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ENRIQUECIMENTO PROTEICO DE RESÍDUOS AGROINDUSTRIAIS PARA ALIMENTAÇÃO DE RUMINANTES

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ENRIQUECIMENTO PROTEICO DE RESÍDUOS AGROINDUSTRIAIS PARA ALIMENTAÇÃO DE RUMINANTES

RESUMO

Esta pesquisa avaliou o enriquecimento proteico de resíduos agroindustriais com levedura (*Saccharomyces cerevisiae*) como fonte alternativa de proteína na alimentação de ruminantes. Três experimentos foram conduzidos: O primeiro estudo avaliou o efeito da inclusão de mandioca fermentada com *S. cerevisiae* no desempenho, consumo de ração, digestibilidade de nutrientes, microflora ruminal e fermentação ruminal em bovinos. Os efeitos da mandioca fermentada com levedura (YFC) nas dietas de bovinos foram avaliados usando a diferença média como tamanho do efeito. Análises de subgrupos e meta-regressão foram conduzidas para investigar a heterogeneidade em um nível de significância de 5%. A meta-análise revelou que a inclusão de mandioca fermentada aumentou significativamente o consumo de matéria seca e a digestibilidade de nutrientes. Houve um aumento nas concentrações de ácidos graxos voláteis ($P<0,001$) e propionato ($P<0,001$), juntamente com um aumento nas populações bacterianas e fúngicas ($P=0,004$) e uma redução na contagem de protozoários ($P<0,001$). Vacas lactantes alimentadas com mandioca fermentada produziram 1 kg/dia a mais de leite ($P=0,025$), com melhorias na composição do leite, incluindo aumentos de gordura ($P<0,001$), proteína ($P<0,001$) e lactose ($P=0,02$). A inclusão de YFC em concentrados bovinos melhorou a fermentação ruminal, a eficiência ruminal, a ingestão de matéria seca, a digestibilidade dos nutrientes, a produção e a composição do leite. O segundo estudo avaliou o uso de resíduos agrícolas como substratos para enriquecimento proteico com *S. cerevisiae*. Utilizou-se um delineamento fatorial 4×2 inteiramente casualizado, com quatro resíduos industriais (bagaço de acerola, raspa de mandioca, bagaço de laranja e casca de abacaxi) e duas avaliações (antes e depois do enriquecimento proteico), com três repetições. A composição química, as frações de carboidratos e proteínas e a produção de gases *in vitro* foram avaliadas. O processo de enriquecimento resultou em aumento significativo do teor de proteína e redução da fração fibrosa nos resíduos analisados ($P<0,001$). Os resíduos enriquecidos apresentaram menor produção total de gases em comparação aos resíduos não tratados ($P<0,001$). O enriquecimento proteico com *S. cerevisiae* é uma alternativa promissora para aumentar o teor proteico de resíduos agroindustriais. O terceiro estudo avaliou o impacto da substituição do farelo de soja por resíduos agroindustriais enriquecidos com levedura na cinética da fermentação ruminal e na produção de gases *in vitro*. Utilizando um delineamento fatorial 3×5 inteiramente casualizado com três repetições, o estudo testou diferentes resíduos agroindustriais (acerola, laranja e abacaxi) e níveis de substituição (0%, 25%, 50%, 75% e 100%) com base na matéria seca. A produção cumulativa de gases foi medida usando o modelo de Gompertz. Os resultados mostraram que a inclusão de resíduos de abacaxi aumentou a produção total de gases linearmente ($P<0,001$), enquanto o resíduo de acerola reduziu essa produção ($P<0,001$). A digestibilidade da matéria orgânica aumentou com a inclusão dos resíduos de laranja e abacaxi ($P<0,001$), enquanto o resíduo de acerola resultou em menores respostas nas variáveis avaliadas em comparação ao tratamento controle ($P<0,001$). Os resultados indicam que resíduos de laranja e abacaxi enriquecidos podem substituir até 100% do farelo de soja em concentrados, beneficiando a fermentação e a digestibilidade ruminal.

Palavras-chave: desempenho; eficiência alimentar; fermentação de resíduos; *Saccharomyces cerevisiae*; valor nutricional.

PROTEIN ENRICHMENT OF AGROINDUSTRIAL WASTE FOR FEEDING RUMINANTS

ABSTRACT

This research aimed to evaluate the potential of protein enrichment of agro-industrial residues with yeast (*Saccharomyces cerevisiae*) as an alternative protein source in ruminant feed. Three experiments were conducted: The first study assessed the effect of including cassava fermented with *S. cerevisiae* on performance, feed intake, nutrient digestibility, ruminal microflora, and ruminal fermentation in cattle. The effects of yeast-fermented cassava (YFC) in cattle diets were evaluated using the mean difference as the effect size. Subgroup analyses and meta-regression were conducted to investigate heterogeneity at a 5% significance level. The meta-analysis revealed that including fermented cassava significantly increased dry matter intake and nutrient digestibility. There was an increase in volatile fatty acid concentrations ($P<0.001$) and propionate ($P<0.001$), along with an increase in bacterial and fungal populations ($P=0.004$), and a reduction in protozoa count ($P<0.001$). Lactating cows fed with fermented cassava produced 1 kg/day more milk ($P=0.025$), with improvements in milk composition, including increases in fat ($P<0.001$), protein ($P<0.001$), and lactose ($P=0.02$). The inclusion of YFC in cattle concentrates improved ruminal fermentation, ruminal efficiency, dry matter intake, nutrient digestibility, milk production, and milk composition. The second study aimed to assess the use of agricultural residues as substrates for protein enrichment with *S. cerevisiae*. A completely randomized 4 x 2 factorial design was used, with four industrial residues (acerola bagasse, cassava root, orange bagasse, and pineapple peel) and two evaluations (before and after protein enrichment), with three repetitions. The chemical composition, carbohydrate and protein fractions, and *in vitro* gas production of the residues were evaluated. The enrichment process resulted in a significant increase in protein content and a reduction in the fibrous fraction in all analyzed residues ($P<0.001$). Enriched residues showed lower total gas production compared to untreated residues ($P<0.001$). Protein enrichment with *S. cerevisiae* is a promising alternative for increasing the protein content of agro-industrial residues. The third study evaluated the impact of replacing soybean meal with agro-industrial residues enriched with yeast on ruminal fermentation kinetics and *in vitro* gas production. Using a completely randomized 3×5 factorial design with three repetitions, the study tested different agro-industrial residues (acerola, orange, and pineapple) and levels of replacement (0%, 25%, 50%, 75%, and 100%) based on dry matter. Cumulative gas production was measured using the Gompertz model. Results showed that including pineapple residue increased total gas production linearly ($P<0.001$), while acerola residue reduced this production ($P<0.001$). Organic matter digestibility increased with the inclusion of orange and pineapple residues ($P<0.001$), while acerola residue resulted in lower responses in the evaluated variables compared to the control treatment ($P<0.001$). The results indicate that enriched orange and pineapple residues can replace up to 100% of soybean meal in concentrates, benefiting ruminal fermentation and digestibility.

Keywords: feed efficiency; nutritional value; performance; *Saccharomyces cerevisiae*; waste fermentation.

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1 CONSIDERAÇÕES INICIAIS

O aumento da demanda por produtos alimentícios de origem animal exige que sistemas de produção de ruminantes utilizem ingredientes alimentares não convencionais ou subprodutos agroindustriais que permitam um desempenho produtivo adequado e sejam economicamente viáveis (Mottet *et al.*, 2018).

O uso adequado de resíduos agroindustriais pode representar uma alternativa sustentável aos ingredientes convencionais, contribuindo para a redução dos custos de alimentação e melhorando a eficiência alimentar dos ruminantes (Azevêdo *et al.*, 2012). Entretanto, é importante destacar que muitos desses resíduos possuem composição nutricional heterogênea, o que tem limitado sua utilização na alimentação animal.

Uma solução promissora para enfrentar esse desafio é melhorar a qualidade nutricional dos resíduos agroindustriais por meio do processo de fermentação para gerar proteína microbiana, conhecida como "single cell protein" (SCP), produzida por fungos, algas ou bactérias (Nasseri *et al.*, 2011). As leveduras são consideradas uma escolha preferencial devido ao seu rápido crescimento, alto teor de proteína, baixo risco de contaminação e por serem uma fonte equilibrada de aminoácidos, além de fornecerem vitaminas, especialmente do grupo B (Ferreira *et al.*, 2010; Nasseri *et al.*, 2011; Øverland *et al.*, 2013).

Os substratos de origem agrícola amplamente empregados na produção de proteína microbiana incluem resíduos de casca de laranja, bagaço de cana-de-açúcar, resíduo de mandioca, polpa de beterraba, resíduos de manga, entre outros (Mensah e Twumasi, 2017; Spalvins et al., 2018). Os resíduos de frutas, devido ao alto teor de açúcares fermentáveis e outros nutrientes essenciais, também representam uma opção valiosa para o crescimento microbiano (Saheed *et al.*, 2016; Thiviya *et al.*, 2022).

Assim, o enriquecimento protéico de resíduos agroindustriais por meio de leveduras (*S. cerevisiae*) e sua utilização como fonte protéica em dietas de ruminantes parece ser uma tecnologia promissora. No entanto, as implicações do uso de resíduos agroindustriais enriquecidos com leveduras no desempenho de animais ruminantes ainda não são totalmente compreendidas.

2 HIPÓTESE CIENTÍFICA

O enriquecimento proteico de resíduos agroindustriais com leveduras pode melhorar a sua composição química;

Resíduos agroindustriais enriquecidos com leveduras podem substituir parcialmente as fontes convencionais de proteína na dieta de ruminante;

3 OBJETIVO

3.1 OBJETIVO GERAL

Avaliar o potencial dos resíduos agroindustriais enriquecidos com leveduras como uma fonte proteica alternativa na alimentação de ruminantes.

3.2 OBJETIVOS ESPECÍFICOS

Realizar uma revisão sistemática e meta-análise sobre o uso de mandioca fermentada com leveduras como fonte de proteína na alimentação de bovinos;

Promover o enriquecimento proteico de resíduos agroindustriais utilizando leveduras (*Saccharomyces cerevisiae*);

Determinar a composição química, fracionamento de proteínas e carboidratos, além da degradabilidade ruminal *in situ* dos resíduos agroindustriais;

4 REFERENCIAL TEÓRICO

4.1 PRODUÇÃO AGRÍCOLA E GERAÇÃO DE RESÍDUOS

A produção global de frutas tem apresentado um crescimento contínuo na última década, atingindo uma estimativa de 909,6 milhões de toneladas em 2021 (FAO, 2023). De maneira similar, o Brasil exportou mais de US$ 1,2 bilhão em frutas em 2023 (ABRAFRUTAS, 2023), consolidando-se como um dos líderes na produção de frutas no mercado internacional.

Os dados mais recentes do IBGE indicam que a área colhida de acerola no Brasil foi de 5.753 hectares, abrangendo 6.646 propriedades rurais (IBGE, 2023). O Brasil alcançou uma produção de acerola de quase 70 mil toneladas, gerando um valor de produção de R$ 91.6 milhões de reais. Estima-se que cerca de 40% da produção total da acerola seja gerada como resíduos, incluindo cascas e sementes, o que resulta em aproximadamente 28 mil toneladas de resíduos (Junior *et al.*, 2006). O estado de Pernambuco destaca-se como o maior produtor, com 21.351 toneladas, seguido pelo Ceará, que produziu 7.578 toneladas, e Sergipe, com 5.427 toneladas de frutas (IBGE, 2023).

Em relação ao abacaxi, a área colhida foi de quase 64 mil hectares (IBGE, 2023). No mesmo ano, foram produzidos 1,59 bilhão de frutos. Considerando que cerca de 30% da produção de abacaxi é composta por resíduos, como cascas e folhas, isso resulta em cerca de 477 mil toneladas de resíduos (Lima, P. C. *et al.*, 2017; Rogério *et al.*, 2007). O estado do Pará lidera a produção, com um valor de R$ 3,89 bilhões, seguido pela Paraíba, com R$ 471,61 milhões, e Tocantins, que alcançou R$ 346,1 milhões (IBGE, 2023).

Quanto à laranja, as informações apontam que a área colhida ultrapassou 575 mil hectares (IBGE, 2023). A produção brasileira de laranja em 2023 excedeu 17,6 milhões de toneladas, gerando um valor de produção de quase R$ 20 bilhões. Aproximadamente 50% dessa produção corresponde a resíduos, como cascas e bagaços, totalizando cerca de 8,8 milhões de toneladas de resíduos (Wikandari, 2014). O estado de São Paulo é o maior produtor, com um valor de R$ 15,43 bilhões, seguido por Minas Gerais, com R$ 1,36 bilhões, e Paraná, com R$ 951,1 milhões (IBGE, 2023).

Por fim, no que diz respeito à mandioca, o último censo revelou que a área colhida ultrapassou 1,2 milhões de hectares (IBGE, 2023). A produção brasileira foi superior a 18,5 milhões de toneladas, resultando em um valor de produção de R$ 19 bilhões. O estado do Pará é o maior produtor, com um valor de R$ 4,39 bilhões, seguido pelo Paraná, com R$ 3,03 bilhões, e Santa Catarina, com R$ 1,49 bilhões (IBGE, 2023).

A indústria de beneficiamento de frutas gera uma quantidade considerável de resíduos, compreendendo, em geral, a porção não comestível, como cascas, vagens, sementes e películas,

que representam cerca de 10 a 60% do peso total dos produtos frescos (Lalramhlimi *et al.*, 2022). As cascas são o principal subproduto, correspondendo a quase 30% do peso total, e podem alcançar proporções ainda maiores em algumas frutas, como banana (35%), mamão (10–20%), abacaxi (33%), manga (45%) e laranja (50%) (Lalramhlimi *et al.*, 2022).

Os resíduos de frutas têm sido aproveitados como ração animal, fonte de combustível, fertilizantes e até mesmo para a produção de novos produtos de valor agregado (‘Aqilah *et al.*, 2023; Ray, 2022). Porém, geralmente, devido ao seu baixo valor nutricional, para reduzir custos de produção, esses resíduos de frutas são frequentemente deixados sem uso ou sem tratamento no meio ambiente, gerando odores desagradáveis e ocasionando enormes problemas ambientais e de saúde (Sadh, Duhan e Duhan, 2018).

Aproveitar resíduos agroindustriais por meio de técnicas de fermentação utilizando leveduras pode ser uma abordagem promissora para reduzir desperdícios e impactos ambientais, além de gerar um recurso valioso para a dieta de ruminantes, beneficiando a produção animal e o meio ambiente.

4.2 LEVEDURAS (*Saccharomyces cerevisiae*)

As leveduras, pertencentes ao Reino Fungi e amplamente representadas pelo gênero *Saccharomyces*, como a espécie *Saccharomyces cerevisiae*, são fungos unicelulares de importância histórica e econômica (Chavez *et al.*, 2023; Spasov, Blagoeva e Zapryanova, 2023). Descobertas há séculos, essas leveduras se destacam pela capacidade de fermentar açúcares, sendo utilizadas na produção de alimentos e bebidas e, mais recentemente, em suplementos alimentares para ruminantes (Chaucheyras-Durand, Walker e Bach, 2008; Dijken, van, Weusthuis e Pronk, 1993). A *S. cerevisiae* é uma levedura anaeróbia facultativa, adaptando-se a ambientes com e sem oxigênio (Luzia *et al.*, 2023), o que facilita sua utilização no rúmen dos ruminantes, onde auxilia na remoção do oxigênio e no suporte às condições anaeróbias favoráveis aos microrganismos ruminais (Bakory, 2014; Wallace, 1994).

As leveduras são produzidas industrialmente por meio de cultivos em meios ricos em açúcares, geralmente obtidos a partir de fontes como melaço de cana (Muller *et al.*, 2023). A inclusão de leveduras na dieta de ruminantes já demonstrou diversos benefícios: aumento na digestibilidade de nutrientes, modulação da proporção de ácidos graxos voláteis no rúmen, redução na amônia ruminal e diarreia em bezerros, além de promover o crescimento da população de microrganismos ruminais, resultando em melhorias no desempenho produtivo, especialmente em vacas leiteiras (Alugongo *et al.*, 2017; Chaucheyras-Durand, Walker e Bach, 2008; Poppy *et al.*, 2012).

Embora os mecanismos de ação das leveduras ainda não estejam totalmente esclarecidos, acredita-se que suas contribuições para a melhora do desempenho nutricional dos ruminantes estejam associadas ao aumento do consumo de alimento, remoção do oxigênio do rúmen, aceleração da taxa de digestão da celulose, aumento do fluxo de proteína microbiana, estabilização do pH ruminal e otimização dos processos de fermentação e digestão (Bakory, 2014; Wallace, 1994).

4.3 METABOLISMO DA *Saccharomyces cerevisiae*

As leveduras, especialmente *S. cerevisiae*, utilizam uma variedade de fontes de carbono para seu crescimento, sendo os carboidratos as mais comuns (Soares Rodrigues *et al.*, 2023). Embora *S. cerevisiae* seja mais conhecida por sua capacidade de metabolizar carboidratos de configuração alfa, como monossacarídeos (frutose, glicose e galactose) e dissacarídeos (maltose e sacarose), sua atividade enzimática para degradar carboidratos complexos, como o amido, em açúcares simples de forma independente é limitada (Gupta *et al.*, 2003; Nurmalasari e Maharani, 2020; Soares Rodrigues *et al.*, 2023). No entanto, estudos demonstraram que *S. cerevisiae* possui atividade pectinolítica e hemicelulolítica, permitindo a degradação de alguns carboidratos de configuração beta presentes nas fibras vegetais, como pectinas e hemiceluloses (Afifi, 2011; Haske *et al.*, 2023; Sun *et al.*, 2021; Takeyama *et al.*, 2022)**.** Isso amplia a gama de substratos que podem ser utilizados por essa levedura, tornando-a capaz de metabolizar também resíduos vegetais que contêm essas fibras (Dunuweera, Nikagolla e Ranganathan, 2021).

Sob condições aeróbicas, o metabolismo de *S. cerevisiae* ocorre principalmente por meio da respiração aeróbica (Jouhten *et al.*, 2008), na qual os açúcares (geralmente glicose) são completamente oxidados a dióxido de carbono (CO_2) e água (H_2O), liberando energia na forma de ATP (adenosina trifosfato) para sustentar o crescimento celular (Nelson e Cox, 2017). Esse processo é composto por três etapas principais: primeiro, a levedura capta os açúcares e os converte em piruvato pela glicólise; em seguida, o piruvato é transportado para a mitocôndria, onde é metabolizado no ciclo de Krebs, produzindo intermediários ricos em elétrons. Esses elétrons são transferidos à cadeia de transporte de elétrons, resultando em fosforilação oxidativa e geração de ATP. A energia liberada é então utilizada na síntese de proteínas, lipídeos e componentes da parede celular, promovendo o crescimento e a multiplicação celular (Nelson e Cox, 2017).

Sob condições anaeróbicas, a *S. cerevisiae* realiza a fermentação alcoólica como alternativa para gerar energia (Luzia *et al.*, 2023; Nelson e Cox, 2017). Nesse processo, o

piruvato gerado na glicólise é convertido em etanol e CO_2, liberando ATP. Apesar de ser menos eficiente em termos de geração de energia comparada à respiração aeróbica, a fermentação permite que a levedura sobreviva e continue a produzir biomassa em ambientes com baixa disponibilidade de oxigênio (Luzia *et al.*, 2023; Nelson e Cox, 2017).

Além das fontes de carbono, o nitrogênio é um elemento essencial para o crescimento da *S. cerevisiae*, pois desempenha um papel fundamental na biossíntese de aminoácidos, proteínas e ácidos nucleicos (Kingsbury, Goldstein e McCusker, 2006; Magasanik e Kaiser, 2002). O nitrogênio é incorporado nas células de *S. cerevisiae* a partir de fontes como a ureia e o sulfato de amônio (Cruz, Da *et al.*, 2001; Godard *et al.*, 2007). A ureia, por exemplo, é hidrolisada pela enzima urease, convertendo-se em amônia (NH_3), que é então assimilada pela levedura e utilizada na síntese de aminoácidos. O processo de biossíntese de aminoácidos envolve a incorporação de grupos amina (NH_2) a esqueletos de carbono provenientes dos intermediários metabólicos gerados durante a glicólise e o ciclo de Krebs (Milne *et al.*, 2015; Mobley, Island e Hausinger, 1995).

