novembro de 2022 intitulado "U**TILIZAÇÃO DE SILAGEM DE GRÃOS DE MILHO RECONSTITUÍDO EM SUPLEMENTOS PARA BOVINOS DE CORTE EM TERMINAÇÃO A PASTO: AVALIAÇÃO DA ESTABILIDADE AERÓBIA DO SUPLEMENTO E DO DESEMPENHO E METABOLISMO DOS ANIMAIS"**, foi registrado com o **protocolo nº 005/2022,** está sob a responsabilidade do Pesquisador Científico **Dr. Gustavo Rezende Siqueira** e envolve a produção, a manutenção e a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica e encontra-se de acordo com os preceitos da lei nº 11.794, de oito de outubro de 2008; do decreto nº 6.899, de 15 de julho de 2009; e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), sendo então **APROVADO** pela **Comissão de Ética no Uso de Animais da Apta Regional**, na X Reunião Extraordinária realizada no dia **30 de novembro de 2022**.

Finalidade: (X) Pesquisa científica – ( ) Ensino	
Vigência da autorização	01/12/2022 a 31/12/2023
Espécie/linhagem e/ou raça	Bovino Nelore
Nº de animais	960
Sexo/Idade/Peso aprox.	960 M e 24 meses / 380 Kg
Localização/Origem	Unidade Regional de Pesquisa e Desenvolvimento de Colina
Responsável Técnico	Dr. Gustavo Rezende Siqueira

Maria Izabel Merino de Medeiros
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RECONSTITUTED CORN GRAIN SILAGE UTILIZATION IN SUPPLEMENTS FOR FINISHING BEEF CATLE ON PASTURE: EVALUATION OF SUPPLEMENT AEROBIC STABILITY AND CATTLE PERFORMANCE

ABSTRACT – Two experiments were conducted aiming to: 1) evaluate the combination of *Lactobacillus buchneri* + *Lactobacillus hilgardii* and *Lactobacillus buchneri* + *Lactobacillus plantarum* in the ensiling of reconstituted corn grains and their effects on silage fermentation, nutritional value, and aerobic stability of the silage and the supplement made with these silages; 2) to determine the impacts of providing fresh and exposed reconstituted corn grain silage supplements on the intake and performance of pasture-finished Nellore cattle with high supplementation. Experiment one evaluated reconstituted corn grain silages non-inoculated and inoculated with *L. buchneri* + *L. hilgardii* both in concentration of 1.5 $x10^5$ cfu/g and *L. buchneri* + *L. plantarum at* 2 $x10^5$ cfu/g and 1 $x10^5$ cfu/g respectively, stored for 60 days and supplements for beef cattle using these silages. Inoculated silages presented a higher number of lactic acid bacteria, pH and aerobic stability ($P < 0.01$) and lower number of yeast ($P < 0.01$) in comparison to non-inoculated and did not differ between both inoculated ($P > 0.01$). Treated supplements had higher aerobic stability and lower yeast and mold counts than control supplement and did not differ between them. In experiment 2, 70% of dry rolled corn was substituted to reconstituted corn grain silage. Cattle consuming reconstituted corn grain silage supplement until 6 days of aerobic exposure had higher average daily gain and final body weight ($P \leq 0.06$) tended to consume less supplement ($P \leq 0.09$) and were more efficient in putting weight per kg of dry matter ingested ($P \leq 0.04$). Aerobic exposition had no linear effect and did not impact cattle performance in comparison to fresh supplement. Inoculation with *L. buchneri* + *L. hilgardii* or *L. buchneri* + *L. plantarum* guarantee aerobic stability for 9 days in silage and for 6 days in supplement under laboratory conditions. Cattle consuming reconstituted corn grain silage supplement exposed until 6 days had better performance than cattle consuming dry corn supplement.

Keywords: finishing beef cattle, high supplementation on pasture, reconstituted corn grain silage

UTILIZAÇÃO DE SILAGEM DE GRÃOS DE MILHO RECONSTITUÍDOS EM SUPLEMENTOS PARA BOVINOS DE CORTE EM TERMINAÇÃO A PASTO: AVALIAÇÃO DA ESTABILIDADE AERÓBICA DO SUPLEMENTO E DO DESEMPENHO DOS BOVINOS

RESUMO – Foram conduzidos dois experimentos com o objetivo de: 1) avaliar a combinação de *Lactobacillus buchneri* + *Lactobacillus hilgardii e Lactobacillus buchneri* + *Lactobacillus plantarum* na ensilagem de grãos de milho reconstituídos e seus efeitos na fermentação, valor nutricional e estabilidade aeróbica da silagem e do suplemento feito com essas silagens; 2) determinar os impactos da oferta de suplementos de grãos de milho reconstituídos frescos e expostos sobre o consumo e desempenho de bovinos Nelore em terminação a pasto com alta suplementação. O experimento um avaliou silagens de grãos de milho reconstituídos não inoculadas e inoculadas com *L. buchneri* + *L. hilgardii ambas na concentração de* 1,5 x 10^5 ufc/g *e L. buchneri (2* x 10^5 ufc/g) + *L. plantarum (*1 x 10^5 ufc/g) armazenadas por 60 dias, e suplementos para bovinos utilizando essas silagens. As silagens inoculadas apresentaram maior número de bactérias ácido lácticas, pH e estabilidade aeróbia ($P < 0,01$) e menor número de leveduras ($P < 0,01$) em comparação a silagem não inoculada, não diferindo entre silagens inoculadas ($P > 0,01$). O suplemento com silagem inoculada apresentou maior estabilidade aeróbia e menor contagem de leveduras e fungos do que o suplemento controle, e não diferiu entre eles. No experimento 2, 70% de milho seco moído foi substituído por silagem de grãos de milho reconstituídos. Os bovinos consumindo suplemento de silagem de grãos de milho reconstituídos por até 6 dias de exposição aeróbia apresentaram maior ganho médio diário e peso corporal final ($P \leq 0,05$), tenderam a consumir menos suplemento ($P \leq 0,09$) e foram mais eficientes em ganhar peso por kg de matéria seca ingerida. A exposição aeróbica não teve efeito linear e não impactou o desempenho dos bovinos em comparação com o suplemento fresco. A inoculação com *L. buchneri* + *L. hilgardii ou L. buchneri* + *L. plantarum* garante estabilidade aeróbica da silagem por pelo menos 9 dias e por 6 dias no suplemento em condições de laboratório. Os bovinos consumindo suplemento de silagem de grãos de milho reconstituídos expostos por até 6 dias tiveram melhor desempenho do que os bovinos consumindo suplemento com milho moído.

Palavras-chave: bovinos de corte, alta suplementação a pasto, silagem de grãos de milho reconstituídos

CHAPTER 1 – GENERAL CONSIDERATIONS

1. Introduction

Brazil was the second biggest bovine meat producer with 10.8 million tons of equivalent carcass in 2022 and the first in exportation (3.02 million tons). To achieve this number, 42.3 million animals were slaughtered and 81.8% came from pasture yielding 297 kg of carcass on average (ABIEC, 2023). Tropical pastures rarely provide the necessary nutrients to achieve high gains and fat deposition in bovines in the fattening phase (REIS et al., 2011). Feedlot fattening during this phase emerges as an alternative for producers to attain satisfactory results in weight gain in a short time, however, properties with feedlot facilities are scarce and the high investment necessary to build them makes it unfeasible for many producers. Thus, fattening with high supplementation on pasture becomes a viable option.

The main energy resource used in Brazil to fatten beef cattle is corn. In the last 2 years, corn prices have varied a lot, and the exchange ratio of carcass:corn sack hit 3.42 sacks in 2022 (CEPEA, 2024). An alternative to protect against these low rates periods is improving the nutrient availability to the animal. Reconstituted corn grain silage (RCGS) increases starch degradability, thereby improving animal feed efficiency (FERRARETTO; CRUMP; SHAVER, 2013; HOFFMAN et al., 2011).

In a general way, supplements utilized in the finishing phase are prepared in advance to be provided throughout the following days. The use of RCGS in this type of supplement is a challenge due to the high susceptibility of aerobic spoilage in comparison with the dry ingredients traditionally used. Thus, to use RCGS in supplements, it is necessary to guarantee the aerobic stability (AS) of the supplement during the whole time before use, avoiding losses in sanitary and nutritional quality, and, consequently, reducing of animal consumption and performance (HOFFMAN; OCHER, 1997; WHITLOCK et al., 2000).

One way to guarantee AS is by inoculating obligatory heterofermentative bacteria during ensilage. This type of bacteria will produce mainly weak acids (e.g., acetic and propionic acids) that have antifungal action, inhibiting yeasts and molds (OUDE ELFERINK et al., 2001). Among the species in this group, *L. buchneri* is

recognized as an extensively studied bacteria and established as an effective silage inoculant, while L. hilgardii, though recently investigated as a silage inoculant and showing promising results, has not yet been fully established.(DA SILVA et al., 2021; DROUIN; TREMBLAY; CHAUCHEYRAS-DURAND, 2019). With this, there is the possibility of using wet ingredients to guarantee a more efficient and profitable supplementation strategy in high-supplementation grass-fed cattle finishing systems.

2. Literature review

2.1. Corn grain physiology

The high grain yield and energy content of corn grain are among the primary reasons it is widely used in animal nutrition. Corn grain can be divided into three main parts: the pericarp (5~7% of the grain) is a fibrous layer covering the grain; germen (11~12% of the grain) is the protein and fat proportion of the grain responsible for starting germination; and the endosperm (80~85% of the grain) responsible for accumulating the grain energy reserves that will be used in the germination process (GARCÍA-LARA; CHUCK-HERNANDEZ; SERNA-SALDIVAR, 2019) (Image 1).

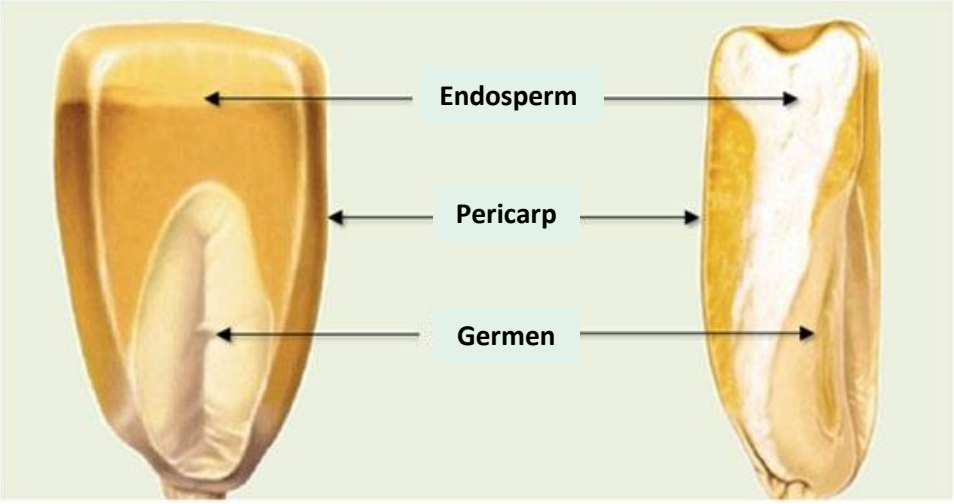

Figure 1: Basic composition of corn grain. Available on: https://www.milkpoint.com.br/colunas/thiago-fernandes-bernardes/conhecendo-e-escolhendo-hibridos-de-milho-para-silagem-80791n.aspx?acao=8f27852b-6ad2-4fc8-a673-01c24aae0ac8

Corn grain stores energy in starch form (70% of dry matter (DM) content). The main varieties of corn planted worldwide for animal nutrition propose are classified as flint or dent corn, according to the percentage of vitreous texture endosperm. Dent corn

has a predominantly white and opaque floury endosperm (51.8% - 80%) (CORREA et al., 2002; ZHANG; XU, 2019) which means most of the starch granules are disposed as smaller spheres with a discontinuous and disorganized protein matrix in the endosperm interspace (GAYRAL et al., 2016; ZHANG; GAO; DONG, 2011). Flint corn has an organized and compacted protein matrix that covers and strongly links with larger polygonal starch granules in the majority part of the endosperm (~73%) (ZHANG; GAO; DONG, 2011) conferring a yellow and translucent apparence and making the grain harder and denser, hindering the access of water and microbes to the starch granules (Image 2). A greater proportion of vitreous endosperm also leads to higher electricity use during the processing of the grain.

The vitreous endosperm has a higher concentration of amylose than the floury endosperm (ZHANG; XU, 2019) which is associated with higher retrogradation after the gelatinization process. The flat organization of glucose in amylose allows it to go into amylopectin forming hydrogen bonds within the starch molecule impacting enzyme activity and ability to expand and this may make it impossible to return to the original structure after retrogradation, thus decreasing degradability (ROONEY; PFLUGFELDER, 1986).

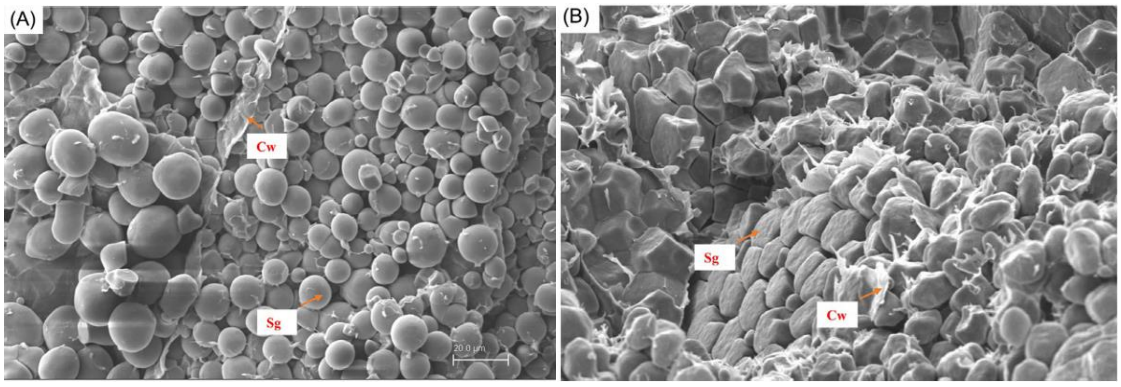

Figure 2: Scanning electron micrograph of fracture yellow dent corn endosperm, showing round (A), thigh-packed starch granules (B), cell walls (Cw), and starch granules (Sg) (x1500). Available at García-Lara et al., (2019).

Flint corn has been predominantly used in Brazil (CORREA et al., 2002) due to better adaptability to tropical weather and higher resistance to insect attack during the storage period. The organizational structure of starch granules in vitreous endosperm makes more difficult its degradation by microorganisms in the rumen. The predominant

protein (~75% among starch granules) in the corn endosperm is called zein, which is part of the prolamin group, followed by glutelin (~14%) (GAYRAL et al., 2016). Philippeau et al., (2000) described the vitreous endosperm as starch granules surrounded by protein storage bodies (zeins) in a protein matrix (glutelin) embedded in a dense matrix of endosperm cells (Image 3). The better organization of starch granules with protein filled in the interspace gives more density to *flint* grains (PEREIRA et al., 2008). Zein is divided in α, β, γ and δ subclasses. The vitreous endosperm has a higher concentration of protein than floury endosperm, principally due to a higher concentration of α-zein. (α, β, δ)-Zeins are positively correlated with grain vitreousness and negatively correlated with ruminal degradability (PEREIRA et al., 2008; PHILIPPEAU; LANDRY; MICHALET-DOREAU, 2000).

When the zein barrier is covering starch granules, rumen microorganisms will have to hydrolyze the prolamin first to then have access to the starch. This process costs time and depending on the grain processing degree, starch will not be available and will go towards omasum. PHILIPPEAU; LANDRY; MICHALET-DOREAU, (2000) found greater concentration of rumen slowly degradable starch in flint corn when compared to dent corn (80.7 vs 73.4% of total starch). CORREA et al., (2002); PEREIRA et al., (2004) observed that starch degradability in vitreous corn decreased as grain maturity increased even after the black line point, directly affecting the nutritional value of the grain, probably because of increasing cross-linking between zeins. With advancing maturity, β and α zeins cross-link, and γ and δ zeins penetrate their net encapsulating starch granules into a hydrophobic starch-protein matrix (BUCHANAN; GRUISSEM; JONES, 2000; HOFFMAN et al., 2011).

