Full Length Article

Critical Harvest Stage Improves Alfalfa Production Performance, Overwintering Rate and Economic Benefits under Drip Irrigation

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Abstract

Alfalfa is an important legume forage with high nutritional quality. Harvest stage has an essential impact on alfalfa hay yield and nutritional quality. However, there is no clear indication about the effects of harvest time on the production performance, overwintering rate and economic benefits of alfalfa under drip irrigation. Therefore, this study was conducted to evaluate the impact of harvesting at bud stage (F_B), 5% flowering stage ($F_{5\%}$), 15% flowering stage ($F_{15\%}$), and full bloom stage ($F_{50\%}$) on hay yield and quality, and economic benefits of two alfalfa cultivars *i.e.*, WL354HQ and Magnum551. Harvesting time had significant effect on hay yield of both cultivars ($P \leq 0.05$) and the order of total dry matter yield (TDMY) was: $F_{5\%} > F_{15\%} > F_{50\%} > F_B$. The alfalfa crude protein (CP) content of each cutting in alfalfa decreased gradually with the advance of the harvest stage, while the neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents gradually increased. A comprehensive analysis of alfalfa dry matter yield (DMY), quality and overwintering rate was used to analyze the fuzzy similarity priority ratio, and the priority ranking was as follows: $F_B > F_{5\%} > F_{15\%} > F_{50\%}$. Maximum economic benefits of both alfalfa cultivars were obtained at $F_{5\%}$ harvest time, reaching 2703 \$ ha^{-1} (WL354HQ) and 2585 \$ ha^{-1} (Magnum551), followed by F_B, $F_{15\%}$, and $F_{50\%}$. Therefore, in order to get more hay yield and economic returns, harvesting at 5% flowering stage of alfalfa seemed viable option under drip irrigation. © 2021 Friends Science Publishers

Introduction

Alfalfa (*Medicago sativa* L.), as a kind of excellent legume widely cultivated in the world, is known as the "king of forage" for its high hay yield and nutritional quality (Gu *et al*. 2018; Zhang *et al*. 2020a). It not only provides a large amount of high-quality forage grass for animal husbandry, but also plays an important role in improving regional economic level (Staniak and Harasim 2018), increasing soil fertility (Jia *et al*. 2006), preventing soil erosion (Zhao *et al*. 2004), *etc*. Xinjiang is an important alfalfa planting area in the northwest of China. It is of great significance to carry out high-quality and efficient alfalfa production under the arid climate of Xinjiang (Zhang *et al*. 2020b).

The growth and development of alfalfa plants are closely linked to the harvest stage. Alfalfa varies greatly in morphological structure, and physiological and biochemical characteristics at different harvest stages (Lenssen *et al*. 1990). Additionally, some reports have shown that alfalfa has higher crude protein content and lower crude fiber content at budding stage and early flowering stage than at its full bloom stage, while the forage yield of alfalfa at flowering stage is higher, but the feeding value of alfalfa at flowering stage is lower than that at budding stage and early flowering stage (Lamb *et al*. 2003; Testa *et al*. 2011). In the actual production of alfalfa, many factors are affecting the nutrient quality of alfalfa hay, and the harvest time is one of the most important factors (Karayilanli and Ayhan 2016). Alfalfa hay with excellent quality and quantity can be obtained by harvesting it at appropriate period (Yari *et al*. 2012). However, once the optimal harvest period is missed, the quality of alfalfa will sharply decline, which is mainly manifested as the decrease in crude protein content, the increase in stem-leaf ratio and the contents of crude fibers, and the aging of leaves (Martiniello *et al*. 1997). Therefore, according to the dynamic changes of hay yield and nutrient quality of alfalfa in different growth periods, it is a key measure to determine the optimal harvest stage to improve the economic benefit of alfalfa production.

Drip irrigation technology is widely used in alfalfa field production due to its advantages of water-saving, high irrigation efficiency, and difficult hardening of soil after irrigation (Trejo *et al*. 2010). Moreover, the use of drip irrigation technology consumes a series of drip irrigation

materials, which greatly increase farmers' planting costs. Therefore, how to improve the economic benefit of alfalfa production in arid areas of Xinjiang under the condition of drip irrigation becomes a key problem to be solved in the process of production. At present, in the actual production of drip irrigation alfalfa, the optimal harvest stage of high quality and efficient production of alfalfa is still uncertain owing to the fluctuation of market price, the lack of quality pricing policy and other factors. Consequently, this study was carried out to explore the change characteristics of dry matter yield and nutritional quality of alfalfa, and analyzed the economic benefit of alfalfa at different harvest stages, so that to determine the best harvest stage for the good-quality and high-yield in alfalfa under drip irrigation. We hypothesized that: (1) Harvesting at the early flowering stage of alfalfa will get higher total hay yield and crude protein yield. (2) Overwintering rate of alfalfa will decrease with the increase of cutting frequency. (3) The highest economic benefits of alfalfa may occur at the early flowering stage.