A amônia (NH_3), após ser captada pela célula, pode ser incorporada ao ácido alfa-ceto-glutárico, formando glutamato, que, por sua vez, pode ser utilizado como precursor para a biossíntese de outros aminoácidos essenciais (Milne *et al.*, 2015; Mobley, Island e Hausinger, 1995). A síntese de proteínas envolve a ligação de aminoácidos para formar polipeptídeos, os quais, após dobramento e modificações pós-traducionais, se tornam proteínas funcionais, necessárias para a multiplicação celular e o funcionamento metabólico da levedura (Dever, Kinzy e Pavitt, 2016; Mustapha Abdulsalam *et al.*, 2024). A utilização eficiente de fontes de nitrogênio, como a ureia e o sulfato de amônio, é crucial para o crescimento da levedura, permitindo que ela produza biomassa rica em proteínas para sustentar sua proliferação e a manutenção de suas funções celulares (Brabender *et al.*, 2018; Yang *et al.*, 2021).

4.4 FERMENTAÇÃO EM ESTADO SÓLIDO E SUBSTRATO PARA PRODUÇÃO DE SCP

A fermentação em estado sólido é o processo de fermentação onde os microrganismos crescem em um substrato sólido com um ambiente sem água livre ou com teor de água livre muito baixo (o teor de água é geralmente inferior a 60%), de modo a melhorar a qualidade nutricional e a digestibilidade (Soccol *et al.*, 2017; Yang, Zeng e Qiao, 2021).

A escolha dos microrganismos e dos substratos são os fatores mais importantes a serem considerados durante o desenvolvimento de uma fermentação em estado sólido (Behera e Ray, 2016). Os fungos filamentosos e as leveduras são considerados os microrganismos mais apropriados para a fermentação em estado sólido, devido a capacidade de crescerem em

ambientes de baixa atividade de água (López-Pérez e Viniegra-González, 2016; Singhania *et al.*, 2009; Yang, Zeng e Qiao, 2021). No entatanto, algumas espécies de bactérias, por exemplo, *Bacillus subtilis*, *Bacillus thuringiensis* e *Lactobacillus* sp. foram relatadas capazes de produzir enzimas durante a fermentação em estado sólido (Singhania *et al.*, 2009; Yang, Zeng e Qiao, 2021).

Os ingredientes mais promissores para a fermentação em estado sólido incluem compostos formados basicamente por celulose, hemicelulose, lignina, amido, pectina e outras fibras. Resíduos agroindustriais, como bagaço de cana-de-açúcar, bagaço de mandioca, farelos de cereais como farelo de trigo, farelo de arroz, polpa e casca de café, cascas e polpas de frutas, espigas de milho, palhas e cascas de diversas origens são exemplos de substratos que podem ser utilizados na fermentação em estado sólido (Godoy *et al.*, 2018; Olukomaiya *et al.*, 2019; Vandenberghe *et al.*, 2021). Além disso, esses resíduos agrícolas não são apenas um suporte sólido para o crescimento da biomassa microbiana, mas também são uma fonte de carbono e nutrientes (Farinas, 2015; Lizardi-Jiménez e Hernández-Martínez, 2017).

Diversos estudos recentes têm investigado a produção de proteína microbiana para alimentação animal a partir de resíduos agroindustriais de frutas e vegetais (Table 1). Esses estudos, que comparam o teor de proteína bruta (PB) antes e depois do enriquecimento proteico com levedura, mostram um aumento significativo no conteúdo de PB na maioria dos casos, reforçando o potencial nutricional desses resíduos como ingredientes para rações animais. Por exemplo, resíduos de abacaxi, que inicialmente continham entre 4,6% e 7,6% de PB, apresentaram teores entre 14% e 20,2% após o processamento (Alexandre *et al.*, 2013; Neto *et al.*, 2017). De forma similar, resíduos de laranja e acerola mostraram aumentos expressivos, passando de 9,9% para 59,1% de PB no caso do resíduo de acerola (Araújo *et al.*, 2021) e de 14,1% para 26,7% no resíduo de laranja (Lima, V. F. de *et al.*, 2017). Esses resultados evidenciam a eficiência do processo de fermentação no enriquecimento proteico de subprodutos, promovendo uma alternativa sustentável para a alimentação animal.

Table 1 – Conteúdo de proteína bruta em resíduos de frutas antes e após enriquecimento por levedura (*S. cerevisiae*).

Referência	**Substrato (Resíduo de Fruta)**	**Conteúdo de PB Antes**	**Conteúdo de PB Depois**
Stabnikova et al., (2005)	Melancia, mistura de resíduos de frutas	—	37,5 – 44,4%
Abarshi et al., (2017)	Melancia, abacaxi	Melancia (6,4%), abacaxi (3,5%)	20% em ambos
Aruna et al., (2017)	Casca de inhame	4,4	13,4%
Bacha et al., (2011)	Mistura de cascas de batata, laranja, cenoura e maçã	Batata (5,1%), laranja (10,1%),	49,3%

		cenoura (8,7%), maçã (1,6%)	
Mondal, S. Sengupta e Bhowal, (2012)	Casca de pepino, casca de laranja	—	Pepino (53,4%), laranja (30,5%)
Umesh et al., (2017)	Mamão	—	27,9%
Res, Kandari e Gupta, (2012)	Maçã, mamão, banana	—	35,7%
Muniz et al., (2020)	Casca de goiaba, bagaço de caju	Goiaba (3,6%), caju (1,6%)	Goiaba (11%), caju (12%)
Khan et al., (2022)	cascas de banana, citros, batata e cenoura	—	47,8%
Alexandre et al., (2013)	Abacaxi	7,6%	20,2%
Neto et al., (2017)	Abacaxi	4,6%	20,1%
Araújo, Aguiar e Pessoa Coelho, (2019)	Abacaxi	12,2%	30,6%
Araújo et al., (2021)	Acerola	9,9%	59,1%
Lima, V. F. de et al., (2017)	Laranja	14,1%	26,7%
Silva et al., (2016)	Abacaxi	5,3%	14%
Boonnop et al., (2009)	Mandioca	3,4%	32,5%

PB: proteína bruta

Nos últimos anos, diversos estudos *in vivo* e *in vitro* têm investigado o potencial de resíduos enriquecidos com leveduras, como resíduos cítricos, mandioca e seringueira, como substitutos do farelo de soja em dietas para ruminantes (Table 2). Wanapat et al. (2011) demonstraram que a mandioca fermentada pode substituir o farelo de soja em até 100%, resultando em melhorias na produção e composição do leite. Em outro estudo, Wanapat et al. (2011) não observaram diferença significativa na produção de leite, mas encontraram aumento nos teores de proteína e gordura do leite em vacas em lactação suplementadas com feno de mandioca e mandioca fermentada. Promkot et al. (2013) relataram que vacas alimentadas com mandioca fermentada durante o período de 14 dias antes e 60 dias após o parto apresentaram aumento no consumo de ração e na digestibilidade de PB e FDN nos primeiros dois meses de lactação.

Table 2 – Resumo de estudos sobre o uso de resíduos enriquecidos com levedura na alimentação de ruminantes.

Referência	**Genótipo**	**Animal**	**Residuo enriquecido**	**Tratamento**	**Oferta diaria concentrado**	**Impacto de resíduos enriquecidos na dieta de ruminantes**
Boonnop et al. (2010)	Holstein Friesian	Novilhos leiteiros	Mandioca	Substituição do farelo de soja em 0%, 33%,	1% PV	↑ consumo de ração e digestibilidade dos nutrientes;

				67% e 100% no conc. MS		↑ fermentação ruminal e população microbiana ruminal; ↑ balanço de N e síntese de Pmic.
Khampa et al. (2010)	N.D.	Novilhas leiteiras	Mandioca	Substituição do conc. em 0%, 25%, 50% e 75% MS	1.5% PV	↑ desempenho; ↑ população microbiana ruminal. N.S. consumo de ração; N.S. fermentação ruminal.
Promkot et al. (2013) - prepartum	Holstein cruzados	Novilhas leiteiras	Mandioca	Adição em 11.9% do conc. MS	1.3% PV	↑ digestibilidade dos nutrientes; N.S. peso e escore corporal antes do parto; N.S. metabólitos sanguíneos.
Promkot et al. (2013) - postpartum	Holstein cruzados	Vacas em lactação	Mandioca	Substituição do farelo de soja em 100% no conc. MS (26% do conc. MS)	Volumoso: concentrado 30:70	↑ consumo de ração e digestibilidade dos nutrientes; ↑ pico de produção de leite; N.S. peso e escore corporal depois do parto; N.S. produção e composição do leite; N.S. metabólitos sanguíneos.
Promkot et al. (2017)	Brahman	Novilhas de corte	Mandioca	Adição em 0%, 10%, 20% e 30% do conc. MS	1.5% PV	N.S GMD, consumo de ração e digestibilidade dos nutrientes; N.S pH, NH-N_3 e população microbiana ruminal.
Promkot et al. (2020)	Brahman	Novilhas de corte	Mandioca	Substituição do farelo de soja em 0%, 20%, 25% e 30% no conc. MS	1.5% PV	↑ consumo de ração; N.S. digestibilidade dos nutrientes; N.S. fermentação ruminal e população microbiana ruminal; N.S. síntese de Pmic.
Sommai et al. (2020)	Thai cruzados	Novilhos de corte	Mandioca	Suplementação com a MFL em 0, 100, 200 e 300 g/dia	0.5% PV	↑ consumo de ração e digestibilidade dos nutrientes; ↑ fermentação ruminal e população microbiana ruminal; ↑ balanço de N.
Wanapat et al. (2011)	Holstein cruzados	Vacas em lactação	Mandioca	Substituição do farelo de soja em 100% no conc. MS (25.5% do conc. MS)	Proporção de concentrado para produção de leite em 1:2	↑ digestibilidade dos nutrientes; ↑ fermentação ruminal e população microbiana ruminal; ↑ composição do leite; N.S. produção de leite; N.S. consumo de ração.

Wanapat et al. (2011)	Holstein cruzados	Vacas em lactação	Mandioca	Substituição do farelo de soja em 0%, 33%, 67% e 100% no conc. MS	Proporção de concentrado para produção de leite em 1:2	↑ consumo de ração e digestibilidade dos nutrientes; ↑ fermentação ruminal e população microbiana ruminal; ↑ produção e composição do leite.
Gunun et al. (2022)	Holstein cruzados	Novilhas leiteiras	Semente de seringueira	Substituição do farelo de soja em 0, 100, 150, 200 e 250 g/kg MS no conc.	1% PV	↑ consumo de ração e digestibilidade dos nutrientes (EE); N.S. ácidos graxos voláteis e pH; N.S. síntese de Pmic e população microbiana ruminal.
Suriyapha et al. (2021)	Holstein cruzados: doadores liquido ruminal	Novilhos leiteiros	Resíduos Cítricos	Substituição do farelo de soja em 0%, 25%, 50%, 75% e 100% no conc. MS	Ensaio *in vitro*	Pode ser incluído em até 75% sem impacto negativo na cinética dos gases, parâmetros ruminais e digestibilidade *in vitro*.
Gunun et al. (2023)	Anglo Nubian cruzados	Caprinos	Mandioca	(1) Controle (concentrado); (2) substituição do concentrado por casca de mandioca fermentada por levedura; e (3) substituição do concentrado por casca de mandioca fermentada por mistura de microrganismos.	1.5% PV	N.S. consumo de ração e digestibilidade dos nutrientes; N.S. fermentação ruminal e ácidos graxos voláteis; N.S. ganho de peso; Menor custo financeiro por quilograma de dieta.
Suriyapha et al. (2022)	Holstein cruzados	Vacas em lactação	Resíduos Cítricos	0.4 kg/100 kg PV de farelo de soja; e 0.4 kg/100 kg PV resíduo cítrico enriquecido	Proporção de concentrado para produção de leite em 1:1	N.S. consumo de ração e digestibilidade dos nutrientes; ↑ amônia e nitrogênio ureico do sangue; N.S. ácidos graxos voláteis e pH; N.S. síntese de Pmic e balanço de N; N.S. produção e composição do leite; Menor custo financeiro por quilograma de dieta.

MFL: mandioca fermentada por levedura; Conc.: concentrado; MS: matéria seca; N.D.: não disponível; ↑: melhor/aumento; N.S.: não significativo; Pmic: proteína microbiana; GMD: ganho médio diário; PV: peso vivo; N: nitrogênio.

Além disso, Suriyapha et al. (2021) and Suriyapha et al. (2022) observaram que a inclusão de resíduos cítricos pode substituir até 75% do farelo de soja na matéria seca do concentrado, sem prejudicar a cinética dos gases, os parâmetros ruminais ou a digestibilidade *in vitro*, além de reduzir o custo por quilograma da dieta. De forma similar, Gunun et al. (2022) constataram que os resíduos da seringueira podem ser utilizados como uma fonte proteica, substituindo até 86% do farelo de soja no concentrado, o que resultaria em uma redução significativa nos custos de produção.

Há também relatos que a inclusão total ou parcial da mandioca fermentada por leveduras em dietas de vacas em lactação e novilhas leiteiras melhoraram a digestibilidade de nutrientes, ganho médio diário, fermentação ruminal, síntese de proteína microbina e aumenta a população de bactérias e fungos, mas reduz a população de protozoários (Boonnop, Wanapat e Navanukraw, 2010; Khampa *et al.*, 2010). Porém, os ensaios realizados com novilhos(as) de corte apresentaram resultados inconsistentes para ganho de peso médio diário, consumo de ração, digestibilidade dos nutrientes, fermentação ruminal e população microbiana ruminal (Promkot *et al.*, 2017; Promkot e Pornanek, 2020; Sommai *et al.*, 2020).

Esses resultados reforçam o potencial dos resíduos agroindustriais, como os cítricos, a seringueira e a mandioca fermentada, como alternativas promissoras para substituir parcialmente ou totalmente o farelo de soja na dieta de ruminantes. Além disso, o enriquecimento proteico com leveduras mostra-se uma estratégia eficaz para ampliar as opções de substitutos ao farelo de soja, possibilitando o uso de uma variedade ainda maior de resíduos agroindustriais. No entanto, a variabilidade nos resultados, especialmente em dietas para novilhos(as) de corte, sugere que mais estudos são necessários para otimizar as condições de inclusão e avaliar os efeitos a longo prazo dessas substituições. Dessa forma, o uso de resíduos enriquecidos com leveduras pode representar uma abordagem sustentável e econômica para a produção animal, contribuindo para a valorização de subprodutos agrícolas e para a redução da dependência de fontes proteicas convencionais.

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5 CAPÍTULO I – YEAST-FERMENTED CASSAVA AS A PROTEIN SOURCE IN CATTLE FEED: SYSTEMATIC REVIEW AND META-ANALYSIS

MANDIOCA FERMENTADA POR LEVEDURA COMO FONTE DE PROTEÍNA NA ALIMENTAÇÃO DE BOVINOS: REVISÃO SISTEMÁTICA E META-ANÁLISE

RESUMO

O presente estudo avaliou o efeito da inclusão de mandioca fermentada com leveduras *Saccharomyces cerevisiae* sobre o desempenho, consumo de ração, digestibilidade de nutrientes, microrganismos do rúmen e fermentação ruminal de bovinos por meio de uma revisão sistemática e meta-análise. Os efeitos da mandioca fermentada com levedura (YFC) na dieta de bovinos foram avaliados usando a diferença média como medida do tamanho do efeito, considerando um intervalo de confiança de 95%. Análises de subgrupo e meta-regressão foram realizadas para investigar a origem da heterogeneidade. O banco de dados incluiu oito experimentos. Três estudos foram relacionados a novilhas leiteiras, três relacionados a vacas leiteiras e os dois estudos restantes foram associados a novilhas de corte. A inclusão de YFC na dieta bovina aumentou o consumo de matéria seca %PC ($P < 0,01$) e a digestibilidade dos nutrientes ($P < 0,05$). Observamos um aumento no pH ruminal médio ($P < 0,01$), na concentração de ácidos graxos voláteis ($P < 0,01$) e no ácido propiônico ($P < 0,01$). Houve aumento significativo na população de bactérias ($P < 0,01$) e fungos ($P < 0,01$), e redução na contagem de protozoários no fluido ruminal ($P < 0,01$) nos animais alimentados com YFC. Vacas lactantes alimentadas com YFC produziram 1,02 kg/d a mais ($P < 0,01$) de leite do que vacas não suplementadas. Além disso, houve aumento de 7,4% na gordura ($P = 0,03$), 6,3% na proteína ($P < 0,01$) e 2,8% na lactose ($P = 0,02$) do leite de vacas suplementadas com YFC. Os resultados da presente meta-análise mostraram que a inclusão total ou parcial de YFC no concentrado bovino melhora a eficiência da fermentação e do rúmen, a ingestão de matéria seca, a digestibilidade dos nutrientes, a produção e a composição do leite.

Palavras-chave: Alimentação de ruminantes; Digestibilidade de nutrientes; Fermentação ruminal; População microbiana ruminal; Produção de leite

YEAST-FERMENTED CASSAVA AS A PROTEIN SOURCE IN CATTLE FEED: SYSTEMATIC REVIEW AND META-ANALYSIS

ABSTRACT

The present study evaluated the effect of the inclusion of cassava fermented with *Saccharomyces cerevisiae* yeasts on performance, feed intake, nutrient digestibility, rumen microorganisms and ruminal fermentation of cattle through a systematic review and meta-analysis. The effects of yeast-fermented cassava (YFC) in the diet of cattle were evaluated using the mean difference as a measure of the effect size, considering a confidence interval of 95%. Subgroup and meta-regression analysis were performed to investigate the origin of heterogeneity. The database included eight experiments. Three studies were related to dairy heifers, three related to dairy cow and the remaining two studies were associated to beef heifers. The inclusion of YFC in the bovine diet increased the dry matter intake %BW ($P < 0.01$) and nutrient digestibility ($P < 0.05$). We observed an increase in mean ruminal pH ($P < 0.01$), volatile fatty acid ($P < 0.01$) and propionic acid concentration ($P < 0.01$). There was a significant increase in the population of bacteria ($P < 0.01$) and fungi ($P < 0.01$), and a reduction in the protozoan count in the rumen fluid ($P < 0.01$) in the animals fed with YFC. Lactating cows fed YFC produced 1.02 kg/d more ($P < 0.01$) milk than non-supplemented cows. In addition, there was an increase of 7.4% in the fat ($P = 0.03$), 6.3% in the protein ($P < 0.01$) and 2.8% in lactose ($P = 0.02$) of milk of cows supplemented with YFC. The results of the present meta-analysis showed that the total or partial inclusion of YFC in cattle concentrate improves fermentation and rumen efficiency, dry matter intake, nutrient digestibility, milk yield, and milk composition.

Keywords: Ruminant feeding; Nutrient digestibility; Rumen fermentation; Rumen Microbial Population; Milk yield

5.1 Introduction

Cassava (*Manihot esculenta* Crantz) is a very common crop in tropical and subtropical areas, basically because of its resistance to hostile environments, dry climate and nutrient-poor soils (Sagrilo et al., 2008), in addition to representing an important source of fermentable carbohydrate (roots) and an excellent source of protein (leaves) in the ruminant diet. (Zeoula et al., 2002; Wanapat, 2009). However, cassava root has a low level of crude protein (2 – 4%) (Zeoula et al., 2002; Ramalho et al., 2006).

The process of protein enrichment using microorganisms in semi-solid culture to increase the concentration of protein in rations and food formulas has been the focus of many studies (Oboh and Akindahunsi, 2003; Oboh, 2006; Aro, 2008). Thus, the inclusion of cultures of microorganisms in the cassava root, mainly *S. cerevisiae*, can improve its nutritional value (Thongkratok et al., 2010; Kaewwongsa et al., 2011; Polyorach et al., 2013).

