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Research Article

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Determining Effects of Different Additives on Methane Production of Tobacco Straws and Silages

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Abstract This study was carried out to determine the effects of tobacco stalks (TS) (*Nicotiana tabacum*) and their silages with different additives (6% molasses (TSM), 3% urea (TSU) and 6% molasses+3% urea; TSUM) on *in-vitro* gas production parameters, methane production, organic matter digestibility (OMD) and energy values. The Hohenheim gas test was used to determine *in-vitro* gas production of the feeds and methane measurements were done with an infrared gas analyzer. The data were analyzed by using a complete randomized design. The highest CP content was found in TSUM. While methane production was decreased in TS and TSM, it was not affected by urea added treatments. TSU and TSUM had higher OMD values than the others. Also, it was found that TSU had higher energy content than the others. In conclusion, harvest residues of tobacco and their silages can be used in ruminant nutrition. This study was carried out to determine the effects of tobacco stalks (TS) (*Nicotiana tabacum*) and their silages with different additives (6% molasses (TSM), 3% urea (TSU) and 6% molasses+3% urea; TSUM) on *in-vitro* gas production parameters, methane production, organic matter digestibility (OMD) and energy values. The Hohenheim gas test was used to determine *in-vitro* gas production of the feeds and methane measurements were done with an infrared gas analyzer. The data were analyzed by using a complete randomized design. The highest CP content was found in TSUM. While methane production was decreased in TS and TSM, it was not affected by urea added treatments. TSU and TSUM had higher OMD values than the others. Also, it was found that TSU had higher energy content than the others. In conclusion, harvest residues of tobacco and their silages can be used in ruminant nutrition.

Keywords Tobacco, silage, gas production, straw, methane

1. Introduction

Roughages have a great importance in ruminant nutrition. However, it is difficult to get good quality roughage in all seasons of the year. The main reason for this is that the forage crops cultivation areas are insufficient. For this reason, it is important to increase the cultivation areas for forage crops, improve the pasture areas, and transform industrial by-products and field harvest wastes into animal production as alternative roughage sources to close the roughage gap. These wastes have an important feed potential in terms of the nutrients they contain. Knowing the feed values of these wastes will make significant contributions to their use in animal production as an alternative feed source [1, 2]. Grain harvest wastes are used as a source of roughage to close the roughage deficit, especially in winter. However, many researchers have recently conducted studies on grape wastes [3], pomegranate peels [4], sugar beet harvest wastes [5], hazelnut harvest wastes [6, 7].

It can be seen that the tobacco crop field residues, which have great potential, are used as fertilizers due to their high organic matter and low toxic element content, but some farmers burn the tobacco stalks. For this reason, tobacco harvest wastes that cannot be used effectively, studies about its use as fertilizer or alternative feed sources have recently intensified [2, 8]. To close the roughage gap, besides the production of alternative feed crops, drying and ensilage of roughages are emphasized in terms of their usability in winter.

In the assessment of nutrient content, gas production, energy value and digestibility of tobacco stalks and its pulp taken from different locations in our country, it has been reported that tobacco seed meal which has high protein content (38.61% CP) can be used as concentrate source where the stems (4.69% CP) as a roughage [2].In terms of condensed tannin (CT) contents, it was determined that tobacco straws (4.62%) and tobacco seed meal (3.28%) were lower than the maximum desired values (10%) for ruminants in feeds [2, 8].

It is believed that these residues can be consumed by ruminants after making silage with some treatments; adding molasses, urea, and pelleting them. One of the biggest advantages in silage production is to make bitter plants a palatable with an aromatic taste in which livestock can consume voluntarily. This study was aimed to increase the quality of roughage by ensiling tobacco straws. This will contribute to the economy. In this study, it was hypothesized that ensiling tobacco straws with molasses and urea as an additives will reduce enteric methane production in the rumen environment.

