ANIMAL BIOSCIENCE

Forage quality and fermentation dynamics of silages of Italian ryegrass (*Lolium multiflorum* Lam.) wilted for varying periods

Yan Fen Li[1], Li Li Wang[2], Young Sang Yu[1], Xaysana Panyavong[1], Li Zhuang Wu[1], and Jong Geun Kim[1,2,*]

* **Corresponding Author:** Jong Geun Kim
Tel: +82-33-339-5728,
E-mail: forage@snu.ac.kr

[1] Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang 25354, Korea

[2] Research Institute of Eco-friendly Livestock Science, Institute of GreenBio Science Technology, Seoul National University, Pyeongchang 25354, Korea

ORCID
Yan Fen Li
https://orcid.org/0000-0002-7318-7318
Li Li Wang
https://orcid.org/0000-0002-6925-7248
Young Sang Yu
https://orcid.org/0000-0002-0046-5807
Xaysana Panyavong
https://orcid.org/0000-0002-9152-076X
Li Zhuang Wu
https://orcid.org/0009-0004-4600-9913
Jong Geun Kim
https://orcid.org/0000-0003-4720-1849

Objective: This trial was conducted to explore the impact of different wilting time of Italian ryegrass (IRG) in the field on the nutritional quality and fermentation characteristics of its silage.
Methods: The harvested IRG was directly wilted in the field for 0 day (W0), 1 day (W1), 2 days (W2), and 3 days (W3), respectively, and tedded every 6 hours. And the preserved IRG was sampled at 1, 2, 3, 5, 10, 20, 30, and 45 days after ensiling and three replicates per treatment.
Results: With the extension of wilting, the dry matter (DM) content and pH value of wilted IRG gradually increased (p<0.05). There was a downward trend in; neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose with the increase of wilting time, but only W2 and W3 were significantly different from W0 (p<0.05). Crude protein (CP), *in vitro* DM digestibility), total digestible nutrients (TDN), and relative feed value decreased significantly with the increase of wilting time (p<0.05), except for W1. After 45 days of ensiling, W1 had the highest CP, TDN, and the lowest ADF and NDF. During ensiling, the increase of acetic acid and the decrease of WSC in W0 and W1 were similar, but the accumulation rate of lactic acid in W0 was faster than that in W1, resulting in the lowest pH value in W0. After 5 days of ensiling, the ratio of lactic acid to acetic acid in W1 stabilized at around 3:1, while W0 kept changing.
Conclusion: Italian ryegrass that wilted in the field for 1 day effectively improved the dynamic changes in CP, TDN, ADF, and NDF and fermentation quality of silage. Therefore, in practice, W1 was more recommended in production of IRG silage.

Keywords: Fermentation; Forage Quality; Italian Ryegrass Silage; Wilting Time

INTRODUCTION

Italian ryegrass (*Lolium multiflorum* Lam., IRG) is considered an important seasonal forage for the maintenance of ruminants due to its high nutritional qualities and is widely cultivated worldwide, such as in temperate, subtropical and tropical plateau regions [1], including Korea. Recently, in Korea, the utiʟization of IRG has been well confirmed and the cultivated area has been gradually increased [2]. Livestock farms have increasingly preferred IRG, which has superior silage quality and palatability, over existing forages such as rye and barley, making it one of the winter forage crops in Korea. In 2018, for winter forage crops, the area of IRG cultivation was 169,000 hectares, and the remaining 6,000 hectares were planted with winter crops such as rye and barley, of which IRG accounted for more than 96.6% [3]. Unfortunately, the best time to harvest IRG is the rainy season, so its storage is an enormous challenge for farmers. The hay making often fails due to rain, silage becomes

the best option.

At present, ensiling has become a common method of forage preservation. High-quality silage can effectively maintain nutrients and palatability of forage. It mainly relies on the fermentation of lactic acid bacteria (LAB) to ferment the carbohydrates in fresh forage into organic acids mainly lactic acid, lower the pH value, and limit the activities of undesirable bacteria [4-6]. Ensiling is an ideal method for maintaining sufficient forage, as it serves as a significant component in the ration of ruminants, not only to effectively alleviate the winter feed shortage problem, but also to help overcome the seasonal imbalance between livestock feed consumption and available forage throughout the year [6,7].

Italian ryegrass silage has become an indispensable source of forage for farms in Korea. However, it is difficult to naturally ferment high-moisture forage to produce high-quality silage. During the ensiling, high-moisture not only causes a large loss of nutrients, but even results in the feed to deteriorate and produce toxic substances, which eventually not only damages the animals' health but also leads to irreparable and significant economic losses to the farm [4,8]. Field wilting prior to ensiling has been widely performed as it is an effective method of reducing moisture content and enhancing silage characteristics and avoiding silo seepage losses [4]. Achieving rapid field wilting is crucial for avoiding the loss of dry matter (DM) and nutritional value. However, the degree of moisture loss and wilting conditions vary, depending on weather conditions, especially temperature, solar radiation, wind conditions, and crop characteristics [9]. If the harvested forage is immediately conditioned and spread in the field, the drying rate can be accelerated and the loss of DM and nutrient can be reduced [4].

Liu et al [10] concluded that wilting before ensiling reduces the content of acetic acid and ammonia nitrogen and improves the fermentation quality, especially heavy wilting limits the production of ammonia nitrogen (NH_3-N) in stylo silage to a greater extent. Many studies have shown that wilting can effectively inhibit the growth of undesirable microorganisms to reduce nutrient loss [4,10,11]. However, some chemical changes may occur during the field wilting process, resulting in nutrient loss, such as the reduction of carbohydrates, protein decomposition, and changes in microbial communities. The current researches have mainly explored factors that affect the wilting speed and quality, and the impact of wilting or not on silage quality, but there are no more references on the influence of wilting time on ensiling dynamics in nutrition value and fermentation profiles of IRG. Thus, this experiment aimed to compare the effects of different wilting time on the forage quality and fermentation dynamics of IRG silage.

