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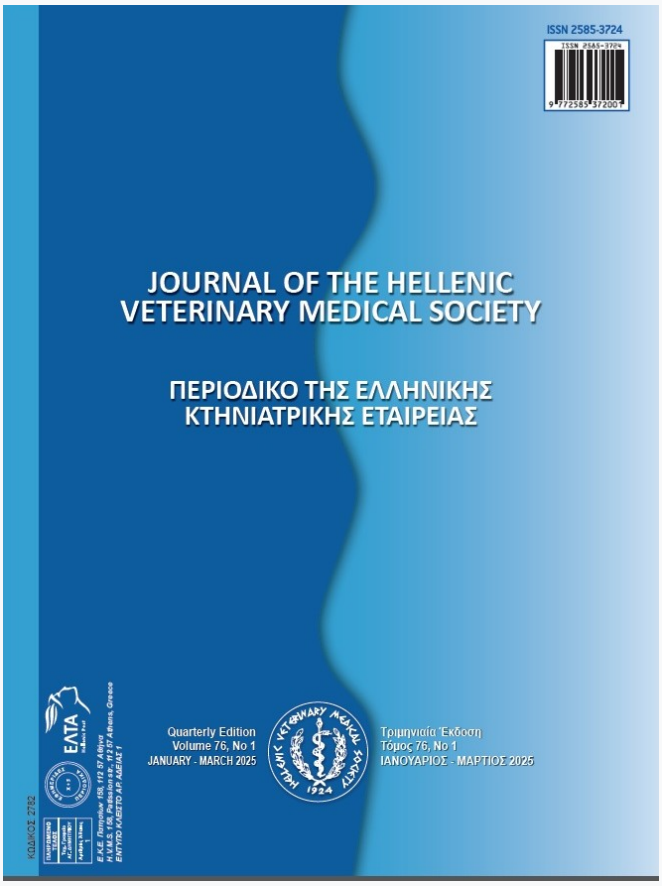

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The evaluation of nutrient compositions of alfalfa hay and corn silage used in dairy farms in Marmara Region, Türkiye

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ABSTRACT: The study aimed to demonstrate the nutritional variations of alfalfa hays and corn silages used in dairy farms in Marmara Region, Türkiye. In addition, the present study was conducted to evaluate the relative feed value (RFV) and total digestible nutrients (TDN) levels of these forages. Twenty diverse alfalfa hay and twenty-six diverse corn silage samples were taken from dairy farms (n=17) with a minimum of 50 lactating cows in Marmara Region throughout 12 months. The highest variations in nutrient composition for alfalfa hay samples were found for neutral detergent fiber (NDF) and acid detergent fiber (ADF) levels. The highest variations concerning the nutrient content of corn silage samples were observed for starch, NDF and ADF values. Correlations between RFV and TDN equations and RFV and crude protein (CP) were significant for alfalfa hay ($P<0.001$). Correlations between RFV and TDN equations and RFV and starch content were significant for corn silage ($P<0.001$). RFV calculation can be used as an indicator for evaluating the quality of alfalfa hay according to our results of the correlations between RFV and TDN equations and the RFV and CP content. In addition, RFV for corn silage can be considered with respect to the quality evaluation because of the significant correlations between RFV and TDN equations and RFV and starch content. The results obtained from our study will contribute to the database of nutrient compositions of alfalfa hay and corn silage.

