Effect of Fruit Seeds on *In Vitro* Gas Production Analysis

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Abstract

The increasing demand for animal products requires a sustainable expansion of feed resources. This study explored the potential of fruit seeds as a non-competitive feed source, evaluating the nutritional composition of durian, champeden, salak, avocado, and jackfruit seeds. Proximate, Van Soest fibre, and *in vitro* gas production analyses were conducted. Proximate analysis revealed that durian seeds exhibited significantly ($P < 0.05$) higher dry matter (DM) and ash content and lower crude fibre (CF) compared to other seed sources. Avocado seeds had the highest crude protein (CP) content ($P < 0.05$), while jackfruit seeds had the lowest. Champeden seeds showed significantly ($P < 0.05$) higher neutral detergent fibre (NDF) and CF. A primary limitation for livestock feed use is the high moisture content of these seeds. *In vitro* fermentation data indicated that gas production was influenced by substrate degradability and rumen microbial activity. Cumulative gas production over 72 hours showed that salak seeds produced the highest volume, while avocado seeds produced the lowest.

Keywords: Fruit seeds, Animal feed, Proximate analysis, Van Soest fibre analysis, *In vitro* gas production.

Introduction

The global population is projected to exceed 9 billion by 2050 (Smith et al., 2024). Consequently, the agricultural industry is rapidly expanding to meet the increasing demand for food and animal feed, while also serving as a vital source of income for farmers (Raihan et al., 2024). To address this demand, industries focus on enhancing plant and animal production. Malaysia, a tropical Southeast Asian nation, is renowned for its rich biodiversity, encompassing rainforests, mangrove ecosystems, and coral reefs (Khan et al., 2024). Furthermore, Southeast Asia boasts a remarkable diversity of fruits, with over 500 species distributed throughout the region (Abedin et al., 2025). The commercialization of exotic tropical fruits presents significant income-generating opportunities for producers.

However, fruit processing generates substantial seed waste, contributing to ecological issues such as increased insect and rodent populations (Diniso et al., 2024; Sandoval-Contreras et al., 2023). Recognising the nutritional potential of these by-products, researchers are investigating their use as food or medicinal sources. Fruits such as durian, champeden, jackfruit, salak, and avocado are known for their carbohydrate and vitamin content. Fruit seeds are being evaluated for industrial and food applications, including novel oil sources (Allaqaband et al., 2022; Alves et al., 2021). However, research on the utilisation of fruit seeds for livestock feed is limited. Livestock feed rations often rely on imported raw materials, with feed costs accounting for up to 70% of farmers' budgets (Zamani et al., 2024). Indonesia is a leading producer of various seed wastes, with annual production rates of 164,323 tons for durian, 68,542 tons for jackfruit, 82,200 tons for avocado, and 298,673 tons for salak (Kumoro et al., 2020). Ranking second in seed waste production after Indonesia, Malaysia has explored the use of these wastes as a supplementary feed in total mixed rations, leveraging their abundant carbohydrates, vitamins, and antioxidants (Liu, 2003). Given the abundance of discarded fruit seeds in Malaysia, there is a need to explore their potential as animal feed. While research on fruit seed utilization for human consumption is prevalent, their application in animal feed remains underdeveloped.

Fruit seeds, often discarded as agricultural waste, are now being explored for their potential nutritional and functional benefits in animal feed (Malenica et al., 2023; Mnisi et al., 2022). Rich in fibre, proteins, lipids, and bioactive compounds, these seeds may influence rumen fermentation dynamics, thereby impacting digestibility and microbial activity (Malenica et al., 2023). Evaluating their fermentation characteristics is essential to determine their viability as alternative feed resources (Bature et al., 2024). *In vitro* gas production analysis is a widely used technique to assess the fermentability of feedstuffs, offering insights into nutrient degradation, volatile fatty acid production, and microbial efficiency (Suassuna et al., 2023). While studies have investigated the effects of fruit by-products such as peels and pulp on rumen fermentation, limited research has focused specifically on fruit seeds. Understanding their role in gas production and fermentation kinetics could provide valuable data for optimizing livestock diets.

Therefore, this study aims to analyze the effects of different fruit seeds on in vitro gas production, contributing to the growing body of research on sustainable and efficient animal nutrition. The general objective of this study is to evaluate the effects of fruit seeds on rumen fermentation through *in vitro* gas production analysis. The findings will help determine whether these seeds can serve as viable feed ingredients, potentially reducing feed costs and enhancing nutrient utilization in ruminant production systems.

