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Original article

The impact of wheat bran and molasses addition to caramba mix silage on feed value and *in vitro* organic matter digestibility

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A B S T R A C T

Background: The fermentation process in silage is an inherently uncontrolled process that results in significant nutritional losses. Supplementing silage with different additives may boost nutrient and energy recovery during fermentation, which in turn can boost animal productivity. However, the impact of wheat bran and molasses addition on feed value of caramba mix silage is unknown.
Methods: This study determined the impact of molasses and wheat straw addition on fermentation and *in vitro* organic matter digestibility of caramba mix silage. Caramba was cut at harvest maturity and subjected to five different treatments, i.e., control, addition of 5% and 10% molasses, and addition of 5% and 10% wheat bran. Caramba mix and additives were mixed and ensiled into laboratory type 1 L glass containers. Silage fermentation, chemical and microbiological composition, and *in vitro* metabolic energy content in silages were determined 60 days after the initiation of treatments.
Results: Different chemical and microbial properties of silage were significantly altered by the addition of molasses and wheat bran. The addition of 10% molasses resulted in the highest values of all measured traits of Caramba mix silage. The dry matter was 27.00%, 28.44%, 33.93%, 32.67%, and 30.61% in control, 5% molasses, 10% molasses, 5% wheat bran and 10% wheat bran, respectively. Similarly, pH was 5.32, 4.89, 4.56, 4.62, and 4.39 in control, 5% molasses, 10% molasses, 5% wheat bran and 10% wheat bran, respectively. Likewise, crude protein was 14.58%, 13.03%, 16.23%, 14.75%, and 16.42% in control, 5% molasses, 10% molasses, 5% wheat bran and 10% wheat bran, respectively.
Conclusion: It is concluded that addition of molasses and wheat bran to the caramba mix silage increased the chemical, and microbiological qualities. Particularly molasses improved the digestibility of silage. Therefore, it is recommended that 10% molasses should be added during the fermentation of the silage to improve chemical, and microbiological properties of Caramba mix silage.

1. Introduction

Caramba mix (*Lolium multiflorum* Lam., var. *italicum)* is used as fodder for livestock in the Mediterranean region with a history dating back to the 12th century. It is grown in temperate climates almost all over the world. Caramba mix is an annual forage in the Poaceae family. Therefore, it has good palatability, highly digestible metabolic energy, and fiber. On the other hand, Caramba mix can be produced more than once a year (Lenuweit and Gharadjedaghi, 2002; Özelçam et al., 2015). Caramba mix is usually used as forage for green or preserved in feeding of dairy cows. It is suggested for cattle due of their high nutritional value and great digestion. However, the nutritional composition of grasses is not always enough. A nutritional imbalance brought on by low water-soluble carbohydrate (WSC) concentrations or low WSC to crude protein (CP) ratios (WSC:CP) prevents ruminal microorganisms from producing microbial protein (Nocek and Russell, 1988; Kingston-Smith and Theodorou, 2000). Therefore, a higher WSC:CP ratio in grasses, which improves nutritional balance, may result in a host animal that uses nitrogen more effectively.

The quality of the silage depends on the crop's maturity during harvest. Silage's nutritional value is reduced by fermentation because less voluntary consumption and use of digestible nutrients occurs. The main objective of silage research up to this point has been to identify strategies for raising the feeding value of the finished product while reducing losses from the original crop.

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Bacterial inoculants, molasses, enzymes, grains etc. additives are used to provide feeding value and silage preservation (Keady, 2000). Especially the addition of carbohydrate-rich molasses increases the activity of epiphytic (lactic acid) bacteria. WB (wheat bran), which is used as an additive in silage, has a positive effect on the dry matter content and feed value of silages (Gül et al., 2019).

Well-fermented silage is characterized by its dry matter content, the physiological characteristics of epiphytic bacteria, and, most importantly, its abundance of water-soluble carbohydrates. The reduction in pH levels in the silage prevents the growth of bacteria that cause rotting, allowing the nutrient content to be preserved. The best silage diets include a high concentration of water-soluble carbohydrates to speed up fermentation and provide enough acid to protect the silage. Good fermentation, preservation, and acid production all depend on the quantity of water-soluble carbohydrates in the silage diet (Zanine et al., 2010). The nutritional value of silage, according to Fariani et al. (1994), is a result of the physiological and morphological changes that occur throughout different stages of development. The quality is significantly altered by plant type, age of growth, soil structure, pasture management, and climate (Aganga et al., 2004).