Keywords: Alfalfa hay; corn silage; nutrient compositions

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INTRODUCTION

Forages are crucial for ruminant rations in point of providing energy, protein and minerals as well as fiber for rumination and rumen health (Ranjbar, 2007; Linn et al., 2014; Guo et al., 2022). In many ruminant rations, forages can contribute more than half of the total dry matter intake and are major sources of dietary neutral detergent fiber (NDF). High quality forages in respect to nutrient composition, digestibility and palatability can promote feed intake, ration digestibility and animal productivity and lead to more profits (Allen, 1996; Guo et al., 2022). Alfalfa hay (Radović et al., 2009; Farhang et al., 2010; Kahyani et al., 2019) and corn silage (Fernandez et al., 2004; Kahyani et al., 2019; Wang et al., 2023) are widely used as forage sources in dairy cattle diets. Alfalfa is the most cultivated legume forage worldwide and is used for dairy cattle nutrition in different forms. Alfalfa is stored as hay or silage for using in the dairy farms (Radović et al., 2009; Li and Brummer, 2012). Alfalfa is considered to be the most important forage crop for providing protein to dairy cattle (Radović et al., 2009; Blume et al., 2021). The previous data have shown that nutrient composition of alfalfa hay is greatly variable (Abaş et al., 2005; İnal et al., 2020; Zhang et al., 2023). The factors affecting nutrient composition of alfalfa hay are field conditions, irrigation, soil structure, fertilization, plant variety, growing stage at harvest, different cutting stages of vegetation, drying method, the duration of drying phase, storage conditions and the duration of storage (Linn et al., 2014; Blume et al., 2021; Filik and Ertürk, 2023). Corn silage is palatable forage that provides effective fiber, starch and moisture to the total mixed ration (Leonardi et al., 2005; Chávez et al., 2022). Most lactating dairy cow rations include high amount of corn silage as the main forage source (Khan et al., 2015; Zardin et al., 2017). In the previous data, it has been shown that nutrient content of corn silage is quite variable (Khan et al., 2015; Tharangani et al., 2021; Chávez et al., 2022). Nutrient composition of corn silage is dependent on field conditions, irrigation, soil structure, fertilization, plant variety, the stage of maturity at harvest, kernel processing, theoretical length of cut, cutting height, the use of silage additives, storage condition and the length of storage (Ferraretto et al., 2018; Tharangani et al., 2021; Özkan, 2024).

Different evaluation systems have been developed to predict the quality of forages given to ruminants (Moore and Undersander, 2002; Hackmann et al., 2008). Relative feed value (RFV) is the one of parameters used to describe forage quality. RFV is determined by neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of the forage. The NDF content is correlated with dry matter intake since it evaluates the bulkiness of the forage and the ADF content is closely related to digestibility of the forage. RFV is calculated by using dry matter intake and digestible dry matter values. Dry matter intake is calculated by using NDF and digestible dry matter is calculated by using ADF (Rohweder et al., 1978; Moore and Undersander, 2002; Ward, 2014). In addition, the term of forage quality includes nutritive value referring to total digestible nutrients (TDN) and nutrient contents. TDN equation used for estimating energy content of individual feedstuffs or diets is considered as an indicator in defining forage quality. Different equations based on research results have been used for calculating TDN of feeds (NRC, 2001; Owens et al., 2010).

The Marmara Region, located in the west of Türkiye and bordering Greece and Bulgaria, is one of the most important livestock production areas in Türkiye. The study aimed to demonstrate the nutritional variations of alfalfa hays and corn silages used in dairy farms in Marmara Region. In addition, the present study was conducted to evaluate relative feed value (RFV) and total digestible nutrients (TDN) levels of these forages.

MATERIALS AND METHODS

This study was supported by Bursa Uludag University in Bursa-Türkiye, located within 40° north latitude and 29° east longitude, The Unit of Scientific Research Projects (Project number: AYP(V)-2016/5). In this study, twenty diverse alfalfa hay and twenty-six diverse corn silage samples were taken from dairy farms (n=17) with a minimum of 50 lactating cows in Marmara Region, Türkiye throughout 12 months. Each forage sample, both alfalfa hay and corn silage, was collected in different periods of the year. Each alfalfa hay sample was collected from the center of five bales. Corn silage samples were taken from the center of the bunker silo at least 8 weeks after ensiling. Ensiling storage time of silages ranged 8-16 weeks.

