https://doi.org/10.17221/212/2022-CJAS

Effect of urea and molasses supplementation on *in vitro* digestibility, feed quality of mixed forage silages

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Citation: Gürsoy E., Sezmiş G., Kaya A. (2023): Effect of urea and molasses supplementation on *in vitro* digestibility, feed quality of mixed forage silages. Czech J. Anim. Sci., 68: 266–276.

Abstract: This study aims to investigate using different ratios of urea-molasses in silage of food *in vitro* fermentation, feed value and quality production in feed. After being withered a little, the fodder plant was fragmented into 2–3 cm long pieces. 0, 2 and 4% molasses and 0, 0.5 and 1% urea were added. The treatment groups were UM1 (control), UM2, UM3, UM4, UM5, UM6, UM7, UM8 and UM9 supplemented with 0, 0, 0, 1, 1, 1, 1.5, 1.5, 1.5% urea and 0, 2, 4, 0, 2, 4, 0, 2, 4% molasses respectively, and a total of 54 silage samples, 9 × 6 parallel, were prepared, vacuumed in ready-made silage bags, and stored at 25 ± 2 °C for 60 days. After the silages were opened, pH, nutrient contents, Fleig scores (FS), *in vitro* digestibility parameters, energy contents, feed value and feed quality were determined, then the data were subjected to analysis of variance. The effects of urea and molasses additives added to the mixed forage plant silage on all parameters were found to be significant ($P < 0.05$, $P < 0.01$). The crude protein (CP) increased its application by UM7. Cell wall component (insoluble fibre in acid detergent (ADF) and neutral detergent insoluble fibre NDF) reduced its application by UM9. All these applications increased the FS. The addition of molasses increased the *in vitro* gas production, while the addition of urea decreased the production. True organic matter digestibility increased with the addition of molasses. As a result, it was concluded that UM9 application in silages would be appropriate for increasing feed value and feed quality, and the UM3 application in silage would be appropriate for improving true organic matter digestibility (TOMD).

Keywords: *in vitro* digestibility; molasses; urea; feed quality; feed value

In order to eliminate the lack of quality roughage, the cultivation of mixed fodder plants has become widespread recently. Mixed fodder planting is the cultivation and harvesting of two or more plant species together in the same area and simultaneously (Seydosoglu and Bengisu 2019). These mixes help increase feed production by improving water and soil quality in that area. This is also effective in increasing soil nutrients, thus, increasing the feed quality and nutritive value as well compared to lean cultivation (Omokanye et al. 2019).

In some regions, it is impossible to supply quality green grass in all seasons of the year. Therefore, in rainy regions, the method of ensiling the feed is used to dry and store the feed and to keep green feed for ruminants in winter (Guyader et al. 2018). Silage not only meets the green feed needs of ruminants but also protects the nutritional value by reducing the an-

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ti-feeding effects of the feed (Canbolat 2022). Thus, it increases production by ensuring that animals fed with quality silage are healthy. Various additives are used in silage making to reduce silage difficulties and increase the nutritional value of the feed (Tyrolova et al. 2017). Molasses, which are used to improve silage fermentation and quality, increases the carbohydrate content of the feed, reduce the likelihood of spoilage of the silage, and increase the crude protein content (Das et al. 2022). Ramzan et al. (2022) reported that sorghum silage prepared by treating with molasses increased the silage quality by increasing its physical, chemical and fermentation. Urea, another feed additive, improves the nutritional value by increasing the crude protein content of the silage when added to the silage in an appropriate amount. It preserves the soluble carbohydrates in the silage (Kraiprom et al. 2022). It has been reported that urea-treated silage increases nutrient digestibility, feed intake, and fermentation (Hou et al. 2022).

The *in vitro* gas production technique, used to determine the nutritional value of feeds, is widely used today. With the *in vitro* gas production technique, the gas production, energy digestibility, methane production, microbial protein production and actual digestion degree of feeds can be determined. It is known that there is a close relationship between *in vitro* gas production parameters and the energy values of feed raw materials obtained in *in vivo* digestion trials. By determining the degree of digestion of the feeds, more information can be obtained about the feeds, and more accurate ratios can be prepared by considering the fermentation parameters.

