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Complete Feed Silage Innovation: Utilization of Agro-Industry By-Products with Chestnut Tannin as Additives

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Abstract. This study evaluated the effect of chestnut tannin as a silage additive on agro-industrial by-products for animal feed. The research utilized a Completely Randomized Design with five treatments and five replications. The treatments were T1 (Complete Feed as control) and T2, T3, T4, and T5 with chestnut tannins at 0.50%, 1%, 1.50%, and 2% DM, respectively, all fermented for 30 days at room temperature. Observed parameters included proximate analysis (crude protein, crude fat, crude fiber, and ash), temperature, humidity, mold growth, dry matter loss, physical quality of silage (texture, aroma, color), and fresh silage quality. The data were analyzed using variance analysis and DMRT at a 5% significance level. The results showed that the addition of 2% chestnut tannin can produce silage with relatively higher crude protein, while oil fat and crude fiber are relatively the same as other treatments; however, the addition of 2% chestnut tannin tends to produce silage with ash content relatively lower than other treatments. Chestnut tannins significantly affected ($P<0.05$) moisture, texture, aroma, ammonia, and total VFA of fresh silage. In conclusion, adding 1.50-2% chestnut tannins to complete feed silage reduces dry matter loss, protects crude protein, maintains physical quality, inhibits mold growth, and stabilizes temperature, resulting in high-quality silage.

Keywords: Ammonia, chestnut tannin, complete feed, nutrients, silage, Total VFA.

Abstrak. Penelitian ini menginvestigasi dampak penambahan tanin chestnut sebagai aditif silase terhadap pemanfaatan produk samping agroindustri untuk pakan ternak. Rancangan yang digunakan adalah Rancangan Acak Lengkap dengan 5 perlakuan dan 5 ulangan. Perlakuan terdiri dari: T1 (Pakan Komplit sebagai kontrol) dan T2, T3, T4, T5 dengan penambahan tanin chestnut masing-masing sebesar 0,50%, 1%, 1,50%, dan 2% DM, semua di ensilase selama 30 hari pada suhu kamar. Perlakuan terdiri dari: T1 (Pakan Komplit sebagai kontrol) dan T2, T3, T4, T5 dengan penambahan tanin chestnut masing-masing sebesar 0,50%, 1%, 1,50%, dan 2% DM, semua diensilase selama 30 hari pada suhu kamar. Parameter yang diamati meliputi uji proksimat (protein kasar, lemak kasar, serat kasar, dan abu), suhu, kelembapan, pertumbuhan jamur, kehilangan bahan kering, kualitas fisik silase (tekstur, aroma, warna), dan kualitas silase segar. Data dianalisis menggunakan analisis ragam dan uji DMRT pada tingkat signifikansi 5%. %. Hasil penelitian menunjukkan bahwa penambahan tanin chestnut 2% dapat menghasilkan silase dengan protein kasar relatif lebih tinggi, sedangkan lemak kasar dan serat kasar relatif sama dengan perlakuan lainnya, namun demikian penambahan tanin chestnut 2% cenderung menghasilkan silase dengan kadar abu relatif lebih rendah dari perlakuan lain. Tanin chestnut berpengaruh signifikan ($P<0,05$) terhadap kelembapan, tekstur, aroma, amonia, dan total VFA silase segar. Kesimpulannya, penambahan tanin chestnut 1,50-2% pada silase pakan komplit mampu mengurangi kehilangan bahan kering, melindungi protein kasar, menjaga kualitas fisik, menghambat jamur, dan menstabilkan suhu, sehingga menghasilkan silase berkualitas baik.

Kata kunci: Amonia, nutrien, pakan komplit, silase, tanin chestnut, total VFA.

Introduction

Complete feed is a formulated ration composed of various feed ingredients containing all essential nutrients that livestock needs (Gómez et al., 2021). The complete feed has long been applied in the beef and dairy cattle industry (Genís et al., 2021) to meet the nutritional needs of cattle, especially during the growth and production phases (Sabariah et al., 2018). Perishable agro-industrial by-products can be utilized as components in formulating complete feed. For example, mixing wet agro-industrial by-products with relatively dry feed ingredients can be processed into silage to make the complete feed (Sadarman et al., 2019a).

Silage is a high-moisture feed produced through fermentation for ruminant livestock (Rossi et al., 2023). Silage is generally made of the whole plants of grasses (*Gramineae*) like corn, sorghum, and other cereals (Jung et al., 2024), oil palm leaves, cassava, rice, ramie, market waste, and agro-industrial by-products such as soy sauce pulp, tofu pulp, and brewery waste (Sadarman et al., 2019b).

The process of making silage, known as ensilage, involves placing chopped forage and agro-industrial by-products into a silo, sealing it to create anaerobic conditions, and allowing bacterial fermentation to the materials (Carvalho et al., 2024). The main objective of ensilage is to reduce protein degradation (proteolysis), which can be controlled by adding plant metabolites, such as tannins (Irawan et al., 2021; Sadarman et al., 2020) to bind proteins (Santoso et al., 2020). Excessive tannins may lower feed palatability (Palacios et al., 2021), but at an appropriate level, tannins can improve ruminant metabolism (Brutti et al., 2023), inhibit protein degradation or proteolysis in feed materials during ensiling, thus preserving the nutrient content of the feed (Niderkorn et al., 2019). Sadarman et al. (2020) reported that chestnut tannin has evidently improved the physical quality of soy sauce pulp silage, as seen in its aroma, color, mold growth, and dry matter loss. Also, Sadarman et al. (2024) indicate that adding 5% fine rice bran and 1% chestnut tannin into tofu waste silage resulted in the best physicochemical quality, reflected in the lowest dry matter loss compared to other treatments. Therefore, both condensed tannins and chestnut tannins can enhance the physical quality of complete feed silage by inhibiting spoilage microbes, preserving the characteristic fermentation aroma, maintaining texture, and stabilizing color. Additionally, tannins can prevent mold growth, ensuring the safety and quality of the silage during storage.

