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Optimizing the utilization of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) tubers as a replacement for cassava chips in ruminant diets through pelleting: an in vitro gas technique study

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Abstract

Background Enhancing the utilization of winged bean tubers (WBT) in ruminant diets is gaining prominence due to the instability of traditional feeds, like cassava chips. Previous studies have shown limited outcomes in fermentation processes and complex quality improvement methods. Pelleting offers a promising approach to improving tuber utilization, while the in vitro gas technique provides insights into digestion and gas production.

Results This study explored the effects of pelleting WBT on gas production and digestion, supporting their integration as a sustainable alternative to cassava chips in ruminant diets. A completely randomized design (CRD) with a 2 × 3 (+ 1) factorial arrangement was used. Factor A comprised of WBT powder (a1) and WBT pellets (a2). Factor B included three substitution levels for cassava chips: b1 (33%), b2 (66%), and b3 (100%). Replacing cassava chips with 100% WBT powder or 66% and 100% WBT pellets resulted in significantly higher levels of Gas "a" ($P < 0.01$). The greatest gas production was observed when both WBT powder and pellet forms replaced cassava chips at a 33% replacement ($P < 0.01$). The replacement of cassava chips with WBT, either in powder or pellet form, did not result in significant differences in the in vitro degradation of the diet. Cassava chips-based diets produced significantly higher total volatile fatty acids (TVFAs) than the other diets ($P = 0.013$). Replacing cassava chips with 100% WBT pellets yielded the lowest methane gas production ($P = 0.02$).

Conclusion The pelletization of WBT has been found to have no adverse impact on rumen fermentation or digestibility. The WBT pellets have emerged as a notably promising alternative to cassava chips; nonetheless, the impact on TVFAs levels warrant careful consideration when utilizing them.

Keywords Winged bean tubers, Cassava chips, Pelleting, In vitro gas technique, Sustainable alternative

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Introduction

In recent years, there has been a steady rise in demand for animal products worldwide, especially meat and dairy [1]. Consumption of meat and milk tends to rise more rapidly in developing countries than in developed nations. Henchion et al. [2] predicted that the Southeast Asia region (SEARO) would consume more meat and milk, with SEARO's regional evolution displaying the strongest rising trend in terms of protein consumption per capita over time. Consequently, the increased demand has led to a rise in the production and importation of ruminants, thereby creating a need for resources to serve as feed ingredients for animals. Ruminants primarily rely on rumen microbial fermentation to break down complex carbohydrates like cellulose and hemicellulose, as well as to convert non-protein nitrogen sources such as urea into usable nutrients [3]. Cassava is an important economic crop in Thailand. It is mainly used for starch production, animal feed, and exports [4] However, their future use as an energy source faces challenges. Since Thailand is China's primary source of cassava chips, China has an extensive need for this raw material [5]. To deal with future uncertainties and their impacts, we must closely monitor several factors such as fluctuating currency exchange rates, trade disputes, geopolitical factors, and changing consumption patterns in China [6]. Then, we may take the required actions to lower risks and enhance our approach. This involves finding alternative feedstuffs and exploring sustainable alternatives to overcome the shortage of domestic feed production. Proactive adaptation and flexibility will be key to the sustainable use of the strengths of Thailand's biodiversity.

This search for viable alternatives has led us to investigate the potential of winged bean (*Psophocarpus tetragonolobus*) tubers (WBT), which exhibit promising qualities as an alternative energy feed source. Notably, WBT presents an intriguing prospect in terms of both quantity and cost-effectiveness when compared to conventional feed resources such as cassava chips. Sriwichai et al. [7] suggested that selecting appropriate breeds of WBT can lead to production that is sustainable in terms of quantity and quality. Based on an investigation of their proximate composition, the study found that WBT were suitable for use as an alternative energy source for animal feed due to their high energy (up to 16,264 J/g) and protein (up to 25.59%) contents. In an in vitro gas production experiment, Suntara et al. [8, 9] used WBT as a component of a concentrate diet to replace cassava chips. This evaluation was conducted to assess the effects of WBT in diets on the performance of ruminants.

The experiments indicated that using WBT as a novel alternative feed effectively replaced cassava chips without negatively impacting rumen function. However, it was found that replacing 100% of WBT with cassava chips alongside rice straw (as a source of low-quality roughage) can decrease TVFAs in the rumen. The realization of this problem carry out research to improve the quality of WBT for feeds with rice straw as the main roughage source. Unnawong et al. [10] performed an intriguing experiment comparing various starch modifications to evaluate their impact on gas kinetics, ruminal degradation, and ruminal fermentation characteristics by treating cassava chips and WBT with hot steam, sodium hydroxide (NaOH), calcium hydroxide ($CaOH_2$), and lactic acid (LA). The results indicated that the physical improvement method involving hot steam had the most positive effect on the nutritional value of WBT. In the following year, Unnawong et al. [11] found that incorporating 10–20% of steam-treated WBT into beef cattle diets increased ruminal propionic acid concentration compared to diets containing only cassava chips, suggesting that partial replacement with steam-treated WBT can enhance rumen fermentation efficiency. However, considering the practical limitations of implementing hot steam treatment, we propose an alternative approach to further enhance the utilization of WBT through pelleting offers advantages such as ease of production and practicality compared to hot steam treatment, which can be challenging to implement in practice [10, 12]. By adopting the pelleting approach, we aim to optimize the nutritional value and accessibility of WBT as a viable feed option in ruminant diets.

Pelleting has long been recognized as an effective technique for processing feed ingredients, providing numerous benefits such as improved nutrient digestibility, reduced feed wastage, and enhanced feed efficiency [12]. By subjecting WBT to the pelleting process, we hypothesize that the resulting pellets will improve rumen fermentation characteristics, especially when combined with rice straw. Our research focused on assessing the effects of substituting cassava chips with pelletized WBT in ruminant diets that are primarily based on rice straw as roughage. The primary objective was to assess the effect of this substitution on gas production parameters, including the quantification of methane gas., in vitro degradability, and ruminal fermentation. By conducting this study, we aimed to understand how the use of WBT pellets as a replacement for cassava chips can influence these key factors in ruminant degradation and assess the feasibility and potential benefits of incorporating WBT pellets into ruminant feeding practices.

Results

Chemical composition of the diet

In Table 1, the winged bean tubers pellets and cassava chips are compared. As fresh, WBT contained 435 g/kg, with an OM content of 963 g/kg. Notably, as a legume, this plant exhibits a remarkable CP of 189.8 g/kg of dry

Table 1 The chemical composition of winged bean tubers and cassava chips

Item	Winged bean tuber	Cassava chips
$g \cdot kg^{-1}$ fresh matter	435	906
	$g \cdot kg^{-1}$dry matter	
Organic matter	963	972
Crude protein	189.8	26
Ether extract	4.5	5.2
Neutral detergent fiber	168	112
Acid detergent fiber	54.2	43

Table 2 Ingredient and the chemical composition of the experimental diet

Ingredient	WBT-pellet 0%	WBT-pellet 33%	WBT-pellet 66%	WBT-pellet 100%
Rice straw	25	25	25	25
Soybean meal	14.25	14.25	14.25	14.25
Rice bran	10.25	10.25	10.25	10.25
Palm kernel meal	7.25	7.25	7.25	7.25
Salt	1	1	1	1
Premix[a]	0.25	0.25	0.25	0.25
Di-calcium-P	0.5	0.5	0.5	0.5
Cassava chips	40	26.8	9.1	0
WBT pellet	0	13.2	17.7	40
Urea	1.5	-	-	-
$g \cdot kg^{-1}$ fresh matter	915	922	918	920
	$g \cdot kg^{-1}$dry matter			
Organic matter	974	966	951	963
Crude protein	148.6	150.1	152.9	153.5
Ether extract	3.82	4.1	4.15	4.33
Neutral detergent fiber	333.1	333.5	338.3	335.8
Acid detergent fiber	127.6	123.4	127.3	124.8

[a]Premix=Vitamins and minerals; A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g

WBT-pellet 0% means no winged bean tuber replacement cassava chips; WBT-pellet 33%, 66%, and 100% means winged bean tuber replacement cassava chips 33%, 66%, and 100%

matter, which surpasses that of cassava chips. Table 2 presents the ingredients and the chemical composition of the experimental diet. In the first treatment, the WBT pellets did not replace cassava entirely, necessitating the inclusion of urea in the formulation to meet protein requirements. Subsequently, cassava levels decreased by specified proportions of 33%, 6.6%, and 100%, while WBT pellets served as replacements. Remarkably, the protein content levels remained relatively stable within the range of 148.6–153.5 g/kg, alongside other chemical elements.

