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Winged bean tuber as a novel alternative to corn meal in concentrate mixture in Thai native beef cattle diets

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Abstract

Background In times of high feed costs and severe feed competition, it is crucial to explore alternative feedstuffs. Identifying alternative feed sources is essential for developing cost-effective and sustainable solutions to address challenges in livestock production and economic constraints. This study evaluates the effects of replacing corn with different levels of winged bean tuber (WBT) in concentrate mixtures on feed intake, feed utilization, and rumen fermentation in Thai native beef cattle. Animals were randomly assigned to a 4×4 Latin square design, with WBT replacing corn in the concentrate mixture at levels of 0, 33, 67, and 100%.

Results Replacing corn with WBT (0–100%) in the concentrate diet did not affect ($p > 0.05$) dry matter, organic matter, or crude protein intake. However, neutral detergent fiber (NDF) and acid detergent fiber (ADF) intake increased linearly ($p < 0.05$) at 67% and 100% replacement levels. Although nutrient digestibility remained unchanged, ruminal pH, ammonia-nitrogen concentration, and protozoal population increased linearly ($p < 0.05$) at 67% and 100% WBT replacement levels. However, WBT replacement did not significantly affect blood urea nitrogen (BUN) concentrations. As the level of WBT increased in the concentrate diet, the concentration of propionic acid (C3) increased, but the amount of acetic acid (C2) and the ratio of C2 to C3 decreased linearly ($p < 0.05$) without shifting the acetic concentration. Additionally, replacing corn with WBT in the concentrate diet had no effect on nitrogen balance.

Conclusion The WBT can be used as an alternative for corn grain up to 100% in ruminant diets without changing feed digestibility, the BUN, and nitrogen balance. It can also improve the fermentation characteristics in the rumen.

Keywords Alternative feed, Beef cattle, Energy source, Rumen fermentation, Tuberous plant

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Introduction

In Thailand, the corn cultivation area in 2020 was approximately 1.1 million hectares, which corresponded to the production of 4.8 million tons of corn [1]. With nearly 47% of its land dedicated to farming and livestock production, Thailand is a predominantly agricultural country [1, 2]. As a result, Thailand has been marketed as the "Kitchen of the World" by the Thai government [3]. Corn is the fifth-largest plantation crop in Thailand, followed by cassava, rice, rubber trees, and sugar cane. While Thailand's feed production requires 8.517 million tons of corn grain, such plantation regions produce 4.535 million tons of corn each year [4, 5]. According to Singvejsakul et al. [6], the two largest export markets for corn goods are Europe and Asia, and Thailand is the leading exporter of corn products worldwide. The Russia-Ukraine crisis has caused a 20% increase in corn prices due to higher global demand and its substitution for other staple crops [7]. Additionally, factors such as the COVID-19 pandemic, climate change, and cross-border transportation challenges have further driven up corn prices in Thailand. To address regional feed shortages and enhance feed security, the introduction of alternative energy crops or novel plant-based feed sources could be a viable solution for the animal feed industry [8].

The winged bean (*Psophocarpus tetragonolobus*, WB) is a subtropical zone leguminous plant of the *Fabaceae* family and *Papilionoideae* subfamily that is often overlooked. Furthermore, all of Thailand's provinces can cultivate winged beans, which yield nutrient-dense and protein-rich edible pods, seeds, leaves, blooms, and tuberous roots [9, 10]. There is limited research on the protein composition and chemical properties of winged bean tuber (WBT). At the time, Sriwichai et al. [11], discovered the chemical ingredients of the tuber and demonstrated that it might be utilized as an animal feed. WBT has significant nutritional value and may be used in feed formulas as a substitute for corn, offering nutritional advantages due to its composition of approximately 20% protein and 25–60% carbohydrates [12]. In addition to having a high protein content of approximately 190 g/kg DM, ether extract ranges from 2.6 to 11.6 g/kg DM, and NDF ranges from 150 to 300 g/kg DM [13]. WBT has a high gross energy content of 15.98 MJ/kg, which is equivalent to corn grain (16.36 MJ/kg) [14].

