P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2025.049

Evaluation of Carbohydrate Fractions and *In-sacco* Starch Degradation in High-Energy Feed By-Products

Mardiah Rahmadani [1,3], Anuraga Jayanegara [2,3,*], Nahrowi [2], Lilis Khotijah [2], Rusli Fidriyanto [3,4], Rahma Dhani Dwi Prasetya [3] and Irwan Susanto [1,3]

[1]Study Program of Nutrition and Feed Science, Graduate School of Institut Pertanian Bogor, Bogor 16680, Indonesia
[2]Department of Animal Nutrition, Faculty of Animal Science, Institut Pertanian Bogor. Jl. Agatis, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia
[3]Research Animal Feed and Nutrition Modelling Research Group (AFENUE), Faculty of Animal Science, IPB University, Bogor 16680, Indonesia
[4]Research Center for Applied Zoology, National Research and Innovation Agency. Jl. Raya Jakarta-Bogor Km 46, Cibinong, Bogor 16911, West Java, Indonesia
***Corresponding author:** anuraga.jayanegara@gmail.com

Article History: 25-041 Received: 12-Feb-25 Revised: 17-Apr-25 Accepted: 24-Apr-25 Online First: 06-May-25

ABSTRACT

This study evaluated the carbohydrate fractions and *in-sacco* starch degradation of high-energy feed by-products that are typically used in ruminant diets in Indonesia. The analyzed by-products included corn gluten meal (CGM), corn gluten feed (CGF), cassava pulp, rice bran, and wheat bran. Chemical composition, carbohydrate fractions, and *in-sacco* degradability were assessed. The chemical composition analysis revealed significant variability, with cassava pulp exhibiting the highest ($P<0.001$) starch content and rice bran showing the lowest ($P<0.001$). Carbohydrate fractionation showed that cassava pulp was significantly ($P<0.001$) high in slowly fermentable energy (CB1), while CGM had the highest ($P<0.001$) sugar content (CA), highlighting their distinct energy contributions. *In-sacco* studies demonstrated significant variations in dry matter (DM) and starch degradation across incubation periods. Wheat bran exhibited the highest DM degradability ($P<0.001$), while CGF had the highest ($P<0.001$) starch degradability and effective degradability. Additionally, all by-products showed similar DM degradation rates, but starch degradation varied significantly ($P<0.001$), with CGM exhibiting the highest rate and cassava pulp the lowest. Correlation analysis indicated a significant positive relationship ($P<0.05$) between total carbohydrates and CB1, emphasizing their role in rapid energy supply, and a negative correlation with non-structural carbohydrates (CNSC). Finally, cassava pulp provides rapidly available energy, while wheat bran offers sustained energy release, making them complementary feed components. Rice bran contributes to rumen fill, and CGM serves as a protein source with minimal fermentable energy. These findings support the development of precise feed formulations tailored to tropical livestock systems, enhancing production efficiency and sustainability.

Key words: By-products, Carbohydrate, Feeds, *In-sacco*, Starch.

INTRODUCTION

In Indonesia, agricultural by-products are widely utilized as key components in ruminant feed formulations due to their cost-effectiveness. This is made possible by the inherent ability of ruminants to digest a broad range of feed qualities, unlike monogastric animals. The use of such by-products has gained increasing significance in promoting ruminant growth, as documented by several studies growth (Purbowati et al. 2021; Salami et al. 2021; Kabsuk et al. 2024). This practice not only addresses environmental challenges associated with agricultural waste but also represents a strategic approach to enhancing food security, improving production efficiency, and fostering a sustainable agribusiness system. Common by-products utilized in Indonesia include corn gluten meal (CGM), corn gluten feed (CGF), rice bran, cassava pulp, and wheat bran, all of which are often rich in carbohydrates and essential for providing energy to ruminants. However, the incorporation of these by-products necessitates a thorough evaluation of their carbohydrate fractions to optimize feed efficiency and animal performance (Poore 2022; Gierus et al. 2024).

Cite This Article as: Rahmadani M, Jayanegara A, Nahrowi, Khotijah L, Fidriyanto R, Prasetya RDD and Susanto I, 2025. Evaluation of carbohydrate fractions and *In-sacco* starch degradation in high-energy feed by-products. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2025.049

During production phases, ruminants are typically fed high-starch concentrate diets to enhance muscle development and milk production, aligning with the primary objectives of livestock production systems. Rumen degradable starch plays a pivotal role as a critical energy source for both rumen microbes and the host animal (Liang et al. 2023). Starch in ruminant diets can be categorized based on its ruminal degradation characteristics into rumen degradable starch (RDS) and rumen-undegradable starch (RUS), also referred to as bypass starch. The degradation of starch in the rumen is commonly assessed using the *in-sacco* method (Gleason et al. 2022). Moreover, carbohydrate degradation predictions often involve fractionation approaches, such as those described by Sniffen et al. (1992), using the Cornell Net Carbohydrate and Protein System (CNCPS). The CNCPS provides a robust framework for classifying carbohydrate fractions and predicting their nutritional behavior. Carbohydrates are divided into four fractions (A, B1, B2 and C) based on their degradation rates (Gierus et al. 2024), which are derived from non-structural carbohydrates, structural carbohydrates, and indigestible fiber (fraction C). Fraction B2, for instance, is slowly fermented in the rumen by bacteria that utilize ammonia as their sole nitrogen source. Non-structural carbohydrates include sugars (fraction A) and starches (fraction B1).

