



## In Vitro and In Situ Evaluation of Fermented High Moisture Corn and Ear Corn as Alternative Feedstuffs for Feedlot Calves

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### ABSTRACT

High-moisture corn products offer potential advantages in intensive cattle feeding systems by providing high-energy feedstuffs while allowing earlier harvest. This study evaluated the nutritional value and fermentation characteristics of fermented high-moisture grain corn (HMG) and high-moisture ear corn (HME) compared to conventional corn grain (CG) and corn silage (CS) through in vitro and in situ techniques. Six samples from each corn product were collected from commercial farms, pooled, and analyzed in five replicates. Chemical analysis revealed that HME contained higher neutral detergent fiber (19.0% vs. 4.25%) but lower starch (60.95% vs. 66.75%) and crude protein (7.27% vs. 8.32%) compared to HMG ( $p<0.01$ ). While accumulated gas production was similar among HMG, HME, and CG, all significantly exceeded CS values ( $p<0.01$ ). HMG demonstrated the highest gas production rate and metabolizable energy content (12.2 MJ/kg), significantly higher than HME (10.1 MJ/kg). In vitro organic matter digestibility was highest in HMG (751.5 g/kg), while HME showed intermediate values (680.2 g/kg). The in situ evaluation revealed higher effective rumen degradability for HMG compared to HME ( $p<0.01$ ). Ammonia nitrogen concentrations remained above microbial requirements across all treatments, with HMG and HME showing similar patterns. Volatile fatty acid profiles indicated enhanced fiber degradability in high-moisture products compared to CG. In conclusion, fermented high-moisture corn products demonstrated distinct nutritional characteristics compared to conventional corn grain, with HMG showing higher energy content and digestibility values, while HME exhibited increased fiber content. These findings provide quantitative data on the nutritional value of fermented high-moisture corn alternatives for feedlot cattle feeding formulations.

**Keywords:** *effective degradability; high-moisture corn; intensive beef production; in vitro gas production; rumen fermentation*

### INTRODUCTION

Corn is a fundamental energy source in intensive beef cattle production systems, particularly in feedlot operations where high-energy diets are essential for optimal growth performance (Samuelson *et al.*, 2016). The versatility of corn as a feed ingredient allows for various processing methods and harvest stages, each offering distinct advantages for cattle feeding programs (Ferraretto *et al.*, 2013). In Spain's northeastern Ebro Valley, the FAO-700 corn variety achieves an optimal balance between energy content and biomass production when harvested at around 32% dry matter, typically around 120-125 days post-planting. As the crop matures, nutrient deposition into the grain becomes complete at 130-135 days post-planting, when grain humidity reaches 30%-35%, making it suitable for harvesting and ensiling as high-moisture grain (HMG).

Because of its starch granule structure, corn starch is not extensively degraded in the rumen. The

processing of corn improves its digestibility in the rumen and intestine (Freitas *et al.*, 2020; Petzel *et al.*, 2021). Processing methods significantly influence the nutritional value and digestibility of corn in ruminant diets. For instance, while steam-flaked corn (SFC) improves starch digestibility, it involves higher processing costs and may increase the risk of ruminal acidosis due to rapid starch degradation (Elmhadi *et al.*, 2022). Alternative processing methods, such as ensiling high-moisture grain, offer potential advantages for intensive beef production systems. Greater ruminal and total-tract starch digestibility is well established in dairy cows fed high-moisture corn compared with dry corn (Ferraretto *et al.*, 2013). This is related to the breakdown of the hydrophobic starch-protein matrix surrounding starch granules during ensiling, which allows for greater microbial fermentation and enzymatic digestion of starch by ruminants (Moharrery *et al.*, 2014). During the ensiling process, organic acids produced by lactic acid bacteria decrease pH, while plant proteases

degrade prolamin proteins that form the matrix. Additionally, fermentation weakens hydrogen and disulfide bonds within the matrix structure, creating micropores that increase access for ruminal microbes and amylolytic enzymes to the previously encapsulated starch granules (Cueva *et al.*, 2023; Muck *et al.*, 2018). In contrast, high-moisture ear corn (HME), which includes both grain and cob, provides a unique nutritional profile with increased fiber concentration and modified energy content compared to HMG, potentially making it more suitable for use in cattle diets while promoting improved rumen health by reducing the risk of subacute ruminal acidosis through slower starch fermentation kinetics (Shang *et al.*, 2024).