Despite its potential, WBT remains underutilized in animal feed due to the limited research on its nutritional benefits [12]. It was therefore recommended that it be used as a corn substitute so that farmers might profit from it, which would eventually result in a significant increase in the production of animal feed on a large scale. Winged bean tuber (WBT) yield in Thailand can reach 15.5 t/ha [11], with a market price of USD 3–5 per kg, significantly higher than corn grain (USD 0.20–0.60 per kg). Despite its cost, WBT is a new feed resource with potential in animal nutrition. Wider adoption and increased cultivation could lower prices, making it a viable alternative to traditional grains, enhancing feed security, and promoting sustainability in livestock production [8]. Despite the fact that WBT should be an excellent substitute for animal feed due to its high nutritional value, earlier studies have suggested that WBT may be restrictive when fed directly to animals. The astringent flavor of WBT may reduce palatability for some animals. Its aroma, however, can increase palatability and feed intake. By drying, boiling, or pelleting WBT before feeding it to the animals, for example, previous studies have shown different ways to get around these restrictions [9, 15]. Additionally, Suntara et al. [10], discovered that fermenting WBT with ruminal crabtree-negative yeasts (*C. tropicalis KKU20* and *P. kudriavzevii* KKU20) can raise its CP content by approximately 7%. It also boosts its ability to produce gas in vitro and its digestibility. When corn is substituted in the concentrate combination for native beef cattle in Thailand, the claim goes that administering WBT to the cattle will boost feed utilization.

The hypothesis of this study was that WBT might be used as an alternate feedstuff to replace corn without affecting feed utilization in beef cattle production. Hence, the purpose of this study was to assess the impact of WBT substitution for corn in concentrate mixtures on feed intake, feed utilization, and rumen fermentation in Thai native beef cattle.

Results

The experimental diets and chemical content

The chemical composition and components of the experimental diets are indicated in Table 1. The concentrate diets were formulated to have similar CP contents (142 to 145 g/kg DM). Rice straw (RS) had a low CP (44 g/kg DM) and a high content of NDF and ADF (733 and 450 g/kg DM, respectively). WBT contained a CP of 225 g/kg and fiber contents (NDF and ADF) of 314 and 144 g/kg DM, respectively.

Feed, nutrient intake, and digestibility

Table 2 presents the impact of replacing corn with WBT in a concentrate diet on feed intake and nutrient intake, including the apparent digestibility of nutrients. The WBT replacement at 0 to 100% for corn in the concentrate diets had no effect on rice straw intake, concentrate diet intake, or total DM intake ($p > 0.05$), whose total DM intake ranged from 6.44 to 6.77 kg/day. In addition, there was no change ($p > 0.05$) in the intake of OM and CP when replacing corn with WBT (0 to 100%). However, as the level of WBT increased in the concentrate diet, the intake of NDF and ADF significantly increased linearly ($p < 0.01$). Moreover, all parameters of nutrient

Table 1 Ingredients and chemical composition of concentrate, rice straw, and winged bean tuber (WBT)

Item	% Winged bean tuber replacement				RS	WBT	Corn
Ingredient, kg of dry matter (DM)	0%	33%	66%	100%			
Cassava chips	30.0	30.0	34.0	35.0			
Ground corn	20.0	13.4	6.8	0.0			
Soybean meal	15.0	15.0	11.0	10.0			
Rice bran	15.0	15.0	15.0	15.0			
Palm kernel meal	16.0	16.0	16.0	16.0			
WBT	0.0	6.6	13.2	20.0			
Urea	0.5	0.5	0.5	0.5			
Minerals and vitamins*	1.0	1.0	1.0	1.0			
Molasses	1.5	1.5	1.5	1.5			
Salt	1.0	1.0	1.0	1.0			
Chemical composition							
Dry matter (DM), g/kg	927	920	919	922	941	925	898
Organic matter, g/kg DM	952	955	955	946	880	974	982
Crude protein, g/kg DM	142	144	145	145	44	225	71
Neutral detergent fiber, g/kg DM	257	286	308	329	733	314	113
Acid detergent fiber, g/kg DM	116	117	126	136	450	144	33
GE, MJ/kg	15.9	15.6	15.7	3.8	14.2	16.3	15.7

* Minerals and vitamins (each kg contains): vitamin 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

T1, T2, T3, and T4 = corn replaced by WBT at 0, 33, 67, and 100%, respectively; RS = rice straw; WBT = winged bean tuber; GE = Gross energy

