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Correlation between Fall Dormancy and Production Performance of Alfalfa (*Medicago sativa*)

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ABSTRACT

Background: Selection for fall dormancy (FD) in alfalfa (*Medicago sativa*) can impact other traits, including dry matter yield (DMY), winter survival rate (WSR) and nutrient compositions. The current study was aimed to screen alfalfa varieties with greater yield, good quality and strong winter survival ability and thus are more suitable for planting in Heilongjiang, China and may be suitable for similar upper-latitude semiarid environments in the world.

Methods: Eight alfalfa varieties (Zhaodong, Gongnong No. 2, Caribou, Gold Empress, Adrenalin, Sanditi, Sardi and WL525) were planted in regional test based on randomized block design and mowed during the early flowering stage from 2019 to 2021. The DMY, WSR and nutrient compositions were measured by weighing method, field investigation and chemical test, respectively.

Result: There was a significant negative correlation (P<0.01) between the FD rating and WSR, with a correlation coefficient of 0.956. The three-year average DMY of Gongnong No. 2 was the greatest (12,745.65 kg ha^{-1}) and there was a significant negative correlation (0.766, P<0.01) between the FD rating and DMY. The crude protein content of Gongnong No. 2 was greater (19.29%) than the other varieties. The Zhaodong had the least neutral detergent fiber (47.76%) and acid detergent fiber (30.79%).

Key words: Fall dormancy, *Medicago sativa*, Nutrient compositions, Winter survival rate, Yield.

INTRODUCTION

Alfalfa (*Medicago sativa*) is the most widely distributed perennial leguminous forage in the world (Albayrak *et al*., 2018; Ventroni *et al*., 2010) but also the high-quality forage in China, alfalfa occupies the largest planting area with the highest forage value (Liu *et al*., 2021; Shi *et al*., 2017). Alfalfa is rich in nutritional value, especially high in crude protein (CP) content, which can replace the conventional concentrated feed to feed livestock. Alfalfa can also help to solve the problem of insufficient supply of high protein forage during winter in Northeast China. Currently, alfalfa varieties introduced from abroad have the advantage of good quality and high dry matter yield (DMY) (Wang *et al*., 2021). Although, autumn harvest time (Berti *et al*., 2012), snow cover (Leep *et al*., 2001), soil moisture (Ryswyk *et al*., 1993) and soil nutrient content (Macolino *et al*., 2013) influences the DMY and winter survival rate (WSR) of alfalfa, whereas it is unclear whether fall dormancy (FD) also affects the production persistence and WSR of alfalfa. Therefore, it is necessary to understand the correlation among production persistence, WSR and FD of alfalfa varieties. Due to the importance of FD in adaptability and DMY, it is often used as an important reference index for alfalfa variety selection (Fairey *et al*., 1996; Volenec *et al*., 2002). The FD ratings are usually divided into three groups: dormancy (FD rating <4), semi-dormant (FD rating 4-6) and non-dormant (FD rating >6) (Barnes *et al*., 1979). Dormancy type varieties have short branches and extroversion in autumn and slow stem elongation after harvest in summer. Therefore, dormancy type varieties have higher WSR (Haagenson *et al*., 2003). In contrast, non-dormancy type varieties thrive in autumn and the branches stretch rapidly after mowing in summer and autumn, forming long and upright branches (Brummer *et al*., 2002; Haagenson *et al*., 2003). Therefore, FD is not only related to the WSR of alfalfa, but also closely related to its yield and quality. Although there are many factors affecting the DMY and quality of alfalfa during its growth, such as mowing period, cutting frequency, climatic conditions and others (Djaman *et al*., 2020), the relationship among FD, DMY and nutrient components still remains unclear. Therefore, this study aims to explore the correlation between WSR, DMY, nutrient composition and FD of alfalfa varieties and screen alfalfa varieties with greater yield, better quality and stronger winter survival ability, so as to provide reference for introducing the best alfalfa in Heilongjiang Province, China and similar upper-latitude semiarid environments.

