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Silage Quality, Rumen Fermentation Characteristics, and Nutrient Digestibility of *Sorghum bicolor* cv. Samurai 1 Harvested at Different Maturity Stages Treated with Fibrolytic Enzyme

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ABSTRACT

The Samurai 1 variety is a genetically mutated strain with superior agronomic characteristics and enhanced nutrient content. This study aimed to evaluate the effect of harvest maturity stages and fibrolytic enzyme (Sunsonzyme) treatment on the nutrient value, fermentative quality, and nutrient digestibility of *Sorghum bicolor* cv. Samurai 1 silage. The silage quality was assessed using a completely randomized design with a 3 x 3 factorial arrangement. Sorghum was harvested at three different maturity stages, namely 85, 90, and 95 days, with fibrolytic enzyme added at 0%, 0.025%, and 0.05% of the dry matter. *In vitro* fermentability and digestibility were evaluated using a randomized block factorial design, which was also based on the same three harvest stages and enzyme levels. Observed variables included the nutrient composition of the sorghum, physical characteristics and nutrient content of the silage, as well as *in vitro* fermentability and digestibility. The results showed a significant interaction between harvest maturity and enzyme level on lactic acid production and total volatile fatty acid (VFA). Harvest age significantly affected ($p<0.05$) all variables, while the treatment of fibrolytic enzyme significantly increased ammonia (NH_3) and total VFA concentrations, as well as reduced the fiber fraction of the silage ($p<0.05$). The harvest age of 90 days showed the best quality in terms of nutrients, silage, and rumen fermentability. The addition of enzyme levels up to 0.05% improved the fermentative quality of silage, reduced fiber fractions, and enhanced rumen fermentability.

Keywords: enzyme; harvest age; quality; sorghum silage

INTRODUCTION

Some varieties of sorghum (*Sorghum bicolor* (L.) Moench) are used as forage crops in marginal and arid regions of Indonesia (Ardiansyah *et al.,* 2016). The Samurai 1 variety is a genetically mutated strain with superior agronomic characteristics and enhanced nutrient content, which is suitable for feed. Sorghum cv Samurai 1 has a sweeter stem compared to the other varieties. The crop is well adapted to drought and high environmental temperatures (Lyons *et al.,* 2019), efficiently uses water (Yang *et al.,* 2019), and can ratoon from a single seeding (Carvalho *et al.,* 2016). A previous study reported that this crop has a complex nutrient profile (Palealu *et al.,* 2022). Samurai 1 can yield up to 66.83 tons ha^{-1} per year (Malalantang *et al.,* 2023), with a composition of 7.57% ash, 1.09% crude fat, and 14.70% crude protein (Puteri *et al.,* 2015). The whole sorghum plant is increasingly used as a substitute for corn in silage production. Previous studies showed that sorghum silage provided equivalent nutrient content and had a similar impact on milk production and quality compared to corn silage (Cattani *et al.,* 2017; Khosvari *et al.,* 2018). Nusrathali *et al.* (2021) also found that whole-plant sorghum silage had comparable nutrient content to corn silage. However, the primary limitation of Samurai 1 sorghum as a silage feedstock is its high fiber content, with neutral detergent fiber (NDF) reaching 69.46% and acid detergent fiber (ADF) at 43.50% (Palealu *et al.,* 2022). This is higher compared to the Samurai 2 and Patir varieties, which show lower NDF and ADF contents of 52.79% and 35.51%, respectively, with NDF and ADF values of 50.6% and 45.37%.

Harvest time is related to the production, nutrient content, and digestibility of forage (Harper *et al.,* 2017). As the plant ages, production and dry matter content in the forage, increase due to the rate of photosynthesis (DiosLeon *et al.,* 2022). A longer photosynthesis process leads to a greater accumulation of photosynthates in the tissues. As photosynthates are translocated to the stems and leaves, more are also transferred to the

yield components, such as seeds and pods (Putri *et al.*, 2021). The aging process changes the plant structure, affecting nutrient content and digestibility for livestock. The proportion of cell wall components, such as hemicellulose, cellulose, and lignin, increases with plant age (Palealu *et al*., 2022). An increased rate of cell wall development affects the accumulation of fiber content, leading to lower dry matter digestibility (DMD) and organic matter digestibility (OMD) (Sriagtula *et al.*, 2017). Selecting the appropriate and optimal harvest age is crucial to achieve the best quality.

The abundant production of sorghum forage at specific harvest ages requires effective preservation methods to ensure continuous forage availability. Silage is a preserved feed product created through fermentation facilitated by anaerobic microorganisms (Ardiansyah *et al.,* 2016). This method minimizes nutrient loss and extends the shelf life of forage (Khota *et al.,* 2017). The addition of fibrolytic enzymes, such as cellulase and xylanase, aims to reduce the fiber fraction and enhance digestibility for livestock. These enzymes work synergistically to break complex bonds within the fiber and ensure more digestibility. The role of the enzymes is important in degrading the complex structure of the cell wall, thereby improving the efficient use of fiber in ruminant livestock (Desta *et al.*, 2016). According to Xiong *et al.* (2022), incorporating fibrolytic enzymes into silage hydrolyzes the fiber fraction, providing simple sugar substrates that promote bacterial growth during fermentation. Several studies have documented the benefits of adding fiber-degrading enzymes to various silage types, including complete feed (Bhasker *et al.,* 2013), sorghum forage (Khota *et al.*, 2017), mulberry leaves (He *et al.,* 2019), and corn stover (Zhao *et al.*, 2021).

