Morning *vs*. Afternoon Harvest Time of Alfalfa, Clover, and Barley Affect the Chemical Composition and Nutritional Value of Silage

Research Article

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ABSTRACT

The chemical composition of forages varies during the day. The natural fermentation characteristics of silage may be influenced by these variations. A study was conducted to investigate the effect of cutting time (morning *vs*. afternoon) on chemical composition, gas production parameters, and digestibility and fermentation characteristics of alfalfa, clover, and barley silage using the gas production method. Alfalfa, clover, and barley forages were harvested twice in the morning (06:00) and the afternoon (18:00). Forages were ensiled in laboratory silos in triplicate at each cutting time and analyzed after 45 days to determine fermentation characteristics. After 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 hours of incubation, the cumulative gas production was measured. After a 24-hour incubation period, the dry matter digestibility (DMD), organic matter digestibility (OMD), metabolizable energy (ME), pH, and short-chain fatty acids (SCFA) were determined. Gas production at different times, water-soluble carbohydrates (WSC), starch, acid detergent fiber (ADF), and neutral detergent fiber (NDF) content, net energy for lactation (NE_l), and net energy for growth (NE_g) were also measured. The results indicated that afternoon-cut forages had lower levels of NDF and ADF than morning-cut forages ($P<0.05$), as well as greater amounts of starch and WSC. Afternoon-cut ensiled forages had higher dry matter (DM), organic matter (OM), crude protein (CP), NE_l, and NE_g and lower pH, significantly. Afternoon-cut forages had a higher gas production potential than morning-cut forages. In general, it may be concluded that delaying harvest time from morning to afternoon can improve the nutritional value of forage.

KEY WORDS alfalfa, barley, chemical composition, clover, harvest time, nutritional value.

INTRODUCTION

Forage plants are the most important part of ruminant diets, both physiologically and economically. Forages provide 60-70 percent of the total dry matter (DM). In this regard, forage supplies are often seen as limiting factors in Iran's animal husbandry. Adequate water-soluble carbohydrates (WSC) of forges is the main factor influencing the natural fermentation quality of silages (Guo *et al*. 2015), and more than other nutrients in the plant, sugar concentrations undergo diurnal fluctuation. Some plants lose their sugar completely during respiration at night, leading to sugar changes in plants during the day (Thomas *et al*. 2002). Sugars accumulate during the day and are consumed at night when respiration occurs, resulting in higher sugar content in forage in the afternoon than in the morning (Orr *et al*. 2001; Ruckle *et al*. 2018). Orr *et al*. (2001) showed that harvesting forage in the afternoon increased sugar content compared to harvesting in the morning (204 *vs*. 175 g/kg DM) and increased milk production by 5% without increasing forage consumption. Kagan *et al*. (2020) showed that afternoon-harvested clover forage increased 10% WSC and

300% starch (18 *vs*. 52 mg/g) compared to the morning harvest. Higher afternoon concentrations of WSC have been observed in clovers in other studies (Owens *et al*. 2002; Pagano *et al*. 2011; Ruckle *et al*. 2018). Dairy cows prefer forage with high sugar content and high digestibility of organic matter (OM) (Smit *et al*. 2006). Feed intake in early lactating cows was also increased by high-sugar, low-fiber forage (Moorby *et al*. 2006). In general, cows graze in a predictable pattern over the day, with greater intake around sunset (Taweel *et al*. 2004).

Alfalfa has a high crude protein (CP) concentration when compared to other forages, and it provides a substantial portion of the animal's amino acid requirements (Broderick, 1995). If this forage is preserved as silage, it is always available to dairy and beef cattle as an important component of their diets. Low WSC content in legumes (Hartinger *et al*. 2019) makes them difficult to ensile and results in silages with low levels of quickly fermentable carbohydrates. Solely feeding alfalfa silage can cause inefficient microbial N fixation in the rumen, resulting in a large amount of nitrogen being wasted, leading to environmental pollution (Hartinger *et al*. 2019). Increasing soluble carbohydrates in forage will reduce nitrogen loss and increase the synthesis of true protein concentrations (Coblentz and Grabber, 2013).

Studies have investigated the diurnal and seasonal changes in forage nutritive value and indicated that harvest management may significantly improve forage nutritive characteristics including NSC content (Morin *et al*. 2011; Claessens *et al*. 2021). Harvesting forage in the late afternoon might increase the content of unstructured carbohydrates in the forage (Fisher *et al*. 2002; Abrahamse *et al*. 2009; Brito *et al*. 2009). The enrichment of soluble energy in the afternoon-cut forage may represent a 5% increase over rations containing morning-cut forage. Thus, one of the cheapest ways to increase the nutritional value of forage is to harvest it at the end of the day, because during the day plant stores the nutrients from photosynthesis (Owens *et al*. 2002; Brito *et al*. 2008; Brito *et al*. 2009). Only in the western United States, if forage harvest is done in the afternoon, it is predicted that the dairy industry will benefit by approximately 300 million dollars per year (Mayland *et al*. 2005). As a result, cutting forages in the late afternoon can be used to increase DM and sugar content in the forage. Previous studies have also shown that increasing the percentage of DM by wilting could increase true protein concentration in the silage (Edmunds *et al*. 2014; Hartinger *et al*. 2019). The concentration of nonstructural carbohydrate (NSC) in alfalfa and other forages increases by cutting in the afternoon, but the effect of this increased concentration during silage fermentation is not well recognized.