2. Materials and Methods

2.1. Materials

In this study were used stalks of tobacco *(Nicotiana tabacum L.)* plants collected as harvest residue from Samsun province (Cetirlipinar Village) in the Blacksea region of Turkiye. After being dried and ground to a size able to pass 25-30 mm sieve (tobacco straw=TS).In this study, 6% molasses, 3% urea, and 6% molasses+3% urea were added to tobacco straws before ensiling.

Rumen fluid used in *in-vitro* studies collected from Anatolian Black × Brown Swiss bulls (Average 24-30 months age and 400-500 kg live weight) slaughtered at a local slaughterhouse. Then it was brought to the laboratory within 15-20 minutes in a thermos (39°C). Rumen content mixed and it was taken under CO_2 and were filtered through two layers of cheesecloth.

2.2. Methods

2.2.1. Feeds supply and silage making: This study was carried out to determine the effect of stalks of tobacco (*Nicotiana tabacum*) harvest residues (TS) and its silages with different additives (6% molasses (TSM), 3% urea (TSU), and 6% molasses+3% urea; TSUM). Tobacco straws (TS) were chopped to about 2.5 cm. First dry matter contents of the samples were balanced as %25-40 DM by adding waters. Then the samples were ensiled into 5 replicate laboratory type PVC silos [9, 10].Tobacco straw was used completely (100%) in the control group. A total of eight groups (4 groups straws and 4 groups their silages) were prepared. Then all the silos were opened after two months (60 days).

2.2.2. Chemical analyses: The silages were dried in a forced-air oven at 55°C for 72 hours. Then, dried silages were milled in a hammer mill through a 1 mm sieve for chemical analysis and *in-vitro* study assay. The samples were analyzed for dry matter (DM), ash and crude protein (CP) contents according to AOAC [11] procedure. Kjeldahl N and CP were calculated by multiplying N by 6.25. The neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fiber (CF) analysis were done according to the method of Van Soest*et al.* [12] using Ankom2000 semi-automated fiber analyzer (Ankom Technology). The ether extract (EE) content was determined using Ankom ^{XT15} analyzer [13]. The contents of organic material (OM) and nitrogen-free extract (NFE) were determined by calculation. Condensed tannin contents were determined according to Makkar *et al.*, [14]. All chemical analyses of samples were carried out in triplicate.

2.2.3. Determining *in-vitro* gas production*:* In this study, approximately 200 mg dry weights of samples were weighed into 100 ml glass syringes following Hohenheim gas test procedures of Menke and Steingass [15]. The syringes were warmed at 39ºC before the injection of 30 ml rumen fluid-buffer mixture (1:2) into each syringe and incubated in a water bath at 39ºC. Gas volumes were recorded at 0, 3, 6, 9, 12, 24, 48, 72, and 96 h of incubation. Five repetitions of each sample were used in the *in-vitro* gas production experiment. Net gas productions of silages and straws were determined at 24 h after incubation and corrected for blank and hay standard. Cumulative gas production data were fitted to the model of Ørskov and McDonald [16] by the NEWAY computer package program.

Organic matter digestibility, ME, and NE_L contents of the samples were estimated using equations given below:
OMD, % = 14.88+ 0.8893GP + 0.448CP + 0.651 ash [17]
ME, MJ/kg DM = 2.20+0.136GP + 0.057CP + 0.002859 EE^2 [17]
NE_L, MJ/kg DM = 0.101GP + 0.051CP + 0.11EE [18]
Where; GP: 24 h net gas production (ml/200mg DM), CP: Crude protein (%), EE: Ether extract (%)

2.2.4. Determining methane production: Methane contents (%) of total gas produced at 24 h fermentation of the samples were measured using an infrared methane analyzer (Sensor Europe GmbH) [19]. After measuring gas produced at 24 h incubation, gas samples were transferred into the inlet of the infrared methane analyzer. Methane production (mL) was calculated as follows.

Methane production (mL) = Total gas production (mL) × the percent of methane (%)

2.2.5. Determining rumen fluid pH, total volatile fatty acids (TVFA) and ammonia nitrogen (NH_3-N): Rumen fluid pH values were determined using a digital pH-meter (Hanna Instrument) in three replicates. The total volatile fatty acid (TVFA) and NH_3-N analysis of rumen fluids were done according to Markham [20] steam distillation in three replicates.