This study was carried out to determine the effect of adding urea and molasses in different ratios to the mixed feed plant on chemical substances, *in vitro* digestibility and feed quality.

MATERIAL AND THE METHODS

Trial area and material source

The study was carried out in one field (39°36'15.94"N–39°54'18.58"E) located within the borders of Erzincan Province, in the 2021 season, in a five-feed mixture [35% Hungarian Vetch (Tarm white), 35% Feed Pea (Szarvası andrea), 10% Oats (Hero), 10% Triticale (Karma 2000) and 10% Wheat (Sönmez 2000)] were harvested at 50% of forage peas flowering.

Preparation of silage

The fodder plant was fragmented into 2–3 cm long pieces after being withered a little. 0, 2 and 4% molasses and 0, 0.5 and 1% urea were added. The treatment groups were UM1 (control), UM2, UM3, UM4, UM5, UM6, UM7, UM8 and UM9 supplemented with 0, 0, 0, 1, 1, 1, 1.5, 1.5, 1.5% urea and 0, 2, 4, 0, 2, 4, 0, 2, 4% molasses, respectively and a total of 54 silage samples, 9 × 6 parallel, were prepared. The prepared silage samples were vacuumed 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 °C for 60 days. Silages were opened 60 days after they were made. For pH analysis, 250 ml of distilled water was added to 25 g of silage sample and the pH value of the filtrate obtained after shaking for 30 min was measured with a digital pH meter (HI 2211 PH /ORP METER). The Fleig score of the silage samples was calculated with the help of the following formula [with the method and formula reported by Ramzan et al. (2022)];

$$FS = [220 + (2 \times DM\ ratio - 15)] - 40 \times pH \quad (1)$$

where:

FS – Fleig scores;
DM – dry matter.

Collection of rumen fluid

Rumen contents were taken from three adult female Montafon cattle (500–550 kg) coming for slaughter at the Meat and Fish Institution operating in Erzurum province. Approximately 5 min after slaughter, rumen fluid was taken from each animal whose rumen was opened and brought to the feed analysis laboratory with the help of a thermos with a temperature of 39 °C with CO_2 addition (Kilic and Abdiwali 2016).

Determination of nutrient content

Crude protein (CP), dry matter (DM) and crude ash (CA) analyses of the feed mixtures obtained after harvesting the plants used in the experiment were performed according to the method reported by the Kilic and Abdiwali (2016) and ether extract (EE) analysis was performed with the help of an

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AnkomXT15 extraction device (Am AOCS 2005). The analysis of fibrous substances insoluble in acid solvents and fibrous substances insoluble in neutral solvents were determined using an ANKOM2000 Fiber Analyzer (Ankom Technology, Macedon NY), and lignin insoluble in acid solvents was determined according to the method of Van Soest et al. (1991).

Determination of relative feed values, relative feed qualities, OMD, metabolic energy and net energy lactation contents of silages

The relative feed value (RFV) was calculated with the formula developed by Van Dyke and Anderson (2000), which is based on the estimation of the energy value that the feed will provide with the consumption potential by the animals.

$$\%DDM = 88.9 - (0.779 \times \%ADF) \quad (2)$$

$$\%DMI = 120/NDF \quad (3)$$

$$RFV = \%DDM \times \%DMI/1.29 \quad (4)$$

where:
DDM – dry matter digestion;
ADF – insoluble fibre in acid detergent;
DMI – dry matter intake;
NDF – neutral detergent insoluble fibre;
RFV – relative feed value.

Buffer solutions required for the determination of *in vitro* digestibility parameters determined using the Ankom Daisy incubator were prepared as reported using the Ankom Daisy *in vitro* fermentation system. True dry matter digestion (TDMD) of silages obtained in the daisy incubator after 48 h, true NDF digestion (TNDFD), true organic matter digestibility (TOMD), DMI and total digestible nutrients (TDN) values and relative feed quality (RFQ) were determined with the help of the following equations (Van Soest et al. 1991; Adesogan 2005).