Despite its potential benefits, current ruminant feed formulation practices in Indonesia primarily focus on total digestible nutrients (TDN) and have yet to widely adopt strategies involving carbohydrate fractionation or the balancing of rumen-digestible starch (McDonald et al. 2022). A key limitation lies in the scarcity of comprehensive data on starch degradation kinetics and carbohydrate fractionation for locally available by-product feed ingredients. While techniques such as *in-sacco* analysis and the CNCPS have been extensively applied in global contexts to evaluate carbohydrate digestibility but their application to local feed resources in Indonesia remains underexplored. For example, Jayanegara and Sofyan (2009) demonstrated the utility of CNCPS in assessing nutrient balance in lactating dairy cows, yet research specifically targeting local by-products such as CGM, CGF, rice bran, cassava pulp, and wheat bran has been insufficient. This lack of data can hinder the development of precise feed formulations and compromise livestock productivity.

Additionally, significant variability in carbohydrate content across different by-products necessitates detailed analyses to better understand the dynamics of starch degradation in the rumen and the contributions of individual carbohydrate fractions to feed efficiency. Although methods such as CNCPS-based fractionation and *in-sacco* starch degradation analyses hold promise, limited studies have investigated their correlation for by-product feed ingredients. This research aimed to address these gaps by evaluating the *in-sacco* starch degradation characteristics, analyzing carbohydrate fractions, and exploring the correlations between them in locally utilized high-energy feed by-products. Such efforts are critical for enhancing digestibility predictions, optimizing feed formulations, improving production efficiency, and advancing sustainability in livestock nutrition systems.

MATERIALS AND METHODS

Ethical approval

All protocols complied with the guidelines of the Animal Ethical Committee of IPB University, Indonesia (approval number: 266-2024 IPB), dated 19 August 2024.

Sample Preparation

This study was conducted at the Feed and Technology Science Laboratory, Faculty of Animal Science, IPB University, Indonesia. By-product samples were collected from various regions in West Java, Indonesia, including Bogor and Sukabumi regencies. The by-products analysed in this research included corn gluten meal (CGM), corn gluten feed (CGF), cassava pulp, rice bran, and wheat bran. Each material was ground using a grinder and sieved through a 2mm screen before being analysed for its chemical composition and degradability through an *in-sacco* study.

Chemical Composition

The chemical composition of each feed ingredient was analyzed in quadruplicate. Proximate analysis parameters, including dry matter (DM), ash, crude protein (CP), and fat, were determined following the official methods of analysis at the Laboratory of Feed Science and Technology, Department of Animal Nutrition and Feed Science, IPB University. Fiber fractions, such as neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, and lignin, were analyzed using the method described by Van Soest et al. (1991). Additionally, starch and amylose contents were determined using the Association of Official Analytical Chemists (AOAC) method 996.11 (AOAC 2005), while amylopectin content was calculated by subtracting amylose from the total starch content.

Carbohydrate Fraction

The carbohydrate fractions were analyzed following the method by Sniffen et al. (1992). The analyzed carbohydrate fractions included total carbohydrates (CHO), unavailable carbohydrates in fiber (CC), available carbohydrates in fiber (CB2), starch and non-starch polysaccharides (CB1), sugar content (CA) and non-structural carbohydrates (CNSC). The calculations for carbohydrate fractions were based on the following equations by Sniffen et al. (1992):

CHO (%DM) = 100 – CP (%DM) – Fat (%DM) – Ash (%DM)

CC (%CHO) = 100 (NDF (%DM) x 0.01 x Lignin (%NDF) x 2.4) / CHO (%DM)

CB2 (%CHO) = 100 ((NDF (%DM) – NDICP (%CP) x 0.01 x Lignin (%NDF) x 2.4) / CHO (%DM)

CNSC (%CHO) = 100 – CB2 (%CHO) – CC (%CHO)

CB1 (%CHO) = Starch (%NSC) x (100 – CB2 (%CHO) – CC (%CHO)/ 100

CA (%CHO) = (100 – Starch (%NSC)) x (100 – CB2 (%CHO) – CC (%CHO))/100

In-sacco dry matter and starch digestibility

The *in-sacco* degradability study involved three fistulated Friesian Holstein bulls (average body weight: 359±20kg). The bulls were fed twice daily (morning and evening) with a diet consisting of elephant grass forage and commercial concentrate in a 60:40 ratio (w/w). The daily DM requirement was calculated at 2% of body weight. The nutrient composition of the forage included 11.06% CP, 23.61% CF, and 69% TDN, while the concentrate contained 10.04% CP, 23.10% CF and 66% TDN. Water was provided *ad libitum*.

The study employed a 5 × 3 randomized complete block design. The five ingredients tested were CGM, CGF, rice bran, cassava pulp, and wheat bran. Nylon bags (5×10cm) were dried at 60°C for 2 hours and weighed before use. Each nylon bag was filled with 5g of feed sample, sealed using an impulse sealer, secured with cable ties, and incubated in the rumen of the fistulated bulls for intervals of 0, 3, 6, 12, 15, 24 and 48 hours. Each treatment was repeated three times.

The nylon bags for the 0-hour incubation were not placed in the rumen but were rinsed with running water and dried under the same conditions as the incubated bags. After incubation, the nylon bags were removed from the rumen at their respective time intervals. To remove adhering particles, rumen fluid, and microbes, the bags were washed for 5min under running water. They were then dried in an oven at 60°C for 3 days to achieve constant weight. The residues were analyzed for dry matter and starch content following the method by AOAC (2005). DM and starch degradation were determined using the exponential model described by Orskov and Mcdonald (1979).

$$\text{Disappearance} = a + b\,(1 - e^{-ct})$$

Where a = the soluble fraction, b = potential degradation, e = natural log, c = the degradation rate of b component, and t = incubation time. In addition, the effective DM or starch degradability (effective degradation [ED]) was calculated using the equation:

$$ED = a + (bc/\,[k+c])$$

Where a, b, and c are as described above and k is rumen outflow rate, assumed to be $0.06h^{-1}$. While RUS was calculated as RUS = 100 – RDS.

