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Effects of different additions to Italian ryegrass (*Lolium multiflorum* Lam.) on silage quality

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Abstract

This research was carried out to determine the effects of different additions (urea and molasses) used with Italian ryegrass (*Lolium multiflorum* L.) silage on fermentation, *in vitro* gas production, microbiological properties, *in vitro* digestibility parameters, and relative fodder quality (RFQ) in silages made under laboratory conditions. The Italian grass (*Lolium multiflorum* L.) used in the study was chopped to an approximate size of 2–3.0 cm. Amounts of 0, 2, and 4% molasses and 0, 0.5, and 1% urea were added to the fresh material as a percentage of dry matter. Because of the urea, crude protein (CP) of Italian ryegrass silage increased, but the content of neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) decreased. While the addition of urea decreased the acetic acid and butyric acid concentrations of the silage, it increased the pH, lactic acid, and ammonia (NH_3) content. Molasses addition increased *in vitro* gas production and organic matter digestibility (OMD); urea increased metabolic energy (ME) and the net energy lactation (NE_L) values of silages. Urea and molasses both increased *in vitro* digestibility parameters, microbial protein production and synthesis, and relative fodder quality of the silage. As a result of the research, it was determined that urea and molasses could be used at contents of 1.5% and 4%, respectively, in Italian ryegrass silage.

Keywords: Italian ryegrass, *in vitro* gas production, silage fermentation, urea, molasses
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Introduction

Ruminants are an important source of animal protein in human nutrition. The importance of quality coarse fodder in ruminant feeding is high. In recent years, Italian ryegrass, which is considered a high-quality coarse fodder and whose cultivation has become widespread, is a single-annual, fodder plant (Dornelles *et al*., 2022). Due to its high digestibility and high content of nutrients, the plant provides an increase in yield and quality of milk and stock due to the high proportion of dry matter. In addition, it can be mowed every 20 days under suitable ecological conditions (Kaymak *et al*., 2021).

Since it is not possible to provide quality green, coarse fodder in the ration in all seasons of the year, silages made from forages during periods when there is no green grass meet the green forage needs of ruminants during these periods. Silage fodder, which is the cheapest and easiest way, can keep the yield of animals at the same level throughout the year; silage production is increasing day by day (Cheli *et al*., 2013; Can *et al*., 2019). The production of quality silage aims to reduce the cost of animal production and health, as well as to minimize the quality losses in fodder (Bartzanas *et al*., 2013; Yıldız *et al*., 2022). One of the methods used to improve silage quality and control the fermentation process is the use of additions. It is known that additions used in silage production reduce losses and increase silage stability (Yitbarek & Tamir, 2014). Adding carbon and nitrogen sources to the silage improves animal husbandry by preventing rumen deterioration and positively affects silage quality (McDonald *et al*., 2011). Urea, one of these additions, increases the level of dry matter in silage, reduces

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proteolysis, and mould growth. In addition, urea increases the pH and crude protein values of silages. Another addition, molasses, reduces the pH, butyric acid, and ammonia values of silage and increases the amount of lactic acid (Fang *et al*., 2022). Phesatcha *et al*. (2016) reported that silages made with the addition of urea and molasses increase the nutritional value (dry matter, organic matter, neutral detergent fibre, and acid detergent fibre), nutrient digestibility, and rumen fermentation efficiency. Today, the use of the *in vitro* gas production technique has become very common in determining feed value and quality (Ayaşan *et al*., 2021; Al-Baadani *et al*., 2022; Zhu *et al*., 2022).

This study was carried out to investigate the effects of different rates of adding urea and molasses to Italian ryegrass on relative fodder quality and *in vitro* gas and digestibility parameters.

Materials and Methods

The study was conducted in a field located within the borders of Erzincan Province, in the 2021 season. One-year ryegrass (*Lolium multiflorum* L.) samples were taken by mowing during the flowering period. After some withering, the fodder plant was chopped into 2–3 cm lengths. Amounts of 0, 2, and 4% molasses and 0, 0.5, and 1% urea were added to the dry matter. A total of 36 silage samples in the form of six samples × six treatments were included in the analysis; 0% molasses, 0% urea (Control); 0% molasses, 1% urea (M0U1); 5% molasses, 0% urea (M5U0); 5% molasses, 1% urea (M5U1); 10% molasses, 0% urea (M10U0); and 10% molasses, 1% urea (M10U1) were prepared. The prepared silage samples were vacuum-sealed in vacuum bags (25 × 35 cm) in a kitchen-type vacuum machine (Lavion DZ-100SS, Xiamen Yeasincere Industrial Corporation, China) and stored at 25 ± 2 ºC for 60 days.