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MATERIALS AND METHODS

A total of eight alfalfa varieties were collected, the China (Zhaodong and Gongnong No. 2), the United States of America (Gold Empress, Sanditi, Sardi and WL525) and Canada (Caribou and Adrenalin). Zhaodong and Gongnong No. 2 (FD rating 1), Caribou (FD rating 2) and Gold Empress (FD rating 2-3) are all dormancy type varieties, Adrenalin (FD rating 4) and Sanditi (FD rating 5-6) are semi-dormancy type varieties and Sardi (FD rating 7) and WL525 (FD rating 8) are non-dormancy type varieties. The FD ratings are provided by the breeding company and Fang *et al*. (2015). The test plot was conducted at the Branch of Animal Husbandry and Veterinary of Heilongjiang Academy of Agricultural, Qiqihar, Heilongjiang, China (47°15′N, 123°41′E). The climate of the experimental site is mainly characterized by drought and cold. The average temperature and total rainfall were 5.9°C and 559 mm in 2019, 2.6°C and 625 mm in 2020 and 5.2°C and 628 mm in 2021, respectively. Eight alfalfa varieties were planted in May 2018. The soil was aeolian sandy soil and contained (0-30 cm depth) 19.89 g kg^{-1} of organic matter (OM), 1.19 g kg^{-1} of available nitrogen (AN), 124.8 mg kg^{-1} of available potassium (AK) and 10.6 mg kg^{-1} of available phosphorus (AP) and had a pH of 7.4. The test plots were designed as a randomized complete block with three replicates. Each test plot was 5.0 m long and 3.0 m wide, with row spacing 0.3 m. The alfalfa cultivar Zhaodong was planted as a protective plant around the test plot. The seeding rate was 15 kg ha^{-1}. No fertilizer was applied to the test plots before sowing and compaction was performed after sowing. The test plot irrigation was carried out once in the regreening stage, the squaring stage and the pre-wintering period. The irrigation technology adopts sprinkler irrigation and the irrigation quota is 225~375 m^3 hm^{-2}. The test plots were hand weeded during the alfalfa growing period. Three mowing per year were carried out during the early flowering stage from 2019 to 2021 (June 12^{th} -June 18^{th}, July 17^{th}-July 22^{th}, August 25^{th} -August 31^{st}).

To measure the DMY, mowing was carried out during the early flowering stage (10% flowering). The fresh alfalfa yield of test plot was measured, stubble height was 5 cm and repeated 3 times. Fresh alfalfa (2.0 kg) was dried in a drying oven at 65°C until the weight was constant and the DMY per ha was calculated. Fresh sample (1.0 kg) was taken from each test plot and the leaves and stems were separated. The leaves and stems were weighed after drying and then the leaf to stem ratio (LSR) was calculated. From October 20^{th} to November 1^{st} of every year, three replicates of a 1.0 m long sample segment were randomly selected in each test plot. The number of surviving plants in each sample segment was investigated and then the number of surviving plants were recorded in the next year. The winter survival rate.

$$\text{WSR} = \frac{\text{N (number of surviving plants after wintering)}}{\text{N}_1\text{ (number of surviving plants before wintering)}} \times 100\%$$

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

The experiment was conducted for three years (2019, 2020 and 2021) in one location. Years was treated as a fixed effect and two treatments (Years and FD Varieties) interactions were analyzed using Two-factors ANOVA. Analysis of variance ($P<0.05$, $P<0.01$) and correlation analysis were performed using SAS 9.0 software (SAS Institute Inc, 2002, NC USA). The data processing was performed with Excel 2007 (MicroSoft, Redmond, WA USA) and the figure was drawn with Sigma Plot 12.5 software (Systat Software Inc, 2003, San Jose, CA USA).

RESULTS AND DISCUSSION

Dry matter yield

Results of correlation analyses are presented in Table 1. Main effect means and results of statistical analyses for year and FD variety are presented in Table 2. Year and variety were significant for all variables, while the year × FD variety interaction was significant only for DMY and LSR. In addition to the significant main effects of year and variety for DMY (Table 2), the significant year × variety interaction is shown in Fig 1. Previous studies have indicated that there was no definite relationship between FD rating and alfalfa yields (Chen *et al*., 2014; Rimi *et al*., 2012). Our results were contrast with the previous report. Based on the three-year average, FD rating 1 (Zhaodong and Gongnong No. 2) had the greatest DMYs. However, the alfalfa varieties with FD ratings between 2 and 8 (Caribou, Gold Empress, Adrenalin, Sanditi, Sardi and WL525) performed poorly in Heilongjiang, indicating that there was a negative correlation between the FD rating and DMY (Table 1). The DMYs of dormancy type and semi-dormancy type varieties were markedly higher than those of non-dormancy type varieties (Fig 1). One hand, this pattern may be related to low the WSR of alfalfa varieties, the long term evolution and selection for the local varieties maybe the main reason and the foreign varieties were also affected by the cold winter climate conditions in this region (Djaman *et al*., 2020). The other hand, the DMY may be closely related to genetic factors in varieties (Hill and Barnes, 1977; Albayrak *et al*., 2018). The FD rating should not be used as the main indicator for selecting alfalfa varieties in Heilongjiang, which was agreement with the results of Wang *et al*. (2021). Their studies confirmed that the FD rating was not related to the production performance of alfalfa in semi-arid climatic conditions. 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 rating is a secondary factor (Ventroni *et al*., 2010).