Materials and Methods

Site description

The experiment was conducted in the experimental field of agricultural demonstration park at Shihezi Agricultural Research Institute in Shihezi (44°26′N, 85°94′E), Xinjiang, China, in 2017. The region has a temperate continental climate with a dry climate and a large temperature difference between day and night. The annual average temperature is 10–15°C, the annual average precipitation is 200–415 mm, the annual evaporation is 1080–1430 mm, the annual sunshine time is 2310–2730 h, and the frost-free period is 147–191 d. The soil type of the test site is gray desert soil with a pH of 7.65. The physical and chemical properties of 0–20 cm topsoil in the test site are shown in Table 1.

Experimental details

Based on the results of the previous pre-test and the results of the experiment conducted in 2017 this field study was conducted to evaluate the impact harvest stages on hay yield, overwintering rate and quality of hay of two alfalfa cultivars. Four growth periods were chosen to determine the hay yield and nutritional quality of alfalfa. The specific harvest periods were as follows: bud stage (50% of budding, F_B), 5% flowering stage ($F_{5\%}$), 15% flowering stage ($F_{15\%}$), and full bloom stage (50% flowering, $F_{50\%}$). Harvesting according to the above growth periods resulted in 6 cuts in F_B stage, 5 cuts in $F_{5\%}$ stage, 4 cuts in $F_{15\%}$ stage and 3 cuts in $F_{50\%}$ stage. The tested alfalfa cultivars WL354HQ and Magnum551 were widely planted in Xinjiang, and the seeds of alfalfa were sown on April 19, 2015. The sowing mode was artificial seeding, with the sowing rate of 18.0 kg ha^{-1}, the row spacing of alfalfa was 20 cm, and the sowing depth was 1.5–2.0 cm.

The experiment was laid out following randomized complete block design under factorial arrangement with net plot area of 40 m^2 (8 m × 5 m) with three replicates. The harvesting time of alfalfa in different growth periods is given in Table 2. Other management measures, such as field fertilization (N: 105 kg ha^{-1}, P: 100 kg ha^{-1}) and irrigation (6000 m^3 ha^{-1}), were carried out following the local drip-irrigated alfalfa high-yielding fields. All the required irrigation water for each plot was conveyed to each plot through eight inlaid drip irrigation belts with an inner diameter of 12.5 mm and a working pressure of 0.1 MPa. The drip distance of the drip irrigation belt was 20 cm, and the flow rate of the drip irrigation belt was 3.2 L h^{-1}. The drip irrigation belt was buried 8–10 cm below the surface at a distance of 60 cm. Drip irrigation belts were laid with three rows of alfalfa in one tube. The concrete irrigation volume was checked by a water meter at the entrance of the plot.

Measurement index and method

Dry matter yield: The DMY of alfalfa was determined by the sampling method, and the alfalfa plants with the same leaf age were randomly selected. The alfalfa plants (cut to 5 cm) in the sampling square were cut with scissors and weighed, and the yield of fresh alfalfa plants was recorded 3 times for each stage; another 300 g alfalfa plant samples were packed in cloth bags and brought back to the laboratory, dried at 105°C for 10 min in an oven, and then dried at 65°C to a constant weight. The moisture content was measured and the alfalfa DMY (t ha^{-1}) was calculated. The specific calculation formula is as follows:

$$DMY = FGY \times (1 - WC) \quad (1)$$

DMY represents dry matter yield of alfalfa, FGY represents fresh grass yield of alfalfa, and WC represents water content of alfalfa.

Nutritional quality: The CP content of alfalfa plants was analyzed by the semi-micro Kjeldahl method (Hirwitz and Latimer 1995), and using the method as described by Van Soest (Soest *et al.* 1991) to determine the NDF and ADF contents. CP yield (t ha^{-1}) was calculated by the following formula:

$$CPY = CPC \times DMY \quad (2)$$

CPY represents CP yield and CPC represents CP content.

Overwintering rate: After frosting in the experiment area, 1 m^2 (1 m × 1 m) was labeled as a quadrat in the center of the plot on October 14, 2017, and the number of all alfalfa plants in the quadrat was recorded with three replicates. The number of surviving alfalfa plants in the labeled quadrat was then recorded after alfalfa returned to green in 2018 (March 25, 2018), and the overwintering rate (%) of alfalfa was calculated using the following equation:

$$OR = TN_{sp} / TN_p \quad (3)$$

OR represents overwintering rate, TN_{SP} represents total

number of surviving plants, and TN_p represents total number of plants.

Economic benefit: The economic benefit of alfalfa was calculated as the following formula:

$$EB = TI - Ti \quad (4)$$

EB ($ ha^{-1}) represents economic benefit, TI represents total income and Ti represents total input. Ti includes material cost (seeds, fertilizer, drip irrigation belts, water and electricity), management fee (sowing, harvesting, drying, bundling, hauling, labor, land lease), *etc*. The TI was calculated by the following formula:

$$TI = TDMY \times P_u \quad (5)$$

TDMY represents Annual total dry matter yield of alfalfa and P_u represents unit price of alfalfa.