The silages were opened 60 days after they were made. An amount of 250 ml of distilled water was added to 25-g silage samples for pH analysis, and the pH value of the filtrate thus obtained was measured with a digital pH meter (HI 2211 PH /ORP METER) by shaking for 30 min (Anonymous, 1993). The fodder mixtures obtained after the harvest of the plants were used in the experiment; crude protein (CP), dry matter (DM), and crude ash (CA), and ammonia (NH_3) contents were determined according to the methods of the AOAC (1988); crude fat (CF) analysis was determined according to the AOCS (2005) with the help of an AnkomXT15 extraction device. The analysis of insoluble fibrous substances (ADF) in acid solvents, insoluble fibrous substances (NDF) in neutral solvents, and crude cellulose (CS) was determined using an ANKOM2000 Fibre Analyzer (Ankom Technology, Macedon NY) and insoluble lignin in acid solvents (ADL) was determined according to the method of Van Soest *et al*. (1991).

In order to determine the *in vitro* digestibility parameters determined with the Ankom Daisy incubator, buffer solutions were prepared as recommended for the Ankom Daisy *in vitro* fermentation system. The samples' true digestibility range (TDR), true digestion of organic matter (TDOM), true NDF digestion (TNDFD), dry matter intake (DMI), and total digestible nutrition (TDN) values were calculated using the formulae given below, starting from the difference between the amount incubated at the beginning and the amount determined at the end of the NDF procedure.

Daisy 48 Hours (% GSD) = (100- ((The amount of the first sample - The amount of the sample after incubation)/ The amount of the first sample)*100)

Daisy 48 Hours (%TNDFD) = (100-((-((The amount of the first sample - The amount of the sample after NDF analysis) / The amount of the first sample)*100)

Daisy 48 Hours (%TDOMD) = (100-((-((The amount of the first sample - Amount of crude ash after NDF analysis) / Amount of crude ash of the sample (%))*100)

According to Ward & Ondarza (2008), the relative fodder quality was calculated using the equation:

RFQ (relative fodder quality) = (DMI, %DM) × (TDN, % DM) / 1.23

Metabolic energy (ME) and net energy lactation (NEL) values of fodder crude materials were calculated using the equation reported by Menke & Steingass (1988):

$$\text{ME (MJ/kg DM)} = 2.20 + 0.1357\times GP + 0.057\times CP + 0.002859\times CF^2$$

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

where GP: net gas production at the end of the 24-h incubation period of a 200 mg dry fodder sample, CP: %crude protein, CF: % crude fat, and CA: % crude ash.

In glass syringes with a volume of 100 ml, an average of 500 mg of fodder sample was incubated with 40 ml of buffered rumen fluid at 39 ˚C for 24 h (Menke *et al*., 1979). After 24 h of fermentation, the amount of methane (%) in the total gas produced was determined using an Infrared Methane Analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel *et al*., 2008) The actual amount of digested dry matter, partition factor, microbial protein production, and synthesizing activity values were determined in accordance with the method reported by Blümmel *et al* (1997).

$$\text{TDDM (mg)} = \text{Incubated DM (mg)} - \text{Remaining DM (mg)}$$
$$\text{GSD (\%)} = (\text{ADDM /Incubated DM}) \times 100$$
$$\text{The Partition Factor (PF)} = \text{ADDM/GP}$$
$$\text{Microbial Protein (MP) (mg/g DM)} = \text{TDDM} – (\text{GP}\times 2.2 \text{ mg/ml})$$
$$\text{Effectiveness of Microbial Protein Synthesis (EMPS)} = (\text{TDDM} – (\text{GP}\times 2.2 \text{ mg/ml}))/\text{ADDM}$$

Lactic, acetic, propionic, and butyric acids, which are mainly found in silage fodders, were determined using the method specified by Canbolat (2019).

In order to compare the data obtained as a result of the research, the Duncan multiple range comparison tests were applied to compare the groups by subjecting the data to variance analysis using the SPSS 24 (IBM, 2016) package.

