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Effect of *Trichanthera gigantea* (Humb & Bonpl.) Nees Leaf Harvested at Different Stages of Maturity on Its Nutrient and Secondary Metabolites Compounds

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ABSTRACT

Trichanthera gigantea (*ketum ayam*) is a species of flowering plant in the acanthus family, Acathaceae. Younger *T. gigantea* leaf was reported to contain good protein content with positive effect on livestock. This study aimed to determine the harvesting effect of *T. gigantea* leaves at 2 different stages of maturity (young: less than 2 months old, mature: 3 to 4 months old) on the nutrient and secondary metabolites compounds. The significant differences were determined at $p<0.05$. Young leaves showed significantly 5.44% higher protein, 4.04% ash, 3.19% fibre contents and 2.7% lower fat and 0.57% energy compared to mature leaves. Phenol and saponin were detected in both young and mature leaves, with the younger leaves contained 0.011% higher percentage in phenol and 3.22% in saponin. However, there is no significant difference in Ca (4.62%, 4.91%) and (0.31%, 0.19%) for both stages of leaf maturity. In conclusion, the young leaves showed higher nutritional value, suggesting potential as a partial protein source in broiler diets. However, feeding trials are needed to confirm its suitability.

Keywords: Broiler, *ketum ayam*, protein, secondary metabolite, *Trichanthera gigantea*

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INTRODUCTION

Ketum ayam or its scientific name, *Trichanthera gigantea* (Humb & Bonpl.) Nees is a flowering shrub plant species belonging to the Acanthaceae family and is commonly known by the names; Madre de Agua (Latin America), Nacedero, Suiban, Cenicero, Tuno, Naranjilo, and Palo de

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Agua. This plant is native to America and was introduced to Malaysia in 2012 by a local farmer. It grows wild and thrives in tropical regions such as the Philippines, Vietnam, Cambodia, including Malaysia, especially in most parts of Peninsular Malaysia. In fact, it is easy to be grown at high environmental temperatures, poor quality soil, and under minimal management (Tran, 2003), with a short harvesting period, as early as two months after planting. In Malaysia, it is locally known as *ketum ayam* because of its physical appearance which looks like *ketum* or kratom or *biak* (*Mitragyna speciosa*) leaf which belongs to the Rubiaceae family and is mostly used as chicken feed. *Kratom* leaf is listed under the Malaysian Poison Act of 1952 because it contains the psychoactive prohibited compounds mitragynine which has a stimulating, sedative, and euphoric effect that can cause addiction. To date, there has been no discovery of that prohibited compound in the leaf of *ketum ayam*.

The consumption of meat in Malaysia in 2019 was reported to be above the average values stated by the Organization for Economic Cooperation and Development (OECD) for that particular year. The poultry meat per capita consumption in Malaysia was 49.3 kg in the year 2020 and increased to 49.7kg by 2021. Malaysia was listed amongst the top global consumers of poultry meat worldwide, and the intake is expected to increase to 51.28 kilograms per capita in 2025 (Hirschmann, 2022).

The increase in the import price of animal feed ingredients such as corns and soybeans in the international market can inadvertently cause an increase in the price of chicken. In 2016, Malaysia spent RM5.6 billion to import corns and soybeans for their use in livestocks such as poultry, goats, and cattle to meet its protein and energy sources, with RM2.2 billion alone is for soybeans. In the year 2020, the price of soybeans increased almost 1.5 times within 1.5 years which is RM 1480/metric ton in June 2020 to RM 2210/metric ton in January 2022 (New Straits Times, 2022). Various efforts were made by the Government to reduce the dependency on imported ingredients, finding sources by looking into other potential locally available and cheaper sources such as cassava meal, palm kernel cake, and so on.

One of the most important aspects is that the leaves can be considered safe to be used in animal feed. It can be offered directly (fresh) or in dried form. Having an acceptable proximal composition as well as the content of secondary metabolites and the presence of low concentrations of anti-nutrients make it possible for this leaf to be used in livestock production systems in tropical areas (Garcia et al., 2006). However, the nutritive value of *T.* gigantea leaf may vary depending on the ages of leaves (Tran, 2003) and cutting intervals (Kien et al., 2020). *T. gigantea* are usually harvested at the age of 3 to 4 months. Tran (2003) noted that the livestock should have better performance traits when they are fed younger leaves rather than the older leaves. For example, the young leaves contained higher crude protein than the old leaves. A 50 to 60-day cutting interval of the *T. gigantea* tree was recommended by Kien et al. (2020).

Thus, this study aimed to investigate the effect of *T. gigantea* (ketum ayam) leaf harvested at two different stages of maturity (young, mature) on its nutrient and secondary metabolite contents.

MATERIALS AND METHODS

Collection and Sample Preparation

About 2 kilograms of each group of leaves were collected randomly in the morning at a local farm located in Bentong, Pahang, Malaysia. The young leaves were taken from the top, whereas the matured leaves were taken from the bottom of the *T. gigantea* trees. The part of the leaves harvested only includes the leaf (from the basal leaf to the tip) and the stipule. The leaf was plucked manually using plant-cutting scissors, weighed immediately, and sealed in plastic bags. The leaf samples were then transferred to the Nutrition Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia for chemical analysis. The identification and authentication were done by the Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia with voucher no. MFI 0236/21.

Chemical Analysis

Upon arrival at the laboratory, all leaves were washed quickly under running tap water to remove any bruised, soiled, or imperfect leaves before the drying process. The leaves were dried in an oven at a temperature of 50±1°C until a constant weight was obtained. Later, the leaves were ground using a laboratory grinder to obtain a fine meal measuring approximately 2 mm in diameter. These leaves were then placed in a separate airtight bottle and stored in a cool and dry place for further analysis.

Proximate Analysis, Van Soest and Gross Energy

The proximate analysis including dry matter (DM), ash, organic matter (OM), crude fibre (CF), crude protein (CP), ether extract (EE), nitrogen free extract (NFE), acid detergent fibre (ADF), acid detergent lignin (ADL), neutral detergent fibre (NDF), and also gross energy were conducted according to the methods of Association of Official Analytical Collaboration (AOAC), 1984.