MATERIALS AND METHODS

Forage cultivation and silage making

The IRG was harvested on June 6, 2021, in the experimental field of Pyeongchang Campus, Seoul National University (37°32′46.1″ N, 128°26′17.9″ E) in Republic of Korea. The temperature, wind speed and rainfall during the experimental month are presented in Figure 1. The meteorological information is registered as mean temperature 17.73°C, average precipitation 0.13 mm, average wind speed 2.30 m/s, average humidity 75.03% during wilting period (June 6 to 8, 2021). The harvested IRG was directly wilted in the field for 0, 1, 2, and 3 days, respectively, and tedded 4 times every day. Italian ryegrass treatments were as follows: 0-day wilting (W0), 1-day wilting (W1), 2-day wilting (W2), and 3-day wilting (W3). Wilted IRG was chopped into 2 to 3 cm lengths and mixed thoroughly for each treatment. A 400-grams wilted IRG were packed into vacuum polyethylene plastic bags (Food grade, 28 cm×36 cm, Korea) and immediately sealed using a vacuum packer (FM-06; Aostar, Seoul, Korea). The IRG stored at ambient temperature (24°C to 30°C) was sampled at 1, 2, 3, 5, 10, 20, 30 or 45 days after ensiling.

Analytical procedures

About 150-grams fresh material and silage were placed in air - forced drying oven at 65°C for 72 hours to analyze DM. The dried samples were ground into 1-mm particle size using a mill (Thomas Scientific, Swedesboro, NJ, USA) for nutrients analysis. Total nitrogen was determined by an elemental analyzer (Euro Vector EA3000; EVISA Co., Ltd, Milan, Italy) according to Dumas method [12]. The water-soluble carbohydrates (WSC) was analyzed by the anthrone method [13]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were performed via the method of Van Soest et al [14] using an Ankom2000 fiber analyzer (Ankom Technology Corp., Fairport, NY, USA) and sodium sulfite and α-amylase were added for NDF producer. *In vitro* DM digestibility (IVDMD) was quantified after the 48-h incubation by an Ankom DaisyII incubator (Ankom Technologies, Inc., USA), according to the method of Goering and Van Soest [15]. The rumen donor (Holstein) and rumen fluid preparation were described as reported by Ahmadi et al [16]. Total digestible nutrients were got by the equation (88.9 − [0.79× ADF %]) [17]. Relative feed value (RFV) was calculated by the following formula described by Rohweder et al [18]. Digestibility of dry matter (DDM) = 88.9−(0.779×ADF%); dry matter intake (DMI) = 120/NDF%; RFV = (DMI×DDM)/ 1.29.

Forage extract was prepared for the analysis of fermentation profile followed by Wei et al [19]. The pH value of extract was determined immediately by the AB 150 pH meter (Fisher Scientific International, Inc., Pittsburgh, PA, USA). Organic
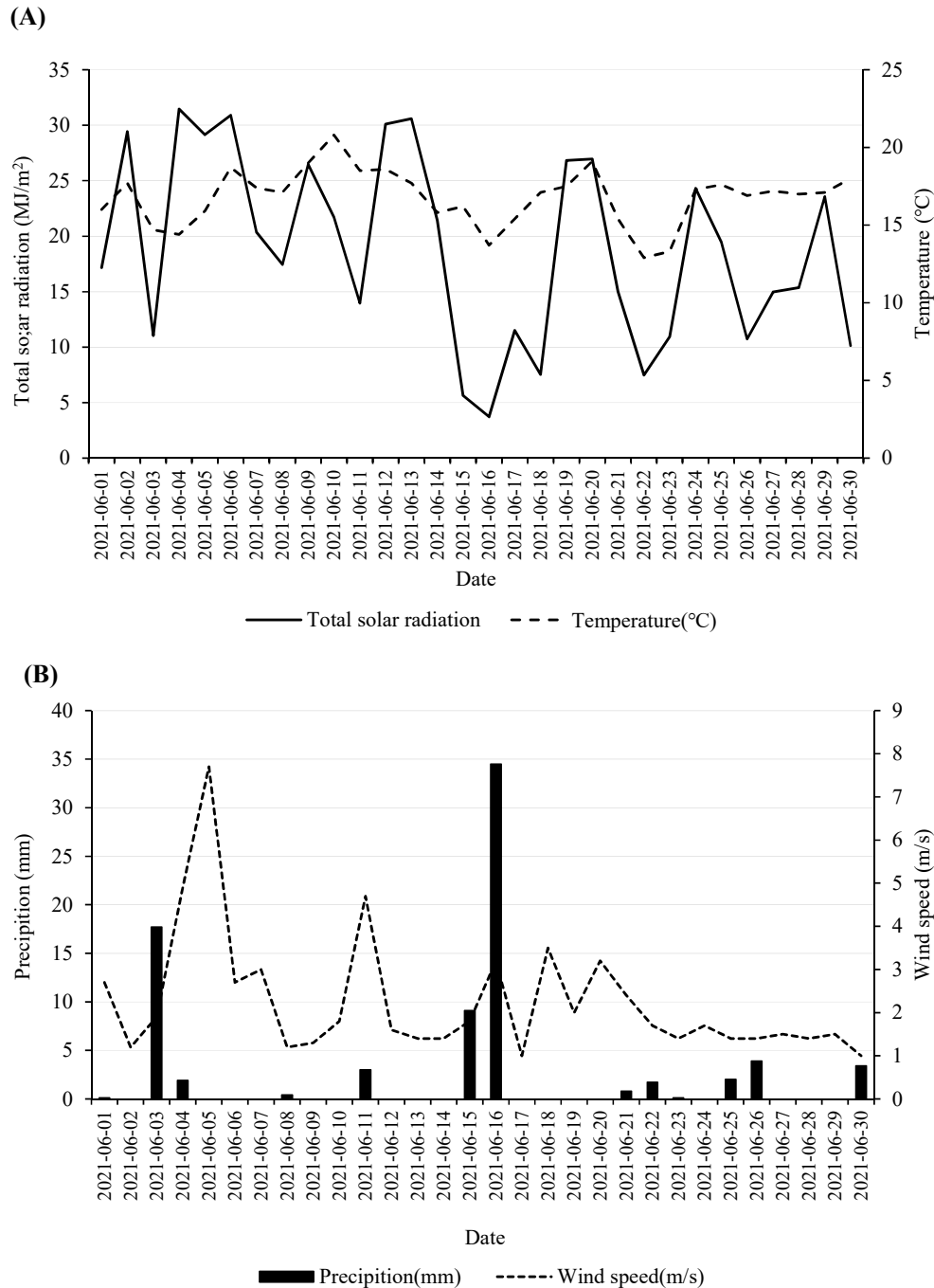
(A)

(B)

Figure 1. Average temperature and total solar radiation (A), wind speed and precipitation (B) during the experimental month (June 1 to 30, 2021). Source: Korean Meteorological Administration.

acids were determined by HPLC system (Detector, RI; Column, Agilent Hi-Plex H; Agilent Technologies 1260 Infinity, Santa Clara, CA, USA) in accordance with the previously prescribed procedures [19]. NH₃-N was measured by a UVIDEC-610 spectrophotometer (Jasco, Tokyo, Japan) [20].