2.2.6. Statistical Analysis: The data obtained from the experiments are analyzed using SPSS 20.0 software package by Ondokuz Mayis University licensed. Nutrient content, *in-vitro* gas production, and silage quality data of the feeds investigated in this study were analyzed by the completely randomized design controlling for normality and variance homogeneity. Duncan's multiple range test was used for the comparison of mean values.

3. Results and Discussion

The nutrient contents of the tobacco harvest waste silages and straws examined in the study and the cell wall structural elements on a dry matter basis are given in Table 1. According to this; In terms of CP content in the roughages used in the study, the groups with the addition of urea+molasses showed the highest value ($P<0.05$). It was determined that CP content decreased for the treatments using only urea in making silage ($P<0.05$). This situation is thought to be caused by the breakdown of proteins in the form of ammonia during silage production [2, 9]. In different studies, CP contents of tobacco straws were reported to be between 5.21% and 15.52% [2, 8, 21, 22]. Therefore, the addition of molasses and molasses+urea in silage increases the nutrient content of feeds. Similarly, Pekpazar [8] reported that CP content increased from 5.21% to 6.14% in the addition of molasses in non-ensilaged tobacco straws; this value increased to 13.25% with the addition of urea+molasses. In this study, CP content showed a similar increase in molasses and urea+molasses additions.

In terms of crude fiber (CF) content, silages showed the highest values in urea or urea+molasses, while lower CF was determined in control group silages ($P<0.01$). In straws, urea treated had higher CF values than others ($P<0.01$). Although it is generally observed that CF content in silage production is numerically higher than straw, this may be due to the use of easily soluble carbohydrate sources in silage fermentation and consequently the increase of high fiber content (cellulose rich) material [9, 10]. Pehlivan and Ozdogan [22], Kilic *et al.*, [2] and Pekpazar [8] reported 32.30-53.53%, and these data were found to be similar to the 43.11% value found in the current study.

Condensed tannin (CT) contents determined in tobacco silage control group and straw were 0.60% and 0.50%, respectively. This value is lower than the values (4.62%) reported by Kilic *et al.*, [2]. In this study, lower than 5% CT contents determined in both silage and straw are acceptable levels. Therefore, it is thought that the percentage of CT in tobacco straw and its silage will not adversely affect ruminant feed intake. However, ruminants show different tolerance to the CT content of feeds. Goats have a higher tolerance level than sheep and it is known that goats can tolerate 8-10% tannins in their diets [23, 24] and it can be said that goats are the most suitable animals for tobacco silage and hay.

In-vitro gas production of tobacco straws and silages and pH values measured after 96 hours of incubation are given in Table 2. According to this, molasses added silages had higher gas production values than silage control group ($P<0.05$), while other treatments were found to be statistically not significant ($P>0.05$). In the study, the gas production determined in the silages during the 6, 9, 12, 24, 48, 72, and 96 hours incubation was higher than the control group in all treatments ($P<0.01$).

Table 1: Chemical compositions of the straws and their silages, % (as DM)