Data Analysis

The obtained data were analysed using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) to determine significant differences (P<0.05). Statistical analyses were performed using SAS On Demand for Academics Edition. Pearson correlation analysis was employed to evaluate the relationship between carbohydrate fractions and *in-sacco* starch digestibility. Additionally, correlation analysis and heat map visualization were conducted using R Studio software.

RESULTS

Chemical Composition

The chemical composition of carbohydrate-based by-products is detailed in Table 1, revealing significant variability across parameters (P<0.001). DM content ranged from 87.462% in Cassava pulp to 92.269% in CGM. OM content displayed a similar pattern, with CGM registering the highest value (90.529%) and rice bran the lowest (74.394%). CP content varied significantly, peaking at 70.116% in CGM and reaching a minimum of 4.325% in cassava pulp. Fat content was highest in rice bran (5.159%) and lowest in cassava pulp (0.543%). Starch content showed substantial differences, with cassava pulp containing the highest proportion (47.320%) and CGM the lowest (13.830%).

Fiber-related parameters also exhibited marked variability. NDF and ADF contents were most pronounced in rice bran (69.880% and 51.736%, respectively) and lowest in CGM (9.163% and 3.556%, respectively). Cassava pulp had the highest cellulose content (37.547%), while CGM contained the least (2.789%). Lignin content varied widely, with rice bran showing the highest concentration (16.682%) and CGM the lowest (0.592%). These findings underscore the substantial compositional diversity among the carbohydrate by-products analyzed, with statistically significant differences observed across all measured parameters (P<0.001).

The starch content and starch characterization of carbohydrate source by-products are presented in Table 1 and 2. Starch content varied significantly (P<0.001) among the by-products, with cassava pulp showing the highest value (47.320%) and CGM the lowest (13.830%). Regarding starch composition, amylose content was highest in cassava pulp (23.47%) and lowest in CGM (2.98%). Amylopectin content was highest in CGF at 31.693%, while CGM had the lowest proportion (10.850%). These findings illustrate the distinct starch profiles of the by-products, which could influence their functional properties and potential applications in feed formulations.

Carbohydrate Fractionation

The carbohydrate fractionation of feed ingredients by-products revealed significant variations (P<0.001) in their composition (Table 3). Cassava pulp showed the highest CHO content (81.804%) and CGM the lowest (26.559%). Among the fractions, rice bran had the highest CC (39.927%), while CGM had the lowest (0.496%). CB2 was

Table 1: Chemical composition of carbohydrate source by-products (%DM)

Feeds	DM	Ash	OM	CP	Fat	Starch	ADF	NDF	NDICP	Lignin	Cellulose
CGF	90.397b	5.548c	84.850b	21.978b	1.964c	36.633b	19.629c	45.405d	4.000b	7.351c	11.177d
CGM	92.269a	1.740d	90.529a	70.116a	1.586d	13.830d	3.556e	9.163e	3.263c	0.592e	2.789e
Rice bran	89.120c	14.726a	74.394d	10.051d	5.159a	24.177c	51.736a	69.880a	4.605a	16.682a	22.394b
Cassava pulp	87.462d	13.328b	74.134d	4.325e	0.543e	47.320a	50.288b	55.460c	3.995b	10.107b	37.547a
Wheat bran	89.538c	5.584c	83.954c	19.122c	4.439b	37.317b	17.287d	59.338b	4.653a	2.780d	12.417c
Total	89.757	8.185	81.572	25.118	2.738	31.855	28.499	47.849	4.103	7.503	17.265
SEM	0.370	1.148	1.467	5.364	0.405	2.680	4.404	4.789	0.118	1.304	2.732
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

DM= Dry matter; OM= Organic matter; CP= Crude Protein; ADF= Acid detergent fiber; NDF= Neutral detergent fiber; NDICP= Neutral detergent insoluble protein; CGF= Corn gluten feed; CGM= Corn gluten meal; SEM= Standard error of the mean; [a-e]= values in the same column with different alphabets are significant (P<0.05).

Table 2: Starch characterization of carbohydrate source by-products (% DM)

Feeds	Amylose	Amylopectin
Corn gluten feed	4.940	31.693
Corn gluten meal	2.980	10.850
Rice bran	4.640	19.537
Cassava pulp	23.47	23.850
Wheat bran	11.05	26.267

most abundant in wheat bran (67.773%) and least in CGM (16.898%), whereas CNSC was highest in CGM (82.607%) and lowest in rice bran (24.037%). CB1 was highest in cassava pulp (47.320%) and lowest in CGM (13.830%), while the CA was significantly higher in CGM (71.182%) compared to wheat bran, which had the lowest value (16.700%).

In-sacco dry matter and starch digestibility

The study evaluated the degradation of DM and starch in five by-products over incubation periods of 0, 3, 6, 12, 15, 24, and 48 hours (Table 4). For DM degradation, at 0 hours of rumen incubation, DM leaching from the bag was highest ($P<0.001$) for wheat bran (8.079%) and lowest ($P<0.001$) for cassava pulp (1.929%). Wheat bran consistently exhibited the highest degradation throughout the incubation periods, achieving a peak of 74.28% at 48 hours, significantly outperforming all other feed ingredients ($P<0.01$). CGF showed moderate DM degradation, reaching 64.88% at 48 hours, while CGM, cassava pulp, and rice bran displayed comparatively lower degradation rates. Notably, cassava pulp exhibited a substantial increase in DM degradability after 24 hours of incubation, indicating a delayed fermentation profile.