Table 2 The effect of the winged bean tuber to replace corn in concentrate mixture on feed intake and on feed intake and digestibility in beef cattle

Item	% Winged bean tuber replacement				SEM	Contrast	
	0%	33%	66%	100%		L	Q
Rice straw intake							
kg of dry matter (DM)/day	4.75	4.85	4.89	5.11	0.19	0.12	0.53
% of body weight (BW)	1.50	1.53	1.54	1.61	0.05	0.36	0.10
g/kg $BW^{0.75}$	63.99	64.45	62.35	67.53	1.99	0.20	0.12
Concentrate							
kg DM/day	1.69	1.68	1.64	1.66	0.06	0.38	0.55
% of BW	0.52	0.51	0.51	0.51	0.01	0.42	0.72
g/kg $BW^{0.75}$	22.73	22.26	20.97	22.01	1.84	0.95	0.60
Total intake							
kg DM/day	6.44	6.53	6.53	6.77	0.14	0.14	0.45
% of BW	2.02	2.04	2.05	2.12	0.05	0.37	0.10
g/kg $BW^{0.75}$	86.72	86.71	83.32	89.54	5.92	0.09	0.50
Nutrient intake, kg/d							
Organic matter	5.79	5.87	5.87	6.07	0.13	0.06	0.72
Crude protein	0.44	0.45	0.45	0.46	0.01	0.13	0.54
Neutral detergent fiber	3.92	44	4.09	4.29	0.51	< 0.01	0.57
Acid detergent fiber	2.33	2.38	2.41	2.53	0.06	< 0.01	0.39
Nutrient digestibility							
Dry matter, g/kg	687	684	685	689	17.82	0.89	0.79
Organic matter, g/kg DM	708	704	709	708	17.02	0.95	0.88
Crude protein, g/kg DM	665	684	673	658	18.27	0.57	0.22
Neutral detergent fiber, g/kg DM	668	653	659	634	17.82	0.13	0.08
Acid detergent fiber, g/kg DM	605	587	596	659	14.25	0.07	0.19

SEM = standard error of the mean; T1, T2, T3, and T4 = corn replaced by WBT at 0, 33, 67, and 100%, respectively; WBT = winged bean tuber digestibility were similar when corn was substituted with WBT (0 to 100%) in the concentrate diet ($p < 0.05$).

Ruminal pH, ammonia-nitrogen, protozoal populations, and blood Urea nitrogen

The ruminal pH, NH_3-N, protozoal populations, and BUN are shown in Table 3. There was no alteration in ruminal pH, NH_3–N concentration, or protozoal population by dietary treatment at 0 h of feeding time ($p > 0.05$). After 4 h of feeding, the ruminal pH, NH_3–N concentration, and protozoal population linearly increased ($p < 0.05$) when corn was substituted with WBT in the concentrate diet at 67% or above. Nonetheless, there were no significant variations in BUN concentrations when corn was substituted with WBT in the concentrate diet ($p > 0.05$).

Total volatile fatty acid concentration and VFA profiles

The effect of WBT replacing corn meal in a concentrated diet on ruminal VFAs is reported in Table 4. There were no changes in total VFA or the proportion of butyric (C4) when corn was replaced with WBT in the concentrate diet ($p > 0.05$). Meanwhile, the proportion of C3 was increased, although the C2 concentration and the C2:C3 ratio were linearly lower with the increasing WBT substitute of corn levels in the concentrate diet ($p < 0.05$).

Nitrogen utilization

The N intake, fecal N excretion, urinary N excretion, N absorption, and N retention did not change ($p > 0.05$) when increasing the WBT level in place of corn in the concentrate diet (Table 5). The N intake, N absorption, and N retention ranged from 0.92 to 0.98, 0.63 to 0.68, and 0.54 to 0.57 g/kg $BW^{0.75}$, respectively.

Discussion

The National Research Council (NRC) [16] demonstrated that growth and maintenance in beef cattle required a weight range of 200 to 450 kg, an average daily gain (ADG) range of 0.50 to 2.50 kg, and daily crude protein requirements for maintenance and gains at shrunken body weights of 142–160 g/kg DM. Similarly, Suntara et al. [10]; Ning et al. [14], found that WBT contains 1.40–2.76% moisture, 0.21–4.53% DM of fat, 17.00–25.00% DM of CP, 2.76-3.0% DM of crude fiber (CF), 25–30% DM of carbohydrate content, and the values of gross energy ranged from 15.81 MJ/kg to 16.24 MJ/kg [11].