The addition of fibrolytic enzymes to local forages is very important. An experiment was conducted on fibrolytic enzymes to determine the nutritive value of sorghum silage, resulting in reduced NDF and ADF (Foster *et al*., 2019). Investigating the combination of harvest age and fibrolytic enzyme supplementation is critical for optimizing forage use. The evaluation of silage quality should also extend beyond nutrient content and physical characteristics to include digestibility testing in livestock. *In vitro* methods provide valuable simulations of the livestock digestive system, providing reliable assessments of feed viability. Therefore, this study aimed to evaluate the effects of harvest age and fibrolytic enzyme levels on the nutrient composition, fermentation quality, and nutrient digestibility of sorghum silage.

MATERIALS AND METHODS

Materials Used and Silage Production

Samurai 1 sorghum seeds were sown on a 1000 m² plot with a planting distance of 25 cm × 25 cm and a mound spacing of 100 cm. Cow manure containing 43% organic matter, 22% organic carbon, 1.22% total nitrogen, 0.80% total phosphorous, and 0.60% potassium was applied during planting at a rate of 10 tons per hectare. The sorghum was fertilized twice during the growth using inorganic fertilizer applied 14 days after planting and the second fertilizer applied 30 days after planting. The application consisted of 200 kg urea (46% nitrogen), 100 kg phosphorus (P), and 60 kg potassium chloride (KCl) per hectare. Sorghum forage was harvested at three different stages, namely 85, 90, and 95 days after sowing, depending on the treatment. The entire plant, including stems, leaves, and panicles, was chopped into 1-2 cm pieces using a chopper with a capacity of 2.5 tons per hour.

The study used a commercial fibrolytic mixture enzyme from Sunsonzyme containing *Enterococcus faecium* bacteria ($5x10^9$ CFU mL^{-1}), xylanase (10,000 units g^{-1}), and cellulase (100 units g^{-1}), produced by Sunhy Biology, China. The enzymes were applied at treatment levels of 0% (0 g), 0.02% (5 g), and 0.05% (12.5 g) in 25 kg of fresh forage. The forage mixture, weighing 25 kg, was fermented in double-layer, air-tight bags, and vacuum-sealed to compact the material. The specifications of the inner plastic layer are 120 × 60 cm in size with a thickness of 100 microns, while the plastic bag has a capacity of up to 40 kg. The silage was stored at room temperature (25 °C to 37 °C) for 21 days.

Experimental Design

A factorial completely randomized design with four replications was used to assess the quality parameters of sorghum forage. The factors included three different harvest ages of Samurai 1 sorghum, namely 85, 90, and 95 days after sowing, and three levels of enzyme fibrolytic 0%, 0.025%, and 0.05% (w/w of sorghum forage).

The fibrolytic enzyme was prepared by dissolving the enzyme powder in 250 mL of distilled water at room temperature, according to the treatment levels of 0, 5, and 12.5 g for 0%, 0.025%, and 0.05%. The enzyme solution was incubated for 1–2 hours at room temperature (25 °C) before use.

Chemical Composition Analysis of Fresh Sorghum and Silage

Forage and silage samples of sorghum at each harvest time were dried at 60 °C for two days and then ground to pass through a 1 mm mesh screen (Khota *et al.,* 2017). Nutrient content was analyzed using Near-Infrared Reflectance Spectroscopy (NIRS) with the Buchi NIRFlex N500 Fourier Transform Near-Infrared (FT-NIR) spectrometer connected to the tropical forage sorghum database. The analyzed components included dry matter, ash, crude protein, crude fiber, NDF, and ADF. Sugar values were measured following the procedure of Wahyono *et al.* (2019), using a refractometer for readings. Water-soluble carbohydrates (WSC) were analyzed using the phenol method, with sample absorbance measured at 490 nm with a UV-VIS spectrophotometer.

The pH measurements were conducted following the procedure of Kaewpila *et al.* (2020) using a pH meter. The Fleig value was calculated based on the silage pH and dry matter content, according to Idikut

et al. (2009). Fleigh's point was categorized into five groups, namely scores of 85-100, 60-80, 40-60, 20-40, and <20, categorized as excellent, good, fair, moderate, and poor. This point was calculated using the following formula: Fleigh= 220 + (2 × DM(%) - 15) - (40 × pH). Lactic acid testing was carried out following the procedure described by Borshchevskaya *et al.* (2016).