The DMYs of the first, second and third mowing of alfalfa are shown in Fig 2. The forage yield of each cutting accounted for 48%, 36% and 16% of the total yield in the whole year, respectively. Wang *et al*. (2009) found that the yield was significantly different with different cutting times and the yield of the first cutting was higher than that of the second cutting and the third cutting. These results of this study showed the same rule. This also in consistent with the typical growth/regrowth pattern of well-watered alfalfa that is driven by high summer temperatures (relative to FD adaptation) and declining daylength after summer solstice, which is exhibited by fall growth upon which fall dormancy categories are based. The yield of the first two cuttings accounted for more than 80% of the total yield in the whole year. In addition, there was a positive correlation between alfalfa yield and WSR (Table 1), which shows that the first cutting yield is very important to the total annual yield of alfalfa. Even though the growth of the first cutting alfalfa is affected by low temperature in winter and spring, it still

Table 1: 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 different alfalfa varieties from 2019 to 2021 average value.

Item	FD rating	CP (%)	NDF (%)	ADF (%)	DDM (%)	RFV	LSR	WSR (%)	DMY (kg ha^{-1})
CP	-0.379								
NDF	0.646**	-0.731**							
ADF	0.661**	-0.549**	0.775**						
DDM	-0.661**	0.549**	-0.775**	-1.000**					
RFV	-0.688**	0.697**	-0.968**	-0.909**	0.909**				
LSR	-0.442*	0.761**	-0.772**	-0.649**	0.649**	0.766**			
WSR	-0.956**	0.304	-0.536**	-0.652**	0.652**	0.613**	0.450*		
DMY	-0.766**	-0.039	-0.179	-0.465*	0.465*	0.300	0.157	0.877**	1.000

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

Table 2: Dry matter yield (DMY), leaf to stem ratio (LSR), winter survival rate (WSR), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and digestible dry matter (DDM) of alfalfa varieties of varied fall dormancy (FD) grown at Heilongjiang, China, from 2019 to 2021 average value.

	FD	CP (%)	NDF (%)	ADF (%)	DDM (%)	RFV (%)	LSR	WSR (%)
Year								
2019	-	18.90 a	48.74 a	32.18 a	63.72 a	121.87 a	0.80 a	68.86 a
2020	-	19.80 a	49.44 a	31.51 a	64.35 a	123.94 a	0.88 a	65.79 a
2021	-	18.21 a	49.44 a	32.75 a	63.39 a	120.15 a	0.73 a	63.56 a
Standard error		0.79	0.40	0.62	0.48	1.89	0.07	2.66
Variety								
Zhaodong	1	19.25 a	47.76 e	30.79 e	64.91 a	126.43 a	0.85 b	96.43 a
Gongnong No. 2	1	19.29 a	48.20 d	31.43 d	64.43 b	124.35 b	0.92 a	94.59 b
Caribou	2	19.15 b	48.49 cd	32.11 c	63.89 c	122.57 c	0.83 b	85.59 c
Gold Empress	2-3	18.78 c	49.07 b	32.21 c	63.81 c	120.97 d	0.74 f	75.10 d
Adrenalin	4	18.43 c	49.78 a	32.84 a	63.32 e	118.33 e	0.69 g	64.08 e
Sanditi	5-6	18.65 c	49.88 a	32.66 ab	63.46 de	118.34 e	0.77 e	64.07 e
Sardi	7	19.14 b	48.90 bc	32.71 ab	63.43 de	120.65 d	0.80 d	24.88 f
WL525	8	19.16 b	48.58 cd	32.42 bc	63.54 d	120.88 d	0.82 c	23.83 f
Standard error		0.32	0.73	0.71	0.56	2.80	0.07	28.50
P-values								
Year	-	**	**	**	**	**	**	**
FD variety	-	**	**	**	**	**	**	**
Year×FD variety	-	NS	NS	NS	NS	NS	**	NS

Note: Means for a treatment level (year or variety) with the same letter in the same column are not significantly different at 0.05 probability level.

