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Effects of Different Additives on Feed Values of Tobacco Straws and its Silages

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Abstract This study was carried out to determine the effect 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 nutrient contents, silage quality and relative feed values (RFV). The data were analyzed by using a complete randomized design. The highest CP content was determined in TSUM. The silage quality of TSU was classified as “moderate” and the others was as “good” as a result of the sensory evaluation. According to the Flieg-point system, TSM had the highest quality. In conclusion, harvest residues of tobacco and their silages can be used as an alternative roughage in ruminant feeding and molasses is the most suitable additive in making tobacco silage among the other treatments.

Keywords Tobacco, silage, roughage, straw, relative feed value, ruminant feeding

1. Introduction

The most important problem that limits economic livestock production particularly ruminants is the high prices of roughage and concentrate feeds. One of the problems encountered in the supply of roughages, which has an important place in the rations of ruminants, is the insufficient quality forage crop cultivation areas. For this reason, it is important to increase the cultivation areas of available fodder crops, improve the meadow pasture areas, and convert industrial by-products, factory and field harvest wastes into animal feed as alternative roughage sources in order to close the forage scarcity, which is seen in Turkiye especially in winter months. With the increase of agricultural production in the worldwide, the amounts of both crop harvest residues and agricultural industry waste have been increasing. In addition to being a serious source of organic matter loss, these wastes also have an important potential in terms of the plant nutrient contains. Knowing the feed values of these wastes will provide significant contributions to their use in animal production as an alternative feed or roughage source [1, 2]. Cereal grains harvest residues take an important place as an alternative roughage source in order to close the forage gap seen especially in the winter season. However, many researchers recently conducted studies about grape seeds and pomace [3], pomegranate peels [4], sugar beet heads and leaves [5], hazelnut harvest wastes such as husk and empty hazelnuts [6, 7].

The annual amount of herbal and agricultural waste in the world is approximately 9.6 billion tons [8]. Due to its high organic matter and low toxic element content, it is possible to use tobacco harvest wastes as a soil conditioner (fertilizer). However, it is also observed that tobacco stubbles are wasted by burning in the field. Therefore, it was investigated the possibilities of using tobacco harvest residues left in the field in different areas. Nowadays, studies on the use of these residues as fertilizers or alternative feed sources have intensified [2, 9]. In addition to the production of alternative feed crops in order to close the roughage gap, making hay and ensilage of forages are emphasized to use in winter months. Ensiling is an important roughage preservation method to fill the quality roughage gap in the winter. In this context, all green plants and alternative roughage sources can be ensiled.

It is known that tobacco stems are ensiled or mixed with straw and given to sheep, where the leaves and seeds left in the field after harvest are consumed by goats. As a result of the evaluation of the nutrient content of tobacco stalks (straw) and bagasse taken from different locations in Turkiye, it was reported that tobacco seed meal can be used as a concentrated feed source with high protein content (38.61% HP) where stems (4.69% HP) as a roughage source [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, 9]. Therefore, it is thought that tobacco stems can be used successfully in goats that tolerate its bitter taste. Thus, it is believed that the silage of these residues can be consumed by other ruminants after treatment of molasses and urea. Indeed, one of the biggest advantages of making silage is to provide bitter plants with an aromatic flavor and make them be consumed by animals [10]. The purpose of this study was to increase the quality of roughage by ensılıng tobacco straws with appropriate additives. Thus, it is aimed to contribute to the economy by using the tobacco harvest residue straws as a part of the roughage needs. The hypothesis of the study was that the molasses and urea used as additives in the ensiling process will increase the quality and relative feed value of tobacco straw silages.

2. Materials and Methods

2.1. Materials

The tobacco (Nicotianatabacum L.) harvest residue stalks used in this study were collected from Samsun province (Cetirlipinar Village) in the Black sea region of Turkiye. After being dried tobacco straws, it was ground to a size that can pass through 25-30 mm sieve.In this study, 6% molasses, 3% urea and 6% molasses + 3% urea were added to tobacco straws duringthe ensiling process.

