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Exploring the potential of tropical fruits waste for livestock feed: effect of germinating durian seed on nutrients, antinutrients, and bioactive compound

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Abstract The highest dry matter content was found without germination followed seed sprouts at 6 cm and 3 cm. The organic matter and total carbohydrate were lowest in germinated seed sprouts 3 cm compared to 6 cm and without germination. The crude protein and fiber content were highest in seed sprouts 6 cm compared to 3 cm and without germination. The oxalate acid, CPAs, antioxidant activity and flavonoid were lower in germinated seed sprouts 6 cm, followed by 3 cm and on the seed without germination. Phenol in germinated seed sprouts 6 and 3 cm was higher compared to the seed without germination. The flavonoid was lower in germinated seed sprouts 6 and 3 cm compared to the seed without germination. Tannin in the seed without germination and germinated seed sprouts 3 cm was the highest as compared to germinated seed sprouts 6 cm. Germination was suitable with the concept of sustainability. Germination can preserve the quality of bioactive chemicals while reducing antinutrients and increasing helpful nutrients. The germinated durian seed could be harvested when the length of sprout 3 cm.

Keywords: Feedstuff, Germination, Sprouts

Introduction

In recent years, there has been growing attention to the need to find sustainable solutions for managing agricultural waste and enhancing the nutritional value of livestock feed. Tropical fruits such as durian, which are well-known for their flavorful and varied nutritional profiles, frequently produce a significant number of residues, such as seeds, peels, and pulp leftovers. FAO (2023) reported Thailand, Malaysia and Indonesia as the key production countries could produce approximately 3 million tons per annum, globally traded quantities increased more than tenfold between 2003 and 2022. From a single durian fruit could generated 20% durian seeds (Sugiarto and Toana, 2018), Up

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until now, these seeds have only been wasted, however, this waste might potentially be converted into biomass for animal feed.

Various studies on durian seeds show that the nutrient content contains 6-10% Crude Protein, 2.4-4.3% Crude Fat, 14% Crude Fiber, 60-80% carbohydrates, and 3-4% ash (Sugiarto and Toana, 2018; Baraheng and T. Karrila, 2019; Mangisah *et al.,* 2020) Based on the nutrient content, they have the potential to be used as a source of energy feed for livestock. However, although durian seeds have good potential to be used for feed, durian seeds contain anti-nutrients, namely cyclopropane fatty acid (CPFAs), and oxalic acid (Mangisah *et al.,* 2020). The CPFAs can interfere with the fat digestion process in livestock by inhibiting fatty acid desaturation and stoeryl-CoA desaturation (Yu *et al.,* 2011). Meanwhile, oxalic acid can interfere with the absorption of calcium and decrease productivity of livestock (Goyal, 2019). These antinutrients can be overcome by processing by boiling. Mangisah *et al.* (2020) reported that boiling can reduce oxalic acid levels by up to 60% and reduce CPFAs by 20%. However, boiling method requires more cost and increase carbon footprint hence running counter to the idea of sustainability. Therefore, it is necessary to study a simple processing method that could applicable for farmer, less cost and in line with sustainability concept, and naturally the durian seeds can be ingested without having a negative impact on livestock.

One simple method was germination. Germination was the process of growing a seed into a new plant. Germination of seeds was a bioprocess of emerging interest to improve nutritional and maintain the bioactive compounds, and decrease of antinutrients of seeds in a natural way (Gan *et al.,* 2017; Ohanenye *et al.,* 2020). To uphold the animal welfare highly recommended to fed the livestock with the high-quality feed not only to support the productivity but also the health status, and the components of bioactive compound in the feedstuff should be considered (Balasubramanian *et al.,* 2023). Regarding the authors literature search the information related to bioactive compound such as antioxidant, phenols, tannin and flavonoid of germinated durian seed still scarce. Therefore, the aim of this study was to investigate the viability of using durian seeds that germinated as a useful component in animal feed.

Materials and methods

Germinated durian seeds and experimental design

The durian seeds used in this study came from local durian on Java Island, Indonesia. Durian seeds were obtained from local fruit processing industries around Semarang, Central Java, Indonesia. The durian seeds were cleansed to isolate them from trash and other contaminants before they germinated. Germination was done after cleaning. After cleaning, germination was carried out. Germination begins by making germination media using a plastic box measuring 10 cm long, 10 cm wide, and 5 cm high. The plastic box was given a cotton pad and 250 ml of water was added. On each germination media, 12 durian seeds were placed.

A completely randomized design was used in this study. There were 3 treatments, the first treatment was durian seeds that had not yet germinated (0 cm) as a control, the second treatment was durian seeds harvested at a sprout length of 3 cm, and the third treatment was durian seeds harvested at a sprout length of 6 cm. Each treatment received 10 replications. The harvested durian seeds were ground using a grinder and the powder was sun drying for 3 days, after that, the samples were taken to be analyzed.