The adoption of high-moisture corn products in livestock feeding systems has gained interest due to several practical advantages. Early harvest of high-moisture corn enables a shortened cultivation cycle, facilitating double cropping systems when combined with winter crops such as ray grass, barley, or triticale. Additionally, farmers can utilize existing silage facilities for storage, making it an economically attractive option for producing local high-energy feed resources. The fermentation process during ensiling generates organic acids that create a stable storage environment by inhibiting undesirable microorganisms, thus preserving nutritional value (Li *et al.*, 2023). Understanding the comparative nutritional characteristics and fermentation patterns of different high-moisture corn products is therefore critical for optimizing their inclusion in ruminant diets while maximizing production efficiency and animal health.

Despite these potential benefits, comparative data on the nutritional value and fermentation characteristics of HMG and HME in beef cattle feeding systems remains limited. Therefore, this study aimed to evaluate and compare the *in vitro* and *in situ* fermentation patterns and digestibility characteristics of high-moisture corn with and without cob (HME and HMG, respectively) against conventional dry corn grain (CG) and whole-plant corn silage (CS) in feedlot cattle diets. We hypothesized that HME would provide a more suitable fermentation pattern for intensive beef production by offering a better balance between readily fermentable carbohydrates and fiber content compared to HMG and CG, potentially reducing the risk of ruminal disorders while maintaining high energy availability.

## MATERIALS AND METHODS

The experimental protocol was approved by the Ethical Committee of the University of Lleida (CEEA. 01-07/16).

### Corn Products Preparation and Analysis

Samples of HMG, HME, CG, and CS were collected from six commercial farms in Ebro's Valley, Lleida, Spain, to ensure a comprehensive representation of the region's corn products. For each corn product, samples obtained from the six farms were pooled together to

create a representative composite sample. HMG and HME samples were obtained from trench silos, while CS samples were collected from fresh silage material. All samples were freeze-dried and ground through a 1-mm screen for subsequent analysis. Each corn product (HMG, HME, CG, CS) was analyzed in triplicate using the methods outlined by AOAC (1990). Dry matter was assessed by drying the samples in an oven at 105 °C until a constant weight was achieved (method 934.01), while ash content was measured by incineration at 550 °C for 4 hours (method 942.05). The crude protein (CP) was measured by the Kjeldahl method (ref. 976.05), and ether extract (EE) was determined using the Soxhlet extraction method with diethyl ether (ref. 920.39). The amyloglucosidase- $\alpha$ -amylase method (ref. 996.11) was used to determine the total starch content. Neutral detergent fiber (NDF) was analyzed following the procedure of Van Soest *et al.* (1991), using  $\alpha$ -amylase without sodium sulfite and correcting for ash content in the residue. Acid detergent fiber (ADF) was determined using the ANKOM Technology protocol in accordance with method 973.18.