In vitro kinetics of gas production

Interactions were observed between WBT and cassava chip replacement levels in terms of gas kinetics and cumulative gas production, as depicted in Table 3. There was an interaction between the WBT form and the level of replacement ($P < 0.01$) in terms of gas production from the immediately soluble fraction (Gas "a"), ranging from −9.97 to -15.92 ml. The replacement of cassava chips with WBT powder at 100% or WBT pellets at 66% and 100% resulted in the highest production of Gas "a", demonstrating a statistically significant linear increase [Interaction (linear); $P < 0.01$]. In terms of the effect on Gas "a", a comparison of feed sources in this study indicated that the utilization of cassava chips resulted in lower Gas "a" compared to WBT ($P < 0.01$). However, absolute gas production (|a|) is ideally used to describe the fermentation of the soluble fraction, and our study did not find any significant differences between the processing patterns of WBT in powder and pellet forms.

The level of gas production from the insoluble fraction (Gas "b") exhibited different patterns when replacing cassava chips with different levels of WBT. The highest gas production was observed when WBT powder substituted cassava chips at 33% (155.81 ml), while the lowest gas production occurred when WBT pellets were used as a complete replacement at 100% (126.63 ml) ($P < 0.01$). Significantly, these findings suggest a linear relationship between the increase in WBT level and the decrease in gas production from Gas "b" (Interaction (linear); $P < 0.01$). Furthermore, the study demonstrated that the use of cassava chips promoted a significantly greater amount of Gas "b" compared to the utilization of WBT ($P < 0.01$).

By replacing cassava chips with WBT pellets at levels of 33%, 66%, and 100%, a replacement increase in the gas production rate constant for the insoluble fraction (Gas "c") was evident when compared to other groups. The Gas "c" showed significant increments of 14.7%, 20%, and 29%, respectively, for each respective WBT pellet replacement level compared to the WBT powder group ($P < 0.01$). However, it is worth noting that despite these increases, the overall gas production rate for Gas "c" was highest when cassava chips were utilized in the feed formulations ($P < 0.01$).

The gas potential, as indicated by the extent of gas production (Gas "|a|+b"), reached its highest level when WBT powder was used to replace cassava chips at a 33% replacement level ($P < 0.01$). In contrast, the results of this experiment demonstrated that Gas "|a|+b" was lowest when WBT pellets replaced cassava chips at all levels ($P < 0.01$). The lowest values were found when WBT pellets fully replaced cassava chips at 100% ($P < 0.01$). Additionally, the use of cassava chips as a feed source resulted in a 10.27% greater Gas "|a|+b" compared to the WBT group ($P < 0.01$).

Cumulative gas production at 96 h

The cumulative gas production at 96 h ranged from 121.75 to 142.35 ml (Table 3). Moreover, the gas production curve, specifically when WBT replaced for

Table 3 The impacts of different winged bean tubers form and cassava chips replacement levels on gas kinetics and cumulative gas production

Process	WBT inclusions (%)	Gas kinetics[1]				Cumulative gas at 96 h, ml
		a, ml	b, ml	c, h/ml	|a|+b, ml	
Control	0	-15.92[b]	152.86[a]	0.073[bc]	168.78[a]	139.48[ab]
Powder	33	-14.78[b]	155.81[a]	0.068[cd]	170.59[a]	142.35[a]
	66	-14.13[b]	139.68[c]	0.065[de]	153.81[cd]	127.05[cd]
	100	-10.36[a]	133.10[d]	0.062[e]	143.46[d]	123.15[d]
Pellet	33	-14.89[b]	147.33[b]	0.078[ab]	162.22[b]	135.40[ab]
	66	-11.18[a]	140.47[c]	0.078[ab]	151.65[bc]	133.25[bc]
	100	-9.97[a]	126.63[e]	0.080[a]	136.60[e]	121.75[d]
SEM		0.53	0.91	0.002	1.24	2.07
P-value	Cassava chip vs. WBT	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$
	Interaction (linear)	$P<0.05$	$P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$
	Interaction (quadratic)	$P<0.01$	0.54	$P<0.05$	0.16	0.62
			Comparison			
Plant	Cassava chip	-15.92[b]	152.86[a]	0.073[a]	168.78[a]	139.48[a]
	WBT	-12.55[a]	140.50[b]	0.071[b]	153.06[b]	130.49[b]
WBT type	Powder	-13.09	142.87	0.06	155.95	130.85
	Pellet	-12.01	138.14	0.08	150.16	130.13
WBT level	33	-14.84	151.57	0.07	166.41	138.88
	66	-12.65	140.07	0.07	152.73	130.15
	100	-10.17	129.87	0.07	140.03	122.45

WBT, winged bean tubers; Cassava chip vs. WBT, mean *p*-value is shown by comparing the mean of the group employing 100% cassava chips (WBT-free) to the mean of WBT replacement at all levels, the mean is shown by the feed source; Interaction, defined as the combined effect between powder and pellets with the WBT level, is represented by the *p*-value as a linear and quadratic trend; SEM: Standard error of the mean; $^{a–e}$ means in the same column with different lowercase letters differ ($p<0.05$, $p<0.01$)

[1]a = The level of gas production from the immediately soluble fraction, b = the level of gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), a + b = the gas potential extent of gas production

cassava chips, exhibited a consistent upward trend until it reached a relatively stable phase after 60 h. The temporal pattern of gas production during the experiment is effectively depicted in Fig. 1. When comparing only the feed sources, the average gas pattern revealed a significantly higher cumulative gas level for cassava chips compared to WBT (both powder and pellets forms) from the 20 h until the 96 h after incubation time, as illustrated in Fig. 2. The highest gas production was noted when cassava chips were replaced with both WBT powder and pellet forms, each at a 33% replacement rate, yielding 142.35 ml and 135.40 ml, respectively. These values were equivalent to the gas production achieved when cassava chips were fully utilized (139.48 ml).

Alterations in in vitro feed degradability and rumen fermentation

The modification of WBT to pellets or its original form as a powder to replace cassava chips in feed formulation exhibited no discernible effect on the dynamics of digestion processes, as demonstrated by the results presented in Table 4. The mean in vitro organic matter digestibility (IVDMD) values of the feed exhibited a progressive increase over time, specifically from 12 h to 24 h, as expected. At the 12-hour mark, the diet degradation ranged from 599 to 685 g/kg DM, while at the 24-hour mark, it extended from 732 to 830 g/kg DM. The IVOMD ranged from 679 to 710 g/kg DM after 12 h of incubation time and showed an increase to a range of 787 to 820 g/kg DM after 24 h of incubation time. Importantly, no statistical differences were observed between the different treatment groups.