Unfortunately, the research on the use of different additives to improve silage quality is not advanced in Turkey. Therefore, this study determined the effects of molasses and wheat bran addition on feeding value and organic matter digestibility of caramba mixed silage. It was hypothesized that addition of molasses would improve feeding value and organic matter digestibility of caramba mixed silage. It was further hypothesized that addition of molasses will result in higher improvement in feeding value and organic matter digestibility of caramba mixed silage compared to wheat bran.

2. Materials and methods

2.1. Study site and experimental treatments

Caramba mix was grown on a farm located in Tekirdağ province situated at the Marmara Sea coast of Turkey (40° 59′ N, 27° 34′ E, elevation 17 m). The annual mean temperature is 10.5 °C. Caramba was harvested at the growing stage in June. Silage treatments were divided into five groups after the Caramba mix was cut into 1.0–1.5 cm lengths. The treatments include, control, 5% molasses, 10% molasses, 5% WB (wheat bran) and 10% WB (wheat bran). The Caramba mix was combined with the additives and ensiled in 50 pieces of 1-liter laboratory type glass containers (Weck, Wher-Oftlingen, Germany) fitted with gas-release-only lids. For a period of 60 days, the silage samples were stored at room temperature (20 ± 1 °C). On the 60th day, samples were taken from three glass jars per treatment from all group for analyzing chemical and microbiological attributes, and cell wall contents.

2.2. Analytical procedure

Three samples were taken from all treatments to analyze chemical properties. The samples were dried at 60 °C for 72 h, ground, and passed through a 1 mm sieve. After drying at 105 °C for 4 h, amount of dry matter (DM), CP (crude protein) and ash contents were analyzed according to the methods described by AOAC (1990). The hemicellulose, cellulose, DM digestibility and relative feed value were also analyzed. Likewise, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the method described by Van Soest et al. (1991). The metabolizable energy contents were calculated as specified in TSE Animal feeds-Determination of Metabolizable Energy (1991). The *in vitro* OM digestibility (OMD) of the silage treatments was computed according to Aufrere and Michalet-Doreau (1988). Similarly, NH_3-N and pH contents of silage treatments were recorded according to MAFF (1986). The lactic acid (LA) contents were calculated using the method described by Barker and Summerson (1941). Water-soluble carbohydrate (WSC) values were determined on spectrophotometer as reported by MAFF (1986). Seale et al. (1990) were followed for the determination of microbiological attributes such as lactic acid bacteria (LAB), yeast, and mould in silage. As stated in the method, MRS and malt extract was used to determine the amount of LAB, yeast, and mould. The amount of LAB, mould, and yeast in all silage groups were calculated as 30°at the end of the three-day incubation period and converted into logarithmic coli form units (cfu/g).

2.3. Statistical analysis

The collected data were statistically analyzed by general linear model (GLM) procedure (Statical Analysis System, 2005). The probability level of $P < 0.05$ was considered significant. When significant differences were denoted by GLM, the means' comparisons for the applied treatments were compared using the Duncan test.

3. Results and discussion

3.1. Nutrient composition

Nutrient composition of caramba mix silage is summarized in Table 1. The addition of molasses and wheat bran had significant ($P < 0.01$) effect on dry matter (DM). The highest DM content was noted for 10% wheat bran (33.93%), followed by 5% molasses (32.67%), 10% molasses (30.61%), and 5% wheat bran (28.44). The dry matter value of control treatment is consistent with the finding of Özelçam et al. (2015). However, DM contents of silage (5–10% wheat bran and molasses) were higher than earlier findings of Baldinger et al. (2012), Shao et al. (2007), and Özelçam et al. (2015).

The crude protein (CP) was significantly ($P < 0.01$) influenced by addition of molasses and wheat bran to the silage. The highest CP (16.42%) was recorded for 10% molasses followed by 10% wheat bran (16.23%), 5% molasses (14.74%), control (14.58%) and (13.03%) 5% wheat bran. The CP values of all traetemnts included in this study were higher than those reported by DLG (1991), Bernard (2003), Aganga et al. (2004), Fonseca et al. (2005), Shao et al. (2007), Baldinger et al. (2012), and Özelçam et al (2015). Caramba mix silage is reported to have <10% CP. The protein content of Italian grass has been reported between 5 and 9% (Bernard et al., 2002). The higher CP content in wheat bran and molasses increased CP content of silages. Fariani et al. (1994) reported that nutritional value of forage is due to physiological and morphological changes that occur during growth periods. Differences with previous studies are due to differences in plant diversity, growth stage, soil structure, pasture management and climate etc. (Aganga et al., 2004).