Our data support earlier studies by demonstrating that cow intake of roughage, concentrate, and total DM was unaffected by raising the amount of WBT in concentrate diets from 0 to 100% as a surrogate for corn. Total feed intake ranged from 2.00 to 2.55% BW, aligning with the recommended intake of 2.5% BW for beef cattle weighing 250 to 350 kg [16]. Similarly, previous studies by Tangjitwattanachai and Sommart [17] and Supapong et al. [18] reported that native Thai beef cattle weighing an average of 320 ± 20 kg consumed a diet containing approximately 2.45% BW. In this study, the greater NDF and ADF intake when WBT replaced the dietary corn-based concentrate at 100% could be due to the WBT containing more NDF and ADF than corn. The amount of nutrient intake depends on the relationship between the feed's nutrients and the total feed intake [19–21]. However, this study showed no effect on the apparent digestibility

Table 3 The effect of the winged bean tuber to replace corn in concentrate mixture on ruminal pH, NH_3-N concentration, and ruminal protozoal population in beef cattle

Item	% Winged bean tuber replacement				SEM	Contrast	
	0%	33%	66%	100%		L	Q
Ruminal pH							
0 h post feeding	7.16	7.17	7.16	7.14	0.02	0.27	0.54
4 h post feeding	6.88	6.93	7.04	7.07	0.03	0.01	0.84
Mean	7.02	7.05	7.10	7.11	0.03	0.01	0.62
Ruminal NH_3-N, mg/dL							
0 h post feeding	16.18	16.43	16.74	16.62	0.33	0.15	0.44
4 h post feeding	17.74	17.88	18.36	19.05	0.30	< 0.01	0.22
Mean	16.96	17.16	17.55	17.83	0.22	< 0.01	0.77
Protozoa, $\log_{10}$ cell/mL							
0 h post feeding	6.02	6.08	6.08	6.09	0.03	0.05	0.06
4 h post feeding	6.05	6.09	6.11	6.14	0.01	< 0.01	0.33
Mean	6.03	6.09	6.10	6.12	0.01	< 0.01	0.19
Blood urea-N concentration, mg/dL							
0 h post feeding	6.75	6.00	6.25	6.00	0.99	0.54	0.73
4 h post feeding	8.00	8.25	8.50	9.75	1.45	0.25	0.64
Mean	7.38	7.13	7.38	7.88	1.14	0.95	0.65

SEM = standard error of the mean; T1, T2, T3, and T4 = corn replaced by WBT at 0, 33, 67, and 100%, respectively; WBT = winged bean tuber

Table 4 The effect of the winged bean tuber to replace corn in concentrate mixture on volatile fatty acids profiles in beef cattle

Item	% Winged bean tuber replacement				SEM	Contrast	
	0%	33%	66%	100%		L	Q
Total volatile fatty acid, mM							
0 h post feeding	95.12	96.19	95.54	97.31	1.45	0.17	0.71
4 h post feeding	105.81	104.54	106.42	107.42	1.49	0.18	0.30
Mean	100.47	100.37	100.98	102.37	1.07	0.13	0.06
Acetic acid, mol/100 mol							
0 h post feeding	69.36	68.17	67.81	66.89	0.59	< 0.01	0.74
4 h post feeding	71.55	70.24	67.21	67.10	0.65	< 0.01	0.11
Mean	70.45	69.20	67.51	67.00	0.41	< 0.01	0.14
Propionic acid, mol/100 mol							
0 h post feeding	18.92	18.96	19.93	20.15	0.52	0.02	0.83
4 h post feeding	19.16	20.73	23.52	24.31	0.69	< 0.01	0.35
Mean	19.04	19.85	21.73	22.23	0.43	< 0.01	0.66
Butyric acid, mol/100 mol							
0 h post feeding	11.72	12.87	12.26	12.96	0.78	0.27	0.55
4 h post feeding	9.29	9.03	9.27	8.59	0.72	0.58	0.56
Mean	10.51	10.95	10.76	10.77	0.56	0.66	0.43
Acetic acid: Propionic acid ratio							
0 h post feeding	3.67	3.59	3.40	3.32	0.11	< 0.01	0.96
4 h post feeding	3.73	3.39	2.86	2.76	0.11	< 0.01	0.14
Mean	3.70	3.49	3.13	3.04	0.08	<0.01	0.28