Materials and methods

Samples collection and preparation

Seed samples of ripe durian, champeden, jackfruit, salak, and avocado were obtained from Malim, Perak, Malaysia. The discarded seeds were separated from any fleshy pulp residue, minced with a greater, and subsequently dried in an oven at 60 °C until a constant weight was achieved. The dried seeds were then pulverised into a fine powder and stored in airtight containers.

Proximate analysis

The AOAC (2023) was applied to determine the proximate compositions of the fruit seeds' moisture, dry matter (DM), crude protein (CP), ash content, organic matter (OM), neutral detergent fibre (NDF) and crude fibre (CF). The proximate analysis samples were carried out in four replicates.

Collection and preparation of rumen fluid

The *in vitro* experiment was conducted using the procedure of Menke & Steingass (1988). The rumen fluid was obtained from the abattoir complex, Department of Veterinary Services, situated in Shah Alam, Selangor, Malaysia. The rumen fluid was collected into a pre-warmed thermos flask and immediately transported to the laboratory. The rumen fluid was pooled and quickly filtered through a cloth strainer into a beaker, and added to the buffer mix medium in a volumetric flask while supplied with carbon dioxide (CO2) in a 39 °C water bath. The flask was stopped with a rubber stopper to retain the anaerobic condition.

Preparation of buffered media

The buffered media was prepared by mixing five different solutions: Solution A (micromineral), Solution B (buffer), Solution C (macro mineral), Resazurin, and a reducing solution (Behan et al., 2024). The strained rumen liquor was added to the media in a ratio of 1:2 (v/v). The mixture was kept stirred and then placed in a water bath at 39 °C under constant CO_2 flushing.

In vitro rumen fermentation analysis

Gas production analysis

The *in vitro* rumen fermentation analysis was carried out according to Menke & Steingass (1988). Briefly, each sample (approximately 200 mg) was placed in a 100 mL calibrated glass syringe fitted with a rubber tube and about 30 mL rumen liquor buffer medium was added. A pre-lubricated piston was inserted into the syringe and pressed forward to remove air from the syringe through the rubber tube. The rubber tube was sealed with a plastic clip, and the initial gas volume was read at the point where the end mark of the piston lies. The *in vitro* gas production was measured by incubating the samples for 72 hours at 39oC in a water bath, and the gas produced was recorded at 2, 4, 6, 8, 10, 12, 24 and 72 hours of incubation. The pH of the rumen fluid was taken with a pH meter (Mettler-Toledo, Ltd, England).

Statistical analysis

The data obtained from the proximate analysis and *in vitro* rumen fermentation analysis were further analysed using one-way ANOVA by a general linear model (GLM) procedure in SAS Software

9.4 Version (SAS Institute Inc., Cary, NC, USA). Tukey's Range Test was used to determine the differences between group means at a P < 0.05 significance level.

Results and Discussion

Proximate analysis

Table 1 presents the proximate composition of durian, champeden, salak, avocado and jackfruit seeds. Durian and champeden seeds exhibited 19% higher DM content (P < 0.05), compared to salak seeds, which had the lowest DM content. No significant differences were observed in OM and ash content across all seeds. Salak and avocado seeds show 70% significantly (P < 0.05) higher CP content compared to champeden and jackfruit, which had the lowest. Champeden contains significantly (P < 0.05) high NDF content as compared to other seeds. Congruently, champeden also contains an 80% significantly (P < 0.05) higher CF content compared to durian seeds, which comprise lower CF content.

Table 1. Proximate composition of durian, champeden, salak, avocado, and jackfruit seeds.