Furthermore, to our knowledge, no research has studied the effect of harvesting time (morning *vs*. afternoon) on the chemical composition and gas production characteristics of barley forage silage. So, we hypothesized that forage harvesting in the afternoon might influence the fermentation characteristics during ensiling. Therefore, the objective of the present study was to investigate the effect of harvesting time (06:00 and 18:00) on the chemical composition and gas production parameters in alfalfa, clover, and barley forage silage.

MATERIALS AND METHODS

Preparation of alfalfa, clover, and barley forages

Alfalfa, clover, and barley forages were harvested manually (traditionally) approximately 5 to 7 cm above the soil surface in the morning (06:00) and afternoon (18:00). Alfalfa and clover forages were cut in the second harvest in the early flowering stage (10% flowering), and barley in the milky-paste stage was harvested from farms around Gonbad city. The fodder was chopped into pieces about 2-5 cm and manually compressed and ensiled in small plastic bags in three replications. For 45 days, the full silos were firmly closed and kept at room temperature. Immediately after opening, samples were taken from the upper, middle, and lower levels of each silo.

DM, ash, crude fat, and CP were determined according to standard methods (AOAC, 2000). Neutral detergent fiber (NDF), and acid detergent fiber (ADF; without amylase) were determined according to Van Soest *et al*. (1991). The amount of WSC was determined based on the Hedge and Hofreiter, (1962) method. Hemicellulose, the total amount of digestible nutrients (TDN), net energy for growth (Ne_{gi}), net energy for lactation (Ne_l) were determined according to the equation proposed by the (NRC, 2001), and the amount of starch was determined according to Thayumanavan and Sadasivam, (1984) methods.

Gas production procedure

The gas production test was performed according to the Menke (1988) method. Rumen fluid was collected from 3 fistulated sheep (45±2 kg). Animals were fed diets containing 70% forage (alfalfa and corn silage in equal proportions) and 30% concentrate (barley, cottonseed meal, bran, and supplement) at the maintenance level and had free access to water. The amount of 200 mg DM of the samples was transferred into glass vials. Four replicates of each sample were used as the test, while four vials with no samples were utilized as the control. Flasks containing the filtered rumen fluid were bubbled with carbon dioxide and incubated at 39 ˚C.

Artificial saliva and rumen fluid were mixed at a ratio of 2:1, and 30 mL of the mixture was added to glass vials containing 0.2 g sample or control. Immediately, each vial was bubbled with carbon dioxide for 10 s and sealed using rubber stoppers and an aluminum cover.

The vials were transferred to a shaking water bath at 39 ˚C and incubated for 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 h, after which the gas pressure was measured using a pressure gauge. The experiment was repeated three times.

Cumulative gas production was determined according to Orskov and McDonald, (1979). The OMD, ME, and NE were determined according to Menke *et al.* (1979) equations. The amount of SCFA was calculated using the Makar's equation (Makar, 2004).

OMD (%)= 14.88 + 0.889 GP + 0.45 CP + 0.0651 XA
ME (MJ/kg DM)= 2.20 + 0.136 GP + 0.057 CP + 0.0029 CF
NE (MJ/kg DM)= –0.36 + 0.114 GP + 0.0054CP + 0.0139 EE – 0.0054 XA
SCFA (mmol)= 0.0222 GP – 0.00425

Where:
OMD: organic matter digestibility.
ME: metabolizable energy.
NE: net energy.
SCFA: short-chain fatty acids.
GP: net gas production after 24 hours (per 200 mg sample DM).
CP: crude protein (%).
XA: ash (%).
CF: crude fiber (%).
EE: ether extract (%).

Gas production parameters were estimated as described by Orskov and McDonald (1979):

$$y= b(1-e^{-ct})$$

Where:
y: gas produced at the time of incubation.
b: gas production from an insoluble fermentable fraction.
e: euler's number.
c: gas production rates for b.
t: incubation time.

The data were analyzed in a completely randomized design using SAS statistical software (SAS, 2003).

In vitro digestibility of dry matter and organic matter

This experiment was similar to the gas production test in the preparation of rumen fluid and the basal diet and treatments. *In vitro* digestibility of DM and OM was measured using the batch culture method (Theodorou *et al.* 1994). After filtering with a 4-layer cloth under anaerobic circumstances, the rumen fluid was transferred to the laboratory and combined with a ratio of 1 (rumen liquid) to 2 (saliva solution), and the pH was adjusted to 6.8 with a buffer. Then, 50 mL of artificial saliva was poured into glass vials containing 500 mg DM of a basal diet. After that, the vials were sealed with a plastic lid and aluminum cover and incubated for 24 hours in a water bath at 39 ˚C. At the end of incubation time, glass vials were taken out of the warm water bath and placed in cold water to inactivate microbial activity, and pH was measured using a pH meter (Model 691, Metrohm Company). The contents of the bottles were filtered using nylon cloth (42-mm pore size) to determine the disappearance of DM. The residue was dried in an oven at 60 ˚C for 48 hours, and the apparent DM digestibility was calculated. Then the residual DM was placed into the oven at 550 ˚C for five h, and ash content was calculated. Five mL of fluid was mixed with an equivalent quantity of 0.2 N hydrochloric acid and kept in a freezer at –20 ˚C to determine the ammonia nitrogen concentration. The phenol-hypochlorite technique was used to determine the levels of rumen ammonia nitrogen (Broderick and Kang, 1980).