Statistical analysis

Microsoft Excel 2019 and DPS 7.05 (Data Processing System, China) software were used for statistical analysis. Two-way ANOVA and DMRT (Duncan's multiple range test) were used for the comparison of the differences between different data sets at $P \leq 0.05$. Based on the DMY, CP content, CP yield, NDF, ADF content and overwintering rate of alfalfa, the optimal harvest stage of alfalfa was analyzed using the fuzzy similarity priority ratio method (by DPS 7.05). The fuzzy similarity priority ratio compares many treatments with a fixed optimal one, and a comprehensive ranking result can be obtained. Lower the similarity, better the comprehensive performance. The figures were prepared by Sigma Plot 14.0 (Systat Software Inc., USA).

Results

Dry matter yield

The DMY of two alfalfa cultivars WL354HQ and Magnum551 increased gradually with the advancement of the growth period at the same cutting times, and reached the maximum at the $F_{50\%}$ (Table 3). The TDMY was the highest (22.68–22.96 t ha^{-1}) when the alfalfa plants were harvested at $F_{5\%}$ (5 cuts), followed by $F_{15\%}$ (4 cuts), $F_{50\%}$ (3 cuts), F_B (6 cuts), and the TDMY of alfalfa increased by 11.0–16.8% when harvesting at $F_{5\%}$ compared with that at $F_{50\%}$. The DMY of alfalfa harvested at $F_{5\%}$–$F_{15\%}$ was significantly higher than that of alfalfa harvested at F_B and $F_{50\%}$ ($P \leq 0.05$). Except for the first and the fifth cut, the interactions of other cuts in DMY were significantly different ($P \leq 0.05$). There was significant difference in alfalfa TDMY between cultivars ($P \leq 0.05$) and among harvest stages ($P \leq 0.01$), while there was no significant difference in their interaction ($P \geq 0.05$). Both alfalfa cultivars had the highest DMY in the first crop at different harvest stages. Except for the third crop, the DMY of alfalfa gradually decreased with the increase of cutting times (Table 3).

Crude protein

The CP content of alfalfa in each cut decreased gradually with the advancement of the growth period during the same cutting times. The alfalfa CP content of each cut was the highest in F_B, while it was the lowest in $F_{50\%}$ (Table 4). Harvest stages had a significant effect on alfalfa CP content ($P \leq 0.05$), while CP content had no significant respond to the interaction of cultivar and harvest stage. During the same growth period, CP content in alfalfa showed a slight decline with the increase of cutting numbers. Compared with the first crop, the CP content of the last crop in each growth period was reduced by a greater degree, ranging from 0.47 to 3.51%. And two alfalfa cultivars WL354HQ and Magnum551 showed the same rule.

To further clarify the effect of harvesting at different growth periods on alfalfa CP yield, the CP yield was calculated by converting alfalfa DMY and CP content. The results showed that harvest stages had a significant effect on CP yield in all cuts of alfalfa ($P \leq 0.05$) (Table 5). There was no significant difference between two cultivars in total CP yield of alfalfa ($P \geq 0.05$), while harvest stage had a significant effect on alfalfa total CP yield ($P \leq 0.05$). However, there was also no significant difference in their interaction ($P \geq 0.05$). According to the total CP yield of each crop in different growth periods, harvesting at $F_{5\%}$ had the highest total CP yield, with 3.97 and 3.90 t ha^{-1} CP yield of cultivar WL354HQ and Magnum551 respectively, followed by F_B and $F_{15\%}$. Compared with $F_{5\%}$, the total CP yield of the alfalfa cultivar WL354HQ at $F_{50\%}$ was decreased by 27.85%, and Magnum551 decreased by 25.24%.

Neutral and acid detergent fiber

There were significant differences with NDF content (Table 6) and ADF content (Table 7) with change in harvest stages ($P \leq 0.01$), while there was no statistically significant difference in cultivars and their interactions with harvest stages ($P \geq 0.05$). The contents of NDF and ADF of alfalfa were both the lowest in F_B, followed by $F_{5\%}$, $F_{15\%}$ and $F_{50\%}$. The NDF contents of WL354HQ and Magnum551 during F_B decreased by 37.8–43.2% and 29.6–35.1% respectively compared with those of full bloom stage, and the contents of ADF were 43.6–54.4% and 30.9–40.3% lower than that of $F_{50\%}$, respectively. The NDF and ADF contents of alfalfa both increased firstly and then decreased with the increase of cutting times during the same growth period (the NDF contents during $F_{50\%}$ and $F_{15\%}$ only showed a gradual increasing trend because only 3 and 4 crops of alfalfa were cut respectively), and the maximum value was concentrated in the third or fourth stubble. The changes of NDF and ADF contents in the two cultivars were the same.

