RESEARCH ARTICLE Legume Research- An International Journal, Volume 44 Issue 1: 15-20 (January 2021)

Correlation Analysis of Alfalfa Varieties Based on Production Performances, Winter Survival Rates and Fall Dormancies

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10.18805/LR-551

ABSTRACT

Selection for fall dormancy (FD) in alfalfa can impact other traits, such as dry matter yield (DMY), winter survival rate (WSR) and chemical compositions. Nine alfalfa varieties were mowed during the early flowering stage from 2015 to 2018. The results showed that the WSRs of both Zhongmu No. 2 and Caoyuan No. 3 exceeded 95% and there was a significant negative correlation between the FD rating and WSR (-0.988; P<0.01). The four-year average DMY of Zhongmu No. 2 was the highest (11,912 kg ha^{-1}). The crude protein contents of Caoyuan No. 3 and Zhongmu No. 2 were both greater than 20%. Zhongmu No. 2 had the lowest acid detergent fiber (30.2%) and neutral detergent fiber (48.4%) contents as well as the highest relative feed values (126). Zhongmu No. 2, Adrenalin, Gold Empress and Caoyuan No. 3 (FD 1-4) were the optimum alfalfa varieties for planting in Hohhot, Inner Mongolia, China.

Key words: Alfalfa, Fall dormancy, Forage quality, Winter survival rate, Yield.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most popular perennial legume forages and is widely planted in China (Chen *et al.*, 2014). Alfalfa varieties introduced from abroad have the advantages of good quality and high yield, but their winter survival rates (WSRs) are low, which could result in reduced forage yields in subsequent years in northern China. In addition, many factors, such as autumn harvest time (Berti *et al.*, 2012) and stand age (Suzuki, 1991), affect the stand persistence and WSR of alfalfa. However, the effect of fall dormancy (FD) on production performance and WSR remains unclear. FD is often used as an important index for the selection of alfalfa varieties due to the importance of FD in terms of adaptability and productivity (Fairey *et al.*, 1996). FD is usually divided into three categories: dormancy (FD 1-3), semidormancy (FD 4-6) and nondormancy (FD >6) (Barnes *et al.*, 1979). Dormant varieties produce short branches and decumbent shoots in the autumn and stem elongation is slow after harvest in the summer; therefore, these varieties have high winter cold resistance (Ventroni *et al.*, 2010). In contrast, nondormant varieties thrive in the autumn, with shoots rapidly elongating after cutting in the summer and autumn to form long, erect shoots (Brummer *et al.*, 2000). Therefore, it is necessary to further understand the correlation between the production persistence, WSR and FD of alfalfa varieties. The objective of this study was to determine the correlation between the production persistence, WSR and FD of alfalfa varieties collected from China, the United States of America and Canada. The identification of varieties with high yield, good quality and strong wintering ability will provide a reference for the introduction of optimum alfalfa varieties to the Hohhot area of Inner Mongolia.

MATERIALS AND METHODS

Material from nine alfalfa varieties was collected from China (Caoyuan No. 3 and Zhongmu No. 2), the United States of America (Queen, Gold Empress, Sanditi, Sardi, WL525HQ and WL903) and Canada (Adrenalin). Caoyuan No. 3 (FD 1), Zhongmu No. 2 (FD 1-2), Queen (FD 2) and Gold Empress (FD 2-3) are all dormant varieties, Adrenalin (FD 4) and Sanditi (FD 5-6) are semidormant varieties and Sardi (FD 7), WL525HQ (FD 8) and WL903 (FD 9) are nondormant varieties. The FD ratings are provided by the breeding company and Fang *et al.* (2015). The field experiment was conducted at the Agricultural Science and Technology Research Center of Inner Mongolia Agricultural University, Inner Mongolia, China (40°39′ N, 111°58′ E) from 2014 to

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How to cite this article: Wang, X., Yan, X., Mi, F. and Li, H. (2021). Correlation Analysis of Alfalfa Varieties Based on Production Performances, Winter Survival Rates and Fall Dormancies. Legume Research. 44(1): 15-20. DOI: 10.18805/LR-551.