In the last decade, research has been carried out to test the effect of cassava fermented by *S. cerevisiae* yeasts in ruminant feed. However, the variation in responses to cassava supplementation fermented by *S. cerevisiae* yeasts is not well understood, and uncertainties remain as to whether supplementation of cassava fermented by S. cerevisiae yeasts can improve performance (Khampa et al., 2010; Wanapat, Boonnop, et al., 2011; Promkot et al., 2017), feed consumption (Wanapat, Polyorach, et al., 2011; Sommai et al., 2020), nutrient digestibility (Promkot et al., 2013; Promkot and Pornanek, 2020), rumen microorganisms (Boonnop et al., 2010; Khampa et al., 2010; Sommai et al., 2020) and rumen fermentation (Wanapat, Polyorach, et al., 2011; Promkot and Pornanek, 2020) in cattle.

This systematic review and meta-analysis therefore aim to better understand the effects of cassava supplementation fermented by *S. cerevisiae* yeasts on performance, feed intake, nutrient digestibility, rumen microorganisms and rumen fermentation in cattle.

5.2 Material and Methods

5.1 Literature search

The review question for carrying out this research was defined based on the principles of the PICO strategy, in which the population studied (P) was beef cattle or dairy cattle; the intervention analyzed (I) cattle fed with fermented cassava with yeast in the diet; comparison (C) was cattle fed without fermented cassava with yeast in the diet; and the result (O) referred to performance indicators, feed intake, nutrient digestibility and rumen fermentation.

A systematic review and meta-analysis were performed according to the PRISMA guidelines (Moher et al., 2009) (Supplementary material). The review protocol was not

registered anywhere. The largest number of scientific articles reporting the influence of YFC (S. cerevisiae) on cattle diets was identified by a complete systematic review. Five electronic databases were consulted for the recruitment of articles: PubMed (https://pubmed-ncbi-nlm-nih.ez14.periodicos.capes.gov.br/), Scopus (https://www-scopus.ez14.periodicos.capes.gov.br/search/form.uri?display=basic#basic), Web of Science (https://www-webofscience.ez14.periodicos.capes.gov.br/wos/woscc/basic-search), EMBASE (https://www-embase.ez14.periodicos.capes.gov.br/search/quick?phase=continueToApp) e Animal Health and Production Compendium (CABI Publishing) (https://www-cabi.ez14.periodicos.capes.gov.br/ahpc/).

The search for studies was performed equally in all databases using Boolean terms and operators in a single cross-reference search: (yeast AND fermented AND cassava AND (rumen OR cow OR cattle)). Articles identified as duplicates were removed, that is, duplicate articles were considered only once.

5.2 Selection criteria

Search results were imported into the free Rayyan web tool for systematic reviews (Ouzzani et al., 2016). Initially, the articles were selected by title and abstract. Inclusion criteria were: (1) studies published in the format of a research article; (2) studies that evaluated the effect of YFC on cattle diets; (3) studies that presented YFC results on performance, feed intake, nutrient digestibility and rumen fermentation; (4) studies published between 2000 and June 2022; (5) studies published in the English language.

For the screening phase, two reviewers independently assessed the articles using these criteria and, when there was disagreement in the choice of articles, a third reviewer was consulted to decide on the inclusion or exclusion of the article. The full texts of the relevant articles were examined in their entirety and selected for eligibility criteria. Microsoft Excel® software was used at all stages of screening.

5.3 Data Extraction

Eligible articles were collected and the following information extracted: Article identification: author, year of publication; Feed intake, nutrient digestibility and average daily gain: average daily gain (ADG) (kg/d), dry matter intake (DMI) (kg/d), DMI (%Body Weight, %BW), dry matter digestibility (DM) (%), organic matter digestibility (OM) (%), crude protein digestibility (CP) (%), ethereal extract digestibility (EE) (%), neutral detergent fiber digestibility (NDF) (%), acid detergent fiber digestibility (ADF) (%); Characteristics of ruminal

fermentation and blood urea nitrogen: rumen pH, ruminal temperature (°C), ruminal NH_3-N (mg/dL), blood urea nitrogen (BUN) (mg/dL), volatile fatty acid concentrations (VFA)) in rumen fluid (mm/L), acetate (%), propionate (%), butyrate (%) and acetate:propionate ratio; Microorganisms in rumen fluid and microbial nitrogen production: bacterial count ($x10^{10}$), protozoa ($x10^5$), fungal zoospores ($x10^5$), total viable bacteria ($x10^9$), cellulolytic ($x10^9$), amylolytic ($x10^7$), proteolytic ($x10^7$) and nitrogen microbial production (g N/d); Milk production and composition: milk production (kg/d), fat (%), protein (%), lactose (%), non-fat solids (%), total solids (%) and milk urea nitrogen (MUN) (mg/dL).

In addition, we collected the mean, standard deviation (SD) and number of animals in the treatment and control groups. Many studies reported a common standard error of the mean (SE) and these estimates were used for control and treatment groups. In publications that reported only the SE for the control and treated groups, the SD was derived from the formula described by Higgins et al. (2019): $SD = SE \times \sqrt{n}$

Where, SD = standard deviation, SE = standard error of the mean and n = number of animals in the treatment and control groups.

5.4 Statistical Analysis

The meta-analysis was performed using the R program, version 3.5.2 with RStudio (R Core Team, 2018), using the “meta” package (Schwarzer, 2012; Schwarzer et al., 2015) and "metafor" (Viechtbauer, 2010). Random effect meta-analysis was performed considering the a priori assumption of heterogeneity between studies. The methods DerSimonian and Laird (2015) were used to estimate the variation between studies. The effects of YFC in the diet of cattle for continuous response variables (performance, feed intake, nutrient digestibility and rumen fermentation) were evaluated using the mean difference (MD), which allows the expression of the effect size with the same unit as the measure, recommended by Takeshima et al. (2014). The MD was calculated as the mean of YFC-supplemented treatment minus the mean of control treatment, and each study was weighted by its corresponding sample variance (Viechtbauer, 2010).

Subgroup meta-analysis and meta-regression were used to assess possible sources of heterogeneity. Subgroup analysis was performed with the categories dairy heifer, lactating cow, pre-calving cow and beef heifer. Meta-regression using the model DerSimonian and Laird (2015) was performed to determine whether the level of YFC supplementation (g/kg DM of concentrate) influenced performance responses, feed intake, nutrient digestibility and ruminal fermentation of cattle using the “metareg” command.

The heterogeneity of the results was analyzed using a χ^2-based Q test and the I2 statistic to assess the actual variation due to heterogeneity (Cochran, 1954; Higgins, 2003; Borenstein et al., 2017). I2 shows the proportion of variance, ranging from 0% to 100%, and looks at the effect of the actual size of all studies in the analysis (Borenstein et al., 2017).

Potential publication bias was assessed by visually inspecting the funnel plots and objectively using the Egger test. Publication bias was assessed in the analysis of subgroups with at least ten articles (Sterne et al., 2011). The "trim-and-fill" method was used to evaluate the best estimate of the unbiased effect size of the variables that presented publication bias (Shi et al., 2019). In all analyses, a value of $P < 0.05$ was considered statistically significant.

5.3 Results

5.3.1 Results of the systematic review

In the initial search, 448 potentially relevant studies were identified. After excluding duplicate publications, 370 articles remained. After primary screening of titles and abstracts, 26 articles were selected for full-text search. Among them, seven full articles were retrieved for eligibility verification and included in this review.

Overall, the database included eight experiments and 40 evaluated animals. Three studies were related to dairy heifers, three related to dairy cattle and the remaining two studies were associated to beef heifers. Although the number of comparisons between the group supplemented with YFC and the control group is low in some variables (i.e., three comparisons), a meta-analysis with a minimum of three comparisons is possible (Valentine et al., 2010). With the exception of the work of Promkot et al. (2013) with prepartum cows that had only one comparison (control vs. treatment), all other categories have at least three comparisons.

The studies included in this review and meta-analysis used a Latin square design, except Promkot et al. (2013) who evaluated two groups of eight animals (control and supplemented with YFC) treated for 14 days prepartum and two groups of eight animals (control and supplemented with YFC) treated for 60 days postpartum. In addition, we observed different YFC products, in which YFC chip protein (YEFECAP) was evaluated in five works and YFC root (YEFECAR) was evaluated in the remaining three.

The inclusion of fermented cassava in the animals' diet occurred by total or partial replacement of soybean meal, or partial replacement of the concentrated mixture. The offer of concentrate was made as a percentage of live weight (1.3% or 1.5% BW), forage: concentrate ratio of 30:70, or as a function of milk production (1 kilo of concentrate for 2 liters of milk).

Dairy crossbreds in early lactation with average milk production ranging from 12.6 to 15.7 kg/day were used. The roughage source of the diets was rice straw or rice straw treated with urea offered ad libitum or in the proportion 30:70 roughage: concentrate. A detailed description of the studies included in this review and meta-analysis reporting the effect of YFC on cattle performance is presented in Table 3.

5.3.2 DMI, nutrient digestibility and average daily gain

The inclusion of YFC in the cattle diet increased DMI %BW (+0.14; $P < 0.01$; I2 = 30%) and nutrient digestibility (DM, MO, CP, EE, NDF and ADF) (Table 4). The DMI (kg/d) of dairy heifers (+0.87; $P < 0.01$; $I^2 = 0\%$), pre-calving cows (+2.00; $P < 0.01$) and lactating cows (+1.96; $P = 0.01$; $I^2 = 68\%$) fed with YFC was higher compared to the control group. Similarly, DMI %BW was higher in dairy heifers (+0.12; $P < 0.01$; $I^2 = 66\%$) and lactating cows (+0.39; $P < 0.01$; $I^2 = 0\%$) compared to pre-calving cows and beef heifers. On the other hand, beef heifers fed YFC reduced feed intake (-0.59; $P = 0.02$; $I^2 = 15\%$) compared to the control group.

Table 3 – Summary of publications included in the meta-analysis.

Author	Study design	Phenotype	Animal	Fermented cassava product	No. of comp.	Treatment	Supply of concentrate per day	Source of roughage	Evaluated variables
Boonnop et al. (2010)	Four animals in 4x4 latin square design	Holstein Friesian	Dairy steers	YEFECAP	3	Soybean meal replacement at 0%, 33%, 67% and 100% of the DM of the concentrate.	1% BW	Urea-treated rice straw	Feed intake, nutrient digestibility, rumen microorganisms and rumen fermentation
Khampa et al. (2010)	Four animals in 4x4 latin square design	Dairy heifers	Dairy heifers	YEFECAP	3	Replacement of concentrate at 0%, 25%, 50% and 75% DM	1.5% BW	Rice straw	Performance, feed intake, rumen microorganisms and rumen fermentation
Promkot et al. (2013) - prepartum	Two groups of eight animals (control and YFC supplemented) treated for 14 days pre-partum.	Holstein crossbred	Dairy heifers	YEFECAP	1	Addition of 11.9% of the DM of the concentrate	1.3% BW	Rice straw	Performance, feed intake, nutrient digestibility and rumen fermentation
Promkot et al. (2013) - postpartum	Two groups of eight animals (control and YFC supplemented) treated for 60 days postpartum.	Holstein crossbred	Dairy cows	YEFECAP	1	Soybean meal replacement in 100% of the DM of the concentrate (26% of the DM of the concentrate)	Roughage: concentrated at 30:70	Rice straw	Performance, feed intake, nutrient digestibility and rumen fermentation
Promkot et al. (2017)	Four animals in 4x4 latin square design	Brahman beef cattle	Non-pregnant female	YEFECAR	3	Addition at 0%, 10%, 20% and 30% of the DM of the concentrate	1.5% BW	Rice straw	Performance, feed intake, nutrient digestibility, rumen microorganisms and rumen fermentation

Promkot et al. (2020)	Four animals in 4x4 latin square design	Brahman beef cattle	Non-pregnant female	YEFECAR	3	Soybean meal replacement at 0%, 20%, 25% and 30% of the DM of the concentrate.	1.5% BW	Rice straw	Feed intake, nutrient digestibility, rumen microorganisms and rumen fermentation
Wanapat, Boonnop, et al. (2011)	Four animals in 4x4 latin square design	Holstein crossbred	Dairy cows	YEFECAP	1	Soybean meal replacement in 100% of the DM of the concentrate (25.5% of the DM of the concentrate)	Ratio of concentrate to milk production at 1:2	Urea-treated rice straw	Performance, feed intake, nutrient digestibility, rumen microorganisms and rumen fermentation
Wanapat, Polyorach, et al. (2011)	Four animals in 4x4 latin square design	Holstein crossbred	Dairy cows	YEFECAP	3	Soybean meal replacement at 0%, 33%, 67% and 100% of the DM of the concentrate	Ratio of concentrate to milk production at 1:2	Urea-treated rice straw	Performance, feed intake, nutrient digestibility, rumen microorganisms and rumen fermentation

No. of comp.: number of comparisons between treatment and control groups; YEFECAP: yeast fermented cassava chip protein; YEFECAR: yeast-fermented cassava root

The inclusion of YFC in the diet of dairy heifers and lactating cows improved the digestibility of dry matter and its components ($P < 0.01$) (Table 4). In dairy heifers, nutrient digestibility was improved by up to 6.60% (CP) and in lactating cows by up to 7.43% (EE). However, there was no significant effect on nutrient digestibility of pre-calving cows and beef heifers fed YFC ($P > 0.05$) (Table 4).

Table 4 – Effects of YFC supplementation in cattle diets on average daily weight gain, dry matter intake and nutrient digestibility.

Group/Sub-group	No. of comp.	Control group mean	Mean Difference (95% CI)		Heterogeneity			Funnel te
			Random effect	*P*-value	χ^2 (Q)	I^2	*P*-value	*P*-value
Average daily gain (kg/d)								
Dairy heifer	3	0.38	0.02 (0; 0.05)	0.071	57.3	97%	<0.001	
Beef heifer	3	0.79	0.11 (-0.21; 0.43)	0.507	0.6	0%	0.740	
Total	6	0.58	0.02 (0; 0.05)	0.060	58.18	91%	<0.001	
Dry matter intake (kg/d)								
Dairy heifer	3	11.80	0.87 (0.24; 1.49)	0.006	0.02	0%	0.989	
Pre-calving cow	1	11.60	2.00 (1.45; 2.55)	<0.001	na			
Lactating cow	5	11.20	1.96 (0.87; 3.04)	<0.001	12.49	68%	0.014	
Beef heifer	6	9.30	-0.59 (-0.15; 1.29)	0.015	5.86	15%	0.320	
Total	15	10.59	0.57 (-0.15; 1.29)	0.118	91.46	85%	<0.001	0.2
Dry matter intake (%BW)								
Dairy heifer	6	2.49	0.12 (0.05; 0.20)	<0.001	14.57	66%	0.012	
Pre-calving cow	1	2.70	-0.10 (-0.65;0.45)	0.724	na			
Lactating cow	4	2.63	0.39 (0.16; 0.62)	<0.001	1.03	0%	0.794	
Beef heifer	6	2.45	0.00 (-0.41; 0.41)	0.996	1.15	0%	0.949	
Total	17	2.52	0.14 (0.08; 0.19)	<0.001	22.96	30%	0.115	0.7
Apparent total tract digestibility, %DM Dry matter								
Dairy heifer	3	64.00	4.73 (2.87; 6.60)	0.003	11.34	82%	<0.001	
Pre-calving cow	1	62.10	2.10 (-1.23; 5.43)	0.216	na			
Lactating cow	5	62.42	6.19 (2.67; 9.71)	<0.001	9.68	59%	0.046	
Beef heifer	6	61.30	1.42 (-0.46; 3.29)	0.138	3.42	0%	0.636	

Total	15	62.30	3.95 (2.55; 5.35)	<0.001	38.59	64%	<0.001	0.61
Organic matter								
Dairy heifer	3	67.80	4.90 (2.04; 7.76)	<0.001	23.56	92%	<0.001	
Pre-calving cow	1	63.40	1.10 (-1.67; 3.87)	0.437	na			
Lactating cow	5	68.28	4.51 (3.20; 5.83)	<0.001	3.6	0%	0.462	
Beef heifer	3	55.40	1.93 (-0.15; 4.01)	0.069	0.14	0%	0.931	
Total	12	64.53	3.78 (2.42; 5.13)	<0.001	39.49	72%	<0.001	0.17
Crude protein								
Dairy heifer	3	65.20	6.60 (3.63; 9.57)	<0.001	26.57	92%	<0.001	
Lactating cow	5	66.48	5.53 (4.14; 6.92)	<0.001	2.95	0%	0.567	
Beef heifer	6	65.80	0.71 (-1.13; 2.55)	0.450	0.75	0%	0.980	
Total	14	65.91	4.54 (2.86; 6.23)	<0.001	63.20	79%	<0.001	0.11
Ether extract								
Dairy heifer	3	61.60	4.53 (2.53; 6.53)	<0.001	32.50	94%	<0.001	
Lactating cow	3	62.70	7.43 (4.94; 9.93)	<0.001	0.6	0%	0.740	
Total	6	62.15	5.36 (3.67; 7.06)	<0.001	38.09	87%	<0.001	
Neutral detergent fiber								
Dairy heifer	3	61.10	5.33 (3.14; 7.53)	<0.001	44.87	96%	<0.001	
Pre-calving cow	1	54.10	2.40 (-1.20; 6.0)	0.192	na			
Lactating cow	5	62.24	3.58 (2.37; 4.78)	<0.001	3.3	0%	0.509	
Beef heifer	6	46.40	1.15 (-1.04; 3.34)	0.305	0.03	0%	1.000	
Total	15	55.13	3.64 (2.37; 4.91)	<0.001	69.34	80%	<0.001	0.06
Acid detergent fiber								
Dairy heifer	3	57.90	6.03 (3.38; 8.69)	<0.001	24.91	92%	<0.001	
Pre-calving cow	1	53.30	0.70 (-2.07; 3.47)	0.621	na			
Lactating cow	5	59.40	2.73 (1.08; 4.38)	<0.001	0.73	0%	0.947	
Beef heifer	6	41.20	1.45 (-0.73; 3.63)	0.193	0.19	0%	0.999	
Total	15	51.41	3.24 (1.67; 4.81)	<0.001	59.63	77%	<0.001	0.00

No. of comp.: number of comparisons between treatment and control groups; na: Not available; χ^2 and I^2 provided to show the degree of heterogeneity among studies included in the meta-analysis.

5.3.3 Rumen fermentation characteristics and blood urea nitrogen (BUN)

Cattle fed with YFC showed an increase in mean rumen temperature (+0.22; P = 0.01; I^2 = 0%), mean ruminal pH (+0.16; P < 0.01; I^2=96%), VFA (+7.68; P < 0.01; I^2 = 70%) and concentration of propionic acid (+3.05; P < 0.01; I^2 = 24%). However, there was a reduction in blood urea nitrogen (-1.84; P < 0.01; I^2 = 84%), acetic acid concentration (-2.33; P < 0.01; I^2 = 0%) and C2:C3 ratio (-0.55; P < 0.01; I^2 = 64%). YFC supplementation did not influence NH_3-N concentration (P > 0.05) and butyric acid concentration (P > 0.05) (Table 5).

Table 5 – Effects of YFC supplementation in cattle diets on rumen fermentation characteristics and blood urea nitrogen.

