



## Chemical Composition, *In vivo* Digestibility and Metabolizable Energy Values of Caramba (*Lolium multiflorum cv. caramba*) Fresh, Silage and Hay

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**ABSTRACT:** The experiment was conducted to determine nutritive values of caramba (*Lolium multiflorum cv. caramba*) fresh, silage and hay by *in vivo* and *in vitro* methods. There was a statistically significant difference ( $p < 0.01$ ) in crude protein content value between fresh caramba (12.83%) and silage (8.91%) and hay (6.35%). According to results of experiment, the crude fiber, neutral detergent fiber, acid detergent fiber (ADF), acid detergent lignin contents of the three forms of caramba varied between 30.22% to 35.06%, 57.41% to 63.70%, 35.32% to 43.29%, and 5.55% to 8.86% respectively. There were no significant differences between the three forms of caramba in digestibility of nutrients and *in vivo* metabolizable energy (ME) values ( $p > 0.05$ ). However, the highest  $ME_{CN}$  (ME was estimated using crude nutrients) and  $ME_{ADF}$  values were found in fresh caramba ( $p < 0.01$ ). As a result, it could be said that, there were no differences between the three forms of caramba in nutrient composition, digestibility and ME value, besides drying and ensiling did not affect digestibility of hay. Consequently, caramba either as fresh, silage or hay is a good alternative source of forage for ruminants. (**Key Words:** Caramba, Chemical Composition, Digestibility, Metabolizable Energy)

### INTRODUCTION

For a long time, there has been a lack of high quality roughage for ruminants in Turkey. In recent years it has been popular to cultivate caramba (*Lolium multiflorum cv. caramba*) as a feed crop. This is a variety of Italian ryegrass, also known as “milk grass” in colloquial speech, which has been well adapted to Turkey’s climatic and soil conditions.

Caramba, which is an annual forage poaceae, is rich in protein, minerals and water soluble carbohydrates compared to medium quality pasture grasses, while it has good palatability, is easily enable to be digested and has a high metabolizable energy (ME) value. Furthermore, it can be harvested more than once in a year (Lenuweit and Gharadjedaghi, 2002; Kesiktaş, 2010; Baldinger et al., 2011). Thus, it seems that caramba may be the solution for Turkey’s national shortage of roughage for ruminants. Although generally it is used in ruminant feeding by pasturing or harvesting in the form of fresh grass, caramba

may be used after it is dried or ensilaged (Hannaway et al., 1999; Bernard et al., 2002; Shao et al., 2005; Cooke et al., 2008). The conducted studies showed that Italian ryegrasses have high (71% to 78%) dry matter (DM) digestibility (Ohshima et al., 1988; Catanese et al., 2009; Amaral et al., 2011) and have a favorable effect on milk yield and composition (McCormick et al., 1990;1998; Miller et al., 2001; Bernard, 2003) and increase the live weight of livestock (De Villiers et al., 2002; Zaman et al., 2002; Van Niekerk et al., 2008).

The object of this study was to compare the forms of fresh, silage and hay of caramba, with respect to their chemical composition, *in vivo* digestibility and ME values.

### MATERIALS AND METHODS

#### Sampling procedures

The first growth of caramba grown on a farm located in Kiraz-Ödemiş-İzmir (38°23’S, 28°20’W, h: 312 m; the average annual rainfall is 1,020 mm), was harvested at the growing stage in early May with a silage cutting machine and used for the preparations of hay and wilted silage. The distance between the farm and the experimental station was

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approximately 200 km. Therefore, a portion of the herbage was carefully stored at 4°C for use as fresh. A portion of the herbage was spread in the field and cured for two days. The dried products were packed into linen bags and kept in airy shade. The caramba was mown and wilted to approximately 25% to 30% DM before being chopped (approximately 5 to 10 cm) and ensiled in sealed tanks of 25 m<sup>3</sup> capacity for 45 d. Before the start of feeding trial, silages were transported in plastic drums of 120 L capacity and stored during the experiment at the room temperature. Plastic drums were closed tightly without contact with air.