Statistical analysis

Data were analyzed by two-way analysis of variance with gen-eral linear model Proc in SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0.; IBM Corp., Armonk, NY, USA). The analysis model was expressed as: $Y_{ij} = \mu + T_i + D_j + (T \times D)_{ij} + \varepsilon_{ij}$, where Y_{ij} = observation, μ = mean, T_i = effect of treatments (wilting time), D_j = day of ensiling, $(T \times D)_{ij}$ = interaction effect of treatment × day of ensiling, and ε_{ij} = error term. Each silage bag was considered the ex-perimental unit in the model. Duncan's multiple range test

was used to assess the significance of differences between treatment groups. If p<0.05, there was a significant difference.

RESULTS AND DISCUSSION

Chemical composition prior to ensiling

The moisture of the forage plays an important role in silage fermentation quality. Alcoholic fermentation may occur if low-moisture silage is prepared with a high-sugar grass [21] and the high moisture content of tropical grasses induces acetic acid fermentation to dominate the silage process [22]. Table 1 shows that there was a positive correlation between DM content and wilting time. With the increase of wilting days, the DM content increased and there was a rapid rise in DM during the first 2 days of wilting. Our result was consistent with Ribas et al [23]. Furthermore, Polak and Jančová [24] wilted grass and mixed forage crops for 2 days and measured the DM content in the morning and afternoon each day and observed that the DM increased as the wilting time increased, and there are disadvantages to excessive wilting. This may be because longer wilting time can result in reduced population of LAB and the proliferation of undesirable bacteria. Pahlow et al [25] indicated that prolonged exposure of forage to sunlight can negatively affect silage quality. Similarly, in our study, it can be found there was an increase in NDF, ADF, and hemicellulose (HEM) and decline in IVDMD before ensiling. The effect of wilting time on digestibility was opposite to that of NDF and ADF, W0 was the highest. This is similar to Keles et al [26], who demonstrated wilting for 72 hours leaded to lower IVDMD compared with 24 or 48 hours. Prolonged wilting can lead to increased microbial activity, which may contribute to further breakdown of soluble components and an increase in fiber content. In addition, wilting results in a concentration of the plant's cell wall components. There was a decline in CP as wilting time increased and the CP dropped significantly after 24 hours. Likewise, Müller et al [27] showed that the CP content

of three different fodder crops decreased with increasing wilting time. Furthermore, studies have proven that CP in forage decreased with increasing wilting time or DM content [23,27]. The decrease in protein content may be due to soluble proteins in the plant material leaching out with water as the forage dries during the wilting process. In a previous study, although different, the pH of all three forage crops increased to 5.48-6.84 as the amount of DM increased [27]. Similarly, the pH value gradually changed from 5.86 to 6.41 as the DM content of IRG increased from 30.61% to 75.66% during wilting in our experiment. This may be due to the proteolysis, the activities of aerobic microbes or the respiration of plant cells during wilting. A similar result where the pH of clover increased with prolonging wilting time was also reported by Orosz [28]. It was clarified that the DM content was considered to vary in parallel with the pH value [29].

The effects of wilting time and ensiling days on the chemical compositions of IRG silage after ensiling

During ensiling, the DM content had similar changing trends for W0, W1, W2, and W3 (Table 2). There was a decrease in DM with increasing ensiling time (p<0.05) but W0 and W1 had a larger drop relative to W2 and W3. This may be attributed to the moisture content at the initial of the IRG silage. The moisture of the forage is an important factor affecting the fermentation quality of silage. Wilting time significantly affected the CP content (p<0.01) but the ensiling days had no significant effect on CP (p>0.05) (Table 2). There were ups and downs in CP content of W0 and W1 changes, but W2 and W3 were almost maintained in a stable state throughout the process, which may be related to the moisture content. This is because water activity affects microbial growth and enzyme activity [30]. Overall, the CP was increased in W1, it may be inferred that special microbial activities synthesize amino acids or peptides into proteins, or dead bacteria are degraded into protein.

During ensiling, there is no significant change in NDF

Table 1. The chemical composition of Italian ryegrass with different wilting time prior to ensiling

| Treatments[1] | DM (% FM) | pH | % DM | | | | | | RFV | TDN (%) |
			WSC	CP	NDF	ADF	HEM	IVDMD		
W0	30.61[d]	5.86[b]	17.67	6.36[a]	66.06[b]	40.28[c]	25.78[ab]	65.87[a]	81.01[a]	57.08[a]
W1	42.78[c]	6.27[a]	15.96	6.31[a]	66.75[b]	41.39[bc]	25.37[b]	61.69[ab]	78.96[a]	56.20[ab]
W2	71.01[b]	6.40[a]	18.96	5.06[b]	70.17[a]	43.27[a]	26.90[a]	58.84[b]	73.17[b]	54.72[c]
W3	75.66[a]	6.41[a]	17.48	4.19[b]	69.41[a]	42.43[ab]	26.99[a]	59.28[b]	74.89[b]	55.38[bc]
Mean	56.43	6.24	18.02	5.48	68.10	41.84	26.26	61.42	77.00	55.85
SEM	0.64	0.13	0.89	0.45	0.72	0.60	0.61	2.36	1.25	0.47

DM, dry matter; FM, fresh weight; WSC, water-soluble carbohydrates; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; HEM, hemicellulose; IVDMD, in vitro dry matter digestibility; RFV, relative feed value; TDN, total digestible nutrients; SEM, standard error of mean.
[1] Treatments were 0-day wilting (W0), 1-day wilting (W1), 2-day wilting (W2), 3-day wilting (W3).
[a-d] Withina column means with different superscripts differ (p<0.05).