Alfalfa hay samples were ground using a laboratory mill through a 1 mm screen for chemical analyses and then dried in an oven at 105 °C overnight. Corn silage samples were dried in a forced-air oven at 60 °C for 72 hours for the determination of the dry matter and ground using a laboratory mill through a

1 mm screen for chemical analyses. Nutrient analyses (crude protein, ether extract, starch and crude ash) of alfalfa hay and corn silage samples were performed according to AOAC (2003) and neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), NDF insoluble protein (NDFIP) and ADF insoluble protein (ADFIP) analyses were performed as described by Van Soest et al. (1991) and Licitra et al. (1996). Nutrient compositions of alfalfa hays and corn silages were analyzed in Department of Animal Nutrition and Nutritional Diseases, Bursa Uludag University Veterinary Faculty. TDN values (TDNnrc) of alfalfa hay and corn silage were calculated by NRC (2001) equation. In addition, other TDN calculations (TDN1 for alfalfa hay and TDN2 and TDN3 for corn silage) were performed according to NFTA (2014). TDNnrc, TDN1, TDN2, TDN3 and relative feed values (RFV) were calculated as follows:

TDNnrc = tdNFC + tdCP + (tdFA × 2.25) + tdNDF - 7

(td: total digestible, NFC: nonfiber carbohydrate, CP: crude protein, FA: FA represents the fatty acid fraction that is estimated as crude fat - 1, NDF: neutral detergent fiber)

TDN1 for alfalfa hay = 82.38 − 0.7515 × ADF

TDN2 for corn silage = 87.84 − 0.7 × ADF

TDN3 for corn silage = 31.4 + (53.1 × (0.94 − 0.008 × ADF))

(ADF: acid detergent fiber)

Relative feed value (RFV) = (DDM x DMI) / 1.29

(DDM: digestible dry matter = 88.9 - 0.779 x ADF), (DMI: dry matter intake as % of body weight = 120 / NDF)

The Pearson's correlation coefficients were calculated between RFV and TDN equations (TDNnrc and TDN1) and RFV and crude protein for alfalfa hay and between RFV and TDN equations (TDNnrc, TDN2 and TDN3) and RFV and starch content for corn silage. Statistical analyses were performed by using the SPSS (2021) package program. Correlations with $P<0.05$ were considered significant. The confidence level was 95% in all analyses.

RESULTS

In this study, seventeen dairy farms in Marmara Region were visited. These dairy farms had lactating cows between 50 and 800 and were chosen to represent an intensive dairy farming system. Lactating cows were fed total mixed rations in the dairy farms that alfalfa hay and corn silage samples were taken. The mean milk production ranged from 23.2 to 33.8 l/day in the dairy farms. 4 of 17, 2 of 17, 1 of 17, 4 of 17, 1 of 17, 1 of 17, 3 of 17 and 1 of 17 dairy farms were from Bursa, Sakarya, Kocaeli, Balıkesir, Edirne, Yalova, Kırklareli and Çanakkale, respectively.

Nutrient contents of alfalfa hays (n=20) and corn silages (n=26) were presented in Table 1 and Table 2 as minimum, maximum and mean values, respectively. Dry matter, crude ash, ether extract, crude protein, NDF, ADF and ADL levels of alfalfa hay samples were between 89.58 and 96.36%, 7.92 and 11.62%, 1.15 and 2.69%, 9.98 and 18.36%, 39.53 and 57.83%, 33.36 and 52.37%, 7.97 and 13.33%, respectively. Dry matter, crude ash, ether extract, crude protein, starch, NDF, ADF and ADL contents of corn silage samples were between 26.39 and 36.50%, 3.47 and 8.85%, 2.27 and 4.94%, 5.97 and 8.71%, 9.67 and 36.43%, 35.80 and 56.64%, 21.62 and 34.75%, 2.92 and 7.28%, respectively.

Relative feed values (RFV) and TDN values of

Table 1. Nutrient contents of alfalfa hay (n=20) on a dry matter basis.