**Significant correlation or interaction at P<0.01; NS- Non significant interaction at P>0.05.

contributes the most to the annual yield of alfalfa (Djaman *et al*., 2020). For less dormant varieties (*i.e*., Sardi and WL525), spring yields are reduced, compared to more dormant varieties, by attempted winter growth that reduces root carbohydrates (Liu *et al*., 2019). Therefore, good field management of the first cutting alfalfa is the key to achieve yield increase. The growing period of the second and third crops of alfalfa is in the season of rising temperature and increasing precipitation (June- August) and the plant grows rapidly. If the field management and protection can be strengthened during this period, alfalfa will have great potential for increasing yield (Chen *et al*., 2014).

Winter survival rate

Significant differences were found in the WSR among the alfalfa varieties (Table 2). The WSR of Zhaodong was highest from 2019 to 2020. The WSR of the alfalfa varieties decreased with the increase of the FD rating. The WSRs of Zhaodong (96.43%), Gongnong No. 2 (94.59%), Caribou (85.59%) and Gold Empress (75.10%) were greater than those of Adrenalin, Sanditi, Sardi and WL525 (FD rating 4-8). Correlation analysis showed that there was a negative

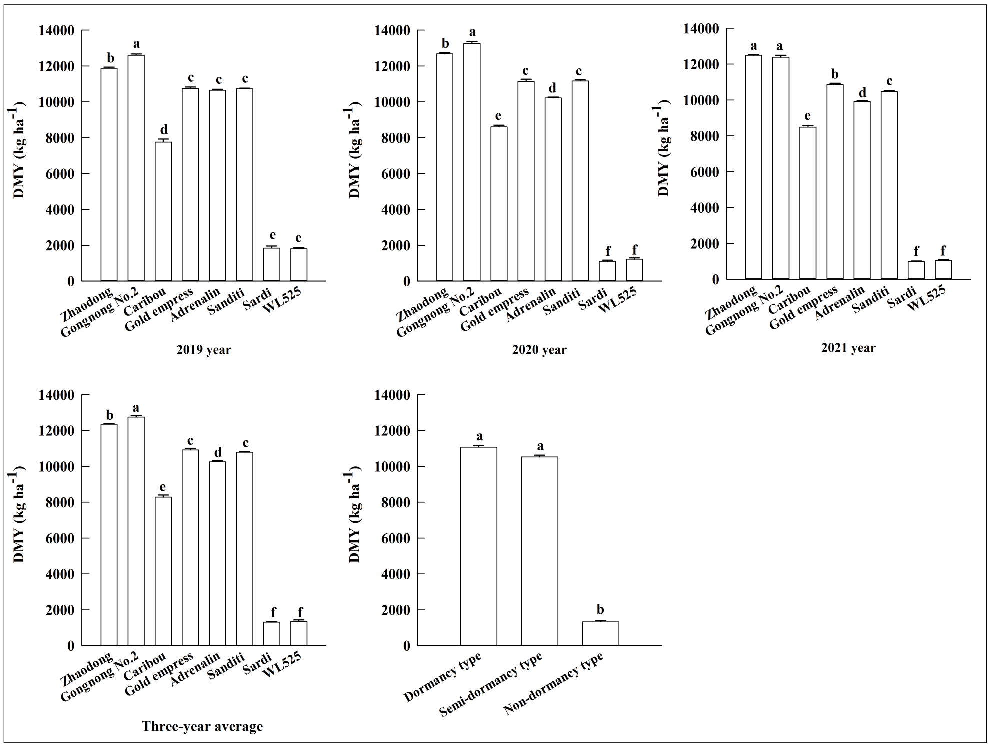

Fig 1: Average values of dry matter yield (DMY) of different alfalfa varieties. Data are presented by mean ± standard error. Dry matter yield (DMY) represented by bars with the same letters in each treatment (each year and three-year average) are not significant difference according to Duncan test at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