To our knowledge, studies on chestnut tannins as silage additives for complete feed are limited, except for ones exploring the general effects of tannins on feed preservation. The specific impact of chestnut tannins on nutrient retention and physical quality in complete feed silage remains understudied. This gap highlights the need for further research to optimize silage formulation for livestock feed and observe the potential of tannin as a silage additive to protect proteins from degradation during ensilage. This study determined the effect of adding chestnut tannins as a silage additive on the nutrient content and quality of complete forage silages, observing the nutrient content, silage temperature and humidity, aroma, color, texture, mold growth, dry matter loss, and fresh silage quality. Results of this study are expected to lend valuable insights into improving silage formulation which contributes to enhanced feed efficiency, livestock health, and sustainability in agro-industrial by-product utilization.

Materials and Methods

The Research Location

The chestnut production and harvest, pH testing, and physical quality assessment were conducted at the Nutrition and Feed Technology Laboratory of the Faculty of Agriculture and Animal Science, Universitas Islam Negeri Sultan Syarif Kasim, Riau. The proximate analysis was carried out at the Animal Nutrition Laboratory, Faculty of Animal Science, Universitas Andalas, Padang. Fresh silage tests were undertaken at the Dairy Cattle Laboratory, Faculty of Animal Science, IPB University, Bogor. Each laboratory was selected for its specialized equipment and expertise in the respective analysis.

The Research Materials

The materials for this were field grass, oil palm fronds, palm kernel cake (PKC), tofu pulp, molasses, salt, urea, and CaCO3. Chestnut tannins were incorporated to inhibit protein degradation and improve silage quality (Irawan et al., 2021). The bark and the wood are the primary part of the chestnut plant (Castanea spp.) to produce tannins. The tannin-rich chestnut bark is frequently used in the leather tanning industry because the tannins provide a rich brown color and is a natural preservative. Tannins are also present in the chestnut seeds but at a much lower concentration than the bark and the wood. The feed ingredients were formulated to meet the required 14% crude protein and 61% Total Digestible Nutrient (McDonald et al., 2022). Other materials were utilized in the proximate analysis and fresh silage testing. The equipment used was silage-making tools like silos and instruments to measure variables such as temperature, pH, and silage quality, including fresh silage.

The Research Designs

This study utilized an experimental approach in a Completely Randomized Design with five treatments and five replications to ensure statistical reliability and account for variability between samples. The treatments involved the preparation of complete feed silage with the addition of tannins as a silage additive. The treatments were T1: Complete Feed Silage (control); T2: T1 + 0.50% chestnut tannins based on dry matter (DM); T3: T1 + 1% chestnut tannins based on DM; T4: T1 + 1.50% chestnut tannins based on DM; and T5: T1 + 2% chestnut tannins based on DM.

Research Procedure

The complete feed silage was prepared according to the method by Sadarman et al. (2023a). The complete feed was wilted and evaluated for its dry matter content, then weighed according to the silo capacity (1.50 kg on the laboratory scale). The feed was then placed into a container, mixed with tannins, stirred thoroughly then poured into the silo. The silo was then sealed tightly to ensure anaerobic conditions. The silo was then kept away from sunlight for 30 days at a controlled room temperature (25°C) throughout the fermentation period. After 30 days, the silage was harvested and presented to 60 untrained respondents. A portion of the silage was dried in an oven at 105°C and ground to 1 mm for proximate analysis.

Measured variables

The physical quality of the silage was assessed by observing 60 untrained respondents, who were healthy students from the Animal Science Program at the Universitas Islam Negeri Sultan Syarif Kasim Riau, and had no impairments in vision, smell, or touch (Sadarman et al., 2024). The use of untrained respondents was intended to simulate the real-world conditions in which livestock caretakers, who may not be experts, assessed the silage quality. Measures were taken to minimize bias by providing clear assessment criteria with the method of Sadarman et al. (2023b). The observation scores are presented in Table 1.

The nutrient content of the silage was analyzed using proximate methods (AOAC, 2019). Temperature was measured with a cleaned thermometer inserted into the silo for 1 minute (Sadarman et al., 2023b). Humidity was calculated by comparing the wet and dry weights of the silage (Moore, 2018). Acidity was measured by blending the silage with distilled water and using a digital pH meter (Bernardes et al., 2019). Ammonia concentrations were measured using the Conway microdiffusion method (Conway, 1993). Total VFA levels were assessed by gas chromatography (Katoch, 2023). Samples were taken at days 30 to monitor changes in temperature, pH, ammonia, and VFA concentrations over time.

Table 1. Score values of vegetable market waste silage criteria

Criteria	Silage Characteristics	Score
Smell	Not fresh	1-1.99
	Fresh	2-2.99
	Distinct aroma of silage	3-3.99
Texture	Fine clumping	1-1.99
	Moderately slightly clumping	2-2.99
	Smooth non-clumping	3-3.99
Colour	Dark brown	1-1.99
	Light dark brown	2-2.99
	Brownish yellow	3-3.99
Fungal Growth	A lot, more than 5% of the total silage	1-1.99
	Sufficient, >2% of total silage	2-2.99
	No mold found in silage	3-3.99

Data analysis

Proximate analysis data were described, while other data was processed using the Statistical Package for the Social Sciences (SPSS) version 26.0. If significant differences were found through analysis of variance, further testing was conducted using Duncan's Multiple Range Test (DMRT) at a 5% significance level.

Results and Discussion

Nutrient Content of Complete Feed Silage

Nutrients in feed are important organic compounds for livestock, absorbed by the post-rumen and small intestine in the forms of energy, crude protein, crude fat, crude fiber, ash, and other nutrients. The primary goal of silage production is not to increase the nutrient content of the silage material, but rather to minimize nutrient loss during the ensiling process. Data on the nutrient content of complete feed silage is presented in Table 2.