Table 2. Effect of different wilting period on the dynamic change of DM, CP, NDF, ADF and hemicellulose content of Italian ryegrass silage

Items	Treatments[1]	Ensiling days (d)								Mean	SEM	p-value[2]		
		1	2	3	5	10	20	30	45			T	D	T×D
DM (% FM)	W0	28.72	28.47	26.50	27.34	27.37	27.93	27.55	27.89	27.72	1.15	<0.001	<0.001	<0.001
	W1	39.40	39.55	39.75	39.95	37.37	39.90	38.99	39.04	38.00				
	W2	71.00	68.53	66.04	70.06	67.41	68.81	69.60	69.28	68.84				
	W3	72.83	73.72	73.54	74.58	72.98	73.24	73.33	75.13	73.67				
	Mean	52.99	52.57	51.46	52.98	48.78	52.47	52.37	52.84	52.06				
CP (% DM)	W0	6.32	7.15	6.90	6.62	6.36	6.03	6.56	6.23	6.52	0.29	<0.001	0.127	0.120
	W1	6.98	7.00	6.66	6.60	6.36	6.73	7.64	7.58	6.94				
	W2	4.96	4.90	5.22	5.06	5.35	5.13	5.06	5.05	5.09				
	W3	4.75	5.37	4.63	4.52	4.38	4.95	4.51	4.82	4.74				
	Mean	5.75	6.11	5.85	5.70	5.61	5.71	5.94	5.92	5.82				
NDF (% DM)	W0	65.33	63.26	63.87	65.53	64.43	65.52	64.31	62.55	64.35	1.68	<0.001	0.300	0.150
	W1	60.08	59.38	60.53	61.18	62.43	62.55	61.88	61.38	61.43				
	W2	62.71	68.44	67.64	73.56	69.96	70.01	72.01	70.59	69.37				
	W3	71.54	72.44	71.28	70.84	70.13	69.40	70.17	71.44	70.91				
	Mean	64.92	65.88	65.83	67.78	67.24	66.87	67.09	66.49	66.51				
ADF (% DM)	W0	40.12	39.38	39.09	40.39	40.68	40.49	40.09	40.65	40.11	1.72	<0.001	0.250	0.500
	W1	36.79	37.19	36.88	38.01	38.68	38.73	38.48	38.01	38.10				
	W2	36.88	42.45	42.23	47.42	44.24	44.52	45.69	45.55	43.62				
	W3	45.03	43.52	44.57	44.45	43.64	43.23	43.55	43.91	43.99				
	Mean	39.71	40.64	40.69	42.57	42.31	41.74	41.95	42.03	41.46				
HEM (% DM)	W0	25.21	23.88	24.77	25.14	23.75	25.02	24.23	21.91	24.24	0.65	<0.001	0.640	0.060
	W1	23.29	22.19	23.65	23.16	22.97	23.83	23.40	23.37	23.23				
	W2	25.83	25.98	25.41	26.14	25.72	25.50	26.32	25.03	25.74				
	W3	26.50	28.91	26.71	26.39	26.49	26.17	26.62	27.53	26.92				
	Mean	25.21	25.24	25.14	25.21	24.73	25.13	25.14	24.46	25.03				

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; SEM, standard error of mean; FM, fresh weight; HEM, hemicellulose.
[1] Treatments were 0-day wilting (W0), 1-day wilting (W1), 2-day wilting (W2), 3-day wilting (W3).
[2] T, treatments; D, the days of ensiling; T×D, the interaction of treatments and ensiling days.
When p-value was smaller than 0.05, means were significantly different.

and ADF of W0, W1, W2, and W3 (p>0.05), among which W1 and W2 had frequent changes and in a wide range (Table 2). The occurrence of this phenomenon may be due to the metabolic activity of microorganisms during ensiling for W1, and may be related to the changes in DM content for W2. W3 and W2 had the higher NDF, ADF, and HEM compared to W0 and W1 (p<0.01) during ensiling. And ADF was the lowest in W1 (p<0.01). This could be explained as the cell wall is degraded by plant enzymes, cellulolytic microorganisms or acid hydrolysis. The higher NDF and ADF concentration were reflected in a lower TDN and RFV for W2 and W3. This is line in with the theory that RFV and TDN are calculated based on NDF and ADF [18]. TDN was relatively stable overall in W0, W1, and W3, but W2 changed significantly. W1 had the highest TDN, followed by W0 (Table 3). British turf with wilting for 24, 48, and 72 hours was ensiled for 295 days, which proved that wilting time had a significant effect on DM and IVDMD [26]. In the experiment, it was reported that IVDMD increased and decreased during the ensiling.

Fermentation quality after ensiling

The effects of the wilting time and days of ensiling on silage pH are illustrated in Figure 2. During the first 10 days of ensiling, the pH had the greatest drop in W0 and W1, but little change in W2 and W3. The rapid drop in pH value can effectively reduce proteolysis, inhibit undesirable microorganisms and reduce nutrient loss [31,32]. The pH value in W0 and W1 dropped to 4.23 on the 10th day of ensiling while W2 and W3 have a higher pH value, 5.50 and 6.03 respectively. It is likely deduced that as forage wilts and loses moisture, the concentration of these buffering agents increases, making it harder for the pH to decrease rapidly once fermentation begins. After that only W0 had the further declines and reached 3.81 after 60 days-ensiling. Studies indicated that a pH between 3.5 and 4.5 indicates a successful fermentation for forage silage [32]. It suggested that W0 and W1 were successfully fermented while there was a poor fermentation in W2 and W3. Furthermore, studies reported that high DM content in forage do not contribute to decreasing silage pH levels [33]. Therefore, our experimental results were further confirmed.

Table 3. Effect of different wilting periods on the dynamic change of RFV, TDN, and IVDMD of Italian ryegrass sampled at days of ensiling

| Items | Treatments[1] | Ensiling days (d) | | | | | | | | Mean | SEM | p-value[2] | | |
		1	2	3	5	10	20	30	45			T	D	T×D
IVDMD	W0	61.55	64.53	62.95	60.77	58.79	62.91	61.95	61.94	61.92	1.31	0.14	<0.001	0.15
(% DM)	W1	62.92	62.53	61.79	61.29	58.79	61.91	58.65	58.00	60.74				
	W2	59.87	60.07	62.45	59.50	58.91	61.11	57.97	64.67	60.57				
	W3	61.56	62.94	63.16	59.71	61.81	63.38	57.80	61.94	61.54				
	Mean	61.48	62.52	62.59	60.32	59.58	62.33	58.83	61.64	61.18				
TDN (%)	W0	57.20	57.79	58.02	56.99	56.76	56.91	57.23	56.79	57.21	1.01	<0.001	0.03	0.15
	W1	59.84	59.52	59.76	58.87	59.05	58.31	58.50	58.87	59.09				
	W2	59.76	58.69	55.54	54.77	53.95	53.73	52.81	52.91	55.27				
	W3	53.32	54.52	53.69	53.79	54.42	54.75	54.50	54.21	54.15				
	Mean	57.53	57.63	56.75	56.10	56.05	55.92	55.76	55.70	56.43				
RFV	W0	82.09	85.67	85.21	81.55	82.61	81.45	83.42	85.10	83.39	3.57	<0.001	0.27	0.38
	W1	93.36	93.91	92.48	90.17	91.08	87.57	89.06	90.09	90.96				
	W2	89.26	77.04	77.65	67.80	72.38	72.04	68.90	70.39	74.43				
	W3	70.02	70.62	70.78	71.27	72.82	74.02	72.95	71.23	71.72				
	Mean	83.68	81.81	81.53	77.70	79.72	78.77	78.58	79.20	80.12				

RFV, relative feed value; TDN, total digestible nutrients; IVDMD, *in vitro* dry matter digestibility; SEM, standard error of mean; DM, dry matter.
[1] Treatments were 0-day wilting (W0); 1-day wilting (W1); 2-day wilting (W2); 3-day wilting (W3).
[2] T, treatments; D, the days of ensiling; T×D, the interaction of treatments and ensiling days.
When p-value was smaller than 0.05, means were significantly different.