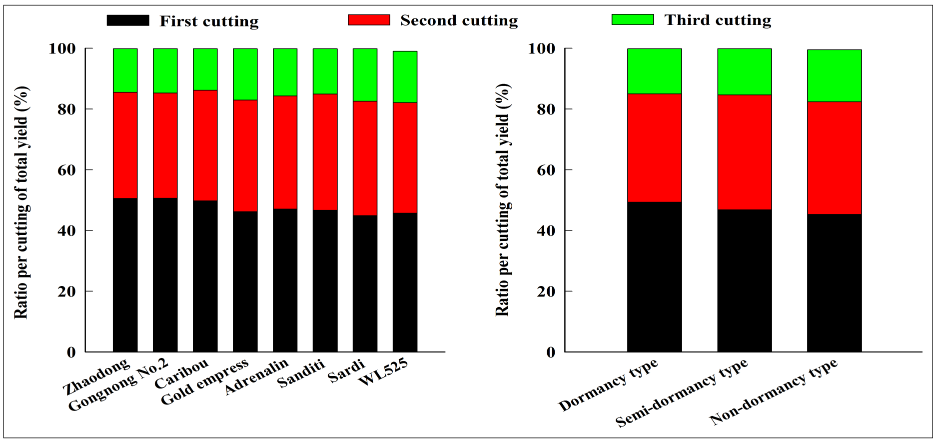

Fig 2: Based on mean of three years, ratio of per cutting dry matter yield (DMY) accounted for the total yield of alfalfa varieties.

correlation between the WSR and the FD rating (Table 1). Similar results were obtained in previous studies (Brummer *et al*., 2002; Wang *et al*., 2021). This indicated that the WSRs of dormancy type varieties (FD rating 1-2 including Caribou) were markedly higher than those of semi-dormancy type (FD rating 4-6) and non-dormancy type (FD rating 7-8) varieties, suggesting that dormancy type varieties have stronger cold tolerance. It is likely that the accumulation of raffinose and amino acid (Threonine, Histidine, Proline and Glycine) contributes for enhancing cold tolerance in dormancy type varieties (Liu *et al*., 2019). This positive association between the WSR and FD has been reported in previous researches (Li *et al*., 2015; Wang *et al*., 2021), which recommended that FD rating be used as an important index for selecting alfalfa varieties for winter hardiness (Barnes *et al*., 1979).

Crude protein content

Differences were examined among the different varieties in terms of CP contents (Table 2). The average CP contents over three years ranged from 18.43% to 19.29%, which is similar to those measured by others researchers (Avci *et al*., 2017; Albayrak *et al*., 2018; Wang *et al*., 2021). Gongnong No. 2 and Zhaodong (FD rating 1) showed greater CP contents than the other varieties. Their superiority, in terms of CP content, suggests that higher CP contents were related to their FD categories. As the FD of varieties has a major effect on the CP content of dry matter, the very dormancy varieties almost always had greater CP contents (Strbanovic *et al*., 2017). Some studies have also shown that the decrease in LSR is one of the main factors causing alfalfa quality depletion due to a decline in crude protein and an increase of fibrous constituents (Rimi *et al*., 2012; Avci *et al*., 2017; Wang *et al*., 2021).

Leaf stem ratio

The variance analysis of the LSR of the alfalfa varieties is shown in Table 2. The greatest LSR was observed in the Gongnong No. 2 (FD rating 1). Alfalfa leaves are the most valuable (*i.e.*, nutritive and CP content) parts of alfalfa plants and varieties with greater LSRs are characterized by better quality (Strbanovic *et al*., 2017). According to the correlation analysis, there was a positive correlation between the LSR and the CP contents (Table 1), suggesting that the LSR is the most important determinant of quality for alfalfa among the dormancy varieties included in our correlation analysis. Leaves account for 55% to 65% (LSR=1.22-1.85) of high-quality alfalfa plants and 35% to 45% (LSR=0.53-0.82) of low-quality alfalfa plants (Putnam *et al*., 2008). According to this assessment, while the Gold Empress, Adrenalin, Sanditi, Sardi and WL525 variety (LSR=0.74-0.82) could be defined as low-quality alfalfa, the quality of Gongnong No. 2, Zhaodong and Caribou (LSR=0.83-0.92) falls between the low- and high-quality classes. Rotili *et al*. (1999) also found 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 high plant quality. When considered from this perspective, Zhaodong and Gongnong No. 2 are high-quality alfalfa varieties (Table 2).