### In Vitro Incubation and Measurements

The in vitro fermentation study was conducted using a randomized complete block design, with five consecutive incubation sets serving as blocks. Each corn product was evaluated using five analytical replicates, one replicate per incubation set. Rumen fluid was collected at slaughter from four beef cattle finished in an intensive feeding system. The donor animals were maintained for one month prior to slaughter on an ad libitum diet consisting of 90% commercial concentrate (50% corn grain, 18% barley, 10% lupines, 10% gluten feed, 6% dry distillery grains, 6% soybean meal) and 10% barley straw offered *ad libitum*. The rumen fluid was stored in pre-heated (39 °C) thermos flasks immediately after collection, transported to the laboratory and filtered through a double layer of gauze. Incubation glass bottles (120 mL total volume) were prepared under continuous CO<sub>2</sub> flow. Each bottle contained 600 mg DM of substrate and 80 mL of incubation medium, comprising rumen inoculum (20% of total volume) combined with mineral, buffer, and reducing solutions as described by (Seradj *et al.*, 2019). Control bottles (blanks) containing only incubation medium were included in each incubation set to correct for gas production in the absence of substrate. All bottles were sealed and incubated at 39 °C in a shaking water bath for 96 h. Gas pressure was measured using a TP704 Manometer (DELTA OHM, Italy) at 2, 4, 6, 8, 12, 24, 48, 72, and 96 h of incubation. Pressure readings were converted to gas volumes using a predetermined linear regression equation established between pressure measurements and known air volumes at the incubation temperature. Gas production was expressed as mL per g of incubated DM, corrected for blank values. The calculations were performed using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

Initial samples were collected from the stock solution at the onset of each incubation set (Time 0), and

subsequent samples were obtained at 24, 48, and 72 h post-incubation from one bottle per treatment immediately following gas pressure measurements. Upon opening each bottle, the contents were filtered through a 1-mm metal sieve. Two aliquots were collected from the filtered fluid: 2 mL for ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) analysis, which was combined with 0.8 mL of 0.5 N HCl, and 4 mL for volatile fatty acid (VFA) analysis, which was added to 1 mL of solution containing 20 mL/L ortho-phosphoric acid and 2 g/L of 4-methylvaleric acid (internal standard) in distilled water. All samples were stored immediately at  $-20^{\circ}\text{C}$  until analysis. The remaining two bottles per treatment were maintained for gas production measurements through 96 h, after which their contents were pooled and sampled following the same procedure.  $\text{NH}_3\text{-N}$  concentration was determined using a colorimetric method based on the phenol-hypochlorite reaction (Weatherburn, 1967). VFA concentrations and molar proportions were measured using gas chromatography (Agilent 7890A, Net Work GC System, Beijing Elmer, Boston, USA) equipped with a flame ionization detector and a capillary column (BP21, 30 m  $\times$  0.25 mm ID  $\times$  0.25  $\mu\text{m}$ , DE, USA) following the sample preparation method of Dhakal *et al.* (2024).

#### *In Situ* Incubation Procedure

The *in situ* study was conducted using six Holstein bull calves (400 kg body weight, 9 months old), all surgically fitted with permanent ruminal cannulas. Animals were individually housed in pens equipped with separate feed bunks and automatic water dispensers. Calves had *ad libitum* access to a corn-based concentrate and barley straw diet, as described for the donor animals in the *in vitro* study, with free access to water throughout the experimental period. Flexible ruminal cannulas (Bar Diamond Inc. 2004; 8 cm diameter) were surgically installed in the dorsal sac of the rumen. Post-surgical recovery was monitored using feed intake as the primary criterion, with animals considered fully recovered when they reached their pre-surgery intake levels, typically seven days post-operation.

Ruminal degradability was determined using the *in situ* nylon bag technique described by Ørskov and McDonald (1979). Corn products (HMG, HME, and CG) were weighed (5-7 g fresh matter) into coded nylon bags (SEFAR, Cardedeu, Spain; 50  $\mu\text{m}$  pore size). The bags were secured to a 40-cm chain anchored to the ruminal cannula to ensure proper placement within the rumen environment. Each corn product sample was tested in duplicate for each animal at each incubation time. Incubation times were 0, 2, 4, 6, 12, 24, 48, and 72 h, with bags being inserted sequentially rather than simultaneously to maintain consistent removal times. The whole plant corn silage was excluded from the *in situ* analysis due to its physical characteristics.