The change in pH value is closely related to the lactic acid concentration, and the rapid decrease in pH value is attributed to the low pKa of lactic acid [34]. Our experimental result likewise indicated this. It was reflected in changes in lactic acid concentration during ensiling. Lactic acid concentrations in both W0 and W1 increased rapidly and were consistent at day 10 (8.16% DM), while there was still a rapid increase in W0 afterward but stabilized in W1 (p<0.001) (Figure 3). This coincided with the decrease in pH. However, the lactic acid concentration of W2 and W3 was quite low, lower than 1% in DM. The lactic acid concentration in IRG

silage exceeded the typical range (20 to 40 g/kg DM) in this experiment, which may be attributed to the higher moisture content (600 to 700 g/kg) of W0 and W1 during ensiling, which promoted the formation of a large amount of lactic acid Kung Jr et al [34]. Wilting level had significant effects on IRG fermentation by limiting microflora activity, as shown by a decrease in fermentation product with increasing DM [35]. In the study, this was reflected in the higher pH (p<0.001) and lower (p<0.01) lactic acid concentration in the more wilted silages (W2 and W3). Light wilted silage (W1) showed more intense fermentation with higher lactic acid
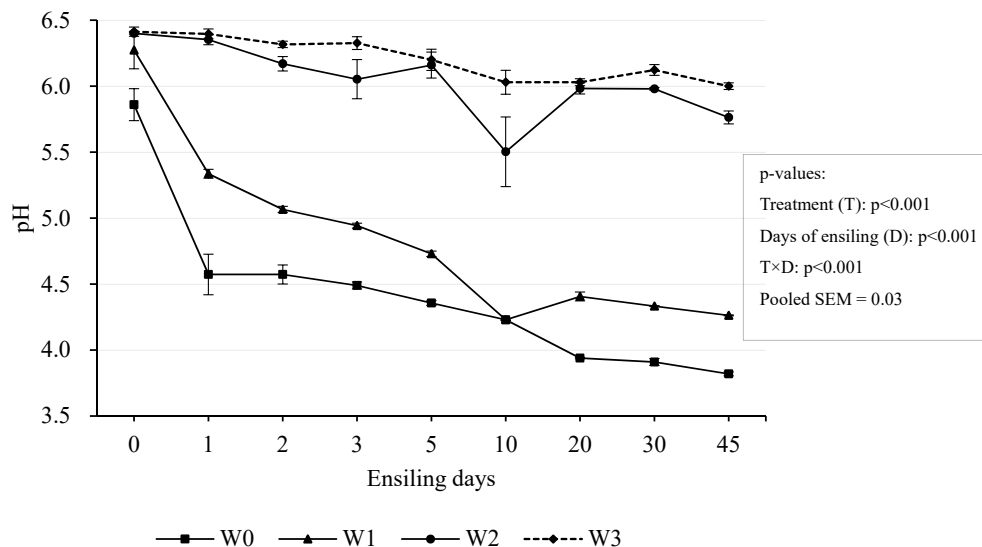

Figure 2. Dynamic changes of pH during ensiling. Treatments were 0-day wilting (W0), 1-day wilting (W1), 2-day wilting (W2), 3-day wilting (W3). T×D, the interaction of treatments and ensiling days. SEM, standard error of mean. If p<0.05/0.001, there was a significant difference.
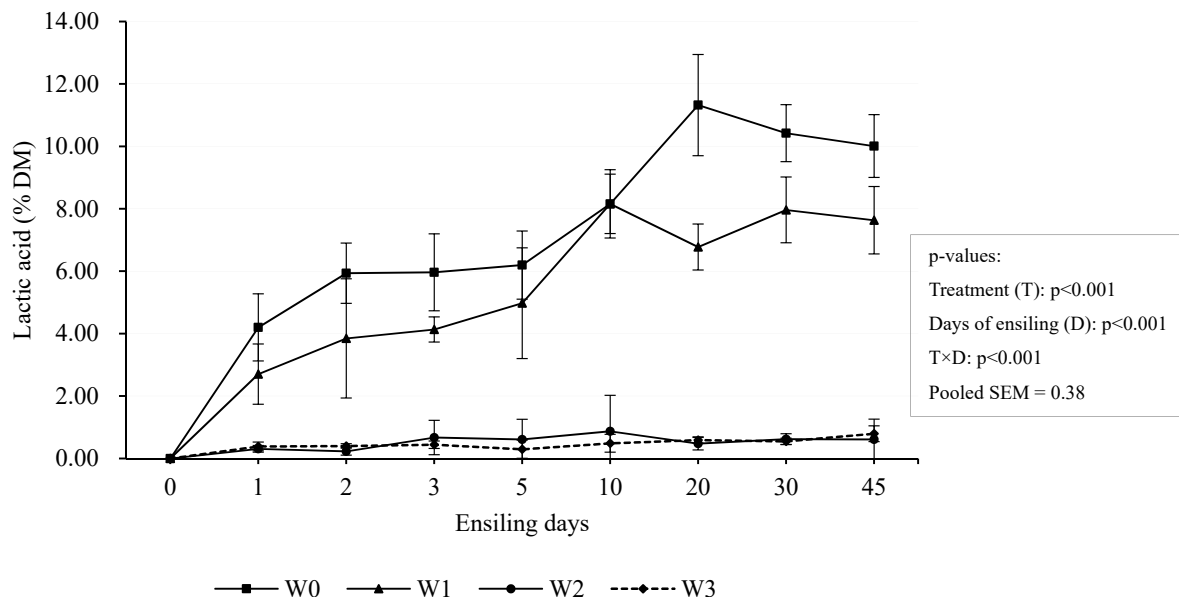
Figure 3. Dynamic changes of lactic acid concentration during ensiling. Treatments were 0-day wilting (W0), 1-day wilting (W1), 2-day wilting (W2), 3-day wilting (W3). T×D, the interaction of treatments and ensiling days. SEM, standard error of mean. If p<0.05/0.001, there was a significant difference.

and acetic acid content.