The data in Table 2 show that the complete feed produced silages with nutrient contents that varied across treatments. The average value of the crude protein was 16.1-17.9%, crude fat was 4.02-4.86%, crude fiber was 14.6-17.8%, and ash was 4.72-5.65%. This result confirms a previous study that chestnut tannins incorporated to complete feed can maintain high protein content by protecting crude protein from degradation (Niyigena et al., 2024). Tannins tend to lower crude fat by reducing fat available for microbial fermentation and by binding to nutrients, which reduces fat degradation by microbes and consequently, less fat is available for digestion (Fonseca et al., 2024). While tannin can optimize crude fibre content by slowing down fibre degradation, it does not significantly affect the ash content, which remains normal (Battelli et al., 2024). Overall, tannin derived from chestnut tannin and other types of tannin can be added to silage making because tannin is able to reduce crude fat, optimize crude fiber by slowing t fiber degradation, and maintain ash content within normal limits according to Zotte et al. (2018).

Table 2. Effect of treatment on nutrient content of complete feed silage

Treatments	Silage Nutrient (%)			
	CP	C Fat	C Fiber	Ash
T1: Complete Feed	17.2	4.86	16.3	5.12
T2: T1 + 0.50% chestnut tannin based on DM	16.2	4.67	17.1	5.02
T3: T1 + 1% chestnut tannin based on DM	17.9	4.84	17.8	5.65
T4: T1 + 1.50% chestnut tannin based on DM	16.1	4.02	14.6	5.04
T5: T1 + 2% chestnut tannin based on DM	17.7	4.29	15.4	4.72

Description: CP = Crude Protein, CF = Crude Fat, CF = Crude Fiber

Table 3. Temperature, moisture, fungi, and DM loss in complete feed silage

Treatments	Silage Temperature (°C)	Silage Humidity (%)	Fungi in Silage	DM Lose (%)
T1: Complete Feed	29.3±0.42	75.4±0.14^{a}	3.69±0.06	1.77±0.87
T2: T1 + 0.50% chestnut tannin based on DM	29.2±0.12	73.3±0.09^{b}	3.71±0.04	1.32±0.09
T3: T1 + 1% chestnut tannin based on DM	29.1±0.13	70.1±0.08^{c}	3.74±0.03	1.31±0.34
T4: T1 + 1.50% chestnut tannin based on DM	29.0±0.05	68.2±0.04^{d}	3.75±0.02	1.23±0.36
T5: T1 + 2% chestnut tannin based on DM	29.0±0.05	63.3±0.01^{e}	3.76±0.04	1.28±0.29

DM: Dry Matter. The presented data are mean values ± standard deviation. Different superscripts in the same column indicate significant differences (P<0.05) in silage moisture among treatments

Apart from that, tannin helps protect nutrients such as crude protein from excessive degradation in order to produce silage with good nutrient quality and support an optimal fermentation process (Ibrahim and Hassen, 2022).

Temperature, Humidity, Fungi, and DM Loss of Complete Feed Silage

The temperature of silage during harvesting can vary depending on several factors, including the type of plants used for silage, weather conditions during harvesting, and the silage storage method. The crop silage temperature, moisture, fungi and DM loss are presented in Table 3.

Temperature of the Complete Feed Silage

The addition of chestnut tannin in making complete feed silage did not show a significant effect (P>0.05) on temperature, indicating that chestnut tannins do not interfere with the natural fermentation process, maintaining stable conditions for microbial activity. The average silage temperature after 30-day anaerobic fermentation ranged between 29 °C (T4 and T5) and 29.3 °C (T1). It shows that chestnut tannins can support the activity of good bacteria in the fermentation of enzylated complete feed.

The increase in temperature during ensiling is due to microbial activity in the silo (McDonald et al., 2022). When substrates are available, microbes become active, and the temperature inside the silo rises (Tian et al., 2020). McDonald et al. (2022) stated that materials in silage production undergo four stages: aerobic state, microbial activity, stabilization, and harvest. Temperature rise in the silo usually occurs in the second stage, where microbial activities are present and needs substrates for feeding (Saha and Patak, 2021).

Sadarman et al. (2024) reported that chestnut tannin, as an additive to tofu bagasse silage, can optimize temperature to a normal range of 27.5-30.5°C, which confirms McDonald et al. (2022) that the normal temperature for silage is 26-30°C. it is evident that chestnut tannins for complete feed silage can keep the temperature within a normal range. The contributing factors to temperature rises during ensiling are the materials for ensilage, the materials' nutrient content, and others (Rossi et al., 2023).

Humidity of the Complete Feed Silage

Moisture content during ensiling represents the feed's humidity level when stored as silage, which is crucial for both ensiling success and silage quality. The results of analysis of variance (see Table 4) shows that chestnut tannins significantly influenced (P<0.05) the humidity of complete feed silage, with an average silage humidity at harvest of 63.3% to 75.4%. Duncan's Multiple Range Test showed that the humidity of T1 (control) was higher than that of T2, T3, T4, and T5, with significant differences observed in T4 and T5. These differences may stem from the impact of chestnut tannins on fermentation and the feed's water absorption and retention capacity.

Tannins possess astringent qualities that lower the water absorption in feed, reducing the moisture of silage with tannins (T2, T3, T4, and T5) compared to the tannin-free control (T1). Tannins inhibit microbial enzymatic activity by forming complexes with proteins and polysaccharides, thus reducing water availability for microbial metabolism and lowering the moisture content in the silage. Tannins can also inhibit microorganisms, like lactic acid bacteria, which are crucial for fermentation. This slows the ensiling process results in less water formation (Jung et al., 2024), so silage added with tannin has lower moisture than the control.

Hynd (2019) highlights that proper moisture in ensiled forage is vital for supporting microbial activity during anaerobic fermentation. Optimal moisture promotes fermentative microbes, like lactic acid bacteria, which aid feed conversion into silage. Inadequate moisture will disrupt microbial processes and lower silage quality. McDonald et al. (2022) note that fungi will grow at a minimum level when silo is maintained at an optimal humidity. Dryden (2021) suggests that 60-70% humidity is ideal for grass silage, while corn silage thrives at 65-70% moisture.