The pH value usually drops through the fermentation of lactic acid (Van Soest, 1994). In this study pH value was 5.32, 4.89, 4.56, 4.62, 4,39 for control, 5% wheat bran, 10% wheat bran, 5% molasses and 10% molasses, respectively. The differences were statistically significant ($P < 0.01$). The results are similar with the findings of Baldinger et al. (2012). However, the values observed in the current study are higher than those reported by Shao et al. (2007). The differences are linked with the addition of various additives (molasses and wheat bran) in the current study. For good quality silage, fermentation aerobic and lower pH must be ensured. The pH value usually drops through the fermentation of lactic acid (Van Soest, 1994). The addition of wheat bran and molasses

Table 1
The impact of wheat bran mixture and molasses' addition on some chemical attributes of Caramba silage.

Treatments	DM (%)	pH	CP (% DM)	Ash (% DM)
Control	27.00 ± 0.28e**	5.32 ± 0.05a**	14.58 ± 0.04b**	15.50 ± 0.04b**
5% WB	28.44 ± 0.28d**	4.89 ± 0.11b**	13.03 ± 0.03c**	14.49 ± 0.05c**
10% WB	33.93 ± 0.29a**	4.56 ± 0.08a**	16.23 ± 0.24a**	13.99 ± 0.22d**
5% M	32.67 ± 0.60b**	4.62 ± 0.03c**	14.75 ± 0.10b**	16.03 ± 0.01a**
10% M	30.61 ± 0.60c**	4.39 ± 0.01d**	16.42 ± 0.02a**	15.32 ± 0.06b**
P value	0.001	0.001	0.001	0.001

**(P < 0.01) DM: dry matter, CP: crude protein W.B.: wheat bran M: molasses, the means followed by different letters within a column denote that these are significantly different from each other.

reduced the silage pH. The differences between earlier reports and findings of the current study are caused by different additives used.

The ash content in control, 5% wheat bran, 10% wheat bran, 5% molasses, and 10% molasses were 15.50%, 14.49%, 13.99%, 16.03%, and 15.32%, respectively. Wheat bran and molasses decreased crude ash content of silages ($P < 0.01$).

The results showed that water-soluble carbohydrates (WSC) contents varied from 10.32% to 19.05% (Table 2). Dry matter content, physiological features of epiphytic bacteria, and most crucially, the quantity of WSC are the attributes of a well fermented silage. By lowering its pH, silage inhibits the development of spoilage microbes, keeping its nutritious content intact. Since more fermentation and acid production are required to maintain silage, the best silage feeds are those with a high proportion of WSC. It is important for a healthy silage feed to include enough WSC to promote fermentation, protect the silage, and generate an appropriate amount of acid (Zanine et al., 2010).

The highest silages lactic acid (LA) (51.61%) was found in 10% wheat bran followed by 5% molasses (49.81), 5% wheat bran (47.32%), control (47.10) and 10% wheat bran (44.87%) (Table 2). The differences among the treatments were statistically significant ($P < 0.05$). Catchpoole and Hanzell (1971) reported that LA values as 46.85–121.76 g/kg[1] DM for Italian ryegrass silages. The results of the current study met these criteria. The LA content of all treatments are consistent with the findings of Shao et al. (2007), while higher than Baldinger et al. (2012). The caramba mix and additives utilized here vary from those in earlier experiments because they include mono- and disaccharides that are easily fermented.

Ammonia nitrogen contents value ranged between 64.64 and 96.41 g/kg TN (Table 2). The highest ammonia nitrogen contents were observed in 5% wheat bran (96.41 g/kg TN), while the lowest contents were recorded for 10% wheat bran (64.64 g/kg TN). These differences were statistically significant (P < 0.01).

3.2. Microbial composition

The microbial contents of the silage recorded in the current study are given Table 3. The LAB, yeast and mould contents between 3.92 and 5.58 log cfu/g DM, 3.94–6.08 log cfu/g DM, and 3.60–5.24 log cfu/g DM respectively. An increase was recorded in LAB and the number of yeasts decreased in the silage treated with wheat bran and molasses compared to control. This experiment clearly showed that the molasses and wheat bran significantly lower mould content compared to control. In this study, wheat bran and molasses were used to reduce WSC loss by undesirable yeast, mould and aerobic bacteria in the first stage of ensiling.

Table 2
The impact of wheat bran mixture and molasses' addition on some chemical attributes of Caramba silage.