Results and Discussion

The effect of urea and molasses addition to Italian ryegrass silage at different rates on the nutrient composition of the fodder was found to be significant (*P* <0.05) (Table 1). The DM content of urea and molasses silage increased and the highest DM content was found in the 4% M application (40.59%). Some studies have reported that urea and molasses additions increase the silage DM (Nursoy *et al*., 2003; Avcı *et al*., 2013; Kebede *et al*., 2018) and some studies do not (Denek *et al*., 2014; Bolakar & Yüksel, 2021). While molasses decreased silage's CA and CF content (Kebede *et al*., 2018), the highest CA and CF were in the 1%U and 4M applications (9.24% and 2.21%). Molasses alone did not affect the CP content of the fodder, whereas the CP content increased with the increase in the amount of urea applied. The CP content of the silage increased in sync with providing urea, carbon, and energy for microbial growth (Salem *et al*., 2013; Kang *et al*., 2018).

Table 1 The effect of urea and molasses addition at different rates on the raw nutrient composition of Italian ryegrass forage crop silage (% DM)

Silage %	DM	CA	CF	CP	ADF	NDF	ADL
Control	32.27^{c}	8.93^{ab}	1.97^{ab}	10.76^{c}	44.05^{b}	72.18^{a}	21.82^{a}
0U and 2M	35.20^{abc}	8.59^{abc}	1.83^{ab}	10.59^{c}	40.87^{de}	67.54^{b}	21.42^{a}
0U and 4M	**40.59^{a}**	7.96^{c}	1.87^{ab}	10.20^{c}	39.94^{de}	65.69^{c}	20.41^{a}
1U and 0M	34.91^{bc}	8.75^{ab}	1.86^{ab}	19.89^{b}	46.32^{a}	72.51^{a}	19.79^{a}
1U and 2M	35.27^{abc}	8.71^{ab}	1.92^{ab}	20.15^{b}	42.99^{bc}	69.07^{b}	18.91^{a}
1U and 4M	35.84^{abc}	**9.24^{a}**	**2.21^{a}**	19.58^{b}	39.82^{de}	64.24^{cd}	**11.31^{b}**
1.5U and 0M	37.87^{ab}	8.95^{ab}	1.60^{b}	29.20^{a}	42.82^{bc}	69.33^{b}	19.06^{a}
1.5U and 2M	33.91^{bc}	8.39^{bc}	1.97^{ab}	29.22^{a}	41.32^{cd}	65.16^{c}	18.90^{a}
1.5U+4M	37.84^{ab}	8.32^{bc}	1.95^{ab}	**29.50^{a}**	**39.42^{e}**	**63.10^{d}**	19.68^{a}
SE	0.64	0.94	0.04	1.50	0.44	0.64	0.66
***P* value**	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

[a-e] Within a row, means with a common superscript did not differ *P* <0.05; SE: standard error, DM: dry matter, CA: crude ash, CF: crude fat, CP: crude protein, NDF: insoluble fibre in neutral detergent, ADF: insoluble fibre in acid detergent, ADL: insoluble lignin in acid detergent

The ADF and NDF values of the cell wall components decreased as the proportion of urea and molasses additions increased, and the lowest ADF and NDF (Naroee, 2019) contents were observed at the 1.5% U and 4M application (39.42% and 63.10%, respectively). The lowest ADL content was determined in the 1% and 4M application (11.31%). It is believed that the fibre contained in molasses increases the activity of microorganisms during fermentation (Lunsin *et al*., 2018). As an energy source, molasses reduces the content of ADF, NDF, and ADL in silage studies (Wanapat & Kang, 2013; Kebede *et al.*, 2018; Musa *et al*., 2020). The effect of urea and molasses additions applied to Italian ryegrass silage at different rates on the fermentation properties of fodder was found to be significant (P <0.05) (Table 2).

Table 2 The effect of different proportions of urea and molasses addition on fermentation properties of Italian ryegrass fodder silage (g/kg, DM)

Silage %	pH	LA	AA	BA	NH_3
Control	5.19^{d}	0.24^{e}	0.39^{bc}	1.35^{a}	**0.34^{e}**
0U and 2M	**4.64^{e}**	1.49^{d}	**0.16^{d}**	0.37^{f}	0.58^{d}
0U and 4M	4.84^{e}	1.41^{d}	0.22^{cd}	0.36^{f}	0.69^{d}
1U and 0M	7.10^{a}	0.03^{e}	0.75^{a}	0.92^{c}	2.16^{a}
1U and 2M	6.24^{b}	3.48^{c}	0.71^{a}	0.70^{e}	2.00^{ab}
1U and 4M	5.76^{c}	3.54^{c}	0.48^{b}	0.83^{d}	1.93^{b}
1.5U and 0M	7.24^{a}	0.02^{e}	0.39^{bc}	1.11^{b}	1.39^{c}
1.5U and 2M	7.19^{a}	4.20^{b}	0.32^{bcd}	0.16^{g}	1.55^{c}
1.5U and 4M	6.22^{b}	**5.22^{a}**	0.19^{d}	**0.09^{h}**	1.39^{c}
SE	0.18	1.89	0.04	0.08	0,12
***P* value**	<0.05	<0.05	<0.05	<0.05	<0.05