In Vitro Ruminal Fermentation Test

In the *in vitro* fermentability and digestibility studies, a factorial randomized block design was used with the same treatments as the quality parameter experiments. Quality parameters include fermentative quality (fleig value, pH, and lactic acids) and chemical composition of silage (DM, ash, CP, CF, NDF, and ADF). The blocks were based on different rumen fluid collection dates: December 11, 2023, January 11, 2024, January 22, 2024, and February 1, 2024. The rumen fluid was collected from fistulated dairy cows for *in vitro* analysis, according to standard laboratory procedures by Standard Operating Procedure (RUM_020 Rumen Sampling of Cattle via a Rumen Fistula) from The University of Queensland. Gloves covered both hands, and a thermos was filled to approximately three-quarters capacity with hot water. The sample (rumen content) was collected by hand, taking multiple small handfuls from various areas to ensure a representative sample. The hot water was then emptied from the thermos, and the sample was placed inside. *In vitro* fermentation and digestibility were conducted using the method of Tilley and Terry (1963). A total of 0.5 g of silage sample was placed into a tube, mixed with 40 mL of McDougall's solution and 10 mL of rumen with CO_2. At the end of the first fermentation at 39 ^oC for four hours, microbial activity was stopped with 2-3 drops of $HgCl_2$ and it was centrifuged for 10 minutes at 3000 rpm. The solid residue was mixed with 50 mL of 0.2% pepsin-HCl solution. Each silage sample was tested in duplicate, using two tubes as blanks. The concentration of NH_3 and total VFA were analyzed using the microdiffusion Conway (1942) and steam distillation method, respectively.

Statistical Analysis

The collected data were analyzed using analysis of variance (ANOVA) in Minitab version 18.2. Significant differences were assessed by the Tukey test, with a significant level set at $p<0.05$ (Steel & Torrie, 1997).

RESULTS

Nutritional Quality of Samurai 1 Forage

The ANOVA showed that harvest time had a significantly increased ($p<0.05$) on the dry matter, ash, crude fiber, crude fat, nitrogen-free extract (NFE), while significantly decreased ($p<0.05$). The dry matter, crude fiber, and ash content in this study ranged from 22.68% to 36.53%, 26.06% to 28.46%, and 2.92% to 5.12%, respectively (Table 1). Additionally, harvest time significantly influenced ($p<0.05$) the reduction in crude protein, Total Digestible Nutrient (TDN), and WSC content of Samurai 1 sorghum.

Fermentative Characteristics of Samurai 1 Silage

The results in Table 2 showed a significant interaction effect between harvest time and fibrolytic enzyme levels on the Flieg silage value ($p<0.05$), suggesting that all silage samples had very good quality. Both harvest time and enzyme levels significantly affected pH values ($p<0.05$), although no interaction was observed between these factors. However, an interaction effect was found for lactic acid content ($p<0.05$), influenced by both harvest time and enzyme addition. The inclusion of enzymes decreased silage pH and increased lactic acid content.

Nutritional Quality of Samurai 1 Silage

Silage quality, often evaluated based on nutrient content, is shown by the nutrient composition of Samurai 1 sorghum silage at various harvest times and with different levels of fibrolytic enzyme addition (Table 3). The result showed a significant interaction effect of harvest time and enzyme addition on the dry matter content of the silage ($p<0.05$). Furthermore, harvest time and enzyme addition levels significantly increased ash content ($p<0.05$), but no interaction was observed between the two factors. Harvest time also significantly affected crude protein content ($p<0.05$), but fibrolytic enzyme addition did not show a significant effect ($p>0.05$), and no interaction was detected between harvest time and enzyme addition. Harvest time had a significant effect on crude fiber, NDF, and ADF contents of sorghum silage ($p<0.05$). Additionally, the level of enzyme addition significantly reduced crude fiber, NDF, and ADF contents ($p<0.05$), but there was no interaction between harvest time and enzyme levels.

In Vitro Ruminal Fermentability

Sorghum forage silage, harvested at different times and with varying levels of enzyme addition,

Table 1. Quality of samurai 1 sorghum forage harvested at different time

Variables	Harvest time (d)		
	85	90	95
Dry matter	22.68±1.23^c	29.60±0.87^b	36.53±1.21^a
Ash (% DM)	2.92±0.06^c	3.15±0.02^b	5.12±0.03^a
Crude fiber (% DM)	26.06±0.04^c	27.53±0.08^b	28.43±0.16^a
Crude protein (% DM)	11.32±0.57^a	10.23±0.59^a	7.64±0.24^b
Ether extract (% DM)	1.11±0.10^b	2.15±0.27^a	2.58±0.18^a
NFE (% DM)	59.19±1.04^a	56.94±1.74^{ab}	54.66±2.31^b
TDN (%)	60.18±0.08^a	59.68±0.10^b	58.70±0.34^b
WSC (% DM)	24.49±3.84^a	16.38±2.91^b	9.67±0.82^c
Sugar (%)	14.00±0.82^c	16.50±0.58^b	20.75±0.96^a

Note: Means in the same row with different superscripts differ significantly ($p<0.05$). NFE= nitrogen free extract; TDN= total digestible nutrient; WSC= water soluble carbohydrate.