Daisy 48 h (%TDD) = [100 – ((initial sample amount-Post-fer sample amount)/initial sample amount) × 100] (5)

Daisy 48 h (%TNDFD) = [100 – (– (initial sample amount – NDF post-analysis sample amount)/initial sample amount) × 100] (6)

Daisy 48 h (%TOMDD) = [100 – (– (initial sample amount – crude ash amount after NDF analysis)/crude ash amount of sample (%)) × 100] (7)

This was calculated with the equation

$$RFQ = [DMI\ (\%DM)] \times [TDN\ (\%DM)]/1.23 \quad (8)$$
(Ward and Ondarza 2008)

where:
TDD – true digestion degree;
TNDFD – true NDF digestion;
TOMDD – true degree of organic matter digestion;
RFQ – relative feed quality;
DM – dry matter;
DMI – dry matter intake;
TDN – total digestible nutrient.

The RFV, TOMD, TNDFD, DMI, RFQ and TDN values of the samples were calculated with the following formulas, starting from the difference between the amount incubated at the beginning and the amount determined at the end of the NDF procedure.

ME and NEL values of feed ingredients were calculated using the equation reported by Menke et al. (1979).

$$ME\ (MJ/kg\ DM) = 2.20 + 0.135\,7 \times GP + 0.057 \times CP + 0.002\,859 \times EE2 \quad (9)$$

$$NEL\ (MJ/kg\ DM) = 0.101 \times GP + 0.051 \times CP + 0.112 \times EE \quad (10)$$

where:
ME – metabolic energy;
DM – dry matter;
GP – net gas production at the end of the 24 h incubation period of 200 mg dry feed sample;
CP – % crude protein;
EE – % eter extract;
NEL – net energy lactation;
CA – % crude ash.

Determination of *in vitro* gas productions and true digestion degrees

Total gas and methane production of these forage mixtures were determined using the Hohenheim gas test (HGT) according to the procedure described by Menke et al. (1979). In the experimental groups,

https://doi.org/10.17221/212/2022-CJAS

approximately 500 mg of feed samples were incubated in 100 ml glass syringes with 40 ml of buffered rumen fluid in a water bath 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 with an Infrared Methane Analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel et al. 2008). After the gas measurements, the remaining rumen fluid in the syringe and feed samples were boiled for 1 h in NDF solution prepared as reported by Van Soest et al (1991). After boiling, the actual amount of dry matter digested, the actual degree of digestion, partition factor, microbial protein production and synthesis efficiency values were determined using the equations published by Blummel et al. (1997).

$$\text{TDDM (mg)} = \text{incubated DM} - \text{remaining DM} \quad (11)$$

$$\text{PF (mg)} = \text{TDDM/GP} \quad (12)$$

$$\text{MY (mg)} = \text{TDMM} - [\text{GP (24 h)} \times 2.2 \text{ mg/ml}] \quad (13)$$

$$\text{MPPE (\%)} = (\text{MY/TDDM}) \times 100 \quad (14)$$

where:
TDDM – true amount of dry matter digested;
DM – dry matter;
PF – partition factor;
GP – net gas production at the end of the 24 h incubation period of 200 mg dry feed sample;
MY – microbial yield;
MPPE – microbial protein production efficiencies;
MY – microbial yield.

Statistical analysis

In order to compare the data obtained as a result of the research, the Duncan comparison test was applied to compare the groups by applying variance analysis (GLM) in the SPSS v24 (IBM Corp., Armonk, NY USA) package program. In the research randomised plots, 3 (supplement) × 4 (supplement dose) according to factorial trial design planned and the mathematical model is given below

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk} \quad (15)$$

where:
Y_{ijk} – 1st dose of j^{th} essential booster of i^{th} supplement observation value;
μ – overall average;
α_i – effect of ith supplement (i = 2; 1 = U, 2 = M);
β_j – effect of the jth booster dose (j_U= 3; 1 = 0.2 = 0.5%, 3 = 1%, j_M = 3; 1 = 0.2 = 4%, 3 = 4%);
$\alpha\beta_{ij}$ – effect of interaction between doses of i^{th} supplement and j^{th} supplement;
ε_{ijk} – trial error.

RESULTS

Nutrient content

The effects of urea and molasses supplementation to mixed forage silage at different rates on nutrient composition (Table 1) were statistically significant (P < 0.05 and P < 0.01). With the addition of urea and molasses, the CA content of the mixed fodder silage decreased, while it increased (9.24%) in the UM6 application compared to the control (8.93%). The EE and CP content of the silage increased with the addition of urea and molasses. The highest EE was seen in the UM8 application, the highest in the HP UM7 application. While ADF and NDF contents decreased with urea and molasses additives, it was determined that there was an increase in ADL content.