Submitted: 18-01-2020 **Accepted:** 15-03-2020 **Online:** 15-05-2020

2018. Nine alfalfa varieties were planted in May 2014. The soil was mainly chestnut and contained (0-30 cm depth) 15.97 g kg^{-1} of organic matter (OM), 348.0 mg kg^{-1} of available nitrogen (AN), 54.0 mg kg^{-1} of available phosphorus (AP) and 328.0 mg kg^{-1} of available potassium (AK) and had a pH of 8.30 (1:2.5 soil: water). Each plot was 5.0 m long and 2.0 m wide, with row spacings 0.4 m. The test plots were designed as a randomized complete block with three replicates. The alfalfa variety Caoyuan No. 3 was planted as a protective plant around the plot. The seeding rate was 15 kg ha^{-1}. No fertilizer was applied to and test plots before sowing and compaction was performed after sowing. During the management of the test plot, irrigation was carried out once in the regreening stage, the squaring stage and before the wintering period. The plots were hand weeded during the alfalfa growing period. Three cuttings per year were carried out during the early flowering stage from 2015 to 2018 (June 10th - June 16th, July 14th - July 20th, August 23rd - August 29th). The average temperature and total rainfall were 7.9°C and 395 mm in 2015, 7.6°C and 482 mm in 2016, 8.1°C and 306 mm in 2017 and 7.7°C and 390 mm in 2018, respectively.

To measure the DMY, cutting was carried out during the early flowering stage. Fresh alfalfa (1.0 kg) was dried in an oven at 65°C until the weight was constant and the DMY per ha was calculated. Fresh alfalfa (0.5 kg) was taken from each plot and the stems and leaves were separated. The stems and leaves were weighed after drying and then the leaf to stem ratio (LSR) was calculated. The WSR was calculated by the as follow (Chen *et al.*, 2014).

$$WSR\ (\%) = \frac{N\ (\text{plant numbers after wintering})}{N_1\ (\text{plant numbers before wintering})} \times 100$$

The crude protein (CP) content was determined by the AOAC method (AOAC, 1990). The acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were measured with ANKOM fiber analyzer filter bag method (Anonymous, 1997). The dry matter intake (DMI), digestible dry matter (DDM) and relative feed value (RFV) were calculated as follows (Albayrak *et al.*, 2018; Avci *et al.*, 2017). Dry matter intake (DMI) = 120/NDF; digestible dry matter (DDM) = 88.9 - (0.779 × ADF) and RFV = DMI × DDM/1.29.

The experiment was conducted for four years (2015, 2016, 2017 and 2018) in one location. Analysis of variance ($P<0.05$, $P<0.01$) and correlation analysis (Table 5) were performed using SAS software (SAS, 9.1). The data processing was performed with Excel 2010 and the figure was drawn with Sigma Plot software (Sigma Plot, 12.5).

RESULTS AND DISCUSSION

Winter survival rate

Significant differences ($P<0.05$) were observed in the WSR among the varieties (Table 1). The WSR of Caoyuan No. 3 was highest from 2015 to 2018. The four-year average WSR of the alfalfa varieties decreased with the increase of the FD rating. The WSRs of Caoyuan No. 3, Zhongmu No. 2, Queen, Gold Empress and Adrenalin (FD 1-4) were significantly higher ($P<0.05$) than those of Sardi, WL525HQ and WL903 (FD 7-9). There was a significant negative correlation between the FD rating and the WSR, with a correlation coefficient of -0.988 (Table 5). Similar results were obtained in previous studies (Brummer *et al.*, 2002). This indicated that the WSRs of fall-dormant alfalfa were markedly higher than those of nondormant alfalfa. It is likely that the accumulation of raffinose and amino acid contribute to enhancing cold tolerance in fall-dormant alfalfa (Liu *et al.*, 2019). This positive association between winter survival and FD has been reported in previous studies (Brummer *et al.*, 2000; Liu *et al.*, 2019), which recommended that FD be used as an important indicator for selecting varieties of winter hardiness (Barnes *et al.*, 1979).