Group/Sub-group	No. of comp.	Control group mean	Mean Difference (95% CI)		Heterogeneity			Funnel tes
			Random effect	*P*-value	χ^2 (Q)	I^2	*P*-value	*P*-value
pH								
Dairy heifer	6	6.52	0.20 (0.10; 0.30)	<0.001	108.27	95%	<0.001	
Beef heifer	6	6.75	0.03 (-0.05; 0.12)	0.437	5.48	9%	0.361	
Lactating cow	4	6.35	0.20 (0.09; 0.31)	<0.001	100	97%	<0.001	
Total	16	6.56	0.16 (0.08; 0.23)	<0.001	343.51	96%	<0.001	0.24
Ruminal temperature (°C)								
Dairy heifer	3	39.20	0.20 (0.01; 0.39)	0.041	0	0%	1.000	
Lactating cow	4	39.13	0.30 (-0.07; 0.66)	0.109	0.19	0%	0.979	
Total	7	39.16	0.22 (0.05; 0.39)	0.011	0.41	0%	0.999	n
NH_3-N (mg/dL)								
Dairy heifer	6	13.70	3.11 (1.54; 4.68)	<0.001	23.12	78%	<0.001	
Beef heifer	6	11.49	-0.04 (-0.81; 0.73)	0.920	0.42	0%	0.995	
Lactating cow	4	17.43	-1.54 (-3.37; 0.29)	0.100	5.84	49%	0.119	
Total	16	13.80	0.63 (-0.74; 2.01)	0.367	113.57	87%	<0.001	0.03
Blood urea nitrogen (mg/dL)								
Dairy heifer	6	12.50	-1.27 (-3.04; 0.50)	0.160	52.38	90%	<0.001	
Pre-calving cow	1	11.50	0.70 (-1.24; 2.64)	0.480	na			
Lactating cow	5	15.62	-3.11 (-3.91; -2.30)	<0.001	4.91	19%	0.296	
Total	12	13.72	-1.84 (-2.93; -0.75)	<0.001	70.96	84%	<0.001	0.00

Molar proportion of VFA (mol/100 mol)								
VFA (mmol/L)								
Dairy heifer	3	104.70	8.23 (3.33; 13.13)	<0.001	6.49	69%	0.039	
Beef heifer	3	75.60	-2.83 (-12.44; 6.77)	0.563	0	0%	0.998	
Lactating cow	4	91.35	13.14 (2.07; 24.20)	0.020	15.68	81%	<0.001	
Total	10	90.63	7.68 (3.52; 11.84)	<0.001	29.99	70%	<0.001	0.31
Acetate %								
Dairy heifer	3	63.40	-3.07 (-5.36; -0.77)	0.009	2.85	30%	0.241	
Beef heifer	3	65.80	-1.83 (-12.72; 9.05)	0.741	0	0%	0.998	
Lactating cow	4	65.48	-1.24 (-3.61; 1.14)	0.308	0.27	0%	0.965	
Total	10	64.95	-2.33 (-3.81; -0.85)	0.002	4.51	0%	0.874	0.74
Propionate %								
Dairy heifer	3	22.00	3.67 (1.08; 6.26)	0.006	7.26	72%	0.027	
Beef heifer	3	24.00	1.73 (-1.79; 5.25)	0.335	0.47	0%	0.789	
Lactating cow	4	22.63	2.89 (2.37; 3.41)	<0.001	2.55	0%	0.466	
Total	10	22.85	3.05 (2.21; 3.88)	<0.001	11.86	24%	0.222	0.99
Butyrate %								
Dairy heifer	3	14.60	-0.53 (-1.49; 0.43)	0.276	0.12	0%	0.942	
Beef heifer	3	10.20	0.77 (-1.47; 3.01)	0.502	0.9	0%	0.638	
Lactating cow	4	11.90	-1.37 (-4.15; 1.41)	0.335	0.42	0%	0.936	
Total	10	12.20	-0.43 (-1.27; 0.41)	0.321	3.02	0%	0.964	0.83
C2:C3 ratio								
Dairy heifer	3	3.30	-0.90 (-1.69; -0.91)	<0.001	6.63	70%	0.036	
Beef heifer	3	2.70	-0.27 (-0.75; 0.21)	0.286	0.26	0%	0.878	
Lactating cow	4	2.90	-0.40 (-0.59; -0.22)	<0.001	2.72	0%	0.437	
Total	10	2.96	-0.55 (-0.78; -0.32)	<0.001	22.66	60%	0.007	0.49

No. of comp.: number of comparisons between treatment and control groups; na: Not available; χ^2 and I^2 provided to show the degree of heterogeneity among studies included in the meta-analysis.

In dairy heifers, YFC supplementation in the concentrate provided a higher mean ruminal pH (+0.20; $P < 0.01$; $I^2 = 95\%$), increased mean rumen temperature (+0.20; $P = 0.04$; $I^2 = 0\%$), NH_3-N (+3.11; $P < 0.01$; $I^2 = 78\%$), VFA (+8.23; $P < 0.01$; $I^2 = 69\%$) and concentration

of propionic acid (+3.67; P < 0.01; I^2 = 72%). However, there was a reduction in the concentration of acetic acid (-3.07; P < 0.01; I^2 = 30%) and in the C2:C3 ratio (-0.90; P < 0.01; I^2 = 70%).

Lactating cows fed YFC increased rumen pH (+0.20; P < 0.01; I^2 = 97%), VFA (+13.14; P = 0.02; I^2 = 81%), and propionic acid concentration (+2.89; P < 0.01; I^2 = 0%), but decreased BUN (-3.11; P < 0.01; I^2 = 19%) and the C2:C3 ratio (-0.40; P < 0.01; I2 = 0%). Regarding beef heifers, there was no significant effect on variables related to ruminal fermentation characteristics (P > 0.05) and blood urea nitrogen (BUN) (P > 0.05) (Table 5).

5.3.4 Microorganisms in the rumen fluid and on the production of microbial nitrogen

There was a significant increase in the population of bacteria (+1.05; P < 0.01; I^2 = 96%) and fungi (+1.85; P < 0.01; I^2 = 91%), and a reduction in the population of protozoa in the rumen fluid (-2.27; P < 0.01; I^2 = 79%) in animals fed with YFC (Table 6).

Table 6 – Effects of YFC supplementation in cattle diets on rumen microorganism population and microbial nitrogen production.

Group/Sub-group	No. of comp.	Control group mean	Mean Difference (95% CI)		Heterogeneity			Funnel test
			Random effect	*P*-value	χ^2 (Q)	I^2	*P*-value	*P*-value
Bacteria ($x10^{10}$)								
Dairy heifer	6	236.00	3.11 (0.29; 5.92)	0.031	175.14	97%	<0.001	
Beef heifer	6	11.50	0.80 (-2.06; 3.66)	0.584	0.64	0%	0.986	
Lactating cow	4	0.53	0.18 (0.12; 0.25)	<0.001	4.93	39%	0.177	
Total	16	93.1	1.05 (0.63; 2.48)	<0.001	389.24	96%	<0.001	0.019
Protozoa ($x10^5$)								
Dairy heifer	3	15.40	-6.40 (-9.66; -3.14)	<0.001	6.85	71%	0.033	
Beef heifer	6	27.50	0.58 (-4.02; 5.18)	0.804	0.49	0%	0.992	
Lactating cow	4	4.70	-0.39 (-0.64; -0.14)	0.002	3.23	7%	0.358	
Total	13	17.70	-2.27 (-3.64; -0.90)	<0.001	56.34	79%	<0.001	0.078
Fungi ($x10^5$)								
Dairy heifer	6	26.6	3.29 (1.48; 5.10)	<0.001	21.81	77%	<0.001	
Beef heifer	6	4.00	0.67 (-0.77; 0.21)	0.361	0.42	0%	0.995	
Lactating cow	4	1.96	1.62 (-5.31; 3.29)	0.058	31.03	91%	<0.001	
Total	16	11.9	1.85 (0.58; 3.13)	0.004	163.58	91%	<0.001	0.005

Ruminal bacteria group (CFU/mL)								
Total viable bacteria (x10^9)								
Dairy heifer	3	3.00	3.10 (1.04; 5.16)	0.003	57.81	96.5%	<0.001	
Lactating cow	4	3.95	2.51 (-0.04; 5.06)	0.054	18.83	84.1%	<0.001	
Total	7	3.54	2.77 (1.18; 4.36)	<0.001	107.27	94.4%	<0.001	na
Cellulolytic bacteria (x10^9)								
Dairy heifer	3	190.00	2.60 (1.21; 3.99)	<0.001	31.2	93.6%	<0.001	
Lactating cow	4	20.10	0.32 (0.01; 0.63)	0.043	42.86	93%	<0.001	
Total	7	92.90	1.22 (0.65; 1.80)	<0.001	279.71	97.9%	<0.001	na
Amylolytic bacteria (x10^7)								
Dairy heifer	3	29.00	9.33 (2.90; 15.77)	0.004	2.8	28.5%	0.247	
Lactating cow	4	3.01	1.53 (0.14; 2.92)	0.031	52.3	94.3%	<0.001	
Total	7	14.20	2.05 (0.59; 3.51)	0.006	65.12	90.8%	<0.001	na
Proteolytic bacteria (x10^7)								
Dairy heifer	3	19.00	15.67 (2.02; 29.31)	0.024	23.25	91.4%	<0.001	
Lactating cow	4	3.58	1.58 (0.16; 3.01)	0.029	29.71	89.9%	<0.001	
Total	7	10.2	4.68 (2.36; 7.00)	<0.001	109.94	94.5%	<0.001	na
Microbial-N yield (g N/day)								
Dairy heifer	3	90.80	24.03 (10.16; 37.91)	<0.001	28.42	93%	<0.001	
Beef heifer	6	79.00	0.70 (-3.14; 4.54)	0.720	0.3	0%	0.998	
Total	9	82.93	8.66 (-1.04; 18.35)	0.080	102.63	92%	<0.001	na

No. of comp.: number of comparisons between treatment and control groups; na: Not available; χ^2 and I^2 provided to show the degree of heterogeneity among studies included in the meta-analysis.

The inclusion of YFC in the dairy heifer concentrate increased the ruminal population of bacteria (+3.11; P = 0.03; I^2 = 97%) and fungi (+3.29; P < 0.01; I^2 = 77%), but reduced the population of protozoa (-6.40; P < 0.01; I^2 = 71%). Likewise, lactating cows supplemented with YFC increased the bacterial population (+0.18; P < 0.01; I^2 = 39%) and reduced the protozoan

population (-0.39; P < 0.01; I^2 = 7%). There was no significant effect of YFC on the fungal population of lactating cows (P > 0.05).

Beef heifers supplemented with YFC did not show significant changes in bacterial, protozoal, and fungal populations in the rumen (P > 0.05). There was no influence of YFC on microbial nitrogen production in cattle (P > 0.05). However, dairy heifers supplemented with YFC showed a 26.5% increase (+24.03; P < 0.01; I^2 = 93%) in microbial nitrogen yield.

5.3.5 Milk yield and milk composition

In the present meta-analysis, dairy crossbreeds were used in early lactation with an average milk production of 13.62 kg/day (Table 7). Lactating cows fed YFC produced 1.02 kg/d more milk than non-supplemented cows (+7.5%; P < 0.01; I^2 = 0%). In addition, there was an increase of 7.4% in fat (+0.28; P = 0.03; I^2 = 39%), 6.3% in protein (+0.20; P < 0.01; I^2 = 0%) and 2.8% in lactose (+0.13; P = 0.02; I^2 = 0%) from milk from cows supplemented with YFC. However, there was no significant difference in the percentage of non-fat solids (P > 0.05) and total solids (P > 0.05) (Table 7). Milk urea nitrogen was significantly reduced in cows fed YFC compared to the control group (MD=-2.24; P < 0.01; I^2= 0%).

Table 7 – Effects of YFC supplementation in bovine diets on milk yield and milk composition.

Group/Sub-group	No. of comp.	Control group mean	Mean Difference (95% CI)		Heterogeneity		
			Random effect	*P*-value	χ^2 (Q)	I^2	*P*-value
Milk yield (kg/d)							
Total	5	13.62	1.02 (0.60; 1.44)	<0.001	3.68	0%	0.451
Milk composition %							
Fat	5	3.78	0.28 (0.04; 0.53)	0.025	6.57	39%	0.160
Protein	5	3.18	0.20 (0.11; 0.29)	<0.001	2.78	0%	0.596
Lactose	4	4.60	0.13 (0.02; 0.25)	0.020	0.68	0%	0.877
Solids not fat	5	8.38	0.14 (-0.22; 0.49)	0.449	0.44	0%	0.979
Total solids	4	12.40	0.51 (-0.70; 1.72)	0.406	0.05	0%	0.997
Milk urea nitrogen (mg/dL)							
Total	4	15.08	-2.24 (-2.86;-1.62)	<0.001	0.89	0%	0.827

No. of comp.: number of comparisons between treatment and control groups; χ^2 and I^2 provided to show the degree of heterogeneity among studies included in the meta-analysis.

5.3.6 Meta-regression

The variables that presented significant heterogeneity were investigated and the meta-regression results indicated that the covariate supplementation with YFC in the animals' diet (Sup. YFC) contributed to explain the statistical heterogeneity of the variables.

The result of the meta-regression showed that the covariate Sup. YFC explained the heterogeneity of DMI (kg/d) of lactating cows and DMI %BW in dairy heifers. Lactating cows supplemented with YFC increased DMI by up to 2.98 kg/d ($P < 0.001$; $I^2 = 0.0\%$; Model 2.1) and dairy heifers increased DMI %BW by up to 0.42% ($P < 0.01$; $I^2 = 0.0\%$; Model 3.1). Similarly, there was an improvement in the digestibility of OM (+7.44%; $P < 0.01$; $I^2 = 0.0\%$; Model 5.1), NDF (+7.26%; $P < 0.01$; $I^2 = 62.26\%$; Model 8.1) and ADF (+8.30 %; $P < 0.01$; $I^2 = 60.12\%$; Model 9.1) of dairy heifers supplemented with YFC. However, Sup. YFC did not explain the heterogeneity observed in ADG, DM, CP, and EE digestibility ($P > 0.05$) (Table 8).

Table 8 – Meta-regression of the effects of YFC supplementation in cattle diets on average daily weight gain, dry matter intake and nutrient digestibility.

Model	Variable	YFC (g/kg DM)[a] (min. to max.)	Meta-regression				
			Intercept	Sup. YFC	Adj. R^2 (%)	*P*-value	I^2 (%)
	Average daily gain (kg/d)						
1.1	Dairy heifer	0.25 to 0.75	0.012	0.020	0.00	0.805	98.15
	Dry matter intake (kg/d)						
2.1	Lactating cow	0.07 to 0.28	-0.343	11.851	100.00	0.001	0.00
	Dry matter intake %PV						
3.1	Dairy heifer	0.07 to 0.75	0.027	0.519	100.00	0.001	0.00
	Apparent total tract digestibility, %DM						
	Dry matter						
4.1	Dairy heifer	0.07 to 0.28	2.899	10.584	0.00	0.366	83.98
4.2	Lactating cow	0.07 to 0.28	10.406	-20.213	0.00	0.396	60.91
	Organic matter						
5.1	Dairy heifer	0.07 to 0.28	0.779	23.774	100.00	<0.001	0.00
	Crude protein						
6.1	Dairy heifer	0.07 to 0.28	3.302	19.027	15.76	0.247	91.19
	Ether extract						
7.1	Dairy heifer	0.07 to 0.28	2.191	13.511	29.64	0.183	91.48
	Neutral detergent fiber						
8.1	Dairy heifer	0.07 to 0.28	2.210	18.020	92.30	<0.001	62.26

	Acid detergent fiber						
9.1	Dairy heifer	0.07 to 0.28	2.346	21.276	86.84	0.003	60.12

[a] = levels of yeast-fermented cassava in replacing concentrate or soybean meal; YFC: yeast-fermented cassava; Sup. YFC: supplementation with YFC in the animals' diet; I^2 = percentage of residual variation due to heterogeneity; Adj. R^2 = adjusted R^2: proportion of between-study variance explained.

The rumen pH of lactating cows supplemented with YFC was up to 4.7% higher ($P < 0.001$; $I^2 = 0.0\%$; Model 10.2) compared to cows not supplemented with YFC. On the other hand, YFC supplementation reduced the ruminal pH of dairy heifers by up to -0.11 units ($P = 0.021$; $I^2 = 95.26\%$; Model 10.1), with no significant reduction in statistical heterogeneity (Table 9).

Table 9 – Meta-regression of the effects of YFC supplementation in cattle diets on rumen fermentation characteristics and blood urea nitrogen.

Model	Variable	YFC[a] (g/kg DM) (min. to max.)	Meta-regression				
			Intercept	Sup. YFC	Adj. R^2 (%)	*P*-value	I^2 (%)
	pH						
10.1	Dairy heifer	0.07 to 0.75	0.362	-0.627	0.00	0.021	95.26
10.2	Lactating cow	0.07 to 0.28	0.034	0.956	100.00	<0.001	0.00
	NH_3-N (mg/dL)						
11.1	Dairy heifer	0.07 to 0.75	3.873	-2.924	0.00	0.489	82.15
	Blood urea nitrogen (mg/dL)						
12.1	Lactating cow	0.07 to 0.28	-1.518	-7.976	100.00	0.098	0.00
	Molar proportion of VFA (mol/100mol)						
	VFA (mmol/L)						
13.1	Dairy heifer	0.07 to 0.28	1.615	38.181	100.00	0.019	0.00
13.2	Lactating cow	0.07 to 0.28	11.788	8.423	0.00	0.918	85.11
	Propionate %						
14.1	Dairy heifer	0.07 to 0.28	0.060	20.808	100.00	0.011	0.00
	C2:C3 ratio						
14.2	Dairy heifer	0.07 to 0.28	-1.299	2.300	0.00	0.371	72.84

[a] = levels of yeast-fermented cassava in replacing concentrate or soybean meal; YFC: yeast-fermented cassava; Sup. YFC: supplementation with YFC in the animals' diet; I^2 = percentage of residual variation due to heterogeneity; Adj. R^2 = adjusted R^2: proportion of between-study variance explained.

The heterogeneity observed in the total concentration of VFA and propionate in dairy heifers was explained by the inclusion of Sup. YFC in meta-regression. Dairy heifers supplemented with YFC increased total VFA concentration by up to 12.31 mm/L (P = 0.02; I^2 = 0.0%; Model 13.1) and propionate concentration by up to 5.31% (P = 0.011; I2 = 0.0%; Model 14.1). However, Sup. YFC did not explain the heterogeneity of VFA in lactating cows (P = 0.918). There was no association between Sup. YFC and NH3-N, BUN and C2:C3 ratio of dairy heifers (P > 0.05).

The Sup. YFC was associated with ruminal bacterial counts of dairy heifers, but did not reduce heterogeneity (P = 0.005; I^2 = 96.61%; Model 15.1). YFC supplementation in dairy heifers promoted an increase of up to 9x1010 bacterial cells/mL of rumen fluid compared to heifers not supplemented with YFC. Similarly, the cellulolytic bacteria count of dairy heifers also increased (+3.76x10^9; P = 0.017; I^2 = 78.46%; Model 19.1) as a function of YFC supplementation (Table 10).

Table 10 – Meta-regression of the effects of YFC supplementation in cattle diets on rumen microorganism population and microbial nitrogen production.

Model	Variable	YFC[a] (g/kg DM) (min. to max.)	Meta-regression				
			Intercept	YFC	Adj. R^2 (%)	*P*-value	I^2 (%)
	Bacteria counts (cells/mL)						
15.1	Dairy heifer	0.07 to 0.75	-6.03x10^{10}	5.368x10^{11}	0.00	0.005	96.61
	Protozoa counts (cells/mL)						
16.1	Dairy heifer	0.07 to 0.28	-2.69 x10^5	-2.14 x10^6	29.18	0.099	63.21
	Fungal zoospores count (cells/mL)						
17.1	Dairy heifer	0.07 to 0.75	7.10 x10^4	1.47 x10^6	8.80	0.137	66.02
17.2	Lactating cow	0.07 to 0.28	1.39 x10^5	1.30 x10^5	0.00	0.915	92.08
	Ruminal bacteria group (CFU/mL)						
	Total viable bacteria						
18.1	Dairy heifer	0.07 to 0.28	4.13 x10^8	1.55 x10^{10}	59.88	0.053	91.8
18.2	Lactating cow	0.07 to 0.28	2.15 x10^9	2.11 x10^9	0.00	0.911	88.08
	Cellulolytic bacteria						
19.1	Dairy heifer	0.07 to 0.28	7.21 x10^8	1.08 x10^{10}	75.05	0.017	78.46
19.2	Lactating cow	0.07 to 0.28	2.27 x10^8	5.05 x10^8	0.00	0.828	94.53
	Amylolytic bacteria						
20.1	Lactating cow	0.07 to 0.28	1.49 x10^7	2.44 x10^6	0.00	0.981	95.48

	Proteolytic bacteria						
21.1	Dairy heifer	0.07 to 0.28	4.65 x10^7	6.35 x10^8	0.00	0.509	93.82
21.2	Lactating cow	0.07 to 0.28	1.33 x10^7	1.40 x10^7	0.00	0.893	91.88
	Microbial-N yield (g N/day)						
22.1	Dairy heifer	0.07 to 0.28	3.708	117.262	100.00	<0.001	0.00

[a] = levels of yeast-fermented cassava in replacing concentrate or soybean meal; YFC: yeast-fermented cassava; Sup. YFC: supplementation with YFC in the animals' diet; I^2 = percentage of residual variation due to heterogeneity; Adj. R^2 = adjusted R^2: proportion of between-study variance explained.