The observed data in Table 5 reveals significant shifts in the rumen fermentation process, attributed to the interaction of diverse forms of WBT alongside different degrees of cassava replacement ($P<0.01$). At 8 h post-incubation, a significant disparity in ruminal pH was evident. The inclusion of WBT as a feed source resulted in a notably higher pH in the rumen fluid compared to the utilization of cassava chips (7.03 vs. 6.99, respectively) ($P=0.04$). In contrast, there were no significant differences in ruminal pH between the unprocessed and pellet forms of WBT. The examination of mean pH values in the rumen fluid revealed not statistically significant.

Significantly elevated concentrations of ammonia nitrogen were discerned in the rumen fluid when WBT pellets were integrated into feed formulations, manifesting a remarkable increase of 44.6% relative to the utilization of WBT powder ($P<0.01$). This notable finding highlights the profound influence of incorporating WBT pellets on ammonia nitrogen in the rumen fluid, particularly during the 4-hour interval following the incubation

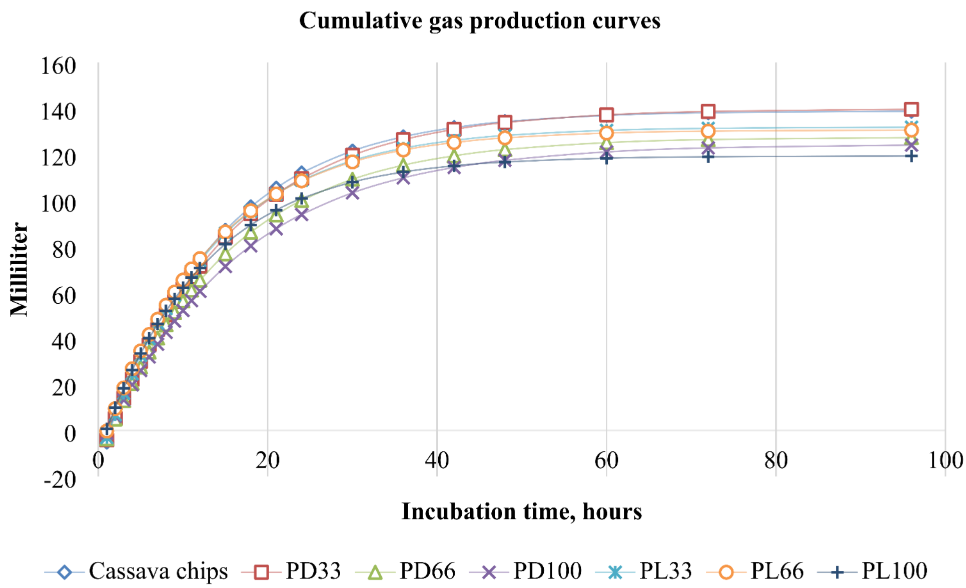

Fig. 1 Fitting a gas production model to the observed data of the combined effect between different winged bean tubers (WBT) and cassava chips replacement on cumulative gas production curves, reflecting the incubation period of 0 to 96 h; PD33, PD66, and PD100 referred to WBT as a powder replacement cassava chip at 33, 66, and 100%, respectively; PL33, PL66, and PL100 referred to WBT as a pellet replacement cassava chip at 33, 66, and 100%, respectively

period. Furthermore, at 8 h after the incubation period, an interaction effect was observed between the different forms of WBT combined with cassava chips at all levels (16.4 to 20.4 mg/dL) ($P<0.01$), except for the 100% WBT pellet replacement for cassava chips (12.9 mg/dL). Remarkably, in these cases, the ammonia nitrogen levels were similar to those of the control group (13.6 mg/dL), indicating no significant differences. The overall average ammonia nitrogen levels were found to be significantly higher ($P<0.01$) in the pellet forms compared to the powder forms. There was no observed influence between the form of WBT and the levels of cassava chip replacement on the population of rumen protozoa.

No interaction effect was observed between WBT and cassava chip replacement levels on TVFAs levels. However, distinct factors associated with the specific forms of WBT resulted in variations in TVFAs concentrations within the rumen fluid ($P<0.01$). The mean TVFAs concentration was significantly higher when WBT powder was utilized in the feed formulation, exhibiting a 6.12% increase compared to WBT pellets ($P=0.04$). Furthermore, cassava chips were identified as a significant contributor to TVFAs production in the rumen fluid, resulting in a noTable 12.1% increase compared to WBT ($P=0.013$). The methane concentration was derived by calculating the three principal volatile fatty acids (VFAs), and the specific values can be found in Table 3. There was no interaction between WBT and cassava chip replacement levels on methane concentrations at the 4th hour after incubation, but there were differences between the methane concentrations at the 8th hour after incubation. A significant quadratic interaction effect (Interaction (quadratic); $P=0.03$) was observed between WBT and cassava chip replacement levels. Interestingly, when cassava chips were replaced at 100% by WBT pellets replacement for, it resulted in the lowest methane gas production recorded at 20.8 mmol/L ($P=0.02$, Fig. 3). These findings highlight the pronounced non-linear relationship between WBT and cassava chip replacement levels, emphasizing the potential of utilizing WBT pellets as an effective approach to reduce methane emissions during rumen fermentation processes.

Volatile fatty acid profile

The proportions of VFAs profiles (acetic acid and butyric acid) in the rumen fluid exhibited significant variations, as outlined in Table 6. Processing of WBT to powder and pellets, and their inclusion in ruminant diets to replace cassava chips at different levels led to notable changes in the mean acetic acid values. Significantly, the greatest impact were observed when the WBT pellets was employed as a full replacement for cassava, leading to a significant decline in acetic acid content ($P<0.05$). The comparison of feed sources revealed that incorporating WBT into the diet resulted in a notable rise in the proportion of acetic acid ($P=0.03$). At the 66% cassava chip replacement level, the proportion of butyric acid displayed a significant variation depending on the different forms of WBT used, as revealed by the interaction effect. Specifically, the utilization of WBT powder was associated with a lower quantity of butyric acid (6.9 mol/100mol), whereas the application of WBT