Item	Minimum	Maximum	Mean	Standard error	Standard deviation
Dry matter (%)	89.58	96.36	93.43	0.40	1.82
Crude ash (%)	7.92	11.62	9.91	0.21	0.96
Ether extract (%)	1.15	2.69	1.89	0.09	0.43
Crude protein (%)	9.98	18.36	14.71	0.43	1.93
NDF (%)	39.53	57.83	47.26	1.12	5.02
ADF (%)	33.36	52.37	41.68	1.01	4.52
ADL (%)	7.97	13.33	10.58	0.40	1.80

NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin

alfalfa hays were given in Table 3 as minimum, maximum and mean values. In Table 4, RFV and TDN values of corn silages were presented as minimum, maximum and mean values. Results of correlation coefficients between RFV and TDN equations (TDNnrc and TDN1) and RFV and crude protein of alfalfa hay were given in Table 5. Correlations between RFV and TDN equations and RFV and crude protein were significant for alfalfa hay (P<0.001). Results of correlation coefficients between RFV and TDN equations (TDNnrc, TDN2 and TDN3) and RFV and starch content of corn silage were presented in Table 6. Correlations between RFV and TDN equations and RFV and starch content were significant for corn silage (P<0.001).

Table 2. Nutrient contents of corn silage (n=26) on a dry matter basis.

Item	Minimum	Maximum	Mean	Standard error	Standard deviation
Dry matter (%)	26.39	36.50	31.24	0.58	2.95
Crude ash (%)	3.47	8.85	5.65	0.25	1.28
Ether extract (%)	2.27	4.94	3.67	0.12	0.66
Crude protein (%)	5.97	8.71	7.06	0.11	0.57
Starch (%)	9.67	36.43	24.92	1.28	6.52
NDF (%)	35.80	56.64	44.38	1.09	5.59
ADF (%)	21.62	34.75	28.02	0.69	3.53
ADL (%)	2.92	7.28	5.31	0.19	0.99

NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin

Table 3. RFV and TDN values of alfalfa hays (n=20).

Item	Minimum	Maximum	Mean	Standard error	Standard deviation
RFV	77.38	147.01	112.84	4.10	18.35
TDNnrc	41.99	58.06	51.50	0.82	3.67
TDN1	43.02	57.31	51.05	0.76	3.40

RFV: relative feed value, TDN: total digestible nutrients, TDNnrc: TDN calculated by NRC (2001) equation, TDN1: TDN calculated according to NFTA (2014).

Table 4. RFV and TDN values of corn silages (n=26).

Item	Minimum	Maximum	Mean	Standard error	Standard deviation
RFV	101.55	185.03	143.25	4.45	22.68
TDNnrc	59.24	72.59	65.50	0.63	3.23
TDN2	63.52	72.71	68.22	0.49	2.47
TDN3	66.55	72.13	69.41	0.29	1.50

RFV: relative feed value, TDN: total digestible nutrients, TDNnrc: TDN calculated by NRC (2001) equation, TDN2 and TDN3: TDN calculated according to NFTA (2014).

Table 5. Correlation coefficients between RFV and TDN equations and RFV and CP content of alfalfa hay.

	TDNnrc	TDN1	CP	P-value
RFV	0.87	0.92	0.81	<0.001

RFV: relative feed value, TDN: total digestible nutrients, TDNnrc: TDN calculated by NRC (2001) equation, TDN1: TDN calculated according to NFTA (2014), CP: crude protein.

Table 6. Correlation coefficients between RFV and TDN equations and RFV and starch content of corn silage.

	TDNnrc	TDN2	TDN3	Starch	P-value
RFV	0.70	0.91	0.91	0.83	<0.001

RFV: relative feed value, TDN: total digestible nutrients, TDNnrc: TDN calculated by NRC (2001) equation, TDN2 and TDN3: TDN calculated according to NFTA (2014).