Fungal Growth in Complete Feed Silage

Fungi are heterotrophic organisms without chlorophyll and can be either unicellular or multicellular. The results of variance analysis in Table 4 shows that chestnut tannins used as a silage additive do not significantly impact (P>0.05) fungal growth. The average fungal growth in this study ranged from 3.69 to 3.76, with the highest score in complete feed silage without chestnut tannins, and the lowest in treatments with 2% chestnut tannins (DM). The reduced fungal growth may be attributed to chestnut tannins accelerating LAB growth, which acidifies the silo and speeds up ensiling.

Irawan et al. (2021) noted that fast LAB growth is necessary for acidic conditions in the silo. McDonald et al. (2022) explained that an acidic environment in the silo suppresses harmful bacteria like *Clostridia sp.*, which coincides with lower fungal growth in ensiled materials. Sadarman et al. (2019b) demonstrated that tannins from acacia and chestnut significantly reduced fungal growth in soy pulp silage. Similarly, Chen et al. (2021) found that tannic acid enhances alfalfa silage quality by inhibiting microbial metabolism, thus reducing fungal growth. The level of fungal growth in silage can be influenced by the high moisture content of the material (McDonald et al.,2022) and protein degradation during ensiling. Chestnut tannins are a component of silage additives that can act as inhibitors of harmful microbial growth during the ensiling process within the silo (Sadarman et al., 2020).

Dry Matter Loss of Complete Feed Silage

Dry matter loss in silage refers to the reduced dry weight of feed during fermentation, which affected by factors including silage additives. In this study, the addition of chestnut tannin did not show a statistically significant effect on dry matter loss in complete feed silage (P>0.05) but tend to reduce dry matter loss. The average dry matter loss ranged between 1.23% (chestnut tannin treatments) and 1.77% (control). The 1.50% DM tannin treatment showed the lowest dry matter loss across treatments, suggesting that chestnut tannin can effectively reduce dry matter loss during the ensiling. A lower dry matter is believed to be due to the inhibitory effect of chestnut tannin on microbial metabolism, which helps maintain the silage quality.

Table 4. Physical quality of complete feed silage

Treatments	Smell	Texture	Colour
T1: Complete Feed	2.96±0.10^{b}	2.81±0.02^{b}	2.98±0.05
T2: T1 + 0.50% chestnut tannin based on DM	3.01±0,01^{b}	2.86±0.03^{b}	2.98±0.93
T3: T1 + 1% chestnut tannin based on DM	3.05±0.02^{b}	2.97±0.07^{a}	2.99±0.14
T4: T1 + 1.50% chestnut tannin based on DM	3.09±0.08^{a}	2.98±0.06^{a}	3.03±0.08
T5: T1 + 2% chestnut tannin based on DM	3.11±0.12^{a}	3.04±0.15^{a}	3.07±0.02

DM: Dry Matter. Data presented means ± standard deviation. Different superscripts in the same column indicate significant differences (P<0.05) in silage aroma, texture and color between treatments.

Chen et al. (2021) reported the addition of tannic acid can increase dry matter in ensiled materials by limiting microbial activity during anaerobic fermentation. Tian et al. (2022) further explained that proteolysis contributes to DM loss as plant proteases convert proteins into peptides and amino acids, which are then broken down into amines, amides, and NH3 by microbes. Significant proteolysis during ensiling can lower silage quality and subsequently increase dry matter loss (Moore, 2018). Leon-Tinoco et al. (2022) report critical need to minimize dry matter losses in silage, as excessive losses can compromise its quality and suitability for livestock consumption. Maintaining low DM loss is essential to ensure the nutritional value and overall effectiveness of silage as feed. Hassoun et al. (2021) recommended DM forage for ruminants to maximize intake and reduce bulkiness, which slows rumen digestion and causes shallow breathing due to the pressure on the diaphragm. McDonald et al. (2022) noted DM loss between 1-5% during ensiling is linked to excessive fungal growth.

The Physical Quality of Complete Feed Silage

The physical quality of complete feed silage assessed from smell, fungal growth, texture, and colour. The scores of each variable are presented in Table 4.

The Smell of Complete Feed Silage

The results of the Duncan's test showed that the aroma of the complete feed silage with the addition of 0.50-1% DM chestnut tannins was not different from that of the control. However, as the level of chestnut tannins increased, the silage aroma became more yeasty. Therefore, the addition of 1.50% and 2% DM chestnut tannins resulted in better aroma than the control and the 0.50-1% DM treatments. It proves that adding chestnut tannins can enhance the yeasty aroma of the silage.

Previous studies reported that chestnut tannins can produce silage with distinctive fermentation aroma (Niyigena et al. (2024) that using two types of tannins (hydrolysable and condensed) produced soy sauce waste silage with sour aroma (Sadarman et al., 2019b). Chestnut tannins work by limiting protein degradation & minimizing the population of harmful bacteria (McDonald et al., 2022).

Texture of Complete Feed Silage

Texture is a physical characteristic of feed that can influence digestibility (Sadarman et al., 2024). This study showed the significant effect (P<0.05) of incorporating chestnut tannin into the complete feed silage on the silage texture, which ranged between medium and soft (2.81-3.04). The Duncan's test results showed that the texture of the complete feed silage was not different across treatments, but a significant difference was observed in the texture of control and 0.5% DM chestnut tannin from that of 1%, 1.50%, and 2% DM. The best results were obtained in the 2% DM chestnut tannin although not significantly different from that of 1% and 1.50%. Therefore, a good silage texture is achievable by adding chestnut tannins in the range of 1-2% DM. Pereira da Silva et al. (2021) stated that soft texture of silage added with chestnut tannin is more palatable and digestible by the ruminants, and can improve feed intake.