In terms of starch degradation, at 0 hours of rumen incubation, the percentage of starch loss ranged from 11.583 to 41.564%. The greatest starch loss ($P<0.001$) was observed in CGF, while the lowest ($P<0.001$) was found in wheat bran. Consistently, CGF demonstrated the highest degradability across all incubation times, with a maximum of 92.98% at 48 hours, followed by cassava pulp (89.45%) and wheat bran (84.82%). CGM and rice bran exhibited moderate starch degradation, reaching 88.43% and 79.01% at 48 hours, respectively. Significant differences in starch degradability were observed among the by-products ($P<0.0001$), highlighting variability in their fermentability.

The *in-sacco* degradation kinetics of DM and starch in carbohydrate source feeds showed significant variations across feed types (Table 5). For DM, cassava pulp exhibited the highest degradation potential (a + b = 80.760%) and effective degradability (22.830%) but had the lowest soluble fraction (a = 0.067%). In contrast, wheat bran had the highest soluble fraction (a = 12.227%) and effective degradability (ED6 = 51.810%), while CGM displayed the lowest degradation potential (a + b = 27.100%) and ED6 (13.700%). Additionally, the ruminal digestion of by-product feeds revealed no significant differences ($P>0.05$) in the degradation rate of DM. For starch, cassava pulp showed the highest degradation potential (a + b = 91.983%) but the lowest degradation rate (c = 0.030%/hour), whereas CGF exhibited the highest soluble fraction (a = 45.497%) and effective degradability (73.414%).

Table 3: Carbohydrate fractionation of feed ingredients by-products

Feeds	CHO (%DM)	CC (%CHO)	CB$_2$ (%CHO)	CNSC (%CHO)	CB$_1$ (%CHO)	CA (%CHO)
CGF	70.511c	11.365c	33.704c	54.931b	36.633b	34.805b
CGM	26.559d	0.496e	16.898d	82.607a	13.830d	71.182a
Rice bran	70.064b	39.927a	36.036b	24.037e	24.177c	18.219d
Cassava pulp	81.804a	16.446b	33.263c	50.291c	47.320a	26.490c
Wheat bran	70.855c	5.593d	67.773a	26.634d	37.317b	16.700d
Total	63.958	14.765	37.535	47.700	31.855	33.479
SEM	4.412	3.142	3.817	4.907	2.680	4.578
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

CHO= Total carbohydrates; CC= Non-degradable fraction; CB2= Insoluble and slowly degradable fraction, CNSC= Non-structural carbohydrates; CB1= Soluble and slowly degradable fraction; CA= Readily soluble fraction; CGF= Corn gluten feed; CGM= Corn gluten meal; SEM= Standard error of the mean; $^{a-e}$= Values in the same column with different aphabets are significantly significant ($P<0.05$).

Table 4: Ruminal residual dry matter and starch (%) of by-products feed at different times of ruminal incubation

Feeds	0	3	6	12	15	24	48
Dry matter							
CGF	3.365b	24.416b	29.403b	40.677b	41.092b	45.453b	64.879b
CGM	2.926b	4.605c	8.199c	13.984c	15.691c	20.739d	24.653d
Rice bran	2.017b	4.460c	5.392d	7.385d	7.721d	8.077e	9.015e
Cassava pulp	1.929b	4.644c	7.272cd	18.534c	19.879c	33.123c	54.181c
Wheat bran	8.079a	37.864a	44.682a	54.939a	57.109a	67.060a	74.275a
SEM	0.637	3.711	4.165	4.822	4.910	5.535	6.604
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Starch							
CGF	41.564a	62.507a	68.052a	74.344a	78.410a	83.172a	92.979a
CGM	21.253b	46.993b	65.739a	70.564b	75.101a	79.479a	88.431bc
Rice bran	15.074c	24.832c	43.472c	51.961d	59.654c	70.131b	79.007d
Cassava pulp	25.965b	29.187c	34.411d	42.307e	51.409d	69.067b	89.452ab
Wheat bran	11.583c	43.878b	51.605b	62.962c	66.769b	77.479a	84.821c
SEM	2.869	3.636	3.458	3.199	2.720	1.698	1.356
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

CGF= Corn gluten feed; CGM= Corn gluten meal; SEM= Standard error of the mean; $^{a-e}$= Values in the same column with different alphabets are significantly significant ($P<0.05$).

Table 5: *In-sacco* degradation kinetic parameters of dry matter and starch in carbohydrate source feed materials

Variables	CGF	CGM	Rice bran	Cassava pulp	Wheat bran	SEM	P-value
DM							
a (%)	8.840b	1.957c	2.223c	0.067c	12.227a	1.299	<0.001
b (%)	55.813b	25.140c	6.900d	80.693a	58.263b	6.995	<0.001
a + b (%)	64.653c	27.100d	9.123e	80.760a	70.490b	7.372	<0.001
c (%/hour)	0.337	0.053	0.113	0.023	0.127	0.056	0.346
ED_6 (%)	37.423b	13.700d	6.587e	22.830c	51.810a	4.371	<0.001
Starch							
a (%)	45.497a	20.840b	14.540c	22.220b	16.023c	3.043	<0.001
b (%)	44.640c	61.813b	65.957b	91.983a	65.300b	4.094	<0.001
c (%/hour)	0.097c	0.176a	0.077d	0.030e	0.123b	0.013	<0.001
ED_6 (%)	73.414a	66.924b	51.534d	51.967d	59.967c	2.294	<0.001

DM= Dry matter; a= Soluble fraction; b= Insoluble but potentially soluble fraction; a + b= Degradation potential; c= Degradation rate; ED_6= Effective degradability at a degradation rate of 6%/hour; RDS= Rumen degradable starch; RUS= Rumen undegradebale starch; CGF= Corn gluten feed; CGM= Corn gluten meal; SEM= Standard error of the mean; [a-e]= Values in the same row with different alphabets are significantly significant (P<0.05)

Table 6: Estimated rumen degradable starch (RDS) and rumen undegradable starch (RUS)

Feeds	RDS (% of starch)	RUS (% of starch)
Corn gluten feed	26.894a	9.739d
Corn gluten meal	9.256e	4.574e
Rice bran	12.459d	11.718c
Cassava pulp	24.591b	22.729a
Wheat bran	22.378c	14.939b
SEM	1.865	1.613
P-value	<0.001	<0.001

SEM= Standard error of the mean; [a-e]= Values in the same column with different alphabets are significantly significant (P<0.05).