DISCUSSION

In the current study, nutrient contents of alfalfa hay samples collected from dairy farms varied at different levels. The highest variations in nutrient composition for alfalfa hay samples were found for NDF and ADF values. Similarly, nutrient compositions of corn silage samples collected from dairy farms were variable at different levels. The highest variations concerning nutrient composition of corn silage samples were observed for starch, NDF and ADF levels.

Alfalfa is the most valuable forage used as crude protein source in ruminant nutrition (Mirzaei-Aghsaghali et al., 2008; Radović et al., 2009; Blume et al., 2021). In our study, it was observed that different alfalfa hay samples collected from dairy farms had a wide variety with regard to CP content as well as NDF and ADF levels. The factors affecting nutrient composition of alfalfa hay are field conditions, irrigation, soil structure, fertilization, plant variety, growing stage at harvest, different cutting stages of vegetation, drying method, the duration of drying phase, storage conditions and the duration of storage (Linn et al., 2014; Blume et al., 2021; Filik and Ertürk, 2023). Minimum and maximum values of CP and crude ash for alfalfa hays in our study were in agreement with the results reported by Ünalp (2014), who analyzed nutrient compositions of alfalfa hays in different cutting and growth stages of vegetation in Aydın, Türkiye. We observed similar results for crude ash, ether extract and ADL contents of alfalfa hay samples compared with minimum and maximum values for these parameters of alfalfa hays produced in Kırıkkale, Türkiye (Güngör et al., 2008). In the study conducted by İnal et al. (2020), the ranges between minimum and maximum values of crude protein, ether extract, crude ash, NDF, ADF and ADL contents of alfalfa hay samples were wider than those found in our study, which was due to more number of samples in that study (İnal et al., 2020) compared with our study. Our result for mean crude protein level of alfalfa hay samples was lower than that reported by İnal et al. (2020) while mean values for NDF and ADL contents of alfalfa hay samples in our study were similar to those found in the study conducted by İnal et al. (2020). Minimum and maximum values of NDF and ADF for alfalfa hay samples in our study were higher than the results reported by Boğa and Ayaşan (2022), who analyzed different alfalfa varieties and lines at Eastern Mediterranean Agricultural Research Institute in Adana, Türkiye. Mean values for crude protein, ether extract and ADL contents of alfalfa hay samples in our study were similar to those found in the study conducted by Filik and Ertürk (2023), who analyzed different forage sources cultivated in different locations in the Western Mediterranean of Türkiye.

After harvesting, alfalfa is generally dried in the field. In the current study, all alfalfa hays collected from dairy farms had been dried in the field. The nutritional value of alfalfa hay is affected by different drying methods, the duration of drying phase and weather variations after harvesting (Farhang et al., 2010; Neres et al., 2010; Sengul et al., 2019). Especially, leaf loss during the drying process is associated with poorer quality for alfalfa hay in respect to nutrient content (Neres et al., 2010; İnal et al., 2020). In addition, alfalfa moisture content at the time of baling and storage duration of baled alfalfa hay affect nutritional quality by microbial activity and the subsequent generation of heat (Coblentz et al., 1996; Yuan et al., 2022). Aforementioned conditions are the one of main reasons of variations regarding chemical compositions of alfalfa hay samples taken from dairy farms in our study.

Corn silage is one of the main sources of forage used in the diets of dairy cows in many parts of the world (Khan et al., 2015; Zardin et al., 2017; Ferraretto et al., 2018) and quality corn silage is palatable forage that supplies digestible NDF, high level of starch and net energy and moisture to total mixed ration. These characteristics allow producers and nutritionists to prepare an economical and a proper total mixed ration (Leonardi et al., 2005; da Silva et al., 2015). The factors influencing nutrient composition of corn silage are field conditions, irrigation, soil structure, fertilization, plant variety, the stage of maturity at harvest, kernel processing, theoretical length of cut, cutting height, the use of silage additives, storage condition and the length of storage (Ferraretto and Shaver, 2012; Tharangani et al., 2021). According to NRC (2001), corn silage is typically composed of 25-35% starch and 40-50% NDF on a dry matter basis. Although similar results were observed for NDF level of corn silages in our study, starch contents of corn silage samples that we studied were in a wider range (9.67-36.43%) compared with NRC (2001). de Oliveira et al. (2017) reported that mean dry matter, crude ash, crude protein, NDF and starch contents of corn silage samples taken from the center of silo were 35.1%, 3.64%, 7.62%, 50.3% and 30.8%, respectively. Our result for mean crude protein level of corn silage was similar to that reported by de Oliveira et