Determination of M, DM, Ash and OM

About 1 g of each sample was weighed and placed in a pre-weighed crucible. Then, these crucibles containing the samples were dried in an oven at 105°C for 24 hours, cooled in a desiccator for 30 minutes, and was weighed. Next, the crucibles containing the dried sample were burned in a muffle furnace at 550°C for 3 hours, cooled in a desiccator and then weighed. The M, DM, ash and OM contents were determined based on calculation as stated in the method.

Determination of Crude Protein (CP)

Approximately 1 g of each sample was placed in a digestion tube. The digestion tube was then added with a Kjeldahl tablet, followed by 12 mL of concentrated sulfuric acid (H_2SO_4) and 3 mL of 30% hydrogen peroxide (H_2O_2). The samples were digested, distilled and titrated using the AOAC (1984) methods. The protein percentage was calculated by the stated formula in this method.

Determination of Crude Fibre (CF)

Briefly, 1 g of each dried sample was weighed and loaded in a fiber bag (Gerhardt®) and inserted with a glass spacer. Then, each fibre bag was loaded onto the carousel and inserted into a 600 mL beaker. Three hundred sixty (360) mL 0.13 mol/L sulfuric acid (H_2SO_4) was poured into the beaker and mixed it by rotating the carousel for about 1 min. Next, the beaker was placed on the hot plate that had been preheated for about 5 minutes. The hotplate was set up to full before boiling (about 3-5 min) before reducing it to obtain a gentle simmer for about 30 minutes. The beaker was removed from the hotplate, and the carousel was taken out. The solution and soluble within the beaker were discarded, and the carousel was rinsed with hot water several times. The previous steps were repeated with 360 mL 0.313 mol/L natrium hydroxide (NaOH). After boiling with NaOH, the fibrebags were rinsed with hot water and dried with fibre-free tissue before putting into pre-ashed crucible and dried in an oven at a temperature of 105°C for 4 hrs. Next, the crucible contained fibrebags were taken out from the oven, cooled in desiccator, weighed, incinerated at 600°C for 4 hours, cooled at 105°C for 30 min in an oven, and finally cooled in desiccator before weighing. The calculation for crude fibre determination followed the formula described by Gerhardt®.

Determination of Ether Extract (EE)

The ether extract determination was conducted using a manual system, Soxhlet apparatus Gerhardt®. Approximately 1 g of each sample was weighed into an extraction thimble (33x80mm)‑and then inserted into the Soxhlet chamber. A 250 mL pre-weighed round bottom flask was loaded with 200 mL of petroleum ether boiling range of 40-60°C before being fitted into the Soxhlet apparatus. The extraction process was conducted for about 6 hours before removing the round bottom flask containing the ether extract residue. Next, the round bottom flask was dried in an oven at 105°C overnight, cooled in a desiccator and weighed. The ether extract content was calculated using the difference between the weight of the sample and the residue, as stated in the methods.

Nitrogen free extract of each sample was calculated using the stated formula.

NFE (%) = 100 - % CP - % EE - % CF - % Ash

Determination of Neutral Detergent Fibre (NDF)

About 1 g of each sample was weighed and placed in a pre-weighed Gerhardt® fibrebags inserted with a glass spacer. Next, the fibrebag containing the sample was attached to the carousel prior to boiling with the NDF solution. After a gentle simmer for about an hour, the solution was discarded, and the fibrebag was rinsed with hot water before wiping it with fibre-free tissue. Later, the fibrebag was put into a pre-weighed crucible and dried in an oven at 105°C for 4 hours prior to ash in a muffle furnace at 600°C for 4 hours. To cool the sample after ashing, the sample was placed in an oven at 105°C for 30 min, followed by cooling in desiccator for 30 min. The NDF was calculated by a formula.

Determination of Acid Detergent Fibre (ADF)

One gram of each sample was weighed and loaded in a pre-weighed Gerhardt® fibrebag that was already inserted with glass spacer. The fibrebag then was loaded into carousel in a beaker prior to boiling with ADF solution in a hotplate. The fibrebags were drained to free from detergent after one hour boiling, rinsed with hot water, wipped with fibre-free tissue and placed into a pre-weighed crucible before drying in an oven at 105° for 4 hours. After 4 hours, the fibrebag was allowed to cool in an oven at 105°C for 30 min before cooling in desiccator for 30 minutes, then weighed before incinerating in a muffle furnace at 600°C for 4 hrs. Next, the fibrebag was taken out from the muffle furnace and allowed to cool in an oven at 105°C for 30 minutes followed by cooling in desiccator about 30 min. The calculation for determining the percentage of ADF was calculated by a formula.

Determination of Acid Detergent Lignin (ADL)

To determine the ADL content, some steps used in ADF are required before soaking the sample with 72% H_2SO_4 solution. Briefly, approximately 1 g of each sample was weighed and loaded into a pre-weighed Gerhardt® fibrebags containing a glass spacer. Then, the fibrebag was inserted into the carousel in a beaker prior to an hour of gentle simmering with ADF solution. Next, the fibrebags was free from the detergent solution by rinsing with hot water. The rinsed fibrebag was later inserted again into the carousel in a beaker. A 360 mL of ADL solution was poured slowly into the beaker containing the sample, and mixed by rotating the carousel carefully for about 1 min. The fibrebag was allowed to soak in the solution for 3 hours before rinsing it with hot water. After being free from detergent, the fibrebag was wiped with fibre-free tissue and loaded in a pre-ashed crucible. Then, it was placed in an oven at 105°C for 4 hours to dry the fibre bag, cooled in a desiccator for 30 minutes, and weighed. To ash the sample, the fibrebag was incinerated in a muffle furnace at 600° for 4 hours. The fibrebag was allowed to cool in the furnace overnight before

transferring it in an oven at 105°C for 30 minutes and further cooled in a desiccator for about 30 minutes. The cooled pre-ashed sample was weighed and recorded. The calculation for ADL was determined using a formula.

Gross Energy

The gross energy in the samples was determined using an automated bomb calorimeter IKA® C5000 that had been calibrated with benzoic acid prior to analysis.