Fermentation characteristics and Fleig score

The effects of urea and molasses additives at different ratios to the mixed forage plant silage on the fermentation characteristics and Fleig score (Table 2) were found to be statistically significant (P < 0.05 and P < 0.01). While the urea and molasses additives increased the DM content of the silage, the pH of the silage decreased with the addition of urea and increased with the addition of molasses. The highest FS calculated by DM and pH (117.8) was seen in the UM3 application. While the NH_3 value of the silage was not affected only by the addition of urea, this value increased with the addition of molasses.

Gas production, methane, OMD, ME and NEL contents

The effects of urea and molasses additives at different ratios to the mixed fodder silage on *in vitro* gas production, methane (ml), methane (%), OMD,

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Table 1. The effect of urea and molasses additives at different ratios on the raw nutrient composition of the mixed forage crop silage (% DM)

Silage %	CA	EE	CP	ADF	NDF	ADL
UM1	8.93[ab]	0.86[e]	17.39[e]	42.80[a]	67.09[a]	4.89[e]
UM2	8.59[abc]	1.11[d]	19.39[d]	40.68[ab]	64.68[ab]	8.35[ab]
UM3	7.96[c]	1.47[c]	16.11[f]	36.20[de]	59.62[d]	9.59[a]
UM4	8.75[ab]	1.16[d]	28.47[b]	41.27[ab]	62.72[bc]	9.48b
UM5	8.71[ab]	1.63[bc]	26.86[c]	37.23[cd]	61.44[cd]	6.33[cde]
UM6	9.24[a]	1.53[bc]	27.75[ab]	35.79[de]	59.57[d]	7.23[bc]
UM7	8.95[ab]	1.69[bc]	30.31[a]	40.51[ab]	60.88[cd]	7.77[bc]
UM8	8.39[bc]	2.09[a]	27.84[ab]	39.59[bc]	59.43[d]	6.65[cd]
UM9	8.32[bc]	1.71[b]	27.00[c]	34.39[e]	55.22[e]	5.56[de]
SEM	0.09	0.07	0.99	0.58	0.67	0.33
Statistical significance	*	**	**	**	**	**

ADF = insoluble fiber in acid detergent; ADL = lignin insoluble in acid detergent; CA = crude ash; CP = crude protein; EE = ether extract; NDF = neutral detergent insoluble fibre; SEM = standard error of mean; UM1 = control; UM2–9 = supplemented with 0, 0, 0, 1, 1, 1, 1.5, 1.5, 1.5% urea and 0, 2, 4, 0, 2, 4, 0, 2, 4% molasses respectively

*$P < 0.05$; **$P < 0.01$

[a–f]The differences between the averages shown with different letters in the same column are significant

Table 2. The effect of urea and molasses additives in different ratios of mixed forage crop silage on fermentation characteristics and Fleig score (g/kg, KM)

Silage %	DM	pH	FS	NH_3
UM1	35.88[c]	4.94[cd]	78.91[b]	0.02[bc]
UM2	39.86[bc]	4.77[d]	93.79[b]	0.02[bc]
UM3	43.08[ab]	4.33[e]	117.8[a]	0.01[c]
UM4	41.66[ab]	5.78[b]	56.87[c]	0.05[a]
UM5	46.44[a]	5.14[c]	92.29[b]	0.04[ab]
UM6	45.82[a]	5.10[c]	92.51[b]	0.04[abc]
UM7	43.95[ab]	6.50[a]	32.90[d]	0.05[a]
UM8	39.99[bc]	5.18[c]	77.79[b]	0.04[ab]
UM9	42.19[ab]	4.98[cd]	90.17[b]	0.04[abc]
SEM	0.74	0.11	4.76	0.00
Statistical significance	**	**	**	*

DM = dry matter; FS = Fleig score; NH_3 = ammonia; SEM = standard error of mean; UM1 = control; UM2–9 = supplemented with 0, 0, 0, 1, 1, 1, 1.5, 1.5, 1.5% urea and 0, 2, 4, 0, 2, 4, 0, 2, 4% molasses respectively