Table 2. Fermentative characteristics of samurai 1 sorghum silage harvested at different times and levels of fibrolytic enzymes

Variables	Harvest time (d)	Level fibrolytic enzymes (%)			Mean of harvest time
		0	0.02	0.05	
Flieg value	85	92.42±2.21^{e}	105.89±2.61^{cd}	110.88±9.54^{bc}	103.06±8.40
	90	110.27±1.55^{bc}	118.68±1.93^{ab}	120.16±1.93^{a}	116.37±4.89
	95	99.00±1.98^{de}	105.24±4.84^{cd}	105.03±4.64^{cd}	103.09±4.75
Mean of enzyme level		100.56±7.90	109.94±7.19	112.02±7.08	
pH	85	3.83±0.04	3.61±0.01	3.56±0.02	3.66±0.12^{b}
	90	3.61±0.06	3.41±0.05	3.39±0.05	3.47±0.11^{c}
	95	4.04±0.06	3.89±0.16	3.90±0.06	3.94±0.11^{a}
Mean of enzyme level		3.82±0.19^{a}	3.64±0.22^{b}	3.61±0.23^{b}	
Lactic acid	85	8.60±0.95^{de}	11.56±0.81^{cde}	16.73±4.21^{abc}	12.30±4.19
	90	14.00±1.61^{b}	17.39±0.63^{ab}	20.75±0.37^{a}	17.38±3.02
	95	6.67±1.41^{e}	8.91±1.93^{de}	8.02±2.75^{e}	7.87±2.13
Mean of enzyme level		9.76±3.46	12.62±3,87	15.17±6.14	

Note: Means in the same row or column with different superscripts differ significantly ($p<0.05$).

Table 3. Nutrient contents of samurai 1 sorghum silage harvested at different times and levels of fibrolytic enzymes

Variables	Harvest time (d)	Level fibrolytic enzymes (%)			Mean of harvest time
		0	0.02	0.05	
Dry matter	85	20.21±0.99^{d}	22.55±1.51^{c}	24.09±0.86^{bc}	22.28±1.93
	90	24.84±0.54^{b}	25.09±1.05^{b}	25.28±0.30^{b}	25.07±0.66
	95	27.75±0.36^{a}	27.92±0.42^{a}	27.97±0.95^{a}	27.88±0.11
Mean of enzyme level		24.26±3.28	25.19±2.49	25.78±1.83	
Ash	85	3.80±0.13	3.74±0.27	3.81±0.23	3.78±0.20^{c}
	90	3.51±0.18	3.61±0.08	3.72±0.10	3.61±0.15^{b}
	95	3.93±0.25	4.06±0.16	4.19±0.30	4.06±0.25^{a}
Mean of enzyme level		3.75±0.25^{b}	3.80±0.26^{ab}	3.91±0.30^{a}	
Crude protein	85	11.84±0.69	11.16±0.36	11.19±0.85	11.40±0.69^{a}
	90	11.28±0.29	11.70±0.74	12.11±0.82	11.70±0.69^{a}
	95	8.93±0.43	10.33±0.78	10.78±1.35	10.01±1.18^{b}
Mean of enzyme level		10.69±1.39	11.06±0.84	11.36±1.10	
Crude fiber	85	24.57±0.40	24.20±0.04	23.70±0.41	24.16±0.48^{b}
	90	26.53±0.87	26.17±0.72	26.15±0.08	26.28±0.62^{a}
	95	26.77±0.33	26.50±0.34	26.31±0.07	26.53±0.32^{a}
Mean of enzyme level		25.96±1.16^{a}	25.62±1.14^{b}	25.39±1.27^{b}	
NDF	85	65.70±0.51	64.15±0.70	63.76±0.95	64.53±1.10^{b}
	90	66.12±0.48	65.50±0.82	65.49±0.36	65.70±0.61^{b}
	95	66.90±1.03	66.06±1.02	65.42±0.48	66.13±0.94^{a}
Mean of enzyme level		66.24±0.83^{a}	65.24±1.14^{b}	64.99±1.09^{b}	
ADF	85	34.63±1.21	33.42±1.06	33.17±1.44	33.74±1.31^{b}
	90	35.90±0.97	35.87±0.44	33.82±3.46	35.19±2.14^{a}
	95	36.43±0.83	34.16±1.40	34.15±1.43	34.91±1.59^{ab}
Mean of enzyme level		35.65±1.21^{a}	34.48±1.43^{ab}	33.71±2.14^{b}	

Note: Means in the same row or column with different superscripts differ significantly ($p<0.05$). NDF= neutral detergent fiber; ADF= acid detergent fiber.

significantly increased NH_3 and VFA concentrations ($p<0.05$), but pH values were not influenced by the treatments (Table 4). The pH values ranged from 6.89 to 6.97, within the normal rumen pH range (McDonald *et al.*, 2010). These pH results suggested that none of the treatments disrupted the rumen condition.