Gas pressure was recorded using pressure indicators at 2, 4, 6, 8, 10, 12, and 24 hours after incubations, and the accumulated gas was released. The following equation was used to calculate estimates of gas production efficiency (Getachew *et al.* 2004).

$$G_y= GP_{24} / (0.5\text{–DM weight after oven drying})$$

Where:
G_y= gas production efficiency.
GP_{24}= gas production after 24 h of incubation.

The microbial mass production was estimated using the following equation (Blümmel *et al.* 1997):

MCP (mg)= (GP×PF) – 2.2

Where:
MCP= microbial mass production.
GP= pure gas Production after 24 hours (mL).
PF= partitioning factor (mg of OM digested/mL of pure gas volume).

The efficiency of microbial protein was estimated using the following equation:

Microbial mass production efficiency= MCP / disappeared OM.

The data were analyzed using the GLM procedure of SAS statistical software version 9.1 in a completely randomized design (SAS, 2003).

RESULTS AND DISCUSSION

Effect of harvest time on the chemical composition

Afternoon-cut alfalfa silage exhibited significantly higher DM and CP and a lower final pH ($P<0.05$), as indicated in Table 1. Although harvest time had no significant effect on WSC, afternoon-cut alfalfa had numerically higher WSC and starch.

Afternoon-cut clover caused a significant increase in DM, OM, CP, NE_l, NE_g, and decreased pH compare to the morning-cut of clover silage. The WSC concentration in the afternoon-cut clover silage was almost 20% higher, but this difference was not statistically significant.

Afternoon-cut barley forage had significantly lower NDF and higher NFC compared to morning-cut ($P<0.05$). In afternoon-cut barley forage silage, pH reduction occurred faster than morning-cut (0.51 *vs.* 0.36). Afternoon-harvested alfalfa, clover, and barley forages contained lower concentrations of NDF and ADF and greater amounts of DM and OM than morning-harvested forages. These findings are similar to the results of Owens *et al.* (2002), who found that harvesting alfalfa and red clover in the afternoon increased silage DM. Sauve *et al.* (2010) also found that DM and N content increased in gamagrass baleage when harvest in sunset compared to sunrise. Huntington and Burns, (2007) similarly found that afternoon-cut gamagrass and switchgrass baleage had higher DM than morning-cut. The increase in DM in afternoon harvest silage is due to surface evaporation during daylight and increased carbohydrate synthesis as a result of photosynthesis.

All three forages exhibited an increase in CP concentration when harvested in the afternoon. These data indicate that forage N content increased as the day progressed and did not decline in response to carbohydrate accumulation (Burns *et al.* 2007). The NH_3-N concentration did not differ between morning and afternoon cutting in alfalfa and barley silages, which agrees with other studies (Trevaskis *et al.* 2001; Brito *et al.* 2008). Ammonia is an indicator of protein degradation by plant and microbial proteases before pH stabilization and forages silage stability. In general, forage sources are rich in rumen-degradable protein (RDP) but poor in nonstructural carbohydrates (NSC), resulting in imbalanced NH_3-N and fermentable energy supply in the rumen (Brito *et al.* 2016). In legumes such as alfalfa (Brito *et al.* 2008) and red clover (Antaya *et al.* 2015), delaying cutting from early morning to late afternoon has been shown to increase NSC concentrations. This diurnal increase in forage NSC is generally accompanied by a reduction in NDF, ADF, and N concentrations, as well as an increase in *in vitro* true digestibility of DM (Pelletier *et al.* 2010; Morin *et al.* 2011). A simultaneous increase in NSC and CP led to an increase in DM of afternoon harvest silage in this experiment.

According to the findings of this investigation, forages collected in the afternoon exhibited greater levels of WSC and starch. The ruminal bacteria needed WSC and starch as a food source. Soluble carbohydrates provide suitable substrates for the beneficial bacteria in the silage, i.e., lactic acid-producing bacteria. However, many factors including, cutting time, length of the day, air temperature, the intensity of sunlight, and storage method, can affect the amount of accumulated soluble sugars (Brito *et al.* 2008; Brito *et al.* 2009).

The overall diurnal increase in starch and WSC is consistent with published literature for afternoon samples of alfalfa (Burns *et al.* 2007; Brito *et al.* 2008; Brito *et al.* 2009), timothy baleage (Brito *et al.* 2016), and perennial ryegrass (Orr *et al.* 2001). The influence of harvest time and nitrogen levels on barley carbohydrate content was investigated by Henry *et al.* (2000), who found that forage harvested in the afternoon increased carbohydrate content at each level of nitrogen.