Crude protein content

Significant differences ($P<0.05$) were observed among the alfalfa varieties in terms of CP contents (Table 2). The average CP contents of the varieties over four years ranged from 17.27 to 21.17%. Zhongmu No. 2 and Caoyuan No. 3

Table 1: Averaged values of winter survival rate (WSR) for alfalfa varieties tested.

Varieties	WSR (%)				
	2015	2016	2017	2018	Mean
Caoyuan No. 3	98.2 a*	97.9 a	98.5 a	96.9 a	97.9 a
Zhongmu No. 2	98.1 a	96.8 ab	95.6 b	94.7 b	96.3 a
Queen	97.4 ab	96.5 ab	94.6 b	90.9 c	94.8 ab
Gold Empress	96.5 b	96.1 b	91.3 c	89.1 d	93.2 ab
Adrenalin	93.6 c	92.5 c	90.6 c	87.5 d	91.1 ab
Sanditi	90.6 d	90.5 d	86.5 d	84.7 e	88.1 bc
Sardi	88.2 e	86.4e	79.2 e	74.5 f	82.1 c
WL525	88.1 e	86.7 e	78.6 e	71.7 g	81.3 cd
WL903	84.1 f	76.6 f	72.2 f	67.0 h	74.9 d
Mean	92.7 A+	91.1 AB	87.5 AB	84.1 B	88.9

*Means with the same letter in the same column are not significantly different at 0.05 probability level. +Means with the same letter in the same line are not significantly different at 0.05 probability level.

showed significantly higher (P<0.05) CP contents than the other varieties. Their superiority, in terms of CP content, suggests that higher CP contents were related to their FD. As the FD of alfalfa varieties has a major effect on the CP content of dry matter, the dormant alfalfa varieties almost always had higher CP contents (Avci *et al.*, 2017). The CP contents of alfalfa were determined to be 20.99% (Al-Ghumaiz, 2012), 17.90-19.70% (Avci *et al.*, 2017) and 16.58-21.24% (Albayrak *et al.*, 2018); our results are generally similar to those of the aforementioned studies.

Leaf to stem ratio

The means of the LSR for the alfalfa varieties are shown in Table 2. The four-year average LSRs of the different alfalfa varieties ranged from 0.81 to 0.89. The highest LSR was recorded for Zhongmu No. 2. Alfalfa leaves are the most valuable (*i.e.*, nutritive) parts of alfalfa plants and varieties with higher leaf to stem ratios are characterized by better quality (Strbanovic *et al.*, 2015). Correlation analysis showed that there was a significant positive correlation (0.673, P<0.05) between the LSR and the CP contents (Table 5), suggesting that the ratio of leaves to stems is the most important determinant of quality for alfalfa. Leaves account for 35 to 45 % of low-quality alfalfa plants (LSR=0.53-0.82) and 55 to 65 % (LSR=1.22-1.85) of high-quality alfalfa plants (Putnam *et al.*, 2008). According to this assessment, while the Queen variety (LSR=0.81) is low quality, the quality of Zhongmu No. 2, Caoyuan No. 3, Adrenalin, Gold Empress and WL903 (LSR=0.85-0.89) falls between the low- and high-quality classes. Rotili *et al*. (1999) also reported that the quality of alfalfa is mainly influenced by LSR and that plants with an LSR from 0.85 to 1.0 are defined as having optimal plant quality.