Chestnut tannin as silage additives can inhibit the metabolism of harmful microbes (Sadarman et al., 2020), so that good microbes such as LAB can grow optimally (Irawan et al., 2021). The anaerobic fermentation process by microbes produces heat (McDonald et al., 2022), which helps change the texture of the material from hard to soft (Yin et al., 2021). Chen et al. (2021) reported that using tannic acid as a silage additive can improve the quality of alfalfa silage, especially the texture.

Color of Complete Feed Silage

The color of the silage material can be an indicator of silage quality. The analysis of variance showed that the addition of chestnut tannin up to 2% DM had no significant effect (P>0.05) on the color of complete feed silage. Lack of significant color differences suggests that the addition of chestnut tannins does not alter the visual appeal of the silage, which remains within acceptable ranges for livestock feed. It may be due to the inherent color of the feed ingredients, which dominate over any changes caused by tannins. The color score in this study ranged from 2.98 to 3.07, indicating a change in silage color from brownish to dark brown due to ingredients.

According to McDonald et al. (2022), silage color is substantially influenced by the color of the additives. Silage made of soy sauce dregs with the addition of chestnut tannins is dark brown to almost black (Sadarman et al., 2019b), while silage made from tofu dregs and fine rice bran with chestnut tannin had a brownish color that resembled the chestnut tannin (Sadarman, 2024). In addition, heat produced from also affects the color (Tian et al., 2020).

Quality of Fermentation

The quality of silage fermentation reflects the success of the fermentation process in preserving feed ingredients. Good quality silage is characterized by a low pH (3.80-4.20), a predominance of lactic acid, and low levels of ammonia-N and butyric acid, which indicate minimal protein damage and undesirable fermentation. Good silage also has a fresh sour aroma, soft texture, and is free from mold and damage. These factors ensure that silage is safe and nutritious to feed livestock. The quality of fermentation can be assessed based on pH, ammonia, and VFA total (see Table 5).

pH Content of Fresh Silage

The addition of chestnut tannin did not significantly affect the pH of the complete feed silage (P>0.05) because chestnut tannins maintain the ensiling process consistently, regardless of their concentration. In this study, the pH of the complete feed silage ranged from 4.49 to 4.52, which is within the optimal range for good silage. McDonald et al. (2022) highlight that silage quality is often evaluated based on pH levels. Acidification occurs through the enhancement of lactic acid bacteria (Dryden, 2021), and the higher the bacteria population the faster the bacteria reduce pH and undesirable bacteria (Saha and Pathak, 2021). Chestnut tannins act as inhibitors, limiting harmful bacterial growth and thus preserving protein substrates. As a result, the pH was acidic.

Table 5. Fermentation Quality of Tanin Chestnut Fermentation

Treatments	pH Silage	Ammonia (mM)	VFA Total (mM)
T1: Complete Feed	4.49±0.04	3.41±0.15[c]	34.4±5.53[a]
T2: T1 + 0.50% chestnut tannin based on DM	4.49±0.01	3.69±0.25[d]	52.2±4.52[b]
T3: T1 + 1% chestnut tannin based on DM	4.47±0.02	2.81±0.23[b]	58.6±4.52[bc]
T4: T1 + 1.50% chestnut tannin based on DM	4.49±0.05	2.64±0.15[ab]	62.6±4.52[c]
T5: T1 + 2% chestnut tannin based on DM	4.52±0.03	2.48±0.19[a]	64.7±5.53[c]

DM: Dry Matter. Data presented means ± standard deviation. Different superscripts in the same column indicate significant differences (P<0.05) in ammonia and total VFA between treatments.

Sadarman et al. (2020) found that both hydrolyzable and condensed tannins can produce soy sauce pulp silage with pH 5.88-6.40, indicating a shift from weakly acidic to near-neutral pH. According to Sadarman et al. (2022), adding up to 2% chestnut tannin did not significantly affect the pH of corn cob silage, but the resulting silage still maintained good quality with an acidic pH of about 3.64 to 3.78. These studies suggest that chestnut tannins can act as additives that inhibit the growth of undesirable bacteria, thus helping to maintain the silage pH in an acidic range. McDonald et al. (2022) classify silage additives into two types: stimulants, which have high glucose content and stimulate the growth of lactic acid bacteria and other beneficial bacteria, and inhibitors, which suppress the growth of Clostridia sp. and allow beneficial bacteria to thrive during ensiling.

The pH of silage can vary towards either acidic or basic levels, depending on the type of feed and the materials being ensiled. According to Hartinger et al. (2024), silage made from grasses typically becomes more acidic, while silage made from legumes with high protein content may trend towards a more basic pH. The pH can be influenced by the rate of fermentation, which is supported by the availability of adequate feed sources for microbes (Dryden, 2021). Additionally, Hynd (2019) noted that extensive fungal growth and leaks in the silo can also affect the pH of silage. McDonald et al. (2022) reported that leaks in the silo can lead to aerobic fermentation, allowing various microbes and fungi to thrive. This results in higher protein and nutrient degradation, and increased ammonia production in the silage.

Ammonia Content of Fresh Silage

The addition of chestnut tannin up to 2% significantly affected the ammonia levels in the complete feed silage (P<0.05). Based on the 5% DMRT test, ammonia levels in T1 were different from those in T2, T3, T4, and T5. Ammonia in T2 varied from T3, T4, and T5, whereas T3 was similar to T4 but different from T5, while T4 showed no difference from T5. The highest ammonia was produced in T2 (3.69 mM) followed by T1 (3.41 mM). Increasing chestnut tannin addition to T3, T4, and T5 resulted in 2.81 mM, 2.64 mM, and 2.48 mM ammonia, respectively. The reduction in ammonia levels observed with increasing tannin levels aligns with the study's goal to minimize nutrient loss during ensiling, demonstrating that chestnut tannins can effectively protect the protein from degradation. The average ammonia levels in this study ranged from 2.48 to 3.69 mM, which falls within the normal range for silage ammonia.