**$P < 0.01$

[a–d]The differences between the averages shown with different letters in the same column are significant

ME and NEL values (Table 3) were found to be statistically significant ($P < 0.05$ and $P < 0.01$). The mixed silage of molasses and urea increased the *in vitro* gas production (IVGP) and the highest value (79.26 ml) was in the UM3 application. Adding urea and molasses increased the silage's CH4 production (ml and %). It was determined that silage increased the OMD, ME and NEL values, the lowest values were in the control (37.28%, 6.46 and 3.51 MJ/kg) and the highest values were in the UM3 (43.62%) and UM5 (7.59 and 4.29 MJ/kg) applications, respectively.

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Table 3. Effect of urea and molasses additives in mixed forage crop silage on *in vitro* gas production, methane (ml, %), OMD, ME and NEL values (DM)

Silage %	IVGP (ml)	CH_4 (ml)	CH_4 (%)	OMD (%)	ME (MJ/kg)	NE_L (MJ/kg)
UM1	60.22[d]	11.83[de]	19.69[ab]	37.28[d]	6.46[e]	3.51[d]
UM2	65.27[bcd]	14.07[abcd]	21.55[a]	39.14[bcd]	6.85[d]	3.78[c]
UM3	79.26[a]	15.96[a]	20.14[ab]	43.62[a]	7.42[abc]	4.19[ab]
UM4	62.55[cd]	13.41[bcd]	21.45[a]	38.66[cd]	7.22[abcd]	4.02[abc]
UM5	71.10[b]	15.02[ab]	21.12[ab]	41.49[ab]	7.59[a]	4.29[a]
UM6	63.72[bcd]	14.00[abcd]	21.95[a]	39.07[bcd]	7.24[abcd]	4.04[abc]
UM7	58.67[d]	10.81[e]	18.50[b]	37.49[d]	7.12[bcd]	3.94[bc]
UM8	59.44[d]	12.55[cde]	21.08[ab]	37.62[d]	7.02[cd]	3.88[c]
UM9	68.38[bc]	14.88[abc]	21.71[a]	40.45[bc]	7.45[ab]	4.19[ab]
SEM	1.39	0.36	0.31	0.44	0.07	0.05
Statistical significance	**	**	*	**	**	**

CH_4 = methane; IVGP = *in vitro* gas production; ME = metabolic energy; NE_L = net energy lactation; OMD = organic matter digestibility; SEM = standard error of mean; UM1 = control; UM2–9 = supplemented with 0, 0, 0, 1, 1, 1, 1.5, 1.5, 1.5% urea and 0, 2, 4, 0, 2, 4, 0, 2, 4% molasses respectively

*$P < 0.05$; **$P < 0.01$

[a–e]The differences between the averages shown with different letters in the same column are significant

True digestibility values and other parameters

The effects of urea and molasses additives at different ratios to the mixed forage plant silage on the true digestibility values and other parameters (Table 4) were found to be statistically significant ($P < 0.05$ and $P < 0.01$). The addition of urea and molasses increased the silage TDDM value and the highest value was observed in the UM5 (274.31 mg) application. It was observed that the highest (93.06 mg) TOMD value was obtained with the UM2 application, while other applications decreased this value. The PF, MY and EMP values were found to be high-

Table 4. The effect of urea and molasses additives at different rates on TDDM, TOMD, PF, MP, EMP and RFQ values in mixed forage crop silage

Silage %	TDDM (mg)	TOMD (mg)	PF (mg/ml)	MY (mg)	MPPE (%)
UM1	229.46[d]	92.58[ab]	4.43[bc]	115.79[c]	50.41[bc]
UM2	240.97[cd]	93.06[a]	4.31[cd]	117.77[c]	48.75[c]
UM3	260.66[abc]	91.51[abc]	3.84[d]	111.06[c]	42.61[d]
UM4	246.12[bcd]	90.37[bc]	4.58[bc]	128.06[bc]	51.91[abc]
UM5	274.31[a]	92.08[abc]	4.50[bc]	140.11[ab]	51.04[bc]
UM6	269.85[a]	91.69[abc]	4.93[ab]	149.58[ab]	55.37[ab]
UM7	258.76[abc]	89.72[c]	5.14[a]	148.03[ab]	57.23[a]
UM8	264.10[ab]	90.47[bc]	5.19[a]	151.90[a]	57.55[a]
UM9	258.83[abc]	86.65[d]	4.43[bc]	129.77[bc]	50.10[bc]
SEM	3.27	0.41	0.90	3.43	0.99
Statistical significance	**	**	**	**	**