Dry Matter and Organic Matter Digestibility

DMD shows the proportion of the feed that can be digested and used by the animal, while OMD represents the digestibility of organic compounds. Table 5 shows the average DMD and OMD values for Samurai 1 sorghum forage silage at different harvest times and enzyme addition levels. Harvest time significantly

Table 4. *In vitro* fermentation profile of samurai 1 sorghum silage harvested at different times and levels of fibrolytic enzymes

Variables	Harvest time (d)	Level fibrolytic enzymes (%)			Mean of harvest time
		0	0.02	0.05	
pH	85	6.97±0.23	6.93±0.13	6.92±0.08	6.94±0.02
	90	6.90±0.08	6.90±0.08	6.93±0.12	6.91±0.02
	95	6.89±0.09	6.92±0.08	6.89±0.09	6.90±0.01
Mean of enzyme level		6.92±0.04	6.92±0.01	6.91±0.02	
NH_3 (mM)	85	8.85±0.61	9.36±0.71	9.34±0.95	9.18±0.74^{b}
	90	10.96±1.42	10.27±1.62	11.89±1.39	11.04±1.51^{a}
	95	9.00±1.19	9.67±0.48	10.19±1.31	9.62±1.08^{b}
Mean of enzyme level		9.60±1.17^{b}	9.77±0.46^{b}	10.47±1.29^{a}	
VFA (mM)	85	103.97±1.99^{c}	104.31±2.56^{c}	125.08±1.48^{a}	111.12±10.48
	90	114.05±2.79^{b}	112.93±0.68^{b}	122.77±1.01^{a}	116.58±4.86
	95	111.61±1.47^{b}	111.49±1.80^{b}	123.23±1.12^{a}	115.44±5.91
Mean of enzyme level		109.88±4.89	109.58±4.28	123.69±1.52	
Acetate (%)	85	73.75±1.13	59.45±8.26	67.01±6.46	66.73±8.22
	90	70.57±5.82	57.12±11.95	59.91±5.61	62.53±9.67
	95	64.94±4.17	60.31±8.56	68.66±7.44	64.64±7.25
Mean of enzyme level		69.75±5.37	58.96±8.92	65.19±7.13	
Propionate (%)	85	18.36±0.86	30.77±7.95	25.52±5.99	24.88±7.45
	90	22.25±5.28	32.06±10.07	31.68±5.63	28.66±8.15
	95	27.45±2.59	30.24±9.43	24.11±7.69	27.27±7.00
Mean of enzyme level		22.68±4.98	31.02±8.35	27.10±6.80	
Iso butyrate (%)	85	0.60±0.09	0.83±0.24	0.65±0.28	0.69±0.22
	90	0.68±0.31	1.23±1.06	0.70±0.21	0.87±0.64
	95	0.54±0.14	0.64±0.13	0.61±0.12	0.60±0.13
Mean of enzyme level		0.61±0.19	0.90±0.63	0.65±0.20	
N butyrate (%)	85	6.56±0.84	8.00±2.28	6.09±0.78	6.88±1.58
	90	5.79±1.52	8.50±2.49	6.96±0.17	7.08±1.92
	95	6.37±1.63	7.93±1.40	5.87±0.99	6.73±1.54
Mean of enzyme level		6.24±1.29	8.14±1.93	6.31±0.82	
Iso valerate (%)	85	0.36±0.06	0.50±0.17	0.37±0.02	0.41±0.12
	90	0.39±0.03	0.61±0.42	0.40±0.07	0.47±0.25
	95	0.35±0.10	0.45±0.14	0.39±0.06	0.39±0.11
Mean of enzyme level		0.37±0.07	0.52±0.26	0.38±0.05	
N valerate (%)	85	0.37±0.08	0.46±0.10	0.36±0.03	0.40±0.08
	90	0.32±0.08	0.48±0.19	0.36±0.06	0.39±0.13
	95	0.36±0.07	0.43±0.07	0.36±0.07	0.38±0.07
Mean of enzyme level		0.35±0.07	0.46±0.12	0.36±0.05	

Note: Means in the same row or column with different superscripts differ significantly ($p<0.05$). VFA= volatile fatty acid.

Table 5. Dry matter and organic matter digestibility of samurai 1 sorghum silage harvested at different times and levels of fibrolytic enzymes

Variables	Harvest time (d)	Level fibrolytic enzymes (%)			Mean of harvest time
		0	0.02	0.05	
DMD (%)	85	55.79±0.94	58.89±3.62	59.03±3.59	57.35±3.91^{a}
	90	54.33±1.73	54.12±0.42	55.21±2.73	53.98±2.82^{b}
	95	53.37±4.04	53.22±3.53	53.68±4.82	53.43±3.77^{b}
Mean of enzyme level		54.50±2.56	54.29±4.63	55.97±4.17	
OMD (%)	85	54.32±0.99	58.14±3.88	58.58±3.73	57.15±3.45^{a}
	90	52.22±0.67	53.50±0.39	54.45±2.97	52.40±3.99^{b}
	95	52.12±3.82	52.97±3.93	53.03±5.05	52.71±3.92^{b}
Mean of enzyme level		52.89±2.34	54.02±5.53	55.35±4.38	

Note: Means in the same column with different superscripts differ significantly ($p<0.05$). DMD= dry metter digestibility; OMD= organic matter digestability.

affected both DMD and OMD values, as evidenced by $p<0.05$. However, enzyme addition levels did not have a significant effect, and there was no interaction between harvest time and enzyme levels on DMD and OMD.