Neutral detergent fiber

The variance analysis of the NDF contents of the different alfalfa varieties is shown in Table 3. The NDF contents of alfalfa ranged from 48.4 to 51.0%, on average, over four years. The lowest NDF content was found in Zhongmu No. 2. In previous studies (Albayrak *et al.*, 2018; Avci *et al.*, 2017; Spandel and Hesterman, 1997), the NDF contents of alfalfa were reported to be 41.7-44.8, 44.8-49.6 and 39.4-47.8%, depending on the variety and year. Low NDF contents are desirable and are associated with increased animal intake. Correlation analysis showed there was a significant negative correlation (-0.849, P<0.05) between CP and NDF contents

Table 2: Averaged values of crude protein (CP) and leaf to stem ratio (LSR) for alfalfa varieties tested.

Varieties	CP (%)					LSR				
	2015	2016	2017	2018	Mean	2015	2016	2017	2018	Mean
Caoyuan No. 3	20.17 a*	20.71 ab	21.22 b	20.20 b	20.57 b	0.89 ab*	0.88 ab	0.87 ab	0.85 ab	0.87 ab
Zhongmu No. 2	20.23 a	21.38 a	22.18 a	20.89 a	21.17 a	0.91 a	0.90 a	0.90 a	0.86 a	0.89 a
Queen	18.06 b	18.96 d	18.65 d	19.00 de	18.67 e	0.82 d	0.82 d	0.82 d	0.79 d	0.81 e
Gold Empress	19.34 a	19.20 cd	18.77 d	19.15 d	19.11 de	0.87 bc	0.86 bc	0.86 bc	0.85 ab	0.86 bc
Adrenalin	19.73 a	20.48 ab	19.18 cd	19.41 c	19.70 c	0.89 ab	0.88 ab	0.88 ab	0.84 abc	0.87 ab
Sanditi	19.74 a	21.49 a	18.38 d	18.88 e	19.62 cd	0.85 cd	0.84 cd	0.86 bc	0.83 bc	0.84 cd
Sardi	17.26 c	17.84 e	16.89 e	17.39 g	17.27 f	0.84 cd	0.84 cd	0.84 cd	0.83 bc	0.84 cd
WL525	19.76 a	20.82 ab	19.02 cd	18.42 f	19.50 cd	0.84 cd	0.84 cd	0.84 cd	0.82 c	0.83 d
WL903	19.34 a	20.08 bc	19.84 c	18.81 e	19.52 cd	0.86 bc	0.86 bc	0.85 bc	0.83 bc	0.85 cd
Mean	19.29	20.11	19.35	19.13	19.46	0.86	0.86	0.86	0.83	0.85

*Means with the same letter in the same column are not significantly different at 0.05 probability level.

Table 3: Averaged values of neutral detergent fiber (NDF) and acid detergent fiber (ADF) for alfalfa varieties tested.

Varieties	NDF (%)					ADF (%)				
	2015	2016	2017	2018	Mean	2015	2016	2017	2018	Mean
Caoyuan No. 3	49.9 cd*	48.3 d	48.0 de	49.4 e	48.9 de	30.6d e*	31.4 b	30.9 c	31.2 d	31.0 e
Zhongmu No. 2	48.9 d	48.2 d	47.8 e	48.6 f	48.4 e	29.7 e	29.8 c	30.0 d	31.1 d	30.2 f
Queen	50.8 bc	52.6 a	49.9 bc	49.9 d	50.8 ab	32.1 bc	33.7 a	34.4 a	34.3 bc	33.6 b
Gold Empress	51.2 b	48.8 cd	52.3 a	51.8 a	51.0 a	32.6 ab	33.5 a	35.1 a	35.2 ab	34.1 ab
Adrenalin	49.3 d	50.1 bc	48.9 cde	49.3 e	49.3 cde	30.2 de	33.4 a	30.1 d	34.0 c	31.9 d
Sanditi	49.7 cd	50.6 b	49.8 bcd	50.1 cd	50.1 bc	31.2 cd	31.0 b	32.6 b	34.1 c	32.2 d
Sardi	53.6 a	49.7 bcd	49.7 bcd	50.4 bc	50.9 ab	33.4 a	34.1 a	34.8 a	35.3 a	34.4 a
WL525	48.8 d	49.8 bcd	48.8 bcde	49.5 e	49.2 cde	30.6 de	30.4 bc	32.5 b	34.1 c	31.9 d
WL903	49.1 d	48.7 cd	50.5 b	50.8 b	49.8 cd	31.2 cd	33.6 a	32.9 b	34.3 bc	33.0 c
Mean	50.1	49.7	49.5	50.0	49.8	31.3 B+	32.3 AB	32.6 AB	33.7 A	32.5