MPPE = microbial protein production efficiencies; MY = microbial yield (mg); PF = partition factor; SEM = standard error of mean; TDDM = true amount of dry matter digested; TOMD = true organic dry matter; UM1 = control; UM2–9 = supplemented with 0, 0, 0, 1, 1, 1, 1.5, 1.5, 1.5% urea and 0, 2, 4, 0, 2, 4, 0, 2, 4% molasses respectively

*$P < 0.05$; **$P < 0.01$

[a–d]The differences between the averages shown with different letters in the same column are significant est in the UM8 application (5.19 mg/ml, 151.90 mg and 57.55%) and lowest in the UM3 application (3.84 mg/ml, 111.06 mg and 42.61%).

Feed value and feed quality

The effects of urea and molasses additives at different ratios to the mixed forage plant silage on the RFV and RFQ (Table 5) were found to be statistically significant ($P < 0.01$). Addition of urea and molasses increased silage RFV, DMI, TDN, Daisy NDF digestion (TNDFD) and RFQ values. The highest RFV, DMI, TDN and RFQ values (114.12, 2.37, 60.89 and 117.36) were determined in the UM9 application, while the TNDFD (53.99%) was determined in the UM5 application.

DISCUSSION

Nutrient contents

In the aforementioned study, the effects of urea and molasses additives at different rates in the mixed forage plant silage on the nutrient composition were found to be significant for all parameters. Crude protein (CP) was found 17.39% in the UM1; Different levels of urea and molasses additives led to an increase in the CP value of the silage. As a result of using urea as a nitrogen source, the CP value of the silage increased. Canbolat et al. (2014) found that the addition of urea to silage caused an increase in CP value.

Urea and molasses additives increased the ash content of the silage. It was different from the results of some other study (Sanchez-Santillan et al. 2020). The reason for this difference is thought to be due to the different plant material used for silage. The ADF and NDF content were decreased with the addition of urea and molasses (Sanchez-Santillan et al. 2020; Abo-Donia et al. 2022). This decrease is due to the fact that urea and molasses lack the fibrous fraction and do not contribute to structural carbohydrates. Li et al. (2022) stated that the NDF ratio, which was 57.45% in the control group, in silage opened on the 60th day decreased to 55.52% with the addition of molasses to the silage. The same researcher also determined that the ADF ratio, which was 31.04% in the control group, in the silage opened on the 60th day, decreased to 29.08% with the addition of molasses to the silage.

The researcher also stated that the increase in molasses additive contributes positively to digestibility by reducing the NDF ratios of silages, unlike urea. Kang et al. (2018) found that both urea and molasses additives to silages reduce the NDF ratios of silages, but the addition of molasses is

Table 5. The effect of urea and molasses additives in different ratios of mixed forage crop silage on RFV, DMI, TDN, TNDFD and RFQ values

Silage %	RFV	DMI	TDN	TNDFD (%)	RFQ
UM1	80.69^{g}	1.87^{e}	50.41^{d}	48.03^{c}	76.82^{e}
UM2	85.79^{g}	1.93^{de}	51.18^{d}	47.86^{c}	80.55^{de}
UM3	103.02^{ab}	2.19^{b}	56.94^{b}	51.41^{abc}	101.01^{b}
UM4	89.56^{ef}	2.03^{cd}	54.15^{c}	49.38^{bc}	89.74^{cd}
UM5	102.33^{bc}	2.20^{b}	57.38^{b}	53.99^{a}	102.81^{b}
UM6	106.20^{b}	2.24^{b}	57.27^{b}	53.34^{ab}	104.64^{b}
UM7	94.94^{de}	2.13^{bc}	55.84^{bc}	50.97^{abc}	97.08^{bc}
UM8	99.64^{cd}	2.21^{b}	57.47^{b}	51.98^{abc}	103.46^{b}
UM9	114.12^{a}	2.37^{a}	60.89^{a}	52.11^{abc}	117.36^{a}
SEM	2.03	0.03	0.65	0.54	2.51
Statistical significance	**	**	**	**	**