$^{a\text{-}h}$ Within a row, means with a common superscript did not differ P <0.05; SE: standard error, LA: lactic acid, AA: acetic acid, BA: butyric acid, NH_3: ammonia

Silage pH values varied between 4.64 and 7.24. Despite the fact that the molasses addition reduced the silage pH value, urea significantly increased the pH value (Kang *et al*., 2018; Naroee, 2019). Urea prevents a pH decrease by increasing NH_3, which has high buffering properties (Kung *et al*., 2018). Molasses was not able to reduce the pH to the desired level. The amount of LA varied between 0.22 and 5.22, and the urea and molasses additions increased the amount of LA. Studies have reported that using only urea decreases the lactic acid level, and the lactic acid level increases with molasses addition (Ishida & Hassan, 1997; Lunsin *et al*., 2018). It has been reported that the amount of acetic acid in silages should not exceed 0.8% (Alçiçek & Özkan, 1996), and the values obtained in this study were below 0.8%. Urea and molasses reduced the amount of AA in silage, with the lowest contribution of 2%M (0.16 g/kg). Urea and molasses additions reduced the amount of BA, and the lowest was observed in the 1.5%U and 4M group (0.09 g/kg). Contrary to this research, Lunsin *et al*., found that urea and molasses addition to sugar cane pulp silages increased the amount of AA and BA. It is believed that these differences are due to the difference in plant material from which silage is made. Urea and molasses additions increased the amount of NH_3 compared to the control group (Kung *et al*., 2018).

The effects of urea and molasses addition to Italian ryegrass silage at different rates on *in vitro* gas production of fodder, methane (ml & %), OMS, ME, and NEL values were found to be significant (P <0.05) (Table 3).

While IVGP and OMD were not affected by urea and molasses addition, molasses only increased gas production and OMD, and the highest values (87.95% and 46.29%, respectively) occurred in silages with 4% molasses addition. Cherdthong *et al*. (2011), Sweeny *et al*. (2014), Kang *et al*. (2018), and Naroee (2019) reported that the addition of urea and molasses increased the *in vitro* gas production of silage. Similarly, Hunter *et al*. (2013) concluded that the addition of molasses to corn stalks increased the OMD by affecting silage fermentation.

Methane production in the silages increased with urea and molasses addition, while the lowest methane (10.81 ml and 14.29%) was observed in the control group. Kaya *et al*. (2020) reported that urea and molasses added to wheat straw increase methane production. Urea and molasses addition increased the ME and NE_L values of silages compared to the control group, and the highest (8.05 and 4.77 MJ/kg) values were determined in silages with only 1.5% Urea. The reason for this can be attributed to the increase in the ME and NE_L contents of the silages due to the increase in the urea added to the silage, and the decrease in the NDF and ADF levels as a result of the increase in the CP levels of the silages (Canbolat *et al*., 2014).

Table 3 The effect of urea and molasses addition at different ratios on Italian ryegrass forage silage on *in vitro* gas production, methane (ml & %), OMD, ME, and NE_L values (DM)

Silage %	IVGP ml	CH_4 ml	CH_4 %	OMD %	ME MJ/kg	NE_L MJ/kg
Control	75.53^{bc}	**10.81^{c}**	**14.29^{d}**	42.16^{cd}	6.92^{e}	3.82^{c}
0U and 2M	84.05^{a}	13.80^{ab}	16.40^{c}	45.01^{ab}	7.37^{d}	4.14^{b}
0U and 4M	**87.95^{a}**	14.66^{a}	16.69^{c}	**46.26^{a}**	7.56^{cd}	4.28^{b}
1U and 0M	73.05^{bc}	12.82^{b}	17.56^{bc}	41.85^{cd}	7.30^{d}	4.17^{b}
1U and 2M	74.82^{bc}	14.12^{ab}	18.87^{ab}	42.47^{cd}	7.42^{d}	4.26^{b}
1U and 4M	75.17^{bc}	14.30^{ab}	19.02^{ab}	42.59^{cd}	7.41^{d}	4.28^{b}
1.5U and 0M	76.95^{b}	14.10^{ab}	18.33^{ab}	43.74^{bc}	**8.05^{a}**	**4.77^{a}**
1.5U and 2M	70.92^{c}	13.69^{ab}	19.31^{a}	41.66^{d}	7.73^{bc}	4.57^{a}
1.5U and 4M	73.05^{bc}	14.38^{ab}	19.67^{a}	42.39^{cd}	7.86^{ab}	4.67^{a}
SE	1.13	0.25	0.34	0.33	0.06	0.05
***P* value**	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