*Means with the same letter in the same column are not significantly different at 0.05 probability level.

+Means with the same letter in the same line are not significantly different at 0.05 probability level.

(Table 5). Avci *et al*. (2017) also found that CP and NDF are inversely related and that the varieties highest in CP had the lowest in the NDF contents, while the varieties lowest in CP typically had the highest NDF contents. When considered from this perspective, Zhongmu No. 2 is a high-quality variety.

Acid detergent fiber

The variance analysis of the ADF contents of the different alfalfa varieties is shown in Table 3. The four-years average of the alfalfa ADF contents ranged from 30.2 to 34.4%. In previous studies (Albayrak *et al.*, 2018; Avci *et al.*, 2017; Spandel and Hesterman, 1997), the ADF contents for alfalfa were found to be 30.5-34.4; 36.8-40.4; 25.2-39.2%. The ADF contents of Zhongmu No. 2 and Caoyuan No. 3 were significantly lower (P<0.05) than those of the other varieties. ADF content has a negative effect on digestibility and intake (Mader *et al.*, 1991), indicating that the nutritional values of Zhongmu No. 2 and Caoyuan No. 3 were better than those of the other alfalfa. On the one hand, this pattern may be related to ecological factors, such as the relatively low precipitation and the high evaporation in Hohhot; it is possible that the foreign alfalfa varieties were affected by the arid climate conditions in this region (Rimi *et al.*, 2012). On the other hand, fiber content may be closely related to genetic factors in alfalfa varieties (Albayrak *et al.*, 2018; Hill and Barnes, 1977). These findings indicate that the chemical composition of alfalfa mainly depends on the variety and many ecological factors (Albayrak *et al.*, 2018; Karayilanli and Ayhan, 2016).

Digestible dry matter

Significant differences (P<0.05) were found in the DDM content among the alfalfa varieties (Table 4). The DDM value of Zhongmu No. 2 was the highest in 2015 and 2016. In 2018, the DDM values of Zhongmu No. 2 and Caoyuan No. 3 were significantly higher (P<0.05) than those of the other varieties. The four-year average of the DDM contents of the alfalfa varieties ranged from 62.10 to 65.40%. Zhongmu No. 2 and Sardi had the highest and the lowest DDM contents, which was mainly due to these varieties having the highest and lowest LSRs, respectively. As already noted, alfalfa leaves have relatively higher nutritive value and intake than stems. Julier and Huyghe (1997) also reported that the digestibility of alfalfa varieties showed differences depending on their LSRs. The DDM content determined for the alfalfa varieties in our study was similar to that reported in a previous study by Avci *et al*. (2017).

Table 4: Averaged values of digestible dry matter (DDM) and relative feed value (RFV) for alfalfa varieties tested.