### Animal trials

In the study, 4 Sakız sheep (2 years old, 67±2 kg W) were used for the *in vivo* digestion trials of feeds. Sheep were placed in individual pens (77×133×110 cm) during the *in vivo* digestion trials. The animals were fed in two equal portions at 08:00 am and 16:00 pm each day while licking stones and water were provided *ad libitum* during the experiment. The forms of fresh, silage and hay of caramba were provided to 1.2 times maintenance. Each experimental period was conducted for 14 days in which the first 7 day period was a preliminary period and the last 7 days were the collection period. The total fecal collection was made over the last 7 days. Feces were collected daily in a manure bag from individual sheep, weighed and stored (portion of 10%) in capped glass jars supplemented with 2 to 3 mL chloroform at 4°C until the samples were required for analysis.

### Analytical methods

Dried feeds and fresh feces samples were ground in a laboratory mill to pass through a 1 mm screen for chemical analyses. Dry matter, crude ash, crude protein (CP), ether extract (EE) contents of samples were determined according to AOAC (1990) procedures and crude fiber (CF) concentration was determined using the method of

Crampton and Maynard (1938). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were determined using the methods of Goering and Van Soest (1970), hemicellulose contents was calculated the difference in NDF-ADF. Then, the equations developed by Kirchgessner and Kellner (1981) and proposed by TSI (2004) were used to calculate the *in vitro* ME values of feed for ruminants.

$$\text{ME [kcal/kg organic matter (OM)]} \\ = 3,260 + (0.455 \times \text{CP}) + (3.517 \times \text{EE}) - (4.037 \times \text{CF})$$

CP, EE, CF quantities, g/kg OM is at

$$\text{ME (MJ/kg DM)} = 14.70 - 0.150 \times \text{ADF (\%)}$$

Digestibility was determined by accurately measuring feed intake and excreta output. From these measurements, together with chemical analysis for nutrients, the digestibility was calculated at the end of the digestion trials, then *in vivo* ME values (Schiemann et al., 1971) were calculated.

$$\text{ME (MJ/kg DM)} = 0.0152 \times \text{DCP} + 0.0342 \times \text{DEE} \\ + 0.0128 \times \text{DCF} + 0.0159 \times \text{DNFE}$$

DCP, digestibility CP; DEE, digestibility EE; DCF, digestibility CF; DNFE, digestibility N-free extract, g/kg DM<sup>2</sup> is at.

### Statistical analysis

All data obtained from the experiment were evaluated according to the analysis of variance using general linear model. Tukey's multiple comparison test (SPSS, 2002) was used to determine differences between the means.

**Table 1.** Chemical composition of caramba (% DM)

Groups	Fresh	Silage	Hay	SEM	Significance
Crude nutrients					
Dry matter	22.27	25.43	90.77	-	-
Organic matter	90.29 <sup>b</sup>	88.80 <sup>c</sup>	91.91 <sup>a</sup>	0.27	0.00
Crude protein	12.83 <sup>a</sup>	8.91 <sup>b</sup>	6.35 <sup>c</sup>	0.66	0.00
Ether extract	2.49 <sup>b</sup>	2.83 <sup>a</sup>	1.84 <sup>c</sup>	0.09	0.00
Crude fiber	30.90 <sup>b</sup>	35.06 <sup>a</sup>	30.22 <sup>b</sup>	0.50	0.00
N-free extract	44.09 <sup>b</sup>	42.67 <sup>b</sup>	53.56 <sup>a</sup>	1.29	0.00
Cell wall contents					
Neutral detergent fiber	57.41 <sup>c</sup>	63.70 <sup>a</sup>	59.08 <sup>b</sup>	0.81	0.00
Acid detergent fiber	35.32 <sup>c</sup>	43.29 <sup>a</sup>	38.26 <sup>b</sup>	1.00	0.00
Hemicellulose	22.09 <sup>a</sup>	20.41 <sup>b</sup>	20.82 <sup>ab</sup>	0.28	0.00
Acid detergent lignin	8.86	5.55	7.30	0.61	0.068

DM, dry matter; SEM, standard error of means.