DISCUSSION

Nutritional Quality of Samurai 1 Forage

The highest dry matter content, recorded at the 95-day harvest, can be attributed to the extended photosynthesis period, leading to a greater accumulation of photosynthates. The increase in dry matter content with plant maturity was also due to the decreasing water content. Savitri *et al.* (2013) reported that as plants age, water content decreases, resulting in higher dry matter. The result of water content in this study is higher than the 28.84% reported by Palealu *et al.* (2022) during the hard dough stage.

Increasing the harvest age also leads to higher fiber content. The increase in fiber and ash content with plant maturity is attributed to lignification, leading to cell wall thickening in older plants. This thickening increases the proportion of cell wall components, such as hemicellulose, cellulose, and lignin, thereby elevating the fiber fraction content. Palealu *et al.* (2022) reported that cell wall thickening occurs at older harvest ages, leading to the accumulation of crude fiber and inorganic materials, as evidenced by the plant's ash content (Sriagtula *et al.,* 2017). The increase in ash content was associated with mineral absorption, suggesting that more minerals are absorbed when the plant is allowed to grow. Older harvest ages are associated with higher concentrations of magnesium and calcium. According to Widiyanto *et al.* (2016), these minerals are primarily found in the stem, and the concentrations increase as the stem matures.

In this study, the average crude protein content ranged from 7.64 to 11.32%, which remained within the typical range for sorghum crude protein values (Tasie & Gebreyes, 2020). The highest crude protein content of 11.32% was observed at the 85-day harvest age. A slight increase in protein content was observed at 85 and 90 days with increasing concentrations of the added fibrolytic enzyme. This modest increase in protein content with enzyme addition and harvest age was due to several factors, such as enhanced accessibility of cellular proteins from fiber breakdown, a concentration effect as dry matter increases, and a shift in the nutrient profile as fiber content decreases. These factors have a collective contribution to the marginal improvement in the protein percentage observed in the silage.

Crude protein levels decline as the plant ages, primarily due to cell wall thickening and a reduction in the proportion and functionality of leaves. The decrease in leaf proportion associated with aging inhibits protein synthesis due to the reduced photosynthetic capability of older plants (Baloyi *et al.,* 2013; Sriagtula *et al.,* 2016). Studies by Dios-Leon *et al.* (2022) and Zailan *et al.* (2016) further show that harvest age significantly affects crude protein content, correlating with an increased stem-to-leaf ratio as the plant matures.

At 80 days, the NFE was the highest at 59.19%, and this value declined progressively to 56.94% and 54.66% at 90 and 95 days, respectively. Non-structural carbohydrates, such as sugars and starches, constituted NFE, were progressively converted into structural carbohydrates to support plant growth and seed formation. The increase in structural components reduces the digestibility and energy content of the forage. The data collected in this study showed the typical trend of declining forage quality with increasing maturity. Forage cut at 80 days provided the highest nutritional value due to its high NFE, while 90 and 95 days sacrificed quality for higher biomass yield. The optimal cutting stage should be based on livestock energy requirements and the balance between forage quality and quantity.

The TDN and WSC content at the 95-day harvest age showed the lowest levels. This decrease in TDN and WSC corresponds with a reduction in non-structural carbohydrates at older harvest ages and an accumulation of structural carbohydrates that are more difficult to digest. According to Sriagtula *et al.* (2016), the decline in WSC was associated with an increase in structural carbohydrate fractions. This result was consistent with the report of Sriagtula *et al.* (2017) that crude protein and WSC content decreases as harvest age increases, while crude fat, ash, and crude fiber content increases.

The differences in harvest age influence the sugar content in sorghum. Older harvest ages provide the plant with a longer photosynthetic period, leading to a higher accumulation of carbohydrates. During the transition from the soft dough to the hard dough stage, carbohydrates shift from the stems to the grain, while sugars accumulate in the stems (Wahyono *et al.,* 2019). The change in plant age from 80 to 85 and then to 90 days allows for the dynamics of non-structural carbohydrates, such as WSC. This is because WSC is a simple carbohydrate that is immediately used as an energy source when the plant needs to form seeds, perform respiration, and support other metabolic processes. Meanwhile, fiber serves as a rigid matrix that is relatively persistent and stable as the plant ages further. As plants mature, carbon allocation gradually shifts from producing soluble carbohydrates to synthesizing structural components (Luo *et al.*, 2020). However, this process is slow and not prominent over a short period.