Varieties	DDM (%)					RFV				
	2015	2016	2017	2018	Mean	2015	2016	2017	2018	Mean
Caoyuan No. 3	65.09 ab*	64.45 b	64.78 b	64.58 a	64.73 b	121 a*	124 ab	126 a	122 b	123 b
Zhongmu No. 2	65.73 a	65.69 a	65.54 a	64.65 a	65.40 a	125 a	127 a	127 a	124 a	126 a
Queen	63.88 cd	62.61 c	62.09 d	62.17 bc	62.69 e	117 b	111 e	116 c	116 de	115 e
Gold Empress	63.54 de	62.79 c	61.58 d	61.51 cd	62.36 ef	115 b	120 cd	110 d	110 g	114 e
Adrenalin	65.38 ab	62.88 c	65.46 a	62.40 b	64.03 c	123 a	117 d	125 a	118 c	121 bc
Sanditi	64.58 bc	64.73 b	63.48 c	62.35 b	63.79 c	121 a	119 cd	119 bc	116 e	119 cd
Sardi	62.91 e	62.30 c	61.78 d	61.41 d	62.10 f	109 c	116 d	115 c	113 f	113 e
WL525	65.07 ab	65.25 ab	63.57 c	62.31 b	64.05 c	124 a	122 bc	121 b	117 cd	121 bc
WL903	64.58 bc	62.75 c	63.23 c	62.19 bc	63.19 d	122 a	120 bcd	116 c	114 f	118 d
Mean	64.53 A+	63.72 AB	63.50 AB	62.62 B	63.59	120	119	119	117	119

*Means with the same letter in the same column are not significantly different at 0.05 probability level.

+Means with the same letter in the same line are not significantly different at 0.05 probability level.

Table 5: Correlation analysis for fall dormancy (FD), dry matter yield (DMY), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), digestible dry matter (DDM), relative feed value (RFV), winter survival rate (WSR) and leaf to stem ratio (LSR) of alfalfa varieties from 2015 to 2018.

Item	FD	CP (%)	NDF (%)	ADF (%)	DDM (%)	RFV	LSR	WSR (%)
CP	-0.430							
NDF	0.145	-0.849**						
ADF	0.327	-0.991**	0.963**					
DDM	-0.328	0.912**	-0.963**	-1.000**				
RFV	-0.224	0.888**	-0.991**	-0.990**	0.990**			
LSR	-0.333	0.673*	-0.680*	-0.649	0.649	0.678*		
WSR	-0.988**	0.440	-0.178	-0.374	0.374	0.283	0.325	
DMY (kg ha^{-1})	-0.657	0.548	-0.364	-0.447	0.447	0.418	0.833	0.627

*Significant correlation at P<0.05; **Significant correlation at P<0.01.