Means with different superscripts within a row are significantly different ( $p < 0.01$ ).

## RESULTS AND DISCUSSION

### Chemical compositions of feeds

Chemical composition of feeds is given in Table 1. According to the findings, the highest CP content value (12.83%) was found in the form of fresh and followed by silage (8.91%) and hay (6.35%). These differences are statistically significant ( $p < 0.01$ ). The CP content of caramba fresh is consistent with the findings of relevant studies (DLG, 1991; Amrane and Michalet-Doreau, 1993; Redfearn et al., 2002; Bernard, 2003; Aganga et al., 2004; Repetto et al., 2005). The finding regarding CP content of caramba silage is consistent with that found by Bernard (2003), Aganga et al. (2004), Fonseca et al. (2005) and Baldinger et al. (2011) while it is lower than the value found by DLG (1991) (11.7%). However, approximately 10% lower CP contents have mostly been reported for silage. Thus, Bernard et al. (2002) reported CP values as 5.8% to 9.9% for Italian ryegrass silages. The finding regarding CP content of caramba hay is lower than the value found by Ohshima et al. (1988 and 1991), DLG (1991), Amrane and Michalet-Doreau (1993), Fariani et al. (1994) and Hannaway et al. (1999). However, this value is consistent with the result of the study (McCormick et al., 1998) reporting that Italian ryegrass hay contains less protein compared to its silage form.

Some of the differences between reports may be based on the difference between plant variety, growth age, soil structure, climate and pasture management (Amrane and Michalet-Doreau, 1993; Aganga et al., 2004). Likewise, it is reported that nutritive value of roughage depends on morphological and physiological changes occurred during growth period (Fariani et al., 1994).

We found that CF content was highest in the silage form (35.06%) and followed by fresh (30.90%) and hay (30.22%). These values are statistically significant ( $p < 0.01$ ). Findings about CF content of caramba fresh (DLG, 1991; Lenuweit and Gharadjedaghi, 2002; Ximena and Rene Anrique, 2011), silage (DLG, 1991; Nishino et al., 1995; Ridla and Uchida, 1998; Boyd et al., 2008; Cooke et al., 2008) and hay (Ohshima et al., 1988; Fariani et al., 1994) are generally higher than those reported in the literature. On the other hand, it is reported that Italian ryegrasses are rich in water soluble carbohydrates and this richness is correlated with a generally slow growth rate and less dry content yield (Marais and Goodenough, 2000). In the present experiment, the highest N-free extract (NFE) value (53.50%) was found in the hay. The results are similar to reported by DLG (1991), Ohshima et al. (1988), Fariani et al. (1994) and Nishino et al. (1995). Lower NFE content especially in silage form (42.67%) indicates nutritive matter loss depending on silo water while the fact that it is high in the hay form may be associated with low N content in mid

climate meadow grasses (Marais and Goodenough, 2000).

The high NDF contents of feed are negatively related with digestibility (Redfearn et al., 2002). The present results found the highest value for NDF and ADF contents in silage (63.70% and 43.29% respectively) and followed by hay (59.08% and 38.26% respectively) and fresh (57.41% and 35.32% respectively). These values are statistically significant ( $p < 0.01$ ). The caramba was wilted before ensiled and NDF content was increased with the wilting process. Also, non fiber carbohydrate content decreased due to loss of silo water. In this manner, NDF content was increased relatively. Findings about NDF and ADF contents of caramba fresh are consistent with those found by Ridla and Uchida (1998), Redfearn et al. (2002), and Aganga et al. (2004) while they are higher than findings of Fulkerson et al. (1998), Ben-Ghedalia et al. (2001), Montossi et al. (2001), and Ximena Valderrama and Rene Anrique (2011). Our results for NDF and ADF contents of caramba silage are similar to those from the study of Fonseca et al. (2005) that are NDF (63.8%) and ADF (39.3%). Results for NDF content of caramba hay are consistent with that found by Amrane and Michalet-Doreau (1993) and Fariani et al. (1994) (52.4% and 64.4% respectively). However, although the value for ADF content is similar to that found by Fariani et al. (1994) (35.4% to 46.4%) it is higher than the value found by Amrane and Michalet-Doreau (1993) (21.9% to 27.4%).