Minerals

Calcium and phosphorus were determined using the In-house Method, SGS-SOP-LAB-028, based on AOAC 986.15, 975.03, and 922.02, APHA 3120B and APHA 3125B (ICP-OES).

Qualitative Analysis of Secondary Metabolites

The presence of secondary metabolite compounds such as phenols, saponins, tannins, flavonoids, cardiac glycosides, anthocyanides and terpenoids was conducted according to the methods as reported by Raipuria et al. (2018), steroids (Aliyu et al., 2017), alkaloids and antraquinones (Abdullahi et al., 2020); Perveen and Zaid, 2013), Phlobatannins (Ezeonu and Ejikeme, 2016) and oxalates (Kgosana, 2019).

Extraction of Leaves Meal

Approximately 100 mg of each dried sample was kept overnight in 25 ml of ethanol. The extracts were filtered using Whatman filter paper no. 1 and used for phytochemical screening as follows:

Phenol

Two ml of the above filtrate was taken into a 15 ml glass test tube and 1 ml of 1% Ferric Chloride ($FeCI_3$) was added to it. Brown haziness indicated the presence of phenols.

Saponins

One ml of the filtrate was mixed with 5 ml of H_2O, and then shaken vigorously using IKA vortex mixer. The persistence of frothing showed the presence of saponins.

Tannins

Two ml of the filtrate was taken into a 15 ml glass test tube followed by 2 ml of 0.1% $FeCI_3$. Brownish color indicated the presence of tannins.

Flavanoids

The test for flavonoid adopted is as stated by Ezeonu and Ejikeme (2016). Briefly, 300 mg of each sample was mixed with 30 ml of H_2O for 2 hours. The solutions were filtered with Whatman filter paper no. 42 (90 mm). Each 5 ml of extract was added with 2.5 ml of 1.0M ammonia (NH_3) solution followed by 2.5 ml of concentrated H_2SO_4. Yellow color indicated the presence of flavonoid.

Cardiac Glycosides

One mL glacial acetic acid was added into each 2 ml filtrate followed by addition of 1 ml glacial acetic acid (CH_3COOH), 1 ml of 1% $FeCI_3$ and 1 ml concentrated H_2SO_4. The presence of cardiac glycosides was indicated by a brown ring.

Anthocyanides

Five ml of 10-12% hydrochloric acid (HCI) was added to 1 ml of each above filtrate. The presence of pale pink color, indicating the presence of anthocyanides.

Terpenoids

Five mL of each extract was added by 2 ml of chloroform ($CHCI_3$) followed by 3 mL of concentrated H_2SO_4. The terpenoids were shown by the presence of a reddish-brown coloration at interface formed.

Steroids

Three mL of $CHCI_3$ was added into 0.5 mL of each extract followed by 3 ml of concentrated H_2SO_4. With green fluorescence, the presence of steroids was indicated when the upper layer turned red whereas the H_2SO_4 layer turned yellow.

Alkaloids

Two grams of dried *T. gigantea* leaves was extracted by boiling it for 2 minutes in a solution contained 20 ml 5% H_2SO_4 in 50% ethanol. The sample was filtered using Whatman Filter paper no. 42, and the filtrate was made alkaline using 5 ml of 28% NH_3 in a separating funnel. The filtrate then was extracted again with 5 ml chloroform and 5 ml of 1M H_2SO_4. The final filtrate (2 ml) was added with 1 ml of Mayer's reagent and shaken. No emergence of whitish precipitate confirmed the presence of alkaloids (Ezeonu and Ejikeme, 2016; Abdullahi et al., 2020).

Quantitative Analysis of Secondary Metabolites

The total phenol was determined according to the methods used by Singleton and Rossi (1965) and Sreeramulu and Raghunath (2011) while saponins (Nwosu et al., 2010) with slight modification.

Total Phenols

The phenol content was determined based on the methods used by Singleton and Rossi (1965) and Sreeramulu and Raghunath (2011). One g of the sample was placed in a 50 mL centrifuge tube, followed by 20 mL of 70% methanol (MeOH) containing 0.1% hydrochloric acid (HCI) for sample extraction. The tube was shaken vigorously for 4 hours at room temperature using an IKA® vortex mixer. The sample suspension was centrifuged at 10000 g for 15 minutes at 10°C. The supernatant was collected using a Pasteur pipette and then filtered it with a Whatman filter paper no. 1. The resultant filtrate was kept at -20°C for phenol determination. Gallic acid was used as a standard and it was prepared in a 100 mL volumetric flask by dissolving 500 mg of dry gallic acid (Merck®) in 10 mL of 99.9% MeOH and was topped up to the volume with distilled water (H_2O). A gallic acid calibration curve was prepared by adding 0, 5, 10, 15, 20, and 25 mL of the above phenol stock solution into 100 mL volumetric flasks, and then diluting to the mark with H_2O (Figure 1). The resulting solutions will have phenol concentrations of 0, 0.25, 0.50, 0.75, 1.0 and 1.25 mg/mL. To prepare 20% sodium carbonate (Na_2CO_3) solution, 100 g of anhydrous Na_2CO_3 was dissolved in 400 mL H_2O by heating it to boiling. After cooling, a few crystals of Na_2CO_3 were added into the solution. Twenty-four hours later, it was filtered into a 500 mL volumetric flask using a Whatman filter paper no. 1. The filtrate was made up to the mark with H_2O. Next, 20 µL of each calibration solution, sample and blank were pipette into separate cuvettes, followed by 1.58 mL H_2O and 100 µL Folin-Ciocalteu