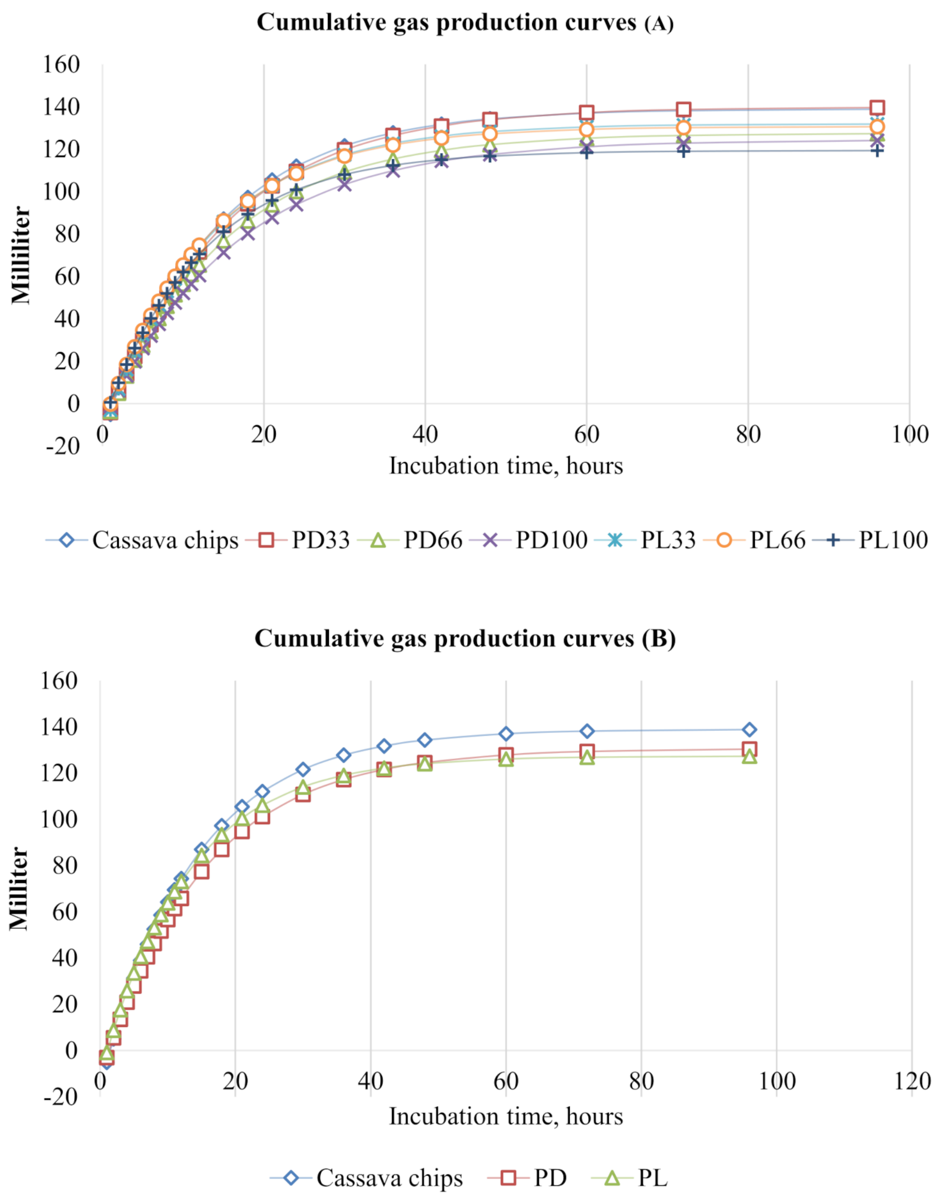

Fig. 2 Illustrates the application of a gas production model to the observed data, examining the impact of substituting cassava chips with WBT. Subfigures A present the fitting results for different scenarios: PD33, PD66, and PD100 represent the use of WBT powder as replacements for cassava chips at 33%, 66%, and 100%, respectively, while PL33, PL66, and PL100 represent the utilization of WBT pellets as replacements at the same respective levels. The influences of different feed sources are denoted by cassava chips, PD (WBT powder), and PL (WBT pellet)

pellets led to a higher concentration (14.2 mol/100mol). These two treatments exhibited a statistically significant difference when compared ($P = 0.04$).

Discussions

The chemical constituents of WBT contribute to differentiation during rumen fermentation compared to cassava chips [9]. Our experimental results revealed that the chemical composition of WBT aligned closely with the findings of Sriwichai et al. [7]. Specifically, we observed the presence of CP, EE, NDF, and ash at estimated proportions of approximately 19%, 0.3%, 16.8%, and 3.7%, respectively. The chemical composition was standardized across treatments to make it easier to see the differences caused by feedstuffs.

The starch content and composition of starch constituents in WBT have received limited research attention. However, according to Unnawong et al. [10], WBT is reported to contain approximately 53% non-fiber carbohydrates (NFC), whereas cassava chips are estimated to have around 67% NFC using the calculation method proposed by Weiss et al. [13]. The 53% of NFC in WBT can

Table 4 The impacts of different winged bean tubers form and cassava chips replacement levels on in vitro degradability

Treatments	WBT inclusions (%)	In vitro dry matter degradability (IVDMD), g/kg DM		In vitro organic matter degradability (IVOMD), g/kg/DM	
		12 h	24 h	12 h	24 h
Control	0	663.0	789.9	701.6	818.9
Powders	33	679.5	795.1	683.0	817.6
	66	685.8	809.9	734.5	819.1
	100	673.8	830.8	710.8	820.5
Pellets	33	599.9	803.3	690.6	800.2
	66	648.3	750.9	679.9	787.6
	100	630.9	732.2	688.3	799.7
SEM		22.5	54.3	33.7	15.0
p-value	Cassava chips vs. WBT	0.92	0.85	0.12	0.52
	Interaction (linear)	0.94	0.58	0.18	0.30
	Interaction (quadratic)	0.30	0.76	0.77	0.93
		Comparison			
Feed sources	Cassava chips	663.0	789.9	701.6	818.9
	WBT	653.0	787.0	697.9	807.5
WBT form	Powders	679.7	812.0	709.4	819.1
	Pellets	626.4	762.1	686.3	795.8
WBT levels	33	639.7	799.2	686.8	808.9
	66	667.1	780.4	707.2	803.4
	100	652.3	781.5	699.6	810.1

WBT, winged bean tubers; Cassava chip vs. WBT, mean *p*-value is shown by comparing the mean of the group employing 100% cassava chips (WBT-free) to the mean of WBT replacement at all levels, the mean is shown by the feed source; Interaction, defined as the combined effect between powder and pellets with the WBT level, is represented by the *p*-value as a linear and quadratic trend; SEM: Standard error of the mean

result in altered fermentation patterns and nutrient utilization in the rumen.

The gas production from the immediately soluble fraction, also known as Gas "a," is directly associated with the lag phase, which represents the initial stage of microbial incubation characterized by a delay in the colonization of microorganisms on the substrate [14]. This phase is characterized by slower microbial activity as the microorganisms adapt to the new environment and establish their populations [15]. The hallmark of this phase is a deceleration in microbial activity as microorganisms undergo a phase of acclimatization, progressively colonizing the substrate and building their populations. This means that the degradation of feed has an initiation period before the actual degradation begins [16]. Our findings highlight the significant influence of the replacement of cassava chips with WBT pellets on Gas "a". The use of cassava chips resulted in a more negative Gas "a" value compared to WBT. Conversely, refining WBT through pelleting and increasing the amount of WBT used instead of cassava chips in the feed formulation played a key role in increasing Gas "a", suggesting enhanced fermentation activity.

Negative gas "a" values are often observed in in vitro gas production studies and may result from early microbial adaptation phases or delayed fermentation activity of certain feed components [17], and similar findings have been reported in studies utilizing cassava chips as feed ingredients. The utilization of various energy sources in an in vitro gas production experiment by Srakaew et al. [18] revealed that cassava chips resulted in a significantly more negative Gas "a" value compared to corn, suggesting variations in their rumen fermentation patterns. However, the experimental results did not reveal a statistically significant difference between the two materials in terms of the Gas "a" value. In a more comprehensive study conducted by Sommart et al. [19], the effect of varying levels of cassava chips in the feed formulation was examined. Results showed that regardless of the substitution level (15%, 30%, or 40%), Gas "a" value consistently exhibited negativity, with recorded values of -35.2, -25.7, and −16.1, respectively. It is noteworthy that the higher level of cassava chips in the diet corresponded to an increase in the volume of Gas "a". This observation implies that the presence of carbohydrates in the feed (the greater the proportion) facilitates microbial fermentation, occurring prior to the rapid proliferation of microbial populations. This finding aligns with the experiment conducted by Cherdthong et al. [20], which demonstrated that the inclusion of easily fermentable carbohydrates in the diet or any process that leads to the breakdown of feedstuffs, resulting in an increase in water-soluble carbohydrates, also leads to an elevation in Gas "a". The addition of molasses and cellulase enzymes to rice straw was found to increase Gas "a", whereas the introduction of