Treatments	DM*	OM	Ash	CP	EE	CF	NFE	NDF	ADF	ADL
Silages										
Control	63.01	84.89±1.36c	15.11±1.36a	8.80±0.31d	3.17±0.81a	46.06±3.22bc	26.86±2.50ad	60.70±1.88cd	44.51±.171d	34.36±2.05d
Molasses	60.67	86.68±1.07b	13.32±1.07b	11.09±0.47c	2.14±0.40b	50.24±3.31ab	23.20±3.36ce	62.53±0.59bc	48.12±0.32c	38.23±0.52bc
Urea	61.71	89.20±0.73a	10.80±0.73c	11.43±0.48c	1.74±0.36b	54.59±5.50a	21.43±5.54e	67.15±0.51a	53.02±0.07a	42.77±0.42a
Molasses+Urea	63.61	89.09±0.31a	10.91±0.31c	13.29±0.67a	1.47±0.40b	51.44±1.10a	22.88±1.60de	62.94±0.09b	50.80±1.23b	39.63±1.01b
Straws										
Control	93.70±0.52	83.92±0.14c	16.08±0.14a	8.28±0.73d	3.64±0.26a	43.11±1.19c	28.89±2.19ab	59.23±1.18d	42.68±0.81e	34.15±0.78d
Molasses	92.38±0.14	87.52±0.40b	12.48±0.40b	11.68±0.68bc	1.96±0.17b	43.50±1.24c	30.38±1.45a	60.42±1.06cd	44.70±1.02d	37.44±1.01c
Urea	93.06±0.22	89.81±0.71a	10.19±0.71c	12.18±0.46b	1.69±0.07b	51.03±2.26a	24.90±2.20be	65.72±1.05a	51.18±0.52b	42.11±0.70a
Molasses+Urea	92.65±0.09	87.90±0.61b	12.10±0.61b	13.26±0.50a	1.78±0.12b	44.87±3.10c	27.99±2.13ac	60.47±1.64cd	45.70±0.62d	36.51±0.52c
Significantly	-	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000

*DM: dry matter (Natural form), OM: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fibre, NFE: nitrogen free extracts NDF: neutral detergent fibre, ADF: acid detergent fibre ADL: acid detergent lignin, P<0.001; a,b,c: Means with different superscripts in the same column are significantly different.

In terms of *in-vitro* gas production, only urea added treatments showed the lowest numerical values among straws, and molasses added groups showed the highest values. In terms of 24-hour gas production, the addition of molasses had a higher gas production value than other treatments (P<0.001).

At the 24th, 48th and 72nd hours of *in-vitro* incubation there was a decrease of gas production in the control group and the groups with only molasses (P<0.001), the effect of ensiling on *in-vitro* gas production was no significant in other treatments. In the study, when using molasses in silage making, the rapid consumption of lactic acid bacteria in the silo and the formation of gas may have caused the decrease of total gas production value in the silage. Thus, it has been found that the highest gas losses determined in silages added with molasses (3.00%). Besides, the use of molasses is a matter of lactic acid formation that reduces gas production value. These situations reveal the reason for the low gas production value in molasses added silages. Low gas production means that there is a decrease in the ruminal breakdown rates of nutrients. High protein feeds generally lead to low ruminal gas production. In addition, ammonia produced as a result of ruminal fermentation of proteins prevents CO_2 gas production by neutralizing H+ ions to from VFAs. Low ruminal gas production can also be explained by the high fat content in feed material [25].

Kilic *et al*. [2] reported the 24-hour gas production of tobacco straws as 29.95 ml / 200 mg DM, while Pekpazar [8] reported this value as 23.41 ml / 200 mg DM. The value found in this study was similar to the literature reports. The same researchers calculated the 96 hour gas production value as 37.83 ml / 200 mg DM and 36.88 ml / 200 mg DM respectively, and these values were higher than the value in the present study (27.79 ml / 200 mg DM). It can be said that the difference arises from the type of feed, harvest time, applied methods, rumen fluid properties, etc. [26]. The 24-hour *in-vitro* gas production of tobacco straws with the addition of molasses and urea+molasses was found to be 22.44 ml / 200 mg DM and 30.79 ml / 200 mg DM respectively. These values were determined as 31.28 ml / 200 mg DM and 21.17 ml / 200 mg DM in non-ensiled materials in the present study.