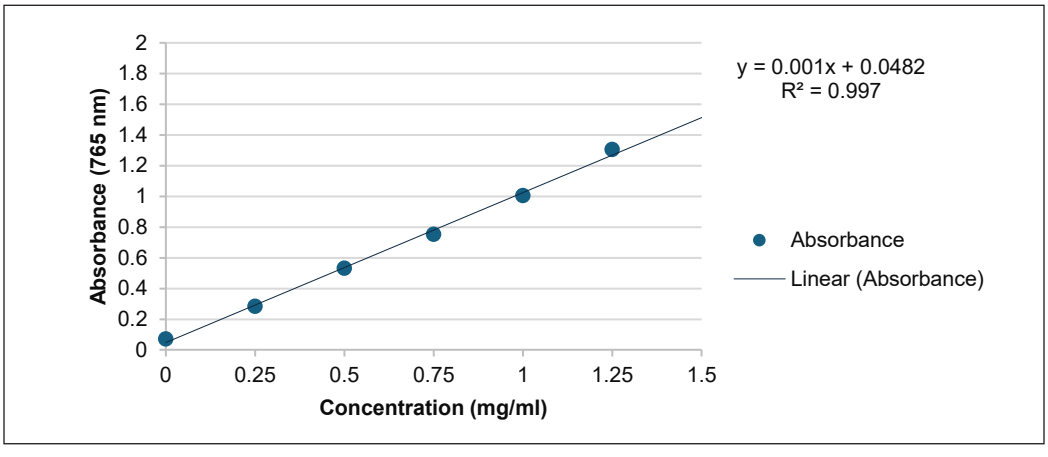

Figure 1. Standard calibration curve of gallic acid for the quantitation of phenols

reagent (Merck®). The solutions in the cuvettes were mixed well. After 4 minutes, 300 µL of the Na_2CO_3 was added to each cuvette and shaken vigorously. The solution in the cuvettes was incubated at 40°C for 30 minutes before reading the absorbance at 765 nm against the blank (the '0 mL' solution) using the TECAN Multimode Microplate Reader Infinite M200 PRO instrument. The phenol concentration of the T. *gigantea* dried leaves meal was determined from the graph plotted absorbance versus concentration of gallic acid. Gallic acid was used in the phenol determination because it is the most used standard, more stable, provide consistent and reproducible results, a pharmacologically active antioxidant, and quantitatively equivalent to other phenolics (Claudine et al., 2005).

Total Saponins

The saponin content in the samples were analyzed based on the methods stated by Nwosu et al. (2010). Briefly, 1 g of the fried sample was weighed and placed into a cellulose thimble and covered with cotton wool. The thimble bearing the sample was inserted into Soxhlet extractor chamber before pouring 200 mL of petroleum benzine (boiling point 40-60°C into a pre-weighed 250 mL round bottom flask. The extraction was conducted for around 3 hours and the defatted material in the thimble was used for second extraction. The extraction was repeated using the same procedure with a new pre-weighed round bottom flask containing 150 mL 99.9% MeOH. Later, the flask was removed from the apparatus when the saponin content was almost dried. The flask was then dried in an oven at 70±1°C for 2 hours, cooled in a desiccator, and weighed. The calculation of saponin was as follows:

Saponin (%) = (Mass of saponin (g) / Mass of sample) x 100

Statistical Analysis

All data were analyzed using T-test in R to compare the means between two groups. The statistical significance was determined at $p<0.05$.

RESULTS AND DISCUSSION

Table 1 below shows the proximate analysis and gross energy content in young and matured dried *T. gigantea* leaves meal. The dry matter, organic matter, crude fat, nitrogen free extract, neutral detergent fibre and gross energy content of the leaves increased with age whereas the remaining components were vice versa. Tran (2003) indicated that the dry matter and nitrogen content of *dried T. gigantea* leaves meal in Vietnam were higher in younger than older leaves. In that experiment, the DM content of young and old leaves were 17.1% and 18.5% respectively, while the N content were 4.32% and 2.48%. In this current experiment, the dry matter of young and mature leaves was 12.15% and 17.72% (Table 1).

Table 1
Proximate analysis of Trichanthera gigantea *meal at different stages of leaves*

No.	Components	Stages of Maturity		SEM	*P* value
	Proximate analysis	**Young Leaves**	**Mature Leaves**		
1	[x]Moisture (%)	87.85[a]	82.28[b]	0.45	<0.001
2	[x]Dry matter$_{fw}$ (%)	12.15[b]	17.72[a]	0.45	<0.001
3	Moisture (%)	6.32	6.20	0.42	0.791
4	Dry matter (%)	93.68	93.80	0.42	0.791
3	Ash (%)	24.34[a]	20.30[b]	0.26	<0.001
4	Organic matter (%)	82.40[b]	86.31[a]	0.29	<0.001
5	Crude Protein (%)	21.24[a]	15.80[b]	0.58	<0.001
6	Crude Fat (%)	1.35[b]	4.05[a]	0.03	<0.001
7	Crude Fibre (%)	17.64[a]	14.45[b]	0.44	<0.001
8	Nitrogen free extract (%)	42.38[b]	51.30[a]	0.90	0.001
9	Neutral detergent fibre (%)	44.82[b]	59.72[a]	0.41	<0.001
10	Acid detergent fibre (%)	46.63[a]	39.55[b]	0.36	0.002
11	Acid detergent lignin (%)	19.53[a]	18.95[b]	0.12	0.040
12	Gross energy (MJ/kg)	15.57[b]	16.14[a]	0.06	<0.001

Note. Mean within the same rows with different superscript letters indicate statistical significance (p<0.05). Based on dry matter, SEM: Standard error of mean, *P*: Probability, [x]: fresh weight

Previous findings noted that the minimum and maximum values of *T. gigantea* leaves ranges were as follows; Berdos et al. (2019) stated that on dry matter basis, the leaves presented 93.3% DM, 19.59 % CP, 11.89% CF, 2.33 % EE, 20.15% ash, 39.34% NFE, 3665 kcal/kg gross GE and 2310 kcal/kg ME while Balraj et al. (2018) reported that based on dry matter, the OM, CP, CF, EE, NDF and ADF of dried *T. gigantea* leaf were 80.3%, 18.1%, 18.4%, 2.97%, 28.8% and 21.4% respectively. Heuze et al. (2016) showed DM, 13 to 26.3%, CP, 12 to 21.7%, CF, 13 to 25.1%, NDF, 28.2 to 65.5%, ADF, 24.5 to 56.3%, ADL, 2.7 to 9.5%, EE, 1.5 to 5.8%, Ash, 9.3 to 32.3% and gross energy, 15.2 to 18.4 MJ/kg. When expressed on fed basis, proximate analysis showed that the leaves contained 15.80% CP, 15.42% CF, 1.15% EE, 18.07% ash and 35.86 NFE (Saguidan, 2015). The *T. gigantea* leaves dried in an oven at 60°C for 72 hours contained 94.1% residual DM, 72% OM, 20.3% CP and 49.9% NDF (Rodriquez et al., 2014). Edwards et al. (2012) demonstrated that the cutting interval from 6 to 12 weeks had no effect on the content of OM, ADF, NDF, ADL, soluble condensed tannins (SCT) and insoluble condensed tannins (ICT) of *T. gigantea* leaves at three different locations except on DM, CP and acid detergent insoluble nitrogen (ADIN). The minimum and maximum chemical composition values for the locations were DM, 85.6 to 87.7%, OM, 72.2 to 74.9%, CP, 18.5 to 22.6%, ADIN, 28 to 38%, ADF, 30 to 53.8%, NDF, 62.2 to 65.5%, ADL, 24 to 28%, SCT, 0 to 0.01% and ICT, 0 to 0.01%. The fresh leaves contained 13.7% dry matter, 15.1% ash, 21.6% crude protein, 4.3% crude fat,