Nutrients and antinutrients content analysis

The dry matter (DM), crude protein (CP), ether extract (EE), and ash of durian seed were analyzed following the Association of Official Analytical Chemists (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by Van Soest method (2018). Oxalic acid content was determined using titration method according to Sa *et al.* (2019) and CPFAs was analyzed by gas chromatography-mass spectrometry [GC-MS] method according to Lolli *et al.* (2018). Carbohydrate and hemicellulose were determined with the formula according to Xin *et al.* (2021).

$$\text{Total carbohydrate} = 100 - \text{EE} - \text{CP} - \text{ash}$$

$$\text{Hemicellulose} = \text{NDF} - \text{ADF}$$

Bioactive compound analysis

Antioxidant activity was analyzed using the 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH RSA) method according to the guidelines of Baliyan *et al.* (2022). Phenols was analyzed using Folin-C (Folin and Ciocalteu) Colorimetric Method according to Kupina *et al.* (2019). Tannins was analyzed using UV–Vis Spectrometry according to Loum *et al.* (2020). Flavonoid was analyzed using colorimetric method according to Zubaydah *et al.* (2021).

Statistical analysis

The collected data were analyzed using analysis of variance according to Gomez and Gomez (1984) using SPSS 26 version. The significance level was determined at 5%. If the parameter was significance, then continue to analyzed by Duncan multiple range test.

Results

Nutrients and antinutrients content

The result showed that obtaining the sprout with a length of 6 cm needed more time than the sprout length of 3 cm. Based on the data on nutrient content, the germination was affected on the dry matter content. The lowest dry matter content was in the germinated seed sprouts at 3 cm, followed by 6 cm, and the highest in without germination. The organic matter and total carbohydrate were lowest in germinated seed sprouts 3 cm compared to germinated seed sprouts 6 cm and without germination. Increasing the length of sprouts increased the crude fiber and protein content, the crude fiber and protein content was highest in sprouts 6 cm compared to 3 cm and without germination. The NDF content was similar in germinated seed sprouts 3 cm and 6 cm, but high compared to those without germination, however, the ADF was highest in germinated seed sprouts 6 cm, followed by 3 cm and the lowest on the seed without germination.

Table 1. The nutrients and antinutrient content of germinated durian seed

Nutrients content	The length of sprout			P Value
	0 cm	3 cm	6 cm	
Days for germination (day)	0.00 ± 0.00^a	4.40 ± 0.26^b	7.00 ± 0.33^c	0.000
Dry matter (%)	88.76 ± 0.07^c	87.11 ± 0.08^a	88.12 ± 0.18^b	0.000
Ash (%)	11.99 ± 0.47	12.67 ± 0.62	11.98 ± 0.48	0.407
Organic matter (%)	76.76 ± 0.43^b	74.43 ± 0.66^a	76.14 ± 0.44^b	0.012
Ether extract (%)	2.04 ± 0.24	1.92 ± 0.16	2.22 ± 0.20	0.593
Crude protein (%)	7.44 ± 0.19^a	8.38 ± 0.14^b	8.24 ± 0.18^b	0.000
Total carbohydrate (%)	78.73 ± 0.55^b	77.26 ± 0.58^a	77.81 ± 0.58^b	0.020
Crude fibre (%)	3.27 ± 0.14^a	3.46 ± 0.06^a	4.17 ± 0.14^b	0.000
NDF (%)	30.77 ± 1.86^a	42.65 ± 0.56^b	44.00 ± 1.25^b	0.000
ADF (%)	5.43 ± 0.31^a	13.48 ± 0.39^b	14.89 ± 0.99^c	0.000
Hemicellulose (%)	25.33 ± 1.83	29.17 ± 0.55	29.10 ± 1.16	0.074

^{a,b,c} Different superscript in the same line show significantly different (P>0.05)
NDF (Neutral Detergent Fibre)
ADF (Acid Detergent Fibre)

The germination process did not affect ash content and ether extract, however, the hemicellulose tended to be significant. Germination was affected on the antinutrient content. Increasing the length of sprout on germinated seed was decreased the oxalate acid and cyclopropane fatty acids. The lowest oxalate acid and cyclopropane fatty acids (CPAs) was shown in germinated seed sprouts 6 cm, followed by 3 cm and on the seed without germination.