SEM = standard error of the mean; T1, T2, T3, and T4 = corn replaced by WBT at 0, 33, 67, and 100%, respectively; WBT = winged bean tuber

Table 5 The effect of the winged bean tuber to replace corn in concentrate mixture on nitrogen (N) balance in beef cattle

Item	% Winged bean tuber replacement				SEM	Contrast	
	0%	33%	66%	100%		L	Q
Nitrogen (N) intake							
g/day	70.94	71.94	71.56	73.55	1.29	0.08	0.51
g/kg $BW^{0.75}$	0.97	0.96	0.92	0.98	0.12	0.75	0.11
N excretion, g/day							
Fecal N excretion	24.67	23.00	24.73	25.53	1.98	0.50	0.40
Urinary N excretion	6.34	6.54	5.64	5.82	0.44	0.10	0.98
N absorption,							
g/day	46.27	48.94	46.83	48.02	2.20	0.62	0.70
g/kg $BW^{0.75}$	0.63	0.66	0.68	0.65	0.08	0.80	0.60
N retention							
g/day	39.93	42.40	41.19	42.20	2.44	0.45	0.73
g/kg $BW^{0.75}$	0.54	0.57	0.54	0.57	0.04	0.70	0.96

SEM = standard error of the mean; T1, T2, T3, and T4 = corn replaced by WBT at 0, 33, 67, and 100%, respectively; WBT = winged bean tuber

of nutrients. This study found no significant impact on apparent nutrient digestibility, likely due to the carbohydrate content in WBT (57.72%) [22], which serves as an energy source for ruminal microorganisms, promoting microbial proliferation and digestion.

This study showed a greater ruminal pH at 4 h after feeding and a mean value when corn replacement with WBT was at 67% or above. This is probably due to WBT containing more fiber than corn (1.70-3.0% DM of CF) [14], which may result in slower degradation and a higher ruminal pH in the rumen. The cellulose and hemicelluloses in WBT affect slow degradation and increase the pH to over 6.0, and may positively affect ruminal pH [24], and Orskov and Fraser [25], advise that the average ruminal pH should be between 6.2 and 7.0 to maintain proper fiber rumen fermentation. The ruminal pH values observed in this study (6.88–7.16) suggest that high levels of WBT inclusion do not negatively impact rumen fermentation. Similarly, Vargas et al. [26] showed that substituting corn meal with rice bran in a lamb diet increased ruminal pH while applying up to 100% beet pulp to corn raised ruminal pH and NH_3-N concentration in Egyptian buffaloes [27]. Moreover, it could be due to the greater NH_3-N, which is alkaline and whose buildup contributes to a higher ruminal pH [23, 28]. Suriyapha et al. [29] demonstrated that increasing ruminal pH correlates with higher ruminal NH_3-N levels in Thai-native cattle fed high-protein ingredient feedstuffs in the concentrate diet.

The higher NH_3-N concentration at 67% or greater WBT replacement levels may be attributed to WBT's higher crude protein content compared to corn. Feed protein could be metabolized in the rumen by microbes, increasing the NH_3-N concentration [15]. Similarly, previous studies by Criscioni and Fernandez [30] and Vargas et al. [26] found that increasing the substitution of rice bran in corn at 100% resulted in an increase in NH_3-N concentration in dairy goats. In addition, the increased NH_3-N concentration reveals an increased rate of proteolysis and amino acid metabolism in animals [31]. Generally, ruminal NH_3-N at 5 mg/dL is the minimum level required for optimal ruminal microbial synthesis in the rumen [32]. We may conclude from this work that the rumen microbiota has adequate NH_3-N to enable microbial protein production.