Parameters (%) *	D	C	S	A	J	*P-value*
DM	54.21±0.61^{a}	53.19±0.61^{a}	43.08±0.58^{c}	47.05±0.58^{b}	48.18±0.61^{b}	<.0001
OM	95.92±0.30^{b}	95.91±0.27^{b}	96.43±0.09^{ab}	96.66±0.23^{ab}	96.78±0.31^{a}	0.1013
Ash	4.08±0.30^{a}	4.09±0.27^{a}	3.57 ± 0.09^{ab}	3.34±0.23^{ab}	3.22±0.31^{b}	0.1013
CP	10.38±1.61^{b}	5.80±0.04^{c}	13.32±0.14^{a}	14.64±0.12^{a}	4.13±0.13^{c}	<.0001
NDF	51.08±3.61^{c}	79.94±1.51^{a}	42.86±2.27^{c}	47.93±3.29^{c}	63.37±3.04^{b}	<.0001
CF	2.70±0.38^{d}	14.52±0.70^{a}	6.61±0.45^{b}	4.16±0.21^{c}	4.76±0.93^{c}	<.0001
ADF (%)	99.29±1.03^{c}	79.73±1.26^{d}	184.03±2.64^{a}	131.66±1.89^{b}	80.58±6.29^{d}	<.0001
ADL (%)	157.22±4.06^{b}	100.63±5.11^{c}	82.50±13.75^{c}	229.84±0.95^{a}	103.62±6.55^{c}	<.0001
ME (MJ/kg DM)	18.39±0.05^{b}	18.52±0.03^{a}	18.26 ±0.00^{c}	18.21 ±0.03^{c}	18.48±0.01^{a}	<.0001

Sufficient nutrient intake is an important criterion for livestock production (Hossain et al., 2016). In the present study, the evaluation of discarded seeds as potential nutrient sources in livestock found that durian and champeden seeds have lower moisture content, which could supply partial DM requirements. DM content is an indicator of the nutrients available for absorption; a higher DM signifies a higher nutrient potential (Fasuyi, 2006). A substantial amount of DM is important in ruminants in maintaining healthy ruminal function and subsequently provides the substrate for weight gain (Simeanu & Radu-Rusu, 2023). The ash content in potential feedstuff is crucial for identifying mineral availability and the inorganic matter portion in the feedstuff (Quirino et al., 2023). It can also be used to calculate non-fibre carbohydrates and the potential energy in seeds (Buzna et al., 2024). In the present study, although insignificant, all seeds contain at least 3% ash, which is lower compared to the known ash value for common feedstuff Napier grass (9%) (Zailan et al., 2016). However, the low ash value will not directly affect animals as the seeds are intended to be included in the total mixed ration supplementary feed (Quirino et al., 2023).

Sufficient CP content supplied in the animal diet is important in maintaining muscle metabolism and as a food source for the rumen microbes (Rosmalia et al., 2022). Although rumen microbes can synthesize protein, providing sufficient protein in the diet will ensure a healthy microbial population in the rumen for various nutrient utilization from feedstuff (Shi et al., 2020). A deficient protein supply in the diet will cause a reduction in metabolizable protein flow to the duodenum (Diao et al., 2019). In the present study, avocado and salak seeds contain a high amount of CP, 14.64% and 13.32%, respectively, which is comparable to conventional feedstuff such as Napier grass, which contains 9% - 14% of CP (Zailan et al., 2016).

Fibre is an important aspect of ruminants' diets as it stimulates rumination and is used by cellulolytic bacteria to convert fibre to energy (Hall & Mertens, 2008). Determining the amount of digestible fibre in feedstuff allows efficient feeding ration formulation to cater to daily NDF and DM requirements (Mirzaei-Aghsaghali, 2011). NDF fibre fraction considers the total cellulose, hemicellulose and lignin content in the seeds, while CF determines the partial cellulose and lignin content in the seeds (AOAC, 2023). In the present study, champeden seeds contain a high amount of NDF and CF compared to other seed types, signifying very high cellulose, hemicellulose and lignin content in the seeds. In this study, champeden and jackfruit seeds were found to have a high content of neutral detergent fibre (NDF) and crude fibre (CF) compared to other seed types. Despite this, their fibrous nature also indicates a significant quantity of carbohydrates that could be utilised as a source of soluble dietary fibre in animal feed (Dasaesamoh & Seechamnanturakit, 2014). To enhance their nutritional availability and ease of incorporation into concentrate formulations, these seeds can be processed into a flour. This method is effective, with studies demonstrating that seed flour can be incorporated at levels up to 40% in total mixed rations for cattle without detrimentally affecting milk production (Zhang et al., 2025). In comparison, durian seeds contain 51% NDF and a low 2.7% CF, signifying a high digestible fibre content which the animal can fully utilise. Whereas salak seed was observed to contain low-digestible fibre. Evaluating digestible fibre content is important in ensuring the animal consumes adequate daily fibre requirements to ensure sufficient energy feed-to-energy conversion (Yunilas et al., 2023).