The *in-sacco* estimated RDS and RUS values of by-product feeds are presented in Table 6. The RDS and RUS values of each feed ingredient differed significantly (P<0.001). Estimated RDS and RUS values were derived from ED and starch composition. The highest RDS value was observed in CGF (26.89% of starch), while the lowest value was recorded in CGM (9.26% of starch). Conversely, cassava pulp exhibited the highest RUS value (22.73% of starch), whereas CGM displayed the lowest (4.57% of starch). Rice bran demonstrated relatively balanced RDS and RUS values.

Correlation of carbohydrate fraction and starch digestibility

The correlation analysis between carbohydrate fractions and starch digestibility demonstrated significant associations, shedding light on the intricate dynamics of starch utilization (Fig. 1). Total carbohydrate content (CHO) exhibited a strong positive correlation with CB1 (r=0.87, P<0.001), underscoring its critical role in RDS fractions. Conversely, CHO was negatively correlated with CNSC (r=-0.77, P<0.001), indicating a trade-off between rapidly and slowly digestible carbohydrate components. RDS showed a positive correlation with the degradation rate constant "c" (r = 0.56, P<0.01), highlighting its importance in facilitating efficient starch hydrolysis, while its negative association with CHO and CB2 emphasizes its selective role in starch breakdown. Furthermore, CB1, a pivotal starch fraction, was positively associated with total carbohydrate digestibility and metrics such as CC (r=0.48, P<0.05), reinforcing its significance in enhancing starch availability. These findings provide valuable insights into the relationships between carbohydrate fractions and starch digestibility.

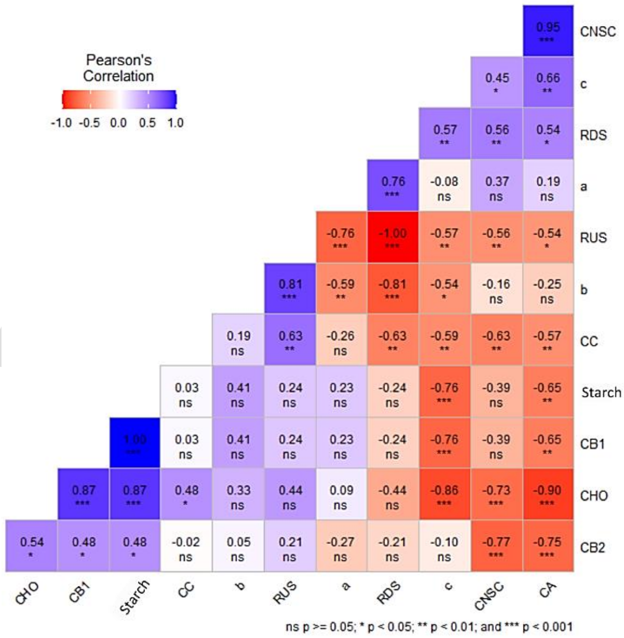

Fig. 1: Correlation between carbohydrate fraction and *in-sacco* starch degradation.

DISCUSSION

A comprehensive and accurate database on the chemical composition and degradation kinetics of DM and starch in feed by-products is increasingly essential for optimizing ruminant nutrition. This study provides a robust dataset outlining the chemical profiles, carbohydrate fractions, and degradation dynamics of DM and starch in energy-dense feed ingredients commonly used in tropical regions. Feed samples were sourced from local feed industries in Indonesia, ensuring their relevance to regional agricultural practices and feed formulations. These findings offer valuable insights to enhance feed evaluation and formulation strategies tailored to tropical livestock systems.

The chemical composition of the carbohydrate-based by-products analysed in this study revealed significant variability across measured parameters (Table 1), reflecting diverse nutritional values and feed-processing characteristics. Rice bran, with its high NDF and ADF content alongside moderate lignin levels, shows the potential to enhance rumen fermentation and improve fiber digestibility. This observation aligns with previous studies by Heidari et al. (2022) and Manlapig et al. (2024) that reported the positive effects of rice bran on rumen microbial activity and feed efficiency. In contrast, CGM exhibiting low fiber content may have a limited capacity to stimulate acetate production in rumen, typically derived from fiber fermentation (Agwaan 2023). However, its high CP content and low lignin levels could support microbial protein synthesis and provide an efficient energy supply.

The by-products also displayed substantial variability in starch content, with cassava pulp containing the highest levels and CGM the lowest. This variability can significantly influence energy density and digestibility. Differences in starch profiles, particularly the amylose-to-amylopectin ratio, were also observed. In this study, cassava pulp comprises approximately 23% amylose and 24% amylopectin, contrasting with cereal-based by-products like CGF and rice bran, which generally have higher amylose content due to their role as energy reserves for germination. These structural differences in starch composition affect the physical and functional properties of the by-products, including gelatinization temperature and pasting behaviour, which influence feed processing characteristics and animal performance (Han et al. 2022; Rahmadani et al. 2024).