The highest value with respect to ADL content was found in the fresh form (8.86%) and followed by the hay (7.30%) and silage (5.55%). These values are not statistically significant ( $p > 0.05$ ). Our results regarding caramba fresh are higher than those produced by Ridla and Uchida (1998), Montossi et al. (2001) and Amaral et al. (2011). Our value for caramba silage is close to that produced by Fonseca et al. (2005) (5.0%). On the other hand, ADL content of caramba hay is higher than that found by Amrane and Michalet-Doreau (1993) and Fariani et al. (1994) (3.5% to 5.1% and 2.7% to 5.0% respectively).

### Digestion coefficients of feeds

Digestion coefficients of feeds are given in Table 2. According to the findings shown in Table 2, there is no

**Table 2.** Digestion coefficients of caramba (%)

Groups	Fresh	Silage	Hay	SEM	Significance
Dry matter	73.07	73.01	79.58	1.79	0.245
Organic matter	75.13	74.44	81.37	1.74	0.212
Crude protein	75.03	67.14	65.16	2.61	0.286
Ether extract	77.89	80.01	76.89	2.71	0.909
Crude fiber	74.77	78.34	77.88	1.68	0.683
N-free extract	75.25 <sup>ab</sup>	72.34 <sup>b</sup>	85.42 <sup>a</sup>	2.22	0.021

SEM, standard error of means.

Means with different supercripts within a row are significantly different ( $p < 0.01$ ).

statistically significant variation between all three forms of caramba with respect to digestion coefficients ( $p > 0.05$ ) but the most digestible CP exists in fresh while the highest digestible NFE content is in the hay. This result may dependent on harvest and drying of Italian ryegrass (Miyashige et al., 1989).

The maturation of the plant reduces its digestibility. The reduction occurs by interaction between different factors such as high cellulose concentration, lignification and stem-leaf ratio (Valente et al., 2000). Furthermore, it has been reported that CP and DM digestion decreases in Italian ryegrass hay with maturity but cell wall fractions increase (Aganga et al., 2004). However, the digestibility of Italian ryegrass silages is typically high. This is associated with the fact that although cold climate poaceae are rich in structural carbohydrates, their lignin content is low. They are easily broken down due to their straight edge structure their leaves' epidermis layer, the leaves' easily digestible mesophyll cell ratio (57% to 66%) and air gap ratio between cells (10% to 35%) is high and thus, digestive bacteria can easily invade the leaves (Tan and Menteşe, 2003).

Experimental results indicate that there were no differences between the three forms of caramba in digestibility ( $p > 0.05$ ). The average digestion coefficients of DM, OM, CP, EE, CF, and NFE are ranged between 73.01% to 79.58%, 74.44% to 81.37%, 65.16% to 75.03%, 76.89% to 80.01%, 74.77% to 78.34%, and 72.34% to 85.42%, respectively. Values of DM and OM digestibility for caramba fresh and silage are higher than those reported by DLG (1991), Oshima et al. (1988), Zhang et al. (1995), Catanese et al. (2009) while lower than those reported by Nishino et al. (1995), van Dorland et al. (2006), Amaral et al. (2011). However, OM digestibility of silage was similar to reported by DLG (1991) value (74%). The value for caramba hay is higher than that reported by Ohshima et al. (1991) (68.3%) while the finding about its silage is close to that reported by the researcher (73.4%). The results can be explained by the differences in harvest season and silage process.