[a,b,c] Within a row, means with a common superscript did not differ P <0.05; SE: standard error, CH_4: Methane, OMD: organic matter digestion, ME: metabolizable energy, NE_L: net energy lactation

The effect of urea and molasses addition to Italian ryegrass silage at different rates on fodder TDMD, TDOM, PF, MP, MPSA, TNDFD, and RFQ values was found to be significant (P <0.05) (Table 4).

Table 4. The effect of different ratios of urea and molasses addition to Italian ryegrass forage silage on TDMD, TDOM, PF, MP, MPSA, TNDFD, and RFQ values

Silage %	TDMD mg	TDOM mg	PF mg/ml	MP mg	MPSA %	TNDFD %	RFQ
Control	167.00^{g}	93.94^{a}	2.35^{i}	10.80^{h}	6.46^{i}	28.86^{cd}	43.02^{e}
0U and 2M	194.66^{e}	93.74^{abc}	2.41^{h}	17.20^{g}	8.83^{h}	35.96^{abc}	57.02^{bc}
0U and 4M	204.33^{cd}	93.27^{bc}	2.56^{g}	29.07^{f}	14.22^{g}	38.64^{ab}	63.69^{ab}
1U and 0M	184.66^{f}	93.62^{abc}	2.64^{f}	31.40^{f}	17.00^{f}	26.48d	39.13^{e}
1U and 2M	199.66^{de}	93.60^{abc}	2.80^{d}	42.73^{d}	21.40^{d}	35.37^{abc}	52.47^{cd}
1U and 4M	208.00^{bc}	**94.06^{a}**	2.87^{c}	48.87^{c}	23.49^{c}	32.61^{bcd}	58.05^{bc}
1.5U and 0M	198.66^{e}	93.80^{ab}	2.75^{e}	39.53^{e}	19.91^{e}	33.30^{abcd}	47.91^{de}
1.5U and 2M	210.00^{b}	93.36^{bc}	3.15^{b}	63.33^{b}	30.16^{b}	37.04^{abc}	59.60^{abc}
1.5U and 4M	**236.33^{a}**	93.19^{c}	**3.39^{a}**	**83.07^{a}**	**35.14^{a}**	**42.00^{a}**	**67.89^{a}**
SE	3.52	0.07	0.06	4.16	1.72	1.15	1.92
***P*-value**	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

[a-i] Within a row, means with a common superscript did not differ P <0.05; SE: standard error, TDMD: true dry matter digestibility, TDOM: true digestion of organic matter (%), PF: partition factor (mg), MP: microbial protein (mg), MPSA: Microbial protein synthesis activity (%), TNDFD: true NDF digestion, RFQ: relative fodder quality

Additions increased the *in vitro* fermentation values compared to the control group. OMD was the highest in the 1% and 4M (94.06%) group, while TDMD, PF, MP, MPSA, TNDFD, and RFQ were the highest in the 1.5% and 4M group (236.33, 3.39, 83.07, 35.14, 42.00, and 67.89, respectively). The higher the gas production of the fodder, the lower the microbial protein production. Therefore, determining other parameters other than gas in this type of *in vitro* experiment allows us to make healthier and more accurate decisions about fodder (Ceren, 2021). Microbial protein is an important source of amino acids for ruminants. In this study, the opinion that the decline in gas production increases the production of MP was confirmed. The increase in digestibility parameters, PF and RFQ, of urea and molasses added to silage was consistent with the low fibre content obtained in these groups. Ahmed *et al*. (2013) reported that the urea addition substantially increased the digestibility of NDF.

Lunsin *et al*. (2018) concluded that urea and molasses addition to sugarcane pulp increased the nutritive value of the fodder by increasing the CP, IVDMD, and IVOMD values.

Conclusions

The pH value of the silage decreased with the addition of molasses to Italian ryegrass, and the CP, fermentation properties, *in vitro* digestibility parameters, and RFQ value increased with the addition of urea and molasses. It is concluded that the addition of 1.5%U + 4M to the Italian ryegrass could be useful in increasing the nutritional value of silage.

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Authors' Contributions

Esra G conceived the study design, data acquisition, and performed the experiments together with Adem K. Adem K carried out the data analysis. Gürkan S and Ali K proofread the manuscript.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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