Sadarman et al. (2020) reported that both hydrolyzed and condensed tannins can produce soy sauce waste silage with pH 5.88-6.40 (from weakly acidic to near-normal pH). According to Sadarman et al. (2022), adding up to 2% chestnut tannin did not significantly affect the pH of corn cob silage, which remained of good quality with a pH between 3.64 and 3.78. It suggests that tannins can act as inhibitors to the growth of undesirable microbes and minimize protein degradation in the silo.

Ammonia in silage is triggered by proteolysis or the degraded proteins in the ensiled material (Pereira da Silva et al., 2021), which is due to undesirable bacteria inhibiting the growth and proliferation of beneficial bacteria (Irawan et al., 2021). As a result, undesirable bacteria grow exponentially and fermentation rates fall significantly (Kriswantoro and Chu, 2024). According to McDonald et al. (2022), a slow fermentation can lead to increased substrate degradation, thereby raising ammonia levels in the silage. This study indicates that the higher the chestnut tannins addition in the silage, the lower the ammonia production, suggesting that chestnut tannins can inhibit the growth of undesirable bacteria during ensiling.

Total VFA Production in Fresh Silage

The addition of up to 2% chestnut tannins significantly affected the total volatile fatty acids

(VFA) in complete feed silage (P<0.05). The average VFA in this study ranged from 34.4 to 64.7 mM, with T5 producing the highest VFA. The Duncan test showed different FVA levels in T1 from all other treatments due to the absence of chestnut tannins, which inhibit harmful microbes and slow fermentation. VFA levels in T2 were similar to T3 but different from T4 and T5, whereas VFA levels in T3 were the same as in T4 and T5. Therefore, adding up to 2% chestnut tannins helps reduce substrate degradation and optimize VFA production.

The study by Sadarman et al. (2020) concluded that using chestnut tannins as an additive in soy sauce pulp resulted in a total VFA of 58.5 mM. This indicates that chestnut tannins effectively protect the substrate in soy sauce pulp, minimizing substrate degradation and leading to high VFA levels. Total VFA is the end product of fermentation in the silo or rumen (McDonald et al., 2022), and carbohydrate fermentation produces VFAs like acetate, propionate, lactate, and butyrate, which serve as energy sources for livestock (Makkar and Vercoe, 2007), However, it takes adequate energy sources for microbes to convert the ensiled material into high-quality silage. Abdullah (2023) found that using chestnut tannins to ensile a mixture of fresh tofu dregs and fine rice bran resulted in silage with total VFA values ranging from 44.6 to 65.4 mM.

According to McDonald et al. (2022), high levels of volatile fatty acids (VFAs) are essential for good-quality silage. VFAs are by-products of carbohydrate fermentation in the silo, so total VFA production can indicate the quality of the silage. High VFA concentrations generally indicate effective fermentation and good silage quality, while low levels suggest poor fermentation and potential nutrient loss. Monitoring VFA production helps assess silage quality and ensures it provides optimal energy for livestock. The levels of VFAs can vary based on the type of material being ensiled, the amount of soluble carbohydrates in the material, and the inhibition of undesirable microbial growth during the ensiling process.

Conclusions

The addition of chestnut tannin up to 2% in complete feed silage significantly reduces dry matter loss, enhances crude protein retention and stabilizes silage texture and aroma. Tannins help reduce dry matter loss while preserving crude protein and maintaining the physical integrity of the silage. Although the temperature remains stable, tannins may inhibit mold growth, ensuring good fermentation quality. Further research is necessary to identify the optimal tannin concentration for enhancing feed efficiency and livestock performance.

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References

AOAC. 2019. *Official Methods of Analysis*, 21st edn. Rockville, Maryland. USA.

Battelli, M., S. Colombini., G.M. Crovetto., G. Galassi., F. Abeni., F. Petrera., M.T. Manfredi, and L. Rapetti. 2024. Condensed tannins fed to dairy goats: Effects on digestibility, milk production, blood parameters, methane emission, and energy and nitrogen balances. *Journal of Dairy Science*, Volume 107, Issue 6, Pages 3614-3630, ISSN 0022-0302, https://doi.org/10.3168/jds.2023-24076.

Bernardes, T.F., J.R.S. Gervásio., G. De Morais, and D.R. Casagrande. 2019. Technical note: A comparison of methods to determine pH in silages. *Journal Dairy Science*, 102: 9039–9042.

Brutti, D.D., M.E.A. Canozzi., E.D. Sartori., D. Colombatto, and J.O.J. Barcellos. 2023. Effects of the use of tannins on the ruminal fermentation of cattle: A meta-analysis and meta-regression. *Animal Feed Science and Technology*, Volume 306, 115806, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2023.115806.

Carvalho, M.G.M., J.M. Bragatto., S.C. Buttow., A.F. Silva., L.S. Silva., N.G. Silva., H.U. Auerbach., L.G. Nussio, and J.L.P. Daniel. Applying the fermentability coefficient concept in tropical grass silages. *Animal Feed Science and Technology*, Volume 314, 115995, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2024.115995.

Chen, L., X. Bao., G. Guo., W. Huo., Q. Xu., C. Wang, and Q. Liu. 2021. Treatment of alfalfa silage with tannin acid at different levels modulates ensiling characteristics, methane mitigation, ruminal fermentation patterns and microbiota. *Animal Feed Science and Technology*, Volume 278, 114997, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2021.114997.

Conway, E. 1993. *Microdiffusion of Analysis of Association Official Analytical Chemist*. Georgia (US): Georgia Press.

Dryden, G.M. 2021. *Fundamentals of Applied Animal Nutrition*. CABI Press. England.

Fonseca, N.V.B., A. da. S. Cardoso., Y.T. Granja-Salcedo., D. Siniscalchi., K.D.V. Camargo., I.A. Dornellas., M.L.C. Silva., L. dos. S.D. Vecchio., R.K. Grizotto, and R.A. Reis. 2024. Effects of condensed tannin-enriched alternative energy feedstuff supplementation on performance, nitrogen utilization, and rumen microbial diversity in grazing beef cattle. *Livestock Science*, Volume 287, 105529, ISSN 1871-1413, https://doi.org/10.1016/j.livsci.2024.105529.