Burns *et al.* (2007) harvested alfalfa forage from 07:00 to 16:00 at various times and observed that total non-structural carbohydrate concentrations were altered by cut time, increasing from 85 g/ kg at 07:00 h to 97 g/ kg by 16:00 h. In the study of Guo *et al.* (2015), the WSC concentration of fresh napiergrass was also increased from 80.6 to 107 g/kg DM from sunup to sundown. Therefore, changing the harvest time from morning to afternoon, increased the concentration of soluble carbohydrates (Burns *et al.* 2007; Huntington and Burns, 2007; Brito *et al.* 2008; Brito *et al.* 2009; Brito *et al.* 2016; Claessens *et al.* 2021). The contents of WSC and NSC in red clover (Owens *et al.* 2002) and alfalfa silages (Tremblay *et al.* 2014) cut in the afternoon and stored in laboratory silos indicated substantial decreases as a result of fermentation processes during storage. Differences in soluble carbohydrates content at the time of ensiling due to afternoon cutting decrease during silage fermentation. Starch, on the other hand, is not utilized as much during silage fermentation, and variations in its content at the time of ensiling are more stable during the fermentation process than differences in soluble carbohydrate content.

Table 1 The effect of harvest time (morning *vs.* afternoon) on the chemical composition (% DM) of alfalfa, clover, and barley silage

Chemical composition	Alfalfa silage				Clover silage				Barley silage			
	Morning	Afternoon	SEM	P-value	Morning	Afternoon	SEM	P-value	Morning	Afternoon	SEM	P-value
DM (%)	20.81[b]	31.19[a]	5.19	≤ 0.0001	25.68[b]	29.62[a]	1.97	0.0015	28.87	30.06	0.594	0.781
Ash (%)	13.16[b]	13.45[a]	0.145	0.0084	12.75[b]	14.38[a]	0.815	0.0002	8.68[b]	10.83[a]	1.075	0.0237
CP (%)	14.79[b]	16.65[a]	0.93	≤ 0.0001	12.48[b]	14.62[a]	2.569	≤ 0.0001	11.81	11.88	0.035	0.8650
OM (%)	86.83[a]	86.54[b]	0.145	0.0131	87[a]	85.74[b]	0.629	0.0002	92.01[a]	89.16[b]	1.425	0.0239
ADF (%)	41[a]	32.5[b]	4.25	0.0009	39.25	35.5	1.875	0.0947	37.5	35	1.25	0.0856
NDF (%)	56.25	52	2.125	0.0691	56.75	54.5	1.125	0.2914	60.5[a]	53.5[b]	3.5	0.0005
TDN	58.79	56.95	0.92	0.2506	54.57[b]	59.31[a]	2.368	0.0128	54.6	56.54	0.97	0.0809
NFC (%)	16.53	16.38	0.075	0.9446	19.51	14.99	2.259	0.0731	23.49[b]	28.28[a]	2.395	0.0173
NE_g (MJ/kg)	0.645	0.697	0.026	0.2583	0.5725[b]	0.71[a]	0.0687	0.0118	0.575	0.630	0.0275	0.0853
NE_L (MJ/kg)	1.32	1.27	0.025	0.3660	1.21[b]	1.33[a]	0.06	0.0138	1.21	1.26	0.0011	0.0788
N-NH_3 (mg/dL)	2.63	2.66	0.015	0.889	3.13	2.67	0.23	0.1279	1.70	1.21	0.245	0.0958
HEMI	15.25	19.5	2.625	12.5	17.5	19	0.749	0.6798	23[b]	18.5[a]	10.12	0.0094
NDS (%)	46	48	1	0.3660	43.25	45.5	1.124	0.2914	39.5[b]	46.5[a]	3.5	0.0005
WSC (%)	58.07	68.63	5.28	0.1103	47.1	58.55	5.72	0.1110	61.66	69	3.67	0.3165
Starch (Mg/g)	93.52	97.82	2.15	0.8609	260.57	268.17	3.8	0.8056	363.93	380.75	8.41	0.1105

DM: dry matter (%); OM; organic matter (%); ADF: acid detergent fiber (%); NDF: neutral detergent fiber (%); CP: crude protein (%); TDN: total digestible nutrients; NFC: non-fiber carbohydrates (%); NE_g: net energy for growth (MJ/kg); NE_L: net energy for lactating (MJ/kg); HEMI: hemicellulose; NDS: neutral detergent solubles (%) and WSC: water soluble carbohydrates (%).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Due to the starch's relatively constant level during the fermentation process, higher silage NSC concentrations with afternoon cut alfalfa can be attributed mostly to starch (Tremblay *et al.* 2014). High levels of WSC and starch, as substrates required by microorganisms, improve the fermentation conditions in silage, which seems to be provided in the afternoon-cut forage silage.

The effect of harvest time on the chemical composition of alfalfa, clover, and barley forage silage showed that afternoon-cut forage improves the traits that are important for ensiling. For example, the decrease in pH of afternoon-cut silage was faster (2.27 *vs.* 2.46 units for alfalfa, 1.9 *vs.* 2.39 units for clover, and 0.36 *vs.* 0.51 units for barley). The silage pH reduced with fermentation time for all treatments as predicted; this decrease was rapid in days 1 to 5, followed by a gradual decrease until the end of the fermentation period (Figure 2). This decrease in pH indicates the appropriate ensiling conditions for afternoon-cut forage because well-preserved silage is characterized by reduced pH, greater lactic acid concentration, and reduced ammonia concentration (Downing *et al.* 2008; Tremblay *et al.* 2014; Kung, 2018). Owens *et al.* (2002), Downing *et al.* (2008), and Tremblay *et al.* (2014) in alfalfa and Downing *et al.* (2008) in perennial ryegrass also reported lower silage pH with afternoon-cutting. One of the benefits of rapid pH reduction is the decrease in plant enzyme activity. Overall, enzymes have a restricted pH range, and for each enzyme, there is an optimal pH range where the enzyme is most active. A rapid decrease in silage pH reduces the activity of plant enzymes and thus reduces the breakdown of protein into non-protein nitrogen. This rapid decrease in the pH of silage can reduce nutrient losses (Kung, 2018).