The heterogeneity observed in the production of microbial nitrogen in dairy heifers was explained by the inclusion of Sup. YFC in meta-regression. YFC supplementation promoted higher microbial nitrogen production (+36.5g N/day; $P < 0.001$; $I^2 = 0\%$; Model 22.1) in dairy heifers. There was no association between Sup. YFC and counts of fungi, protozoa, total viable bacteria, amylolytic and proteolytic bacteria from dairy heifers and lactating cows.

5.3.7 Publication bias

There was no evidence of publication bias for most of the variables evaluated ($P > 0.05$). However, visual inspection of the funnel plot and statistical analysis by the Egger test suggested the presence of publication bias for the digestibility of ADF, NH_3-N, BUN, rumen population of bacteria and fungi ($P < 0.05$). The trim-and-fill method adjusted the mean estimate of the variables, resulting in a change in the treatment effect obtained in the random effects meta-analysis for ADF digestibility (5.42; 95% CI 3.98 to 6.86; $P < 0.01$), NH3- N (2.39; 95% CI 1.09 to 3.68; $P < 0.01$), BUN (-3.34; 95% CI -4.52 to -2.16; $P < 0.01$), ruminal bacterial population (1.06x10^{10}; 95% CI 0.58x10^{10} to 1.54 x10^{10}; $P < 0.01$) and fungi (1.26x10^5; 95% CI 1.84x10^4 to 2.32x10^5; $P < 0.01$).

5.4 Discussion

5.4.1 DMI and nutrient digestibility

The higher DMI in dairy heifers and lactating cows fed the YFC-containing diet is possibly due to higher fiber digestibility, which could increase the passage rate and therefore improve the daily feed intake (NASEM, 2021). The higher digestibility can be attributed to the ability of *S. cerevisae* to secrete some extracellular enzymes during cassava fermentation, resulting in an improvement in the nutritional value of the food (Oboh and Akindahunsi, 2003). In addition, alternative mechanisms of action of YFC supplementation on rumen metabolism,

such as stabilization of rumen pH and increase in the population of cellulolytic microorganisms, may also have contributed to the improvement of DM digestibility and nutrient digestibility (NASEM, 2021).

The reduction in DMI of beef heifers supplemented with YFC may be associated with lower DM digestibility and nutrient digestibility, resulting in physical limitations in intake due to reduced digestion rate and rumen passage (NASEM, 2021). Furthermore, the loss of appetite in the animals may be related to the concentration of HCN in the YFC (approximately 47.3 mg/kg) (Boonnop et al., 2009; Paulinus and Obaika, 2013). The presence of HCN in ruminant feed leads to higher levels of intake of sulfur or sulfur amino acids, such as methionine and cysteine, for HCN detoxification (Promkot et al., 2007; Promkot and Wanapat, 2009; Cherdthong et al., 2018). YFC contains low levels of methionine and cysteine (Polyorach et al., 2013), therefore, a high level of YFC in the feed results in an inadequate sulfur level for HCN detoxification and nutritional requirements (<0.4% DM) (Promkot et al., 2007; Promkot and Wanapat, 2009; Cherdthong et al., 2018), which will likely decrease feed intake.

5.4.2 Rumen fermentation characteristics and blood urea nitrogen (BUN)

The higher mean ruminal pH of dairy heifers and lactating cows supplemented with YFC may be a consequence of the action of YFC yeasts in the rumen environment. Yeast can compete with S. bovis and Lactobacillus for fermentable carbohydrates or to stimulate the growth of lactate-using bacteria (*Selenomonas ruminantium* and *Megasphaera elsdenii*), which results in low lactate accumulation and consequently a higher pH (Lynch and Martin, 2002; Chaucheyras et al., 1996; Chaucheyras-Durand and Fonty 2001; Chaucheyras-Durand and Fonty, 2006; Bach et al., 2007). Furthermore, the increase in lactate-using bacteria modifies individual VFA production, as they convert lactate to propionate, with their growth stimulated by yeast supplementation (Jiang et al., 2017; Sartori et al., 2017), resulting in increased propionate concentration and reduced concentrations of acetate, butyrate, and acetate to propionate ratio (Table 5).

The increase in total rumen VFA concentration due to YFC supplementation in dairy heifers (+8.23 mmol/L) and lactating cows (+13.14 mmol/L) is not surprising given the effects on pH discussed above. It is known that the increase in rumen pH favors the fibrolytic activity of ruminal bacteria (Mould et al., 1983) which may explain, at least partially, the effect in this study.

The increase in ruminal NH_3–N concentration in dairy heifers could be associated with the ability of YFC yeast to stimulate growth and proteolytic activity of rumen bacteria (Yoon

and Stern, 1996), whereas the population of proteolytic bacteria from dairy heifers supplemented with YFC was 82.5% (+15.67x107 CFU/mL) higher compared to non-supplemented dairy heifers (Table 4). The result obtained is within the optimal ruminal NH3-N between 15-30 mg/dL to increase microbial protein synthesis, diet digestibility and voluntary DM intake in ruminants fed on low quality roughage (Wanapat and Pimpa, 1999).

The lower concentration of BUN (-20%) and MUN (-15%) in lactating cows fed YFC compared to the control group can be attributed to the better utilization of NH3-N in the rumen (Table 3). It is likely that the inclusion of YFC in the diet provides a suitable rumen environment to stimulate microbial protein synthesis, resulting in decreased milk or ruminal concentration of NH_3-N and BUN (Erasmus et al., 1992; Chiquette et al., 2015).

5.4.3 Microorganisms in rumen fluid and microbial nitrogen production

YFC supplementation in dairy heifers and lactating cows significantly increased the population of rumen microorganisms (bacteria and fungi). The increase in the number of bacterial cells in the rumen can be attributed to the ability of the yeasts present in the YFC to improve the rumen environment by reducing the concentration of oxygen present in the rumen fluid, providing important nutrients and cofactors (B vitamins), and stimulating the cellular activities of proteases, α-amylase, β-glucosidase and xylanase (Newbold et al., 1995; Jouany, 2006). Furthermore, *S. cerevisiae* leads to increased germination of zoospores of a ruminal fungal strain of Neocallimastix frontalis as shown in an *in vitro* study (Chaucheyras et al., 1995).

In contrast, the protozoan count in the rumen fluid of dairy heifers and lactating cows was decreased by YFC supplementation ($P < 0.01$) (Table 6). Similarly, Polyorach et al. (2014) observed that the use of YFC as a source of concentrate with a roughage: concentrate ratio of 40:60 increased the total bacterial count and ruminal fungal zoospores, while the population of protozoa and methanogenic microorganisms was reduced. The reported effects of *S. cerevisiae* supplementation on the number and genera of ruminal protozoa were often inconsistent (Newbold et al., 1998; Kowalik et al., 2011, 2012; Phesatcha et al., 2022). However, previous studies have reported a linear reduction in ruminal protozoan counts as a result of increasing YFC in the diet of cows (Boonnop et al., 2010; Wanapat, Boonnop, et al., 2011; Wanapat, Polyorach, et al., 2011).

Dairy heifers that received YFC in the concentrate increased microbial nitrogen synthesis (+26.5%) compared to non-supplemented animals ($P < 0.01$) (Table 6). Meta-regression results also showed an increasing linear increase in microbial nitrogen synthesis

because of increasing YFC in the diet ($P < 0.01$; $I^2 = 0.0\%$; Model 22.1). More protein nitrogen is incorporated into rumen microbial protein only if more non-fibrous carbohydrates (NFC), known to be the main energy substrate for rumen microorganisms, are supplied to the animals (Schwab et al., 2005). Therefore, the high starch content (NFC) in YFC and the increase in the population and activity of rumen proteolytic microorganisms explain the higher production of microbial nitrogen in animals fed with YFC.

5.4.4 Milk yield and milk composition

The higher milk yield, fat, protein, and lactose content of milk from cows fed with YFC may be related to higher DM intake, nutrient digestibility, and fermentation characteristics. Several studies investigated the effects of including yeast in the diets of lactating cows and the results obtained showed an improvement in milk production (Desnoyers et al., 2009; Poppy et al., 2012), higher concentration of milk fat (Dias et al., 2018; Nasiri et al., 2019; Elaref et al., 2020) and increased milk protein (Jiang et al., 2017).

The increase in milk fat production can be attributed to an increase in the population of cellulolytic bacteria (+1.6%; $P = 0.043$) and an increase in NDF (+5.8%; $P < 0.001$) and ADF (+4.6%; $P < 0.001$) digestibility in animals supplemented with YFC (Table 4 and Table 6).

The positive response in the percentage of protein in the milk of cows fed YFC suggests a more efficient metabolism of N, which may be related to a greater supply of microbial protein or an improvement in the profile of amino acids supplied to the duodenum (Doepel et al., 2004; NASEM, 2021).

The higher percentage of lactose in milk observed in cows supplemented with YFC can be explained by the increase in ruminal propionate (Table 5). Propionate is the main substrate for gluconeogenesis in ruminants (Reynolds et al., 2003). Thus, it is likely that increased ruminal production of propionate has increased blood glucose concentration via gluconeogenesis and, consequently, increased milk lactose.

5.5 Conclusion

The results of the present meta-analysis showed that the total or partial inclusion of YFC in the concentrate of dairy heifers and lactating cows provides improvement in rumen fermentation, dry matter intake, nutrient digestibility. Furthermore, the total or partial inclusion of YFC in the diet of dairy crossbreeds with an average milk production of 13.62 kg/day showed potential benefits in milk production and milk components. However, we did not observe

effects on performance, nutrient digestibility, rumen microorganisms and rumen fermentation of beef heifers supplemented with YFC.

We emphasize that these results should be interpreted with caution, as they were based on a group of eight experiments. Finally, we suggest that future experiments be carried out with high production dairy cows and confined beef cattle, in order to explore the potential of YFC in total or partial replacement of high-performance animal concentrate.

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6 CAPÍTULO II – PROTEIN ENRICHMENT OF AGRO-INDUSTRIAL RESIDUES FOR RUMINANT FEED

ENRIQUECIMENTO PROTEICO DE RESÍDUOS AGROINDUSTRIAIS PARA ALIMENTAÇÃO DE RUMINANTES

RESUMO

O objetivo deste estudo foi avaliar o uso potencial de resíduos agrícolas como substratos para enriquecimento proteico com *Saccharomyces cerevisiae*, analisando a composição química e a cinética de produção de gases. Utilizou-se um delineamento inteiramente casualizado em esquema fatorial 4 x 2, consistindo em quatro resíduos industriais (bagaço de acerola, raspa de mandioca, bagaço de laranja e casca de abacaxi) e duas avaliações (antes e depois do enriquecimento proteico), com três repetições. As matérias-primas foram submetidas ao processo de enriquecimento nutricional com levedura (*S. cerevisiae*). Avaliaram-se a composição química, fracionamento de carboidratos e proteínas, composição mineral e produção de gases *in vitro* dos resíduos, antes e após o enriquecimento. Os dados foram analisados por ANOVA utilizando o delineamento fatorial 4×2 ao nível de significância de 5%. Os resultados do processo de enriquecimento mostraram um aumento significativo no teor de proteína ($P<0,001$) e uma redução na fração fibrosa ($P<0,001$) em todos os quatro resíduos, especialmente no FDNcp ($P<0,001$), HEM ($P<0,001$) e CEL ($P<0,001$). Além disso, os resíduos enriquecidos apresentaram uma diminuição no teor de carboidrato total em comparação com os resíduos não tratados ($P<0,001$), principalmente devido a uma redução na fração de carboidratos não fibrosos (A+B1) ($P<0,001$). Houve também um aumento significativo nos níveis de proteína bruta ($P<0,001$) e mudanças nas frações de PB ($P<0,001$), com aumento na fração A e redução nas frações B1+B2 ($P<0,001$). Em termos de cinética de produção de gases *in vitro*, os resíduos não tratados geralmente produziram mais gás total em comparação com os resíduos enriquecidos ($P<0,001$). No entanto, os resíduos enriquecidos exibiram um maior tempo até o ponto de inflexão ($P<0,001$) e maior tempo de atraso ($P<0,001$). Esses achados sugerem que o enriquecimento proteico de resíduos agroindustriais com *S. cerevisiae* é uma alternativa promissora para aumentar o teor de proteína.

Palavras-chave: Alimentação Animal; Fermentação; *Saccharomyces cerevisiae*.

PROTEIN ENRICHMENT OF AGRO-INDUSTRIAL WASTES FOR RUMINANT FEED

ABSTRACT

The aim of this study was to evaluate the potential use of agricultural wastes as substrates for protein enrichment with *Saccharomyces cerevisiae* by analyzing the chemical composition and gas production kinetics. Completely randomized design was used in a 4 x 2 factorial scheme, consisting of four industrial wastes (acerola bagasse, cassava chip, orange bagasse and pineapple peel) and two evaluations (before and after protein enrichment), with three replicates. These raw materials were subjected to the nutritional enrichment process using yeast (*S. cerevisiae*). The chemical composition, carbohydrate and protein fractionation, mineral composition, and *in vitro* gas production of the wastes were evaluated before and after enrichment. Data were analyzed by ANOVA using a randomized 4×2 factorial design at 5% significance. The results of the enrichment process showed a significant increase in the protein content ($P<0.001$) and a reduction in the fibrous fraction ($P<0.001$) across all four wastes, especially in the NDFap ($P<0.001$), HEM ($P<0.001$) and CEL ($P<0.001$). Additionally, the enriched wastes exhibited a decrease in total carbohydrate content compared to untreated wastes ($P<0.001$), primarily due to a reduction in the non-fibrous carbohydrate fraction (A+B1) ($P<0.001$). There was also a significant increase in crude protein levels ($P<0.001$) and changes in CP fractions ($P<0.001$), with an increase in fraction A and a reduction in fractions B1+B2 ($P<0.001$). In terms of *in vitro* gas production kinetics, untreated wastes generally produced more total gas compared to enriched wastes ($P<0.001$). However, enriched wastes exhibited a longer time to the inflection point ($P<0.001$) and a longer lag time ($P<0.001$). These findings suggest that protein enrichment of agro-industrial wastes with *S. cerevisiae* is a promising alternative for increasing protein content.

Keywords: Animal Feed; Fermentation; *Saccharomyces cerevisiae.*

6.1 Introduction

The growing demand for livestock products, particularly in developing countries, has led to a surge in animal feed production (Bajić et al., 2023). Consequently, agricultural activities and the food industry generate significant quantities of wastes rich in organic matter (Capanoglu et al., 2022; Dunuweera et al., 2021; Nath et al., 2023), presenting an opportunity for the creation of value-added products (Capanoglu et al., 2022; Santeramo and Lamonaca, 2021).

One promising approach involves the utilization of agro-industrial wastes, particularly from the juice processing industry. This not only tackles waste management challenges but also helps minimize raw material costs (Hoehn et al., 2021; Santeramo and Lamonaca, 2021). These wastes contain considerable amounts of fermentable and non-fermentable sugars (Dunuweera et al., 2021), which can be used as substrates in solid-state fermentation (SSF) methods to enrich the protein level, intending to be used in animal feed (Nath et al., 2023).

While various microorganisms have been employed for protein enrichment, yeasts emerge as a favored choice, alongside bacteria, algae, and fungi (Bajić et al., 2023). Yeast cells, such as *Saccharomyces cerevisiae*, offer distinct advantages, including their larger size facilitating separation, high levels of vitamins (especially group B), and ease of harvest compared to bacteria (Nasseri et al., 2011; Ritala et al., 2017). Furthermore, yeast is rich source of high-quality protein (~40-50% of dray basis) (Bertolo et al., 2019; Nasseri et al., 2011), and the concentration of essential amino acids (lysine, tryptophan, threonine, and methionine and cysteine) is satisfactory when used as feed additives (Yamada and Sgarbieri, 2005), otherwise these can be added at the end of the process with few costs.

It is hypothesized that *S. cerevisiae* can use the carbohydrates present in agro-industrial wastes for its growth, resulting in an increase in the protein content of these materials. Based on this, the objective of the present study was to evaluate the potential use of agricultural wastes as substrates for protein enrichment with *S. cerevisiae*, analyzing the chemical composition and kinetic of gas production.

6.2 Material and Methods

6.2.1 Location and Ethics Committee

Protein enrichment of agro-industrial wastes and chemical analyses were conducted at the Federal University of Maranhão (UFMA) in Chapadinha, Maranhão, Brazil. *In vitro* Gas production kinetic analyses were conducted at the Federal University of Agreste de Pernambuco (UFAPE) in Garanhus, Pernambuco, Brazil. Mineral analyses were conducted at the Brazilian Agricultural Research Corporation (Embrapa Semiárido) in Petrolina, Pernambuco, Brazil. All the animals were cared for in accordance with guidelines of the National Council for the Control of Animal Experimentation (CONCEA, 2023).

6.2.2 Experimental Design and Treatments

A completely randomized design in a 4 x 2 factorial scheme was adopted for data analysis, considering four industrial wastes (acerola bagasse, cassava chip, orange bagasse and pineapple peel) and two evaluations (before and after protein enrichment), each with three replicates. The choice of industrial wastes was based on their availability and potential use, aiming to optimize the protein enrichment process. The two evaluations allowed a direct comparison of the effects of enrichment, contributing to a more robust understanding of the impacts of different wastes on the nutritional and functional characteristics of the final product.

6.2.3 Protein enrichment of agro-industrial wastes

The agro-industrial wastes used as raw materials were obtained from local agro-industries or businesses. The wastes obtained were acerola pomace, cassava chip (obtained from cutting and drying the roots), orange pomace, and pineapple peel. For protein enrichment of the substrate, yeast (*Saccharomyces cerevisiae*), urea and brown sugar were used. Commercial grade urea, brown sugar, and yeast (Instant Dry Yeast, Angel Yeast Co., Ltd) were purchased from a local shop.

The residues were dried in a forced-ventilation oven at 60°C for 48 hours, or until fully dried, prior to the experiment. This procedure was carried out to standardize the dry matter content of the residues. The yeast culture used in this experiment contains as an effective agent non-pathogenic live yeast of *S. cerevisiae*.

Approximately 1 kg of agro-industrial wastes, with a dry matter content between 85% and 95%, was used in the protein enrichment process. The moisture content of the wastes was

adjusted to 50% by adding a solution composed of 10% urea, 1.25% molasses, and 5% *S. cerevisiae*, resulting in a solution containing approximately 1.2 x 10^8 yeast cells per milliliter (mL) (KAEWWONGSA et al., 2011). The enriched material samples underwent a 5-day incubation period at 27 °C (KHAN et al., 2022), were subsequently dried, ground with a 2 mm sieve, and stored for subsequent analyses.

6.2.4 Chemical analysis

The samples were subjected to pre-drying for 72 hours, in a forced ventilation oven at 60±5°C and, subsequently, ground in a mill with 1 mm (Wiley mill, Marconi, MA-580, Piracicaba, Brazil) sieves to determine the dry matter (DM; method 967.03), protein crude (CP; method 988.05), and mineral matter (MM; method 942.05) using the Association of Official Analytical Chemists (AOAC, 2016). The quantification of the ether extract (EE) content was analyzed using a fat extractor (ANKOM TX-10, Macedon - NY, United States), according to the method of the American Oil Chemists' Society (AOCS, 2017). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991) with modifications proposed by Senger et al. (2008), in which he used an autoclave at 110 °C for 40 minutes.

The organic matter (OM) content was obtained by the equation, OM = 100 – MM. The concentration of neutral detergent fiber corrected for ash and protein (NDFap) was determined by the equation: %NDFap = %NDF - (% neutral detergent insoluble CP + % neutral detergent insoluble ash) (DETMANN et al., 2021). Protein-corrected acid detergent fiber (ADFp) was calculated by subtracting ADF from acid detergent insoluble CP (ADICP) (DETMANN et al., 2021). Lignin was determined according to Van Soest et al. (1963). The hemicellulose content (HEM) was calculated by subtracting the NDFp from the ADFp, and cellulose (CEL) from the subtraction of the ADFp by the lignin.

