



Determining The Feed Value, Feed Quality, and *in Vitro* Fermentation Properties of Italian Ryegrass (*Lolium multiflorum* L.) Harvested in Different Periods

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ABSTRACT

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The purpose of this study was to determine the chemical content, *in vitro* gas and methane production, relative feed value (RFV), relative feed quality (RFQ), *in vitro* digestibility parameters, and microbial protein production of the Italian ryegrass plants harvested in different periods of the 2020 production season in Erzincan. In this study an ANKOM Daisy Incubator was used to determine the *in vitro* digestibility parameters. *In vitro* gas production technique was used to determine gas production and predicted parameters. It was found that there were significant differences between the Italian ryegrass plants harvested in different periods in terms of composition, RFV, RFQ, and *in vitro* digestibility and fermentation parameters. The RFV, metabolizable energy (ME), net energy (NE), true NDF digestibility (TNDFD), organic matter digestibility degree (OMDD), total digestible nutrient (TDN), dry matter intake (DMI), and RFQ values of the Italian ryegrass plants were found to be 247.32, 9.13, 5.68, 62.26%, 54.15%, 55.35%, 4.82%, and 215.81, respectively. On the other hand, after 24 hours of incubation it's *in vitro* gas (GP), and methane production (ml and %), true dry matter digestibility (TDMD) values, partitioning factor (PF), microbial protein (MP), microbial protein synthesis efficiency (MPSE), and true digestibility (TD) were found to be 105.41, 17.35, 16.42, 281.72, 2.68, 57.68, 20.32, and 59.82, respectively. In conclusion, determining the digestibility of plants via the measurement of RFV, RFQ, digestion parameters, and gas production in different harvest periods provided insights into the potential of Italian ryegrass as a feed material.

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Introduction

Today, in order to eliminate the deficit of quality roughage for ruminants, cultivation of ryegrass as a forage crop is becoming widespread thanks to its high content of water-soluble carbohydrates. Ryegrass is rich in some vitamins and minerals (Bernard et al., 2002; Humphreys et al., 2006). It is an annual forage plant that can be reaped more than once in a season and produce high quality forage when harvested in the flowering period (Soya et al., 1997). While it is called “*sütotu*”, literally meaning “milkgrass”, in Turkey because it increases milk production, its variety Caramba has other names such as Trinova (shorter version of caramba), annual grass, and Italian grass in some regions (Çetinkaya, 2019).

In addition to *in vivo* studies, chemical analyzes are recommended in determining the feed quality (Rivera and Parish, 2010). Relative feed value (RFV) has been the first parameter used to determine the quality. RFV is calculated

using digestible dry matter (DDM) and dry matter intake (DMI). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) values are also used in calculations (Boman, 2003). The scale developed by Rohweder et al. (1978) is used to determine the feed value. While ADF and NDF values are used to determine RFV (Newman et al., 2006); total digestible nutrients (TDN), *in vitro* fiber digestibility estimates, and dry matter intake (DMI) [instead of digestible dry matter (DDM)] are used to determine RFQ. Therefore, it is recommended to use RFQ rather than RFV in determining feed production potential. (Ward and Ondarza, 2008). RFQ has been used to estimate the voluntary intake of available energy in case of feed being the sole source of energy and protein. It has been included in the RFV system as an alternative quality index (Moore and Undersander, 2002a and 2002b).

In vitro gas production technique is used to determine the energy digestibility of feeds. This technique is also used to determine methane production, microbial production, and true digestibility in feeds (Vercoe et al., 2010; Lin et al., 2013; Thang et al., 2012; Ayaşan et al., 2021a; Ayaşan et al., 2021b). Methane, the most rapid greenhouse gas after carbon dioxide, is produced in significant amounts by ruminant animals (an adult the amount of methane formed in the rumen of the cattle is 300 liters / day is around), causing global warming (Carlin, 2006). Another disadvantage associated with methane production is that about 2% to 12% of the digestible energy intake by ruminants is lost via enteric methane production (Johnson and Johnson, 1995). Microbial synthesis (Leng, 1993), an important process for ruminants, is used to determine the performance of animals (NRC, 2001). In previous studies, feeds were evaluated by taking into account the amount of gas production and using fewer parameters. However, in recent studies, feeds were evaluated by taking into account not only the amount of gas, but also the microbial production and true digestibility (Blümmel and Lebzien, 2001; Cengiz and Kamalak, 2020). This evaluation makes it possible to make more accurate decisions in feeds preferences.