At the end of the ensiling process, WSC concentration in the afternoon harvested forage was higher than in morning harvested, indicating the protective effect of pH and silo conditions on the preservation of nutrients, including WSC. There was also a protective effect on other compounds such as CP, especially in clover and alfalfa forage, which reduces CP loss by about 2%. To support this, Downing *et al.* (2008), and Tremblay *et al.* (2014) found that afternoon-cut silages had a lower pH than morning-cut silages. On the other hand, in *in vivo* studies, Brito *et al.* (2009) reported that ruminal pH was significantly higher in lactating dairy cows fed afternoon-cut alfalfa vs. morning-cut at 2, 3, 4, 6, and 8 h post-feeding. A significant increase in the pH of the rumen was also observed when steers were fed ryegrass with higher WSC content (Lee *et al.* 2002). As a result, it is expected that differences in NSC concentration between morning and afternoon-cut alfalfa at the time of ensiling will be decreased during silage fermentation, but the fermentation process and the ensuing silage conservation characteristics may improve (Owens *et al.* 2002).

Effect of harvest time on the gas production parameters

Table 2 shows the effect of silage harvest time (morning *vs.* afternoon) on gas production parameters for alfalfa, clovers, and barley silage. Harvest time had no significant effect on gas production potential and gas production rate of alfalfa silage (P>0.05). However, afternoon harvested barley and clover silage showed significantly higher gas production potential than morning harvested silage. In terms of estimated parameters, all three afternoon harvested fodder had higher metabolizable energy compared to morning harvested (P<0.05).

Table 2 The effect of harvest time (morning *vs*. afternoon) on the gas production parameters (GPP) of alfalfa, clover, and barley silage

GPP	Alfalfa silage				Clover silage				Barley silage			
	Morning	Afternoon	SEM	P-value	Morning	Afternoon	SEM	P-value	Morning	Afternoon	SEM	P-value
(a+b)	282.97±10	482±0.9			281.9±9.44	276.7±9.91			434.6±12.32	453.3±11.36		
c	0.0306±0.0027	0.0306±0.0024			0.029±0.0022	0.027±0.0022			0.032±0.0022	0.028±0.0016		
SCFA	0.59	0.59	0	1	0.54	0.57	0.015	0.4272	0.93	0.88	0.025	0.0693
ME	10.7[b]	12.03[a]	0.665	0.0001	6.25[b]	11.85[a]	2.8	≤0.0001	5.95[b]	6.89[a]	0.47	≤0.0001
OMD	38.73	38.74	0.005	0.9964	36.81	37.96	0.575	0.4047	52.7	50.36	1.17	0.0629

[1] (a+b): gas production potential (mL/gDM); c: gas production rate (mL/h); SCFA: short chain fatty acids (mmol); ME: metabolizable energy (MJ/kg) and OMD: organic matter digestibility (% DM).