Total carbohydrates (TC) were calculated from the equation: TC = 100 – (%CP + %MM + %EE), according to Sniffen et al. (1992). The concentration of non-fibrous carbohydrates (NFC) was obtained by the equation, NFC = 100 - (%CP + %NDF + %EE + %MM), as proposed by Detmann et al. (2021). Estimates of total digestible nutrients (TDN) were made according to the equations proposed by NRC (2001) and Detmann et al. (2006):

TDN (%) = CPd + NDFd + NFCd + (EEd * 2,25) – 7 (NRC, 2001);

Digestible non-fibrous carbohydrate (NFCd) = 0,98 * NFC (NRC, 2001);

Digestible crude protein (CPd) = [1 – 0,4 * (ADICP / CP)] * CP (NRC, 2001);

Digestible ether extract (EEd) = 0,8596 * EE – 0,18 (DETMANN et al. (2006));

Digestible neutral detergent fibre (NDFd) = 0,75 * [(NDF – NDICP) – Lignin] * [1 – (Lignin / NDF – NDICP)0,667] (NRC, 2001).

The carbohydrate fractions were determined according to Sniffen et al. (1992). The C fraction was obtained by the equation: C = 100 * NDF(%DM) * 0.01 * (Lignin(%DM) * 2.4) / CT(%DM). The B2 fraction was obtained by the equation: B2 = 100 * [(NDF(%DM) – PIDN(%CP) * 0.01 * CP(%DM)) – (NDF(%DM) * 0.01 * Lignin(%DM) * 2.4)] / TC(%DM). The fraction with a high ruminal degradation rate (A+B1) was determined by equation 100 – (C + B2).

To determine the fractionation of CP, the recommendations of Licitra et al. (1996). Fraction A, known as non-protein nitrogenous compounds (NPN), was obtained as follows: 0.5g of the sample was treated with 50mL of water for 30 minutes. Then, we added 10mL of a 10% trichloroacetic acid (TCA) solution and left it to rest for another 30 minutes. The mixture was filtered using rapid filtration filter paper (Whatman 54), with the waste washed using 50mL of a diluted 1% TCA solution. Subsequently, the nitrogen content in the waste and paper was determined. Fraction A or NPN (%CP) was calculated by the difference between the total nitrogen content and the insoluble nitrogen in TCA, as described by Sniffen et al. (1992).

Fraction B3 was obtained by the difference between neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN), while fraction C corresponds to ADIN, determined according to Silva and Queiróz (2006). To calculate the fractions B1 + B2, we follow the equation B1 + B2 = 100 - (A + B3 + C), according to standards by Sniffen et al. (1992) and Licitra et al. (1996).

6.2.5 Mineral profile

Mineral analyses were conducted following the methodology described by Araújo et al. (2023). Briefly, the samples were subjected to digestion using 65% nitric acid and 70% perchloric acid, as described by Malavolta et al. (1997). Potassium (K) and sodium (Na) were measured by flame emission spectrometry (SP-500F/59; SP Labor), calibrated with standards of 0 and 50 mg l−1 for K, and 0 and 10 mg l−1 for Na. Phosphorus (P) was analyzed by molecular spectrometry (K37-VIS; Kasvi) at 420 nm, while calcium (Ca) and magnesium (Mg)

were determined by atomic absorption spectrometry. Sulfur (S) was analyzed using turbidimetry, and boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were measured by atomic absorption spectrophotometry (Analyst 100; Perkin Elmer). Chlorides (Cl) were determined using the argentometric Mohr's method (AZMAT et al., 2021).

6.2.6 Kinetics of gas production

Gas production was determined using an *in vitro* technique with a pressure transducer (LOGGER AG100–Agricer), following Theodorou et al. (1994). Cumulative gas production was measured at 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 30, 36, 42, 48, 54, 60, 66, and 72 hours after incubation. Gas volume was recorded by displacing the syringe plunger until internal pressure returned to ambient, as indicated by a zero reading. The measurement process took 10–15 seconds per bottle, ensuring temperature stability. The total gas produced by control bottles was subtracted from each sample. The equation used to convert pressure (psi) to gas volume (mL), developed at the Federal University of Agreste of Pernambuco (UFAPE) from 937 observations (1 psi = 4859 mL gas), was: gas production (mL) = 5.1612 × psi − 0.3017, with an R^2 value of 0.9873.

Cumulative gas production data were analyzed using the Gompertz model (Lavrenčič et al., 1998a) by the following equation:

$$Y = ae^{-be^{-ct}}$$

Where, Y is equal to the accumulated gas production at time x, $a > 0$ is the maximum gas production, the parameter $b > 0$ is the difference between the initial gas and the final gas at time *t*, and the parameter $c > 0$ describes the specific gas accumulation rate.

The following fermentation indicators were calculated: time to point of inflection (HPI, hours), gas inflection point (GPI ml), maximum gas production rate (TMPG, ml/h), and lag phase (FL or microbial accommodation h). To estimate the biological parameters, the following equations were used: HPI = b/c; GPI = a/*e*; TMPG = (a*c)/*e*; and FL = ((b/c) − (1/c)); where *e* is Euler's number, which equals approximately 2.718281828459.

6.2.7 Statistical analysis

Data were analyzed by analysis of variance (ANOVA), using a completely randomized design in a 4 x 2 factorial scheme. This scheme consisted of four industrial wastes (acerola

bagasse, cassava chip, orange bagasse and pineapple peel) and two evaluations (before and after protein enrichment), with three replicates per treatment. The focus of the analysis was exclusively on the interaction between industrial wastes and the evaluations, without considering the main effects of the wastes or evaluations. The statistical model used was:

$$Yijk = \mu + (W \times E)ij + \varepsilon ijk$$

where $Yijk$ represents the observed response variable, μ is the overall mean, (W×E)ij corresponds to the interaction between waste and evaluation, and εijk is the experimental error. The analysis was conducted using the R program (R Core Team, 2023), applying Tukey's test at a 5% significance level and considering a normal distribution of errors with constant variance.

6.3 Results

6.3.1 Chemical composition

The results of the enrichment process were highlighted by a significant increase in protein content and a reduction in the fiber fraction of the agro-industrial residues from cassava, acerola, pineapple, and orange ($P<0.001$). However, no difference was observed in the NDFap content for pineapple or in the cellulose content for cassava and pineapple ($P<0.05$). A significant difference in EE content was observed only for the acerola residue ($P<0.001$) (Table 11).

A significant reduction in dry matter content was observed in all agro-industrial residues following protein enrichment with *S. cerevisiae* ($P<0.001$). Similarly, protein enrichment led to reductions in organic matter and increases in mineral content specifically in pineapple peel ($P<0.001$), while no significant changes were found in acerola, cassava, or orange residues ($P<0.001$) (Table 11).

Table 11 – Chemical composition of agro-industrial wastes (g/100g DM).

Item	Cassava chip		Acerola pomace		Orange pomace		Pineapple peel			
	In nat[1]	Enriched	In nat	Enriched	In nat	Enriched	In nat	Enriched	SE	P-value[5]
DM	88.66 ^{AB}	78.50 ^{C}	90.54 ^{A}	69.13 ^{D}	86.96 ^{B}	61.05 ^{E}	85.17 ^{B}	67.38 ^{D}	1.25	<0.001
OM	96.96 ^{A}	96.56 ^{AB}	96.14 ^{B}	96.41 ^{AB}	95.39 ^{C}	95.20 ^{C}	93.03 ^{D}	91.97 ^{E}	0.20	<0.001
MM	3.04 ^{E}	3.44 ^{DE}	3.86 ^{D}	3.59 ^{DE}	4.61 ^{C}	4.80 ^{C}	6.97 ^{B}	8.03 ^{A}	0.20	<0.001
CP	1.75 ^{G}	4.75 ^{F}	13.53 ^{D}	31.15 ^{C}	7.61 ^{E}	40.12 ^{A}	9.45 ^{E}	33.52 ^{B}	0.70	<0.001
NDFap	7.12 ^{F}	3.78 ^{G}	56.37 ^{A}	51.08 ^{B}	36.00 ^{D}	27.63 ^{E}	42.02 ^{C}	40.41 ^{C}	0.98	<0.001
ADFp	3.73 ^{E}	3.68 ^{E}	43.50 ^{A}	38.23 ^{B}	20.85 ^{C}	19.82 ^{C}	17.34 ^{D}	18.93 ^{CD}	1.00	<0.001
CEL	1.40 ^{E}	0.75 ^{E}	24.09 ^{A}	18.07 ^{B}	16.10 ^{C}	14.40 ^{D}	14.47 ^{D}	14.65 ^{D}	0.39	<0.001
HEM	3.56 ^{E}	0.41 ^{F}	14.09 ^{C}	9.98 ^{D}	15.45 ^{C}	7.95 ^{D}	24.67 ^{A}	21.48 ^{B}	1.20	<0.001
TC	92.43 ^{A}	89.52 ^{B}	79.82 ^{E}	63.37 ^{F}	85.99 ^{C}	54.23 ^{H}	82.87 ^{D}	58.17 ^{G}	0.39	<0.001
NFC	85.63 ^{A}	85.24 ^{A}	23.89 ^{E}	12.86 ^{G}	51.37 ^{B}	25.73 ^{D}	41.04 ^{C}	17.77 ^{F}	0.50	<0.001
EE	2.53 ^{AB}	2.67 ^{AB}	1.69 ^{BC}	2.93 ^{A}	1.43 ^{CD}	1.82 ^{BC}	0.66 ^{D}	0.66 ^{D}	0.26	<0.001
LIG	0.52 ^{C}	1.03 ^{C}	17.20 ^{A}	19.73 ^{A}	5.32 ^{B}	5.81 ^{B}	3.25 ^{BC}	3.62 ^{BC}	1.48	<0.001
TDN	85.50 ^{A}	87.22 ^{A}	47.29 ^{E}	53.01 ^{D}	71.16 ^{C}	74.86 ^{B}	69.64 ^{C}	70.82 ^{C}	1.19	<0.001
PIC[3]		2.71		17.62		32.51		24.07		
IPC[4]		2.57		2.3		5.27		3.55		

[1]*In natura*; [2]Standard error; [3]Protein Increase Ratio; [4]Increase in Protein Content. [5]P-value of interaction agro-industrial wastes x protein enrichment.

Protein enrichment with *S. cerevisiae* led to significant reductions in fiber components across various agro-industrial wastes (Table 11). Cassava chip showed decreases of 46.5% in NDFap ($P<0.001$), 42.9% in CEL ($P<0.001$), and 88.9% in HEM ($P<0.001$). Acerola pomace exhibited reductions in NDFap (10.4%), ADFp (13.9%), CEL (33.1%), and HEM (41%). Similarly, orange pomace showed decreases of 23.3% in NDFap, 10.6% in CEL, and 48.4% in HEM. Pineapple peel showed a significant reduction in HEM content by 10.6% ($P<0.001$), while no significant differences were observed for NDFap ($P>0.05$) or CEL ($P>0.05$) (Table 11).

A significant reduction in NFC ($P<0.001$) was observed in the acerola (53.8%), orange (46.2%), and pineapple (43.3%) residues. In contrast, no significant difference was found in the cassava chip, which maintained an average value of 85.44 g/100g DM (Table 11). Furthermore, protein enrichment with *S. cerevisiae* led to a significant increase in TDN for acerola (12.1%) and orange wastes (5.2%) ($P<0.001$), while no significant changes were observed for cassava and pineapple wastes ($P>0.05$) (Table 11).

6.3.2 Carbohydrate fractionation

Protein enrichment with *S. cerevisiae* led to varied effects on carbohydrate fractions across different wastes (Table 12). In cassava chip, a 3.3% reduction in TC content ($P<0.001$) and a 3% increase in the A+B1 fraction ($P<0.001$) were observed, along with 38.1% decreases in the B2 ($P<0.001$) and 26.2% indigestible C ($P<0.001$) fractions (Table 12). Acerola pomace showed a 20.6% reduction in TC content, driven by a 21.5% decrease in the A+B1 fraction, with no significant change in the B2 fraction but a 19.8% increase in the indigestible C fraction. Similarly, enrichment of orange pomace led to a 36.9% reduction in TC and an 18% decrease in A+B1, while the B2 and indigestible C fractions increased by 22.3% and 62.8%, respectively. Pineapple peel showed a 29.8% reduction in TC content and a 36% decrease in A+B1, accompanied by increases in the B2 (33.2%) and indigestible C (66.4%) fractions.

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Table 12 – Carbohydrate fractionation of agro-industrial wastes (%TC and g/100g DM).

Item	Cassava chip		Acerola pomace		Orange pomace		Pineapple peel			
	In nat[1]	Enriched	In nat	Enriched	In nat	Enriched	In nat	Enriched	SE	P-value
TC (%DM)	92.43 ^{A}	89.52 ^{B}	79.82 ^{E}	63.37 ^{F}	85.99 ^{C}	54.23 ^{H}	82.87 ^{D}	58.17 ^{G}	0.386	<0.001
A+B1 (%TC)	92.58 ^{B}	95.36 ^{A}	30.89 ^{E}	24.24 ^{F}	59.81 ^{C}	49.03 ^{D}	49.75 ^{D}	31.85 ^{E}	0.556	<0.001
A+B1 (g/100g)	85.57	85.37	24.66	15.36	51.43	26.59	41.23	18.53		
B2 (%TC)	7.00 ^{F}	4.33 ^{G}	38.43 ^{D}	39.48 ^{D}	35.70 ^{E}	43.66 ^{C}	46.62 ^{B}	62.11 ^{A}	0.442	<0.001
B2 (g/100g)	6.47	3.88	30.67	25.02	30.7	23.68	38.63	36.13		
C (%TC)	0.42 ^{G}	0.31 ^{G}	30.29 ^{B}	36.28 ^{A}	4.49 ^{E}	7.31 ^{C}	3.63 ^{F}	6.04 ^{D}	0.146	<0.001
C (g/100g)	0.39	0.28	24.18	22.99	3.86	3.96	3.01	3.51		

[1]In natura; [2]Standard error; CT= total carbohydrates (%DM); A+B1= non-fibrous carbohydrates (%TC); B2= available components corresponding to the potentially degradable fraction (%CT); C= indigestible fraction of the cell wall (%CT). [5]p-value of interaction agro-industrial wastes x protein enrichment.

6.3.3 Protein fractionation

For cassava chip enriched with *S. cerevisiae*, there was a significant increase in CP content ($P<0.001$), rising from 1.75% to 4.75% (Table 13). This increase was accompanied by a reduction in non-protein nitrogen content ($P<0.001$), which decreased from 34.36% to 18.77%. Additionally, the B1+B2 fractions increased from 65.20% to 81.07% ($P<0.001$). Simultaneously, there were significant reductions in both the slow B3 fraction and the indigestible C fraction ($P<0.001$), decreasing from 0.11% to 0.09% and from 0.33% to 0.06%, respectively.

In enriched acerola, there was an increase in CP, reaching 31.15%, compared to the 13.53% in untreated acerola (Table 13). This increase was accompanied by a significant increase in non-protein nitrogen content, increasing from 4.24% to 46.56%, and a significant reduction in the fractions B1+B2, decreasing from 95.27% to 53.19%. The fraction B3 and fractions C also decreased significantly, from 0.21% to 0.07% and from 0.28% to 0.18%, respectively.

The enriched orange showed a substantial increase in crude protein (CP) content, rising from 7.61% to 40.12% (Table 13). This increase was accompanied by a significant rise in non-protein nitrogen content, from 46.62% to 79.72%, while the B1+B2 fractions significantly decreased from 53.19% to 20.23%. Additionally, fractions B3 and C also exhibited significant reductions, with B3 decreasing from 0.13% to 0.02% and C from 0.07% to 0.03%.

The enriched orange exhibited an increase in CP content, rising from 7.61% to 40.12% (Table 13). This elevation was accompanied by a rise in non-protein nitrogen content, increasing from 46.62% to 79.72%, and a significant reduction in the fractions B1+B2, decreasing from 53.19% to 20.23%. Furthermore, fraction B3 and fraction C also reduced significantly, from 0.13% to 0.2% and from 0.07% to 0.03%, respectively.

Table 13 – Protein fractionation of agro-industrial wastes (%CP and g/100g DM).

Item	Cassava chip		Acerola pomace		Orange pomace		Pineapple peel			
	In nat[1]	Enriched	In nat	Enriched	In nat	Enriched	In nat	Enriched	SE[2]	P-value
CP (%DM)	1.75 ^{G}	4.75 ^{F}	13.53 ^{D}	31.15 ^{C}	7.61 ^{E}	40.12 ^{A}	9.45 ^{E}	33.52 ^{B}	0.699	<0.001
A (%CP)	34.36 ^{D}	18.77 ^{E}	4.24 ^{F}	46.56 ^{C}	46.62 ^{C}	79.72 ^{A}	65.15 ^{B}	82.11 ^{A}	1.810	<0.001
A (g/100g)	0,60	0,89	0,57	14,50	3,55	31,98	6,16	27,52		
B1+B2 (%CP)	65.20 ^{C}	81.07 ^{B}	95.27 ^{A}	53.19 ^{D}	53.19 ^{D}	20.23 ^{F}	34.66 ^{E}	17.82 ^{F}	1.813	<0.001
B1+B2 (g/100g)	1,14	3,85	12,89	16,57	4,04	8,12	3,27	5,97		
B3 (%CP)	0.11 ^{C}	0.09 ^{D}	0.21 ^{A}	0.07 ^{F}	0.13 ^{B}	0.02 ^{H}	0.08 ^{E}	0.05 ^{G}	0.002	<0.001
B3 (g/100g)	0,002	0,004	0,028	0.01	0,01	0,008	0,01	0,17		
C (%CP)	0.33 ^{A}	0.06 ^{E}	0.28 ^{B}	0.18 ^{C}	0.07 ^{E}	0.03 ^{F}	0.12 ^{D}	0.01 ^{G}	0.005	<0.001
C (g/100g)	0,006	0.003	0,038	0,005	0,012	0,01	0,01	0,003		

[1]In natura; [2]Standard error; CP= crude protein (%DM); A= non-protein nitrogen (%CP); B1+B2= degradable protein (%CP); B3= slowly degradable protein (%CP); C= unavailable protein (%CP). [5]p-value of interaction agro-industrial wastes x protein enrichment.

Similarly, the enriched pineapple experienced a significant increase in CP content, reaching 33.52%, compared to the untreated pineapple which had 9.45% ($P<0.001$; Table 13). This was accompanied by an increase in non-protein nitrogen, increasing from 65.15% to 82.11% ($P<0.001$), and a decrease in the fractions B1+B2, dropping from 34.66% to 17.82% ($P<0.001$). A significant reduction was observed in both fraction B3 and fraction C, with their values decreasing from 0.08% to 0.05% and from 0.12% to 0.01%, respectively ($P<0.001$).

6.3.4 Mineral profile

Yeast-enriched cassava chip showed significant reductions in Cu, Mn, and Zn, and an increase in the Na content ($P<0.001$), with no differences observed for P, K, Ca, Mg, B, and Fe ($P>0.05$). In acerola waste, no significant changes were found in P, Ca, B, Fe, or Na content, but there were significant reductions in both macronutrients (K, Mg, S) and micronutrients (Cu, Mn, Zn) ($P<0.05$). Similarly, orange waste showed no significant differences in P, K, Mg, S, B, Fe, or Mn ($P>0.05$), but enrichment led to a significant reduction in Ca, Cu, and Zn ($P<0.05$). For pineapple peel, no significant changes were observed in P, K, Ca, Mg, B, or Fe content ($P>0.05$), although significant increase was noted in Na content ($P<0.001$), alongside decreases in S, Cu, Mn, and Zn ($P<0.05$; Table 14).

6.3.5 Kinetics of gas production

The exponential model parameters were significantly affected by waste type and protein enrichment, with greater differences between cassava chip and the acerola pomace (Figure 1 and Table 15). There were differences in accumulated gas production (a), with higher values observed for cassava chip (in natura: 385.7 mL, enrich: 317.2 mL). Likewise, the higher b (in natura: 6.19, enrich: 5.80) and c (in natura: 0.12, enrich: 0.13) coefficients were observed in the cassava chip compared to the other wastes ($P<0.05$; Figure 1).

Table 14 – Mineral composition of agro-industrial wastes.