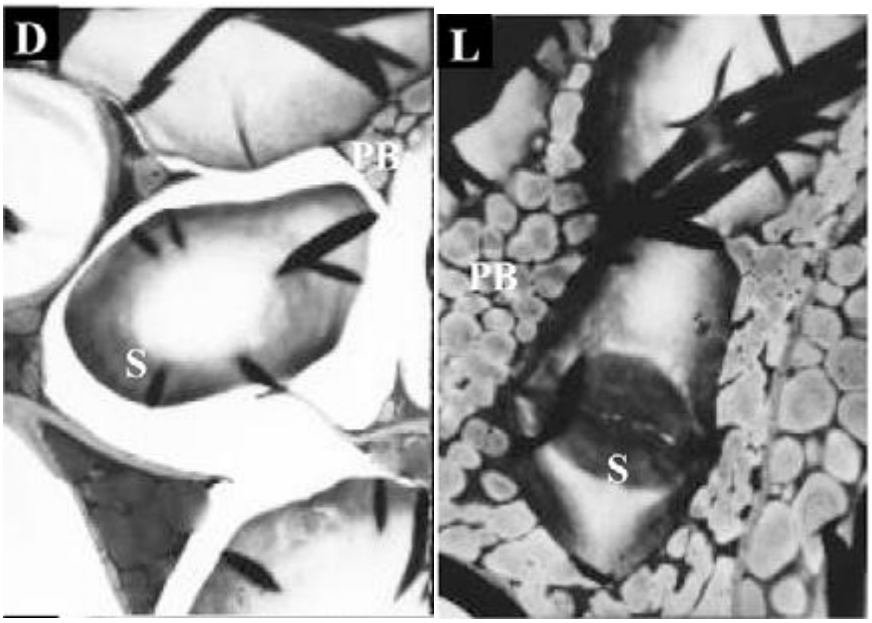

Figure 3 Transmission electron microscopy showing starch granule (S) and protein body (PB) in dent corn (D) and popcorn (L). Available in Zhang et al., (2011).

2.2. Corn use and processing methods

The main objective of processing grain is to increase nutritional value. When corn is processed, the pericarp is damaged, particle size is reduced, and starch surface area increases, facilitating microbial and enzymatic access to the starch granules, bosting digestibility. The effects of processing are more noticeable in grains with lower degradability (e.g., sorghum and flint corn) (JACOVACI et al., 2021; OWENS; BASALAN, 2013).

2.2.1. Whole corn and dry processing methods

Whole corn is the simplest way to feed corn to ruminants. Usually, it is cheaper than any other corn processing since no energy is required to process the grain, but it requires that the animal chews the grain to damage its pericarp. Conflict in literature has been found about the advantage of using whole corn in beef cattle nutrition. GOROCICA-BUENFIL; LOERCH, (2005) fed whole and ground dent corn to cattle and got similar average daily gain (ADG), hot carcass weight (HCW), and dry matter intake (DMI) however more fecal starch were found for whole corn-fed steers. According to

the same author, 91.04% of the whole ingested kernels disappeared and 86.01% of the initial starch in a kernel was recovered in non-damaged excreted kernels. FREITAS et al., (2021), in contradiction with the previously mentioned experiment, found better ADG, HCW, lower DMI, and similar feed efficiency for animals eating ground corn diets in comparison with whole corn diets. OWENS; SODERLUND, (2006) in a compilation of data, concluded that the ruminal disappearance of whole corn grain starch is 68.34%, while dry-rolled corn starch is 63.8%. However, when total tract starch disappearance is analyzed, this value reverses to 91.03% for starch coming from dry rolled corn and 87.08% for whole corn grain starch. The lower ruminal starch degradability of rolled corn may be explained by the faster rate of passage of small particles, which can be accelerated when a diet contains high levels of physically effective fiber.

Ground and rolled corn are the most common processing methods adopted in Brazil due to their facility (SILVESTRE; MILLEN, 2021) and to enhance the surface area exposed to microbial and enzymatic attack. Rolling corn consists of breaking the kernel using two rolls while grinding normally uses a hammer mill with a sieve. Rolled grains are characterized to have more uniform and larger particle size than ground grains (CORONA et al., 2005; NIKKHAH, 2012). No difference in ADG, feed efficiency (FE), and rumen pH was reported when ground corn was compared to dry rolled corn, still, starch digestion (86.3% vs 92%) and excretion (25% vs 16% of DM) were better for ground corn due to its smaller particle size (CORONA et al., 2005). SCHWANDT et al., (2016) observed a quadratic tendency for ADG and FE during the acclimatization time when cattle were fed medium-rolled corn (3.76 um) but no differences were seen when the entire feedlot period was analyzed, even with a linear increase in fecal starch as particle size increases. It is important to mention that the previous experiments were performed using dent corn. GOUVÊA et al., (2019) evaluating two different geometrical particle sizes of flint corn noticed a tendency of better ADG, final body weight, and HCW when corn was coarsely ground (2.12mm vs 1.66mm) without differences in starch digestibility (87%) and fecal excretion (21% of DM).

Finer particles are quickly degradable in the rumen and might cause subacute acidosis reducing intake and performance (OWENS; SODERLUND, 2006). Rumen fermentation is less energy efficient (80%) due to heat losses and methane production.

The better energy efficiency of small intestine starch digestibility (97%) may compensate for the smaller rumen degradability rate of larger particles that go through it (HARMON; MCLEOD, 2001; HUNTINGTON; HARMON; RICHARDS, 2006). The small intestine (SI) in ruminants has limitations in amylase production and glucose absorption that contribute to the passage of intact starch and glucose towards the large intestine which is less energy efficient and increases starch rate in feces meaning in loss of potential energy for the ruminant (OWENS; SODERLUND, 2006). Therefore, excess of coarsely particles might be inefficient.

Dry processing has shown a less efficiency in corn digestibility as values around 13 and 7% of starch remain indigestible even on how fine the grain is ground (GOUVÊA et al., 2019; SCHWANDT et al., 2016; ZINN; SHEN, 1998). HUNTINGTON; HARMON; RICHARDS, (2006) summarized that when more than 75% of starch in the small intestine is digestible there is an increase in the yield of digestible energy by shifting starch degradation in the rumen to be digestible in SI but values above 70% digestion were found only when 700g or less of starch was going to SI.

2.2.2. Ensiled corn

Ensiling corn grain has a low storage cost and can be done in two ways, high moisture corn silage (HMC) and reconstituted corn grain silage (RCGS), both with satisfactory performance results in comparison with dry-processed corn (DA SILVA, 2016). Both methods lead to an increase in ruminal and total digestion, where flint corn may have the same digestibility values as ensiled dent corn. Increases in degradability are noticed to continue at least until 300 days of ensiling but it is more accentuated until 60 days (DA SILVA et al., 2019; FERNANDES, 2014; GERVÁSIO et al., 2023). The ruminal starch degradability was summarized as 86.55% for HMC and 99.25% for total tract starch digestion, avoiding almost 100% of losses (OWENS; SODERLUND, 2006).

High moisture corn grain silage is made when corn is harvested between 60% and 65% of grains DM. Corn kernels are passed to a roll or grinder to damage the pericarp and expose the endosperm to enzymatic and bacterial attack in the silo. The advantage of this method is the no need for water addition before ensiling and lower

field losses due to early harvest, but, in contrast, there is a short harvest window to hit the grain's ideal moisture content. On a large scale, this short period can be a hindrance when an extensive amount of land must be harvested, also, moist grains force more the machine during harvest. Small farms might face problems of land availability to plant corn, which directs these farmers to the market. Farmers that buy corn to make HMC often report a large variety of moisture content and may need to add water in the silo. When a huge silo is used, the amount of grain available per day to unload on the farm may be an issue and might take too much time to close the silo, leading to aerobic deterioration and loss of water through evaporation.

When dry corn is ground with water addition and then ensiled, it is called reconstituted corn grain silage or rehydrated corn grain silage. The advantage over the HMC is the strategic grain purchase in periods when it is cheaper, less DM variability regardless of resources, and no need for planting. In contrast with these advantages, a large amount of water is necessary to moisten the silo mass until it reaches 65~60% DM, which can be a limiting factor in regions with low amounts of water availability.

JACOVACI et al., (2021) did a metanalysis evaluating the effects of ensiling flint corn grain on feedlot beef cattle and found positive results for DM intake (8.85 vs 10.3; kg), feed efficiency (0.194 vs 0.164), total tract starch digestibility (0.991 vs 0.959) and diet metabolizable energy (ME; 12.9 vs 11.7; MJ/kg DM) with no difference in weight gain (1.58 vs 1.61 kg/day). The similar gain with lower intake can be supported by the higher grain digestibility and higher ME that might activate chemical mechanisms of satiety earlier in comparison to dry corn. The higher rumen fermentation also increases the risk of acidosis in non-well-balanced diets, but, on the other hand, the inclusion of corn grain silage stimulates a decline in meal size and increases in number of meals. In conclusion, ensiled corn grain has a feed value improvement of 25.7% in comparison with dry corn grain (JACOVACI, 2019).

Low rumen pH is found in animals eating diets with corn grain silage (GODOI et al., 2021). The risk of subacute acidosis can reduce intake and negatively affect performance (MILLEN et al., 2016). Formulating diets with different resources of starch may lead to a better synergy with different protein resources in the rumen and may reduce the risk of acidosis. STOCK; ERICKSON, (2006) summarized data from different experiments using ensiled corn and dry corn and found that the inclusion of

70% of ensiled corn could increase performance and efficiency. In contrast, COULSON et al., (2023) and GERVÁSIO, (2021) evaluated diets with 50:50 inclusion of HMC and dry corn and observed better efficiency for animals eating 100% roll HMC. For ground HMC, 100% inclusion of HMC provides similar efficiency to cattle eating 50:50 inclusion of rolled HMC and DRC (COULSON et al., 2023). In only one found study using RCGS for animals finished on pasture, Nellore bulls receiving *ad libitum* supplements containing RCGS had better performance and efficiency than the ones consuming dry-ground corn supplement (GONÇALVES, 2018).

2.2.2.1. Reconstituted corn grain silage

To make reconstituted RCGS, corn must be ground or rolled, moistened, mixed and then compacted to achieve ideal conditions (e.g., moisture and anaerobic environment) so enzymes and microorganisms start consuming residual O_2. In anaerobic conditions, bacteria added in the silo or even epiphytic from the corn kernel begin a fermentation process that reduces the pH and has a great effect on the breakdown of prolamins (JUNGES et al., 2017)(MCDONALD; HENDERSON; HERON, 1991).

Bacteria are responsible for 60.4% of proteolysis in the corn grain silo, while 29.5% are attributed to kernel enzymes, and only 4.8% is resulted from acid activity (JUNGES et al., 2017). In addition to being responsible for proteolysis in the silo, bacteria are responsible for the fermentation and indirect responsible for aerobic stability of the silo post-opening. To ferment properly, reconstituted corn grain silos must be in ideal conditions for bacteria. Besides anaerobic conditions and grain processing, moisture content directly impacts silage quality. The best moisture content for ensiling grain to achieve a satisfactory fermentative profile, aerobic stability, and DM degradability is between 35 and 40% (GOMES et al., 2020). The grain silage particle size also affects the DM degradability and the retention of water as water may percolate and not be absorbed by the coarse particles, resulting in inferior silage moisture content.

Grains particle size is in function of mill settings. Finer particles have larger contact surfaces and expose more starch granules to microbial and enzymatic attacks

but require more energy and time to be processed. Even though RCGS ferments slower and less extensively when compared to typical whole plant forage silage (TAYLOR; KUNG, 2002), it has an adequate fermentation profile independent of particle size (DA SILVA, 2015; GERVÁSIO, 2021) but longer storage periods are recommended for coarse silages, principally to have similar DM degradability results as fine ground silage (DA SILVA et al., 2019; GERVÁSIO, 2021).

Storage time affects positively the aerobic stability and starch degradability. Proteolysis is noted to be constant during the storage time, but it is more intensive at the beginning of the period (DA SILVA et al., 2019; HOFFMAN et al., 2011). Greater gains in DM degradability in inoculated RCGS are found until 57 days of ensiling, even though it continues as the silo remains stable (DA SILVA et al., 2019). Longer storage times lead to the accumulation of fermentation products that kill undesirable microorganisms. Expressive increases in inoculated RCGS aerobic stability vary and are found between 120 and 240 days of storage, proximally (DA SILVA et al., 2019; GERVÁSIO et al., 2023). After this time, the substrate for acid production starts to decrease followed by the beneficial microorganism population and the increment in AS becomes less expressive. Longer storage periods can also be a problem for farmers who have a high rate of silage use and for those in times of feed deficit. The use of inoculants can be a strategy to accelerate and improve the desirable effects in corn grain ensiling.

2.3. Corn grain silage inoculants

The use of inoculants in silage is strategic to correct some mistakes in the confection (e.g., moisture, compacting) but in the case of RCGS, it works as an insurance as it boosts fermentation and aerobic stability. Cereal silo is a huge amount of immobile capital and nutrients for yeast and molds, thus, additives become an indispensable tool.

Inoculants are mainly composed of lactic acid bacteria, which are divided in three major groups according to carbohydrate metabolism: homofermentative bacteria, facultative heterofermentative, and obligatory heterofermentative bacteria. Homofermentative bacteria metabolize hexoses by glycolysis pathway producing 2

lactic acids as the only product of their metabolism. Facultative heterofermentative ferments hexoses like homofermentative bacteria and pentoses by phosphoketolase pathway which produces mainly acetic and lactic acid and ethanol. The third group uses hexoses and pentoses throw the phosphoketolase pathway (REIS et al., 2018).

MORAIS et al., (2017) made a review comparing the characteristics of corn grain silage (CGS) inoculated with different inoculants: Corn grain silage is characterized as moderate fermentation reaching pH 4.42 when no inoculant is used, the addition of homolactic bacteria improves pH drop to 4.16 but reduces aerobic stability by 40 hours. Comparing non-inoculated CGS against silage treated with heterofermentative bacteria, the inoculated ones were more than 100 hours more stable and pH was equal but tended to increase the fermentative DM loss by 0.5 points; the combination of both types of bacteria increased the aerobic stability by 40 hours and decrease the pH by 0.15.

The exclusive use of homolactic bacteria will direct sugars to be converted into lactic acid (pKa = 3.86) making a faster and deeper pH drop which avoids undesirable fermentation and decreases DM loss during fermentation. However, this acid can be used by yeast in the opening phase in which will increase pH, making the environment adequate for molds, leading to the loss of the aerobic stability. On the other hand, the exclusive use of obligatory heterofermentative bacteria produces less lactic acid slowing down the pH drop which gives time to secondary fermentation increasing DM losses, but, it produces weak acids (e.g. acetic and propionic) that will increase silage aerobic stability as they have antifungal activity (MOON, 1983). Weak acids, when are in their non-dissociate form, penetrate yeast, and mold membranes and dissociate in there leading to the death of the microorganisms (MCDONALD; HENDERSON; HERON, 1991).

Tropical countries have higher temperatures, therefore, the use of obligatory heterofermentative bacteria makes more sense since undesirable microorganisms grow faster in higher temperatures, making silage spoiling easier. Meanwhile, a combination of the previously mentioned group plus facultative heterofermentative may be ideal in case of mistakes during ensiling (e.g., wrong moisture).

Among facultative heterofermentative bacteria, we can highlight *L. plantarum*, however, the use of this bacteria alone in RCGS has not been showing positive results

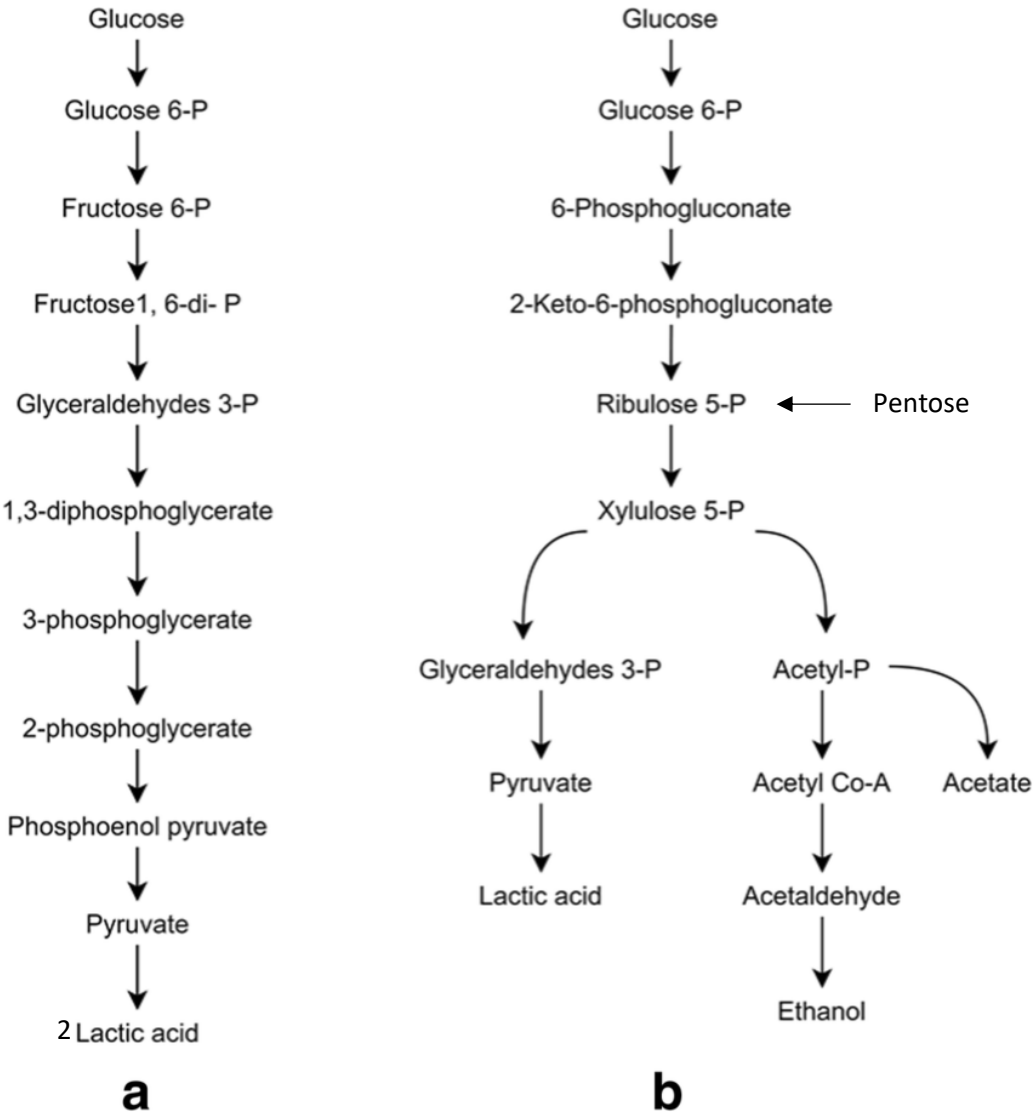

Figure 4: A Glicolyse pathway. B Phosphketalse pathway. Adapted from Chung et al., (2021)

in aerobic stability (da Silva et al., 2018; Saylor et al., 2020). In the obligatory heterofermentative group, *L. buchneri* is one of the most famous bacteria and the mainly used to preserve grain silage under aerobic exposure. Corn grain silages inoculated with *L. buchneri* start to show significant improvement in AS between 45 and 60 days of ensiling (KLEINSCHMIT; KUNG, 2006a; SCHMIDT et al., 2009), with even better effects after 60 days tending to stabilize around 150 days reaching around 250 h of AS (DA SILVA et al., 2019). In anaerobic conditions, *L. buchneri* has the capacity to convert lactic acid into acetic acid (OUDE ELFERINK et al., 2001). Another bacteria that belongs to the same group, *L. hilgardii* was first studied as an inoculant by ÁVILA et al., (2014) in sugar cane silage, being the most promising among 58

strains of 3 different species. SANTOS et al., (2017) studying the same bacteria reported a similar fermentative profile to *L. buchneri*. In only one study found until the moment using *L. hilgardii* in corn grain silage, its inoculation showed good results in aerobic stability after 10 ensiled days and when it was inoculated with *L. buchneri*, the results were even better (DA SILVA et al., 2021) being an excellent tool in an emergency, when the silo must be opened before the minimum recommended time of 60 days and still with good results in AS.