The acetic acid content in W0 and W1 had the largest increase on the first day but then increased slowly while acetic acid was almost not detected in W3 (0.06% DM) (Figure 4). After 45 days-ensiling, W0 and W1 had the highest acetic acid concentration of 2.47% and 2.59% in DM respectively. Acetic acid plays a vital role in silage. The appropriate amount of acetic acid content in silage can not only inhibit yeast and

improve the aerobic stability of silage, but can also be absorbed by the rumen and used for energy or incorporated into milk or body fat. Thus, less is not always better, acetic acid usually accounts for 1% to 3% DM in silage [34]. The acetic acid content in this experiment was just within this range.

The effects of wilting time and days of ensiling on WSC of the IRG are presented in Figures 5. There was gradual de-
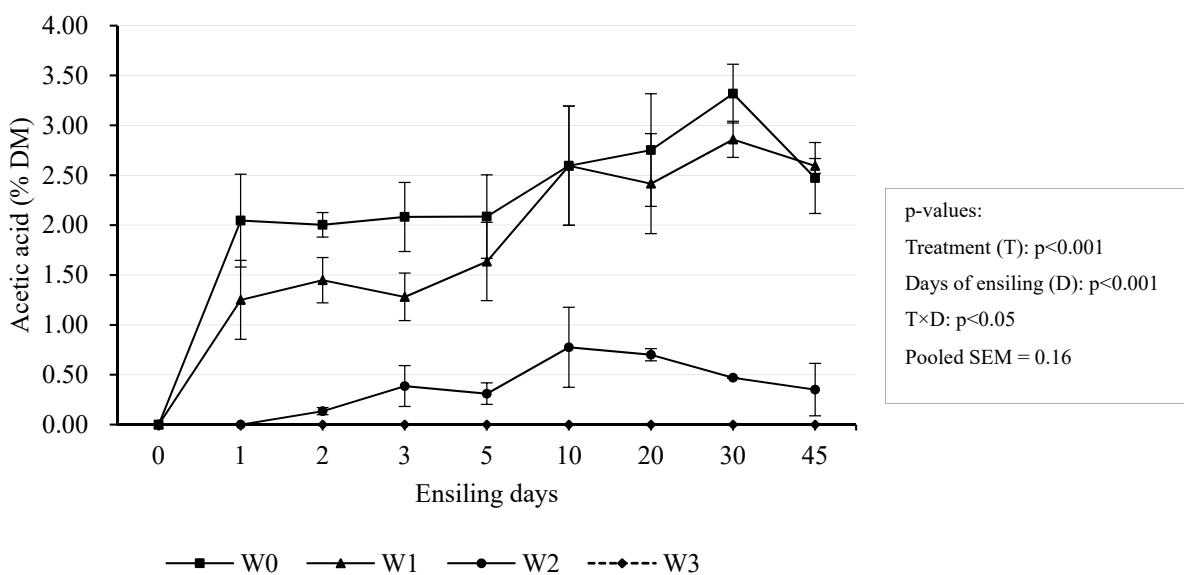

Figure 4. Dynamic changes of acetic acid concentration during ensiling. Treatments were 0-day wilting (W0), 1-day wilting (W1), 2-day wilting (W2), 3-day wilting (W3). T×D, the interaction of treatments and ensiling days. SEM, standard error of mean. If p<0.05/0.001, there was a significant difference.
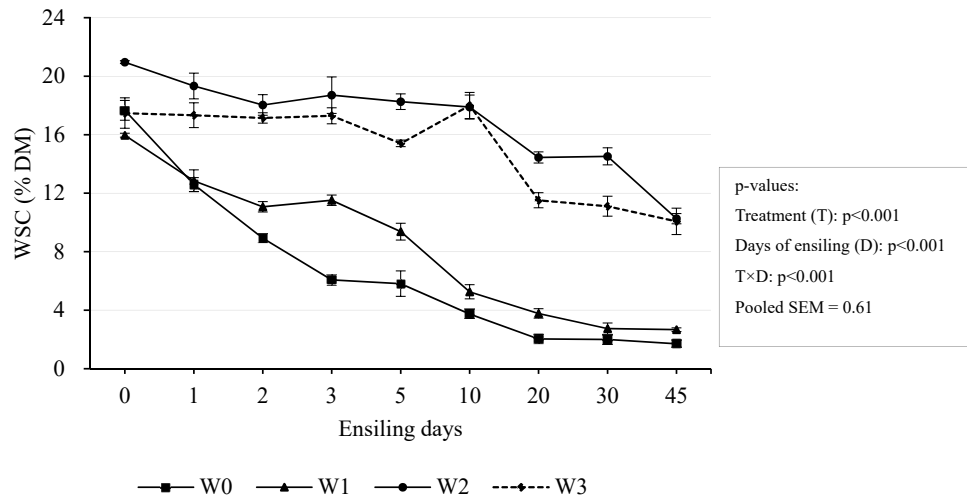
Figure 5. Dynamic changes of WSC concentration during ensiling. Treatments were 0-day wilting (W0), 1-day wilting (W1), 2-day wilting (W2), 3-day wilting (W3). T×D, the interaction of treatments and ensiling days. SEM, standard error of mean. If p<0.05/0.001, there was a significant difference.

crease in WSC concentration in all silages, and the decline was relatively rapid during the first 5 days of fermentation, followed by a slower decline. W0 had the largest decrease in WSC followed by W1, W2, and W3 (p<0.001). After 45 days-ensiling, WSC consumption rates varied, with W0 reaching the maximum of 90.27%, followed by W1 at 83.27%, then W2 at 51.96%, and finally W3 at 42.39%. This corresponded to the level of wilting. As the wilting time increased, the WSC consumption rate decreased. This may be because moisture content affects bacterial growth and thus prevents WSC from being utilized. Depletion of WSC during ensiling is generally due to the consumption of LAB, converting it into organic acids (mainly lactic acid) [32]. Alternatively,

WSC may also be consumed by undesirable bacteria and LAB, with the net result of a low lactic acid level that may not be sufficient to acidify the silages strongly and thus retard the growth of undesirable bacteria [32]. In our experiments, WSC concentration was negatively correlated with lactic acid and acetic acid but positively correlated with pH value. This further showed that the wilting time was prolonged and the moisture content was reduced, which affected microbial activity and inhibited fermentation. This is line in with Valente et al [35].