Genís, S., M. Verdú., J. Cucurull, and M. Devant. 2021. Complete feed versus concentrate and straw fed separately: Effect of feeding method on eating and sorting behavior, rumen acidosis, and digestibility in crossbred Angus bulls fed high-concentrate diets. Animal Feed Science and Technology 273 (2021) 114820.

Gómez, J.F.M., D.S. Antonelo., M. Beline., B. Pavan., D.B. Bambil., P. Fantinato-Neto., A. Saran-Netto., P.R. Leme., R.S. Goulart., D.E. Gerrard, and S.L. Silva. 2021. Feeding strategies impact animal growth and beef color and tenderness. Meat Science, Volume 183, 2022-108599, https://doi.org/10.1016/j.meatsci.2021.108599.

Guimarães, L.J., M. Zundt., M.P. Tsujiguchi., F.M. Giotto., M.J.P.T. Barbosa., F.A. Grandis., I.G. Silva., M.C.S. Pereira, and E.L.A. Ribeiro. The use of condensed tannin in lambs' diet alters the rumen protozoa population without affecting growth performance. *Small Ruminant Research*, Volume 229, 107122, ISSN 0921-4488, https://doi.org/10.1016/j.smallrumres.2023.107122.

Hartinger, T., T. Gruber., K. Fliegerová., G. Terler, and Q. Zebeli. 2024. Mixed ensiling with by-products and silage additives significantly valorizes drought-impaired whole-crop corn. *Animal Feed Science and Technology*, Volume 309, 115899, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2024.115899.

Hassoun, P., M.A. Cordoba., S. Parisot., D. Portes., J. Pradel, and F. Bocquier. 2021. Effects of different supplement amounts on dry matter intake, milk production and milk composition of high-producing Lacaune dairy ewes. *Livestock Science*, Volume 251, 104664, ISSN 1871-1413, https://doi.org/10.1016/j.livsci.2021.104664.

Hynd, P.I. 2019. *Animal Nutrition from Theory to Practice*. CABI Publisher.

Ibrahim, S.L. and A. Hassen. 2022. Effect of non-encapsulated and encapsulated mimosa (Acacia mearnsii) tannins on growth performance, nutrient digestibility, methane and rumen fermentation of South African mutton Merino ram lambs. *Animal Feed Science and Technology*, Volume 294, 115502, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2022.115502.

Irawan, A., A. Sofyan., R. Ridwan., H.A. Hassim., A.N. Respati., W.W. Wardani., Sadarman., W.D. AstutI, and A. Jayanegara. 2021. Effects of different lactic acid bacteria groups and fibrolytic enzymes as additives on silage quality: A meta-analysis. Bioresource Technology Reports, Volume 14, June 2021, 100654.

Jung, J.S., J.W.C. Wong., I. Soundharrajan., Ki-W. Lee., H.S. Park., D. Kim., Ki-C. Choi., S.W. Chang, and B. Ravindran. 2024. Changes in microbial dynamics and fermentation characteristics of alfalfa silage: A potent approach to mitigate greenhouse gas emission through high-quality forage silage. *Chemosphere*, Volume 362, 142920, ISSN 0045-6535, https://doi.org/10.1016/j.chemosphere.2024.142920.

Katoch, R. 2023. *Techniques in Forage Quality Analysis*. Springer. Singapore.

Kriswantoro, J.A and C.Y. Chu. 2024. Biohydrogen production kinetics from cacao pod husk hydrolysate in dark fermentations: Effect of pretreatment, substrate concentration, and inoculum. *Journal of Cleaner Production*, Volume 434, 140407, ISSN 0959-6526, https://doi.org/10.1016/j.jclepro.2023.140407.

Leon-Tinoco, A.Y., B.C. Guimarães., S.T.R. Almeida., D.C. Reyes., S. Rivera., M. Killerby., C. Wu., B. Perkins., C. Knight, and J.J. Romero. 2022. Effect of lignosulfonates on the dry matter loss, nutritional value, and microbial counts of high moisture alfalfa silage. *Animal Feed Science and Technology*, Volume 290, 115346, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2022.115346.

Makkar, H.P.S and P.E. Vercoe. 2007. Measuring Methane Production from Ruminants. Springer Science & Business Media. New York, United State.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A, and Wilkinson, R.G. 2022. Animal Nutrition 8th Edn. Pearson. Singapore.

Moore, R. 2018. Principles of Animal Nutrition. Scientific e-Resources Publisher.

Niderkorn, V., E. Barbier., D. Macheboeuf., A. Torrent., I. Mueller-Harvey and H. Hoste. 2019. In vitro rumen fermentation of diets with different types of condensed tannins derived from sainfoin (Onobrychis viciifolia Scop.) pellets and hazelnut (Corylus avellana L.) pericarps. Animal Feed Science and Technology.

Niyigena, V., K.P. Coffey., D. Philipp., M.C. Savin., J. Zhao., H.D. Naumann., J.M. Diaz., S.P. Park., R.L. Rhein, and S.L. Shelby. 2024. Intake, digestibility, rumen fermentation and nitrogen balance in sheep offered alfalfa silage with different proportions of the tannin-rich legume sericea lespedeza. *Animal Feed Science and Technology*, Volume 308, 115863, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2023.115863.

Palacios, C.E., A. Nagai., P. Torres., J.A. Rodrigues and A. Salatino. 2021. Contents of tannins of cultivars of sorghum cultivated in Brazil, as determined by four quantification methods. Food Chemistry, Pp. 337.

Pereira da Silva, R., K. dos Santos Nascimento., A. R. de Sousa., O.T. Medeiros., M.F.A. Macêdo., T.P. Dias-Silva., R.L. Edvan, and M.J. de Araújo. 2021. Influence of the phenological stage at harvest of sesame (Sesamum indicum) on silage quality, Animal Feed Science and Technology, Volume 281, 2021- 115102, https://doi.org/10.1016/j.anifeedsci.2021.115102.