Few experiments evaluated the aerobic stability of cattle diets, but none of them included grain silage. BERNARDES et al., (2007) in laboratory conditions observed that combination of *L. plantarum* + *Propianobacterium* unsured aerobic stability of a diet made with *Brachiaria* silage for 6 days. BERNARDES et al., (2007) and TAYLOR et al., (2002) did not observe loss of aerobic stability for at least 120 hours in diets made with *Brachiaria* silage (pre wield to 30% MS) and whole plant barley silage, respectively, both inoculated with *L. buchneri*. Taylor et al., (2002), in field conditions observed a reduction in aerobic stability to 79 h for inoculated silage ration against 46 h for control ration. SEPPÄLÄ et al., (2013) demonstrated in their experiment how high hygienic ingredients can affect the aerobic stability of the ration as they got a 50 greater AS for ration made with good-hygienic ingredients and it could have been more, but they stop measuring.

2.4. Beef cattle finishing phase on pasture with high supplementation

Beef cattle high supplementation fattening on pasture consists of providing supplemental levels above 1.2% of body weight (BW), allowing for high stocking rate and performance, reaching the slaughter point in approximately 120 days. This technical management aligns with short-cycle livestock that has been recommended in Brazil for producing better meat quality and enabling greater profitability per area.

In the finishing phase on pasture, the forage mass comprises the dietary roughage to provide ruminal health, and the supplement is provided in a feed trough eliminating the necessity for feedlot pens (SIQUEIRA; RESENDE; MORETTI, 2014). It is important to highlight that the minimum forage requirement must be

available for the animals throughout the whole phase (GALYEAN; HUBBERT, 2014) and the amount might influence results. Cattle coming from the growing phase with a low forage allowance (1.9 kg DM/kg BW) have better average daily gain and feed efficiency when it is finished with a high forage allowance (2.9 kg DM/kg BW), however, forage allowance in finishing phase does not affect cattle coming from a growing phase with high forage allowance (starting at 4.1 DM kg BW) (MOTA et al., 2020).

Cattle finished on pasture can perform similarly to cattle in feedlots. (MORETTI, 2015). The supplement provided is similar to the concentrate used in Brazilian feedlots where 97.22% utilize corn as the main grain in the diet (SILVESTRE; MILLEN, 2021). The dry corn grain used in the supplement may be rehydrated and ensiled serving as a low cost stock.

One experiment conducted in APTA Colina evaluated high-moisture corn grain silage in supplements for finishing animals on pasture, completely substituting dry corn grain. The inclusion of high-moisture corn reduced supplement intake by 8.1 % (650g / day), increased weight gain by 120 g/day, and improved feed efficiency by 19% (151 vs 180 g). Even though forage intake was not measured, forage mass and stocking rate were greater in pasture for animals consuming HMCS which can be an effect in the reduction of pasture consumption due to the same mechanism of feed regulation applied in the reduction of the supplement intake (GONÇALVES, 2018)

Cattle on pasture with low supplementation have larger rumen size and TGI fill compared with animals eating high-energy diets. When we compare finishing cattle on pasture against finishing animals on feedlot, animals on pasture have smaller rumen and TGI fill due to the possibility of selecting better quality forage and the amount ingested while animals on feedlot must consume the mixed diet (MORETTI, 2015). Smaller rumen size and TGI fill tend to lead to a better carcass dressing. A disadvantage of animals on pasture is the necessity to spend more energy to collect forage increasing net energy for maintenance while feedlot animals walk short distances saving more energy for gain. In general , studies show good efficiency for cattle finished on pasture with high supplementation being a solution for Brazilian livestock.

3. Objective and Hypothesis

The experiment hypothesis is: RCGS inoculation with *L. buchneri* + *L. hilgardii* or *L. buchneri* + *L. plantarum* is capable of ensuring the aerobic stability of the RCGS and the supplement containing this silage for at least 72 h, preserving nutrients and ensuring performance similar to animals treated with a supplement provided immediately after preparation. Replacing 70% of DRC with RCGS will improve cattle efficiency and performance.

The aims of this study are: I) to evaluate the combination of *L. buchneri* + *L. hilgardii* and *L. buchneri* + *L. plantarum* in the ensiling of reconstituted corn grains and their effects on silage fermentation, nutritional value, and aerobic stability of the silage and the supplement made with these silage; II) to determine the impacts of providing fresh and exposed supplement on the intake and performance of pasture-finished Nellore bulls with high supplementation.

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CHAPTER 2 – EXPERIMENT 1: EVALUATION OF SILAGE INOCULANTS FOR RECONSTITUTED CORN GRAIN SILAGE AND SUPPLEMENT AEROBIC STABILITY.

1. Introduction

In 2019, 32% of feedlots in Brazil used any type of matured grain silage (e.g HMC, RCGS, snaplage) (BERNARDES; CASTRO, 2019) and this number might increase as the benefits of these technologies become more known among producers. Hence, producers using other strategies to fatten cattle might want to use it too. It is recommended to unload the silo daily and to use the silage immediately after unloading as it can start to spoil quicker but in some business models, some of these recommendations may not be followed and extra care must be taken.

Dry corn kernels used to make RCGS have a low concentration of water-soluble carbohydrates, a low count of desirable epiphytic LAB, and may have high count of undesirable microorganisms (CARVALHO, 2014). This combination can be a factor that will imply poor fermentation, high losses, and short aerobic stability. Aerobic spoilage is a phenomenon where the silage mass has contact with air and aerobic microorganisms start to multiply and consume nutrients (Pahlow et al., 2003). This is one of the main aggravating factors in silage production. With the advance of studies on silage fermentation and conservation, technologies were developed to control these problems, and DM loss due to poor fermentation and spoilage after opening becomes inadmissible, principally in cases where the silage will be exposed for some time for sure.

Facultative heterofermentative bacteria are indicated for silage with fermentation problems because they produce mainly lactic acid and obligatory heterofermentative bacteria are indicated to improve aerobic stability as weak acids are the main product of their fermentation. Even with low substrate to be fermented and a smaller capacity to decrease pH obligatory heterofermentative bacteria are capable of providing a good fermentation profile in RCGS silage (DA SILVA et al., 2021, 2024, 2018, 2019). *L. Buchneri* is one of the most studied bacteria in this

group of obligatory heterofermentative bacteria and shows excellent improvement in aerobic stability (KLEINSCHMIT; KUNG, 2006c; KUNG et al., 2007; TAYLOR; KUNG, 2002). *L. Hilgardii* is in the same group and it seems to have a similar metabolism that *L. Buchneri* with an earlier start of actuation (ÁVILA et al., 2014; DA SILVA et al., 2021; SANTOS et al., 2017). A combination of facultative and obligatory heterofermentative bacteria can be an option to guarantee fermentation and aerobic stability, and *L. Plantarum* is the main bacteria studied with good results to represent the facultative heterofermentative group(DA SILVA et al., 2024).

When silage cannot be immediately used, it can be mixed with other ingredients and the ration or supplement will have to remain stable until being provided. In this situation, acids in the silage will reduce concentration, and undesirable microorganisms will increase due to the addition of other ingredients and contact with machines (SEPPÄLÄ et al., 2013). On the other hand, DM will increase, and water activity decrease, making the environment less favorable to microbial growth (JOBIM et al., 2007). All of these factors affect the aerobic stability of the supplement or ration, and studies are requested to understand this technique and see how it will work.

This study aims to evaluate the combination of *L. buchneri* + *L. hilgardii* and *L. buchneri* + *L. plantarum* in the ensiling of reconstituted corn grains and their effects on silage fermentation, nutritional value, and aerobic stability of the silage and the supplement made with those silage. The hypothesis is inoculation with those bacteria will guarantee aerobic stability of the supplement for at least 72 hours (3 days).

2. Material and Methods

The Experiment was conducted at the São Paulo State Agency for Agribusiness Technology – Alta Mogiana Polo, Colina – SP from October 2022 to December 2022. It was evaluated RCGS inoculated with the following treatments: RCGS without inoculation (CON); RCGS inoculated with *L. hilgardii* CNCM I-4785 (1.5×10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5×10^5 cfu/g) (LBLH, Lallemand Inc); RCGS inoculated

with *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB, Volac International Lim.). At the silo opening, a supplement was prepared using the silage and it was evaluated.

2.1. Ensiling Process and Supplement Preparation

Dry corn grain was acquired from the local market, ground in a hammer mill (TN 9) with a 5 mm sieve and stored in a mixer wagon. A sample was collected to check the dry matter (DM) in a forced ventilation oven at 105° C for 12 h. With the dry matter result, approximately 115 kg of ground corn were transferred to a concrete mixer and chlorine-free water at room temperature was added and mixed for 5 minutes to increase mass moisture to 37.5%. Six piles of 25 kg of rehydrated corn were made above a pre-cleaned canvas. The inoculant was diluted in the same water used to rehydrate the corn and each pile was inoculated using a hand spray filled with 300 ml of diluted inoculant (12 l / ton). The pre-ensiled mass was hand-homogenized while it was inoculated. Control treatment received only water. After homogenization, the rehydrated corn from each treatment was sampled and compacted in pre-cleaned and weighed 23 L plastic buckets to achieve 1050 kg / m^3, sealed with a lid and plastic tape and then weighed. This process was repeated 3 times and rehydrated corn from each repetition was sampled for analysis. The experimental silos were stored for 60 days.

After the storage period, the buckets were weighed again and opened. Approximately 2 cm of silage from the top were discarded and the remaining was homogenized and sampled for further analysis. Three kilograms of silage were taken for aerobic stability evaluation. The remaining content from each bucket was individually used in the preparation of a supplement similar to that would be provided to cattle in experiment 2. The supplement consists of 20.19% ground dry corn, 47.12% RCGS, 11% cottonseed, 17.4% cottonseed cake, and 4.29% mineral premix (Table 2, Exp. 2).

The supplement was manually mixed on a tarp and sampled for analysis. After mixed, 10 kg of the supplement were allocated to 2 new buckets (5 kg/bucket) previously identified, cleaned, and weighed with a data logger (Pro 2.07.09, Escort

Console, Buchanan, VA, United States) inside the bucket, totaling 36 buckets (2 supplement buckets/silage bucket).

2.2. Fermentation losses, aerobic stability, and aerobic exposure losses

After 60 days of ensiling, the silos were weighed again to determine dry matter losses during the fermentation process (Jobim et al., 2007). Approximately 3 kg of silage was placed in plastic bags covered with sheet paper to prevent external contamination (Tabacco et al., 2009). The trays were kept in a closed room at a controlled temperature of 25° C for 9 days. The silage temperature was recorded every 30 minutes using a data logger positioned in the geometric center of the silage. Aerobic stability was defined as the number of hours the silage remained stable before reaching 2°C above room temperature (Ranjit & Kung, 2000).

The buckets with supplements were kept open in the same temperature-controlled room and it was checked if aerobic stability was maintained for 72 h (3 days) or 144 h (6 days), following the same protocol of Ranjit & Kung, (2000) described in the previous paragraph. The buckets were weighed after the predetermined exposure time for calculations of dry matter losses due to aerobic exposure (Jobim et al., 2007).

2.3. Chemical analyses

A pre-ensiled corn sample from each treatment was dried in a forced ventilation oven at 55°C for 72 h to estimate partial dry matter (DM) and then ground in a Willey mill using a 1 mm mesh sieve. The ground samples were used for quantification of dry matter in a 105°C oven for 12 h (method 934.01, AOAC, 2012) and total nitrogen by the Kjeldahl method (method 981.10, AOAC, 2012), crude protein (CP) content was calculated by multiplying total nitrogen content by 6.25. Water-soluble carbohydrates (Hall et al., 2015) was also quantified. Another pre-ensiled sample from each treatment was added to plastic bags containing distilled water at a 1:10 ratio and then homogenized in a Stomacher® device (400 Circulator, West Sussex, UK) for 4 minutes. From the solution, pH was checked using a potentiometer (DM-22, Digimed,

São Paulo, SP, Brazil) and ammonia nitrogen (N-NH3) content by following the procedures described in the INCT (Detmann et al., 2012).

Silage sample was collected in each bucket on the silo opening day and dried in a forced ventilation oven at 55°C for 72 h to estimate partial DM and then ground in a Willey mill with a 1 mm mesh sieve. The ground samples were used for DM quantification, CP, and sugars all following the protocols described above. Additionally, ash analysis (method 930.05, AOAC, 2012), fats (method 945.16, AOAC, 2006) and starch (Hall et al., 2015) were performed. Another sample collected from each bucket was diluted with distilled water (1:10) and homogenized in a stomacher® for 4 minutes, then pH and N-NH3 were analyzed following the previously described protocols; additionally, the levels of alcohols, esters, and volatile fatty acids were analyzed using gas chromatography with mass detector (GCMS) (GCMS QP 2010 plus, Shimadzu®, Kyoto, Japan), with a capillary column (Stabilwax, Restek®, Bellefonte, USA; 60 m, 0.25 mm ø, 0.25 µm crossbond carbowax polyethylene glycol) and analytical parameters as recommended by the manufacturer.

Samples of the supplement collected at the time of preparation and from each bucket after the aerobic exposure period were analyzed for DM, CP, fat, ash, sugars, starch, and pH. All analyses will follow the protocols referenced at the beginning of this section. Analyses of neutral detergent fiber with heat-stable amylase (NDF) and acid detergent fiber (ADF) were performed in the supplement samples (Van Soest et al., 1991) and total digestible nutrients (TDN) (NASEM, 2001).

Table 1: Silo density and chemical composition of pre-ensiled reconstituted corn without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB)

Item[a]	CON	LBLH	LPLB
Silo Density (ton/m³)	1.04	1.03	1.03
DM (g/kg DM)	619	619	620
CP (g/kg DM)	98.4	98.0	98.2
WSC (g/kg DM)	16.1	16.1	16.1
pH	5.99	6.00	6.03
N-NH3(g/kg DM)	0.19	0.15	0.15

[a]DM = dry mater; CP = crude protein; WSA = water soluble carbohydrates; $N\text{-}NH_3$ = ammonia nitrogen

2.4. Microbiological Analyses

Samples collected from pre-ensiled material, post-opening silage, supplement ingredients, and supplement at times 0, 72, and 144 were placed in sterile bags and diluted with peptone water (1 g/L, 1:10 peptone water) and then homogenized for 4 minutes in a stomacher®.

Surface plating technique on YGC Agar medium was carried out for yeast and filamentous fungi counting. Serial dilutions (10^{-1} to 10^{-6}) were prepared and plated in duplicate (Tabacco et al., 2009). After incubation at 28° C for three and five days for yeast and filamentous fungi, respectively, colonies were counted separately, based on their macro-morphological characteristics. The same technique described for yeast and filamentous fungi was used for LAB counting, with MRS Agar medium and incubation at 35°C for three days for subsequent counting.