Table 2. The antinutrients content of germinated durian seed

Antinutrients content	**The length of sprout**			**P Value**
	0 cm	3 cm	6 cm	
Oxalate acid (%)	7.35 ± 0.05[c]	3.20 ± 0.04[b]	2.51 ± 0.03[a]	0.000
Cyclopropane fatty acids (%)	5.88 ± 0.04[c]	2.40 ± 0.03[b]	1.51 ± 0.02[a]	0.000

[a,b,c] Different superscript in the same line show significantly different (P>0.05)

Bioactive compounds

The germination was affected on the antioxidant activity in germinated durian seed. Antioxidant activity in germinated seed sprouts 6 and 3 cm was lowest compared to the seed without germination. Phenol in germinated seed sprouts 6 and 3 cm was high compared to the seed without germination, however, otherwise flavonoid was low in germinated seed sprouts 6 and 3 cm compared to the seed without germination. Tannin level in the seed without germination was similar with the germinated seed sprouts 3 cm, the tannin level of both was highest compared to germinated seed sprouts 6 cm.

Table 3. The bioactive compounds of germinated durian seed

Antinutrients content	The length of sprout			P Value
	0 cm	3 cm	6 cm	
Flavonoid (mg QE/100 g)	8.53 ± 1.05[b]	4.28 ± 0.05[a]	4.11 ± 0.55[a]	0.000
Phenol (mg GAE/100g)	4.34 ± 0.36[a]	7.57 ± 0.71[b]	7.83 ± 0.42[b]	0.000
Tannin (mg GAE/100g)	65.01 ± 2.89[b]	59.64 ± 1.18[b]	40.87 ± 3.25[a]	0.000

[a,b] Different superscript in the same line show significantly different (P>0.05)

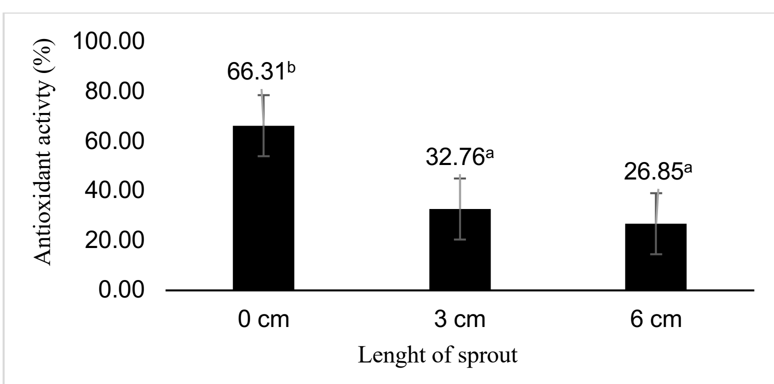

Figure 1. Antioxidant activity of germinated durian seed

Discussion

Large seeds go through three stages of water uptake during germination. During the first phase, the dry seed material quickly absorbs water until the entire matrix and cell contents are filled. Water consumption was then restricted in the second phase as the sprouts started to develop. Then, while not as much as in the first phase, there was another increase in water extraction in the third phase (Nonogaki *et al.,* 2010). Based on that theory, that was the reason the dry matter content was lower in the germinate seed sprout 3 cm than 6cm. The result of this study was in line with the study reported by Zinia *et al.* (2022) on germinated soybeans on day 4th the length of the sprout was 3 cm and on day 6th the length of the sprout was more than 5 cm, and the dry matter content decrease during the germination time.

The lowest content of organic matter and carbohydrates in germinated seed sprouts 3 cm was due to the important role of carbohydrates in the initial phase of the germination process. Organic matter including carbohydrates is the main component that is broken down at the beginning of the germination process. Carbohydrates are needed for the sprout formation process and the breakdown of endosperm cell walls so that sprouts can grow (Ma *et al.,* 2017). Carbohydrates that decrease during germination are generally followed by an increase in crude protein and crude fiber. Like the result of the germinating seed was 3 cm and 6 cm the crude protein fiber was higher than the seed without germination. This is because these carbohydrates are used for the synthesis of protein into amino acids for sprout growth, and protein increases rapidly after 72 hours after germination (Nkhata *et al.,* 2018). The result of this study was in line with the study reported by Uppal and Bains (2012) which was studied various legumes, that germination could increase crude protein.

Carbohydrates are also used during the germination process to form pectic polysaccharides and cellulose which causes an increase in the crude fiber content in the germinated seeds (Hettiarachchi and Gunathilake, 2023). Polysaccharides synthesis from carbohydrate could impact on NDF and ADF, the longer germination time the higher NDF and ADF content (Megat *et al.,* 2016). The ADF content was highest in germinated seeds at 6 cm, it was allegedly because the cell walls began to strengthen the roots of sprouts so that the content of insoluble fiber increased, this was also confirmed by the hemicellulose content which tended to increase. The results of this study are different from those reported by Duenas *et al.* (2016) in that in germinated lentils the NDF and ADF content decreased. It shows that seed varieties and characteristics respond differently during germination, it depends on the structure, composition, and cell tissue of the seed.