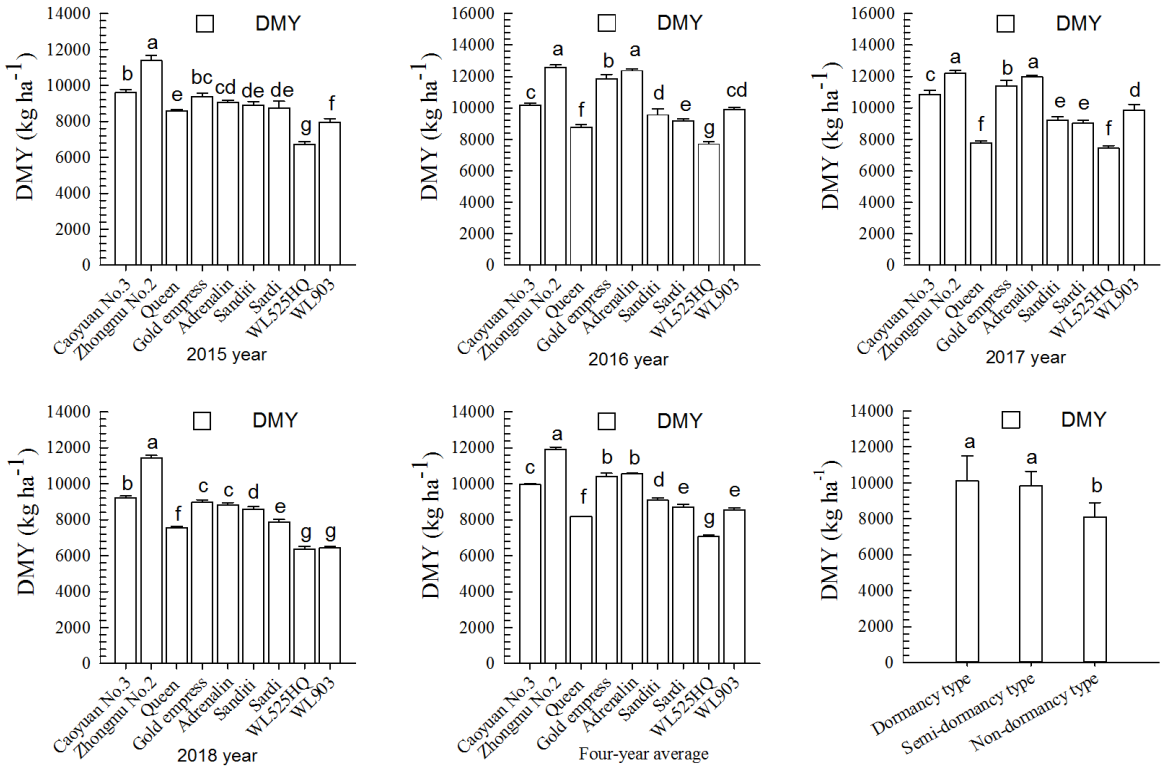

Fig 1: Average values of dry matter yield (DMY) of different alfalfa varieties. Data are presented by mean ± standard error. Dry matter yield (DMY) represented by bars with the same letters in each treatment (each year, four-year average and fall-dormancy type) are not significant difference according to Duncan Test at 0.05 probability level.

Relative Feed Value

Significant differences (P<0.05) were observed in the RFVs among the varieties (Table 4). The four-year average RFVs of the alfalfa varieties ranged from 113 to 126. The highest and the lowest RFV values were found for Zhongmu No. 2 and Sardi, respectively. This indicated that the RFVs were significantly different among the varieties, which may be mainly due to the large amount of variation in the DDM and DMI (not shown here). RFV is derived from the DMI and DDM contents of alfalfa (Avci *et al.*, 2017). Forages with RFVs between 150-125, 124-103, 102-87 and 86-75 are categorized as premium, good, fair and poor, respectively (Kiraz, 2011). According to this assessment, Zhongmu No. 2 is classified as premium, while the other varieties are classified as good.

Dry Matter Yield

The means of the DMY for the alfalfa varieties are shown in Fig 1. Based on the four-year average, the four varieties (FD 1-4) with the highest DMYs were Zhongmu No. 2 (11,912 kg ha^{-1}), Adrenalin (10,559 kg ha^{-1}), Gold Empress (10,406 kg ha^{-1}) and Caoyuan No. 3 (9,960 kg ha^{-1}). However, the varieties with FD ratings between 7 and 9 (Sardi, WL525HQ and WL903) performed poorly in Hohhot, indicating that there was not a significant correlation between the FD rating and DMY (Table 5). Previous studies have reported that there was no definite relationship between FD and annual forage yields (Chen *et al.*, 2014; Rimi *et al.*, 2012). Our results provide solid evidence to support these claims. FD should not be used as the main index for selecting alfalfa varieties in Hohhot. Previous studies have also reported that in areas with warmer winters, the establishment of suitable harvesting systems is the main factor affecting alfalfa production and FD is a secondary factor (Berti *et al.*, 2012; Ventroni *et al.*, 2010).

CONCLUSION

The FD rating of alfalfa had no significant effect on alfalfa production performance, including the DMY and chemical compositions, but it was significantly negatively correlated with the WSR. Zhongmu No. 2, Adrenalin, Caoyuan No. 3 and Gold Empress, which were more suitable for planting in the Hohhot area, had optimal DMYs, nutritional quality and WSRs. Using the FD rating, priority should be given to the selection of alfalfa varieties with FD values of 1 to 4.

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