Table 2: Microbial counts of supplement ingredients and pre-ensiled corn without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB)

Item[a]	CON	LBLH	LPLB	DGC	Cottonseed cake	Cottonseed whole
LAB (log CFU/g)	3.71	4.11	4.46	-	-	-
Yeast (log CFU/g)	2.82	3.05	3.01	3.11	2.59	2.30
Molds (log CFU/g)	2.91	3.10	3.23	4.08	3.18	2.44

[a]LAB: Lactic acid bacteria; CFU, colony-forming unity
DGC: dry ground corn

2.5. In vitro and In vivo degradability and microscopy analyses

Samples of RCGS, and supplement at all 3 times of evaluation were collected and dried in forced-air ovens at 55°C for 72 hours and then ground to 2 mm for in-vitro digestibility tests at a commercial laboratory (IFM® Alltech, Maringá – PR; Meads et al., 2021). The total gas production, and volatile fatty acids (VFA) production, were evaluated for 48 hours. Rumen fluid was collected from a Holstein cow consuming whole plant corn silage and minerals.

Five grams of dry silage from each bucket, in the original particle size, were placed in pre-weighed nylon bags to analyze DM degradability for 12 hours in

canulated Nellore bulls receiving a diet of 15% Brachiaria hay, 68% rolled corn, 7% soybean meal, 7% citrus pulp, and 3.1% mineral supplement. After 12 hours, bags were taken out and rinsed with water until have gotten crystal clear water and then dried in an oven at 105°C for 12 hours. DM degradability was calculated as: (post-incubated dried total bag weight – bag weight) / [(pre-incubated total bag weight – bag weight) x sample DM %].

Furthermore, dry 1mm ground samples from pre-ensiled corn and post ensiled corn of each treatment were taken to The University of Adelaide and visually analyzed in a scanning electron microscopy model Ultim Max 65 (Oxford Instruments, Oxon - UK) to observe starch granules following recommendations described by Hoffman et al., (2011).

2.6. Statistical analysis

Corn silage data were analyzed as a randomized block design using the MIXED procedure of SAS (SAS Institute, INC., Cary, NC). Each one of the 3 rehydrations of ground corn was considered as one block. The model considered treatment as fixed effect and block as random effect. Means were confronted in orthogonal contrasts for 5% probability: C1: CON vs LBLH + LPLB; C2: LBLH vs LPLB.

For the supplement, data were analyzed as repeated measures using the MIXED procedure of SAS. Treatment, day, and treatment*day were considered fixed effects, block and bucket were considered random effects and bucket(day) was the subject. The following covariance structures: ANTE1 AR1 ARH1 ARMA11 CS CSH FA1 FA2 HF TOEP2 TOEPH2 UN UN1 UN2 VC were tested for all parameters. The covariance structure with the lowest Bayesian information criteria was chosen separately for each parameter. All means were compared using the t-test at a 5 % probability. Polynomial contrasts (linear and quadratic) were performed for days and C1 and C2 for treatments when no interaction effect was detected. When the interaction effect was significant, contrast for days (linear and quadratic) in treatments and treatments (C1 and C2) in days were performed. The significance level adopted was $P < 0.05$ and $P \leq 10$ was considered tendency.

3. Results

DM loss was two times higher for inoculated silages (C1, P < 0.01) as pH was 0.5 points higher for these silages (C1, P < 0.01). No differences for both characters were found in inoculated treatment silages (C2, P ≥ 0.11). Counting for LAB was approximately 8.08% higher in inoculated treatments (C1, P < 0.01) and similar for both inoculated treatments (C2, P < 0.01). Molds were not detected in all treatments and yeast was noted only in control silage (C1, P < 0.01). DM degradability at 12 hours was higher in inoculated silages (C1, P < 0.01) and higher in LBLH than in LBLP (C2, P < 0.01). N-NH_3 was higher in inoculated silages (C1, P < 0.01) and tended to be higher in LBLH treatment (C2, P = 0.08) (Table 3).

Table 3: DM loss, pH, microbial counts, dry matter digestibility and N-NH_3 of RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) on the opening day.

Item[a]	Treatments			SEM	P-Value[b]	
	CON	LBLH	LBLP		C1	C2
DM loss (g/kg DM)	22.4	47.8	42.4	0.13	<0.01	0.11
pH	3.95	4.45	4.45	0.01	<0.01	0.90
LAB (log CFU/g)	5.63	6.13	6.04	0.09	<0.01	0.52
Yeast (log CFU/g)	3.56	ND	ND	0.07	<0.01	1.00
Molds (log CFU/g)	ND	ND	ND	0.12	1.00	1.00
DMD 12h (g/kg DM)	745	782	757	0.35	<0.01	<0.01
N-NH_3 (g/ kg DM)	0.91	1.40	1.35	0.19	<0.01	0.08

[a]DMD = dry matter degradability; LAB = lactic acid bacteria; DM loss = DM loss during storage period.
[b]C1: CON vs LBLH + LPLB; C2: LBLH vs LPLB
ND = counts lower than 2 CFU

The production of lactic acid was 3.7 times greater for CON silage and differed from inoculated silages (C1, P < 0.01) which were equal between inoculated ones (C2, P = 0.13). Acetic acid were 4.15 times greater for inoculated silages (C1, P < 0.01) and LBLH produced 1.3g/kg DM more than LPLB (C2, P < 0.01). Butyric acid and ethanol had higher concentration in CON silage than in inoculated silages (C1, P < 0.01) and were similar in both inoculated silages (C2, P ≥ 0.14).1,2 propanediol was 9 times more

concentrate in inoculated silages than in non-inoculated silage (C1, P < 0.01) and LPLB had greater concentration than LBLH (C2, P < 0.01).

Table 4: Fermentation products of RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) on the opening day.

Iten (g/kg DM)	Treatments			SEM	P-Value[a]	
	CON	LBLH	LPLB		C1	C2
Lactic acid	27.6	7.03	7.88	0.71	<0.01	0.13
Acetic acid	2.95	12.9	11.6	0.17	<0.01	<0.01
Butyric acid	0.06	0.01	0.01	0.01	<0.01	0.41
Ethanol	2.95	2.33	2.18	0.07	<0.01	0.14
1,2 propanediol	0.348	2.67	3.76	0.28	<0.01	<0.01

[a]C1: CON vs LBLH + LPLB; C2: LBLH vs LPLB

Aerobic stability was more than four times greater in inoculated treatments (C1, P < 0.01) and it was not lost in inoculated silages. DM after 9 days was greater in inoculated treatments (C1, P < 0.01) and similar in both inoculated (C2, P < 0.75). DM loss in this period was 6 times higher in non-inoculated silage (C1, P < 0.01) and similar in both inoculated (C2, P=0.70). Yeast and Molds were detected in only non-inoculated silage after 9 days (C1, P < 0.01) (Table 5).

Table 5: Aerobic stability, DM, DM loss, microbial counts, and pH of RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) after AS period.

Item	Treatments			SEM	P-value[b]	
	CON	LBLH	LBLP		C1	C2
AS (hours)	49.8	>216	>216	0.93	<0.01	1.00
DM (g/kg)	610	629	630	0.01	<0.01	0.75
DM loss (g/kg DM)	120	19.6	20.6	0.01	<0.01	0.70
pH	4.98	4.48	4.52	0.05	<0.01	0.62
Yeast (log CFU/g)	5.96	ND	ND	0.06	<0.01	1.00
Molds (log CFU/g)	5.46	ND	ND	0.07	<0.01	0.67

[a] AS = aerobic stability; DM = dry matter; DM loss = DM loss during exposing period.
[b]C1: CON vs LBLH + LPLB; C2: LBLH vs LPLB
ND: counts lower than 2 CFU

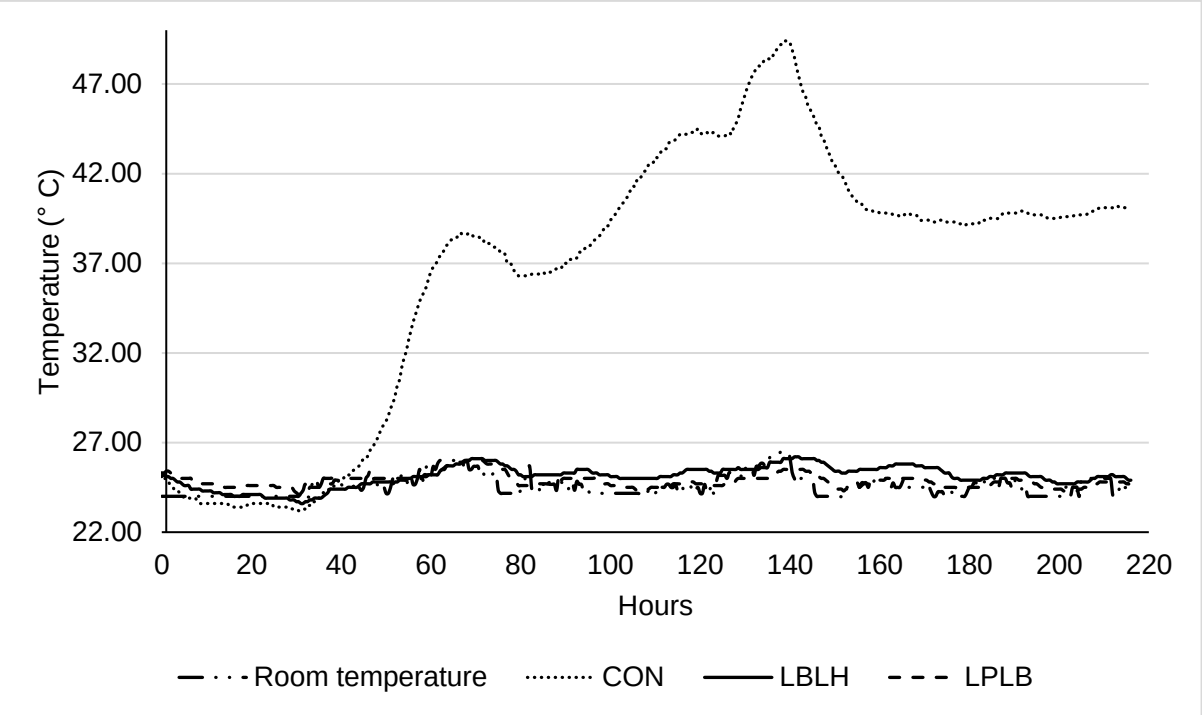

Figure 5: Temperature during aerobic stability test of RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB).

DM at the opening was higher for non-inoculated silage (C1, P = 0.06) and similar for inoculated ones (C2, P = 0.64). PB was slightly higher in inoculated silages (C1, P < 0.01) and was higher in LPLB silage than in LBLH (C2, P < 0.01). Soluble protein was similar in inoculated and non-inoculated silage (C1, P = 0.52) and tended to be lower in LBLP compared to LBLH (C2, P = 0.07). Starch was similar for all silages (P ≥ 0.21) and sugar was lower in inoculated silages (C1, P < 0.01). Ash, fat, and TDN were similar for all treatments (P ≥ 0.12) (Table 6). Total gas production and VFA production did not differ between inoculated silages and control silage (P ≥ 0.16) (Table 7).

Pre ensiled starch granules looks more aggregate than starch granules after storage period. In the 0d image is possible to notice the dense protein matrix unifying starch granules. After storage period, the protein matrix is reduced and fragmented, what facilitate microbial remen access and starch degradation (Figure 6)

Table 6: Chemical composition of RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) on the opening day.

Item[a]	Treatments			SEM	*P*-value[b]	
	CON	LBLH	LBLP		C1	C2
DM (g/kg)	601	598	601	2.03	0.06	0.64
CP (g/kg DM)	100	103	109	1.51	<0.01	<0.01
SP (g/kg CP)	336	348	327	30.7	0.52	0.07
Starch (g/kg DM)	742	752	726	8.56	0.82	0.21
WSC (g/kg DM)	5.41	3.66	4.02	0.41	0.01	0.57
Fat (g/kg DM)	27.6	30.6	28.8	0.21	0.34	0.46
Ash (g/kg DM)	12.5	12.4	12.0	0.42	0.56	0.53
TDN (g/kg DM)	872	881	873	3.33	0.23	0.12

[a]DM = dry mater; CP = crude protein; SP = soluble protein; WSC = water soluble carbohydrates; TDN = total digestible nutrients.
[b]C1: CON vs LBLH + LPLB; C2: LBLH vs LPLB

Table 7: Total gas and VFA production of RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) on the opening day.

Item	Treatments			SEM	*P*-value[b]	
	CON	LBLH	LBLP		C1	C2
Total gas production (ml/g DM)	209	212	221	6.72	0.16	0.17
Total VFA[a] (mM/g DM)	27.77	28.87	28.99	1.42	0.32	0.92

[a]VFA = volatile fat acids
[b]C1: CON vs LBLH + LPLB; C2: LBLH vs LPLB

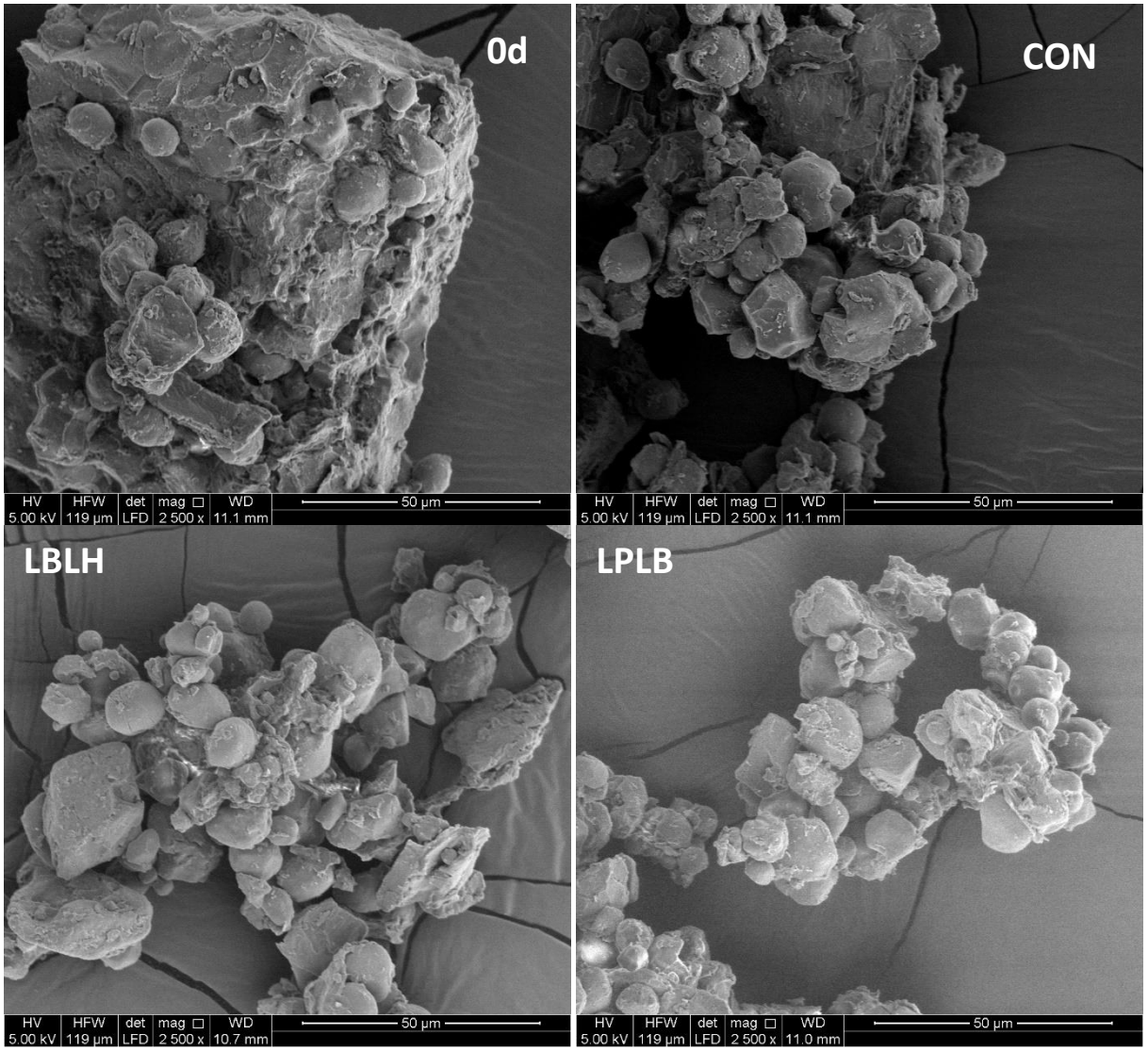

Figure 6: Starch granules of pre ensiled corn (0d), no inoculated RCGS (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) on the opening day.