#### Calculations and Statistical Analysis

Cumulative gas production data from 2 to 96 h of incubation were fitted to the modified McDonald

(1981) model using nonlinear regression (PROC NLIN procedure of SAS):

$$y = a \times (1 - e^{-b \times (t - c)})$$

where y represents the cumulative gas production (mL) at time t, a is the potential cumulative gas production (mL), b is the gas production rate (mL/h), t is the fermentation time (h), and c represents the discrete lag time (h).

*In vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) were calculated using equations developed by Menke and Steingass (1988):

$$\text{IVOMD (\%)} = 14.88 + (0.889 \times \text{GP}) + (0.045 \times \text{CP}) + (0.0651 \times \text{ash})$$

$$\text{ME (MJ/kg DM) for forage feeds (CS and HME)} = 2.20 + (0.136 \times \text{GP}) + (0.057 \times \text{CP}) + (0.0029 \times \text{EE})$$

$$\text{ME (MJ/kg DM) for concentrate feeds (CG and HMG)} = 1.06 + (0.157 \times \text{GP}) + (0.084 \times \text{CP}) + (0.22 \times \text{EE}) - (0.081 \times \text{ash})$$

where GP represents gas production (mL) from 200 mg substrate after 24 h incubation, and CP and EE represent crude protein and ether extract content, respectively.

Dry matter degradation data from the *in situ* study were fitted to a first-order kinetic model:

$$y = a + b \times (1 - e^{-ct})$$

where a represents the soluble fraction, g/kg; b is the insoluble but potentially degradable fraction, g/kg; and c is the fractional degradation rate, g/h. Effective rumen degradability (ERD; %) was also calculated using the following equation considering different passage rates ( $k = 0.02, 0.05, \text{ or } 0.08; \text{ %/h}$ ) based on physiological states as defined by ARC (1980).

$$\text{ERD} = a + \frac{b \times c}{c \times k}$$

Chemical composition data were analyzed using the GLM procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC). *In vitro* fermentation parameters, including gas production kinetics, IVOMD, and ME, were analyzed using PROC MIXED of SAS. The model included corn product as the fixed effect and incubation set as the random block effect. For  $\text{NH}_3\text{-N}$  and VFA concentrations, data were analyzed as repeated measures using a mixed model that included corn product, incubation time, and their interaction (corn product  $\times$  incubation time) as fixed effects, with incubation batch as a random block effect. The time was included in the model as a repeated measure. *In situ* degradation parameters were analyzed using the PROC GLM procedure of SAS with corn product as the fixed effect and animal as the experimental block. The means were compared using Tukey's multiple comparison test. Statistical significance was declared at  $p \leq 0.05$ .

## RESULTS

### Chemical Composition of Corn Products

The chemical composition of the corn products is presented in Table 1. Dry matter content was

similar between HMG and HME, but lower than CG and higher than CS ( $p<0.01$ ). Crude protein content differed significantly among products, with the highest concentration in CG, followed by HMG, HME, and CS in decreasing order ( $p<0.01$ ). Ether extract concentrations were similar between HMG and HME, and both were higher than CG and CS ( $p=0.02$ ). The NDF and ADF concentrations were similar between HMG and CG, but lower than HME ( $p<0.01$ ). CS exhibited the highest fiber fractions, with NDF and ADF values significantly higher than all other products ( $p<0.01$ ). In contrast to the fiber content pattern, starch concentration followed an inverse relationship across feed types, with the highest starch content in CG, followed by HMG, then significantly lower levels in HME, and CS containing the least starch of all treatments ( $p<0.01$ ). Ash content varied significantly among all corn products ( $p<0.01$ ).