Item	Cassava chip		Acerola pomace		Orange pomace		Pineapple peel		SE	p-value
	In nat[1]	Enriched	In nat	Enriched	In nat	Enriched	In nat	Enriched		
				g kg^{-1}						
P	0.91 ^{A}	0.91 ^{A}	1.19 ^{A}	1.59 ^{A}	0.94 ^{A}	1.16 ^{A}	1.01 ^{A}	1.36 ^{A}	0.518	0.762
K	9.00 ^{AB}	8.00 ^{AB}	11.50 ^{A}	4.67 ^{B}	9.83 ^{AB}	6.67 ^{AB}	11.67 ^{A}	12.17 ^{A}	2.184	<0.001
Ca	2.50 ^{B}	2.29 ^{B}	5.08 ^{B}	1.67 ^{B}	12.04 ^{A}	4.41 ^{B}	5.27 ^{B}	2.56 ^{B}	1.374	<0.001
Mg	1.29 ^{BC}	1.07 ^{C}	3.02 ^{A}	1.98 ^{B}	1.56 ^{BC}	1.36 ^{BC}	1.52 ^{BC}	1.23 ^{BC}	0.266	<0.001
S	0.73 ^{D}	0.96 ^{CD}	3.69 ^{A}	1.40 ^{CD}	2.56 ^{ABC}	2.04 ^{BCD}	3.67 ^{A}	3.28 ^{B}	0.567	<0.001
				mg kg^{-1}						
B	16.19 ^{A}	19.55 ^{A}	28.54 ^{A}	29.57 ^{A}	31.29 ^{A}	22.92 ^{A}	35.68 ^{A}	16.81 ^{A}	11.897	0.507
Cu	22.44 ^{B}	9.46 ^{C}	29.50 ^{A}	11.25 ^{C}	25.21 ^{AB}	8.81 ^{C}	27.58 ^{AB}	10.84 ^{C}	2.220	<0.001
Fe	344.33 ^{BC}	332.48 ^{BC}	526.59 ^{BC}	207.00 ^{C}	627.26 ^{ABC}	306.47 ^{C}	756.38 ^{AB}	972.94 ^{A}	150.196	<0.001
Mn	12.50 ^{D}	6.61 ^{F}	23.58 ^{C}	11.73 ^{DE}	11.89 ^{DE}	7.17 ^{EF}	85.25 ^{A}	48.71 ^{B}	1.798	<0.001
Zn	40.04 ^{B}	16.02 ^{C}	63.99 ^{A}	19.46 ^{C}	49.55 ^{B}	11.85 ^{C}	45.92 ^{B}	13.66 ^{C}	4.136	<0.001
Na	140.00 ^{BC}	320.00 ^{A}	330.00 ^{A}	296.67 ^{A}	266.67 ^{A}	336.67 ^{A}	100.00 ^{C}	243.33 ^{AB}	42.180	<0.001

[1]In natura; [6]Standard error. [5]P-value of interaction agro-industrial wastes x protein enrichment.

Conversely, lower accumulated gas production was observed in acerola (in natura: 148.8 mL, enrich: 113.4 mL), followed by pineapple peel (in natura: 270.2 mL, enrich: 201.7 mL), and orange (in natura: 306.2 mL, enrich: 233.9 mL). This pattern was similarly observed for parameters *b* and *c* of acerola, pineapple peel, and orange (Table 15).

A longer inflection time was observed in the enriched wastes compared to the in natura materials (acerola: 52.7 vs 35.7 h; pineapple: 38.5 vs 28.2 h; and orange: 35.8 vs 26.2 h). However, the cassava chip presented a longer inflection time in the in natura material compared to the enriched material (53 h vs 46.3 h) ($P<0.001$; Table 15). However, the maximum gas production rate and gas production at the inflection point were higher in in natura wastes, especially cassava chip (in natura: 141.9 mL, enrich: 116.7 mL) ($P<0.001$; Table 15). Orange and pineapple wastes showed moderate values for these parameters, with a consistent reduction from fresh to enriched conditions. The colonization time was shorter in enriched wastes, especially orange (in natura: 16.7 h, enrich: 24.2 h) and pineapple (in natura: 16.9 h, enrich: 25.5 h) wastes ($P<0.001$; Table 15; Figure 1).

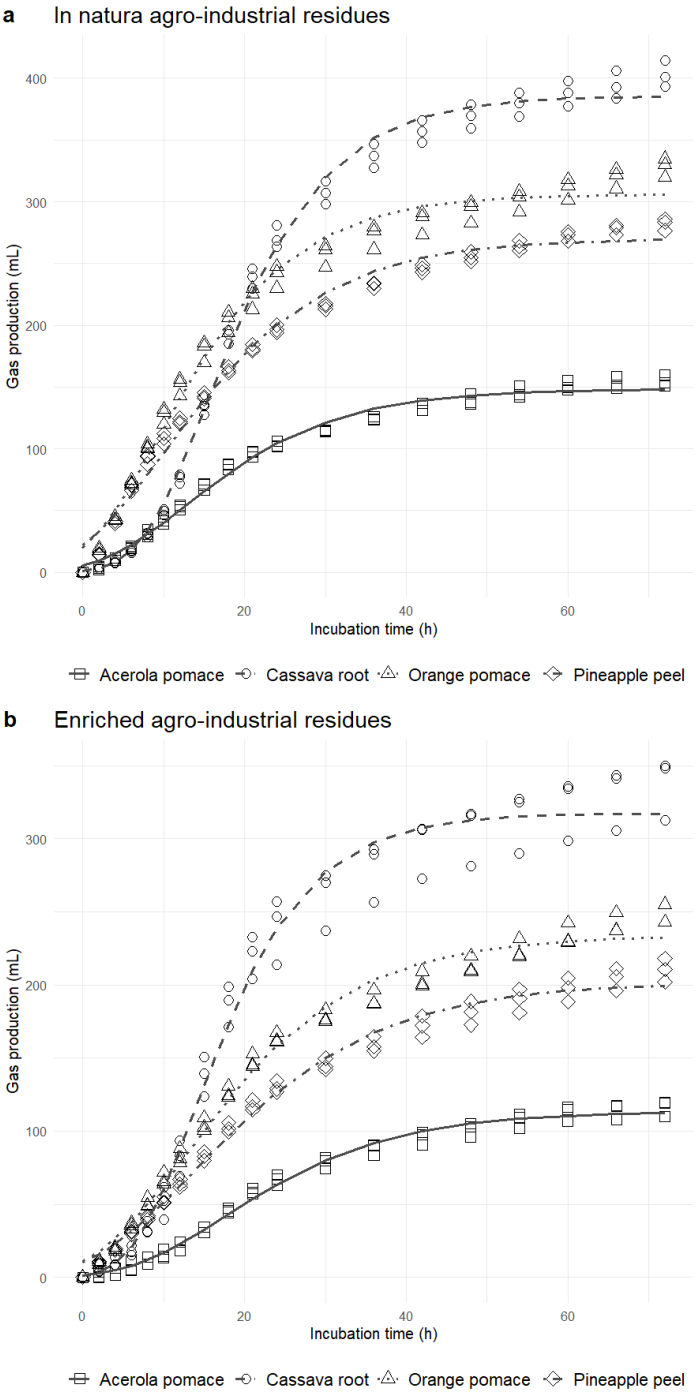

Figure 1 – Predicted cumulative gas production of the agro-industrial wastes per gram of incubated dry matter. Symbols: observed values; lines: predicted values.

Table 15 – Parameters of the Logistic model to produce gas observed in the agro-industrial wastes.

Coefficients	Cassava chip		Acerola pomace		Orange pomace		Pineapple peel		SE	P-value
	In nat[1]	Enrich	In nat	Enrich	In nat	Enrich	In nat	Enrich		
a	385.65 ^{A}	317.21 ^{B}	148.78 ^{F}	113.43 ^{G}	306.16 ^{B}	233.85 ^{D}	270.20 ^{C}	201.69 ^{E}	9.92	<0.001
b	6.19 ^{A}	5.80 ^{A}	3.32 ^{C}	4.50 ^{B}	2.75 ^{CD}	3.08 ^{CD}	2.50 ^{D}	2.96 ^{CD}	0.21	<0.001
c	0.12 ^{A}	0.13 ^{A}	0.09 ^{BC}	0.09 ^{BC}	0.10 ^{B}	0.09 ^{BC}	0.09 ^{BC}	0.08 ^{C}	0.01	<0.001
R^2	99.7%	99.3%	99.2%	99.6%	98.9%	99.1%	98.7%	99.3%		
TIP (h)	53.0 ^{A}	46.3 ^{B}	35.7 ^{C}	52.7 ^{A}	26.2 ^{D}	35.8 ^{C}	28.2 ^{D}	38.5 ^{C}	1.88	<0.001
GIP (mL)	141.87 ^{A}	116.69 ^{B}	54.73 ^{F}	41.73 ^{G}	112.63 ^{B}	86.03 ^{D}	99.40 ^{C}	74.20 ^{E}	3.65	<0.001
MRGP (mL/h)	16.56 ^{A}	14.64 ^{B}	5.11 ^{E}	3.55 ^{F}	11.81 ^{C}	7.39 ^{D}	8.82 ^{D}	5.72 ^{E}	0.52	<0.001
LP (h)	44.45 ^{A}	38.30 ^{B}	24.93 ^{C}	40.98 ^{AB}	16.65 ^{D}	24.17 ^{C}	16.94 ^{D}	25.48 ^{C}	1.89	<0.001

TIP time to inflection point (h), GIP gas to the inflection point (ml), MRGP maximum rate of gas production (ml/h), LP lag phase (h).

6.4 Discussion

The results of this study highlight that enriching agro-industrial wastes with S. cerevisiae not only led to a significant increase in crude protein content but also induced specific changes in the carbohydrate, protein, and mineral composition of each waste analyzed. While the significant increase in crude protein content can be attributed to the utilization of carbohydrate sources present in the wastes by *S. cerevisiae* to support its cellular growth and biomass production (De-Barros et al., 2023), it is important to note that this enrichment also resulted in an increase in non-protein nitrogen (NPN) content. This suggests that not all of the nitrogen was converted into true microbial protein, as some remained in the form of NPN compounds, such as free amino acids or peptides. *S. cerevisiae* secretes enzymes like cellulases and pectinases, which hydrolyze complex polysaccharides into simple sugars that can be assimilated (Afifi, 2011; Haske et al., 2023). These sugars are subsequently metabolized through the glycolytic pathway, providing energy for cellular growth (De-Barros et al., 2023; Pinu et al., 2014).

Additionally, previous studies highlight the biotechnological potential of using *S. cerevisiae* to increase protein and amino acid content while reducing fiber content in various fruit and vegetable wastes (including banana, cassava, potato, sugar beet, orange, mango, apple, pineapple, carrot, etc.) (Bacha et al., 2011; Bajić et al., 2023; Khan et al., 2022; Khejornsar and Khejornsart, 2021; Razzaq et al., 2020).

Moreover, it is crucial to highlight the fundamental role of nitrogen sources in facilitating efficient cellular growth (Khan et al., 2022; Razzaq et al., 2020). Nitrogen is indispensable for yeast metabolism, serving as the building blocks for amino acid synthesis. Without adequate nitrogen sources, yeast faces constraints in amino acid production, thereby impeding its growth process and metabolic functions (Afifi, 2011; Albers et al., 1996; Khan et al., 2022; Razzaq et al., 2020).

The increase in protein content led to distinct alterations in the protein fractions within each agro-industrial waste. There was a significant increase in non-protein nitrogen content, possibly due to the contribution of *S. cerevisiae* itself, both in terms of protein value and amino acid profile (Yamada et al., 2003; Yamada and Sgarbieri, 2005). This yeast can represent between 20% and 30% of the total nitrogen content in the form of NPN, consisting mainly of nucleic acids (Yamada et al., 2003; Yamada and Sgarbieri, 2005). Furthermore, previous studies have shown a significant increase in amino acid fractions after waste enrichment, with

leucine, followed by valine and lysine, being the most prominent (Khan et al., 2022; Razzaq et al., 2020). Thus, wastes enriched with *S. cerevisiae* not only boast a substantial protein content but also may exhibit a high-quality amino acid profile, rendering them a viable alternative for animal nutrition.

Controversially, it is crucial to note that, quantitatively (g/kg dry matter), protein fractions B3 and C displayed a decrease, whereas both fraction A and fractions B1+B2 exhibited significant enhancement post-yeast enrichment. These results strongly suggest a redistribution of nitrogenous fractions within the wastes, likely influenced by the incorporation of nucleic acids and other constituents produced by yeast during the biomass production process (Razzaq et al., 2020).

The observed increase in protein content seems to be correlated with the results of the carbohydrate fractionation analysis, especially carbohydrates from fractions A+B1, indicating an inverse relationship between protein and carbohydrate contents. As explained previously, this yeast can secrete enzymes that hydrolyze complex polysaccharides into simple sugars that can be assimilated, generating energy for cell growth (Afifi, 2011; De-Barros et al., 2023; Haske et al., 2023). However, it is interesting to note that, despite cassava root being an excellent source of starch (Unnawong et al., 2023), there was no significant reduction in the A+B1 fractions. This occurrence could be attributed to the limited amylolytic activity inherent in *S. cerevisiae,* which commonly lacks the inherent capability to enzymatically degrade complex carbohydrates like starch into more readily accessible simple sugars independently (Gupta et al., 2003).

The significant decrease in the mineral profile of wastes enriched with *S. cerevisiae* can be attributed to the utilization or absorption of these minerals by yeast during the cell growth process (Chen et al., 2021; Stanly Pradeep and Pradeep, 2013). It is important to emphasize that many minerals are essential for yeast metabolism, participating in metabolic reactions or acting as enzyme cofactors to ensure proper cellular functioning (Chen et al., 2021; Gaensly et al., 2014; Stanly Pradeep and Pradeep, 2013). Therefore, *S. cerevisiae* is expected to utilize not only carbohydrates and nitrogen sources present in waste, but also minerals, to maximize its growth and biomass production (Stanly Pradeep and Pradeep, 2013; Yamada and Sgarbieri, 2005). These discoveries emphasize the crucial role minerals play in yeast metabolism and the generation of biomass in a wide range of agro-industrial substrates.

The accumulated gas production during the 72-hour incubation period indicates significant differences in gas production across wastes and treatments. Cassava chip generally exhibits the highest production and gas rates, while acerola pomace shows the lowest. Additionally, we noticed that the enriched condition typically presents lower values for gas production parameters compared to the natural condition.

The higher values of gas production parameters in cassava chip are possibly caused by the higher fraction of non-fibrous carbohydrates and rapidly fermentable carbohydrates, which provide more fermentable substrate for ruminal microorganisms (Rodríguez et al., 2014; Wanapat and Kang, 2015). On the other hand, the lower values of total gas production observed in acerola waste may be due to the higher concentration of lignin and the indigestible fraction of carbohydrates, which markedly limit microbial access (Grabber, 2005; Lousada et al., 2005).

In general, enriched residues showed lower gas production and lower values for Gompertz model parameters, such as GIP, MRGP, and LP, compared to untreated residues. During the protein enrichment process, yeast primarily utilized carbohydrates—especially non-fibrous and readily degradable ones—for its growth and microbial biomass production (De-Barros et al., 2023). This suggests that the reduced total gas production and Gompertz parameters in enriched residues, relative to untreated residues, were due to the consumption of carbohydrates by *S. cerevisiae* during enrichment, which decreased the availability of these substrates for rumen microorganisms.

6.5 Conclusion

The protein enrichment of acerola, orange, and pineapple wastes with *S. cerevisiae* significantly enhances protein content—up to five times—while simultaneously reducing fibrous fractions, which improves digestibility. The enriched agro-industrial wastes exhibit lower total gas production, with cassava chip showing higher gas production rates and acerola pomace displaying lower rates. In conclusion, these findings indicate that enriching agro-industrial wastes with *S. cerevisiae* offers a promising source of nutrients and presents a viable alternative to conventional protein ingredients in animal feed.

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7 CAPÍTULO III – RUMINAL FERMENTATION KINETICS AND *IN VITRO* GAS PRODUCTION OF AGRO-INDUSTRIAL BY-PRODUCTS AS ALTERNATIVES TO SOYBEAN MEAL IN RUMINANT

CINÉTICA DA FERMENTAÇÃO RUMINAL E PRODUÇÃO DE GASES *IN VITRO* DE SUBPRODUTOS AGROINDUSTRIAIS COMO ALTERNATIVAS AO FARELO DE SOJA EM DIETAS PARA RUMINANTES

RESUMO

Este estudo teve como objetivo avaliar o impacto da substituição do farelo de soja por resíduos agroindustriais de acerola, abacaxi e laranja enriquecidos com levedura na cinética da fermentação ruminal e na produção de gás *in vitro*. Utilizando um delineamento fatorial 3×5 em um delineamento inteiramente casualizado com três repetições, o estudo testou diferentes resíduos agroindustriais (acerola, laranja e abacaxi) e níveis de substituição (0%, 25%, 50%, 75% e 100% de matéria seca). A produção cumulativa de gás foi medida ao longo do tempo e analisada usando o modelo de Gompertz. Contrastes polinomiais lineares e quadráticos foram usados para examinar as respostas dos alimentos ao aumento dos níveis de adição dos resíduos. A inclusão de resíduo de abacaxi aumentou linearmente a produção total de gás (P=0,038), enquanto o resíduo de acerola resultou em uma diminuição linear (P=0,004) e o resíduo de laranja não teve efeito significativo na produção de gás (P=0,244). A inclusão do resíduo de laranja reduziu a fase lag (P=0,028), enquanto a inclusão do resíduo de acerola a aumentou (P=0,009). No entanto, a inclusão do resíduo de abacaxi não teve efeito significativo na fase lag (P=0,567). A digestibilidade da matéria orgânica aumentou com a inclusão de resíduos de laranja (P=0,045) e abacaxi (P=0,049), mas diminuiu linearmente com maior inclusão de acerola (P=0,008). No geral, a substituição do farelo de soja por resíduos enriquecidos com levedura afeta positivamente a fermentação ruminal, a produção de gás e a digestibilidade da matéria orgânica. Os resíduos de abacaxi e laranja são substitutos eficazes, capazes de substituir até 100% do farelo de soja no concentrado. No entanto, a inclusão do resíduo de acerola não foi eficiente, resultando em respostas menores nos parâmetros avaliados em comparação ao tratamento controle.

Palavras-chave: Alimentação animal, Fermentação ruminal, Resíduos de frutas,

Saccharomyces cerevisiae.

RUMINAL FERMENTATION KINETICS AND *IN VITRO* GAS PRODUCTION OF AGRO-INDUSTRIAL BY-PRODUCTS AS ALTERNATIVES TO SOYBEAN MEAL IN RUMINANT DIETS

ABSTRACT

This study aimed to evaluate the impact of replacing soybean meal with yeast-enriched acerola, pineapple, and orange agro-industrial residues on ruminal fermentation kinetics and *in vitro* gas production. Using a 3×5 factorial design in a completely randomized design with three replicates, the study tested different agro-industrial residues (acerola, orange, and pineapple) and replacement levels (0%, 25%, 50%, 75%, and 100% dry matter). Cumulative gas production was measured over time and analyzed using the Gompertz model. Linear and quadratic polynomial contrasts were used to examine responses of feeds to increasing addition levels of the residues. The inclusion of pineapple residue linearly increased total gas production ($p=0.038$), while acerola residue resulted in a linear decrease ($p=0.004$) and orange residue had no significant effect on gas production ($p>0.05$). Orange residue linearly reduced lag phase ($p=0.048$), acerola showed a quadratic effect with an increase up to 75% inclusion ($p=0.009$), while pineapple had no significant effect on the lag phase ($p>0.05$). Digestibility of organic matter increased with the inclusion of orange and pineapple residues ($p<0.05$), but decreased linearly with higher acerola inclusion ($p=0.008$). Overall, replacing soybean meal with yeast-enriched residues affects positively ruminal fermentation, gas production, and digestibility of organic matter. Pineapple and orange residues are effective substitutes, capable of replacing up to 100% of the soybean meal in the concentrate. However, the inclusion of acerola residue was not efficient, resulting in lower responses in the evaluated parameters compared to the control treatment.

Keywords: Animal Feed, Fruit waste, Ruminal fermentation, *Saccharomyces cerevisiae*.

7.1 Introduction

Agro-industrial by-products, which consist of waste from agricultural crops or fruit and vegetable processing industries, pose significant environmental challenges due to their potential as pollutants (Kumar et al., 2022). Globally, it's estimated that about 22% of the total food losses and waste produced by the food chain come from fruits and vegetables (Santos et al., 2022), highlighting the magnitude of the issue.