The significant variations in chemical composition among feed by-products were closely linked to their carbohydrate fractionation. Cassava pulp had the highest total CHO content, primarily attributed to its high CB1 fraction, indicative of slow fermentable energy in the rumen. This aligns with cassava's natural composition, which is rich in starch, low in fiber, and predominantly composed of amylopectin. The branched structure of amylopectin facilitates enzymatic degradation in the rumen (Piao et al. 2021), explaining why cassava pulp has a high proportion of soluble carbohydrates. This characteristic makes cassava pulp a valuable energy-dense feed ingredient but requires careful dietary balancing to avoid rumen acidosis due to its limited fiber content and fast fermentation rate. Conversely, CGM, with the lowest total CHO content, exhibited the highest CNSC and highest CA fractions. The high CA fraction indicates that a significant portion of its fraction is sugar (Rodríguez-Espinosa et al. 2021). These characteristics highlight the limited role of CGM as a carbohydrate source but underscore its potential as a protein supplement, contributing minimal fermentable energy to ruminant diets.

Rice bran, with its high CC fraction, reflects the possible presence of rice husks in this sample study. Rice bran contaminated with rice husk will contain lignin-bound fiber and non-starch polysaccharides, which reduce its digestibility (Rosani et al. 2024). Although rice bran contains some starch, its lignocellulose and indigestible fiber components limit its value as a rapidly fermentable energy source but contribute to rumen fill. In contrast, wheat bran, characterized by its highest CB2 fraction and low CA value, balances fermentable carbohydrates with insoluble fiber. Its moderate starch content, combined with relatively higher amylose levels, contributes to a slower degradation rate, making it a favorable energy source for microbial fermentation and potentially reducing the incidence of acidosis (Pan et al. 2021).

These findings also highlight the importance of understanding the chemical composition of feed ingredients, particularly fiber and starch profiles, to predict their functional roles in the rumen more effectively. Balancing rapidly fermentable carbohydrates (e.g., CB1 in cassava pulp) with slower-degrading carbohydrates (e.g., CB2 in wheat bran) and accounting for indigestible fractions (e.g., CC in rice bran) is critical to optimize energy supply, enhance microbial efficiency, minimize methane emissions, and reduce the risk of metabolic disorders (Mulakala et al. 2022; Herliatika et al. 2024). This detailed characterization further underscores the complementary nature of these feedstuffs, enabling precise inclusion rates in ruminant diets to meet specific energy requirements and enhance overall animal performance.

Understanding *in-sacco* DM and starch digestibility is also vital for optimizing the utilization of these feed by-products in the rumen. Nutrient degradation at 0 hours represents the portion of nutrients that dissolve in water prior to microbial fermentation. The DM loss at 0 hours ranged from 1-8%, likely due to physical factors such as feed grinding, which directly influence particle size distribution (Darma et al. 2023). The starch loss at 0 hours, ranging between 11-41%, indicates the predominance of soluble and small-particle starch.

Across all incubation periods, DM degradability after 48 hours was below 75%, indicating that not all DM was available for rumen microbial activity. Wheat bran exhibited the highest DM degradability (74.28%), while rice bran showed the lowest (9.02%). These differences are attributed to variations in chemical composition that influence microbial activity. Wheat bran's high starch and NDF content, with a predominance of amylopectin, likely contributed to its higher degradability, as reported by Feyisa et al. (2024). However, González et al. (2015) reported slower wheat bran degradation compared to CGF, highlighting the influence of genotype and processing methods (Seifried et al. 2017). For starch, almost all starch from the by-products was degraded after 48 hours of incubation. CGF exhibited the highest starch degradability, while rice bran had the lowest. Differences in nutrient solubility significantly influenced this outcome, as higher solubility improves nutrient availability in the rumen. The high digestibility of CGF aligns with findings from Zhang et al. (2021), attributing its solubility to its composition as a wet corn milling by-product.

The degradation kinetics of DM and starch revealed clear distinctions in the a, b, and ED fractions. Although all by-products showed similar DM degradation rates, starch degradation varied significantly, with CGM exhibiting the highest rate and cassava pulp the lowest. This difference may be explained by amylose, with its linear chain structure, being more resistant to enzymatic degradation than amylopectin (Ma et al. 2024). Cassava pulp's high amylose content (Jumare et al. 2024) likely contributed to its lower degradation rate. Additionally, protein matrices and fat content surrounding starch granules can influence degradation rates (Rahmadani et al. 2023). ED at a 6%/hour degradation rate varied for DM and starch. Wheat bran had the highest ED for DM, while rice bran had the lowest. For starch, CGF exhibited the highest ED, with cassava pulp and rice bran showing the lowest. The differences in ED reflect the overall nutrient composition and starch fractionation of the by-products.

Ruminants consume starch-rich feed to meet energy demands for milk production and muscle growth (Liang et al. 2023). RDS leads to quick fermentation, resulting in the accumulation of short-chain fatty acids and a subsequent pH decline, potentially causing subacute ruminal acidosis. This study highlights the balance between RDS and RUS across by-products. CGF had the highest RDS, while cassava pulp exhibited the highest RUS. The balanced composition of RDS and RUS in cassava pulp supports efficient energy utilization without reducing rumen pH (Zheng et al. 2020).

The substantial variability in carbohydrate fractions emphasizes their influence on starch degradation. A significant positive correlation between total CHO and CB1 highlights the role of carbohydrates in providing RDS for rapid energy production. Conversely, the negative correlation between CHO and CNSC underscores the trade-off between fast and slow starch digestion, necessitating the inclusion of effective fiber sources to balance rumen fermentation and organic matter digestibility (Wang et al. 2021). Furthermore, the positive correlation between RDS and degradation rate constants confirms the role of RDS in enhancing starch hydrolysis efficiency (González et al. 2015).