DMI = dry matter intake; RFQ = relative feed quality; RFV = relative feed value; SEM = standard error of mean; TDN = total digestible nutrients; TNDFD = true NDF digestion; UM1 = control; UM2–9 = supplemented with 0, 0, 0, 1, 1, 1, 1.5, 1.5, 1.5% urea and 0, 2, 4, 0, 2, 4, 0, 2, 4% molasses respectively

**$P < 0.01$

$^{a–f}$The differences between the averages shown with different letters in the same column are significant

https://doi.org/10.17221/212/2022-CJAS

more effective in reducing NDF ratios than urea additives.

In our study, the addition of urea and molasses increased the ether extract content of the silage. Canbolat (2022) stated that the effect of different levels of urea additives in silage on EE (ether extract) is important, while there is a decrease in EE level as the urea additive increases (0U and 2.5U respectively, 4.40 and 4.29).

Many studies have also been conducted to determine the silage quality of urea and molasses additives with different materials (Sanchez-Santillan et al. 2020; Abo-Donia et al. 2022).

Fermentation characteristics and Fleig score

The pH value used in the Fleig scoring method is a numerical measure that determines whether the feeds are sufficiently fermented. A high ratio of DM reduces the pH levels and negatively affects lactic acid fermentation, thus reducing the quality of silage.

In the current study, the DM ratio, which was determined as 35.88% in the control group, was found to be higher in the urea and molasses added groups, and a statistical difference was found between the groups. Kraiprom et al. (2022) stated that urea and molasses additives to silage increased the DM level from 60.18 to 69.59%. Kang et al. (2018) reported that the contribution of molasses and urea on the KM level of silage did not make a statistical difference.

While the addition of urea and molasses increased the KM level of silage, molasses decreased the pH level of silage and urea increased it (Hou et al. 2022). The reason for this is that urea prevents pH decrease by increasing NH_3, which has high buffering properties (Kun et al. 2018). In one study, it was reported that molasses decreased the pH value, and the pH value (4.5 and lower) required in good silage was obtained in the UM3 application (Das et al. 2022). In another study, it was determined that the urea additive to the silage increased the pH value of the silage (Canbolat 2022). Li et al. (2022) found that the pH ratio, which was 4.32 in the control group, in the silage opened on the 60th day decreased to 4.25 with the addition of molasses to the silage.

The best Fleig score was in the UM3 application with the addition of plain molasses. In the same application, NH_3 obtained its lowest score. Ramzan et al. (2022) reported that the addition of molasses in sorghum-sudan grass silage increased the Fleig score.

Fleig score is one of the methods used to determine silage quality; the higher the Fleig score, the higher the quality of that silage. In the current study, the Fleig score, which was 78.19 in the control group, increased with the addition of urea and molasses. In our study, the highest Fleig score was found in the group that did not include urea and added molasses at the level of 4% (117.8). The lowest Fleig score was observed in the UM7 group. Similarly, Abo-Donia et al. (2022) reported that the addition of urea and molasses to rice straw increased the Fleig score.

Gas production, methane, OMD, ME and NEL contents

The *in vitro* gas production technique is used to determine the *in vitro* degradation rate, amount, ME and OMD degree of feeds. This technique is an indirect method based on the measurement of gases released as a result of feed fermentation. Also, the amount of produced gas serves for the determination of many parameters of feed. It is a well-known fact that there is a close relationship between *in vitro* gas production parameters and the energy values of feed raw materials obtained in *in vivo* digestion trials.

Results showed that the addition of urea and molasses increased the levels of GP, OMD, ME and NEL (Abo-Donia et al. 2022). The decrease in ADF caused these values (GP, OMD, ME and NEL) to increase (Ramzan et al. 2022). The fact that the highest *in vitro* gas production value and TOMD were observed in the UM3 application may be due to the fact that the addition of molasses increased the water-soluble carbohydrate content in the silage and decreased the ADF and NDF content. However, the addition of urea and molasses increased the CH_4 level (Sanchez-Santillan et al. 2020). This is thought to be due to the fermentation of cellulose. The increased gas production kinetics may be due to the addition of urea to silage, which can improve rumen fermentation and nutrient digestibility.