The germination was an effective way to decrease oxalate acid in seeds (Gunathunga *et al.,* 2024). During germination, the oxalate acid was catabolized by oxalate oxidase into carbon dioxide, an impact decreasing the oxalate acid in the seed (Pal *et al.,* 2016). That information could explain the reduction of oxalate acid in germinated durian seeds with different lengths of sprouts. The result of this study was similarly reported by Anaemene and Fadupin (2022) in legumes that germination could decrease 89% of oxalate acid in germinated legumes during 48 hours compared to 24 hours. Enzyme cyclopropane fatty acid synthase (CPS) may be expressed to create CPA. S-adenosyl-Met is needed as a methylene group donor for CPS to convert a monounsaturated fatty acid that has been esterified to phosphatidylethanolamine (PE) or phosphatidylcholine (PC) into Cyclopropane fatty acids (Yu *et al.,* 2019). It was suspected that during the germination time the CPS enzyme activity also decreases so that the CPAs content also decreases as the germination process progresses.

Proteins and carbohydrates are broken down in the early stages of germination. As a result, bound phenolics conjugated with the components of the cell wall are also liberated. As the germination period lengthens, more plant cells divide, construct new cell walls, and secrete the synthesized soluble phenolics to the cell wall to create new bound phenolics (Gan *et al.,* 2017). That theory also inline with the result of this study, the length of the germination time and sprout of seed the increase the phenol content of germinated durian seed.

Tannin content of durian seed without germination and germinated seed sprouts 3 cm was similar, however in germinated seed sprout 6 cm the tannin content was lower. It was caused the catabolic enzyme was active with increase the time of germination. Numerous catabolic enzymes are activated during germination, most notably polyphenol oxidase, which catalyzes the breakdown of polyphenolic substances. Consequently, phenoloxidase enzymes hydrolyze tannins, causing a decrease in tannin content in germinated seed (Avezum *et al.,* 2022; Liu *et al.,* 2022). The result of this study was not in line with Anaemene and Fadupin (2022) who study in legumes and Bassey *et al.* (2023) who study in cereals, they reported that the tannin content was significantly decrease after 24 hours until 72 hours of germination. The reduction of tannin content in seed was affected by differences in the duration of germination, the temperature conditions applied, and the types of seed (Gunathunga *et al.,* 2024).

Antioxidant capacity and total flavonoid content were known to be strongly correlated; the higher the antioxidant capacity, the more flavonoids that were extracted from plants (Zhang *et al.,* 2011; Bordin-Viera *et al.,* 2023). That report also inline with the result of this study, the trend of flavonoid and antioxidant was decrease in germinated durian seed compare to without germination. As a part of their growing process, seeds expel gas and water during germination. The content of certain flavonoids in the seeds may drop as a result of dissolving in the released water or becoming stuck in gaseous form (Pinheiro *et al.,* 2021). Based on that explanation, the length of the sprouts the longer the time to germinate, followed by the time suspected the flavonoid content was released in the water or became stuck in gaseous form and the effect on decreased flavonoid content and antioxidant activity.

Based on the result of the nutrients content of germinated durian seed were almost similar with rice bran and corn bran. Nuraina *et al.* (2020) reported that the rice bran was containing 86.6% of dry matter, 11.1% of ash, 8.0% of crude protein, 3.9% of crude fiber and, 8.0% of crude protein. The corn bran was containing 90.4% of dry matter, 2.4% of ash, 7.9% of crude protein, 3.8% of crude fat and, 6.9% of crude fiber. Based on that the germinated durian seed could used as a feedstuff for livestock such as the rice bran and corn bran. Besides the nutrient content, the antinutrient was decreased more than 50% with germinated method, and the feedstuff containing 1-3% was no negative effect in livestock such as ruminants (Goyal, 2018). The bioactive compound such as tannin that origin from tropical plant was effectively affect on reduce the methane emission in ruminants (Cardoso-Guiterrez *et al.,* 2021). The other bioactive compound such as phenol and flavonoid Phenolic compounds carry various pleiotropic functions including antibacterial, antioxidant, anti-inflammatory, antiviral, hepatoprotective, anti-allergic, antithrombotic, oncostatic, and immune-modulating activities, and could have a beneficial effect not only on livestock productivity but also on health and welfare (Hajam *et al.,* 2020; Mahfuz *et al.,* 2021). Based on that information, therefore the germinated durian seed could be potential to use as a feedstuff for a livestock. However, it was needed more research through in vitro and in vivo method to have better understanding the maximum level of durian seed could be given for the livestock such as poultry and ruminants livestock.

Germination was an easy and cheap method to increase the quality of nutrient content of tropical fruit seeds such as durian, and this method was suitable with the concept of sustainability. Germination could increase beneficial nutrients such as organic matter, protein, crude fiber, carbohydrate and decrease the antinutrients, and maintain the quality of bioactive compounds. The germinated durian seed could be harvest when the length of sprout 3 cm.

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Conflicts of interest

The authors declare no conflict of interest.

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