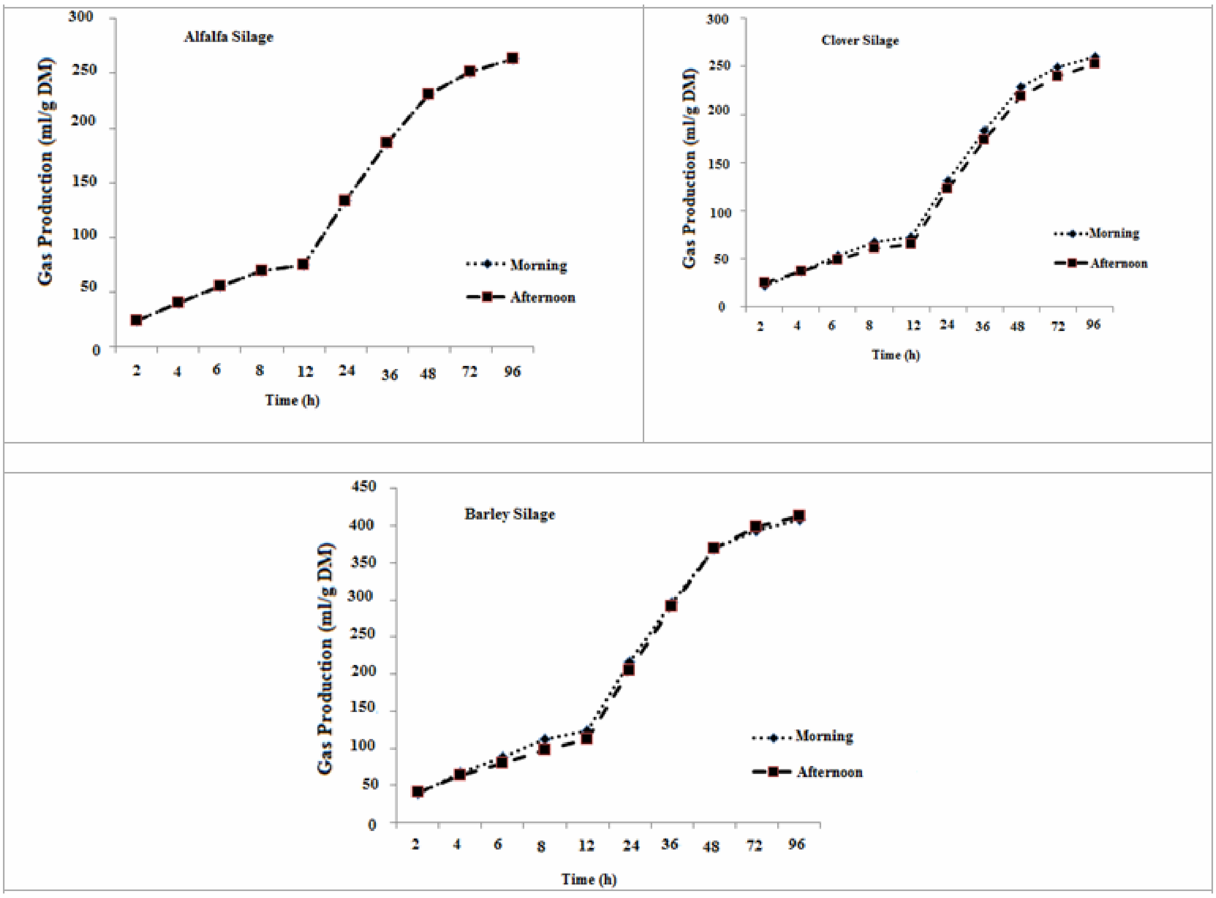

Figure 1 The effect of morning and afternoon harvest time on gas production of alfalfa, clover and barley silage

There was no significant difference in gas production at different times after incubation between alfalfa, clover, and barley forage harvested in the morning and afternoon (P>0.05; Figure 1).

Barley and clover silage harvested in 18:00 had higher gas production potential than silage harvested at 06:00. Gas production techniques indirectly reflect the general condition of fermentation, and the amount of gas produced depends on the feedstuff chemical composition. Gas is produced mainly due to the digestion of soluble and insoluble carbohydrates, and the presence of the protein more than rumen fluid microbes requirements, due to the conversion to ammonia, can have an inhibitory effect on fermentation and consequently gas production (Cone and van Gelder, 1999; Getachew *et al*. 2004).

Cone and van Gelder (1999) reported that with increasing grass age, the gas production potential increased due to a decrease in CP content and an increase in structural carbohydrates (increased 2.5 mL gas production per 1% reduction in protein content).

Cutting alfalfa in the afternoon could increase soluble carbohydrates, leaf content, and true protein compared to the morning (Fisher *et al*. 2002; Fisher *et al*. 2005; Huntington and Burns, 2007; Brito *et al*. 2009), which may affect the pattern of fermentation by gas production method. increases in alfalfa NSC concentration at ensiling due to afternoon cutting were reduced during silage fermentation mostly through a reduction in soluble carbohydrate concentration (Tremblay *et al*. 2014). Due to diurnal variations in light intensity, temperature, angle of sunlight, and humidity, harvest time is effective in the accumulation of nutrients, particularly structural carbohydrates, soluble carbohydrates, and protein synthesis in the plant. Low levels of indigestible carbohydrates, and greater contents of soluble carbohydrates, and non-structural fibers may be contributing to improved gas production potential in afternoon-cut forages.

Effect of harvest time on digestibility, ammonia nitrogen, pH, and fermentation parameters

Harvest time had no significant effect on the digestibility of DM, OM, ammonia nitrogen concentration, and pH of alfalfa silage (Table 3 and Figure 2). However, the partitioning factor (PF) and Efficiency of Microbial CP were significantly higher in alfalfa and barley silage harvested in the afternoon ($P<0.05$). Afternoon-cut clover forage significantly reduced OM digestibility, gas production efficiency, and ammonia nitrogen concentration compared to morning-cut ($P<0.05$). Afternoon-cut barley silage also had a lower digestibility of OM compared to morning-cut (Table 3).

In vitro OM digestibility was lower in the afternoon *vs.* morning-cut clover and barley forages. This study's findings are in contrast with those of Brito *et al.* (2009), who showed that feeding p.m. cut alfalfa was more digestible than a.m. cut alfalfa by 0.8 kg/d. Burns *et al.* (2005) also reported no difference in digestibility of DM with steers fed afternoon or morning alfalfa hay. Similar results were observed by Huntington and Burns (2007), who observed apparent DM digestibility of gamagrass and switchgrass baleage and fiber components were not affected by cutting in the morning or afternoon. Conversely, some studies reported significant increases in DM digestibility by feeding high vs. low WSC ryegrass to dairy cows (Miller *et al.* 2001; Moorby *et al.* 2006; Brito *et al.* 2008). In mid-lactation dairy cows, the effective degradability of total carbohydrates in the rumen was also higher with p.m.-cut alfalfa hay than a.m.-cut alfalfa hay (Yari *et al.* 2014). Differences in the degradability or digestibility of different plant species can be due to differences in their chemical composition.