The purpose of this study was to determine the chemical composition, relative feed value (RFV), relative feed quality (RFQ), *in vitro* digestibility parameters, gas production potential, methane production, true digestibility, and microbial protein production of the Italian ryegrass plants harvested in different periods.

Material and Method

Plant Material

This study was carried out in a field in Erzincan in the 2020 vegetation period. Ryegrass (*Lolium multiflorum L.*, genotype: Devis) procured from a private seed company was used as the plant material of the study.

The information on the temperature, precipitation, and humidity of the Province of Erzincan was obtained from the Directorate General of Meteorology (Table 1). In the study period, the lowest temperature was observed in February, while the highest was in June. Precipitation was low and the highest humidity was observed in February. Precipitation was low in 2020 compared to the previous years. We think that this extreme situation was caused by climate change due to global warming (Kibar et al., 2014).

The field was left fallow for 1 year before planting, and then plowed, raked, and leveled using a harrow. The seeds were sown using a sowing machine (15 kg of seeds per decare) in early spring (March 05, 2020). Diammonium phosphate (15 kg per hectare) was applied along with the sowing. Moreover, 15 kg of urea per decare was applied to the field on April 28, 2020. The grasses were reaped twice due to the low precipitation and early sowing (Annual grass seeds should be sown in autumn in the climate of Erzincan). In the first reaping, the plants were harvested three times on: May 16, 2020 (before flowering), May 28, 2020 (during flowering), and June 15, 2020 (after flowering). Also in the second reaping, they were harvested three times on: June 15,

2020 (before flowering), June 01, 2020 (during flowering), and July 15, 2020 (after flowering). A buffer zone of 0.5 m between the boundaries of the plots was not harvested to eliminate the edge effect. In both reaping, flood irrigation was provided after harvesting in the flowering period.

Determining the Nutrient Contents

The analyzes of dry matter (DM), crude protein (CP), and crude ash (CA) were carried out as specified by AOAC (1998), and the ether extract (EE) analysis was carried out in line with AOCS Am 5-04 using AnkomXT15 extraction system. The acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude fiber (CF) analyses were carried out using an ANKOM2000 Fiber Analyzer (Ankom Technology, Macedon NY), and the acid detergent lignin (ADL) was determined using the method reported by Van Soest et al. (1991).

Determining the Relative Feed Value, *in Vitro* Digestibility, and Relative Feed Quality

Relative feed value was calculated using the following equation (Van Dyke and Anderson, 2000).

$$\begin{aligned} \text{RFV} &= \text{DMD}\% \times \text{DMI}\% \times 0.775 \\ \text{\%DMD} &= 88.9 - (0.779 \times \text{ADF}\%) \\ \text{DMI}\% &= 120 / \text{NDF} \end{aligned}$$

In vitro digestibility parameters were determined using an ANKOM Daisy incubator, and the buffer solutions were prepared in line with the recommendations for the Ankom Daisy *in vitro* fermentation system. The rumen content was taken from 2 mature female cattle brought to be slaughtered at the Meat and Fish Institution operating in Erzurum Province. The rumens of the animals were opened about 5 minutes after the slaughter and the rumen fluid taken put into a thermos that already contained CO₂ at 39°C. Then, the sample was brought to Atatürk University, Faculty of Agriculture, Animal Science Department, Feed Laboratory as soon as possible, and filtered with four layers of gauze in a CO₂ environment at 39°C. The pH value of the rumen fluid was 6.35.