Overwintering rate

With the advancing of the growth period, the overwintering

Table 1: Physicochemical properties of 0–20 cm soil in the experiment

Total nitrogen (g kg⁻¹)	Available nitrogen (mg kg⁻¹)	Total phosphorus (g kg⁻¹)	Available Phosphorus (mg kg⁻¹)	Available Potassium (mg kg⁻¹)	Organic matter (g kg⁻¹)	Soil bulk density (g cm⁻¹)	Field capacity (%)
1.63	72.5	0.19	15.2	139.6	25.3	1.46	24.6

Note: units rendered as $g\ kg^{-1}$, $mg\ kg^{-1}$, $g\ cm^{-1}$, etc.

Table 2: Harvesting time of each cut at different harvest stages (Month-Day) (2017)

Harvest stage	First cut	Second cut	Third cut	Fourth cut	Fifth cut	Sixth cut
F_B	05-04	06-03	07-01	07-25	08-19	09-18
$F_{5\%}$	05-12	06-15	07-16	08-19	09-20	—
$F_{15\%}$	05-25	06-28	08-05	09-15	—	—
$F_{50\%}$	06-10	07-30	09-18	—	—	—

F_B, bud stage; $F_{5\%}$, 5% flowering stage; $F_{15\%}$, 15% flowering stage; $F_{50\%}$, full bloom stage

Table 3: Effect of harvest stage on dry matter yield (t ha⁻¹) of two alfalfa cultivars

Cultivars	Harvest stage	First cut	Second cut	Third cut	Fourth cut	Fifth cut	Sixth cut	TDMY
WL354HQ	F_B	4.81 ± 0.17d	3.19 ± 0.14d	4.19 ± 0.14d	3.01 ± 0.16c	2.48 ± 0.13	1.52 ± 0.16	19.20 ± 0.31c
	$F_{5\%}$	5.96 ± 0.18c	4.78 ± 0.17c	5.20 ± 0.16c	4.47 ± 0.17b	2.55 ± 0.16	—	22.96 ± 0.34a
	$F_{15\%}$	6.65 ± 0.19b	5.14 ± 0.18b	5.48 ± 0.17b	4.73 ± 0.19a	—	—	22.01 ± 0.29b
	$F_{50\%}$	7.47 ± 0.25a	6.23 ± 0.24a	5.95 ± 0.24a	—	—	—	19.64 ± 0.21c
Magnum551	F_B	4.88 ± 0.17d	3.44 ± 0.16d	4.42 ± 0.19d	3.07 ± 0.18c	2.32 ± 0.17	1.58 ± 0.15	19.71 ± 0.42c
	$F_{5\%}$	6.00 ± 0.26c	5.21 ± 0.19c	5.21 ± 0.16c	4.29 ± 0.15b	2.42 ± 0.18	—	22.68 ± 0.32a
	$F_{15\%}$	6.80 ± 0.19b	5.48 ± 0.22b	5.47 ± 0.23b	4.47 ± 0.16a	—	—	22.23 ± 0.28a
	$F_{50\%}$	7.60 ± 0.20a	7.16 ± 0.18a	5.66 ± 0.19a	—	—	—	20.43 ± 0.27b
Cultivar		**	**	**	**	**	ns	*
Harvest stage		**	**	**	**	ns	—	**
Interaction		ns	**	**	**	ns	—	ns

The different lowercase letters in the same column indicate significant differences among harvest stage levels under the same cultivar ($P \leq 0.05$)
**= Significant at $P \leq 0.01$; *= Significant at $P \leq 0.05$; ns= Non-significant; FB, bud stage; F5%, 5% flowering stage; F15%, 15% flowering stage; F50%, full bloom stage

Table 4: Effect of harvest stage on crude protein contents (%) of two alfalfa cultivars

Cultivar	Harvest stage	First cut	Second cut	Third cut	Fourth cut	Fifth cut	Sixth cut
WL354HQ	F_B	21.19 ± 0.49a	20.54 ± 0.52a	20.08 ± 0.48a	19.78 ± 0.50a	19.07 ± 0.56a	18.33 ± 0.54
	$F_{5\%}$	18.20 ± 0.58b	17.68 ± 0.48b	17.38 ± 0.52b	16.53 ± 0.53b	15.73 ± 0.55b	—
	$F_{15\%}$	17.01 ± 0.60c	16.70 ± 0.54c	16.10 ± 0.41c	15.18 ± 0.48c	—	—
	$F_{50\%}$	15.11 ± 0.58d	14.43 ± 0.58d	14.20 ± 0.56d	—	—	—
Magnum551	F_B	20.47 ± 0.50a	19.61 ± 0.39a	18.87 ± 0.50a	18.95 ± 0.51a	18.92 ± 0.54a	18.63 ± 0.46
	$F_{5\%}$	17.87 ± 0.48b	17.41 ± 0.49b	16.98 ± 0.52b	17.15 ± 0.43b	16.60 ± 0.49b	—
	$F_{15\%}$	16.55 ± 0.52c	15.65 ± 0.46c	15.47 ± 0.45c	15.09 ± 0.44c	—	—
	$F_{50\%}$	14.67 ± 0.48d	14.17 ± 0.49d	13.67 ± 0.48d	—	—	—
Cultivar		*	**	**	ns	ns	ns
Harvest stage		**	**	**	**	**	—
Interaction		ns	ns	ns	ns	ns	—