Table 2: *In vitro* gas productions (ml/200 mg DM) and pH values after 96.h incubation for samples

Treatments	Incubation times, hour								
	3	6	9	12	24	48	72	96	pH*
Silages									
Control	4.95±3.36b	8.80±3.42bc	10.42±3.46e	12.68±3.65d	16.57±5.30d	18.40±5.50d	18.48±5.88c	20.32±5.23d	6.71±0.10ab
Molasses	5.34±1.33a	11.60±1.77a	14.96±2.05cd	18.12±2.10bc	22.22±1.62bc	24.50±1.74bc	25.31±1.58b	25.82±1.60c	6.62±0.13b
Urea	4.04±1.68ab	11.77±1.24a	16.03±1.52b	19.83±1.85ab	25.71±2.88bc	27.95±3.30bc	31.22±4.49b	30.97±2.16b	6.64±0.18b
Molasses+Urea	4.49±0.58ab	11.51±0.71a	16.41±1.48ab	20.09±1.67ab	26.65±2.05ab	29.55±2.24b	31.31±2.87b	31.75±1.96b	6.49±0.25b
Straws									
Control	2.66±1.11bc	8.40±1.19bc	13.84±2.12cd	17.63±1.45bc	23.41±1.70bc	25.69±2.33bc	26.14±1.34b	27.79±2.92bc	6.60±0.04b
Molasses	4.56±0.66ab	9.78±2.65ab	19.54±3.78a	23.31±0.86a	31.28±1.48a	34.94±1.04a	38.68±1.62a	37.67±1.35a	6.45±0.10b
Urea	0.36±0.62c	6.52±0.27c	11.84±1.23de	16.04±2.04c	21.17±4.22cd	23.85±4.73c	26.09±4.34b	25.64±4.96c	6.91±0.21a
Molasses+Urea	3.26±0.54ab	9.69±0.92ab	16.34±0.46ab	20.77±0.59ab	26.15±1.01bc	28.51±1.85bc	29.68±0.89b	30.32±1.31bc	6.57±0.08b
Significantly	0.038	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.038

*Measured pH values after 96.hours incubation. P<0.001; a,b,c..: Means with different superscripts in the same column are significantly different.

The pH values measured at the end of the incubations in the study should be at a suitable value for the rumen micro-organisms to perform their activities [26]. This situation is tried to be achieved by adding a buffer. If the buffer had been depleted, the measured pH value would have shifted to acidity. Under rumen conditions, the optimum pH value is considered to be between 5.5-7.0. Therefore, the pH values obtained in this study was the optimum to provide appropriate rumen conditions and indicate that the buffer substance is not consumed during the study. However, a higher pH value (6.91) in tobacco straws with only urea added, and lower pH values in those with only molasses (6.45) were expected. As an easily soluble energy source, molasses lead a decrease in the rumen pH value, but urea increase the pH value due to the ammonia formation.

In-vitro gas production parameters, methane productions, OMD, ME, and NE_L contents of tobacco straws and silages are given in Table3. In the study, the highest numerical value of "a value" was determined in the control group silage, while the lowest values were determined in the straw with urea and molasses+urea. The "a value" increased for all groups in silage ($P<0.001$). In terms of “b value” and total gas production (a+b value), the highest numerical values were found in the straws with molasses added, while the lowest b values were found in the control group silage. It was determined that silage making decreased the “b value” and “a+b” values in the control group and only molasses added groups ($P<0.001$), but had no effect on other treatments.There was no statistically significant difference between treatments in terms of gas production rate (c value) ($P> 0.05$). In terms of the amount of methane, straws with the highest numerical value was molasses added, where the lowest values were shown by the silage control groups.

Methane production in tobacco straws was determined as 1.85 ml by Pekpazar [8] and 4.62 ml by Kilic *et al.*, [2], and these values were similar to the value found in the present study (3.52 ml). Methane production in tobacco straw treated with molasses and urea+molasses was found to be 2.11 ml and 4.30 ml, respectively, by Pekpazar, [8], while in the current study, these methane production values were determined as 5.34 ml and 3.51 ml.