The buffer solutions with the amounts specified in the procedure were mixed homogeneously in a 2-liter flask until reaching 39°C. 1600 ml of buffer solution mixture was filled into each digestion unit of the incubator. Then, the digestion units were placed in the incubator, 400 ml of rumen fluid was added to the buffer solution in each digestion unit at 39°C, and the prepared bags were placed in the ANKOM Daisy incubator. CO₂ gas was added to the digestion units to maintain the anaerobic environment, and then the mixture was incubated for 48 hours. After the incubation, the incubation medium in the digestion units was removed by pouring. The bags were washed under running tap water until being cleaned completely and taken to the ANKOM fiber analyzer (Kılıç and Abdiwali, 2016). Then, NDF procedure was applied. The OMDD, TNDFD, DMI, and TDN of the samples were calculated based on the difference between the amount incubated initially and the amount after the NDF procedure.

The relative feed quality was calculated using the following equation:

$$\text{RFQ (Relative feed quality)} = (\text{DMI, DM\%}) * (\text{TDN, DM\%}) / 1.23$$

(Ward and Ondarza, 2008).

$$\text{TDN} = (\text{NFC} \times 0.98) + (\text{CP} \times 0.93) + (\text{FA} \times 0.97 \times 2.25) + \text{NDFn} \times \text{NDFD} / 100 - 10$$

$$\text{FA} = \text{Ether extract} - 1$$

$$\text{NDFn} = \text{NDF} \times 0.93$$

$$\text{NDFD} = 48 \text{ hours in vitro NDF digestibility}$$

Metabolizable energy (ME) and net energy lactation (NEL) of the feed raw materials were calculated using the equation reported by Menke and Steingass (1988).

$$\text{ME, MJ/kg DM} = 2.20 + 0.1357 \times \text{GP} + 0.057 \times \text{CP} + 0.002859 \times \text{EE}$$

$$\text{NEL (MJ/kg DM)} = 0.101 \times \text{GP} + 0.051 \times \text{CP} + 0.112 \times \text{EE}$$

(GP: Net gas production from 200 mg dry feed sample at the end of a 24-hour-incubation, CP: % Crude protein, EE: % Ether extract; CA: % Crude ash).

Determining In Vitro Gas Production and True Digestibility

About 0.2 grams of the feed samples were incubated with 30 ml of solution (10 ml of rumen fluid + 20 ml of artificial saliva) in 100 ml glass syringes at 39 °C for 24 hours (Menke et al., 1979). The amount of methane (%) was determined using an Infrared Methane Analyzer (Sensors Europe GmbH, Erkrath, Germany) after a 24-hour-fermentation (Goel et al., 2008).

Then, the residual materials remaining in the syringes were transferred to a beaker, 50 ml of NDF solution was added into it, and the mix was left to boil for one hour. The filtration was carried out using a crucible (Por.2). True dry matter digestibility, partitioning factor, microbial protein production, and synthesis efficiency were calculated using the method reported by Blümmel et al. (1997).

$$\text{TDMD (mg)} = \text{Incubated DM (mg)} - \text{Remaining DM (mg)}$$

$$\text{TD (\%)} = (\text{TDMD} / \text{Incubated DM}) \times 100$$

$$\text{Partitioning Factor (PF)} = \text{TDMD} / \text{GP}$$

$$\text{Microbial Protein (MP) (mg/g DM)} = \text{TDMD} - (\text{GP} \times 2.2 \text{ mg/ml}),$$

$$\text{Microbial Protein Synthesis Efficiency (MPSE)} = (\text{TDMD} / (\text{GP} \times 2.2 \text{ mg/ml})) / \text{TDMD}.$$

Statistical Analysis

The data was analyzed using the analysis of variance, and the differences between the means were determined using Duncan multiple comparison tests (SPSS, 24).