Control supplement remained stable when exposed to air for 107.5 hours while inoculated treatments remained stable for the whole experimental period (Figure 7). DM loss was similar between treatments on day 3 ($P > 0.18$) and differ for non-inoculated supplement in day 6 ($P = 0.04$) it also differs between day 3 and day 6 ($P < 0.01$) (Figure 8). pH, yeasts, and molds had an interaction between days and treatment ($P < 0.01$) (Figures 9, 10 and 11). pH differs between inoculated and non-inoculated treatments and type of inoculant on day 0 and between inoculated and non-inoculated supplement on day 6 ($P < 0.01$). In all days, all treatments had a linear and quadratic

effect (P < 0.01). Yeasts were higher in all days for control (P> 0.01) and did not differ between inoculated treatments (P > 0.53). In treatment, yeasts had a linear effect in non-inoculated treatment. Mold counting was higher on all days for control treatment and tended to be higher in LPLB supplement on day 6; In days, it had a quadratic effect in control (Table 8).

Total gas production was lower in supplements using RCGS (P < 0.01) and similar for inoculated supplements (P = 0.68). It also presented a quadratic behavior as exposure days increase (P = 0.03). Total VFA production differ only between inoculated supplements (P = 0.04), and no differences were found in the other contrasts (P > 0.72) (Table 9).

DM tended to differ between non-inoculated and inoculated supplements (P = 0.09) and to have a linear comportment of reduction through days for DM (P < 0.01). No differences in bromatological analyses for supplements were found between inoculated treatments (P > 0.25) but fat (P < 0.01) was higher for inoculated treatments in comparison to non-inoculated and tended to be higher for NDF (P = 0.06). Starch and PB decreased linearly (P < 0.01) and NDF (P = 0.07) tended to decrease linearly while EE had a quadratic effect (P < 0.01) (Table 10).

Table 8: Aerobic stability, DM loss, pH and microbial counts of supplements made with RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB)

Item[a]	Treatment (T)			SEM	Exposing days (D)			SEM	*P*-value[b]						
	CON	LBLH	LBLP		0	3	6		T	D	T x D	C1	C2	L	Q
AS (hours)	107.5	>144	>144		-	-	-		<0.01	-	-	<0.01	1.00	-	-
DM loss (g/kg DM)	32.3	25.7	22.9	0.19	-	22.8	35.4	2.88	0.56	<0.01	0.05	0.40	0.26	<0.01	-
pH	6.03	5.68	5.69	0.02	5.26	5.78	6.36	0.02	<0.01	<0.01	<0.01	-	-	-	-
Yest (log CFU/g)	5.09	0.18	0.33	0.18	1.69	1.69	2.21	0.25	<0.01	<0.01	<0.01	-	-	-	-
Molds (log CFU/g)	1.97	1.94	2.13	0.11	1.77	1.79	2.48	0.11	0.42	<0.01	<0.01	-	-	-	-

[a]AS = aerobic stability, DM loss = dry matter loss during aerobic stability test.
[b]C1: CON vs LBLH + LPLB; C2: LBLH vs LPLB. L: linear contrast effect between exposing days; Q: quadratic contrast effect between days of exposure.

Table 9: Total gas and VFA production of supplements made with RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB)

	Treatment (T)			SEM	Exposing days (D)			SEM	*P*-value[b]						
Item	CON	LBLH	LBLP		0	3	6		T	D	T x D	C1	C2	L	Q
Total gas prod (ml/g DM)	198	186	188	3.52	179	198	193	2.71	0.07	0.01	0.06	-	-	-	-
Total VFA (Mm/g DM)	31.0	33.0	29.6	1.75	31.1	30.8	31.6	1.63	0.13	0.85	0.22	0.74	0.04	0.99	0.75

[b]C1: CON vs LBLH + LPLB; C2: LBLH vs LPLB.L: linear contrast effect between exposing days; Q: quadratic contrast effect between days of exposure.

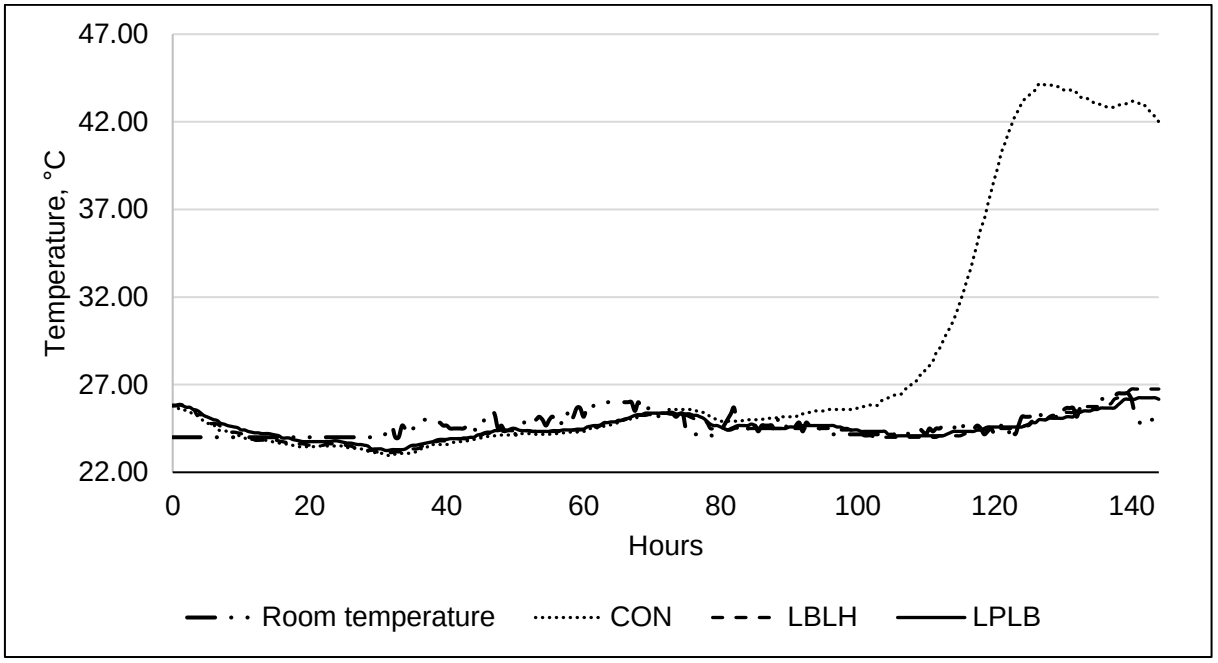

Figure 7: Temperature of supplements made with RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) during aerobic stability test.

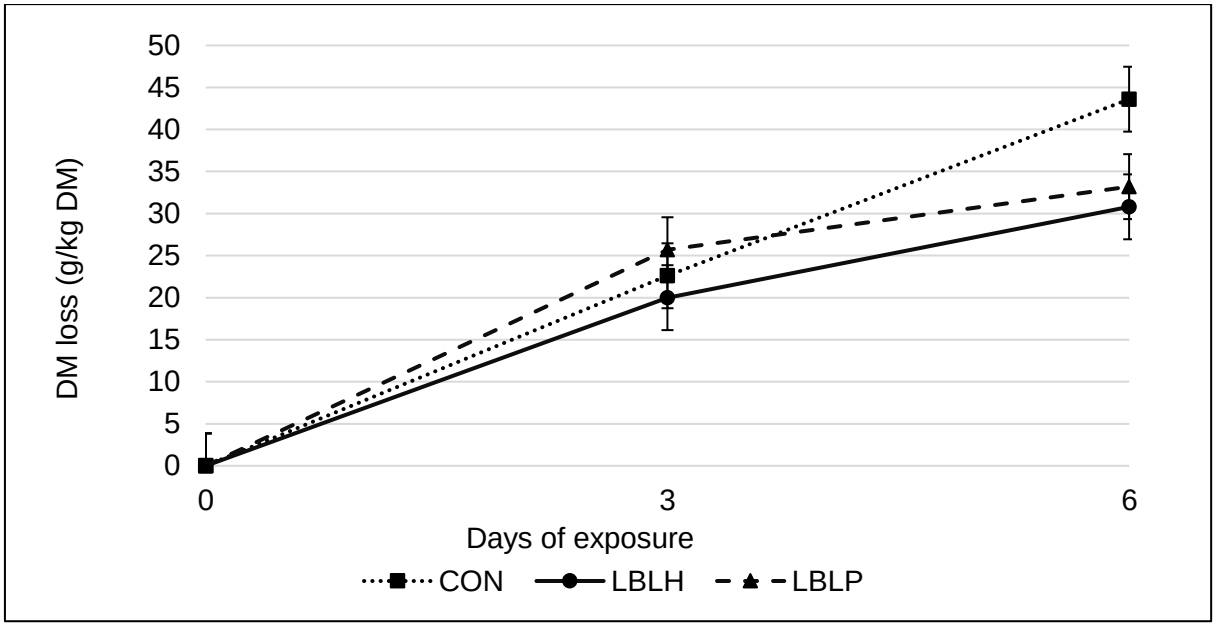

Figure 8: DM loss in supplements made with RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) exposed for 0, 3 and 6 days. Significant contrast effects (C1 and C2) of inoculant over aerobic exposing time: 3 d: NS. 6 d: C1, $P < 0.04$; C2, NS. Significant contrast effects (3 vs 6) of exposing length over treatment: CON: $P < 0.01$. LBLH: $P = 0.01$. LPLB: $P = 0.30$. *NS = P-value not statistically significant.

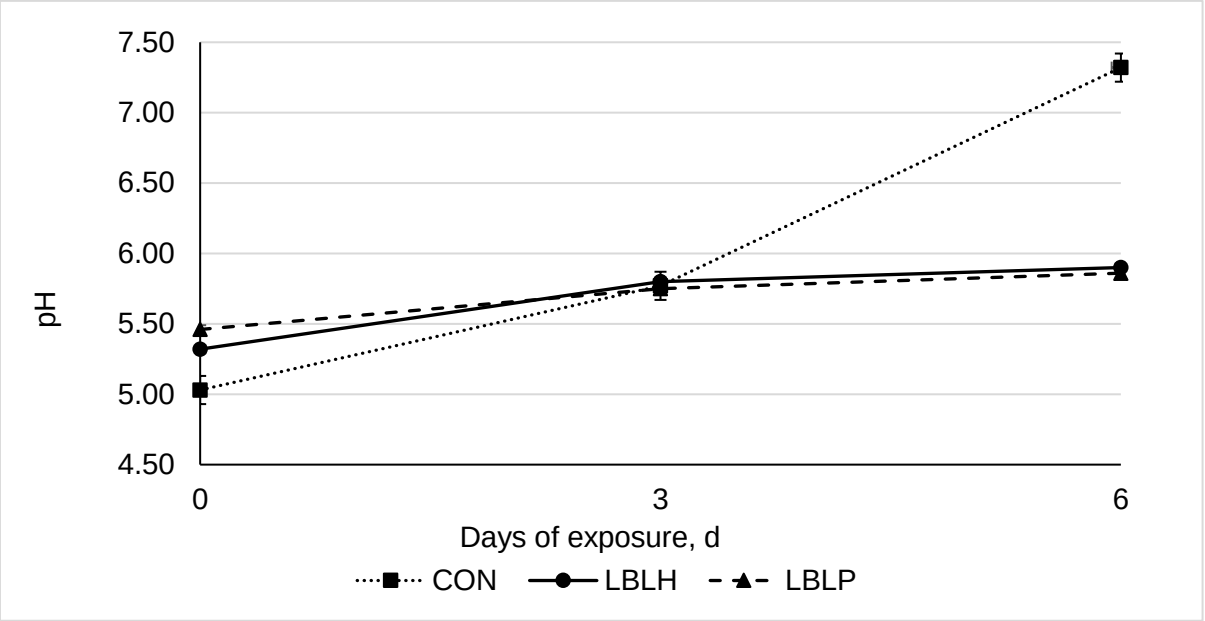

Figure 9. pH in supplements made with RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) exposed for 0, 3 and 6 days. Significant contrast effects (C1 and C2) of inoculant over aerobic exposing time: 0 d: C1, P < 0.01; C2 P < 0.01. 3 d: NS. 6 d: C1, P < 0.01; C2, NS. Significant contrast effects (L and Q) of exposing length over treatment: CON: L, P < 0.01; Q, P < 0.01. LBLH: P < 0.01; Q, P < 0.01. LPLB: P < 0.01; Q, P < 0.01. *NS = P-value not statistically significant.

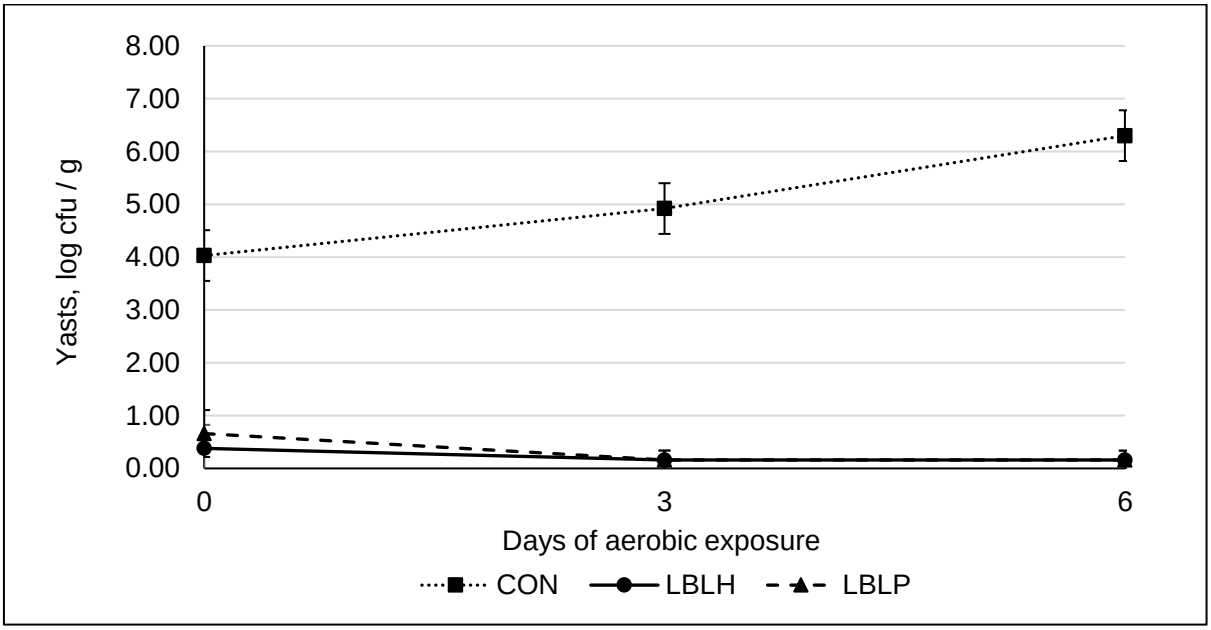

Figure 10. Yeast counts in supplements made with RCGS without inoculant (CON) and inoculated *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) exposed for 0, 3 and 6 days. Significant contrast effects (C1 and C2) of

inoculant over aerobic exposing time: 0 d: C1, P < 0.01; C2, NS. 3 d: C1, P < 0.01; C2, NS. 6 d: C1, P < 0.01; C2, NS. Significant contrast effects (L and Q) of storage length over treatments: CON: L, P < 0.01; Q, NS. LBLH: NS. LPLB: NS. *NS = P value not statistically significant

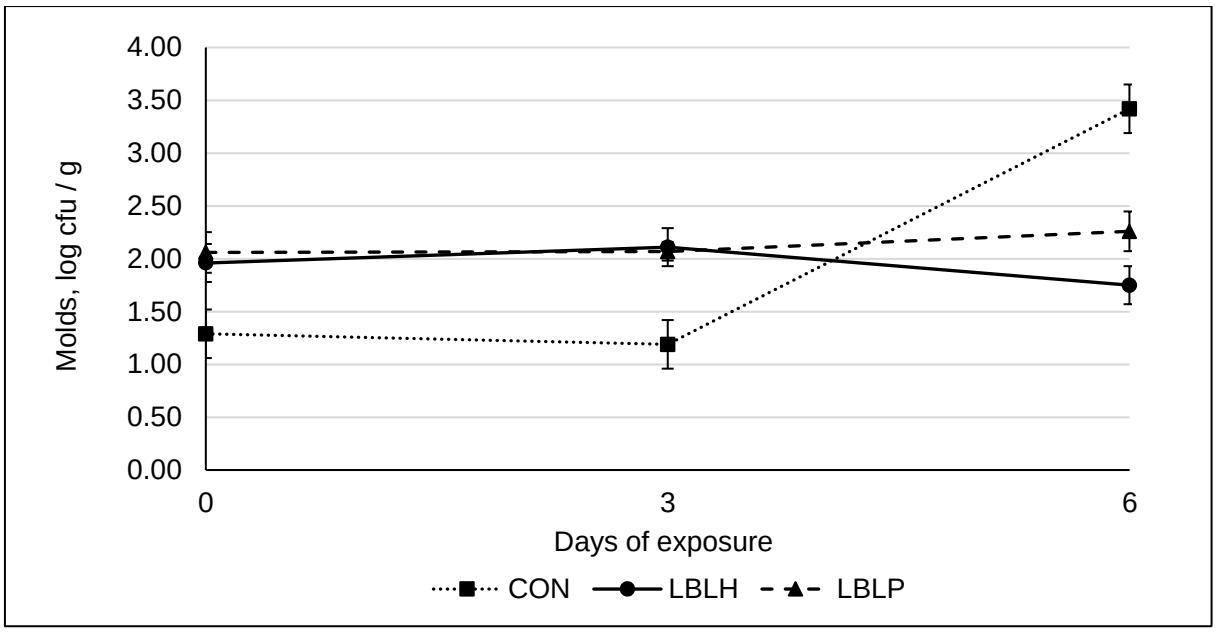

Figure 11. Molds counts in supplements made with RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) exposed for 0, 3 and 6 days. Significant contrast effects (C1 and C2) of inoculant over aerobic exposing time: 0 d: C1, P < 0.01; C2, NS. 3 d: C1, P < 0.01; C2, NS. 6 d: C1, P < 0.01; C2, NS. Significant contrast effects (L and Q) of storage length over treatments: CON: L, P < 0.01; Q, NS. LBLH: NS. LPLB: NS. *NS = P value not statistically significant.