W0 and W1 showed an increase in the lactic acid/acetic acid ratio during the early phase of ensiling, after that W1 tended to be stable but W0 had an increase and drops (Fig-
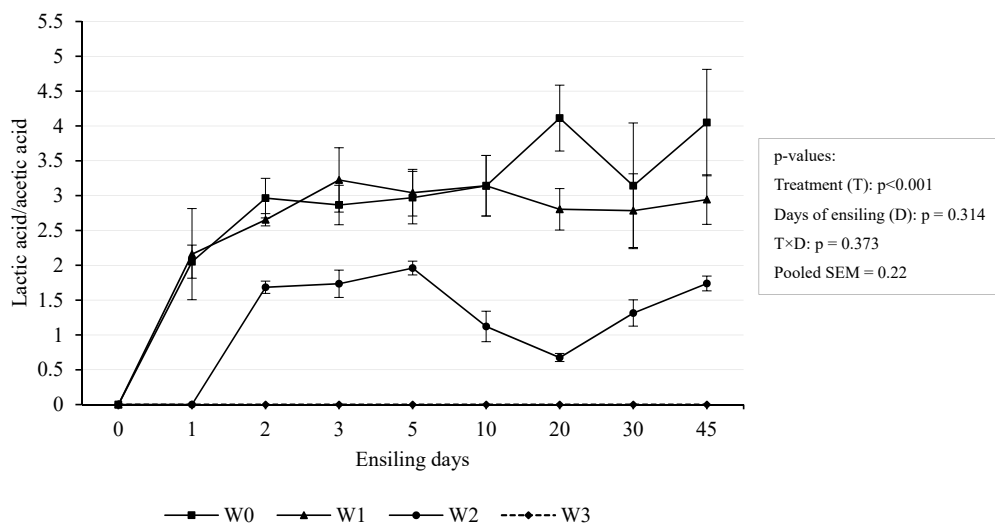

Figure 6. Dynamic changes of lactic: acetic acid ratio concentration during ensiling. Treatments were 0-day wilting (W0), 1-day wilting (W1), 2-day wilting (W2), 3-day wilting (W3). T, treatments; D, the days of ensiling; T×D, the interaction of treatments and ensiling days. SEM, standard error of mean. If p<0.05/0.001, there was a significant difference.

ure 6). After 5 days of ensiling, the lactic acid to acetic acid value of W1 stabilized at around 3:1, while that of W0 stabilized at around 3:1 within 3-10 days and then rose to 4.05:1, and dropped again. It showed that W1 fermentation was basically stable after 5 days of ensiling, while W0 was less stable. W2 had a slower and lower increase and a decrease after 5 days of ensiling, and the maximum value didn't reach 2:1. The ratio of lactic acid to acetic acid is considered an indicator of the type of fermentation. Researches showed that good silage fermentation usually has a ratio of 2.5 to 3.0 [34]. Therefore, it's proved that W1 had good fermentation. Silage with a higher than normal ratio of lactic acid: acetic acid may indicate that it is more unstable because acetic acid at lower concentrations is not sufficient to inhibit lactate-assimilating yeasts. In our study, W0 had a lactic acid: acetic acid ratio higher than 3, which may be attributed to W0 having a higher moisture content relative to the other treatments. It also indicated that W0 silage was more unstable. W3 has an abnormal lactic acid: acetic acid ratio, which may be due to low moisture content causing abnormal fermentation. This is line with Kung Jr et al [34], who reported that a lactic acid:acetic acid ratio below 1:1 usually indicates an abnormal fermentation.

CONCLUSION

In our study, wilted 1-day-wlited IRG silage had greater potential for nutritional value and fermentation profile. There was a significant decline in moisture content, an increase in CP content and a decrease in fiber content (ADF, NDF, and HEM), thereby improving TDN and RFV for 1-day wilted IRG. What's more, the nutritional value of forage that has wilted for 1 day was effectively retained after ensiling, avoiding nutritional losses caused by excessive moisture before ensiling. Moreover, wilted 1-day-wlited IRG silage was more in line with the needs of livestock farmer for forages. Not only did the its acetic acid content fall within the recommended range, but it was mainly homo-fermented by LAB. Above all, secondary fermentation was less likely to occur compared to direct ensiled feed that did not wilt. Hence, in semblable climates, 1-day wilting is recommended as the optimal wilting time for IRG before ensiling to optimize the nutritional value and fermentation characteristics of IRG silage in our experiment.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any organization regarding the materials discussed in the manuscript.

FUNDING

REFERENCES

1. Fluck AC, Costa OAD, Schafhauser Junior J, et al. In vitro and in situ digestibility of annual ryegrass silage from distinct phenological growth stages and predehydration times. Biosci J 2018;34:1239-47. https://doi.org/10.14393/BJ-v34n5a2018-39421

2. Soundharrajan I, Kuppusamy P, Park HS, et al. Lactic acid bacteria mixture as inoculants on low moisture italian ryegrass silage fermentation. J Kor Soc Grassl Forage Sci 2019;39:127-31. https://doi.org/10.5333/KGFS.2019.39.3.127

3. MAFRA. The current situation of forage increase production and supplementation policy. Sejong, Korea: Ministry of Agriculture, Food and Rural Affairs; 2019.

4. Borreani G, Tabacco E, Schmidt RJ, Holmes BJ, Muck RE. Silage review: factors affecting dry matter and quality losses in silages. J Dairy Sci 2018;101:3952-79. https://doi.org/10.3168/jds.2017-13837

5. Eikmeyer FG, Köfinger P, Poschenel A, et al. Metagenome analyses reveal the influence of the inoculant Lactobacillus buchneri CD034 on the microbial community involved in grass ensiling. J Biotechnol 2013;167:334-43. https://doi.org/10.1016/j.jbiotec.2013.07.021

6. Yin X, Zhao J, Wang S, Dong Z, Li J, Shao T. The effects of epiphytic microbiota and chemical composition of Italian ryegrass harvested at different growth stages on silage fermentation. J Sci Food Agric 2023;103:1385-93. https://doi.org/10.1002/jsfa.12232

7. Yan Y, Li X, Guan H, et al. Microbial community and fermentation characteristic of Italian ryegrass silage prepared with corn stover and lactic acid bacteria. Bioresour Technol 2019;279:166-73. https://doi.org/10.1016/j.biortech.2019.01.107

8. Popescu RG, Rădulescu AL, Georgescu SE, Dinischiotu A. Aflatoxins in feed: types, metabolism, health consequences in swine and mitigation strategies. Toxins 2022;14:853. https://doi.org/10.3390/toxins14120853

9. Charmley E. Towards improved silage quality–a review. Can J Anim Sci 2001;81:157-68. https://doi.org/10.4141/A00-066

10. Liu Q, Zhang J, Shi S, Sun Q. The effects of wilting and storage temperatures on the fermentation quality and aerobic stability of stylo silage. Anim Sci J 2011;82:549-53. https://doi.org/10.1111/j.1740-0929.2011.00873.x

11. Nishino N, Li Y, Wang C, Parvin S. Effects of wilting and molasses addition on fermentation and bacterial community in guinea grass silage. Lett Appl Microbiol 2012;54:175-81. https://doi.org/10.1111/j.1472-765X.2011.03191.x

12. Horwitz W. Official methods of analysis of AOAC interna-

tional. 17th ed. Gaithersburg, MD, USA: AOAC international; 2000.