Results and Discussion

Effect of Different Harvest Periods on The Composition of Ryegrass

It was found that different harvest periods significantly affected the composition of the Ryegrass plants ($P < 0.01$) (Table 2). The mean DM, CA, EE, CP, CF, NDF, ADF, and ADL were found to be 93.29%, 10.63%, 2.67%, 27.48%, 17.82%, 55.93%, 28.57%, and 13.43%, respectively. In different studies, the DM, CA, CF, CP, NDF, and ADF contents of dried ryegrass were reported to be within the ranges of 90%, 7.7-13.2%, 2-2.5%, 7.5-24%, 44-65%, and 23-47%, respectively (Çetinkaya, 2019).

The DM contents were found to range between 92.3 % and 94.05 %. The highest DM content was found to be in the 2nd reaping before the flowering period, while the lowest DM content was in the 1st reaping before the flowering period. As the harvest time was delayed, the DM content increased in the 1st reaping, but decreased in the 2nd reaping. The CA contents were found to range between 8.62 % and 13.2%. The lowest CA content was found to be in the 1st reaping after the flowering period, while the highest CA content was in the 2nd reaping before the flowering period. As the harvest time was delayed, the CA content decreased in both the 1st and 2nd reaping. The delay in harvest time leads to maturation, which in return causes an increase in the cellulose content, and this makes the stem coarser and decreases the mineral substance content in the plants. Therefore, the crude ash content decreases with the delay in harvest time (Tenikecier et al., 2020). The EE contents were found to range between 1.86 % and 4.39 % with the lowest in the 2nd reaping in the full flowering period and the highest in the 1st reaping before the flowering period. The CP contents were found to range between 22.06 % and 35.01 % with the lowest in the 2nd reaping after the flowering period and the highest in the 1st reaping before the flowering period. As the harvest time was delayed, the CP content decreased in the 2nd reaping. The CC contents were found to range between 13.44 % and 20.71 %. The lowest CF content was found to be in the 1st reaping before the flowering period, while the highest CC content in the 2nd reaping in the full flowering period. The NDF, ADF, and ADL contents were found to be within the ranges of 49.53% - 61.44%, 20.26% - 34.23%, and 8.95% - 20.91%, respectively. The lowest cell wall contents were found to be in the 1st reaping before the flowering period, while the highest in the 2nd reaping after the flowering period. It was found that the cell wall contents increased as the harvest time was delayed.

The mean DM and EE contents found in the present study were similar to, but the CA and CF contents were lower than those reported in previous studies (Goktepe, 2015; Cetinkaya, 2019). The CP contents found in the present study were similar to those reported in some studies (Teutsch and Smith, 2001; Simic et al., 2009), but higher than those reported in some studies (Seker, 1992; Szyszkowska and Sowinski, 2001; Kallenbach et al., 2003; Meeske et al., 2009; Goktepe, 2015; Cetinkaya, 2019). We are of the opinion that the reason why the CP contents were higher than those reported in other studies is due to the differences in the types and doses of the fertilizers applied in different studies.

Table 1. The temperature, precipitation, and humidity in Erzurum in 2020 by month

Months	Temperature (°C)	Precipitation (mm)	Humidity (%)
January	0.28	0.50	57.76
February	0.08	1.37	63.35
March	8.18	1.78	55.27
April	13.6	0.89	46.12
May	15.92	1.94	47.25
June	26.66	0.12	40.52
July	25.67	0.01	34.63

Table 2. Chemical composition of the ryegrass plants harvested in different periods (% DM)