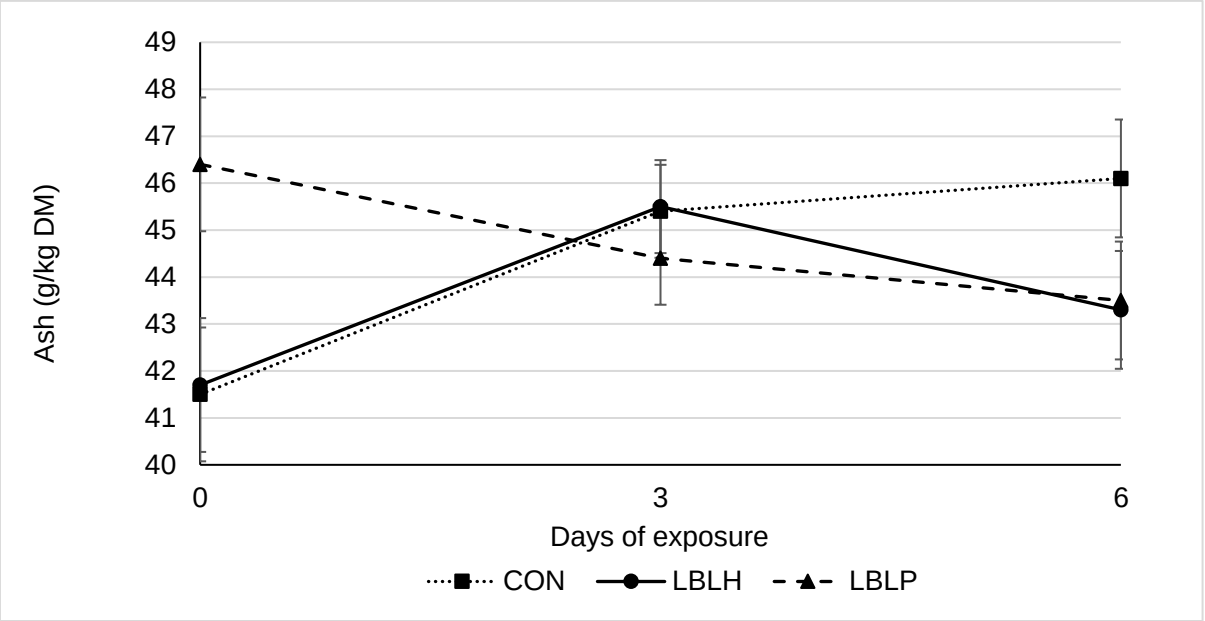

Figure 12: Ash concentration in supplements made with RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) exposed for 0, 3 and 6 days. Significant contrast effects (C1 and C2) of inoculant over aerobic exposing time: 0 d: C1, NS; C2, 0.03. 3 d: C1, NS; C2, NS. 6 d: C1, 0.09; C2, NS. Significant contrast effects (L and Q) of storage length over treatments: CON: L, P = 0.02; Q, NS. LBLH: L, P = 0.09; Q, P= 0.03. LPLB: L, NS; Q, NS. *NS = P value not statistically significant.

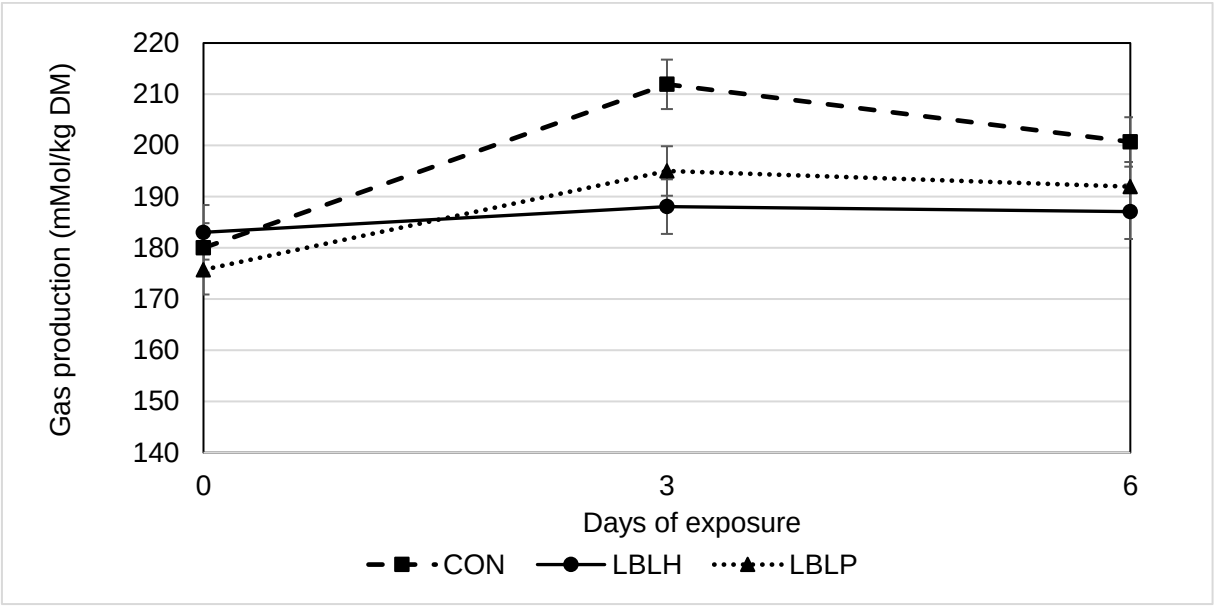

Figure 13: Gas production in supplements made with RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) exposed for 0, 3 and 6 days. Significant contrast effects (C1 and C2) of inoculant over aerobic exposing time: 0 d: C1, NS; C2, NS. 3 d: C1, $P < 0.01$; C2, NS. 6 d: C1, $P < 0.01$; C2, NS. Significant contrast effects (L and Q) of storage length over treatments: CON: L, $P < 0.01$; Q, < 0.01. LBLH: NS. LPLB: L, $P = 0.06$.

Table 10: Chemical composition of supplements made with RCGS without inoculant (CON) and inoculated with *L. hilgardii* CNCM I-4785 (1.5 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1.5 x 10^5 cfu/g) (LBLH) or *L. plantarum*, MTD/1 (1 x 10^5 cfu/g) + *L. buchneri* PJB/1 (2 x 10^5 cfu/g) (LPLB) exposed for 0, 3 and 6 days.

Item[a]	Treatments (T)			SEM	Exposing days (D)			SEM	*P*-value[b]						
	CON	LBLH	LBLP		0	3	6		T	D	T x D	C1	C2	L	Q
DM (g/kg)	721	719	721	0.15	724	718	712	0.10	0.14	<0.01	0.26	0.09	0.77	<0.01	0.88
CP (g/kg DM)	192	194	194	2.10	199	193	188	2.81	0.84	<0.01	0.70	0.55	0.99	<0.01	0.65
NDF (g/kg DM)	194	205	200	3.92	205	198	196	4.31	0.09	0.14	0.39	0.06	0.25	0.07	0.68
Starch (g/kg DM)	501	521	513	9.31	531	514	491	9.91	0.29	0.02	0.57	0.13	0.52	<0.01	0.77
Fat (g/kg DM)	44.2	46.8	46.4	0.87	43.7	48.9	44.8	1.15	0.09	<0.01	0.92	0.02	0.77	0.02	<0.01
Ash (g/kg DM)	44.3	43.5	44.4	0.72	43.2	44.7	44.3	0.76	0.61	0.29	0.01	0.66	0.38	0.16	0.27
TDN (g/kg DM)	781	778	781	2.42	773	787	780	2.43	0.54	0.01	0.25	0.66	0.31	<0.01	<0.01

[a] DM = dry mater; CP = crude protein; NDF = neutral detergent fiber; TDN = total digestible nutrients.

[b] C1: CON vs LBLH + LPLB; C2: LBLH vs LPLB; L: linear contrast effect between exposing days; Q: quadratic contrast effect between days of exposure.

4. Discussion

Higher DM loss in inoculated silage was due to obligatory heterofermentative LAB that produces ethanol and CO_2 which leads to higher DM loss (MCDONALD; HENDERSON; HERON, 1991). DM loss in silage during fermentation is mainly from CO_2 production (BORREANI et al., 2018). Conversion of lactic acid to acetic acid by *L. buchneri* also produces CO_2 (OUDE ELFERINK et al., 2001) in which increase DM loss during fermentation. The higher LAB number in both inoculated silages than the number of yeasts in CON silage also help to explain the higher DM loss during fermentation, once yeasts produce ethanol and CO_2 (DA SILVA et al., 2021). The lower pH in CON silage plus the high number of yeasts at opening indicates the low rate of obligatory heterofermentative LAB and more homofermentative LAB that only produces lactic acid without CO_2 losses. The use of *L. Plantarum* should decrease pH faster due to its metabolism, which avoids secondary fermentation that increases DM loss, however, DM loss data are inconsistent when *L. buchneri* is added to the silage (DA SILVA et al., 2021; KLEINSCHMIT; KUNG, 2006c), and when acetic acid is produced in significant amount, the inclusion of *L. Plantarum* is not capable of subtract *L. buchneri* effects on DM loss (BENJAMIM DA SILVA et al., 2024). Higher values of DM loss in this experiment in comparison with others (DA SILVA et al., 2021, 2024) can be due to enhanced fermentation of finer particle size of grain corn (SAYLOR et al., 2020).

It was expected lower pH in LPLB due to the use of *Lactobacillus Plantarum* as it produces mainly lactic acid (MUCK et al., 2018). Still, *Lactobacillus buchneri* can degrade lactic acid and produce acetic acid under anaerobic conditions as a mechanism of survival in low pH environments (OUDE ELFERINK et al., 2001) and this might act increasing pH in LPLB silages as concentration of *L. buchneri* was greater than *L. plantarum*. As in this experiment, corn grain silages inoculated with LBLH and LPLB had higher pH values than the control silage in other experiments (DA SILVA et al., 2021, 2024).

Plant and microbial enzymes can degrade protein in peptides and amino acids. Microorganisms can deaminate amino acids for their use resulting in $N\text{-}NH_3$ as a subproduct of their metabolism. Greater $N\text{-}NH_3$ concentration in inoculated silages

suggests greater proteolysis and it can be due to higher pH once enzymes are deactivated in low pH. Additionally, even though LAB has low protein activity, *L. Buchneri* may benefit proteolytic microorganisms by consuming lactic acid (JUNGES et al., 2017). The tendency for more N-NH_3 in LBLH silage reinforces the idea that *L. hilgardii* has a similar metabolism and starts its activity earlier.

The main source of protein in corn endosperm is in prolamin form covering starch granules and protecting them from ruminal degradation. Increase in N-NH_3 is associated with a decrease in prolamin as it is a result of deamination (DA SILVA et al., 2018). Greater MS degradability at 12 hours in inoculated silages is correlated with high degradation of prolamin by bacteria, as they are responsible for 60.4% of proteolytic activity (JACOVACI et al., 2021) and inoculated bacteria seem to be essential in this process as epiphyte LAB population is drastically reduced in the dry processing of corn kernels (Carvalho et al., 2015). *L. buchneri* inoculation increased DM degradability in other experiments (DA SILVA et al., 2018) and better degradability for LBLH is related to greater N-NH_3 that can be a result of early start of action by *L. hilgardii* using substrate that could be used for other lactic acid producers decreasing the pH and making it difficult for the action of proteolytic microbes.

Inoculation with specialized bacteria usually reduces bacteria diversity (DA SILVA et al., 2021, 2024). The low pH in control silage coupled with an anaerobic environment prevented mold growth. In inoculated silage, even though the higher pH, acetic acid avoided molds and yeast growth. The absence of acetic acid and the low pH tolerance favored yeast growth in the control silage. The greater concentration of obligatory heterofermentative bacteria in LBLH inoculant promoted greater production of acetic acid. Greater concentration of 1,2 propanediol in inoculated silages indicates the activity of *L. buchneri*. Higher concentration of this bacteria in LPLB led to a higher concentration of 1,2 propanediol when in relation to LBLH. The very low concentration of butyric acid indicates good fermentation during storage period.

Higher acetic acid concentration guarantees the aerobic stability of inoculated RCS for more than 216 hours. After these 9 days of testing, mold and yeast growth were less than 2 CFU and were considered not detectable in inoculated silages and pH remained unchanged. Yeats grow more easily in environments with a low concentration of weak acids and they are pH resistant using lactic acid as substrate for

their metabolism. Consequently, increasing pH opens the way for mold growth as they cannot survive in low pH environments. In a high pH environment with absence of weak acids, undesirable microorganisms start to grow after silage opening, consuming DM and increasing silage temperature which explains the low aerobic stability period in control silages. Inoculation of LBLH guaranteed more than 250 hours of aerobic stability in HMC ensiled for more than 30 days and inoculation with LBLP guaranteed similar results for aerobic stability and only 2.17 yeasts CFU were detectable after 140 hours of aerobic exposure in LPLB HMC (DA SILVA et al., 2021, 2024).

The tendency for lower DM in inoculated silage is due to more DM loss during fermentation as CO_2 produced in the fermentation process can escape as gas and water remains in silage mass. The lower concentration of sugar in inoculated silage indicates the use of these nutrients by LAB to ferment silage. *L. buchneri* has more affinity to ferment WSC when the pH is > 4 which indicates that WSC is more readily used in inoculated silages (KLEINSCHMIT; KUNG, 2006b).

Supplement

Reconstituted corn grain silage was the highest inclusion ingredient in the supplement. The mixture of silage with other ingredients increased the aerobic stability by approximately 115% for control silage. Total ration with sugar cane silage presented aerobic stability results with no more than 10 hours different from the aerobic stability of the sugarcane silage itself (AMARAL et al., 2009). When silage is mixed with other ingredients there are dilutions of organic acids that keep it stable, but, in this experiment, the other ingredients were dry which decreased water activity in the supplement, essential for microorganism growth (JOBIM et al., 2007). No differences in DM loss on day 3 was due control supplement remaining stable for more than 3 days, different on day 6 that control supplement had lost AS and lost more DM. DM losses in inoculated supplements can be mainly soluble nutrients and volatile compounds, principally in the first 3 days.

The lower pH value for the control supplement on day 0 was attributed to the lower pH values in the CON silage, as silage is the main ingredient. Similar pH for all treatments on day 3 indicates the consumption of lactic acid in the CON supplement

by yeast. Similarly, the higher number of yeasts in CON supplement was due to control silage. Under laboratory conditions, in this supplement, yeasts took more than 3 days to consume enough amounts of lactic acid to make the environments available for mold growth, which appeared on day 6. Both inoculants have proven efficient in preventing fungal growth in the supplement due to the fermentation profile of their silages that kept the supplement pH below 5.5.

The decrease in DM over the days of aerobic exposure might be attributed to the consumption of nutrients by microorganisms that produce CO_2, as evidenced by the reduction in the concentration of CP and starch, which are nutrients easily degradable compared to NDF. The reduction in these nutrients led to an increase in EE as it is less degradable by yeast and molds. The increase in EE explains the rise in TDN as it is 2.25x more energetic than CNF in the NDT equation. The control supplement lost aerobic stability after 4.7 days and the test ended 1.3 days after what might be too short time to observe differences in nutritional values as few nutritional values were affected by inoculation or interacted between treatment and inoculation.

It is important to mention that the ingredients and machines hygienic used in the preparation of the ration affect the aerobic stability of the ration or supplement (SEPPÄLÄ et al., 2013), and when it is made using a farm scale silo, the aerobic stability tends to reduce in comparison to ration made using laboratory silage (TAYLOR et al., 2002). Kung Jr, (2010) reported that more than 50% of 30 different TMR lost their stability in less than 12 hours when incubated in a controlled laboratory temperate of 22°C.

5. Conclusion

In laboratory conditions, both inoculants were effective in maintaining the aerobic stability of RCGS and supplement with 47% of RCGS for at least 9 and 6 days, respectively. It is better to store the supplement instead of the silage in aerobic conditions.

Due to the warmer environment in Mato Grosso, where the second experiment would be conducted, and the farm conditions, we decided to select the LBLH treatment

to use in the performance experiment silage due to both bacteria being obligatory heterofermentative bacteria.