The different lowercase letters in the same column indicate significant differences among harvest stage levels under the same cultivar ($P \leq 0.05$)
**= Significant at $P \leq 0.01$; *= Significant at $P \leq 0.05$; ns= Non-significant; FB, bud stage; F5%, 5% flowering stage; F15%, 15% flowering stage; F50%, full bloom stage

rates of alfalfa WL354HQ and Magnum551 increased gradually and reached the highest in the $F_{50\%}$ (cutting 3 crops). However, there was no significant difference with the overwintering rates of alfalfa between $F_{5\%}$ and $F_{15\%}$ ($P \geq 0.05$), and the overwintering rates of alfalfa in $F_{5\%}$, $F_{15\%}$ and $F_{50\%}$ were significantly greater than those in F_B ($P \leq 0.05$) (Fig. 1). The overwintering rate of alfalfa reduced significantly when cutting 6 times. The interaction of cultivar and harvest stage had no significant influence on overwintering rate of alfalfa ($P \geq 0.05$) (Fig. 1).

Fuzzy similarity priority ratio analysis

To determine the optimal harvest stage of drip irrigation alfalfa more accurately, this study analyzed the alfalfa DMY, CP content, CP yield, NDF content, ADF content and wintering rate by fuzzy similarity priority ratio analysis (Table 8). The results showed that cultivars WL354HQ and Magnum 551 were both ranked first in the harvest stage of F_B, followed by $F_{5\%}$, $F_{15\%}$ and $F_{50\%}$, but the similarity of $F_{5\%}$ and F_B was extremely close. We inferred that the stage of F_B or $F_{5\%}$ may be more suitable for harvesting.

Economic benefits

In the actual production of alfalfa, the optimal harvesting period should be determined in combination with the production cost to achieve the maximum economic benefits. The results of economic benefit analysis showed that (Table 9) the maximum economic benefit could be obtained by harvesting at $F_{5\%}$, and the net profit reached 2585.09–2703.13 \$ ha⁻¹, followed by the F_B, $F_{15\%}$ and $F_{50\%}$. And the

Table 5: Effect of harvest stage on crude protein yield (t ha^{-1}) of two alfalfa cultivars

Cultivars	Harvest stage	First cut	Second cut	Third cut	Fourth cut	Fifth cut	Sixth cut	Total crude protein yield
WL354HQ	F_B	0.99 ± 0.03b	0.66 ± 0.02b	0.81 ± 0.04b	0.59 ± 0.02b	0.48 ± 0.01a	0.28 ± 0.01	3.81 ± 0.15ab
	$F_{5\%}$	1.08 ± 0.04a	0.85 ± 0.04a	0.90 ± 0.03a	0.74 ± 0.03a	0.41 ± 0.01b	—	3.97 ± 0.16a
	$F_{15\%}$	1.14 ± 0.04a	0.86 ± 0.03a	0.88 ± 0.03a	0.74 ± 0.04a	—	—	3.61 ± 0.14b
	$F_{50\%}$	1.11 ± 0.04a	0.90 ± 0.04a	0.86 ± 0.04ab	—	—	—	2.86 ± 0.09c
Magnum551	F_B	0.98 ± 0.03b	0.67 ± 0.02c	0.84 ± 0.03a	0.58 ± 0.02b	0.45 ± 0.02a	0.30 ± 0.02	3.82 ± 0.17a
	$F_{5\%}$	1.06 ± 0.04a	0.91 ± 0.04b	0.81 ± 0.04ab	0.72 ± 0.03a	0.39 ± 0.02b	—	3.90 ± 0.12a
	$F_{15\%}$	1.12 ± 0.05a	0.88 ± 0.04b	0.85 ± 0.04a	0.69 ± 0.03a	—	—	3.54 ± 0.15b
	$F_{50\%}$	1.12 ± 0.05a	1.02 ± 0.04a	0.77 ± 0.03b	—	—	—	2.91 ± 0.09c
Cultivar		ns	*	*	*	ns	ns	ns
Harvest stage		**	**	*	**	**	—	**
Interaction		ns	ns	*	ns	ns	—	ns

The different lowercase letters in the same column indicate significant differences among harvest stage levels under the same cultivar ($P \leq 0.05$)
**= Significant at $P \leq 0.01$; *= Significant at $P \leq 0.05$; ns= Non-significant; FB, bud stage; F5%, 5% flowering stage; F15%, 15% flowering stage; F50%, full bloom stage