Treatments	WSC (g/kg DM)	NH_3-N (g/kg TN)	LA (%)
Control	12.77 ± 1.58b**	84.74 ± 4.18b**	47.10 ± 2.58ab*
5% WB	10.32 ± 0.91c**	96.41 ± 1.45a**	47.32 ± 1.32ab*
10% WB	12.30 ± 0.55b**	64.64 ± 3.21c**	51.61 ± 0.30a*
5% M	13.99 ± 0.69b**	85.79 ± 1.70b**	49.81 ± 1.63ab*
10% M	19.05 ± 0.04a**	70.82 ± 1.00c**	44.87 ± 1.03b*
P value	0.000	0.001	0.010

*(P < 0.05), ** (P < 0.01) DM: dry matter, WSC: water soluble carbohydrates, LA: lactic acid, W.B.: wheat bran, M: molasses. The means followed by different letters within a column denote that these are significantly different from each other.

Table 3
The impact of wheat bran mixture and molasses' addition on microbiological analyses of the Caramba silages (log cfu/g DM).

Treatments	LAB	Yeast	Mould
Control	4.84 ± 0.05b**	6.08 ± 0.02a**	5.24 ± 0.06a**
5% WB	4.54 ± 0.01c**	4.67 ± 0.06b**	3.96 ± 0.07c**
10% WB	5.58 ± 0.10a**	4.64 ± 0.21b**	3.75 ± 0.05 cd**
5% M	4.22 ± 0.00d**	3.94 ± 0.06c**	4.45 ± 0.14b**
10% M	3.92 ± 0.03e**	4.49 ± 0.03b**	3.60 ± 0.02d**
P value	0.001	0.000	0.000

*(P < 0.05), ** (P < 0.01) W.B.: wheat bran, M: molasses, LAB: lactic acid bacteria, the means followed by different letters within a column denote that these are significantly different from each other.

3.3. Cell wall composition

Cell wall components of the caramba silages recorded in the current study are given in Table 4. The NDF content ranged between 46.82 and 58.42%. The high NDF value was noted for control treatment (58.42%), while 10% molasses treatment resulted in lower values ($P < 0.01$). Redfearn et al. (2002) reported that high NDF content of feeds are negatively related with digestibility. The NDF content of caramba silages higher than findings of Baldinger et al. (2012). The NDF content recorded in the current study is lower than the findings of Özelçam et al. (2015). Differences between reports may be due to differences in growing age, soil structure, rangeland management, and plant diversity (Amrane and Michalet-Doreau, 1993; Aganga et al., 2004). The reason for the degradation of cell wall components of silage is lower ADF and NDF content of wheat straw. Wheat bran and molasses used as additives in this study became the energy source of LAB that accelerated bacterial activities.

The ADF content ranged between 29.21% and 39.32%. The highest ADF was noted for control (39.32%), whereas the lowest ADF content was recorded for 10% molasses (29.21%) ($P < 0.01$). The ADF content in control treatment is similar with previous study findings of Fonseca et al. (2005), and Shao et al. (2007) (31.00–37.00%). The ADF content was lower than the values reported by Özelçam et al. (2015) (43.29%). The differences among the values are owed to plant variety, pasture management, soil structure and additives (wheat bran, molasses).

Hemicellulose and cellulase contents ranged between 17.61% and 24.80% and 15.46–22.28%, respectively (Table 4). The differences among different treatments were statistically significant ($P < 0.01$). Hemicellulose value of control group was lower than

Table 4
The impact of molasses and wheat bran mixture addition on cell wall contents (% DM) of the Caramba silages.

Treatments	NDF	ADF	HCEL	CEL
Control	58.42 ± 0.45a**	39.32 ± 0.81a**	19.09 ± 0.36ab**	16.55 ± 1.23b**
5% WB	57.45 ± 0.01a**	32.6 ± 0.085c**	24.80 ± 0.10a**	18.45 ± 1.28b**
10% WB	53.62 ± 0.35b**	35.15 ± 0.87b**	18.46 ± 0.51b**	22.28 ± 1.28a**
5% M	53.81 ± 0.47b**	32.88 ± 0.57c**	20.92 ± 1.05b**	15.46 ± 0.46b**
10% M	46.82 ± 0.16c**	29.21 ± 0.72d**	17.61 ± 0.55b**	16.48 ± 1.35b**
P value	0.000	0.000	0.000	0.000

**(P < 0,01) NDF: Neutral Detergent Fiber, ADF: acid Detergent fiber, HCEL: hemicellulose, CEL: cellulase, WB: wheat bran, M: molasses. The means followed by different letters within a column denote that these are significantly different from each other.

those reported by Özelçam et al. (2015). The differences are linked with the variations in plant variety, soil structure and pasture management.