and 39% crude fibre (Leterme et al., 2005) while the dried ones were reported to contain 78.9% dry matter, 23.9% crude protein, 23.8 crude fibre, 2.5% crude fat, 24.3% ash, and 25.5% nitrogen free extract (Sarwatt et al., 2003). It also contained macro elements and microelements of minerals mainly calcium. Ly et al. (2001) found that fresh *T. gigantea* leaf contained 26.30% dry matter while on a dry matter basis, the content of ash, organic matter, NDF and N were 14.6%, 85.4%, 30.8% and 2.72% respectively.

The plant varieties, season of the year (dry or raining season), irrigation, plantation, fertilizer application and harvesting techniques, etc. could contribute to varying in results obtained. The harvesting techniques can influence the production and quality of green fodders. For example, the long cutting interval will increase the proportion of mature leaves, leading to increasing fibre contents in leaves and decreasing of crude protein in leaves, hence declines the quality of feed (Kien et al., 2010; Nouman, 2012; Hien et al., 2013; Hien et al., 2019; Kien et al., 2020). Its special characteristic is that it contains high amounts of crude protein (about 23%) which consists of 17 amino acid components including 10 essential and 7 non-essential amino acids which are required by poultry. Some researchers also reported that the leaves can be incorporated up to 25% in broiler diet. For instance, in 2015, it has widely used in Venezuela, Peru, Equador and Philippines livestock feed, such as in pig, rabbit and poultry as a replacement for the conventional protein sources in the diet.

Bageel and Borthakur (2022) reported that the contents of moisture and CP of giant Leucaena fodder were decreased with maturity while DM and NDF increased as the leaves gets older. Hut et al. (2012) also noted that the nutritional content of cassava leaves was affected by the age of leaves except for ash. DM, NDF and ADF increased with the maturity of the leaves while CP was vice versa. Crude protein reduced significantly ($p<0.05$) with the maturity age of the *Celosia argentea* L. plant leaves (Adediran et al., 2015). This decline can contribute to the accumulation of cell wall material as leaves mature (Edwards et al., 2012). The uppermost eleven leaves of cassava tress were considered very young, young and mature leaves (Ravindran and Ravindran, 1988). Leaves that are one month old were considered young leaves while mature leaves are 3 to 4 months old (Leterme et al., 2005).

No significant ($p>0.05$) difference was seen in Ca and P of young and matured *T. gigantea* leaves as presented in Table 2. The minimum and maximum of Ca and P values of this plant's leaves were 2.45 to 3.80% and 0.25 to 0.47% respectively (Hueze et al., 2016). Berdos et al. (2019) stated that on dry matter basis, the leaves presented 4.47% calcium and 0.25% total P while Saguidan (2015) found it contained 4.7% Ca and 0.32% available P.

In general, the differences in plant nutrient contents may be due to the age, parts, and the development stage of the plant itself. Phosphorus (P) is higher at the beginning and decreases with age while calcium (Ca) is on the opposite. P is a mobile element, and the deficiencies occur in older tissues, whereas Ca is an immobile element, and the deficiencies occur in newer tissues. The Ca and P contents in plants typically range from 0.005 to 5%

Table 2
Macrominerals content of dried Trichanthera gigantea *leaves meal*

No.	Components (%)	Stages of Maturity		SEM	*P* value
		Young Leaves	Mature Leaves		
1	Calcium, Ca	4.62	4.92	0.166	0.411
2	Phosphorus, P	0.31	0.19	0.028	>0.05

Note. Mean within the same rows with different superscript letters indicate statistical significance ($p<0.05$). Based on dry matter, SEM: Standard error of mean, *P*: Probability

and 0.05 to 0.5% of dry weight respectively. However, a similar pattern was observed in *Moringa oleifera* leaves which there was no difference in P content between young and old leaves of Moringa (Andi et al., 2018).

The presence of phenol (450 ppm), saponin and steroid (0.62%) were detected by Leterme et al. (2005). Garcia et al. (2006) revealed that *T. gigantea* leaf contained alkaloids, bitter compounds (B. compounds), condensed tannins, coumarins, cyanogen, flavonoids, phenols, precipitants of proteins, saponins, slimes, steroids, tannins and terpens. Riascos Vallejos et al. (2020) reported that the leaf contains total alkaloids (0.3%), condensed tannnis (0.24%) and phenols (1.15%). Eighteen chemical compounds in *T. gigantea* have been identified by Quan et al. (2022) in their phytochemical screening study consisting of amino acids, betalains, carbohydrates, organic acids, coumarins, flavonoids, carotenoids, mucilages, gum-resins, phlobatannin, xanthoprotein, phenol and essential oil.

However, in this study (Table 3), only phenol and saponin were detected in both cutting intervals of *T. gigantea* leaves meal while the remaining component was unavailable. Edwards et al. (2012) and Hueze et al. (2016) also noted that no tannin was detected in T. *gigantea* leaves. The presence of secondary metabolites compound in those leaves may be vary due to many factors such as temperature, humidity, light intensity, the supply of water, soil fertility, minerals and CO_2 (Akula and Gokare, 2011; Li Yang et al., 2018). For example, deficiencies in potassium, sulfur and magnesium increases phenolic concentration. Genetic, environmental conditions and physiological factors may modify or influence the composition of the secondary metabolites in plants (Hongyan et al., 2012).