13. Yemm EW, Willis AJ. The estimation of carbohydrates in plant extracts by anthrone. Biochem J 1954;57:508-14. https://doi.org/10.1042/bj0570508

14. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583-97. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

15. Goering HK, Van Soest PJ. Forage fiber analyses (apparatus, reagents, procedures, and some applications). In: United States Department of Agriculture, editor. Washington, DC, USA: US Agricultural Research Service; 1970.

16. Ahmadi F, Li YF, Jeong EC, Wang LL. Bharanidharan R, Kim JG. Comparative accuracy of in vitro rumen fermentation and enzymatic methodologies for determination of undigested neutral detergent fiber in forages and development of predictive equations using NIRS. Agriculture 2022;12:1914. https://doi.org/10.3390/agriculture12111914

17. Schroeder JW. Forage nutrition for ruminants. Fargo, USA: North Dakota State University Extension Service; 2004.

18. Rohweder DA, Barnes RF, Jorgensen N. Proposed hay grading standards based on laboratory analyses for evaluating quality. J Anim Sci 1978;47:747-59. https://doi.org/10.2527/jas1978.473747x

19. Wei SN, Jeong EC, Li YF, Kim HJ, Ahmadi F, Kim JG. Evaluation of forage production, feed value, and ensilability of proso millet (Panicum miliaceum L.). J Anim Sci Technol 2022;64:38-51. https://doi.org/10.5187/jast.2021.e131

20. Broderick GA, Kang JH. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J Dairy Sci 1980;63:64-75. https://doi.org/10.3168/jds.S0022-0302(80)82888-8

21. Driehuis F, van Wikselaar PG. The occurrence and prevention of ethanol fermentation in high-dry-matter grass silage. J Sci Food Agric 2000;80:711-8. https://doi.org/10.1002/(SICI)1097-0010(20000501)80:6<711::AID-JSFA593>3.0.CO;2-6

22. Nishino N, Li Y, Wang C, Parvin S. Effects of wilting and molasses addition on fermentation and bacterial community in guinea grass silage. Lett Appl Microbiol 2012;54:175-81. https://doi.org/10.1111/j.1472-765X.2011.03191.x

23. Ribas WFG, Monção FP, Rocha Júnior VR, et al. Effect of wilting time and enzymatic-bacterial inoculant on the fermentative profile, aerobic stability, and nutritional value of BRS capiacu grass silage. Rev Bras Zootecn 2021;50:e20200207. https://doi.org/10.37496/rbz5020200207

24. Polak M, Jančová M. Effectiveness of conditioning herbage at harvesting. In: Proceedings of the 12th International Symposium Forage Conservation; 2006 April 3-5: Brno, Czech Republic. pp. 186-9.

25. Pahlow G, Muck RE, Driehuis F, Elferink SO, Spoelstra SF. Microbiology of ensiling. Silage Sci Technol 2003;42:31-93. https://doi.org/10.2134/agronmonogr42.c2

26. Keles G, O'Kiely P, Lenehan JJ, Forristal PD. Conservation characteristics of baled grass silages differing in duration of wilting, bale density and number of layers of plastic stretch-film. Irish J Agric Food Res 2009;48:21-34.

27. Müller CE, Pauly TM, Udén P. Storage of small bale silage and haylage -influence of storage period on fermentation variables and microbial composition. Grass Forage Sci 2007; 62:274-83. https://doi.org/10.1111/j.1365-2494.2007.00580.x

28. Orosz S, Bellus Z, Kroll B. Alfalfa bale-silage: a new bale-forming technology. In: Rajčáková L, editor. In: Proceedings of the 13th International Conference of Forage Conservation; 2008 September 3-5: Nitra, Slovakia. Nitra, Slovakia: Slovak Agricultural Research Centre, Research Institute of Animal Production, Nitra; 2008. pp. 166-7.

29. Şahar AK, Vurarak Y, Cubukcu P, Yücel H. Determining the effects of wilting time on some quality parameters in soybean haylage. Turk J Field Crops 2020;25:26-31. https://doi.org/10.17557/tjfc.526235

30. Gomez EJ, Delgado JA, Gonzalez JM. Influence of water availability and temperature on estimates of microbial extracellular enzyme activity. Peer J 2021;9:e10994. https://doi.org/10.7717/peerj.10994

31. Gheller LS, Ghizzi LG, Takiya CS, et al. Different organic acid preparations on fermentation and microbiological profile, chemical composition, and aerobic stability of whole-plant corn silage. Anim Feed Sci Technol 2021;281:115083. https://doi.org/10.1016/j.anifeedsci.2021.115083

32. McDonald P, Henderson A, Heron SJE. The biochemistry of silage. 2nd ed. Marlow, England: Chalcombe publications; 1991.

33. Guo XS, Undersander DJ, Combs DK. Effect of inoculants and forage dry matter on the fermentation and aerobic stability of ensiled mixed-crop tall fescue and meadow fescue. J Dairy Sci 2013;96:1735-44. https://doi.org/10.3168/jds.2045-5786

34. Kung L Jr, Shaver RD, Grant RJ, Schmidt RJ. Silage review: interpretation of chemical, microbial, and organoleptic components of silages. J Dairy Sci 2018;101:4020-33. https://doi.org/10.3168/jds.2017-13909

35. Valente ME, Borreani G, Caredda S, Cavallarin L, Sulas L. Ensiling forage garland (Chrysanthemum coronarium L.) at two stages of maturity and at different wilting levels. Anim Feed Sci Technol 2003;108:181-90. https://doi.org/10.1016/S0377-8401(03)00123-8