Table 6: Effect of harvest stage on neutral detergent fiber contents (%) of two alfalfa cultivars

Cultivar	Harvest stage	First cut	Second cut	Third cut	Fourth cut	Fifth cut	Sixth cut
WL354HQ	F_B	28.81 ± 1.36c	30.71 ± 1.23c	33.31 ± 1.14c	35.09 ± 1.37b	32.99 ± 1.10b	31.06 ± 1.34
	$F_{5\%}$	34.67 ± 1.26b	36.35 ± 1.15b	38.13 ± 1.68b	40.53 ± 1.49a	37.96 ± 1.47a	—
	$F_{15\%}$	37.31 ± 1.46b	38.00 ± 1.56b	40.52 ± 1.43b	43.05 ± 2.00a	—	—
	$F_{50\%}$	40.83 ± 1.81a	43.98 ± 1.92a	45.81 ± 1.76a	—	—	—
Magnum551	F_B	29.63 ± 1.29c	32.14 ± 0.94c	33.78 ± 1.01c	34.05 ± 1.30c	31.62 ± 1.20b	29.58 ± 0.77
	$F_{5\%}$	34.79 ± 1.52b	37.53 ± 1.35b	39.83 ± 1.67b	38.90 ± 1.55b	35.78 ± 1.66a	—
	$F_{15\%}$	37.50 ± 1.71a	39.04 ± 1.36b	42.19 ± 2.18b	43.17 ± 1.90a	—	—
	$F_{50\%}$	39.92 ± 1.78a	41.66 ± 1.12a	45.63 ± 1.95a	—	—	—
Cultivar		ns	ns	ns	ns	ns	ns
Harvest stage		**	**	**	**	**	—
Interaction		ns	ns	ns	ns	ns	—

The different lowercase letters in the same column indicate significant differences among harvest stage levels under the same cultivar ($P \leq 0.05$)
**= Significant at $P \leq 0.01$; *= Significant at $P \leq 0.05$; ns= Non-significant; FB, bud stage; F5%, 5% flowering stage; F15%, 15% flowering stage; F50%, full bloom stage

Table 7: Effect of harvest stage on acid detergent fiber contents (%) of two alfalfa cultivars

Cultivar	Harvest stage	First cut	Second cut	Third cut	Fourth cut	Fifth cut	Sixth cut
WL354HQ	F_B	22.39 ± 0.86c	23.65 ± 0.78c	24.85 ± 1.43c	25.69 ± 1.33c	23.81 ± 1.01b	22.30 ± 0.89
	$F_{5\%}$	27.14 ± 1.79b	29.46 ± 1.38b	30.61 ± 1.56b	30.29 ± 0.78b	29.06 ± 1.60a	—
	$F_{15\%}$	28.85 ± 1.29b	30.85 ± 1.53b	31.68 ± 0.90b	33.17 ± 1.86a	—	—
	$F_{50\%}$	32.17 ± 1.73a	36.52 ± 1.54a	36.48 ± 2.06a	—	—	—
Magnum551	F_B	22.95 ± 0.94c	24.82 ± 1.06d	26.85 ± 1.18c	25.74 ± 1.25b	23.45 ± 0.80b	21.60 ± 0.85
	$F_{5\%}$	27.44 ± 1.10b	28.95 ± 1.50c	30.44 ± 1.49b	27.53 ± 0.87b	26.74 ± 1.07a	—
	$F_{15\%}$	29.14 ± 1.04b	31.93 ± 1.57b	32.66 ± 0.99ab	32.64 ± 1.14a	—	—
	$F_{50\%}$	32.21 ± 0.92a	34.55 ± 1.34a	35.15 ± 1.66a	—	—	—
Cultivar		ns	ns	ns	ns	ns	ns
Harvest stage		**	**	**	**	**	—
Interaction		ns	ns	ns	Ns	ns	—

The different lowercase letters in the same column indicate significant differences among harvest stage levels under the same cultivar ($P \leq 0.05$)
**= Significant at $P \leq 0.01$; *= Significant at $P \leq 0.05$; ns= Non-significant; FB, bud stage; F5%, 5% flowering stage; F15%, 15% flowering stage; F50%, full bloom stage

economic benefits of $F_{5\%}$, F_B, and $F_{15\%}$ dramatically higher than that in the $F_{50\%}$. Among them, the profit of alfalfa harvesting at $F_{5\%}$ was 69.9–72.5% higher than that at the $F_{50\%}$ (Table 9).