Table 5 The impacts of different winged bean tubers form and cassava chips replacement levels on rumen fluid fermentation process alteration

Treatments	WBT inclusions (%)	pH			Ruminal Ammonia nitrogen, mg/dL			Protozoa, Log10 cell/ml			Total VFA, mM			Methane prediction, mmol/L		
		4hr	8hr	Mean	4hr	8hr	Mean	4hr	8hr	Mean	4hr	8hr	Mean	4hr	8hr	Mean
Control	0	7.03	6.99	7.01	13.0	13.6^{c}	13.3	7.20	7.29	7.24	92.5	100.1	96.3	26.5	27.5^{a}	27.0
Powders	33	7.13	6.92	7.03	11.4	19.6^{a}	15.5	7.62	7.32	7.47	91.5	90.3	90.9	27.9	25.5^{abc}	26.7
	66	6.99	7.03	7.01	11.9	17.9^{ab}	14.9	7.41	6.84	7.13	87.3	91.0	89.1	26.2	25.8^{abc}	26.0
	100	7.00	7.05	7.02	15.8	20.4^{a}	19.0	7.29	7.02	7.15	85.3	85.1	85.2	24.2	22.9^{bc}	23.6
Pellets	33	7.11	7.07	7.09	21.0	19.1^{a}	20.0	7.23	6.52	6.88	83.9	94.0	88.9	25.8	29.4^{a}	27.6
	66	7.05	7.02	7.04	19.5	16.4^{abc}	19.1	7.21	6.99	7.10	79.3	87.3	83.3	23.9	23.5^{bc}	23.7
	100	7.02	7.08	7.05	16.1	12.9^{c}	14.5	7.24	6.67	6.96	76.7	79.0	77.8	21.6	20.8^{c}	21.2
SEM		0.09	0.05	0.05	1.27	1.02	1.16	0.40	0.63	0.44	3.35	4.21	3.11	1.37	1.57	1.31
p-value	Cassava chips vs WBT	0.74	0.11	0.80	0.66	$p < 0.01$	0.084	0.85	0.65	0.68	0.03	0.03	0.01	0.03	0.02	0.02
	Interaction (linear)	0.61	0.39	0.86	0.15	$p < 0.05$	0.50	0.73	0.75	0.94	0.91	0.22	0.43	0.99	0.50	0.68
	Interaction (quadratic)	0.80	0.06	0.65	0.10	0.14	0.85	0.64	0.52	0.50	1.00	0.12	0.26	0.94	0.03	0.15
Comparison																
Feed sources	Cassava chips	7.03	6.99^{b}	7.01	13.0	13.6^{b}	13.3	7.2	7.3	7.2	92.5^{a}	100.1^{a}	96.3^{a}	26.5^{a}	27.5^{a}	26.9^{a}
	WBT	7.05	7.03^{a}	7.04	15.9	17.7^{a}	17.2	7.3	6.9	7.1	83.9^{b}	87.8^{b}	85.9^{b}	24.9^{b}	24.7^{b}	24.8^{b}
WBT form	Powders	7.04	7.00	7.02	13.0^{b}	19.3	16.5^{b}	7.4	7.1	7.3	88.0^{a}	88.8	88.4^{a}	26.08	24.7	25.4
	Pellets	7.06	7.06	7.06	18.8^{a}	16.1	17.9^{a}	7.2	6.7	7.0	79.9^{b}	86.8	83.3^{b}	23.77	24.6	24.2
WBT levels	33	7.12	7.00	7.06	16.2	19.4	17.8	7.4	6.9	7.2	87.7	92.1	89.9	26.82	27.4	27.1
	66	7.02	7.02	7.02	15.7	17.2	17.0	7.3	6.9	7.1	83.3	89.1	86.2	25.06	24.7	24.9
	100	7.01	7.06	7.03	15.9	16.6	16.8	7.3	6.8	7.1	81.0	82.0	81.5	22.90	21.9	22.4

WBT, winged bean tubers; Cassava chip vs. WBT, mean *p*-value is shown by comparing the mean of the group employing 100% cassava chips (WBT-free) to the mean of WBT replacement at all levels, the mean is shown by the feed source; Interaction, defined as the combined effect between powder and pellets with the WBT level, is represented by the *p*-value as a linear and quadratic trend; SEM: Standard error of the mean; $^{a–c}$ means in the same row with different lowercase letters differ ($p < 0.05$, $p < 0.01$)

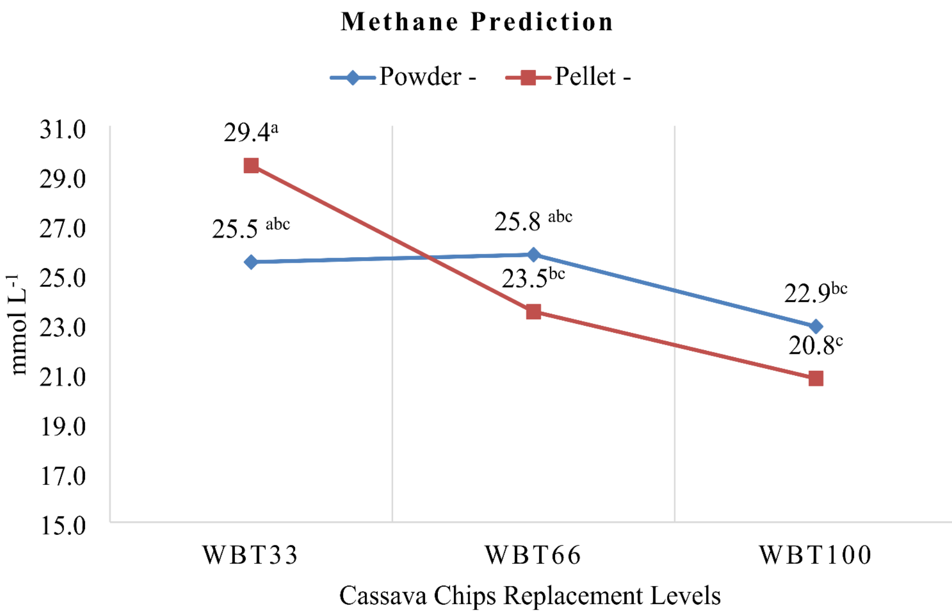

Fig. 3 The effect of winged bean tubers form and cassava chips replacement levels on the average methane prediction. WBT; winged bean tubers, WBT33, WBT66, and WBT100 referred to WBT as a powder replacement cassava chip at 33, 66, and 100%, respective

Table 6 The response of volatile fatty acid characteristics on utilizing different winged bean tubers form and cassava chips replacement levels