In this current study, it was also noted that phenol and saponin compounds in younger leaves were higher than in older leaves which are 0.023% and 0.012 and 9.67% and 6.45% respectively (Table 4). In a study of cassava leaves by Ravindran and Ravindran (1987) found that the levels of some anti-nutritive compounds like tannin and hydrocyanic acid were decreased as the maturity of those leaves increased. The saponin and steroid (0.62%) were low (Leterme et al. 2005) while phenol was 450 ppm. Riascos Vallejos et al. (2020) reported that the leaf contained total alkaloids (0.3%), condensed tannnis (0.24%) and phenols (1.15%). The tannin concentration of giant Leucaena fodder also reduced with maturity (Bageel and Borthakur, 2022). Similar trend was observed for HCN content in

Table 3
Qualitative determination of secondary metabolites in Trichanthera gigantea *leaves meal at different cutting intervals*

No.	Constituents	Test	Stages of Maturity	
			Young Leaves	Mature Leaves
1	Phenols		+	+
2	Saponins	Foam Test	+	+
3	Steroids	Lieberman Burchard's Test	-	-
4	Alkaloids	Mayer's Test	-	-
5	Tannins	Ferric Chloride Test	-	-
6	Flavanoids	Shinoda's Test	-	-
7	Anthraquinones	Bontrager Test	-	-
8	Cardiac Glycosides	Keller Killiani's Test	-	-
9	Oxalates	Oxalates Test	-	-
10	Anthocyanides		-	-
11	Terpenoids	Salkowski Test	-	-
12	Phlobatannins		-	-

Note. + Detected, - Not detected

Table 4
Quantitative determination of secondary metabolites in Trichanthera gigantea *meal at different cutting intervals*

No.	Components	Stages of Maturity		SEM	*P* value
		Young Leaves	Mature Leaves		
1	Total phenols (%)	0.023^{a}	0.012^{b}	0.002	0.012
2	Total saponins (%)	9.67^{a}	6.45^{b}	0.264	0.005

Note. Mean within the same rows with different superscript letters indicate statistical significance ($p<0.05$). Based on dry matter, SEM: Standard error of mean, *P*: Probability

the local variety of cassava which also declined with foliage maturity, but tannin was vice versa (Hue et al., 2012).

Generally, the colour, rigidity and location of the leaves are factors that influence the selection of leaves based on age. The leaves at the top of a tree or shrub are young leaves while the old leaves are at the bottom (Tran, 2003). According to Bageel and Borthakur (2022), young leaves are light green, while mature and old leaves are green and dark green respectively. The position of young leaves is in the middle of the tip of the twig, while mature and old leaves are at the middle of the branch and at the base of the stem, respectively. Young leaves are hairy, smaller, and softer than mature and young leaves.

CONCLUSION

The results indicate that leaf maturity significantly influences nutrient composition and metabolite content, with younger leaves showing higher protein and saponin levels.

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REFERENCES

Abbood, A. A., Azhar, K., Jawab, H. S. A., Abdul Manap, Y., and Sazili, A. Q. (2017). Effects of feeding the herb Borreria latifolia on the meat quality of village chickens in Malaysia. *Poultry Science*. 96 (6), 1767-1782.

Abdullahi, R. A., Ibrahim, H. S., Sani, M. and Abdullahi, H. Y. (2020). Quantititave determination of secondary metabolites of fractions obtained from *Solanum aethiopicum* (L.) fruit. *Dutse Jurnal of Pure and Applied Science* (DUJOPAS). 6(3).

Akula, R. and Gokare, A. R. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signalling and Behavior*. 6(11), 1720-1731.

Aliyu, M. Y., Ismail, S. and Mansur, A. I. (2017). Phytochemical analysis and determination of antioxidant activity in methanol and chloroform etxracts of Moringa oleifera leaves.

Andi, S., Muh, H. and Adriyani, A. 2018. Nutrient contents of Moringa leaves based on leaf age. *Indian Journal of Public Health Research and Development*. 9(1), 397. DOI:10.5958/0976-5506.2018.00073.6

AOAC (1984) Official Methods of Analysis. Association of Official Analytical Chemists. 14th Edition, AOAC, Arlington.

Awad, W. A., Bohm, J., Razzazi-Fazeli, E., and Zentek, J. (2006). Effects of feeding dexynivalenol contamined wheat on growth performance, organ weights and histological parameters of the intestine of broiler chickens. Journal of Animal Physiology and Animal Nutrition. 90 (1-2), 32-37.

Bageel, A. M. and Borthakur, D. (2022). The effects of pH, salinity, age of leaves, post-harvest storage duration, and psyllid infestation on nutritional qualities of giant Leucaena fodder. *Journal of Crop Science and Biotechnology*. 25, 381-392. https://doi. Org/10.1007/s12892-021-00139-9

Balraj, D., Krisnamoorty, U., Nayak, S. and Paul, A. (2018). Effect of partial replacement of commercial sheep ration with Trichanthera gigantea (Nacedero) leaves on feed intake and carcass yield of Barbados blackvalley lambs. *Indian Journal of Small Ruminants*. 24(2), 264-268. DOI: 10.5958/0973-9718.2018.00062.4.

Banerjee, G. C. (1998). Poultry. 3rd edition. Bombay: Oxford and IBH Publishing Co. Pvt. Ldt. p. 104.

Bansal, G. R., Singh vp. And Sachan, N. (2011). Carcass quality characteristics of broilers as affected by dried poultry excreta supplementation. *Asian Journal of Poultry Science*. 5(3):116-123. doi: 10.3923/ajpsaj.2011.116.123.

Basit, M. A., Arifah, A. K., Loh, T. C., Saleha, A. A., Annas, S., Kaka, A. and Banke, I. S. (2020). Effects of graded dose dietary supplementation of Piper bettle leaf meal and Persicaria odorata leaf meal on growth performance, apparent ileal digestibility, and gut morphology in broilers. *Saudi Journal of Biological Sciences*. 27(6). Doi: 10.1016/j.sjbs.2020.04.017.