Protozoal populations increased at 67% and 100% WBT replacement, likely due to the higher levels of insoluble carbohydrates (e.g., cellulose and hemicellulose) in WBT, which provide fermentable substrates for rumen microbes and help maintain optimal ruminal pH [33, 34]. The insoluble carbohydrates and some amount produced from starch that can tolerate ruminal degradation mechanisms were observed to be greater ciliates (e.g., amylolytic bacteria and protozoa) immediately after feeding, which supports the theory of protozoa migration into the rumen in reaction to chemical stimulants from the feed entering the reticulorumen [35]. As a result, it appears that more high-starch feed can increase the protozoal population in a feeding state [15].

The BUN content was not altered when corn was replaced with WBT in the diet. This could be caused by NH_3-N utilization by rumen microbial activity for the synthesis of microbial crude protein, and the protein had no effect on BUN [26, 36]. Normally, BUN values in tropical ruminants range from 6.3 to 25.5 mg/dL, depending on assorted determinants, including nutrition, age, and protein consumption, in particular the concentration of ruminal NH_3-N [29, 37]. This result reveals no significant impact on BUN concentration in diets substituting corn with WBT. Possible causes include analogous degradation kinetic parameters of DM, OM, and DM in the rumen, according to the report by Yahaghi et al. [38]. Similarly, the influence of corn surrogate by sorghum in diets had no effect on BUN in lambs. Lobón et al. [39] reported that field peas with a composition of starch could be added instead of soybean meal or corn to mixtures concentrated in fattening bulls and that the BUN at the initiation and end of each period were unaffected by the inclusion of peas.

The present study discovered that substituting WBT for corn at 67 or 100% greater C3 was observed, whereas lower C2 proportions were observed. According to earlier studies, Lobón et al. [39] and Ma et al. [40] reported that barley replaced corn in the concentrate diet and found no effect on the total VFA, whereas greater C3 and lower C2 proportions were also found without a shift of the rumen pattern fermentation independently of the amount of WBT in the diet. This could be due to the WBT components containing some water-soluble carbohydrates similar to barley [41]. In addition, the WBT might have ruminally degraded starch (RDS) similar to barley, which is higher than corn (55–70%) [42, 43], resulting in a raised amount of fermented starch, offering an augmentation in the total production of propionate [44]. The increased C3 synthesis can be attributed to an increase in the interrelated abundance of *Selenomonas ruminantium*, which is a starch-breakdown bacteria that produces C3 via the succinate or randomizing pathway [40, 45]. However, the proportion of C2 decreased with greater concentrations of C3 and impacted the C2 to C3 ratio by increasing the replacement levels of corn with wheat or barley that contained water-soluble carbohydrates [46]. However, the effects of WBT have not been reported, so proving them will be challenging.

In this study, the N intake ranged from 0.92 to 0.98 g of N/kg $BW^{0.75}$/day, which was higher than the optimal recommended value (0.68–0.75 g of N/kg $BW^{0.75}$/day) and sufficient for maintenance protein intake in Thai-native male cattle fed rice straw-based diets [29, 47]. WTSR [48] recommended the N intake for maintenance of male Thai native cattle be 0.86 g of N/kg $BW^{0.75}$/day. This study discovered that substituting WBT for corn at 67 or 100% was unchanged for N utilization. This could be explained by similar N intake and excretion. The levels of N intake and defecation are correlated with concentrates and higher CP levels, as well as their impact on N absorption and retention [29, 47, 49]. The average values of N absorption and N retention were 48.07 g/d (46.63 to 49.80) and 41.98 g/d (40.71 to 42.97), respectively, which were close to the range for Thai-native cattle fed rice straw-based diets [18, 29]. These results were the same as those reported by Chibisa et al. [50], who found that barley as a substitute for corn is unaffected by the nitrogen balance of dairy cows.

Conclusion

This study suggests that WBT could be used as an alternative feed source for ruminants. Substituting WBT at 100% for corn in the concentrate mixture diet could increase ruminal fermentation characteristics and C3 proportion. Replacing corn with 100% WBT had no negative effects on feed intake, digestibility, BUN, or nitrogen balance in Thai-native cattle. Further research is needed to evaluate the energy value of WBT in different livestock species and production systems. Future research should focus on estimating the digestible energy value of WBT and conducting performance tests to fully assess its energetic potential.