Incorporating agro-industrial by-products as feed ingredients offers a promising solution, not only by mitigating the environmental impact of waste disposal but also reducing competition between animals and humans for land and other resources to grow cereal grains that are fed to animals but could be eaten directly by humans (Fernandes et al., 2022; Wilkinson, 2011), contributing to the sustainability of both agro-industrial and livestock production (Salami et al., 2019).

These by-products are rich in carbohydrates, minerals, and excellent sources of fiber (García-Rodríguez et al., 2019). However, their low protein content limits their effectiveness as a primary component in ruminant diets. To address this limitation, the use of yeast (*Saccharomyces cerevisiae*), a probiotic known for its positive effects on rumen fermentation in ruminants, has been explored (Amin and Mao, 2021; Baker et al., 2022; Desnoyers et al., 2009). Yeast has been employed as a biological method to enhance the protein quality of feedstuffs (Boonnop et al., 2009; Khan et al., 2022). Thus, enriching agro-industrial by-products, particularly residues from acerola, pineapple, and orange, with yeast could potentially improve their nutritional profile by reducing fiber content and boosting protein levels.

Given this context, we hypothesized that replacing soybean meal with yeast-enriched acerola, pineapple, and orange residues in ruminant diets would enhance ruminal fermentation kinetics and *in vitro* gas production while maintaining or improving organic matter digestibility. This enhancement is anticipated to result from the increased availability of fermentable substrates and the beneficial effects of yeast enrichment on microbial activity in the rumen, leading to more efficient fermentation compared to traditional soybean meal. Therefore, the objective of this study was to evaluate the impact of this replacement on ruminal fermentation kinetics, *in vitro* gas production, and *in vitro* organic matter digestibility.

7.2 Material and Method

7.2.1 Location and Ethics Committee Protocol Number

Protein enrichment of agro-industrial wastes and chemical analyses were conducted at the Federal University of Maranhão (UFMA) in Chapadinha, Maranhão, Brazil. *In vitro* Gas production kinetic analyses were conducted at the Federal University of Agreste de Pernambuco (UFAPE) in Garanhus, Pernambuco, Brazil. Mineral analyses were conducted at the Brazilian Agricultural Research Corporation (Embrapa Semiárido) in Petrolina, Pernambuco, Brazil. All the animals were cared for in accordance with guidelines of the National Council for the Control of Animal Experimentation (CONCEA, 2023).

7.2.2 Protein enrichment of agro-industrial residues

The agro-industrial residues utilized in this study, including acerola pomace, orange pomace, and pineapple peel, were sourced from local Fruit Pulp Agroindustry in Chapadinha and Vargem Grande, Maranhão, Brazil. To enrich the substrate with protein, we used yeast (*S. cerevisiae,* Instant Dry Yeast, Angel Yeast Co., Ltd), urea, and brown sugar, all purchased from a local shop. The residues were dried in an oven at 60°C for 48 hours.

For the protein enrichment process, about 1 kg of each residue, with a dry matter content of 85-95%, was used. The moisture content was adjusted to 50% by adding a solution containing 10% urea, 1.25% molasses, and 5% *S. cerevisiae*, resulting in approximately 1.2 x 10^8 yeast cells per milliliter (Kaewwongsa et al., 2011). The enriched material was incubated for 5 days, then dried, ground, and stored for further analysis.

7.2.3 Experimental design and dietary treatments

This study was conducted using an *in vitro* gas production technique at various incubation time intervals. The experimental design was a 3×5 factorial arrangement in a completely randomized design with three replications per treatment. The first factor was the inclusion of these residues enriched with yeast, and the second factor was the levels of substitution of soybean meal by these residues (0%, 25%, 50%, 75%, and 100% of the dry matter).

The diet consisted of corn silage, soybean meal, urea, and mineral premix. The diets were formulated to be isonitrogen according to the NRC (2007) for lambs with an average weight of 22 kg and an average daily gain of 200 g/animal/day. Corn silage was used as the roughage, with a roughage-to-concentrate ratio of 40:60.

Samples of roughage and concentrates were dried at 60 °C, then ground to pass a 1-mm sieve (Cyclotech Mill, Tecator, Sweden) and used for chemical analysis and in the *in vitro* gas

test. The samples were analyzed for dry matter (DM; method 967.03), protein crude (CP; method 988.05), and mineral matter (MM; method 942.05) using the Association of Official Analytical Chemists (AOAC, 2016). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991) with modifications proposed by Senger et al. (2008), in which he used an autoclave at 110 °C for 40 minutes. The chemical composition of the control treatment, enriched residues, and corn silage are shown in Table 16.

Table 16 – Chemical composition of the control diet, yeast-enriched residues, and corn silage used in the experiment on dry matter basis.

Parameters (g/kg)	Control	Corn Silage	Enriched agro-industrial residues		
			Acerola	Orange	Pineapple
Dry matter	604.0	292.2	691.3	610.5	673.8
Organic matter*	955.5	950.3	961.4	952.0	919.7
Mineral matter*	38.1	49.7	35.9	48.0	80.3
Crude protein*	121.9	64.8	311.5	401.2	335.2
Neutral detergent fiber*	365.1	668.5	510.8	276.3	404.1
Acid detergent fiber*	139.2	295.3	382.3	198.2	189.3

* grams per kilograms of dry matter

7.2.4 Ruminal fluid collection and media solution preparation

The ruminal fluid used as inoculum was collected and homogenized from two rumen-fistulated sheep (average body weight of 30 kg) housed in individual pens and fed with elephant grass (*Pennisetum purpureum*) cv. IRI-381, a concentrate based on cornmeal and soybean meal, supplemented with mineral salt (Ovinofós, Tortura, Porto Alegre, Brazil), and provided with ad libitum water. Rumen fluid (solid and liquid) was manually collected from multiple sites within the rumen, transferred into prewarmed (39 ºC) bottles, and immediately transported to the laboratory (Yáñez-Ruiz et al., 2016). In the laboratory, the rumen fluid was ground using a blender with CO_2 flush, filtered through four-layer cheesecloth.

7.2.5 Kinetics of gas production

Gas production was determined using an *in vitro* technique with a pressure transducer (LOGGER AG100–Agricer), following Theodorou et al. (1994). Cumulative gas production was measured at 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 30, 36, 42, 48, 54, 60, 66, and 72 hours after incubation. The equation used to convert pressure (psi) to gas volume (mL) was developed at the Federal University of Agreste of Pernambuco (UFAPE) from 937 observations (1 psi = 4859 mL gas). The equation was the follow: gas production (mL) = 5.1612 × psi − 0.3017 (R^2 = 0.9873).

Cumulative gas production data were analyzed using the Gompertz model (Lavrenčič et al., 1998) by the following equation:

$$Y = ae^{-be^{-ct}}$$

Where, Y represents gas production at time t, 'a' is the maximum gas production, 'b' is the difference between initial and final gas, and 'c' is the rate of gas accumulation.

The following fermentation indicators were calculated: time to point of inflection (TPI, hours), gas inflection point (GPI ml), maximum gas production rate (TMPG, ml/h), and lag phase (FL or microbial accommodation h). To estimate the biological parameters, the following equations were used: TPI = b/c; GPI = a/*e*; TMPG = (a*c)/*e*; and FL = ((b/c) − (1/c)); where *e* is Euler's number, which equals approximately 2.718281828459.

In vitro organic matter digestibility (OMD, %) was estimated using the equation from Menke et al. (1979): OMD (%) = 14.88 + 0.889*GP + 0.45*CP + 0.651*A, where GP is the 24-hour net gas production (mL/200 mg DM), CP is the crude protein content (%), and A is the ash content (%).

7.2.6 Statistical analysis

The experimental design for the *in vitro* analyses of ruminal gas production and fermentation parameters was completely randomized, considering residues enriched with yeast (acerola, orange, and cassava) and the inclusion level (control 0%, 25%, 50%, 75%, and 100% of the dry matter) as fixed factors. The statistical model was:

$$Yijk = \mu + Ri + Lj + (R \times L)ij + \varepsilon ijk$$

Where: Yijk is the response variable; μ is the overall mean; Ri represents the effect of the i-th residue; Lj represents the effect of the j-th inclusion level; (R x L)ij represents the interaction effect between residue and inclusion level; εijk is the random error term associated with the *k*-th replicate for each combination of residue and inclusion level.

Linear and quadratic polynomial contrasts were used to examine responses of feeds to increasing addition levels of the agro-industrial residues. Statistical analyses were performed using R software, version 4.2.3, with RStudio (R Core Team, 2023). A significance level of 5% was considered.

7.3 Results

The results from the gas kinetics analysis and OMD revealed significant and distinct responses following the 72-hour incubation period, where the substitution of soybean meal with

various enriched residues in the diet resulted in specific variations depending on the type of residue and the level of inclusion (Figure 2 and Table 17).

The inclusion of pineapple residue as a replacement for soybean meal led to a quadratic increase in maximum gas production (a), with minimum point at 25% inclusion (P = 0.031) (Figure 2 and Table 17). In contrast, the addition of acerola residue led to a linear decrease in gas production (P = 0.004). On the other hand, the inclusion of orange residue did not result in any significant changes in gas production (P = 0.244) (Figure 2 and Table 17). Furthermore, the time required to reach the inflection point (b), an indicator of the beginning of accelerated gas production, showed a quadratic effect with maximum point was 75% inclusion of acerola residue (P = 0.002; Table 17). In contrast, no such effect was observed for orange (P = 0.972) or pineapple residues (P = 0.878; Table 17). A quadratic effect was observed in the rate of gas accumulation (c) with acerola residue, maximum point was 25% inclusion (P = 0.029), whereas no significant differences were found with orange (P = 0.194) and pineapple residues (P = 0.851; Table 17).

The inclusion of pineapple (P = 0.555) and acerola residues (P = 0.315) did not affect the time to point of inflection (TPI), but a significant reduction in TIP was observed with the inclusion of orange residue (P = 0.024; Table 17). The maximum rate of gas production (MRGP) linear decreased with acerola residue inclusion (P = 0.013), while pineapple (P = 0.146) and orange residue (P = 0.090) inclusion did not significantly affect the maximum gas production rate (Table 17). Similarly, gas at the inflection point (GPI) decreased with the inclusion of acerola residue (P = 0.004), while a quadratic increase was observed with pineapple residue, reaching a maximum at 75% inclusion (P = 0.031). No significant effect was observed for orange residue inclusion on GPI (P = 0.244; Table 17).

The colonization time of diet particles by bacteria, often referred to as the lag phase, decreased quadratically, reaching a minimum at 25% inclusion of orange residue (P = 0.028), and increased quadratically, with a maximum at 31.6% inclusion of acerola residue (P = 0.009). No significant change in the lag phase were observed with the inclusion of pineapple (P = 0.567; Table 17). A positive linear response in OMD was observed with the inclusion of pineapple (P = 0.049) and orange (P = 0.045) residues as replacements for soybean meal. In contrast, acerola residue led to a linear reduction in OMD when it fully replaced soybean meal in the diet (P = 0.008).

Table 17 – Impact of five replacing levels (0%, 25%, 50%, 75%, 100% of dry matter basis) of soybean meal with three yeast-enriched residues (acerola pomace, pineapple pomace and orange pomace) on *in-vitro* rumen gas kinetics parameters, and cumulative gas production and organic matter digestibility after 72 h of incubation.

Residues	**Level**	**Gompertz parameters**			**Gas production parameters**				**OMD**
		a	*b*	*c*	TPI	GPI	MRGP	LP	
Pineapple pomace	*0*	261.6	3.7	0.091	40.53	96.2	8.8	29.5	51.51
	25	232.7	3.9	0.092	42.37	85.6	7.8	31.4	47.39
	50	239.0	4.1	0.098	41.83	87.9	8.6	31.6	49.01
	75	277.4	3.5	0.092	38.55	102.0	9.4	27.7	54.06
	100	268.5	3.8	0.092	41.75	98.8	9.1	30.8	52.26
P-value	Linear	0.038	0.878	0.851	0.555	0.038	0.146	0.567	0.049
	Quadratic	0.031	0.154	0.230	0.994	0.031	0.500	0.499	0.097
Acerola pomace	*0*	261.6	3.7	0.091	40.53	96.2	8.8	29.5	51.51
	25	256.3	3.9	0.096	40.84	94.3	9.1	30.4	51.20
	50	247.9	3.9	0.094	41.72	91.2	8.6	31.1	50.27
	75	237.1	4.0	0.095	42.10	87.2	8.3	31.6	48.65
	100	228.7	3.5	0.085	41.08	84.2	7.2	29.2	47.07
P-value	Linear	0.004	0.364	0.210	0.315	0.004	0.013	0.800	0.008
	Quadratic	0.794	0.002	0.029	0.257	0.794	0.154	0.009	0.497
Orange pomace	*0*	261.6	3.7	0.091	40.53	96.2	8.8	29.5	51.51
	25	256.2	3.7	0.097	37.96	94.3	9.2	27.6	52.62
	50	251.3	3.4	0.090	37.80	92.4	8.3	26.7	50.80
	75	271.4	3.6	0.094	38.11	99.8	9.4	27.5	53.79
	100	270.1	3.7	0.099	37.69	99.4	9.9	27.5	55.10
P-value	Linear	0.244	0.972	0.194	0.024	0.244	0.090	0.048	0.045
	Quadratic	0.305	0.083	0.486	0.093	0.305	0.218	0.028	0.280
Pooled SEM		14.75	0.19	0.005	1.27	5.43	0.79	1.11	2.18
P-value									
Residues		0.022	0.018	0.549	<0.001	0.022	0.056	<0.001	0.002
Levels		0.090	0.322	0.529	0.528	0.090	0.741	0.348	0.240
Residues*Levels		0.009	0.004	0.116	0.006	0.009	0.026	<0.001	0.006

a = maximum gas production, mL; *b* = difference between initial gas and final gas at an x time; *c* = specific gas accumulation rate; TPI = time to point of inflection, h; GIP = gas at the inflection point, mL; MRGP = maximum rate of gas production, mL/h. LP = lag phase, h; OMD = organic matter digestibility at 72 hours, %.

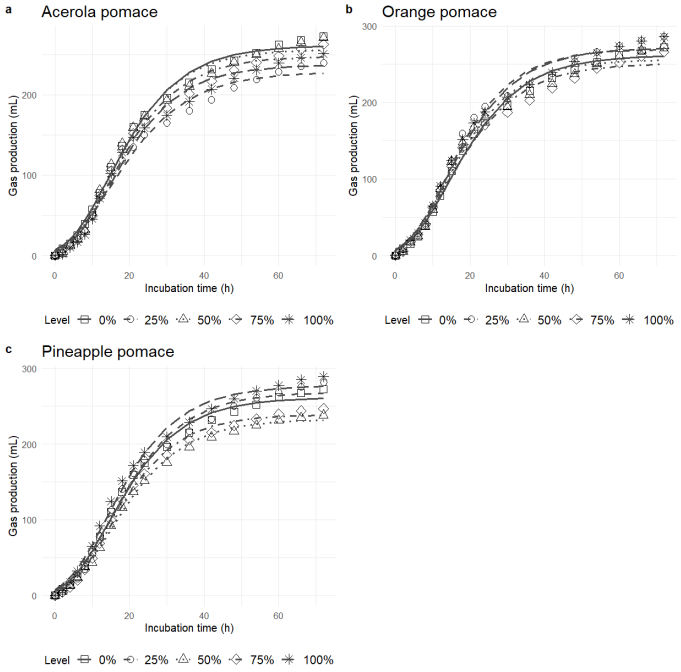

Figure 2 – Impact of five replacing levels (0%, 25%, 50%, 75%, 100% of dry matter basis) of soybean meal with three yeast-enriched residues (acerola pomace, pineapple pomace and orange pomace) on predicted cumulative gas production (mL) from 72h of *in vitro* fermentation. Symbols: observed values; lines: predicted values.

7.4 Discussion

In vitro fermentation kinetics, total gas production and OMD can be influenced by both the chemical composition of the diet and the quality of the fiber (Mertens, 1997). Even with similar chemical compositions, diets with different ingredients can result in different fermentation patterns (Baffa et al., 2023; García-Rodríguez et al., 2019; Getachew et al., 2004; Rymer and Givens, 2002). In this study, the replacement of soybean meal with agro-industrial residues enriched with yeast resulted in variations in fermentation and OMD, mainly due to differences in the chemical composition of the residues analyzed.

The lower gas production, along with the reduced OMD, GPI and MRGP, following the inclusion of acerola residue compared to the control diet, can be explained by its high ADF content, which is less digestible and resistant to degradation in the rumen (Mertens, 1997; Getachew et al., 2004; Lousada et al., 2005). The progressive increase in enriched acerola residue in the diet resulted in a corresponding increase in the total ADF content, which probably compromised the fermentative efficiency of ruminal microorganisms (Jung and Allen, 1995), and impairing the digestibility of organic matter. These results corroborate previous research, which shows that the inclusion of acerola residue in the diet of ruminants may reduce the consumption and digestibility of nutrients, negatively affecting the performance of the animals (Ferreira et al., 2010; Lousada et al., 2005; Mazza et al., 2020).

On the other hand, the increased gas production in diets with pineapple residue, along with the higher OMD, GPI and OMD values, can be attributed to its high-quality fiber, which is rich in soluble and easily fermentable fibers (Getachew et al., 2004; Kiatti et al., 2023; Zhang et al., 2015). This fiber is more easily degraded by ruminal microorganisms, resulting in more efficient fermentation (Getachew et al., 2004).

Yeast-enriched orange residue has also been shown to be an effective alternative to soybean meal. Although the inclusion of orange residue did not improve the evaluated parameters, its ability to maintain constant levels of gas production suggests that it could replace soybean meal without negatively affecting fermentation efficiency. Additionally, the reduction in TIP and lag phase with the inclusion of orange residue may be associated with an increase in the soluble fraction of the diet, which provides a rapidly fermentable energy substrate to the rumen microorganisms (Lee et al., 2003; NASEM, 2021). This facilitates the adhesion and colonization of microorganisms, thereby reaching a peak of gas production in a shorter time and reducing the lag phase (McAllister et al., 1994; Orskov and Mcdonald, 1979).

In addition to the chemical characteristics of the waste, the positive effects on *in vitro* fermentation kinetics, gas production, OMD may be related to the presence of yeast in the enriched waste. Yeasts act as prebiotics in the rumen, supporting microbial growth, stabilizing ruminal pH, and improving fiber degradation (Chaucheyras-Durand et al., 2008). The literature supports this hypothesis, showing that yeast supplementation improves digestibility, fermentation efficiency and overall health of ruminants (Amin and Mao, 2021; Baker et al., 2022; Desnoyers et al., 2009). Thus, the presence of yeast in the residues may have contributed significantly to the positive results, highlighting the usefulness of yeast-enriched residues in ruminant diets.

7.5 Conclusion

In conclusion, our hypothesis is supported by the findings that replacing soybean meal with yeast-enriched residues in ruminant diets enhances ruminal fermentation kinetics and *in vitro* gas production while maintaining or improving organic matter digestibility. Specifically, we found that pineapple and orange residues serve as effective substitutes, capable of replacing up to 100% of soybean meal in the concentrate without adversely affecting total gas production and actually improving OMD. In contrast, the inclusion of acerola residue proved less effective, leading to a reduction in the evaluated parameters, particularly in total gas production and OMD. These results suggest that while pineapple and orange residues are promising alternatives, acerola residue may require further investigation to identify potential improvements for its use in ruminant diets.

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8 CONSIDERAÇÕES FINAIS

O enriquecimento proteico dos resíduos agroindustriais de acerola, abacaxi, laranja e mandioca com leveduras demonstrou um potencial significativo para melhorar sua composição química. Esses resíduos enriquecidos podem substituir parcialmente ou em até 100% as fontes convencionais de proteína na dieta de ruminantes. Além disso, a inclusão de resíduos de abacaxi e laranja não apenas otimiza a cinética de produção de gás, mas também aprimora a fermentação ruminal *in vitro* e a digestibilidade da matéria orgânica. Por outro lado, o resíduo de acerola não demonstrou eficiência quando incorporado à dieta de ruminantes, resultando em uma redução na fermentação ruminal *in vitro* e na digestibilidade da matéria orgânica.