The intracellular contents (soluble compounds) and the structure and size of the cell wall determine the degradability of forage. In other terms, as a result of cell wall lignification and a reduction in the leaf-to-stem ratio, increases in fiber concentrations and decreases in digestibility are common during plant growth.

Therefore, lower OM digestibility in afternoon-cut forages can be attributed to their cell wall content. In this study, the digestibility of barley forage was relatively higher than clover and alfalfa fodder, which can be attributed to the difference in the cell wall structure of the legume with other forages.

Production of SCFA in afternoon-cut barley silage tended to decrease compared to morning-cut silage (P=0.07, 0.93 *vs.* 0.88 mmol). Huntington and Burns (2007) investigated the effect of harvest in the morning (a.m., 06:00) *vs.* the afternoon (p.m., 1800) on the composition and voluntary DMI of gamagrass or switchgrass stored as baleage, and observed that compared with AM, PM-harvested had less acetate (13.0 *vs.* 18.6 g/kg of DM), propionate (0.29 *vs.* 0.82 g/kg of DM), lactate (2.9 *vs.* 3.5 g/kg of DM) and butyrate (3.9 *vs.* 5.1 g/kg of DM).

Microbial mass production or microbial protein synthesis in afternoon-harvested barley forage was higher than in morning-harvested barley forage. Ammonia nitrogen was also lower in clover silage harvested in the afternoon but did not differ in other silages. In an, *in vivo* study, Brito *et al.* (2008) reported that the ruminal ammonia-N concentration did not differ between cows fed p.m. *vs.* a.m. alfalfa. Higher microbial protein production and microbial CP efficiency indicate that: first, the diet was able to produce a higher molar ratio of propionate fatty acid; second, most of the degraded OM has entered into the microbial structure, that was the case in this study also, third, the amount of gas produced, including methane, is lower, resulting in lower energy losses (Makkar, 2005). Ruminal microbes might have benefited from the superior concentration of WSC from afternoon-cut compared with morning-cut forages, and yield more microbial protein (Brito *et al.* 2008). Previous *in vivo* (Lee *et al.* 2002) and *in vitro* (Berthiaume *et al.* 2007) studies found that when steers and dual-flow continuous culture fermenters were fed forages with high amounts of WSC and starch, bacterial N flow increased by 27 and 14 percent, respectively. Also, ruminal ammonia decreased with feeding high WSC diets (Lee *et al.* 2002), indicating that the efficiency of utilization of ammonia by ruminal microbes was increased when more fermentable carbohydrates were available in the rumen (Brito *et al.* 2009). The higher amount of microbial protein production may indicate that the fermentation process has been carried out satisfactorily and nutrients have been used more efficiently. Switching forage cutting from morning to afternoon increased WSC content in forages, which stimulated microbial protein synthesis and ammonia absorption by ruminal bacteria, which then processed it to microbial protein (Brito *et al.* 2009). Increased WSC is expected to improve the ability of ruminal microbes to capture ammonia and synthesize microbial protein (Zheng *et al.* 2018). NSC and CP concentrations are two key indicators of forage nutritive value. Due to the combination of a high concentration of quickly degradable CP and a limited supply of energy to support microbial metabolism, a diet based on alfalfa forage has a low protein efficiency in the rumen (Chen *et al.* 2009).

Table 3 The effect of harvest time (morning *vs.* afternoon) on the digestibility and fermentation parameters (Didest. Frem. Para.) of alfalfa, clover, and barley silage

Didest. Frem. Para.[1]	Alfalfa silage				Clover silage				Barley silage			
	Morning	Afternoon	SEM	P-value	Morning	Afternoon	SEM	P-value	Morning	Afternoon	SEM	P-value
IVDOD	50.5	51.5	0.5	0.5770	42	40	1	0.3142	53	49	2	0.1138
IVOMD	48.5	49	0.25	0.8299	39.25[a]	34[b]	3.125	0.0033	53[a]	45.25[b]	3.875	0.0039
N-NH_3	1.82	1.81	0.005	0.9261	2.23[a]	2.03[b]	0.1	0.0439	2.87	3.15	0.14	0.4629
pH	6.94	6.97	0.015	0.0390	6.95	6.92	0.015	0.2983	6.78	6.81	0.015	0.2044
PF	7.31	7.47	0.08	0.5751	4.6	5.02	0.21	0.3042	5.05[b]	5.26[a]	0.105	0.0327
Gas yield$_{24}$	113.14	111.03	1.05	0.7205	178.01[a]	150.26[b]	13.87	0.0406	174.91[a]	136.83[b]	19.04	0.0114
MCP	146.21	149.56	1.67	0.6165	88.1	82.4	2.85	0.4483	122.67	131.3	4.31	0.5069
EMCP	0.7[b]	0.7025[a]	0.001	0.8335	0.516	0.555	0.0195	0.2416	0.555[b]	0.642[a]	0.0435	0.0351