2.2. Methods

Feeds supply and silage making: This study was carried out to determine the effect of stalks of tobacco (*Nicotiana tabacum*) harvest residues (TR) and their silages with different additives (6% molasses (TRM), 3% urea (TRU) and 6% molasses + 3% urea; TRUM). Tobacco straw (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 [11].Tobacco straw was used completely (100%) in the control groups. Total eight groups (4 groups straws and 4 groups their silages) were prepared. Then all the silos were opened after two months (60 days) of anaerobic preservation.

2.2.1. 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 assays. The samples were analyzed for dry matter (DM), ash and crude protein (CP) contents according to AOAC [12] 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.* [13] using Ankom 2000 semi-automated fiber analyzer (Ankom Technology). The ether extract (EE) content was determined using Ankom ^{XT15} analyzer [14]. 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 [15]. All chemical analyses of samples were carried out in triplicate.

2.2.2. Determining pH and VFA analysis in silages

The pH values of silages were determined at samples obtained from different parts of silages. With this aim, 25 g silage sample was put in a mixer, 100 ml distilled water added and mixed for 5-10 minutes. Then, the fluid part of the mix was filtered to a beaker via a filter paper and after 15-20 minutes the pH was measured using a digital pH-meter in three replicates. Ammonia nitrogen contents of the silages were determined according to the Kjeldahl method [16]. Lactic acid (LA) and volatile fatty acids (acetic acid (AA), butyric acid (BA) and propionic acid (BA)) contents of the samples were determined using a high-performance liquid chromatography

(HPLC) device (Agilent 1100 HPLC; Agilent Technologies) according to Tjardes et al., [16] and Canale et al., [17].

2.2.3. Determining forage quality and silage quality

The relative feed value (RFV) of tobacco silages were calculated as follows [18];

Dry matter digestibility (DMD, %) = 88.9-(0.779 ×ADF %)

Dry matter intake (DMI, live weight, %) = 120/ (NDF%)

Relative feed value (RFV %) = (DMD × DMI)/1.29

The quality class of the silages were determined by using Flieg score (FS) as following formula [10].

$$\text{Flieg score (FS)} = 220+(2 \times \text{dry matter \%} - 15) - 40 \times \text{pH}$$

The required pH value in a silage is related to DM content. In other words, each silage should have a pH value which is determined according to its DM content. The "required pH values" were determined by using following formula [19]. This pH value prevents the proliferation of clostridia and enterobacteria.

$$\text{Required pH (RpH)} = 0.00359 \times \text{DM (g/kg)} + 3.44$$

2.2.4. Statistical Analysis

The data obtained from the experiments were analyzed using SPSS 20.0 software package by OndokuzMayıs University licensed. Nutrient contents and silage quality data of the feeds investigated in this study were analyzed in accordance with 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 and the cell wall structural elements of the tobacco harvest residue silages and straws examined in the study were summarized in Table 4.1 on dry matter basis. According to this, in terms of CP content of the roughages used in the study, the groups with the urea + molasses addition showed the highest values ($P < 0.05$).The high CP content determined in molasses + urea treatment is that the additive effect of molasses in addition to the amount of urea used in the study. Thus, the CP Content was increased proportionally compared to urea addition alone. As it was expected the lowest CP contents were found in the control groups. In the study, it was determined that CP content was decreased in the treatments using only urea during silage making ($P < 0.05$). It is thought that this was due to the breakdown of proteins in the form of ammonia during silage production [2, 11]. In previous different studies, the CP contents of tobacco straws were reported between 5.21-15.52% [2, 9, 20, 21]. The CP value determined in this study was within the range of literature reports. It is thought that the differences observed in the study were due to the variety, fertilization, soil structure, harvest time, applied processes, etc [22].

Nitrogen-free extract (NFE) contents indicate easily soluble carbohydrates in feeds and determine the amount of nutrients that can easily be converted to energy. In the study, the amount of NFE in the silage decreased numerically in all treatments, particularly it was determined that all molasses treated groups statistically decreased ($P < 0.01$).This was due to the fact that easily soluble carbohydrates were used as nutrients by lactic acid bacteria during silage fermentation [10], thus, molasses is a nutrient rich in NFE.