Berdos, J. I., Martin, E. A., Celestino, O. F. and Paragas, E. J. S. 2019. Egg production performance of improved Philippine Mallard ducks (Anas platyrhynchos) fed diets supplemented with fresh Trichanthera (*Trichanthera gigantea*) leaves. *Philippines Journal of Veterinary Animal Science*. 45(1), 48-57.

Bryan, D. D. S. L. and Classen, H. L. (2020). In vitro methods of assessing protein quality for poultry.

Edwards, A., Mlambo, V., Lallo, C. H. O. and Garcia, G. W. (2012). Yield, chemical composition and In Vitro Ruminal Fermentation of the Leaves of *Leucaena Leucocephala*, *Gliricidia Sepium* and *Trichanthera Gigantea* as influenced by harvesting frequency. Journal of Animal Science Adv. 2(3.2), 321-331. ISSN: 2251-7219.

Elbayoumi, Z. H., Shawish R. R., Hamada, M., and Esmail, H. R. (2018). Molecular characterization of Escherichia coli isolated from poultry meat and its products. *Alexandria Journal of Veterinary Science*, 56(2), 39-47.

Ezeonu, C. S. and Ejikeme, C. M. 2016. Qualitative and quantitative determination of phytochemicals contents of indigenous Nigerian softwoods. *New Journal of Science*. http://dx.doi.org/10.1155/2016/5601327

Francisco, F. Buctot, Jr. (2018). Growth response and carcass evaluation of broilers fed with varying levels of *Trichanthera gigantea* leaf. *Journal of Science Technology*, 4, 17-22.

Garcia, D. E., Medina, M. G., Dominquez, C., Baldizan, A. and Luis Cova, J. H. y. (2006). Chemical evaluation of non-leguminous species with fodder potential in Trujillo state, Venezuela. Zootecnia Tropical. Instituto Nacional De Investigaciones Aricolas Venezuela. [online]. 24(4), 401-415. ISSN 0798-7269.

Gehrt, A. J., Caldwell, M. J. and Elmslie, W. P. (1955). Feed digestibility, chemical method for measuring relative digestibility of animal protein feedstuffs. *Journal of Agric. Food Chemistry*. 3, 159-162.

Hien, T. Q., Hoan, T. T., Khoa mai Anh, Kien, T. T., huong, P. T., Nhung, H. T. H. (2017). Nutrient digestibility determination of cassava, Leucaena, Stylosanthes, Moringa and Trichantera gigantea leaf meals in chickens. Bulgarian Jpournal of Agricultural Science. 23(3), 476-480. Hien, Q. T., Kien, T. T. and Hoan, T. T. 2013. "Determination of the appropriate cutting intervals for *Brachiria decumhens* basilisk grown in Thai Nguyen". TNU Journal of Science and Technology. 104(4), 23-27.

Hien, Q. T., Hoang, T. H. N. and Trung, T. Q. (2019). "Determination of optimum harvesting interval for *Moringa oleifera* in first year production". *Vietnam Journal of Science and Technology. Ministry of Science and Technology*. 61(5), 42-47.

Hirschmann, R. (2022). Per capita poultry consumption in Malaysia 2006-2025. Published April 14. Online. https://www.statista.com/statistics/757983/malaysia-poultry-consumption-per-capita/

Hongyan, L., Rong, T. and Zeyuan, D. (2012). Factors affecting the antioxidant potential and health benefits of plant foods. *Canadian Journal of Plant Science*. https://doi.org/10.4141/cjps2011-239.

Hue, K. T., Van, D. T. T., Ledin, I., Wredle, E. and Sporndly, E. (2012). Effect of harvesting frequency, variety and leaf maturity on nutrient composition, hydrogen cyanide content and Cassava foliage yield. *Asian-Aust Journal of Animal Sciece.* 25(12), 1691-1700. http://dx.doi.org/10.5713/ajas.2012.12052

Hueze, V., Tran, G., Boudon, A. and Bastianelli, D. (2016). Nacedero (Trichanthera gigantea). Feedipedia, a program by INRA, CIRAD, AFZ and FAO. http://www.feedipedia.org/node/7270 Last updated on February 25, 2016, 4.53 pm.

Jaya, A. F., Soriano, M. L. and Carpentaro, B. B. (2009). Utilization of Madre de Agua (*Trichantera gigantea* var guianensis) leaf meal as feed for growing-finishing pigs. *Philipines Journal of Veterinary and Animal Science.*

Kgosana, K. G. (2019). The effects of extraction techniques and quantitative determination of oxalates in Nerium oleander and feeds. *Onderstepoot Journal of Veterinary Research*. 86(1), 1-9. https://doi.org/10.4102/ojvr.v86i1.1611.

Kien, T.T., Hien, T. Q., Huan, T. T. and Nhung, T. T. (2010). "Study the effect of different cutting intervals on the ability to receive, the grate of grass used and the digestibility of some imported grass species (*P. atranum, B. brizantha, B. decumhens*) on beef cattle". *TNU Journal of Science and Technology*. 67(5), 109-112.

Kien, T. T., Khoa, M. A., Han, T. T. and Hien, T. Q. (2020). Effect of cutting intervals on yield and quality of the green fodder *Trichantera gigantea*. *AGROFOR International Journal*. 5(1), 22-29.

Law, F. L. (2018). Effects of supplementing protease in low protein and low energy diets on growth performance and physiological responses of broiler chicken under different environmental conditions. PhD Thesis, Universiti Putra Malaysia.

Laudadio, V., Passantino, L., Perillo, A., Lopresti, A., Passantino, A., Khan, R. U. and Tufarelli, V. (2012). Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Production, modelling and education. *Poultry Science*. 91, 265-270.doi:10.3382/ps.2011-01675.

Leterme, P., Angela, M. L, Fernando, E., Wolfgang B. S, and Andre Buldgen (2005). Chemical composition, nutritive value and voluntary intake of tropical free foliage and cocoyam in pigs. Journal of Science Food Agriculture 85, 1725-1732.