Conclusion

In summary, this study revealed considerable variability in the chemical composition, carbohydrate fractions, and degradation kinetics of DM and starch in feed by-products. The positive correlation between total carbohydrates and CB1 highlights their importance in rapid energy production, while the negative correlation with CNSC reflects the balance between fast and slow starch digestion. Cassava pulp and wheat bran provide complementary benefits by balancing quick and sustained energy release, whereas rice bran enhances rumen fill and CGM contributes protein with minimal fermentable energy. Properly balancing these fractions is crucial for optimizing energy utilization, supporting rumen function, and improving animal performance in tropical livestock systems.

Funding: This research was funded by the Directorate General of Higher Education, Ministry of Higher Education, Science, and Technology under the PMDSU program, with grant number 001/E5/PG.02.00/PL.PMDSU/2024.

Acknowledgment: The authors would like to sincerely thank the laboratory technicians of the Laboratory of Feed Science and Technology, Department of Animal Nutrition and Feed Science, IPB University, for their valuable assistance and guidance during the data collection process in the laboratory.

Conflict of Interest: The authors declare that there are no conflicts of interest regarding the publication and/or funding of this manuscript.

Author's Contribution: Mardiah Rahmadani: original draft writing, visualization, software, methodology, investigation, formal analysis, data curation, and conceptualization. Anuraga Jayanegara: review and editing, validation, supervision, resources, project administration, methodology, investigation, funding acquisition, data curation, and conceptualization. Nahrowi and Lilis Khotijah: review and editing, validation, supervision, resources, and conceptualization. Rusli Fidriyanto: review and editing, validation, and supervision. Rahma Dhani Dwi Prasetya: original draft writing, investigation, and data curation. Irwan Susanto: original draft writing, data curation, and methodology. All authors have read and approved the final version of the article.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's Note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Agwaan HWK, 2023. Some physiological effect of different protein sources in ruminants ration: a comparative review. Journal of Applied Veterinary Sciences 8(2): 55–61. https://doi.org/ 10.21608/JAVS.2023.182063.1203

AOAC, 2005. Association of Official Analytical Chemists. 18th Edition. Horwitz W, Latimer GW, editor. Gaithersburg: AOAC International.

Darma ING, Jayanegara A, Sofyan A, Budiartilaconi E, Ridla M and Herdian H, 2023. Evaluation of nutritional values of tree-forage legume leaves from Gunungkidul District, Indonesia. Biodiversitas 24(5): 2733–2745. https://doi.org/10.13057/biodiv/d240527

Feyisa T, Tolera A, Nurfeta A, Balehegn M and Adesogan A, 2024. Availability, distribution and quality of agro-industrial byproducts and compound feeds in Ethiopia. Frontiers in Animal Science 5: 1–14. https://doi.org/10.3389/fanim.2024.1408050

Gierus M, Salama HSA, Lösche M, Herrmann A and Taube F, 2024. Protein and carbohydrate fractionation to evaluate perennial ryegrass (*Lolium perenne* L.) accessions. Agronomy 14(1). https://doi.org/10.3390/agronomy14010168

Gleason CB, Beckett LM, dos Reis BR and White RR, 2022. Evaluating the relationship between in vitro and in situ starch degradation rates. Animal Feed Science and Technology 283: 115175. https://doi.org/10.1016/j.anifeedsci.2021.115175

González J, Mouhbi R, Guevara-González JA, Rodríguez CA and Arroyo JM, 2015. Protein value of cereals and cereal by-products for ruminants: A comparison between crude protein and protein-based estimates. Archives of Animal Nutrition 69(4): 237–250. https://doi.org/10.1080/1745039X.2015.1053263

Han C, Guo Y, Cai X and Yang R, 2022. Starch properties, nutrients profiles, in vitro ruminal fermentation and molecular structure of corn processed in different ways. Fermentation 8(7). https://doi.org/10.3390/fermentation8070315

Heidari M, Ghorbani GR, Hashemzadeh F, Ghasemi E, Panahi A and Rafiee H, 2022. Feed intake, rumen fermentation and performance of dairy cows fed diets formulated at two starch concentrations with either conventional urea or slow-release urea. Animal Feed Science Technology 290:115366. https://doi.org/10.1016/j.anifeedsci.2022.115366

Herliatika A, Widiawati Y, Jayanegara A, Harahap RP, Kusumaningrum DA, Shiddieqy MI, Sasongko WT, Asmairicen S, Hadiatry MC, Putri AS, Handiwirawan E, Kostaman T, Praharani L and Adiati U, 2024. Meta-analysis of the relationship between dietary starch intake and enteric methane emissions in cattle from in vivo experiments. Journal of Advanced Veterinary and Animal Research 11(1): 212–230. https://doi.org/10.5455/javar.2024.k767

Jayanegara A and Sofyan A, 2009. Supplementary feeding on the nutrient balance of lactating dairy cow at contrasting temperature regimes: Assessment using cornell net carbohydrate and protein system (CNCPS) model. Journal of the Indonesian Tropical Animal Agriculture 34(3): 196–204. https://doi.org/10.14710/jitaa.34.3.196-204

Jumare FI, Salleh MM, Ihsan N and Hussin H, 2024. Cassava waste as an animal feed treatment: Past and future. Reviews in Environmental Science and Biotechnology 23(3): 839-868. https://doi.org/10.1007/s11157-024-09701-7

Kabsuk J, Nusri-un J, Binsulong B, Gunha T and Sommart K, 2024. Effects of substituting cassava pulp with broken rice and cassava chips in crossbred holstein diets: rumen fermentation, enteric methane emission, and energy utilization. Animals 14(2257): 1–15. https://doi.org/10.3390/ani14152257

Liang Z, Jin C, Bai H, Liang G, Su X, Wang D and Yao J, 2023. Low rumen degradable starch promotes the growth performance of goats by increasing protein synthesis in skeletal muscle via the AMPK-mTOR pathway. Animal Nutrition 13: 1–8. https://doi.org/10.1016/j.aninu.2022.10.006

Ma W, Tang J, Cheng H, Tian J, Wu Z, Zhou J, Xu E and Chen J, 2024. High-resistant starch based on amylopectin cluster via extrusion: from the perspective of chain-length distribution and structural formation. Foods 13(16). https://doi.org/10.3390/foods13162532

Manlapig JJD, Kondo M, Ban-Tokuda T and Matsui H, 2024. Effect of rice bran fermented with Ligilactobacillus equi on in vitro fermentation profile and microbial population. Animal Science Journal 95(1): 1–8. https://doi.org/10.1111/asj.13955

McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, Sinclair LA and Wilkinson RG, 2022. Animal Nutrition Eighth Edition. Pearson Education Limited, Harlow, England, UK.