Methods

The research for this study was carried out at Khon Kaen University's Tropical Feed Resources Research and Development Center. To ensure animal welfare, all animal methods were approved by Institutional Animal Care No. IACUC-KKU-46/65 issued by Khon Kaen University's Ethical Committee. Our research confirmed that all methods were carried out in accordance with the applicable guidelines and regulations. 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.

Preparation of WBT

This study confirms that all methods were carried out in accordance with relevant Plant guidelines. Winged bean tubers were supplied by the Department of Agronomy, Faculty of Agriculture, Khon Kaen University. From December 2021 to May 2022, the winged bean was cultivated in 1600 m^2 at the Agronomy Research Unit (Department of Agronomy, KKU), and the fresh WBT was harvested by hand at 56.0% DM. The fresh WBT utilized in this trial was chopped to a specific length of 3 to 5 cm before being oven-dried overnight at 60 °C for 72 h. The chemical composition of WBT was defined using the Association of Official Analytical Chemists [51] methods for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), and acid detergent fiber (ADF), as well as the Van Soest et al. [52] method for neutral detergent fiber (NDF). Gross energy (GE) was defined with an adiabatic calorimeter bomb (AC500, LECO Corporation, Michigan-USA), as shown in Table 1.

Animals, treatments, and feeding

Four male Thai native beef bulls (300 ± 20 kg BW; 2–3 years old) were housed in individual pens and fed the experimental diets. The cattle were designated randomly as a 4 × 4 Latin square design representing the amount of corn replaced in the concentrate mixture by WBT at 0, 33, 67, and 100% substitution rates. All animals were fed at 8:00 a.m. and 4:00 p.m. the dietary treatments with access to clean water and a mineral block preparation. Diets were adjusted over four 14-day intervals, with sample collection and digestibility testing conducted during the last 7 days of each period. The concentrate diet prepared by WBT at 0, 33, 67, and 100% corn was fed at 0.5% BW, and rice straw (RS) (a roughage source) was utilized as the only source of roughage and was freely accessible. The chemical composition of the concentrate, RS, and WBT is provided in Table 1.

Feed, feces, urine, rumen fluid, and blood sample collections

Daily feed intake was determined by weighing offered and ort prior to morning feeding. Four periods of 21 days each were used for the investigation. All of the cattle were transposed to metabolism crates during the final seven days of each phase to examine nitrogen excretion and digestibility. The total feces collection was executed using metabolic crates to individually house the animals, facilitating the collection of all fecal output. Fecal samples were collected daily during the last 7 days of each experimental period. The collection process was meticulously monitored to prevent contamination and ensure accuracy in assessing nutrient utilization. The stool samples were divided into two portions, the first for daily DM analysis and the second kept in a refrigerator, and samples for approximately 5% of the total fresh weight were taken. To ensure that the pH stayed below 3.0 and to minimize nitrogen loss, urine was collected in 10-L vessels containing 100 mL of 10% H_2SO_4. The volume of acid was predetermined to effectively lower the pH while preventing excessive acidification. Since daily urine output varied among animals, the final pH of each sample was measured and, if necessary, adjusted by adding small increments of 10% H_2SO_4 to maintain a pH below 3.0. Every 24 h, urine tubes were changed, the amount of acidified urine was quantified, and a sub-sampled sample of 100 mL of each animal's urine was instantly frozen at − 20 °C. At the end of each period, cattle were employed to collect feces and urine for chemical analysis, and the AOAC [51] method was used to impose nitrogen (N) balance. Feed samples (both offered and refused) and feces were compiled during the last 7 days of each period for chemical composition delineation and an estimate of feed digestive capacities. Fecal matter, which made up 5% of the total fresh weight, was compiled and divided into two parts: the first was tested daily for DM, and the second was frozen in a refrigerator and combined by animals on the final day of each period to assess nutritional content.