Neutral detergent fiber and acid detergent fiber

The means of the NDF and ADF contents for the different alfalfa varieties are shown in Table 2. The NDF contents of alfalfa varieties ranged from 47.76% to 49.88%, over three years. The lowest NDF content was recorded for Zhaodong. Previously published reports found that the NDF contents of alfalfa depended on the variety (Albayrak *et al*., 2018; Avci *et al*., 2017; Spandel and Hesterman, 1997; Wang *et al*., 2021). There was a negative correlation between CP and NDF contents (Table 1), which was also reported by Avci *et al*. (2017). When indicated from this perspective, the Zhaodong is a good quality variety. The FD rating 1 varieties had greater nutritive value than less dormant varieties and that within FD rating 1 Zhaodong had better quality than Gongnong No. 2.

The three-year average of the ADF contents ranged from 30.79% to 32.84%, which is consistent to that reported elsewhere for alfalfa (Spandel and Hesterman, 1997; Avci *et al*., 2017; Albayrak *et al*., 2018; Wang *et al*., 2021). The ADF contents of Gongnong No. 2 and Zhaodong (FD rating 1) were significantly lower ($P<0.05$) than those of the other varieties. The lower the ADF value in alfalfa, the lower the content of cellulose and hemicellulose in the forage, so the palatability of Gongnong No. 2 and Zhaodong was good. The ADF content has a negative effect on digestibility and intake (Mader *et al*., 1991), suggesting that the nutritional values of Zhaodong and Gongnong No. 2 were better than those of the foreign varieties (i.e., Caribou, Gold Empress, Adrenalin, Sanditi, Sardi and WL525). This may be related to ecological factors, such as the relatively high evaporation and low precipitation (May and June) in Qiqihar and it is possible that the foreign varieties were affected by the arid climate conditions in this region (Rimi *et al*., 2012). Other studies indicate that the nutrient composition of alfalfa mainly depends on the variety and ecological factors (Karayilanli and Ayhan, 2016; Albayrak *et al*., 2018).

Digestible dry matter and relative feed value

Differences were observed in the DDM content among the varieties (Table 2). The DDM of Zhaodong was the greatest in 2019 and 2020. In 2021, the DDM values of Gongnong No. 2 (63.93%) and Zhaodong (64.42%) were significantly higher ($P<0.05$) than those of the other varieties. The three-year average of the DDM values of the varieties ranged from 63.32% to 64.91%. Zhaodong and Adrenalin had the highest and the lowest DDM values, which was mainly due to these alfalfa varieties having the greatest and least LSRs, respectively. As already noted, alfalfa leaves have relatively higher nutritive value and intake than stems. Julier and Huyghe (1997) also found that the digestibility of alfalfa varieties showed differences depending on their LSRs. The DDM value measured for the varieties in our study was generally similar to that reported in a previous research by Avci *et al*. (2017).

Significant differences were found in the RFVs among the alfalfa varieties (Table 2). The three-year average RFVs of the varieties ranged from 118.33 to 126.43. The highest and the lowest RFVs were recorded for Zhaodong and Adrenalin. This result suggested that the RFVs were significantly different among the alfalfa 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 DDM and DMI values of alfalfa (Avci *et al*., 2017). Correlation analysis showed that there was a significant negative correlation ($P<0.01$) between the RFV and the NDF, ADF contents (Table 1), indicating that the NDF and ADF contents have negative effect on quality and feed value of alfalfa. RFV of full bloom alfalfa variety has a value of 100 and the higher values indicate better quality relative to the base of 100 (Avci *et al*., 2017). Legume 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 aforementioned assessment, Zhaodong is classified as premium, while the other alfalfa varieties are classified as good. The RFV determined for the varieties in our research was similar to that found in a previous study by Wang *et al*. (2021).

CONCLUSION

The FD rating 1 had significant effect on alfalfa production performance, such as the DMY and nutrient composition and it was negatively correlated with the WSR. Gongnong No. 2 and Zhaodong making them more suitable for planting in the Qiqihar, Heilongjiang, China and may be suitable for similar upper-latitude semiarid environments in the world.

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