Parameters	1. Reaping			2. Reaping			Mean	SEM	S L
	BF	FF	AF	BF	FF	AF			
DM	92.13 ^e	93.19 ^c	93.67 ^b	94.05 ^a	93.78 ^b	92.93 ^d	93.29	0.156	**
CA	11.57 ^b	11.36 ^b	8.62 ^e	13.20 ^a	9.76 ^c	9.29 ^d	10.63	0.380	**
EE	4.39 ^a	2.97 ^b	1.92 ^{cd}	2.70 ^{bc}	1.86 ^d	2.21 ^{bcd}	2.67	0.218	**
CP	35.01 ^a	23.54 ^{bc}	25.17 ^b	34.13 ^a	24.97 ^b	22.06 ^c	27.48	1.250	**
CF	13.44 ^c	17.58 ^{ab}	19.04 ^{ab}	20.21 ^a	20.71 ^a	15.91 ^{bc}	17.82	0.666	**
NDF	49.53 ^c	49.64 ^c	59.60 ^a	54.09 ^b	61.44 ^a	61.29 ^a	55.93	1.273	**
ADF	20.26 ^f	25.79 ^e	30.57 ^c	28.10 ^d	32.45 ^b	34.23 ^a	28.57	1.124	**
ADL	8.95 ^b	10.35 ^b	14.36 ^{ab}	10.62 ^b	15.42 ^{ab}	20.91 ^a	13.43	1.102	**

a, b, c, d, e: The means with a different symbol in the same column are different from each other. **: P<0.01, BF: Before Flowering, FF: Full Flowering, AF: After Flowering, SEM: Standard error of mean, SL: Significance Level, DM: Dry Matter, CA: Crude ash, EE: Ether extract, CP: Crude protein, CF: Crude Fiber, NDF: Neutral detergent fiber, ADF: Acid Detergent Fiber, ADL: Acid Detergent Lignin.

Table 3. The RFV, energy, and *in vitro* digestibility of the ryegrass plants harvested in different harvest periods (DM)

Parameters	1. Reaping			2. Reaping			Mean	SEM	SL
	BF	FF	AF	BF	FF	AF			
RFV	188.74 ^c	289.99 ^a	266.76 ^b	198.69 ^c	267.46 ^b	272.31 ^b	247.32	9.463	**
ME (Mj/kg)	9.86 ^a	9.61 ^{ab}	8.40 ^d	9.16 ^c	8.39 ^d	9.34 ^{bc}	9.13	0.138	**
NE _L (Mj/kg)	6.46 ^a	6.03 ^b	5.04 ^d	5.76 ^c	5.02 ^d	5.75 ^c	5.68	0.126	**
TNDFD (%)	67.76 ^a	67.35 ^a	56.74 ^d	61.85 ^b	59.78 ^c	60.10 ^c	62.26	0.986	**
OMDD (%)	56.35 ^b	58.37 ^a	50.23 ^d	52.60 ^c	50.29 ^d	57.06 ^{ab}	54.15	0.804	**
TDN	62.03 ^a	61.47 ^a	51.00 ^c	52.73 ^b	51.66 ^{bc}	53.19 ^b	55.35	1.119	**
DMI	3.33 ^c	5.44 ^{ab}	5.29 ^b	3.83 ^c	5.42 ^{ab}	5.64 ^a	4.82	0.219	**
RFQ	167.96 ^d	271.78 ^a	219.23 ^c	164.00 ^d	227.77 ^c	244.09 ^b	215.81	9.505	**

a, b, c, d: The means with a different symbol in the same column are different from each other. **: P<0.01, BF: Before Flowering, FF: Full Flowering, AF: After Flowering, SEM: Standard error of mean, SL: Significance Level, RFV: Relative Feed Value, ME: Metabolizable energy, NE_L: Net energy lactation, TNDFD: True NDF digestibility, OMDD: Organic matter digestibility degree, TDN: Total digestible nutrient, DMI: Dry Matter Intake, RFQ: Relative Feed Quality.