In-vitro gas production

In vitro gas production and rate of gas production were significant ($P < 0.05$) for the seventy-two hours of incubation. The avocado fruit seeds produced the least amount of gas and significantly ($P < 0.05$) differed from the other fruit seeds at the fourth, sixth, eighth, 10, 12, 24, 48, and 72 hours of incubation. Degradation of the organic fraction of feed by rumen microorganisms is the main source of gas produced by feed in the rumen (Chen et al., 2024). In vitro gas production is an essential measure of feed digestibility (Dijkstra et al., 2005), and the level of *in vitro* gas production can directly reflect the nutritional quality of feed samples (Chen et al., 2024), which indicates the metabolism of rumen microorganisms and measures dietary digestibility (Menke et al., 1979). There is a positive correlation between the magnitude of gas production and fibre digestibility (Lei et al., 2018). This may help explain why avocado fruit seeds produce less gas during incubation than other fruit seeds, since they have a lower digestible fibre concentration.

On the contrary, jackfruit seeds showed elevated *in vitro* gas production from the fourth until the twelfth hours of incubation, which may be attributed to the high NDF and low CF available in the seeds. Although champeden seeds contain a significant amount of NDF, the high CF content prevented the rumen microbe from converting all digestible fibre into energy due to the high lignin content (Zhang et al., 2022).

In this study, the salak seeds exhibited the highest 48 and 72 hours of cumulative gas production, suggesting that a significant portion of fermentable components in them were utilized. In this experiment, the fruit seeds had adequate substrate fermentation, which led to an increase in gas production and an increased gas production rate with time, as the substrate was reduced, and the gas production gradually declined in the later stages. In the pre-fermentation period, the degree of feed degradation by rumen bacteria increased with time; however, in the subsequent period, the amount of degradable material gradually reduced, resulting in a slow increase in gas production.

Table 2: *In-vitro* gas production of different fruit seeds (ml/200 mg).

GP (mL)	D	C	S	A	J	*P-value*
2hr	0.69±0.10[e]	1.86±0.84[a]	1.16±0.47[c]	0.93±0.23[d]	1.39±0.40[b]	<.0001
4hr	3.25±0.93[d]	4.88±0.70[b]	3.95±0.61[c]	2.56±1.16[e]	5.81±0.84[a]	<.0001
6hr	6.28±2.79[c]	9.07±0.81[b]	6.28±0.81[c]	3.49±1.21[d]	12.80±0.93[a]	<.0001
8hr	9.42±1.40[c]	12.21±0.81[b]	9.19±1.30[c]	5.46±1.53[c]	15.01±0.81[a]	<.0001
10hr	13.26±2.41[c]	15.36±1.21[b]	12.80±0.47[d]	8.61±1.23[e]	17.45±0.81[a]	<.0001
12hr	16.52±2.29[c]	16.98±1.42[b]	15.12±1.30[d]	10.47±1.07[e]	18.61±1.01[a]	<.0001
24hr	25.94±3.98[d]	27.34±2.83[a]	26.41±1.23[b]	19.89±0.40[e]	26.18±1.07[c]	<.0001
48hr	39.91±3.37[c]	42.93±3.85[b]	46.89±2.03[a]	29.90±1.63[e]	38.05±2.82[d]	<.0001
72hr	43.05±3.66[c]	47.24±5.00[b]	50.03±2.29[a]	31.65±1.23[e]	41.65±2.62[d]	<.0001

a, b, c, d, e Means with different letters within a column differed significantly ($P < 0.05$). GP = Gas production, D = Durian, C = Champeden, S = Salak, A = Avocado, J = Jackfruit seed

Conclusion

The findings of this study reveal that durian and champeden seeds are excellent sources of dry matter and ash, while durian, champeden, and jackfruit seeds provide a valuable source of fibre. Moreover, salak, avocado, and durian seeds demonstrate crude protein levels comparable to widely used Napier grass. These results strongly suggest that the utilization of these diverse fruit seeds in livestock feed is not only feasible but also holds significant potential to enhance feed resource sustainability. Further research aimed at determining precise inclusion levels within total mixed rations is essential to realise the value of these currently discarded seeds.

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