al. (2017). Corn silages produced in warm climates tend to include higher levels of NDF and less starch in comparison to corn silages produced in temperate areas (Adesogan, 2010). Mean NDF content was greater in the study (de Oliveira et al., 2017) investigating the nutritive values of corn silages grown in Brazil compared with mean NDF level of corn silage in our study. On the other part, mean starch content of corn silage was lower in our study than the study conducted by de Oliveira et al. (2017), which may be related to a wide variety for starch level (9.67-36.43%) of corn silages in our study. While ADF levels of corn silages as minimum, maximum and mean values in our study were similar to those reported by Tharangani et al. (2021), who collected corn silage samples from bunker silos at least 10 weeks after ensiling, lignin contents of silage samples in the study conducted by Tharangani et al. (2021) were lower than those found in our study. Lower lignin levels for corn silage can be obtained by harvesting early in the grain-filling period and / or hybrids modified for lower lignin content (Khan et al., 2015; Chávez et al., 2022). In a previous study (Akdeniz, 2016), corn plant was harvested at 1/4 or 1/2 milk line maturity period and whole plant corn was ensiled for 90 days. Lignin contents (3.36 and 3.39% for 1/4 and 1/2 milk line maturity, respectively) of corn silage samples collected 90 days after ensiling in this study (Akdeniz, 2016) were lower than mean lignin level (5.31%) in our study. Our result for mean crude protein level of corn silage samples was similar to those reported by Akdeniz (2016). Mean NDF, ADF and crude ash values of corn silage samples in our study were similar to those of corn silages harvested at 1/2 milk line maturity period in the study conducted by Akdeniz (2016) but these results in our study were higher than those of silage samples harvested at 1/4 milk line maturity period (Akdeniz, 2016). Partial differences in respect to mean NDF, ADF and crude ash levels of silages between our study and the study mentioned above (Akdeniz, 2016) were due to great variations for NDF, ADF and crude ash contents of silage samples in our study.

Relative feed value (RFV) has been used for years to represent the quality of legume and grass hays and silages (Jeranyama and Garcia, 2004; Hackmann et al., 2008; Kilic, 2010; Li et al., 2022; Filik and Ertürk, 2023). RFV is estimated from NDF and ADF contents of the forage (Rohweder et al., 1978; Moore and Undersander, 2002; Ward, 2014). Therefore, since wide variations were observed in NDF and ADF contents of both alfalfa hay and corn silage samples, RFV were also greatly variable as minimum and maximum values in our study. The RFV is an index used to rank forages relative to nutritive value of full bloom alfalfa hay including 41% ADF and 53% NDF on a dry matter basis and the RFV of full bloom alfalfa hay has a value of 100, which is considered to be a standard score (Rohweder et al., 1978; Moore and Undersander, 2002; Jeranyama and Garcia, 2004). In the current study, RFV was lower than 100 for 5 of 20 alfalfa hay samples and greater than 100 for 15 of 20 alfalfa hay samples. Alfalfa hay is the most important legume forage used as a protein source in the cattle diets (Radović et al., 2009; Blume et al., 2021). In our study, a high correlation was found between RFV and crude protein content of alfalfa hay (r=0.81). Therefore, an idea in respect to crude protein level of alfalfa hay can be obtained by evaluating RFV, although the protein content of the forage is not included in this calculation while RFV is calculated. Consequently, RFV can be used for determining alfalfa hay quality and price.