Table 4. Gas production, microbial protein production, and true digestibility of the ryegrass plants harvested in different periods

Parameters	1. Reaping			2. Reaping			Mean	SEM	SL
	BF	FF	AF	BF	FF	AF			
GP (ml)	110.76 ^b	119.38 ^a	93.85 ^c	98.68 ^c	93.85 ^c	115.93 ^{ab}	105.41	2.561	**
Methane (ml)	18.11 ^{bc}	19.29 ^{ab}	15.44 ^d	16.06 ^{cd}	14.45 ^d	20.77 ^a	17.35	0.569	**
Methane (%)	16.35 ^b	16.15 ^b	16.45 ^b	16.28 ^b	15.41 ^b	17.91 ^a	16.42	0.242	*
TDMD (mg)	314.73 ^a	317.50 ^a	244.03 ^d	278.91 ^b	264.42 ^c	270.74 ^{bc}	281.72	6.483	**
PF (mg/ml)	2.84 ^a	2.66 ^b	2.60 ^b	2.83 ^a	2.82 ^a	2.34 ^c	2.68	0.044	**
MP (mg)	79.32 ^a	63.75 ^b	44.56 ^d	69.17 ^b	64.94 ^b	24.33 ^d	57.68	4.423	**
MPSE (%)	25.20 ^a	20.10 ^b	18.26 ^b	24.80 ^a	24.56 ^a	8.99 ^c	20.32	1.391	**
TD (%)	67.78 ^a	67.25 ^a	51.59 ^d	58.88 ^b	55.87 ^c	57.54 ^{bc}	59.82	1.439	**

a, b, c, d: The means with a different symbol in the same column are different from each other. *: P<0.05, **: P<0.01, BF: Before Flowering, FF: Full Flowering, AF: After Flowering, SEM: Standard error of mean, SL: Significance Level, GP: Net gas production, TDMD: True dry matter digestibility (mg), PF: Partitioning Factor, MP: Microbial Protein (mg), MPSE: Microbial Protein Synthesis Efficiency (%), TD: True Digestibility (%).

Özdemir (2017) reported that the CP content of ryegrass increased with the increase in the amount of nitrogen applied as fertilizer. The mean ADF content was similar to, but the NDF and ADL contents were higher than those reported in previous studies (Teutsch and Smith, 2001; Kallenbach et al., 2003; Goktepe, 2015; Cetinkaya, 2019).

The Effect of Different Harvest Periods on RFV, Energy, and *In Vitro* Digestibility of Ryegrass

It was found that different harvest periods significantly affected the RFV, energy, and *in vitro* digestibility of ryegrass plants (P<0.01) (Table 3). The mean RFV, ME, NE_L, TNDFD, OMDD, TDN, DMI, and RFQ were found to be 247.32, 9.13 Mj/kg (DM), 5.68 Mj/kg (DM), 62.26%, 54.15%, 55.35, 4.82, and 215.81, respectively.

The RFVs were found to range between 188.74 and 289.99. The lowest RFV was found to be in the 1st reaping before the flowering period, while the highest in the 1st reaping in the full flowering period. The ME and NE_L values were found to range from 8.39 to 9.86 Mj/kg (DM) and from 5.02 to 6.46 Mj/kg (DM), respectively. The lowest ME and NE_L values were found to be in the 2nd reaping in the full flowering period, while the highest in the 1st reaping before the flowering period. Harvest time affects nutrient content and digestibility positively or negatively. The plant is highly productive when the plant is young and at the beginning of flowering period. However, if the harvest time is past and the woody parts increase in size, this negatively affects the nutrient content (Çetinkaya, 2019). The TNDFD values were within the range of 56.74% - 67.76%. The lowest TNDFD value was found to be in the 1st reaping after the flowering period, while the highest in the 1st reaping before the flowering period. On the other hand, the OMDD values were found to be within the range of 50.23% - 58.37% with the lowest in the 1st reaping after the flowering period and the highest in the 1st reaping in the full flowering period. The TDN values were found to be within the range of 51.00% - 62.03% with the lowest in the 1st reaping after the flowering period and the highest in the 1st reaping in the full flowering period. The DMI values were found to range from 3.33% to 5.64% with the highest in the 2nd reaping after the flowering period, while the lowest in the 1st reaping before the flowering period. The RFQs were found to range between 164.00 and 271.78. The highest RFQ was observed in the 1st reaping in the full flowering period. When all these values are considered together, the highest values were observed in the 2nd reaping before the flowering period and in the full flowering period. Although the RFVs calculated based on NDF and ADF were much higher than the RFQ values calculated based on TND and DMI, the highest values were observed in the same reaping and periods.