Treatments	WBT inclusions (%)	Acetic acid, mol/100mol			Propionic acid, mol/100mol			Butyric acid, mol/100mol		
		4 h	8 h	Mean	4 h	8 h	Mean	4 h	8 h	Mean
Cassava chips	0	67.7	67.7	67.7^{bc}	19.0	21.5	20.3	13.3	10.8^{ab}	12.1
Powders	33	74.5	70.8	72.6^{ab}	17.9	21.4	19.7	7.6	7.8^{ab}	7.7
	66	73.6	71.6	72.6^{ab}	18.5	21.5	20.0	7.9	6.9^{b}	7.4
	100	73.2	70.1	71.7^{abc}	21.5	21.5	21.5	5.3	8.4^{ab}	6.8
Pellets	33	72.6	73.2	72.9^{a}	16.7	16.6	16.7	10.7	10.3^{ab}	10.5
	66	74.3	65.3	69.8^{abc}	18.5	20.5	19.5	7.2	14.2^{a}	10.7
	100	71.4	64.1	67.1^{c}	20.6	22.0	21.3	8.0	13.9^{ab}	11.0
SEM		2.58	2.91	1.40	1.21	2.45	1.65	2.91	2.03	1.51
p-value	Cassava chips vs. WBT	0.40	0.12	0.03	0.18	0.59	0.37	0.87	0.10	0.19
	Interaction (linear)	0.38	0.18	$p<0.05$	0.35	0.84	0.62	0.26	$p<0.05$	0.19
	Interaction (quadratic)	0.30	0.42	0.89	0.29	0.34	0.28	0.18	0.99	0.20
				Comparison						
Feed sources	Cassava chips	67.7	67.7	67.7^{b}	19.0	21.5	20.3	13.3	10.8	12.1
	WBT	73.3	69.2	71.2^{a}	19.0	20.6	19.8	7.8	10.3	9.0
WBT types	Powders	73.8	70.8	72.3	19.3	21.5	20.4	6.9	7.7	7.3
	Pellets	72.8	67.5	70.1	18.6	19.7	19.2	8.6	12.8	10.7
WBT levels	33	73.5	72.0	72.7	17.3	19.0	18.2	9.1	9.1	9.1
	66	74.0	68.4	71.2	18.5	21.0	19.7	7.6	10.6	9.0
	100	72.3	67.1	69.7	21.0	21.8	21.4	6.7	11.2	8.9

WBT, winged bean tubers; Cassava chip vs. WBT, mean *p*-value is shown by comparing the mean of the group employing 100% cassava chips (WBT-free) to the mean of WBT replacement at all levels, the mean is shown by the feed source; Interaction, defined as the combined effect between powder and pellets with the WBT level, is represented by the *p*-value as a linear and quadratic trend; SEM: Standard error of the mean; $^{a-c}$ means in the same row with different lowercase letters differ ($p<0.05$, $p<0.01$)

Lactobacillus casei TH14 into rice straw reduced Gas "a" due to the sugar consumption by *Lactobacillus*, thereby keeping the Gas "a" level low. Furthermore, it is worth noting that the nutrient composition of WBT pellets, particularly their higher protein content, might have created a conducive environment for enhanced microbial growth and activity. This favorable nutrient composition may have contributed to a shorter lag phase. In a study conducted by Chumpawadee et al. [21], the protein content of various feed sources, including ground corn, rice bran, rice pollard, broken rice, and cassava chips, was determined. The protein content of these feed sources was found to be 8.53%, 14.26%, 8.46%, 7.8%, and 1.89%, respectively. Notably, cassava chips exhibited the lowest protein content among the experimental feed sources. These raw materials were subjected to gas production analysis, revealing a distinct Gas "a" value. Cassava chips displayed a negative Gas "a" value, with the highest recorded at -50.98 ml. Other raw materials, such as ground corn, rice bran, rice pollard, and broken rice, displayed lower Gas "a" value compared to cassava chips. Specifically, the Gas "a" value for these materials were – 32.4, -21.67, -3.39, and – 34.2 ml, respectively.

Despite the WBT pellets having the ability to produce gas "a", cassava chips have a higher concentration of Gas "b," Gas "c," Gas "|a|+b," and cumulative gas at 96 h. This discrepancy can be attributed to the fact that protein fermentation yields notably lower gas production compared to the substantial gas production generated by carbohydrate fermentation. However, cassava chips possess a distinctive characteristic in that they contain a higher quantity of starch, leading to substantial gas production despite their low protein content [22]. Cassava chips outperformed low-starch feedstocks in in vitro gas technique experiments with rumen fluid, confirming previous findings. In a study conducted by Nitipot and Sommart [23], it was concluded that cassava chips had a higher rate and more effective gas-producing capability than cornmeal, broken rice, and other industrial byproducts as a consequence of their research into in vitro gas production techniques.

In terms of the rumen environment, our study demonstrates that utilizing WBT as feed results in a more stable pH than using cassava chips. The dissimilarity in starch content between WBT and cassava chips is a possible factor contributing to the variations in the observed results. Cassava chips contain large amounts of starch that are rapidly digested [24]. Cassava chips contain a high starch concentration, exceeding 80% of dry matter (DM) [25]. Numerous studies have reported that cassava starch exhibits superior digestibility, making it a favorable ingredient in diets aimed at maximizing starch utilization [26]. Due to the presence of this component, the presence of starch in cassava chips in the diet can potentially result in a decrease in rumen pH, attributed to the heightened synthesis of short-chain fatty acids (SCFAs) and lactic acid [27]. The data presented align with the findings of this experiment, indicating that the average TVFAs production from cassava chips was significantly higher compared to WBT (92.5 vs. 83.9, respectively). The glucose molecules released from starch hydrolysis undergo fermentation by various groups of rumen bacteria, particularly amylolytic bacteria [28]. These bacteria metabolize glucose through a series of enzymatic reactions, resulting in the production of VFAs and other by-products [29]. Despite our experiments not quantifying the specific amount of lactic acid produced in the rumen fluid, the utilization of high levels of highly degradable starch was associated with an observed increase in lactic acid production, as reported by Metzler-Zebeli et al. [27]. During glycolysis, glucose is primarily converted into lactic acid by specialized bacteria known as lactic acid-producing bacteria (LAB), which become more metabolically active and utilize glucose for lactic acid synthesis [30]. The main types of LAB involved in lactic acid production in the rumen are *Streptococcus bovis* and *Lactobacillus* spp [31]. Our findings revealed a shift in the VFA profile in WBT-based diet, characterized by an increase in acetic acid concentration. The increase in ruminal acetic acid concentration can be attributed to the stimulation of cellulolytic bacteria, which play a key role in degrading complex carbohydrates and producing volatile fatty acids, particularly acetic acid [32]. Additionally, acetogenic bacteria contribute to acetic acid production as a major end-product of their metabolic activity, forming a crucial component of the VFAs synthesized in the rumen [33]. The stimulation of these microorganisms requires a fiber-rich diet, while other compounds that could potentially enhance microbial activity in WBT remain unknown. Another contributing factor could be the adaptation of the rumen environment, including pH, which may indirectly promote the proliferation of cellulolytic bacteria.

Our study, propionic acid, a crucial intermediate product of rumen fermentation, remained unchanged despite varying levels of WBT pellets. Propionic acid is typically generated through the fermentation of starch, sugars, and other non-structural carbohydrates. However, the starch content of WBT pellets is not comparable to that of cassava, potentially leading to lower levels of propionic acid. This discrepancy may be attributed to the compensation of the food source that serves as the precursor for propionic acid production. Research by Unnawong et al. [10] suggests that crab pellet heads contain a significant proportion (53%) of non-fiber carbohydrates, which could potentially offset the decrease in propionic acid observed in this study. Similarly, reports from Castrillo et al. [34] indicate that the physical form of feed, such as pellets, can influence propionic acid levels in the rumen. According to his findings, the use of pellets resulted in a 22.6% increase in propionic acid, suggesting that this effect may be due to enhanced propionic acid production within the rumen lumen. As a result, propionic acid levels remain stable despite changes in dietary composition.