Discussion

Dry matter yield of alfalfa is an important indicator to measure the production performance of alfalfa, so it can be utilized to directly characterize the economic benefits of alfalfa production when little difference in nutritional quality exists (Rade *et al*. 2012). Dry matter yield of alfalfa is influenced by many factors, such as the variety of alfalfa, fertilization, field management mode, and harvest stage,

which all have a strong relationship with the dry matter yield of alfalfa (Dhont *et al*. 2004). While harvesting time is an influential factor that is relatively easy to control (Lamb *et al*. 2003). Studies have shown that alfalfa forage yield varies significantly at different harvest stages (Moyer *et al*. 1999). The current study found that as the growth period progressed, the DMY of alfalfa showed a gradual upward trend and reached the maximum at the F_B, which was mainly because the DMY of alfalfa was closely related to the plant height, stem diameter, stem-leaf ratio of alfalfa plants (Annicchiarico 1993). With the advancing of the growth period, alfalfa showed the basic growth rule of "slow-fast-slow", which broadly conformed to the "S" growth curve of plants. During the period from the bud

Table 8: Fuzzy similarity priority ratio analysis

Cultivar	Harvest stage	Similarity	Sorting
WL354HQ	F_B	16	1
	$F_{5\%}$	17	2
	$F_{15\%}$	25	3
	$F_{50\%}$	32	4
Magnum551	F_B	16	1
	$F_{5\%}$	17	2
	$F_{15\%}$	25	3
	$F_{50\%}$	32	4

FB, bud stage; F5%, 5% flowering stage; F15%, 15% flowering stage; F50%, full bloom stage

Table 9: Economic benefit ($ ha^{-1}) of alfalfa harvested at different harvest stages

Cultivar	Harvest stage	Earnings	Cost	Profit
WL354HQ	F_B	5344.50	2859.24	2485.26
	$F_{5\%}$	5384.64	2681.51	2703.13
	$F_{15\%}$	4914.54	2503.78	2410.76
	$F_{50\%}$	3892.89	2326.05	1566.84
Magnum551	F_B	5129.89	2859.24	2267.69
	$F_{5\%}$	5266.60	2681.51	2585.09
	$F_{15\%}$	4707.19	2503.78	2203.41
	$F_{50\%}$	3847.57	2326.05	1521.52

FB, bud stage; F5%, 5% flowering stage; F15%, 15% flowering stage; F50%, full bloom stage
Note: Alfalfa hay is priced at 0.237$ per kg containing 17% crude protein (calculated based on the 2017 local market price and the 2017 average exchange rate of RMB USD, 1$ = 6.7518 CNY), each increase or decrease of 1% crude protein content, the price increases or decreases by 0.0148$ accordingly
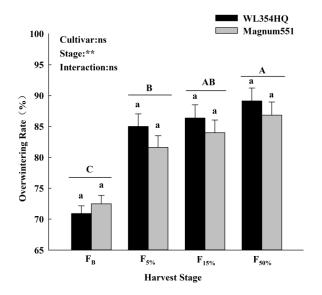

Fig. 1: Overwintering rate of alfalfa harvested at different harvest stages
The same lower case letters on the bars show there is no significant differences between WL354HQ and Magnum551 treatments at $P \leq 0.05$ and bars with different capital letters show there is significant differences among harvest stages at $P \leq 0.05$
ns= Non-significant; F_B= Bud stage; $F_{5\%}$= 5% flowering stage; $F_{15\%}$= 15% flowering stage; $F_{50\%}$= Full bloom stage

stage to the early flowering stage, as a fast-growing leguminous plant, the photosynthetic capacity of alfalfa increased rapidly, so the yield gradually increased. However, the growth rate of alfalfa plants slowed down after growing into the full bloom stage and the DMY increased slightly or unchanged due to the growth rhythm.

In this study, with the increase of harvest times, the DMY of alfalfa showed a slowly decrease during the same growth period. This result may be explained by the fact that alfalfa has accumulated lots of nutrients during a long

winter, and there are enough nutrients to promote alfalfa plants to grow rapidly after the arrival of spring. As for the yield of the last crop corresponding to each growth period was the lowest, a possible explanation for this might be that alfalfa consumes many nutrients in the soil after the first few harvests, resulting in a decrease in the growth of subsequent alfalfa plants (Ventroni *et al*. 2010). Another possible explanation for this is that the last harvest is in autumn, which is lower air temperature and shorter duration of light, thus causing a weaker photosynthetic capacity and a slower

growth rate of alfalfa. The results of a 2-year alfalfa planting experiments conducted in Argentina at cutting intervals of 40, 30 and 20 days respectively showed that the annual total hay yield of alfalfa decreased gradually with the increase of cutting frequency (Ventroni *et al.* 2010), and the same results were also demonstrated in experiments implemented in Kansas, central United States (Min 2016). The results of this study supported those of the previous.