6. References

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CHARPTER 3 – EXPERIMENT 2: INTAKE AND PERFORMANCE OF FINISHING BEEF CATTLE ON PASTURE RECEIVING RECONSTITUTED CORN GRAIN SILAGE SUPPLEMENTATION ON PASTURE EXPOSED FOR DIFFERENT DAYS

1. Introduction

Ensiling corn grain silage increases starch degradability, leading to the improvement of cattle efficiency (CAETANO et al., 2019; GODOI et al., 2021). In the case of flint corn, improvements are even better compared to dry corn processing methods, as it can get similar digestibility as ensiled dent corn (JACOVACI et al., 2021).

When HMC was included in diets for finishing cattle on pasture, in addition to improving efficiency, there was an improvement in ADG and final hot carcass weight (GONÇALVES, 2018). Usually, supplements for finishing cattle on pasture do not have sufficient levels of physically effective fiber, therefore, additives of intake and fermentation modulation are necessary in the diet to help in the control of subacute acidosis. The partial substitution of high-moisture corn with dry ground corn decreases the rate of starch fermentation in the rumen and has a positive effect on ADG and feed efficiency (STOCK; ERICKSON, 2006).

Inclusion of RCGS in beef cattle supplements presents conditions that are favorable to spoilage: it is exposed to air during mixing, has an easily degradable substrate, high moisture level and mixers and other ingredients might have a high number of undesirable microbes. In high-temperature environments, undesirable microbes find it easy to multiply in high moisture feed and, consequently, increase the temperature of high moisture supplement leading to the loss of its aerobic stability. In farm conditions, total mixed ration heating may occur within 12 h after mixing and has the negative potential to lose DM, reduce feed intake, and subsequently reduce carcass gain (KUNG, 2005).

Heterofermentative bacteria used as an inoculant in the silage produces antimicrobial acids during silage fermentation which inhibits mold and yeast growth.

These bacteria might be an alternative to guarantee the aerobic stability of the RCGS supplement guaranteeing similar performance for finishing cattle consuming it after aerobic exposure.

This study aims to determine the impacts of providing fresh and exposed RCGS supplements on the intake and performance of pasture-finished Nellore cattle with high supplementation. The hypothesis of this experiment is: cattle consuming RCGS supplement until 72 h after mixing will have similar performance to cattle consuming it fresh and both would perform better than cattle consuming a dry corn supplement.

2. Material and Methods

The experiment was carried out on Netolândia farm, in Tangará da Serra-MT (14° 39' 02" S 57° 53' 42" W) between March and June 2023. Animals used in this experiment arrived on different days on the farm. All Nellore bulls (N = 864) were fasted from solids for 12 hours, weighed, randomized blocked according to BW and assigned to 12 *Brachiaria Brizantha* cv. *Xaraés* paddocks. Animals from block 3 were weighed on March 14 (414 ± 0.6), block 2 (420 ± 0.5) on March 15 and block 1 on March 17 (394± 0.8). The experiment started 6 days after the final blockage and all animals were fasted and weighed again before sending to paddocks, initial weight was considered in this last weighing. Six paddocks had 8 hectares and the other six had 10 hectares. The paddocks were divided in the 3 blocks. Stock density in each paddock was 8 animals/hectare. Each paddock was equipped with a covered 18 x 0.7 m feed trough and a water trough. The animals could access the feed trough from both sides.

Four treatments were randomized in each block: CON - Cattle receiving supplement compost of dry rolled corn; T0 – animals receiving supplement where 70% of dry corn was replaced by RCGS and provided after preparation; T3 – animals receiving supplement where 70% of dry corn was replaced by RCGS and provided after 72 hours (3 days) of aerobic exposure; T6 - animals receiving supplement where 70% of dry corn was replaced by RCGS and provided after 144 hours of aerobic exposure. The supplement composition was similar to the one made in the first experiment differing only in the mineral supplement composition.

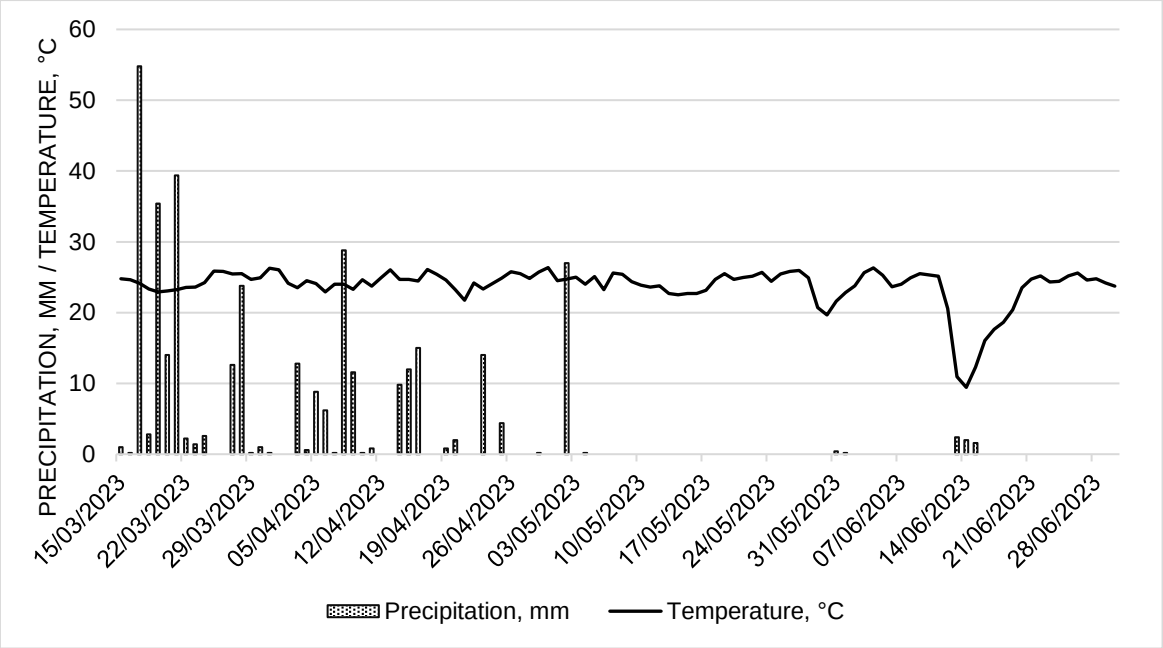

Figure 1: Daily precipitation and average temperature during experimental period.

Tabela 1: Supplements composition

Ingredients, % DM	Control supplement	RCGS supplement
Dry rolled corn	67,31	20,19
RCGS	-	47,12
Cottonseed whole	11,00	11,00
Cottonseed cage	17,40	17,40
Premix Mineral[1]	4,29	4,29

[1]Premix Mineral, guarantee levels: Calcium: 156g/kg, Phosphorus: 10g/kg, Sodium: 51 g/kg, Sulfur: 20 g/kg, Zinc: 1436 mg/kg, Magnesium: 17.3g/kg; Copper:435 mg/kg, Manganese: 446 mg/kg, Cobalt: 57 mg/kg, Iron: 435 mg/kg Iodine: 42 mg/kg, Selenium: 6 mg/kg, Urea: 298 g/kg Monensin: 747 mg/kg, Virginiamicin: 467mg/kg, Amaize: 12000U

Reconstituted corn grain silage used in this experiment was produced using a roller mill grain bagger machine (SGM 20, Multiagro, Porto Alegre, RS). The bagger machine was loaded by a grain buggy and 34.1 corn tons were ground per hour. The silage was inoculated with LBLH inoculant (*L. hilgardii* CNCM I-4785 (1 x 10^5 cfu/g) + *L. buchneri* NCIMB 40788 (1 x 10^5 cfu/g) and stored in a 50 m silo bag. The inoculation rate was 1.37 l per ton of rehydrated corn. Three silos were made and the first one was opened after 60 days. The silo was depleted daily to make the supplement, the average daily advance into the silo was 1.3 m and final DM was 60.7%. Fermentation products

concentration were 31.5 g/kg of lactic acid, 3.3 g/kg acetic acid and 0.001 g/kg of butyric acid.

Table 2: Distribution of RCGS and DRC particle size.

Sieve, mm	% Retained	
	RCGS	DRC
6 (Intact)	1.90	0.56
3.25	45.9	24.1
2	35.2	39.7
1.25	11.6	29.3
Bottom	5.40	6.47
GMD, mm	3.25	2.72
GSD, mm	0.46	0.29

GMD = geometrical mean particle size; GSD = geometrical standard deviation.
GMD equation calculated according to WILCOX; DEYOE; PFOST, (1970).

The supplement ingredients used were mixed in a stationary wagon (Reel Auggie 3136, KUHN, Passo Funso, RS). The amount of supplement daily made was enough to be offered in 3 days: on the day of preparation it was provided to T0 animals and the remaining was stored in a barn to be used after 72 hours and then after 144 hours. The supplement was distributed using a truck with a wagon (RD 25, Siltomac, São Carlos, SP) at 14:00.

Animals were fed for 102 days, divided into 3 periods of 37 days with one silo used in each period. Animals started receiving 1% of BW in supplement DM and 0.4 kg DM/animal was increased every clean trough reding until reaching 2% of BW. After 2%, 0.25 kg of supplement DM/animal was increased per day until leftovers. After the first appearance of leftovers, 0.25 g of supplement DM/animal was added after 3 days of no leftovers or reduced after 3 days of leftovers. Feed trough was managed to get leftovers around 0.3% of the offered and leftovers were mixed with the new distributed supplement. The amount of leftovers was estimated 1 hour before serving and discounted in the next feed furnace once the leftovers were not removed.

2.1. Forage mass and supplement:

The average height in each paddock was calculated on days 17, 52, and 88 (middle of each period) collecting the height of 100 random points across the whole paddock area using a ruler. On the next day, the total mass inside a 0.3 x 0.3 square was collected in 3 average height points in each paddock to estimate the total mass in the paddock. Two sub-samples of this mass were taken, one was used to estimate DM and the other separated into green leaf (GL), green steam (GS), dead leaf (DF), and dead steam (DS) to calculate the proportion of each component in the total forage mass. Samples were dried for 16 hours in a 105° C forced fan oven to get DM results.

On days 19, 54, and 90, to estimate forage nutritional quality, forage samples were collected in each paddock after previous observations on what cattle were grazing following the hand-plucking method (DE VRIES, 1995). Samples were oven-dried at 55° C for 72 hours and analyzed for DM, CP, NDF, ADF and ash.

Table 3: *Brachiaria brizatha* cv. Xaraés pasture quantitative and qualitative characteristics during experimental period.

Item[a]	Treatments				Period		
	CON	T0	T3	T6	1	2	3
Height (cm)	45.0	38.9	38.6	40.5	50.7	43.4	28.1
DM (g/kg)	336	298	325	296	262	269	410
Forage mass (kg DM/ha)	8031	6421	6841	6764	8075	7201	5767
GL (%/mass)	30.9	32.4	31.0	34.9	31.1	41.8	24.0
GS (%/mass)	31.9	31.5	33.6	32.2	31.9	33.0	32.0
DL (%/mass)	18.9	17.9	16.7	16.1	22.9	13.0	16.2
DS (%/mass)	18.3	18.2	18.6	16.9	14.1	12.2	27.7
Leaf mass (kg/ha)	4031	3308	3294	3519	4384	3941	2289
CP (g/kg DM)	153	152	139	156	159	170	120
NDF (g/kg DM)	64.2	625	655	627	669	585	658
ADF (g/kg DM)	295	285	306	290	312	253	317
Ash (g/kg DM)	67.5	65.2	64.9	70.1	65.2	65.8	69.9

[a]GL = green leaf; GS = green steam; DL = dead lead; DS = dead steam; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber. CON, control supplement; T0, RCGS supplement fresh; T3, RCGS supplement exposed for 72 hours; T6 RCGS supplement exposed for 144 hours.

Daily supplement samples were collected to adjust DM and the amount provided to the cattle. Weekly, samples of the supplements were collected and dried at 55°C. At the end of the experiment, weekly samples were proportional

mixed and analyzed for DM, CP, NDF, ADF, EE, ash, starch, and WSC following the methodology described in Exp. 1. NDT was calculated using WEISS; CONRAD; ST. PIERRE, (1992) and metabolizable energy corrected to monensin adjustment as: EM = (NDT*0.044)*0.82*1.023 (NASEM, 2016).

Table 4: Supplements chemical composition

Item	Treatments			
	CON	T0	T3	T6
DM (g/kg)	890	741	755	761
CP (g/kg DM)	171	181	171	170
SP (% CP)	30.9	44.6	41.2	36.9
ADF (g/kg DM)	140	116	118	115
NDF (g/kg DM)	257	215	231	225
Starch (g/kg DM)	449	469	476	473
Sugars (g/kg DM)	25.0	14.6	11.6	9.20
Fat (g/kg DM)	49.3	49.0	50.6	51.3
Ash (g/kg DM)	45.9	50.4	48.1	45.4
TDN (g/kg DM)	793	801	798	800
ME (Mcal/kg DM)	2.93	2.96	2.95	2.96

CON, control supplement; T0, Fresh RCGS supplement; T3, RCGS supplement exposed for 72 hours; T6 RCGS supplement exposed for 144 hours.

2.2. Performance, intake, feed efficiency and starch digestibility:

Performance was calculated by weighing the animals fasted (12 hours of solid fast) on D0 and D105. Average daily gain (ADG) was determined by the difference between final body weight (FBW, D105) and initial body weight (IBW, D0) divided by the finishing period (105 days). Individual supplement DMI was obtained as follows: offered (kg DM/paddock) / number of animals/paddock. Feed efficiency was calculated [(average ADG/paddock) / (average DMI of supplement/paddock)].

Ten different fresh feces from each paddock were collected on day 90 to measure the pH and starch. Feces were oven-dried at 55° for 3 days and then sent to a commercial lab to be analyzed for starch using NIRS and CP. Starch digestibility was calculated according to ZINN et al., (2007) considering the % starch of each supplement. Net energy for gain and maintenance of corn (Nem_{corn} and $Neg_{corn)}$ were

calculated following an equation developed by ZINN et al., (2007): Neg_{corn} =0,877× Nem_{corn} −0,41; Nem_{corn} =2,49−0,0127×FS−0,000292×FS^2; FS = fecal starch

2.3. Slaughtering and carcass characteristics:

At the end of the experiment, all animals were slaughtered in a commercial abattoir located in Tangará da Serra (MT), following the Regulations of Sanitary and Industrial Inspection for Animal Products (BRASIL, 2000). After slaughter, all carcasses were identified and weighed, to obtain the hot carcass weight (HCW). Carcass dressing was determined by the formula: CD = HCW / FBW. Carcass ADG was calculated considering the initial carcass dressing as 50%: HCW – (IBW *0.5) /105. Biological efficiency (Bio F:G) was estimated as the necessary intake to accumulate 15kg of carcass: total DM intake / ((HCW - (IBW * 0.5)) / 15).

2.4. Statistic

The experiment was designed in randomized blocks with 4 treatments, 3 blocks, and 3 repetitions per treatment. Data were analyzed using the PROC MIXED of SAS. Treatment was considered as fix effect and block was considered as random effect. Initial BW was included in the model and considered as covariable. For DMI, G:F, Bio F:G, feces pH, fecal starch, starch digestibility, ME intake, Nem_{corn} and Neg_{corn} the subject was paddock. For the other variables, animal(paddock) was the subject. Orthogonal contrasts were analyzed considering $P<0.05$ as significant effect and $P \leq 10$ was considered tendency: C1: CON vs T0; C2: linear effect of time of aerobic exposition in RCGS suplement; C3: CON vs T3+T6.

3. Results

Animals supplemented with RCGS accumulated 190 kg on average in the experimental period, 5 kg more than CON bulls, so final body weight tended to be greater for the RCGS group (C1, P = 0.05 and C3, P = 0.06) and similar among T0, T3 e T6 (C2, P = 0.85). The substitution of dry corn to RCGS affected intake and cattle

receiving fresh or exposed RCGS supplement tended to ingest 5 and 7.5% less than cattle receiving dry corn supplement respectively (C1, P = 0.09 and C3, P = 0.05). Exposing RCGS supplement before providing it did not affect intake, therefore, there was no linear effect in intake when it was aerobic exposed (C2, P = 0.83). Intake in % of BW was significant smaller for fresh and exposed treatments when compared to control treatment (C1 and C3, P < 0.01) and tented to have a linear effect as it was exposed (C2, P = 0.09). The substitution of dry corn to RCGS in the supplement increased the ADG for animals receiving this supplement fresh (C1, P = 0.03) and tended to increase when it was exposed to air (C3, P = 0.06). Exposing RCGS supplement before providing had no linear effect in ADG (C2, P = 0.52). Feed efficiency was better for RCGS treatments compared to control treatment (C1 and C3, P ≤ 0.03) and it did not have a linear effect over exposed treatments (C2, P = 0.99). Nellore cattle gain 10.5 % more in BW (0.21 vs 0.19 kg) for 1 kg DM RCGS supplement ingested (Table 5).