#### In Vitro Gas Production Variables

Gas production variables of different corn products are presented in Table 2. Accumulated gas production was similar among HMG, HME, and CG, with all three products showing higher values than CS ( $p<0.05$ ). The rate of gas production was highest for HMG, significantly exceeding values observed for CG and CS ( $p<0.01$ ). No significant differences were detected in the gas production rate between HME and HMG. There were no significant differences in lag time among the corn products ( $p=0.390$ ). Metabolizable energy content differed significantly among products ( $p=0.01$ ), with the highest value observed in HMG, followed by CG, HME, and CS in decreasing order. *In vitro* organic matter digestibility was highest in HMG ( $p<0.01$ ), while HME

and CG showed similar intermediate values, and CS exhibited the lowest digestibility.

#### In Vitro Concentrations of $\text{NH}_3\text{-N}$ and VFA

The *in vitro*  $\text{NH}_3\text{-N}$  concentrations were similar between HMG and HME, and both were significantly lower than CG and CS (Table 3). No significant differences in  $\text{NH}_3\text{-N}$  concentration were observed between CG and CS. HMG and HME showed similar total VFA concentrations, which were significantly higher than CS. CG exhibited the lowest acetate and highest propionate and butyrate proportions, differing significantly from all other corn products. However, HMG, HME, and CS showed no significant differences in their proportions of acetate, propionate, and butyrate. The proportions of isobutyrate and isovalerate were similar among HMG, HME, and CG, and all were significantly lower than CS. The acetate-to-propionate ratio showed no significant differences among HMG, HME, and CS, but all were significantly higher than CG. The proportion of valerate remained statistically similar across all corn products. No significant interactions were detected between corn product and incubation time for the *in vitro* concentrations of  $\text{NH}_3\text{-N}$  and VFA.

#### In Situ Fermentation Variables

Table 4 presents the *in situ* fermentation variables of different corn products, excluding CS, which was not included in the *in situ* analysis due to its physical structure. HMG, HME, and CG showed similar soluble fractions ( $p=0.696$ ). Although HME exhibited a numerically higher non-soluble fraction compared to both HMG and CG, no significant differences were

Table 1. Chemical compositions of different corn products

Variables	Corn product <sup>1</sup>				SEM <sup>2</sup>	p-value
	HMG	HME	CG	CS		
Dry matter, % of as fed	65.67 <sup>b</sup>	62.87 <sup>b</sup>	86.07 <sup>a</sup>	33.03 <sup>c</sup>	1.300	< 0.01
Crude protein, % of dry matter	8.32 <sup>b</sup>	7.27 <sup>c</sup>	9.31 <sup>a</sup>	6.32 <sup>d</sup>	0.229	< 0.01
Ether extract, % of dry matter	3.55 <sup>a</sup>	3.53 <sup>a</sup>	3.48 <sup>ab</sup>	3.07 <sup>b</sup>	0.113	0.02
Neutral detergent fiber, % of dry matter	4.25 <sup>c</sup>	19.00 <sup>b</sup>	6.32 <sup>c</sup>	43.80 <sup>a</sup>	0.851	< 0.01
Acid detergent fiber, % of dry matter	0.85 <sup>c</sup>	9.27 <sup>b</sup>	1.22 <sup>c</sup>	27.38 <sup>a</sup>	0.662	< 0.01
Starch, % of dry matter	66.75 <sup>b</sup>	60.85 <sup>c</sup>	76.80 <sup>a</sup>	34.90 <sup>d</sup>	0.938	< 0.01
Ash, % of dry matter	1.70 <sup>b</sup>	1.72 <sup>b</sup>	1.61 <sup>b</sup>	4.68 <sup>a</sup>	0.169	< 0.01

Note: <sup>1</sup>HMG: high-moisture corn grain silage; HME: high-moisture ear corn silage; CG: conventional corn grain; CS: whole plant corn silage. <sup>2</sup> SEM: standard error of the mean. <sup>a-c</sup> Values with different superscripts within a row are significantly different ( $p<0.05$ ).