Mulakala BK, Smith KM, Snider MA, Ayers A, Honan MC and Greenwood SL, 2022. Influence of dietary carbohydrate profile on the dairy cow rumen meta-proteome. Journal of Dairy Science 105(10): 8485–8496. https://doi.org/10.3168/jds.2022-21812

Orskov ER and Mcdonald I, 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science 92(2): 499–503. https://doi.org/10.1017/S0021859600063048

Pan L, Huang KH, Middlebrook T, Zhang D, Bryden WL and Li X, 2021. Rumen Degradability of Barley, Oats, Sorghum, Triticale, and Wheat In Situ and the Effect of Pelleting. Agriculture 11(647). https://doi.org/10.3390/agriculture11070647

Piao MY, Hu F ming, Kong F lin, Liu Y long, Wang S, Cui K, Sun T, Diao Q yu and Tu Y, 2021. Effects of dietary amylose to amylopectin ratio on growth performance, carcass quality characteristics and meat fatty acids in Chinese Qinchuan cattle. Journal of Integrative Agriculture 20(12): 3256–3269. https://doi.org/10.1016/S2095-3119(20)63576-6

Poore MH, 2022. Invited review: Use of byproduct feeds in southeastern US beef production systems. Applied Animal Science 38(5): 441–446. https://doi.org/10.15232/aas.2022-02307

Purbowati E, Sri Lestari CM, Adiwinarti R, Restitrisnani V, Mawati S, Purnomoadi A and Rianto E, 2021. Productivity and carcass characteristics of lambs fed fibrous agricultural wastes to substitute grass. Veterinary World 14(6): 1559–1563. https://doi.org/10.14202/vetworld.2021.1559-1563

Rahmadani M, Susanto I, Prasetya RDD, Kondo M, Nahrowi N and Jayanegara A, 2023. Modification of dietary rumen degradable starch content by chemical processing of feed ingredients: A meta-analysis. Animal Science Journal 94(1): e13834. https://doi.org/10.1111/asj.13834

Rahmadani M, Tryas AA, Susanto I, Nahrowi N, Khotijah L and Jayanegara A, 2024. Enhancing resistant starch in foods through organic acid intervention: A meta-analysis on thermal properties, nutrient composition, and in vitro starch digestibility. Journal of Agriculture and Food Research 15(January): 101037. https://doi.org/10.1016/j.jafr.2024.101037

Rodríguez-Espinosa ME, Guevara-Oquendo VH and Yu P, 2021. Carbohydrates molecular structure profiles in relation to nutritional characteristics of newly developed low and normal tannin faba bean varieties in dairy cows analysed by using standard methods and the vibrational molecular spectroscopy (FT/IR-ATR). Journal of Animal Physiology and Animal Nutrition 105(5): 816–831. https://doi.org/10.1111/jpn.13551

Rosani U, Hernaman I, Hidayat R and Hidayat D, 2024. Use of rice bran as ruminant feed in Indonesia. International Journal of Research and Scientific Innovation 11(1): 489–504. https://doi.org/10.51244/ijrsi.2024.1101036

Salami SA, O'Grady MN, Luciano G, Priolo A, McGee M, Moloney AP and Kerry JP, 2021. Concentrate supplementation with dried corn gluten feed improves the fatty acid profile of longissimus thoracis muscle from steers offered grass silage. Journal of the Science of Food and Agriculture 101(11): 4768–4778. https://doi.org/10.1002/jsfa.11123

Seifried N, Steingass H, Hoffmann N and Rodehutscord M, 2017. In situ starch and crude protein degradation in the rumen and in vitro gas production kinetics of wheat genotypes. Journal of Animal Physiology and Animal Nutrition 101(4): 779–790. https://doi.org/10.1111/jpn.12529

Sniffen CJ, O'Connor JD, Van Soest PJ, Fox DG and Russell JB, 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. Journal of Animal Science 70(11): 3562–3577. https://doi.org/10.2527/1992.70113562x

Van Soest PJ, Robertson JB and Lewis BA, 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10): 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Wang R, Wang M, Lin B, Ungerfeld EM, Ma ZY, Wu TT, Wen JN, Zhang XM, Deng JP and Tan ZL, 2021. Associations of ruminal hydrogen and pH with fiber digestibility and microbiota composition induced by increasing starch intake in beef cattle. Animal Feed Science and Technology 278: 114980. https://doi.org/10.1016/j.anifeedsci.2021.114980

Zhang R, Ma S, Li L, Zhang M, Tian S, Wang D, Liu K, Liu H, Zhu W and Wang X, 2021. Comprehensive utilization of corn starch processing by-products: A review. Grain & Oil Science and Technology 4(3): 89–107. https://doi.org/10.1016/j.gaost.2021.08.003

Zheng Y, Xue S, Zhao Y and Li S, 2020. Effect of cassava residue substituting for crushed maize on in vitro ruminal fermentation characteristics of dairy cows at mid-lactation. Animals https://doi.org/10(5).doi:10.3390/ani10050893