The utilization of WBT in animal feed resulted in a significant reduction in calculated methane content. Although this experiment did not directly measure methane gas, it raises intriguing possibilities regarding the potential of WBT to mitigate the impact of this greenhouse gas. We conducted a methane content assessment due to the informative nature of legume data, which often offers valuable insights into methane reduction. For instance, tropical legumes are known to contain tannins, which are plant compounds that can effectively inhibit methanogenesis, thereby presenting interesting opportunities for mitigating methane emissions [35]. Tannins possess the capability to mitigate methane production by inhibiting the activity of methanogenic archaea within the rumen [36], thereby potentially contributing to the observed reduction in methane emissions in this study. In relation to WBT, previous investigations have demonstrated that each gram of WBT contains approximately 5.32 milligrams of tannins [37]. Despite the availability of significant data on WBT, its impact on rumen fermentation processes has not been extensively tested, emphasizing the need for further research in the future to explore its potential effects.

Conclusions

In summary, the pelletizing process can alter the soluble fraction of WBT, but cassava chips still exhibited exceptional overall gas production and TVFAs production. The WBT pellets and powders have no adverse impact on rumen fermentation and show potential for reducing methane gas production. The digestibility of WBT is comparable to cassava chips, but degradation should be evaluated in both the rumen and acid stomach. Overall, WBT pelletization can be a viable substitute for cassava chips, but consideration of decreased TVFAs levels and animal experiments is crucial for further understanding its impact. Furthermore, while the current data on nitrogen-free extract (NFC) may not offer robust evidence supporting its direct impact on rumen fermentation, its investigation remains pertinent. Therefore, undertaking additional research to scrutinize the specific chemical composition of starch is imperative for a comprehensive understanding of its role as a feed component in rumen function.

Materials and methods

The Institutional Animal Care and Use Committee of Khon Kaen University approved all methods and procedures (record number IACUC-KKU-46/65). We confirm that our procedures for laboratory animals comply with the Code of Conduct for Laboratory Animals and meet international standards. In this study, rumen fluid collected from fistulated dairy cattle was utilized to conduct in vitro gas technique analysis. The animals were released immediately after sampling, and no animals were euthanized or subjected to any harm or pain. The process was conducted by experts and was quick and non-invasive.

Cultivation, harvesting, and processing of winged bean tubers (WBT) for pellet production

The WBT were sourced from Dr. Sompong Chankaew at the Department of Agronomy, KKU. The WBT was cultivated in plots measuring 1 m by 1 m, with rows spaced 1 m apart and plants placed at intervals of 0.5 m. Each plot consisted of ten plants, and there was a 2-meter gap between plots within each row. Two rounds of fertilizer application were carried out: the first at 21 days after planting and the second when the plants were two months old [7]. The crop was regularly subjected to manual weed control throughout its growth. Additionally, the plants received consistent irrigation, and measures were taken as needed to control diseases and pests. When the plants reached eight months of age, the tubers were harvested from the plots, and their fresh weights were immediately recorded. A subset of fresh tubers from each plot, belonging to the winged bean accessions, was promptly sampled. These tubers were washed with tap water, cut into small chips, and subjected to drying in a forced-air drying oven (Memmert UM600, located at Willi-Memmert-Strasse, Germany) at a temperature of 60 ∘C for of 72 h, or until a constant moisture content was achieved.

The WBT samples were finely ground into a powder using a grinder from Arthur H. Thomas Co. (Wiley mill, Philadelphia, PA, USA). Subsequently, the resulting powder was divided into two parts. One portion was designated as WBT powder, while the other part was processed into pellets. After obtaining the powdered form, the material was processed through a pellets preparation procedure to transform it into the final product. The powder was carefully fed into a pellet machine, where it underwent a controlled process of agglomeration. Upon completion of the pellet process, the resulting pellets underwent a subsequent drying step, employing the method, to effectively eliminate any excess moisture and enhance their overall stability. This meticulous drying process ensured that the WBT pellets were suitably prepared and ready for comprehensive laboratory examination.

Experimental design and dietary treatments

A completely randomized design (CRD) was utilized, employing a 2×3 (+1) factorial arrangement in the experiment. Factor A involved the use of two types of WBT: WBT powder (a1) and WBT pellets (a2). Factor B represented the level of WBT substitutions for cassava chips in the diet, with three levels determined as b1, b2, and b3, corresponding to 33%, 66%, and 100% substitutions, respectively. Factors A and B were arranged in a factorial design, with a control group for comparison. The control group utilized cassava chips as a primary ingredient (represented as +1), while the feed formula excluded the use of WBT. The formulation of the animal feed followed the Khon Kaen Complete Feed (KCF) 2020 Program, which considered the ingredient composition based on the National Research Council guidelines NRC [38] for the maintenance of growing Holstein Frisian dairy cattle. Detailed information regarding the composition of feed ingredients and the chemical composition of both raw materials and the concentrate diet can be found in Table 1.

Collecting rumen fluid and preparing artificial saliva

Rumen fluid samples were collected from two rumen-fistulated dairy steers weighing approximately 400 ± 15.0 kg. These animals were individually housed in separate pens and were provided with a feeding regimen consisting of *ad libitum* access to rice straw and a concentrated diet containing 149 g/kg crude protein (CP), in accordance with the nutrient requirements specified for dairy cattle in Thailand [39]. The feeding regimen also included the supplementation of a suitable mineral lick (KNZ mineral lick, Akzo Nobel Functional Chemicals B.V., RS Hengelo, Netherlands) to ensure adequate mineral intake. Furthermore, the steers had continuous access to a clean water supply throughout the entire experimental period, which lasted for at least 14 days. This standardized feeding protocol aimed to provide optimal nutrition and created a consistent rumen environment for the collection of rumen fluid samples.

Before the morning feeding, a volume of 1,500 ml of rumen fluid was collected from the animals via a cannula, allowing for direct access to the rumen. To ensure the purity of the collected fluid, the samples underwent a meticulous filtration process using four layers of cheesecloth, removing any solid particles or debris. To preserve the biological integrity and maintain the rumen fluid's optimal conditions, it was immediately transferred to a container with thermal insulation, where it was kept at a controlled temperature of 39 °C. The container with the rumen fluid was swiftly transported to the laboratory within a period of 15 min. Following established methodologies proposed by Menke [40], a buffering nutritive solution preparation was meticulously formulated. This solution preparation consisted of various components, including distilled water (1,095 ml), a micromineral mixture (0.23 ml) comprising specific proportions of $MnCl_2.4H_2O$, $CaCl_2.2H_2O$, $CoCl_2.6H_2O$, and $FeCl_3.6H_2O$. Additionally, a macromineral mixture (365 ml) was incorporated, consisting of KH_2PO_4, Na_2HPO_4, NaCl, and $MgSO_4.7H_2O$, which contributed essential microminerals [41].

To facilitate accurate measurements and maintain experimental precision, a 0.1% resazurin mixture (1 ml) was added to monitor the redox potential. Moreover, a reduction mixture (60 ml), consisting of $Na_2.S._9H_2O$ and NaOH, was included to create a non-oxygen atmosphere, ensuring optimal conditions for subsequent analyses.

Multivariate analysis of ruminal parameters

Each dietary treatment was accurately weighed to a precision of 0.5 g and subsequently transferred into 50-milliliter bottles. To create a consistent experimental setup, a total volume of 40 ml of rumen fluid medium was added to each treatment bottle, ensuring uniform conditions across all samples. The addition of the rumen liquor medium was carried out meticulously using an 18-gauge × 1.5-inch needle to maintain accuracy and prevent any contamination. To guarantee an airtight environment, all experimental bottles underwent thorough sealing. Butyl rubber stoppers and metal caps were tightly affixed to each bottle, creating a secure and sealed enclosure. The incubation of the experimental bottles took place in a hot-air oven, where they were subjected to a controlled temperature of 39 °C. The oven provided a consistent and optimal environment for the subsequent measurements and analysis.