Table 2. Gas production variables, estimated metabolizable energy, and *in vitro* organic matter digestibility of different corn products

Variables <sup>1</sup>	Corn product <sup>2</sup>				SEM <sup>3</sup>	p-value
	HMG	HME	CG	CS		
Gas production parameters						
<i>a</i> , ml/g DM	329.3 <sup>a</sup>	295.5 <sup>a</sup>	303.5 <sup>a</sup>	227.9 <sup>b</sup>	11.03	< 0.01
<i>b</i> , ml/h	0.22 <sup>a</sup>	0.17 <sup>ab</sup>	0.10 <sup>b</sup>	0.14 <sup>b</sup>	0.019	< 0.01
<i>c</i> , h	0.7	-0.4	-0.1	-0.7	0.58	0.390
ME, MJ/kg	12.2 <sup>a</sup>	10.1 <sup>c</sup>	11.4 <sup>b</sup>	8.2 <sup>d</sup>	0.14	0.01
IVOMD, g/kg	751.5 <sup>a</sup>	680.2 <sup>b</sup>	706.2 <sup>b</sup>	572.9 <sup>c</sup>	9.33	< 0.01

Note: <sup>1</sup> *a*: accumulated gas production; *b*: rate of gas production; *c*: lag time; ME: estimated metabolizable energy; IVOMD: *in vitro* organic matter digestibility. <sup>2</sup> HMG: high-moisture corn grain silage; HME: high-moisture ear corn silage; CG: conventional corn grain; CS: whole plant corn silage. <sup>3</sup> SEM: standard error of the mean. <sup>a-d</sup> Values with different superscripts within a row are significantly different ( $p<0.05$ ).

Table 3. Ammonia nitrogen and volatile fatty acid concentrations of *in vitro* fermented corn products

Variables <sup>1</sup>	Corn product <sup>2</sup>				SEM <sup>3</sup>	p value <sup>4</sup>		
	HMG	HME	CG	CS		Treat	Time	Treat × Time
NH <sub>3</sub> -N, mg/L	317.5 <sup>b</sup>	309.1 <sup>b</sup>	346.4 <sup>a</sup>	366.5 <sup>a</sup>	10.51	<0.01	<0.01	0.40
Total VFA, mM	49.7 <sup>a</sup>	48.9 <sup>a</sup>	45.5 <sup>ab</sup>	41.6 <sup>b</sup>	2.71	<0.01	<0.01	0.12
VFA proportion, mol/100 mol								
Acetate	59.3 <sup>a</sup>	60.7 <sup>a</sup>	55.6 <sup>b</sup>	60.0 <sup>a</sup>	1.37	<0.01	0.22	0.53
Propionate	22.2 <sup>b</sup>	21.4 <sup>b</sup>	24.1 <sup>a</sup>	22.1 <sup>b</sup>	0.35	<0.01	0.10	0.25
Iso Butyrate	1.4 <sup>b</sup>	1.4 <sup>b</sup>	1.4 <sup>b</sup>	1.6 <sup>a</sup>	0.12	<0.01	<0.01	0.50
Butyrate	12.5 <sup>b</sup>	12.1 <sup>b</sup>	14.4 <sup>a</sup>	11.6 <sup>b</sup>	0.73	<0.01	0.16	0.92
Iso Valerate	2.8 <sup>b</sup>	2.6 <sup>b</sup>	2.7 <sup>b</sup>	3.0 <sup>a</sup>	0.21	<0.01	<0.01	0.16
Valerate	1.9	1.8	1.8	1.7	0.10	0.24	0.43	0.96
C2:C3	2.7 <sup>a</sup>	2.8 <sup>a</sup>	2.3 <sup>b</sup>	2.7 <sup>a</sup>	0.09	<0.01	0.11	0.29

Note: <sup>1</sup> NH<sub>3</sub>-N: ammonia nitrogen; VFA: volatile fatty acids; C2:C3: acetate to propionate ratio. <sup>2</sup> HMG: high-moisture corn grain silage; HME: high-moisture ear corn silage; CG: conventional corn grain; CS: whole plant corn silage. <sup>3</sup> SEM: standard error of the mean. <sup>4</sup> Treat: main effect of corn product; Time: main effect of incubation time; Treat × Time: interaction between corn product and incubation time. <sup>a,b</sup> Values with different superscripts within a row are significantly different (p<0.05).