As final body weight, hot carcass weight from T0 animals tended to be heavier than CON HCW (C1, P = 0.09) and it did not present a linear effect until 6 days of exposition (C2, P = 0.18). Carcasses from CON treatment were similar to T3 and T6 carcasses (C3, P = 0.57). Carcass dressing in CON tended to be better than T3 and T6 (C3, P = 0.09) and did not differ in the other contrasts made (C1 and C2, P ≥ 0.43). Carcass ADG tended to be greater for animals consuming fresh RCGS supplement when compared to control group animals (C1, P = 0.09), but no difference was found in the other contrasts (C2 and C3, P ≥ 0.18). In contrast, the substitution of 70% of dry corn to RCGS improved biological efficiency in 6.8 kg for fresh RCGS supplement (C1, P = 0.04) and tended to improve in 5.4 kg for exposed RCGS supplement (C3, P = 0.06) and it had no linear effects over days of exposure (C2, P = 0.64) (Table 5).

Starch digestibility was 3.2% better in animals consuming RCGS supplements (C1 and C3, P < 0.01) and exposing time to air did not affect this digestibility (C2, P = 0.80). With better starch digestibility, starch concentration in feces collected in T0, T3 and T6 was, on average 5.1 points lower than the control treatment (C1 and C3, P < 0.01). Feces pH was 0.5 points lower in the control treatment when compared to feces pH from animals in T0 (C1, P = 0.01) or 0.37 points when contrasted to feces pH in exposed treatments (C3, P = 0.02). ME intake was similar between CON and T0

supplement and it did not have a linear effect for RCGS supplement (C2, P ≥ 0.16), still, it tended to be greater for control group in comparison to exposed supplement group (C3, P = 0.08). Corn Nem and Neg were greater for RCGS supplements (C1 and C3, P < 0.01) and had no linear effect for time of aerobic exposition (C2, P ≥ 0.74) (Table 6).

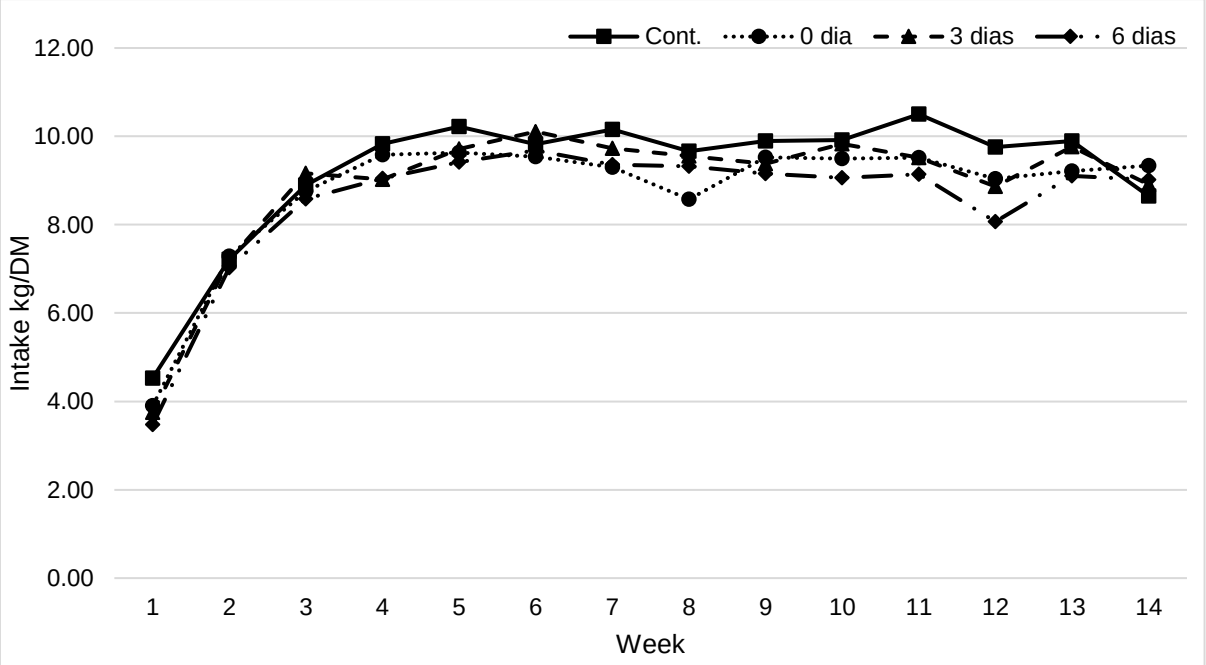

Figure 2: Intake in kg DM/week of Nellore cattle consuming supplement without RCGS (CON) or with RCGS provided after mixing (T0), 3 days after mixing (T3) or 6 days after mixing (T6).

Table 5: Performance and carcass of Nellore bulls finished on pasture consuming DRC supplementation (CON), fresh RCGS supplement fresh (T0); RCGS supplement exposed for 72 hours (T3) or RCGS supplement exposed for 144 hours (T6).

Item[a]	Treatments					P-value[b]		
	CON	T0	T3	T6	SD	C1	C2	C3
Initial BW (kg)	408	408	408	407	7.23	0.98	0.64	0.67
Final BW (kg)	593	598	599	596	7.14	0.06	0.85	0.05
DMI_{Sup} (kg/DM)	9.20	8.74	8.88	8.52	0.25	0.09	0.83	0.05
Sup. Intake (% BW)	1.84	1.74	1.77	1.70	0.00	<0.01	0.09	<0.01
ADG (kg)	1.78	1.85	1.85	1.82	0.09	0.03	0.52	0.06
G:F (kg)	0.19	0.21	0.21	0.21	0.01	0.03	0.99	0.02
HCW (kg)	347	350	347	346	4.91	0.09	0.18	0.57
Carcass Dressing (%)	58.4	58.3	58.0	58.3	0.5	0.50	0.43	0.09
ADGc (kg)	1.38	1.42	1.39	1.39	0.03	0.09	0.18	0.65
Bio F:G (kg/DM)	100	93.2	96.4	92.8	2.11	0.04	0.64	0.06
ME_{sup} intake (Mcal/kg DM)	26.9	25.9	26.2	25.2	0.75	0.16	0.60	0.08

[a]DMI_{sup} = dry matter intake of supplement; Sup. Intake = Supplement intake; ADG = average daily gain; F:G = feed efficiency (ADG/DMI_{sup}); HCW = hot carcass weight; ADGc = carcass average daily gain; BIO F:G = biological efficiency (total DMI / [(final HCW - Iinitial HCW) / 15]); ME_{sup} intake = daily intake of metabolize energy from supplement.
[b]C1, CON vs T0; C2 linear effect of aerobic exposing days; C3, CON vs T3 + T6.

Table 6: Fecal starch, starch digestibility, feces pH, and Nem and Neg of corn grain.

Item	Treatments[a]				SD	P-value[b]		
	CON	T0	T3	T6		C1	C2	C3
Fecal Starch (g/kg DM)	145	95.7	94.8	93.2	1.23	<0.01	0.77	<0.01
Starch digestibility (NIRS)	92.3%	95.2%	95.2%	95.4%	0.56	<0.01	0.82	<0.01
Feces pH	6.15	6.64	6.54	6.5	0.09	0.01	0.33	0.02
Starch digestibility (Zinn et al., 2007)	91.4%	94.3%	94.6%	94.7%	0.68	<0.01	0.60	<0.01
Nem_{Corn} (Mcal/kg DM)	2.24	2.34	2.34	2.35	0.03	<0.01	0.86	<0.01
Neg_{Corn} (Mcal/kg DM)	1.56	1.64	1.64	1.65	0.02	<0.01	0.74	<0.01

[a]CON, control supplement; T0, RCGS supplement fresh; T3, RCGS supplement exposed for 72 hours; T6 RCGS supplement exposed for 144 hours.
[b]C1, CON vs T0; C2 linear effect of aerobic exposing days; C3, CON vs T3 + T6.

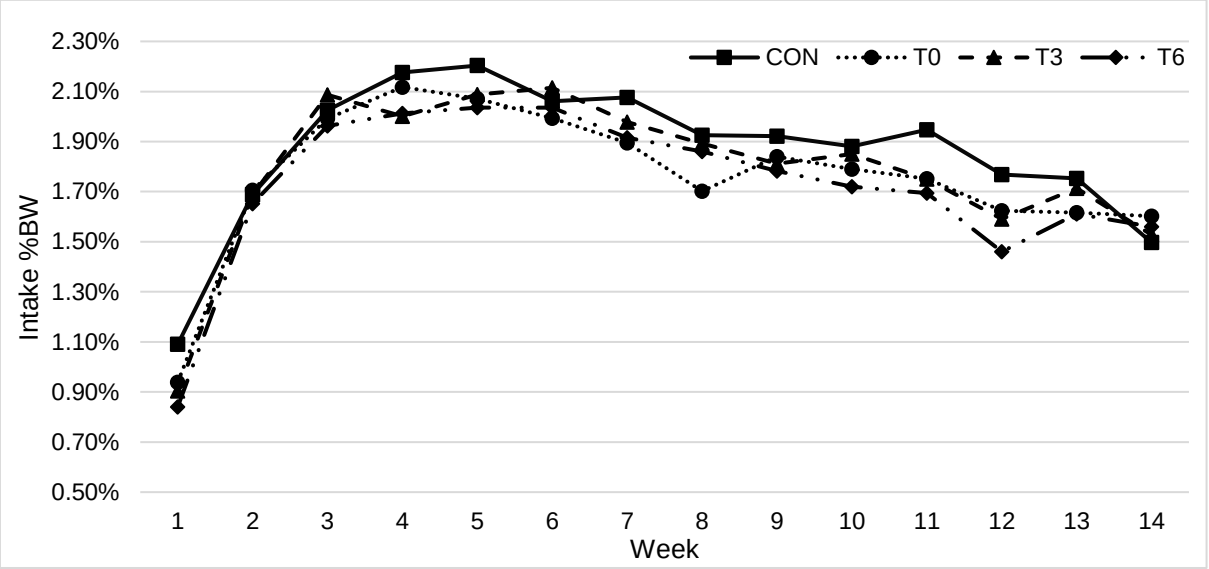

Figure 3: Intake in % of BW per week of Nellore cattle consuming supplement without RCGS (CON) or with RCGS provided after mixing (T0), 3 days after mixing (T3) or 6 days after mixing (T6).

4. Discussion

The idea of substituting 75% of of DRC for RCGS was conceived to maximize gain and feed efficiency as suggested by STOCK; ERICKSON, (2006) and to reduce risk of acidosis by decreasing rate of starch fermentation in the rumen once the roughage was not mixed in the supplement and its intake could not be guaranteed.

The DRC substitution improved ADG in 3.3%, 0.4 percentage points higher than that mentioned by STOCK; ERICKSON, (2006) in this level of substitution. In comparison to control treatment, while ADG was better for D0 supplement, it tended to be better for exposed supplement which might be influenced by T6 that had numerically lower intake probably due to some spoiling effects compromising ADG. OWENS et al., (1997) wrote a review and reported less intake, better efficiency and slight lower ADG for cattle consuming HMC diets attributing the smaller ADG due to the higher fermentation rate in the rumen, which increases subclinical acidosis. Better ADG in this study might be explained by the fact that corn was rolled which can produce larger and more uniform particles but still cracked kernels and, thus, the inclusion of 70% of RCGS provides starch with different fermentation rate and less chance of subclinical acidosis. Forage from pasture might also influenced in this result as cattle can access it when they feel necessary to maintain rumen health. This increase in ADG affected final body weight, however, it was less expressive than results found by GONÇALVES, (2018) where Nellore cattle on pasture consuming HMC supplement were 2.76% heavier than cattle consuming dry corn supplement. In a metanalyses comparing the effects of ensiling flint corn, it did not improve cattle gain (JACOVACI et al., 2021) and when 50% of DRC was replaced to HMC cattle performed numerically worse (Coulson at., 2023).

Ensiling reconstituted corn increases starch degradability, hence diets based on ensiled corn are supposed to have higher energy and capacity to reduce the DMI. Higher ruminal starch fermentability produces more propionate which is oxidized in the liver and induces hypophagia causing satiety and reducing meal size (ALLEN; BRADFORD; OBA, 2009; JACOVACI et al., 2021). In this experiment, reduction on supplement intake was on average 5% (500g), 70% replacement instead of 100% may affect this result as 50% of substitution reduced intake by 3% (COULSON et al., 2023).

When 100% of dry corn is replaced in high corn supplements for beef cattle on pasture the reduction was 7.5% (GONÇALVES, 2018).

Better gain with reduced intake resulted in 10.4% better feed efficiency for animals ingesting RCS supplement, which was 5 percentual points less when 100% of the dry corn was replaced (GONÇALVES, 2018). This better efficiency was attributed to better digestibility of starch after ensiling, which resulted from breakdown of prolamin (HOFFMAN et al., 2011). When dent corn is used, a 50% of substitution did not improve the feed efficiency when compared to a dry rolled corn diet (COULSON et al., 2023; VANDER POL et al., 2008). It is important to mention that dent corn is more digestible than flint corn, thus, the impact of ensiling in starch digestibility is less intense than in flint corn. Biological efficiency was also improved by including of RCGS in the diet, following the same principle of better F:G.

In feedlots, diets made of corn grain silage do not improve carcass when compared to dry corn diets (CAETANO et al., 2015; COULSON, 2021). However, on pasture, it improves carcass weight when there was use of lasalocid (GONÇALVES, 2018). In this experiment, delivery RCGS supplement immediately after mixing tended to improve HCW however, it did not improve HCW when RCGS supplement was exposed. Cursino (2024) observed that bulls consuming diets contaminated with mycotoxins excreted more N in urine, which means less N deposition in carcass. The previous supplement exposure in air might induced the grow of mold and these microorganisms might have produced mycotoxins reproducing similar effects of N excretion.

Calculated NDT and ME in diets were slightly greater for all RCS supplements. In terms of total intake, CON animals tended to ingest more ME than T3 and T6 animals but similar to T0 animals. CAETANO et al., (2019) observed small differences in ME from diets with HMC and finely ground corn and smaller ME intake for Nellore cattle consuming diets with HMC compared to Nellore consuming finely ground corn, even though, all treatments had similar ADG and final body weight but better F:G for HMC animals. Even with a smaller ME intake, ADG and FE were better for RCGS group supporting the great benefit of ensiling corn. The equations used in this experiment can not differ digestibility and ME from corn coming from different processing methods, although an equation developed by (ZINN et al., 2007) can differ Nem and Neg from

corn and explain the better results for RCGS group. Additionally, NASEM (2016) reports dry rolled corn ME and Neg as 3.17 and 1.49 and HMC as 3.27 and 1.56 respectively.

Fecal starch is correlated with total tract starch digestibility and it can explain more than 90% of the variation in starch digestibility (CORONA et al., 2005; ZINN; OWENS; WARE, 2002). By improving digestibility, less starch goes to the large intestine, therefore less substrate is available to be fermented by fecal microorganisms. These microorganisms produce VFAs responsible for decreasing the fecal pH as observed in this experiment and other experiments comparing corn grain silage diets with dry corn grain (CAETANO et al., 2019; COULSON et al., 2023; GODOI et al., 2021). Fecal starch concentration was inversely correlated to G:F (VANDER POL et al., 2008) and fecal pH (CAETANO et al., 2019; NUÑEZ et al., 2013; WHEELER; NOLLER, 1977) supporting results founded in this experiment.

In the premix used in this experiment there was inclusion of 1200 U of amylase. In an experiment comparing diets with RCGS and DRC, using amylase, there was no effect of RCGS on feed efficiency and intake and Nem and Neg were similar in both diets for finishing beef cattle (TRICARICO et al., 2007) differing from other studies without amylase where RCGS decreased intake, increased feed efficiency and Nem and Neg of diet (COULSON et al., 2023; DA SILVA, 2016; GERVÁSIO, 2021). These results suggests that results in this experiment may not have been as expressive as the ones found by GONÇALVES, (2018) due to the use of amylase which could have benefited control diet, once STOCK and ERICKSON, (2006) suggested this rate of inclusion would increase efficiency and gain.

Even with a high ambient temperature of 35° C and less sanitary control, the supplement with RCGS exposed to air for up to 6 days provided better performance than the dry corn supplement and was similar to the fresh supplement. Nonetheless, it is important to mention that exposed RCGS supplement, usually, lost its aerobic stability between 24 and 48 hours after mixing depending on environment temperature and the silo fermentation products were more similar to the CON treatment in the first experiment, in which might be effect of the high environmental temperature during ensiling process, that is prejudicial to LAB (ZHANG et al., 2018). Even though silage remained stable during all the experiment in the front panel, it had partially already

gone through some degree of aerobic exposure and spoilage before being mixed into the supplement (Kung, 2010) which can have some effect in the aerobic stability of the supplement.

5. Conclusion

Substituting 70% of DRC to RCGS in supplements for finishing cattle on pasture increases ADG and improves feed efficiency in Nellore cattle. The inoculation of the silage with LBLH inoculant was enough to guarantee better results of cattle consuming the RCGS supplement for up to 6 days old. No linear effects were detected for exposure time.

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