RFV has been used as an indicator for determining quality of silages in some studies (Kilic, 2010; Kasra, 2014; Sarubbi et al; 2014; Li et al., 2022). In the study conducted by Li et al. (2022), RFV for corn silage samples was calculated between 112.76 and 148.44. Our minimum RFV was lower and our maximum RFV was higher for corn silage samples compared to values found by Li et al. (2022). Minimum and maximum RFV for corn silage samples in our study were higher than the results (74 and 121 as minimum and maximum values) reported by Kasra (2014). Corn silage is the forage used widely as an energy source in the diets of dairy cattle (Ferraretto et al., 2018; Chávez et al., 2022). This energy mainly originates from the starch in the kernel fraction (Jensen et al., 2005; Khan et al., 2015), which is considered as an important characteristic for corn silage (Khan et al., 2015; Ferraretto et al., 2018; Chávez et al., 2022). Since the correlation between RFV and starch content was found significant for corn silage (r=0.83) in our study, RFV can be used with regard to evaluating energy level of corn silage.

Total digestible nutrients (TDN) is an indicator used for estimating energy content of individual feedstuffs and diets (NRC, 2001; Owens et al; 2010). Different equations have been used for calculating TDN of feedstuffs (Owens et al; 2010). In the current study, two and three TDN equations were evaluated for alfalfa hay (TDNnrc and TDN1) and corn silage (TDNnrc, TDN2 and TDN3), respectively. TDNnrc was calcu-

lated by NRC (2001) equation. TDN1 calculation for alfalfa hay and TDN2 and TDN3 calculations for corn silage were performed according to NFTA (2014). In our study, the significant correlations were observed between RFV and all TDN equations for both alfalfa hay and corn silage. The highest correlation for alfalfa hay samples was found between RFV and TDN1 and the highest correlations for corn silage samples were observed between RFV and TDN2 and TDN3 equations, which were due to the fact that TDN1, TDN2 and TDN3 equations are calculated from ADF. Because of the significant correlations found between RFV and TDN equations, RFV may be used as an indicator for evaluating energy content of alfalfa hay and corn silage.

Due to a wide variety in nutrient compositions and energy levels of alfalfa hay (Abaş et al., 2005; İnal et al., 2020; Blume et al., 2021) and corn silage (Khan et al., 2015; Zardin et al., 2017; Tharangani et al., 2021) as the most common forages included in dairy cattle diets, the analyses of nutrient contents for alfalfa hay and corn silage used in the dairy farms are required to prepare the realistic and balanced diet formulations that meet nutrient requirements of animals. In addition, determinations of nutrient composition and RFV calculations of forages are crucial for evaluating quality and / or pricing (Jeranyama and Garcia, 2004; İnal et al., 2020). On the other part, storage conditions, the duration of storage and the physical appearance (color, integrity and molding) of forages (Coblentz et al., 1996; İnal et al., 2020; Yuan et al., 2022) as well as pH value, volatile fatty acids content, ammonia level, chop length and mechanical kernel processing for corn silage (Khan et al., 2015; Tharangani et al., 2021) should be considered regarding nutrient composition, digestibility, dry matter intake and animal health in the dairy farms.

CONCLUSIONS

In an overall conclusion, because of a wide variety in nutrient compositions and energy level of alfalfa hays and corn silages, the analyses of nutrient contents for alfalfa hays and corn silages used in the dairy farms are required to prepare the realistic diet formulations that meet nutrient requirements. RFV calculation can be used as an indicator for evaluating quality of alfalfa hay according to our results of the correlations between RFV and TDN equations and RFV and crude protein content. In addition, RFV for corn silage can be considered with respect to the quality evaluation because of the significant correlations between RFV and TDN equations and RFV and starch content. The results obtained from our study will contribute to the database of nutrient compositions of alfalfa hay and corn silage.

CONFLICT OF INTEREST

There is not conflict of interest with any person or institute/organization regarding this manuscript.

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