Table 4. *In situ* fermentation variables of different corn products

Variables <sup>1</sup>	Corn product <sup>2</sup>			SEM <sup>3</sup>	p-value
	HMG	HME	CG		
a, g/kg	26.0	26.2	23.5	2.20	0.696
b, g/kg	57.5	108.3	63.3	19.32	0.181
c, g/h	0.105 <sup>a</sup>	0.033 <sup>b</sup>	0.047 <sup>b</sup>	0.016	<0.01
ERD 0.02, %	73.5 <sup>a</sup>	62.6 <sup>b</sup>	66.7 <sup>b</sup>	1.39	<0.01
ERD 0.05, %	64.0 <sup>a</sup>	48.4 <sup>c</sup>	53.1 <sup>b</sup>	1.09	<0.01
ERD 0.08, %	57.8 <sup>a</sup>	42.4 <sup>c</sup>	46.1 <sup>b</sup>	1.14	<0.01

Note: <sup>1</sup> a: soluble fraction; b: non-soluble fraction; c: fractional degradation rate; ERD: effective rumen degradability at different passage rates (2, 5, and 8 %/h). <sup>2</sup> HMG: high-moisture corn grain silage; HME: high-moisture ear corn silage; CG: conventional corn grain; CS: whole plant corn silage. <sup>3</sup> SEM: standard error of the mean. <sup>a-c</sup> Values with different superscripts within a row are significantly different (p<0.05).

detected among the tested products (p=0.181). HMG demonstrated the highest fractional rate of degradation, which was significantly higher than both HME and CG (p<0.01). HME and CG showed similar fractional rates of degradation. For effective rumen degradability, significant differences were observed among all corn products (p<0.01), with HMG showing the highest value, followed by CG, and then HME.

## DISCUSSION

Variations in growing conditions, harvest moisture, and location of corn can affect compositional results (García-Chávez *et al.*, 2022). However, we minimized these effects by analyzing six different samples and homogenizing each feedstuff before chemical analysis. The chemical composition of high-moisture corn products in our study aligned with previous research findings, particularly regarding moisture levels of approximately 30% for both HMG and HME (Lardy & Anderson, 2010). While our observed crude protein contents for HMG (8.32%) and HME (7.27%) were lower than those previously reported (10.0% and 8.8%, respectively), they remained within acceptable ranges (Akins & Shaver, 2014). The observed differences in fiber fractions between HME and other grain products (HMG and CG) can be attributed to the inclusion of cob fraction in HME, which naturally increases the NDF and ADF content.

The *in vitro* gas production parameters revealed important differences among corn products. HMG demonstrated superior fermentation characteristics, exhibiting the highest gas production rate and metabolizable energy content. This rapid degradation rate could potentially increase the risk of subacute ruminal acidosis if not properly managed in feeding regimens. The similar accumulated gas production among HMG, HME, and CG, despite their different chemical compositions, suggests that the processing methods influenced the fermentation patterns rather than the total fermentability. These findings were supported by the *in situ* results, where HMG showed the highest effective rumen degradability. This enhanced degradability might be attributed to the processing effect on the protein matrix surrounding starch granules in mature corn grain, which typically limits microbial access (Freitas *et al.*, 2020; Petzel *et al.*, 2021). The estimated metabolizable energy content of CG in our study is aligned with ranges documented by Umucalilar *et al.* (2002) and Abaş *et al.* (2005) of 10.4 to 11.4 and 10.80 to 14.75 MJ/kg DM, respectively.