Table 3*:* *In vitro* gas production parameters, methane production, OMD, ME and NE_L values for samples

Treatments	**a, ml**	**b, ml**	**c, ml/h**	**a+b, ml**	**Methane, ml**	**OMD %**	**ME,MJ/kg DM**	**NE_L, MJ/kg DM**
					Silages			
Control	1.06±3.45a	18.24±5.26d	0.07±0.04	19.30±5.60d	2.25±0.90c	35.82±3.04ab	4.07±0.47d	1.63±0.35bc
Molasses	-1.86±0.88ab	26.98±1.81c	0.11±0.02	25.13±1.50c	3.33±0.40b	38.15±1.57a	4.70±0.24ab	1.93±0.18a
Urea	-3.87±3.87bc	33.93±6.54bc	0.10±0.01	30.06±3.24bc	3.77±0.88b	36.97±1.10ab	4.77±0.17ab	1.92±0.12a
Molasses+Urea	-3.71±1.49bc	34.67±2.99b	0.09±0.01	30.97±2.25b	4.23±0.64b	37.51±0.63a	4.94±0.10a	1.97±0.07a
Straws								
Control	-6.40±0.41cd	33.02±2.22bc	0.11±0.02	26.62±2.22bc	3.52±0.51b	37.83±1.06a	4.51±0.16bc	1.92±0.12a
Molasses	-6.59±2.19cd	43.85±1.09a	0.09±0.02	37.26±1.78a	5.34±0.41a	36.83±2.36ab	4.70±0.36ab	1.83±0.27ab
Urea	-8.54±0.50d	33.70±5.02bc	0.10±0.01	25.16±4.91c	3.51±0.79b	32.24±0.24c	4.26±0.03cd	1.47±0.03c
Molasses+Urea	-7.75±1.86d	37.22±1.26b	0.11±0.01	29.47±1.33bc	4.17±0.39b	35.05±0.81b	4.69±0.12ab	1.80±0.09ab
Significantly	0.000	0.000	0.241	0.000	0.000	0.001	0.000	0.003

a, gas production from the immediately soluble fraction (ml), b, gas production from the insoluble fraction (ml), c: the gas production rate constant for the insoluble fraction (ml/h), a+b: total gas production, OMD: organic matter digestibility, ME: Metabolizable energy, NE_L: Net energy lactation, IVTD: *In vitro* true digestibility. $P<0.001$; a,b,c..: Means with different superscripts in the same column are significantly different

In this study, the lowest OMD value was observed in only urea added straws ($P<0.001$). OMD was increased in silages made with the addition of urea and molasses+urea ($P<0.001$). There was no statistical difference in OMD between silages ($P>0.05$). Therefore, urea and molasses+urea treated groups can be recommended in terms of animal feeding as silage increased the OMD value. Kilic *et al.* [2] determined the OMD content of tobacco straws as 49.42% where Pekpazar [8] reported it as 44.67%. Pekpazar [8] determined the OMD value as 44.55% and 55.20% for molasses and urea+molasses-added straws. In this study, the OMD value (36.32% and 32.24%) determined for molasses and urea+molasses added straw was lower than the literature reports. This situation may be caused by factors such as feed source, conditions of the region where it is grown, climate, irrigation, fertilization, etc. [26, 27].

Ensiling of tobacco straws decreased ME and NEL contents in the control group, whereas these contents increased in urea treatment ($P <0.01$). Control groups of silage and only urea added straw had lower energy content than others (P<0.01). Kilic et al. [2] reported the ME and NEL contents of tobacco straws as 6.55 MJ / kg DM and 3.42 MJ / kg DM, while Pekpazar [8] reported 5.73 MJ / kg DM and 2.88 MJ / kg DM respectively. These values were lower than those found in the current study.

4. Conclusion

As a result, making silage of tobacco field harvest residue improved feed value in terms of nutrient contents, *in-vitro* gas and methane productions, OMD, ME, and NE_L contents. It was concluded that tobacco stalks (straw) and its silage which have significant roughage potential that cannot be utilized economically effective, can be used in ruminant feeding. Particularly molasses and molasses+urea added tobacco residue silages appear to be an important alternative roughage source. However, it is recommended to study the effects of molasses and urea+molasses treated silages of tobacco straw on animals under *in-vivo* conditions and to determine their effects on feed consumption directly.

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