The OMD of the silages was 37.28% in the control group. The addition of urea and molasses increased OMD. Abo-Donia et al. (2022) stated that OMD

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was higher in the urea and molasses-supplemented group than in the control group. Canbolat (2022) stated that different levels of urea additives to silage increase OMD (P < 0.05).

Kang et al. (2018) found that the effect of urea and molasses addition to silage was statistically significant on *in vitro* gas production, which is in agreement with the finding we obtained in the experiment. Similarly, Canbolat (2022) explained that urea supplementation at different levels to silage increases *in vitro* gas production (P < 0.05).

In our study, the ME contents of the silages were found to be 6.46 MJ/kg in the control group, while the addition of urea and molasses to the silages increased the ME content. Li et al. (2014) found the ME of the silage in the control group to be 7.06, and stated that the ME of the silage increased as the additive ratio of molasses increased in the silage. The fact that Li et al. (2014) reported that the addition of urea to silage at different levels increased the ME content of the silage supports the finding we obtained in our study.

True digestibility values and other parameters

Regarding gas measurement techniques, the DDM, OMD, fermentation rate, and fermentation speed of feed materials closely affect the gas ratios produced by the volatile fatty acids (VFAs), which form the end products of fermentation with microbial protein synthesis and microbial activity by strongly affecting the microbial activity of the rumen. Ruminant animals meet their protein needs from microbial and bypass proteins.

While only the UM2 application increased the TOMD value, the addition of urea and molasses increased the TDMD, PF, MP and MPPE values. The addition of urea and molasses to sugarcane silage increased the TOMD and TDMD values, but the addition in plain form had no effect. Abo-Donia et al. (2022) reported that urea and molasses additives added to rice straw increased TOMD and MP values. Microbial protein, which is an important source of amino acids for ruminants, increased with the decrease in gas production. The increase in PF, one of the digestibility parameters of urea and molasses added to silage, was consistent with the low fiber content obtained in these groups. Based on the relevant studies, the PF values of the feeds are the most significant factor determining the microbial protein synthesis efficiency, that their theoretical PF values can be between 2.75 and 4.41 and feeds with high PF values have a high level of microbial protein production efficiencies (Blummel and Lebzien 2001).

Feed value and feed quality

Both RFV and RFQ parameters are used to determine the quality and value of the feed. In order to detect RFV, the chemical analyses are determined in the relevant feed resulting in the use of this value in the evaluation of all plants. Using this value in the evaluation of feeds to be given to dairy animals with high milk production is highly recommended. Feed quality is summarised as the ability of roughage to provide ruminants with the desired nutrients. The RFQ parameter, which is said to be better in identifying feed quality, is determined by confirming the intake of total digestible nutrients (TDN) and dry matter.

The RFV is a parameter used to measure the quality of the feed, calculated using the NDF and ADF values in the feed. The higher the relative feed value, the higher the quality. With the addition of urea and molasses, the feed value and quality of the silage increased. Reducing the ADF and NDF contents by adding urea and molasses resulted in an increase in feed value and feed quality. It has been determined that silages prepared with existing additives increase RFV, TNDFD and RFQ values (Hundal et al. 2021; Abo-Donia et al. 2022; Canbolat 2022; Ramzan et al. 2022).

Abo-Donia et al. (2022) stated that the effect of both urea and molasses additives on RFV was important. Likewise, in similar studies (Abo-Donia et al. 2022; Ramzan et al. 2022), the effect of adding molasses to silage on RFV was statistically significant, and its addition positively affected the RFV of silages by reducing cell wall components that are difficult to digest [NDF andlignin insoluble in acid detergent (ADL)].

CONCLUSION

It may be possible to meet the food shortages that the increasing human population may experience in the future by planting quality roughages,

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which are an important source of protein and energy in ruminant nutrition, both as a mixture in the field and by producing forage crops with preserved nutritional value using additives such as urea and molasses. It was concluded that the UM9 application in silages would be appropriate for increasing feed value and feed quality, and the UM3 application in silage would be appropriate for improving TOMD.

Conflict of interest

The authors declare no conflict of interest.

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Received: December 1, 2022
Accepted: May 22, 2023
Published online: June 16, 2023