On the other hand, the value for caramba fresh CP digestibility is similar to that reported by Amaral et al. (2011) while it is lower than that produced by DLG (1991) (65%). Finding about caramba silage is lower than that reported by Oshima et al. (1988), Nishino et al. (1995), and van Dorland et al. (2006) while it is higher than that reported by DLG (1991) (62%). The CF digestibility of caramba hay is lower than that reported by Oshima et al. (1988) (79.8%) while it is higher than that reported by DLG (1991) (68%). However, CP digestibility of caramba hay is close to that reported by Oshima et al. (1988) while the values for OM, EE, and NFE digestibility are higher than those reported by Oshima et al. (1988) and DLG (1991).

In the study, the digestibility of caramba silage related

NFE content, was lower than the fresh and hay ( $p < 0.01$ ). In fact, Nishino et al. (1995) reported that N of Italian ryegrass silage is evaluated at a lower level compared to its fresh and hay forms and this is associated with chemical changes during ensilaging. Proteins are hydrolyzed after intensive deamination, as a result  $\text{NH}_3$  produced by NPN (non protein nitrogen). This ensures a poor synchronization for microbial synthesis in rumen. The reason is N of silage was broken down rapidly in rumen while easily digestible carbohydrates had been fermented previously during ensilaging.

### ***In vivo* and *in vitro* metabolizable energy values**

*In vivo* ME values ( $\text{ME}_{in vivo}$ ) and *in vitro* ME values ( $\text{ME}_{CN}$  and  $\text{ME}_{ADF}$ ) of caramba fresh, silage and hay are given in Table 3. According to Table 3, there was no statistically significant variation between all three forms of caramba with respect to  $\text{ME}_{in vivo}$  value ( $p > 0.05$ ). The  $\text{ME}_{in vivo}$  value for the caramba hay is higher than that reported by Hannaway et al. (1999) and DLG (1991) (8.62 and 8.53 MJ/kg respectively). This results has been noted that *in vivo* OM digestion coefficient of hay is higher than the other forms. The  $\text{ME}_{in vivo}$  value for the caramba fresh is close to that reported by DLG (1991), Fulkerson et al. (1998), Hannaway et al. (1999), Lenuweit et al. (2002) and De Villiers et al. (2002) for green Italian ryegrass while it is lower than that reported by Ximena Valderrama and Rene Anrique (2011) (11.9 MJ/kg). The value for caramba silage is consistent with the value reported by DLG (1991) while it is lower than that found by van Dorland et al. (2006) (19 MJ/kg).

Highest figures for  $\text{ME}_{CN}$  value were found in caramba's silage and fresh form (7.83 and 7.72 MJ/kg respectively) and this was followed by the hay form (6.77 MJ/kg). This result can be explained by high contents of CP and EE. This finding is consistent with the report by McCormick et al. (1998) that Italian ryegrass hay has lower energy and protein values than its silage form. Highest figure for  $\text{ME}_{ADF}$  value was found in caramba fresh form (9.40 MJ/kg) and this was followed by silage (8.96 MJ/kg) and hay (8.21 MJ/kg) forms ( $p < 0.01$ ). The results can be explained by the low ADF and high hemicellulose contents of caramba fresh. The differences between results obtained from many studies

**Table 3.** *In vivo* and *in vitro* ME (MJ/kg DM) values of caramba

Parameters	Fresh	Silage	Hay	SEM	Significance
$\text{ME}_{in vivo}$	10.45	10.03	11.39	0.27	0.105
$\text{ME}_{CN}$	7.72 <sup>a</sup>	7.83 <sup>a</sup>	6.77 <sup>b</sup>	0.11	0.00
$\text{ME}_{ADF}$	9.40 <sup>a</sup>	8.96 <sup>b</sup>	8.21 <sup>c</sup>	0.15	0.00

ME, metabolizable energy; DM, dry matter; SEM, standard error of means; CN, crude nutrients; ADF, acid detergent fiber.

Means with different supercripts within a row are significantly different ( $p < 0.01$ ).

may depend on plant type, vegetation period, soil and climate, because ME value decreases significantly while CF content increases during the vegetation period.

In conclusion, there is generally no significant variation between different forms of caramba with respect to chemical composition, digestibility and energy value and thus, it may conveniently be used in the form of fresh, silage and hay as an alternative forage source in feeding ruminants.

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