For the experimental setup, three different groups of bottles were established. In the first group, which focused on gas kinetics and gas production measurements, each treatment was assigned three bottles. This allowed for robust data collection and analysis, ensuring statistical significance. In addition to the treatment bottles, three extra bottles were designated as blank controls, providing a baseline for comparison and background measurement. The bottles were gently shaken every 3 h throughout the entire incubation period. Each set comprised three treated bottles and three blank bottles. The blank bottles contained only rumen fluid, and net gas volume was calculated by subtracting the average value of the gas volume from the experimental bottles.

Gas production measurements were conducted using a precise 20 ml glass aloe precision hypodermic syringe (U4520, Becton, Dickinson, and Company, New Jersey, USA). To facilitate the measurement process, the bottles in the incubating chamber (maintained at 39 °C) were punctured with an 18-gauge injection needle, allowing for the accurate collection of gas samples. In Group 2, various parameters, including pH, ruminal ammonia nitrogen (NH_3-N), and VFAs were all examined within the same bottle. To capture the dynamic changes over time, samples were collected at two specific time points: 4 h and 8 h of incubation. Each time point was assessed using three replicate bottles, ensuring robust data collection and analysis. Group 3 focused on measuring nutrient degradability. For this purpose, samples were obtained from three bottle replicates at specific time intervals: 12 h and 24 h after the start of incubation.

In vitro gas production and nutrient degradability

Gas production measurements were conducted at various time intervals throughout the incubation period, including 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 48, 72, and 96 h. The experimental bottles were divided into three different sets, each serving a specific purpose. In the first set, the focus was on gas kinetics and gas production measurement. The second set of bottles was allocated for the measurement of various ruminal parameters at specific time points at 4- and 8-hours post-incubation. These parameters included pH, which was measured using the HANNA Instrument (HI) 8424 microcomputer, Hanna instruments, Inc., Kallang, Singapore. The ruminal NH_3-N levels were determined using the Kjeldahl method as outlined by the AOAC [42]. The VFAs (acetic acid, propionic acid, and butyric acid) were assessed using a gas chromatograph (Nexis GC-2030: SHIMADZU, Shimadzu Corp., Kyoto, Japan) featuring a capillary column (molecular sieve 13X, 30/60 mesh, Alltech Associates Inc., Deerfield, IL, USA) [43]. Additionally, rumen protozoa direct counts were conducted using equipment from Boeckel & Co. GmbH & Co. The final set of bottles was dedicated to assessing in vitro dry matter degradability (IVDMD); IVDMD% = 100 × [(initial dry sample wt-(residue - blank))/initial dry sample wt] and in vitro organic matter degradability (IVOMD); IVOMD% = 100 × [(initial dry organic matter wt-(residue - blank))/initial dry organic matter wt] [44]. CH_4 concentration was estimated according to the equation of Moss et al. [45] [equation: CH_4 estimation = 0.45 (acetic acid)–0.275 (propionic acid) + 0.4 (butyric acid)].

Statistical analyses

Mathematical non-linear models were utilized to estimate the kinetics of ruminal fermentation, as proposed by Ørskov and McDonald [46]. The gas production data collected during the experiment were incorporated into the following equation for gas production recording:

$$\mathrm{Y} = \mathrm{a} + \mathrm{b}\ (1 - \exp\ (-\mathrm{ct}))$$

In this equation, 'a' represents the volume of gas produced from the soluble fraction, 'b' represents the volume of gas produced from the insoluble fraction, 'c' represents the gas production rate constant for the insoluble fraction, 't' represents the incubation time, 'a + b' represents the potential extent of gas production, and 'Y' represents the amount of gas produced at a given time 't'.

To analyze the data effectively, a statistical approach was employed. The experimental design followed a completely randomized design, with a factorial treatment arrangement of 2 × 3 (+ 1), considering the types of WBT (powder and pellet forms), WBT inclusion levels (33%, 66%, and 100% levels), and the control diet. The statistical analysis was carried out using the general linear model (GLM) procedures within the SAS [47] program (Version 9.0, Cary, NC, USA). The least-squares means (LSMEANS) option of the SAS program was utilized to calculate the average values for each treatment, providing a reliable estimation of the treatment effects.

The statistical model used for the analysis can be represented as follows:

$$\mathrm{Yij} = \mu + \alpha\mathrm{i} + \beta\mathrm{j} + \alpha\beta\mathrm{ij} + \varepsilon\mathrm{ij}$$

In this equation, Yij represents the observation for a particular combination of factors, μ denotes the overall mean, αi captures the effect of the types of WBT (powder and pellets forms), βj accounts for the impact of the WBT inclusions level (33%, 66%, and 100%), αβij represents the interaction effect between the roughage source and the levels of WBT, and εij represents the residual error term. To determine the significance of the factors, F-tests were conducted, and when the results indicated significance, orthogonal contrasts were performed to examine specific contrasts between the factors of interest. Additionally, Duncan's new multiple-range test (DMRT) was employed to compare the mean differences between treatments at a significance level of $P < 0.05$ [48], providing valuable insights into the different treatment effects and facilitating further interpretation of the data.

Abbreviations

$CaOH_2$	Calcium Hydroxide
CP	Crude protein
DM	Dry matter
EE	Ether extract
IVDMD	*In Vitro* Dry Matter Degradability
IVOMD	*In Vitro* Organic Matter Degradability
KCF	Khon Kaen Complete Feed
LA	Lactic Acid
Ml	Milliliter
NaOH	Sodium Hydroxide
NDF	Neutral detergent fiber
NH3-N	Ammonia nitrogen
NH_3-N	Ammonia-Nitrogen
NRC	National Research Council
SEARO	South East Asia Regional Office
TVFAs	Total Volatile Fatty Acids
WBT	Winged Bean Tubers

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Author contributions

Planning and design of the study, C.S. (Chanon Suntara) A.C. (Anusorn Cherdthong); Re-sources, S.C. (Sompong Chankaew); Funding acquisition, A.C.; literature search, S.C., W.J. (Winai Jaikan), P.P. (Perapong Phaengphairee), and T.H. (Theerachai Haitook); Feed preparation and Rumen collecting, N.S. (Napudsawun Sombuddee), S.L. (Saow-alak Lukbun), N.K. (Natdanai kanakai) and P.S. (Pachara Srichompoo; Data collection, N.S., S.L., N.K., and P.S.; Sample analysis, N.S., S.L., N.K., and P.S.; Statistical analysis, C.S., A.C., W.J., P.P., and T.H.; Data interpretation, C.S., N.S., S.L., N.K., P.S., S.C., W.J., P.P., T.H., and A.C.; Visualization, N.S., S.L., N.K., and P.S.; Manuscript drafting, C.S., N.S., S.L., N.K., P.S., S.C., W.J., P.P., T.H., and A.C.; manuscript editing and finalizing, C.S., N.K., P.S., S.C., W.J., P.P., T.H., and A.C.All authors have read and agreed to the published version of the manuscript.

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Data availability
The dataset produced and/or examined during the present study is not accessible to the public as it forms an initial component of another study. Nevertheless, interested parties can obtain the data from the corresponding author upon making a reasonable request.

Declarations

Ethics approval and consent to participate
The study received approval from the Khon Kaen University Animal Ethics Committee (Record number IACUC-KKU-46/65). We confirm that our procedures for laboratory animals comply with the Code of Conduct for Laboratory Animals and meet international standards. In this study, we used rumen fluid obtained through oral stomach sampling to conduct in vitro gas technique analysis. The animals were released immediately after sampling, and no animals were euthanized or subjected to any harm or pain. The process was conducted by experts and was quick and non-invasive.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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