Lisnahan, C. V., Wihandoyo, Zuprizal, and Sri Harimurti (2017). Growth performance of native chickens in the grower phase fed methionine and lysine-supplemented cafeteria standard feed. Pak. J Anim Nutr. 16, 940-944.

Li, Y., Kui-Shan, W., Xioa, R., Ying-Xian, Z., Feng, W. and Qiang, W. (2018). Response of plant secondary metabolites to environmental stress. *Molecules.* 23, 762; doi. 10.3390/molecules23040762.

Ly, J., Chhay, Ty., Phiny, C. and Preston, T. R. (2001). Some aspects of the nutritive value of leaf meals of Trichanthera gigantea and Morus alba for Mong Cai Pigs. *Livestock Research for Rural Development*.

Mohanty, S., Mohapatra, S. and Acharya, G. (2020). Comparative haematology and biochemical parameters of indigenous and broiler chicken. *International Journal of Scientific & Technology Research.* 9(4), 972-979.

Morbos, C. E., Espina, D. M., and Bestil, M. E. (2016). Growth performance of Philippine native chicken fed diet supplemented with varying levels of Madre de agua (Trichanthera gigantea Nees) leaf meal. *Annals of Tropical Research* 38(1), 174-182.

Morgan, N. K., Scholey, D. V. and Burton, E. J. (2014). A comparison of two methods for determining titanium dioxide marker content in broiler digestibility studies. *Animal.* 8(4), 529-533. doi:10.1017/S1751731114000068.

New Straits Times (2022). https://www.nst.com.my/opinion/letters/2022/03/776759/ketum-ayam-can-be-new-source-protein-poultry. Published online March 4, 2022.

Nouman, W. (2012). Biomass production and nutritional quality of Moringa oleifera as field crop. Turk Agric Forestry. 37, 410-419.

NRC (1994). Nutrient Requirements of poultry. Ninth Revised Edition, 1994, National Academy Press, Washington D. C.

New Straits Times (2022). https://www.nst.com.my/opinion/letters/2022/03/776759/ketum-ayam-can-be-new-source-protein-poultry. Published online March 4, 2022.

Nouman, W. (2012). Biomass production and nutritional quality of Moringa oleifera as field crop. Turk Agric Forestry. 37, 410-419.

NRC (1994). Nutrient Requirements of poultry. Ninth Revised Edition, 1994, National Academy Press, Washington D. C.

Nwosu, J. N., Ubaonu, C. N., Banigo, E. O. I. and Uzomah, A. (2010). The effects on processing on the anti-nutritional properties of 'oze' (Bosqueia angolensis) seeds. *New York Science Journal* 3(9), 106-111.

Perveen, F. and Zaib, S. (2013). Phytochemical and spectrophotometric analyses of the Hill Toon, Cedrela Serrata Royle methanolic leaves extract and its fraction. International Journal of Agriculture Innovations and Research, 1(5), ISSN (Online) 2319-1473.

Pesti, G. M. (2009). Impact of dietary amino acid and crude protein levels in broiler feeds on biological performance. Journal of Applied Poultry Research 18(3), 477-486.

Pietras, M., Orczewska-Dudek, S., Szczurek, W. and Pieszka, M. (2021). Effect of dietary lupine seeds (Lupinus luteus L.) and different insect larvae meals as protein sources in broiler chicken diet on growth performance, carcass, and meat quality. Livestock Science. 250, 104537. https://doi.org/10.1016/j.livsci.2021.104537

Preston, T. R. (1995). Tropical animal feeding. A manual for research workers. *FAO Animal Production and Health Paper* No. 126. p. 305.

Raipuria, N., Kori, D., Saxena, H. O., Pawar, G., and Choubey, S. K. (2018). Qualitative and guantitative evaluation of secondary metabolites in leaves, roots, and stem of *Cleome viscosa L. International Journal of Green Pharmacy*, 12(4), 285-290.

Ravidran, G. and Ravindran, V. (1988). Changes in the nutritional composition of cassava (*Manihot esculenta* Crantz) leaves during maturity. *Food Chemistry*, 27, 299-309.

Rodriquez, R., Gonzalec, N., Alonso, J., Dominquez, M. and Sarduy, L. (2014). Nutritional value of foliage meal from four species of tropical trees for feeding ruminants. *Cuban Journal Of Agricultural Science.* 48(4), 371-378.

Rosales, M. (1997). *Trichantera gigantea* (Humboldt & Bonpland.) *Nees*: A review Livest. Res. Rural Dev., 9(4).

Riascos-Vallejos, A. R., Reyes-Gonzalez, J. J. and Aquirre-Mendoza, L. A. (2020). Nutritional characterization of trees from amazonian piedmont Putumayo department, Colombia. Cuban Journal of. Agricultural Science 54, 257-265.

Saguidan, J. A. (2015). Growth performance of pigs fed grower diets with madre de agua (Trichanthera gigantea) leaf meal. Thesis/ Dissertation. https://www.ukdr.uplb.edu.ph/etd-undergrad/1431. Retrieved 30 August 2022. 2.01 am.

Sarwatt, S. V., Laswai, G. H., and Ubwe, R. (2003). Evaluation of potential of Trichanthera gigantea as a source of nutrients for rabbit diets under small-holder production system in Tanzania.

Singleton, V. L. and Rossi, J. A. (1965). Calorimetry of total phenols with Phosphomolydic. Phosphotungstic acid reagents. American Journal of Enology and Viticulture. 16(3), 144-158.Sreeramulu, D. and Raghunath, M. 2011. Antioxidant and phenolic content of nuts, oil seeds, milk and milk products commonly consumed in India. *Food and Nutrition Science*. 2, 422-427.

Tran, N. T. B. (2003). Effect of age of leaves from forage trees on nutritive value. MEKARN_Miniprojects_2003.

Wang, F., Zuo, Z., Chen, K., Gao, C., Yang, Z., Zhoa, S., Li, J., Song, H., Fang, J., Cui, H., Ouyang, P., Zhou, Y., Shu, G. and Jing, B. (2018). Histological injuries, ultrastructural changes, and depressed TLR expression in the small intestine of broiler chickens with Aflatoxin B1. *Toxins*. 10, 131. doi:10.3390/toxins10040131