The average digestion coefficients of DDM and DMI ranged between 58.26% and 66.14% and 2.05%-2.56 respectively (Table 5.) (P < 0.01). The highest DDM value was (66.14%) noted for 10% molasses. On the other hand, the lowest DDM value (58.26%) was noted for control treatment. The DDM findings are similar with previous findings of Deutsche Landwirtschafts-Gesellschaft (1991), Zhang et al. (1995), and Catanese et al. (2009). The values recorded in the current study were lower than those reported by Abo-Eid et al. (2016) and Özelçam et al. (2015). Dry matter intake of control group was similar with finding of Abo-Eid et al. (2016). Harvest season, differences in additives (wheat bran, molasses) and silage process are the reasons for differences in DDM and DMI.

The best method to determine the forage quality is to feed the forage directly to animals and measure their production, which is practically impossible. Relative feed value can be valuable for estimating forage quality. To determine the feed quality, the relative feed value (RFV) identified in the United State of America was aimed for clover and forages (Canbolat, 2013; Göktepe and Selçuk, 2017). In the study highest RFV was recorded in 10% molasses. The lowest RFV was noted for control treatment (P < 0.01) (Table 5). The RFV is higher than that found by Göktepe and Selçuk (2017). Differences between reports may be due to different plant varieties and additives (wheat bran, molasses which were used in these trials).

In vitro organic matter digestibility (OMD) and metabolizable energy (ME) values of the study are given in Table 6. The highest (65.66%) OMD was noted for 10% molasses. Similarly, the highest metabolizable energy value (8.28 MJ/kg DM) was noted for 10% molasses (P < 0.01). The OM digestibility of control treatment was lower than the values reported by Deutsche Landwirtschafts-Gesellschaft (1991) and Özelçam et al. (2015). The ME value recorded in the current study is closer to findings of Özelçam et al. (2015) (7.83 MJ/kg DM). The differences between the OMD contents of the current and previous studies can be explained by the differences in wheat bran, molasses and harvest season used in this trial. The low CF content of caramba (at the growing stage) and wheat bran, molasses increased ME content of silages.

Table 5
The impact of molasses and wheat bran mixture addition on digestible dry matter, dry matter intake and relative feed value contents of the Caramba mix silages (% DM).

Treatments	DDM (%)	DMI (%)	RFV
Control	58.26 ± 0.63d**	2.05 ± 0.01c**	92.78 ± 1.74c**
5% WB	63.46 ± 0.06b**	2.08 ± 0.00c**	102.58 ± 0.08b**
10% WB	61.51 ± 0.68c**	2.23 ± 0.01b**	104.44 ± 0.19ab**
5% M	63.28 ± 0.44b**	2.23 ± 0.01b**	109.03 ± 0.19b**
10% M	66.14 ± 0.56a**	2.56 ± 0.01a**	128.62 ± 1.59a**
P value	0.000	0.001	0.000

**(P < 0,01), DDM: digestible dry matter, DMI: dry matter intake, RFV: relative feed value WB: wheat bran, M: molasses. The means followed by different letters within a column denote that these are significantly different from each other.

Table 6
The impact of molasses and wheat bran mixture addition on *in vitro* organic matter digestibility and metabolizable energy contents of Caramba silage.

Treatments	OMD (%)	ME (MJ/kg KM)
Control	51.12 ± 0.44d*	6.46 ± 0.08e**
5% WB	54.99 ± 0.38c*	7.04 ± 0.01d**
10% WB	59.54 ± 0.62b*	7.73 ± 0.01b**
5% M	60.41 ± 0.39b*	7.50 ± 0.01c**
10% M	65.66 ± 0.50a*	8.28 ± 0.08**
P value	0.005	0.000

*(P < 0.05), ** (P < 0.01) OMD. Organic matter digestibility, ME: metabolizable energy, WB: wheat bran, M: molasses. The means followed by different letters within a column denote that these are significantly different from each other.

4. Conclusions

The study revealed that ensiling caramba with wheat bran and molasses improved the nutrient composition, fermentation characteristics and exerted positive effect on cell fiber contents of caramba silages, particularly when the additives included a source of sugar like molasses. Molasses improved the digestibility of silages. Therefore, it is recommended that 10% molasses should be added during fermentation of the silage to improve chemical, and microbiological properties of Caramba mix silage.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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