Rossi, L.G., C.H.S. Rabelo., M.E.B. Andrade., G.R. Siqueira., E.F. Vicente., D.A. Nogueira, and R.A. Reis. 2023. Feed intake, digestibility, ruminal fermentation, growth performance, and carcass traits of lambs fed corn silage treated with *Lentilactobacillus buchneri* and stored for different times. *Animal Feed Science and Technology*, Volume 304, 115751, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2023.115751.

Sabariah, B., F. Norfadzrin., M.I. Noor., A. Aswanimiyuni., H. Haryani., M.H. Ahmad, and A.A.H. Saiful. 2018. Effect of probiotics in fermented palm kernel meal (FPKM) and total mixed ration (TMR) to improve milk production in mafriwal dairy cattle of malaysia veterinary institute (IVM) farm. Malaysian J. Vet. Res. 9(1): 45-51.

Sadarman, Ridla M, Nahrowi, Sujarnoko TUP, Ridwan R and Jayanegara A. 2019a. Evaluation of ration based on soy sauce by-product on addition of acacia tanin: an in vitro study. 9th Annual Basic Science International Conference. Material Science and Engineering 546(2019)022020.

Sadarman, Ridla M, Nahrowi, Ridwan R, Harahap RP, Nurfitriani RA, Jayanegara A. 2019b. Kualitas Fisik Silase Ampas Kecap dengan Aditif Tanin dari akasia (Acacia mangium Wild.). Jurnal Peternakan. 16(2): 66-75.

Sadarman, Ridla M, Nahrowi N, Ridwan R, Jayanegara A. 2020. Evaluation of ensiled soy sauce by-product combined with several additives as an animal feed. Veterinary World. 13(5): 940-946.

Sadarman., Febriana,D., Wahyono,T., Adli, D.N., Qomariyah, N.N., Nurfirtiani, R.A., Murshid, S., Oktafyan, Y.A., Zulkarnain, dan Prasetyo, A.B. 2022. Pengaruh penambahan aditif tanin chestnut terhadap kualitas silase kelobot jagung (*Zea mays*). *Jurnal Nutrisi Ternak Tropis*, 5(1): 37-44. https://doi.org/10.21776/ub.jnt.2021.005.01.4.

Sadarman, Handoko, J., Febrina, D., Febriyanti, R., Purba, R. A., Ramadhan, E. S., Qomariyah, N., Gholib, Nurfitriani, R. A., Adli, D. N, dan Khairi, F. 2023a. Evaluasi Penggunaan Kombinasi Aditif Berbasis Molases dan Sirup Komersial Afkir yang dapat Menstimulasi Pertumbuhan Mikroba Baik terhadap Profil Fermentasi Silase Tebon Jagung. *Jurnal Nutrisi Ternak Tropis,* 6(1), 57-68. doi: 10.21776/ub.jnt.2023.006.01.7.

Sadarman., Febrina, D., Trisna Rinaldi, S., Hendri, H., Ichwan Ilyazar, M., Weno, W., Alfian, A., Amalia Nurfitriani, R., Qomariyah, N., Sukmara, A., Koswara, E., Rachmanto Prihambodo, T., Gholib, G, and Faiz Mohd Azmi, A. 2023b. The Quality of Organic Waste Market Ensiled Using Rejected Commercial Syrup as An Alternative Ruminant Livestock Feed. *Animal Production*, *25*(3), 186-198. https://doi.org/10.20884/1.jap.2023.25.3.257.

Sadarman., R.P. Harahap., A.F.M. Azmi., D. Febrina., R. Febriyanti., Gholib., Yunilas., N. Qomariyah., R.A. Nurfitriani, and F. Khairi. 2024. Physico-chemical Characterization of Tofu By-Product Silage Supplemented with Fine Rice Bran and Chestnut Tannin as Silage Additives. *Jurnal Nutrisi Ternak Tropis,* 7(2), 108-114. https://doi.org/10.21776/ub.jnt.2024.007.02.4.

Saha, S.K and N.N. Pathak. 2021. *Fundamentals of Animal Nutrition, 1st Edn*. Springer Nature. Singapore.

Santoso, B., T.W. Widayati and B.T. Hariadi. 2020. Improvement of Fermentation and the In Vitro Digestibility Characteristics of Agricultural Waste-Based Complete Feed Silage with Cellulase Enzyme Treatment. Adv. Anim. Vet. Sci. 8(8): 873-881.

Theodorou, M.K and A.E. Brook. 1990. *Evaluation of a New Laboratory Procedure for Estimating the Fermentation Kinetic of Tropical Feeds*. Annual Report AFRC Institute, Hurley, Maidenhead, London.

Tian, J., N. Xu., B. Liu., H. Huan., H. Gu., C. Dong, and C. Ding. 2020. Interaction effect of silo density and additives on the fermentation quality, microbial counts, chemical composition and in vitro degradability of rice straw silage. *Bioresource Technology*, Volume 297, 122412, ISSN 0960-8524, https://doi.org/10.1016/j.biortech.2019.122412.

Yin, X., J. Wu., J. Tian., X. Wang, and J. Zhang. 2021. Dried soybean curd residue: A promising absorbent for cleaner production of high-quality silage, Journal of Cleaner Production, Volume 324, 2021, 129300, https://doi.org/10.1016/j.jclepro.2021.129300.

Zotte, A.D., M. Cullere., G. Tasoniero., Z. Gerencsér., Z. Szendrő., E. Novelli, and Z. Matics. 2018. Supplementing growing rabbit diets with chestnut hydrolyzable tannins: Effect on meat quality and oxidative status, nutrient digestibilities, and content of tannin metabolites. *Meat Science*, Volume 146, Pages 101-108, ISSN 0309-1740, https://doi.org/10.1016/j.meatsci.2018.08.007.