Younger plants tend to have higher water content in the stems, leading to lower sucrose concentrations. Meanwhile, plants with lower water content exhibit higher sucrose levels, as evidenced by increased sugar content. This observation is consistent with the result of Erickson *et al.* (2011), who found that younger sorghum has a lower sugar concentration due to higher water content. In general, the average sugar content in the sorghum studied ranged from 14.00% to 20.75%.

Silage Fermentation Characteristics

The silage from the 90-day harvest had the lowest pH value of 3.47, suggesting optimal enzyme activity in

fiber hydrolysis under the dry matter conditions of the 90-day-old sorghum. The addition of enzymes up to a level of 0.02% successfully lowered the pH, showing the presence of simple sugar substrates, which promote the growth of lactic acid bacteria. Enzymes help degrade fiber fractions into simple sugars, serving as substrates for lactic acid bacteria growth (Kaewpila *et al.,* 2020). In this study, the pH values of sorghum silage ranged from 3.39 to 4.04, which were considered ideal for inhibiting the growth of Clostridia bacteria (McDonald *et al.,* 2010).

The lactic acid content is an indicator of a well-functioning fermentation process. The concentration of lactic acid in sorghum silage ranged from 6.67% to 20.75%, which was considered very good (>6.6%) (Khota *et al*., 2017). The highest lactic acid content of 20.75% was produced at the 90-day harvest age with an enzyme addition level of 0.05%. Furthermore, the addition of enzymes promotes the degradation of fibers, leading to the development of substrates in the form of easily soluble carbohydrates for lactic acid fermentation. Mu *et al*. (2023) reported that cellulase enzymes accelerate lactic acid fermentation, leading to a decrease in pH caused by the indirect supplementation of carbohydrates from lignocellulose degradation. According to Pholsen *et al*. (2016), the high lactic acid content in silage was related to the simple sugar content from fiber fraction breakdown, enabling high-level production by bacteria. The increase in lactic acid corresponds with a decrease in pH.

The Flieg scores measure the total silage quality based on dry matter content and pH value. In this study, the score of 92.42 to 120.16 showed that all silage produced was of excellent quality. The highest Flieg score was observed in the treatment with a 90-day harvest age and an enzyme level of 0.05%. This high score was due to the combination of elevated dry matter content and low pH of the silage. According to Kilic (1986), Flieg's points between 81 and 100 represent excellent silage quality, and 61–80 shows good quality. In general, the silages in this study showed preservation quality ranging from good to excellent.

Nutrient Content of Silage

The average dry matter content of the silage ranged from 20.21% to 27.97%. These values are higher than the report of Khota *et al*. (2017) and Sriagtula *et al*. (2019), which were 22.37% and 21.99%, respectively. The dry matter content of silage was related to sorghum forage before ensiling. According to Balo *et al*. (2022), the primary influencing factors were the dry matter content before ensiling and the degradation process during the process. The addition of enzymes up to a level of 0.05% increased the dry matter content of the silage, showing that water was used during the fermentation process. Changes in silage dry matter content can occur due to changes in water content, the growth of lactic acid bacteria, and substrate decomposition. Fermentation occurs due to biochemical reactions that convert dry matter into heat energy, CO_2, and H_2O, where CO_2 is reused by anaerobic microorganisms to support the growth. The addition of fiber-degrading enzymes allows microorganisms to grow more optimally due to the availability of simple substrates resulting from fiber fraction degradation. Microorganisms use water content for protoplasm synthesis and dissolved organic matter, increasing silage dry matter.

Ash content increased with the harvest age and the level of added fibrolytic enzymes. In general, the ash content of the silage in this study ranged from 3.51% to 4.19%. This content reflects the availability of inorganic materials, such as minerals. The addition of enzymes up to 0.05% increased the ash content by 0.16% due to the mineral bonds in the cell walls released by enzymes. Sriagtula *et al*. (2021) reported that high ash content was due to enzyme activity that hydrolyzed organic material bonds, causing the minerals in the material to be expressed as ash content.

The crude protein content of the silage shows the breakdown of protein by aerobic bacteria through proteolysis during the fermentation process (Xiong *et al*., 2022). The older harvest ages resulted in the lowest crude protein content, which was 10.01%. This was because the crude protein content of 95-day-old sorghum was lower compared to 80 and 90 days. The crude protein content of silage was influenced by the protein content of the material (Ardiansyah *et al*., 2016). Furthermore, the total crude protein content ranged from 8.93% to 12.11%, showing that the silage provided sufficient ammonia for microbial activity, which was >7% (Sabertanha *et al*., 2021).