The source of rumen liquid significantly influences fermentation kinetics in *in vitro* incubations. Therefore, we specifically used rumen liquid from fattening calves as inoculum to evaluate these feedstuffs under feedlot conditions. This methodological choice strengthens the practical applicability of our results for feedlot operations, although it may limit direct comparisons

with studies using dairy cattle inoculum. Ammonia nitrogen concentrations remained above the threshold level (50 mg/L) required for microbial protein synthesis (Wang *et al.*, 2018; Zhu *et al.*, 2022) across all corn products. The higher NH<sub>3</sub>-N concentration in CS, despite its lowest crude protein content, can be attributed to the silage processing effect on protein degradability, which may indicate inefficient nitrogen utilization in the rumen environment. While corn silage protein degradability can reach 80%, corn grain typically shows around 40% degradability, with HMG and HME falling between these values (Lardy & Anderson, 2010). This intermediate protein degradability of high-moisture corn products might offer advantages in terms of nitrogen utilization efficiency in feedlot diets.

The VFA concentrations reported in this study represent mean values across three sampling time points (24, 48, and 72 h) during the 96-h incubation period, analyzed using a repeated measures design. This approach was employed to capture the temporal dynamics of fermentation rather than final cumulative VFA production, which explains the relatively lower absolute concentrations compared to endpoint measurements typically reported in *in vitro* studies. Despite these lower mean values, the VFA profiles provided additional insights into the fermentation characteristics. The higher acetate proportion in HMG compared to CG, despite similar NDF concentrations, suggests enhanced fiber degradability in high-moisture corn products. This improved fiber utilization could be particularly beneficial in high-concentrate feedlot diets where maintaining adequate fiber digestion is crucial for rumen health. The higher propionate proportion of CG than other corn products, reflects its greater starch concentration and possibly different patterns of starch degradation. These differences in VFA profiles influence the relationship between rumen VFA concentration and gas production (Cattani *et al.*, 2014; Maccarana *et al.*, 2016), as acetate-producing fermentation pathways yield more CO<sub>2</sub> than propionate pathways (Ungerfeld, 2020). The positive correlation between gas production and VFA concentration corroborates previous findings (Muqier *et al.*, 2023; Tunkala *et al.*, 2023).

The differences in fermentation patterns among corn products might also have practical implications for feeding management. The higher degradation rate of HMG indicates that this feed might require careful management of feeding frequency and dietary inclusion rates to optimize ruminal conditions and prevent potential digestive disorders. Conversely, the slower degradation rate of HME might offer advantages in terms of maintaining more stable ruminal conditions over time. This characteristic makes it particularly valuable during transition periods or as a strategic component in rations designed to reduce the risk of subacute ruminal acidosis in high-producing dairy cattle.

Consistent with the findings of Lardy and Anderson (2010), our results indicate that high-moisture ear corn offers more energy than corn silage with comparable protein levels, but provides less energy than either dry corn grain or high-moisture corn grain. The

lower effective rumen degradability of HME compared to HMG and CG became more pronounced at higher outflow rates, suggesting that the processing method significantly impacts ruminal utilization under typical feeding conditions. This finding has particular relevance for high-producing animals with higher passage rates, where the choice of corn processing method could substantially affect nutrient utilization efficiency. Future research should focus on *in vivo* studies comparing these processing methods across different production stages and under varying dietary regimens to establish optimal inclusion rates for maximizing both productivity and rumen health.

## CONCLUSION

HMG indicated superior metabolizable energy content, digestibility, and fermentation characteristics with rapid gas production rates, while HME showed slightly reduced metabolizable energy compared to HMG due to its cob fraction. HME and HMG exhibited similar fermentation profiles with enhanced total VFA production over conventional alternatives (i.e., CS and CG). Both products provide nutritional benefits for intensive beef production, with HMG preferred for maximum energy density and HME for balanced energy-fiber content.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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