IVDOD: *in vitro* dry matter digestibility (%); IVOMD: *in vitro* organic matter digestibility (%); N-NH_3: ammonia nitrogen (m/dL); PF: partitioning factor (mg OM truly degraded/mL gas produced in 24 h); Gas yield$_{24}$: the amount of gas production after 24 hours of incubation (mL); MCP: microbial crude protein (mg) and EMCP: efficiency of microbial crude protein.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

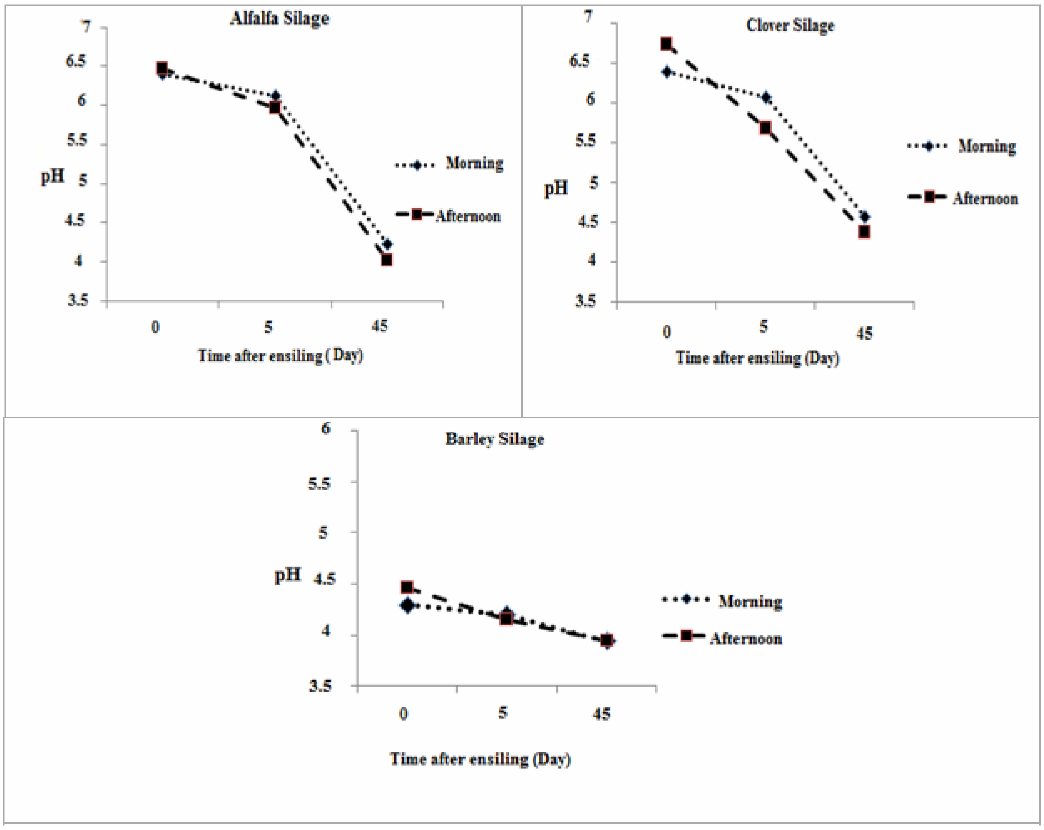

Figure 2 The effect of harvest time in the morning and afternoon on pH reduction during ensiling

As a result, higher microbial protein synthesis in the afternoon-harvested forages may be related to higher crude protein and water-soluble carbohydrate content. Optimizing the energy and protein balance in the forage diet allows for increased microbial protein synthesis efficiency, improved animal performance, and lower nitrogen losses to the environment (Brito *et al.* 2016).

In this study, the amount of PF was higher for barley forage harvested in the afternoon compared to the morning. The PF, which is an indicator of the efficiency of *in vitro* microbial mass synthesis (Blümmel *et al.* 1997), is defined as the ratio of the degraded OM (mg) to the volume of gas produced during the incubation period (mL). For most common feeds, the PF range between 2.74 and 4.65 mg/mL has been reported (Blümmel *et al.* 1997). A higher PF indicates that more nitrogen from degraded OM is absorbed by the microbial mass.

As a result of the higher NSC concentration in the afternoon cut, the silage quality of alfalfa was improved. Higher NSC contents in alfalfa silage, along with improved silage conservation characteristics due to higher NSC concentrations during ensiling, might improve PF.

CONCLUSION

In our research, the time of cutting had a substantial effect on chemical concentrations and nutritional characteristics in alfalfa, clover, and barley. Changing the cutting time from morning to afternoon increased the concentrations of CP in alfalfa and clover silage (by about 11% and 15%, respectively), while decreased NDF in alfalfa silage (around 20%). In general, the harvesting forage in the afternoon compared to morning-cut reduced the NDF, ADF, and pH, and increased the amount of DM, OM, WSC, starch, net energy for lactation, net energy for growth, and TDN. Afternoon-cut forages exhibited better digestibility and microbial protein synthesis indicating that harvest time is an important factor that could improve the nutritional value of forage diets. Delaying forage harvest from morning to afternoon, under the same conditions affecting forage quality and value, seems to be a low-cost management technique for improving forage nutritional content. More study is needed to study how increased NSC content and silage conservation characteristics affect milk production when forages are cut in the afternoon.

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