The fiber fraction content of silage corresponds to its constituent materials. In older plants, lignification occurs in the stems, leading to higher crude fiber, NDF, and ADF content in sorghum silage. The addition of fibrolytic enzymes up to a level of 0.05% significantly reduced the crude fiber, NDF, and ADF content. This reduction was attributed to the breakdown of hemicellulose bonds into digestible carbohydrates. Fiber-degrading enzymes directly bind to the fiber substrate and hydrolyze complex bonds into simple sugars (Phakachoed *et al*., 2013). The results of this study were different from the investigation of McCuistion *et al.* (2017) using brown midrib sorghum, showing that the use of fibrolytic enzymes did not influence the quality of sorghum silage.

In Vitro Fermentability

Ammonia (NH_3) concentration shows the breakdown of dietary protein in the rumen and the synthesis of microbial protein (Lestari *et al*., 2015). In this study, the NH_3 concentration ranged from 8.85 to 11.89 mM, categorized within the normal range of 4.99 to 17.61 mM (McDonald *et al*., 2010). Generally, NH_3 concentration was closely related to the crude protein content of the feed. The highest NH_3 concentration was observed at a harvest age of 90 days, which was due to the availability of digestible protein by microbes up to that age. In older plants, some of the protein was stored in the plant cell walls, bound to the fiber fraction. According to Li *et al*. (2019), the cell walls of older plants contain about 10% protein, which is bound to the fiber protein fraction and plays a role

in supporting secondary plant growth. Some of the proteins found in the cell walls include proline, glycine, and hydroxyproline (Li *et al*., 2019). The addition of fiber-degrading enzymes at a level of 0.05% was able to increase NH_3 concentration. An increase in NH_3 may be associated with the release of some protein embedded in the cell wall matrix. Fibrolytic enzyme activity may break apart the structural matrix of the cell wall, exposing or releasing proteins that were previously trapped or inaccessible (Gemeda *et al*., 2014; Zhang *et al*., 2020).

VFA concentration is the end product of carbohydrate fermentation in the rumen and shows the availability of energy for ruminants (Phakachoed *et al*., 2013). The results of the study showed an interaction between different harvest ages and the levels of added fiber-degrading enzymes on total VFA concentration. Enzyme treatment was able to increase total VFA concentration. The enzyme level of 0.05% produced the highest increase in VFA, by 20.30%, at an 85-day harvest age compared to without enzyme addition. The total VFA concentration in the study ranged from 103.97 to 125.08 mM, which was within the normal range of 80 to 160 mM (McDonald *et al*., 2010). Furthermore, the highest VFA concentration was observed in the treatment with enzyme addition at 0.05% across all forage harvest ages.

The addition of enzymes was able to increase the availability of simple carbohydrates from fiber breakdown. These enzymes hydrolyze complex bonds structurally into simple carbohydrates. The higher the carbohydrate content in the feed, the greater the ability of microbes to ferment it into VFA (Riswandi *et al*., 2017). Changes in total VFA content are related to the fermentation of sugars released through enzymatic hydrolysis of the cell walls. According to Diaz *et al*. (2015), fibrolytic enzymes can enhance gluconeogenesis reactions, thereby improving fermentation efficiency and the substrate produced from fermentation. Fibrolytic enzymes enhance gluconeogenesis indirectly by breaking down plant fiber into fermentable sugars. These fermentable sugars are then converted into VFAs, such as propionate, during microbial fermentation. Propionate serves as a key substrate for gluconeogenesis in the liver, supporting energy metabolism and productivity, particularly in ruminants.

Dry Matter and Organic Matter Digestibility

The result of this study showed no effect of interaction between harvest age and enzyme level on the DMD and OMD values. In this study, the DMD and OMD values ranged from 53.22%–59.03% and 52.12%–58.58%, respectively. These values are higher than those reported by Khota *et al*. (2017), who found DMD and OMD values for sorghum silage of 51.03% and 57.15%, respectively. The effect of harvest age showed that the digestibility of dry matter and organic matter decreases as the harvest age increases. This result is consistent with the fiber fraction content of sorghum silage in this study.

The OMD values were consistent with the DMD values, with older harvest ages significantly reducing OMD. This is due to the high ash accumulation in forages harvested at older ages. The inorganic nature of ash complicates digestion, leading to lower OMD values.

CONCLUSION

In conclusion, the result of this study showed that increasing the harvest age of sorghum reduced the quality of forage, rumen fermentability, and digestibility. The addition of fibrolytic enzymes up to 0.05% improved sorghum silage quality in terms of nutrients, silage fermentative quality, and rumen fermentability, as well as reduced NDF and ADF. However, the addition of enzymes did not improve DMD and OMD. These findings suggest that while fibrolytic enzymes can enhance the fermentation quality and fiber characteristics of sorghum silage, they may not be sufficient to improve overall digestibility significantly. Therefore, to optimize the use of mature sorghum forage in ruminant diets, enzyme application could be considered in combination with other strategies, such as the use of microbial inoculants or alternative enzyme formulations. Moreover, proper harvest timing remains critical in maintaining high forage quality and maximizing feed efficiency.

CONFLICT OF INTEREST

L. Abdullah and N. R. Kumalasari serve as editors of the Tropical Animal Science Journal but have no role in the decision to publish this article. The authors certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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