The RFV, ME, NE_L, and OMDD values of the feeds harvested in different periods were found to be higher than those reported in previous studies (Goktepe, 2015; Kılıç et al., 2015; Cetinkaya, 2019), while the RFV, ME, OMDD, and TNDFD values were higher than those reported in previous studies (Goktepe, 2015; Fluck et al., 2018; Vargas et al., 2018; Alende et al., 2020; Lale, 2020). We are of the opinion that this difference is due to the differences between the present study and previous studies in terms of plant variety, fertilizer type and dose, soil, and climate.

Gas Production, Microbial Protein Production, and True Digestibility of the Ryegrass Plants in Different Harvest Periods

Different harvest periods significantly affected the gas production, microbial protein production, and true digestibility of the ryegrass ($P < 0.05$, $P < 0.01$) (Table 4). The *in vitro* GP and, methane production (ml or %), TDMD, PF, MPSE, and TD were found to be 105.41, 17.35, 16.42, 281.72, 54.15, 55.35, 4.82, and 59.82, respectively.

The amount of gas, which is related with the amount of fermentable carbohydrates, was found to vary between 93.85 and 119.38 ml. The lowest gas amount was observed in the 1st reaping after the flowering period and in the 2nd

reaping in the full flowering period, the highest gas amount in the 1st reaping in the full flowering period. The amount of methane, another gas released as a result of fermentation, was found to be within the range of 14.45-20.77 ml or 15.41%-17.91%. The lowest methane production was observed in the 2nd reaping in the full flowering period, the highest in the 2nd reaping after the flowering period. The TDMD values of the feeds were found to range between 244.02 and 317.50 mg.

The lowest and the highest TDMD values were observed in the 1st reaping after the flowering period and in the full flowering period, respectively. PF, the most important factor determining the microbial protein synthesis efficiency of feeds, was reported to be between 2.75 and 4.41 in the literature (Blümmel and Lebzien, 2001).

In the present study, the PF were found to range between 2.34 and 2.84. The highest PF value was observed to be in the 1st reaping before the flowering period. The MP and MPSE values of the feeds were found to be within the ranges of 24.33-79.32 and 8.99-25.20, respectively. Moreover, it was found that the MP and MPSE values were high in the periods in which the PF was high, and vice versa. The TD values, which determine the amount of fermentable nutrients in the feed, varied between 51.59 and 67.78. The lowest and the highest TD values were observed in the 1st reaping after and before the flowering period, respectively.

The *in vitro* gas and, methane production, TDMD, and TD values of the feeds harvested in different periods were found to be higher than those reported in some studies (Goktepe, 2015; Fluck et al., 2018; Vargas et al., 2018) and lower than those reported in some others (Wang et al., 2020; Fluck et al., 2018; Alende et al., 2020). In their 24-hour *in vitro* study on a perennial grass, Riverro et al. (2020) reported a similar MPSE and higher GP and, methane production, PF values compared those found in the present study. We are of the opinion that this difference is due to the differences between the present study and previous studies in terms of plant species and variety, fertilizer type and dose, soil, and climate.

Conclusion

In this study, it was found that there were significant differences between the Italian ryegrass plants harvested in different periods in terms of chemical composition, RFV, RFQ, and *in vitro* digestibility and fermentation parameters. In general, the parameters desired to be high in both the 1st reaping and the 2nd reaping were found to be high after the flowering period, and the parameters that negatively affect the quality were observed to increase as the plant matured. In conclusion, determining the digestibility of the plants via the measurement of RFV, RFQ, digestion parameters, and gas production in different harvest periods provides insights into the potential of the plant as a feed material.

Conflict of Interest

The authors declare that they have no conflict of interest.

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