Samples were dried for 24 h at 100 °C, and the DM of the meals and excretions was calculated. To prepare the samples of feed and stool for incubation, they were dried in hot forced air ovens at 60 °C. The size of those samples was ground to 1 mm. By burning the samples at 550 °C, the OM of the samples was examined. The methods of the AOAC [51] were used to determine the CP and crude ash of the feed and fecal samples. The ANKOM200 Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA) was used to measure the NDF and ADF contents. according to Van Soest et al. [52] methods. The following formula was used to compute the apparent digestibility of DM, OM, CP, NDF, and ADF as a percentage of the nutrient intake not recovered in the feces:

Apparent digestibility (%) = (Nutrient intake–Nutrient in faeces) × 100/Nutrient intake

On the final day of the feeding trial, 100 mL of ruminal fluid was collected using a stomach tube connected to a vacuum pump at 0 h before feeding and 4 h post-feeding. To minimize saliva contamination, the initial three extractions were discarded, and the ruminal pH was immediately measured using a HANNA Instruments HI 8424 microcomputer (Singapore). The collected ruminal fluid was then divided into two portions: the first part was used for ammonia-nitrogen (NH_3-N) and volatile fatty acid (VFA) analysis, while the second portion was allocated for direct counting of the protozoal population. Based on the procedure of Fawcett and Scott [53], the second was centrifuged at 16,000 g for 15 min to obtain the supernatant fluid, and the first part of NH_3-N in the supernatant was appraised using a spectrophotometer (UV/VIS spectrophotometer, PG Instruments Ltd., London, United Kingdom). A gas chromatography system (Newis GC-2030: SHIMADZU, Shimadzu Corporation, Kyoto, Japan) fitted with a capillary column (molecular sieve 13X, 30/60 mesh, Alltech Associates Inc., Deerfield, IL, USA) was used to measure the volatile fatty acids (VFAs). This system was used to determine the molar proportions of acetic acid (C2), propionic acid (C3), and butyric acid [54].

In the last section, a 9-mL formaldehyde solution and 1 mL of ruminal fluid were combined, and the hemocytometer readings were used to determine the direct counts of the protozoal population [55]. On the final day of each period, blood (10 mL) was taken from the jugular vein and deposited in tubes together with 12 mg of ethylenediaminetetraacetic acid (EDTA) (0 and 4 h after feeding). They were then placed on ice for 30 min; they were centrifuged at 1500 g for 15 min at 4 °C to segregate the plasma and kept at -20 °C until the blood urea nitrogen (BUN) level required to be measured was reached [56].

Statistical analysis and calculations

Statistical analysis accounted for the 4 × 4 Latin square design using the GLM procedure of SAS [57]. The data were analyzed using the following model:

$$\mathbf{Yijk = \mu + Mi + Aj + Pk + \varepsilon ijk}$$

where: Yijk, observation from animal j, receiving diet i, in period k; μ, the overall mean, Mi, effect of the replacement levels (i = 0, 33, 67, 100%), Aj, the effect of animals (j = 1, 2, 3, 4), Pk, the effect of periods (k = 1, 2, 3, 4), and εijk the residual effect. Results are presented as the mean values with the standard error of the means. Trend of replacement levels responses was performed by orthogonal polynomials. Significance was declared at $p < 0.05$ as representing statistically significant differences.

Abbreviations

ADF	Acid detergent fiber
ADG	Average daily gain
BUN	Blood urea nitrogen
BW	Body weight
C2	Acetic acid
C3	Propionic acid
C4	Butyric acid
CF	Crude fiber
CP	Crude protein
DM	Dry matter
EDTA	Ethylenediaminetetraacetic acid
EE;	Ether extract
GE	Gross energy
KKU	Khon Kaen University
LA	Lactic acid
LSD	Latin square design
N	Nitrogen
NDF	Neutral detergent fiber
NH_3-N	Ammonia-nitrogen
NRC	National Research Council
OM	Organic matter
RDS	Ruminally degraded starch
RS	Rice straw
TVFAs	Total volatile fatty acids
WB	Winged bean
WBT	Winged bean tubers

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Author contributions

Planning and design of the study, N.U. and A.C.; conducting and sampling, N.U., A.C., S.C., C.S.; sample analysis, A. C. and N.U.; statistical analysis, A.C., N.U., C.S., C.S., P.C., P.G., N.G., and; S.P.; manuscript drafting, A.C., N.U., and C.S.; manuscript editing and finalizing, A.C., N.U., T.R., S.C., and C.S.; 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). Our study verified that all methods were conducted in adherence to the applicable guidelines and regulations. We affirm that informed consent was obtained from the owner for conducting the study. Furthermore, the study was conducted in compliance with the ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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