Crude protein, NDF and ADF are important indicators for evaluating the nutritional quality of alfalfa forage (Avci *et al.* 2017). The study showed that the content of CP and carotene in alfalfa decreased, but the content of NDF and ADF increased gradually with the development of growth period (Albrecht *et al.* 1987). The nutritional quality of alfalfa hay in different growth periods was best at the bud stage, followed by the initial flowering stage, full flowering stage and pod stage (Albrecht *et al.* 1987). A 4-yr continuous trial conducted in hilly areas of southern Italy concluded that early harvesting resulted in lower forage yield and shorter forage life, but higher quality characteristics such as CP, NDF and ADF (Testa *et al.* 2011). The results of our study were similar to the results of previous researches. Studies have revealed that the protein in alfalfa plants mainly exists in the chloroplast, and as alfalfa reaches the flowering stage, the physiological function of alfalfa matures, the growth mode of alfalfa gradually transforms from vegetative growth to reproductive growth, and the nutrients in plants are more focused on ensuring flowering and subsequent fruiting processes (Lamb *et al.* 2012), the nutrients used for plant growth will be insufficient. This transformation process makes alfalfa more lignified, which in turn leads to the decrease of CP content and the increase of NDF and ADF content.

At the same time, changes in the content of certain small molecule metabolites during the growth process of plants can also cause significant changes in their nutritional value. Studies have illuminated that the contents of carotene, lysine, methionine, arginine and tryptophan decreased to different degrees after alfalfa grew to the full budding stage (Han 2007). Also, with the advancement of the growth period, L-glutamic acid, as an important intermediate product in the synthesis of plant proteins, is gradually reduced, and the contents of purine, pyrimidine and other small molecular substances constituting non-protein nitrogen in plants are gradually reduced (Semel *et al.* 2007; Fan *et al.* 2018), which in turn causes the decrease of crude protein content in alfalfa plants. Besides, the expression of L-phenylalanine, salicylic acid and D-mannose, which are closely related to the synthesis of alfalfa lignin and hemicellulose, is up-regulated with the advancing growth period, and is also a crucial reason for the elevation of NDF content and ADF content of alfalfa (Fan *et al.* 2018).

The overwintering rate has a vital effect on the yield of alfalfa plants in the second year. There are many factors affecting the overwintering rate, such as fall dormancy, cold resistance index, climatic conditions, cutting times, etc.

Studies have shown that the overwintering rate of alfalfa shows a slightly decreasing trend with the increase of cutting times (Sheaffer *et al.* 1992), because frequent cutting can reduce the regeneration ability of alfalfa (Ventroni *et al.* 2010). Meanwhile, the earlier the harvest stage, the more the cutting times, the greater the nutrient consumption of alfalfa plant roots, and then the more serious damage to alfalfa plants. Kallenbach *et al.* (2002) revealed that high harvesting frequency may reduce the resistance of alfalfa plants, which was not conducive to the safe overwintering of alfalfa plants, ultimately leading to the decline of dry matter yield and nutritional quality of alfalfa in the next year. It is also believed that frequent cutting will sharply decrease the hay yield of alfalfa in the coming year (Gramshaw *et al.* 1993), and the results of this study are in agreement with previous results. However, the difference between the two is that the decline of alfalfa hay yield is different in magnitude; this is because the alfalfa varieties used by the two have different fall dormancy, which affects the wintering rate of the two, resulting in an inconsistent decline in the alfalfa hay production next year.

Harvest time is one of the main factors determining the quantitative and quality traits of alfalfa. Early harvest has low yield, high protein content and high digestibility, while there is high yield, low protein content and low digestibility in late harvest. Therefore, both yield and quality of alfalfa must be taken into consideration in the realistic production of alfalfa. Prior studies have proposed that harvesting at 30–35 d cutting intervals maximizes both dry matter yield and nutrient quality of alfalfa (Hoveland *et al.* 1996). In this study, the comprehensive performance of alfalfa was the best at the bud stage and 5% flowering stage. However, a series of input costs in actual production such as seed, drip irrigation belt, water and electricity fee, land lease, harvest fee and field management should be considered comprehensively to determine the optimal harvest stage of alfalfa and further achieve the highest economic benefits. Our hypothesis that the highest economic benefits of alfalfa may occur at the early flowering stage was mostly upheld judging from dry matter yield, nutritional quality, overwintering rate and economic benefits of alfalfa.

Conclusion

The TDMY and CP yield of alfalfa were the highest when harvesting at $F_{5\%}$, followed by $F_{15\%}$, $F_{50\%}$, and F_B. The overwintering rate of alfalfa is decreased with the increase of cutting times. Based on the DMY, nutritional quality and overwintering rate of alfalfa, the priority of the growth period suitable for alfalfa harvesting was: $F_B > F_{5\%} > F_{15\%} > F_{50\%}$. In combination with the input cost, it is concluded that harvesting at 5% flowering stage of alfalfa seemed viable option to grow alfalfa under drip irrigation in the oasis area of Xinjiang, China.

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Author Contributions

Qianbing Zhang designed the study. Xuanshuai Liu and Yanliang Sun interpreted the results. Xuanshuai Liu, Yanliang Sun, Shengyi Li and Junwei Zhao performed the experiments. Xuanshuai Liu participated in writing the manuscript. Qianbing Zhang and Chunhui Ma supervised the study. All authors read and approved the final manuscript for publication.

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