In terms of the cell wall structural contents (NDF, ADF and ADL), only urea added groups showed the highest values among silages and straws ($P < 0.001$).In the study, it was observed that NDF and ADF contents increased in all silage treatments ($P < 0.001$).In this situation, it is thought that it is caused by the proportional increase in fibrous components that occur due to the consumption of microorganisms the easily soluble carbohydrates in silage fermentation and converting to lactic acid [10, 11]. In the study, it was determined that ADL contents increased in the silage of urea + molasses added groups ($P < 0.001$), while silage making had no effect on lignin content in the other groups.

According to this, considering that high NDF content will reduce feed consumption [22], it is expected that intake will be decreased in the feeds containing high NDF levels of dry matter. However, due to the high water content of silages, their natural consumption may be higher than to those of straw. Therefore, the expectation for a reduction in feed take should be evaluated at the dry matter level. In this context, it is seen that the highest feed consumption will be in molasses (on dry matter basis) and molasses + urea groups, followed by the control group. As an indication of digestibility, in terms of ADF content straws will have higher dry matter digestibility than silages; it should be noted that the digestibility on dry matter basis in silage production will be lower than that of straw. However, it was determined that the highest digestibility in both straws and silages would be in the control groups which shown the lowest ADF value.

Different studies determined that the NDF contents of feeds were between 50.36-72.53% and ADF contents between 42.08-53.48% [2, 9, 20, 21]. The NDF and ADF values found in this study remained between the upper and lower limits of the literature reports. Various studies determined that lignin (ADL) content of tobacco stems was varied between 14.77-38.63% [2, 9, 23, 24] and the value found in this study (34.15%) was among the literature report ranges. The differences observed may be due to factors such as variety, soil structure, fertilization, harvest time, treatment of feed etc.

The DMD, DMI and RFV index of feeds are given in Table 2. In the analysis of the feed value after and before ensiling the tobacco field residue straws and silages on the table, the lowest values in terms of DMD, DMI and RFV were determined in the urea only treated (P <0.001).In other words, with the increase of CP content in urea added groups, it was also determined that the quality of roughage decreased by considering the NDF and ADF contents. In terms of DMD in both hay and silage, other treatments had better digestibility than the groups with only urea added, and the best DMD values were determined in the control groups.

Table 1: Nutrient contents of the samples used in the experiment, % (as DM)

Samples	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 the study, it was determined that straw with molasses had similar DMD content to the silage control group (P> 0.05). In the analysis of the values obtained in terms of dry matter intake, it was observed that the DMI value of the straws with only urea added and their silage gave poorer results than the other groups, and the highest DMI values were found in the control groups in both straw and silage. On the other hand, in terms of RFV control groups showed the highest values in all groups (P <0.001). Factors affecting roughage quality are the same as factors affecting NDF and ADF content and feeds with high NDF and ADF contents are expected to have low RFV values.

Condensed tannin (CT) contents determined in tobacco silage and straw were 0.60% and 0.50%, respectively. This value is lower than the values (4.62%) reported by Kilic et al, [2]. It has been reported that the CT content in feeds at the level of 5-10% causes repulse in animals and reduces feed intake. In addition, it is known that CT decreases the body weight gain, digestion and absorption of nutrients, affects performance negatively and causes toxic effects [25]. In this study, less than 5% CT contents determined in both silage and straws are acceptable levels and in this context it will not negatively affect feed intake in ruminants. However, ruminants show different tolerance to the CT content of feeds, goats have a higher tolerance level than sheep, and goats can tolerate 8-10% tannins in their diets [26]. Thus, by considering this, it can be said that the most suitable animals for untreated tobacco silage and straw are goats.

The gas losses during fermentation, pH values, initial dry matter contents and organoleptic (color, odor and structure) results of silage made from tobacco harvest residues are summarized in table 3. According to the table, it was determined that the highest gas loss during fermentation was found in molasses alone treated silages with 3.00%, and the least gas loss was in only urea treated group (0.93%). In good quality silage the pH value is required to be around 4 (3.8-4.2). In the study, it was determined that the closest silage to this value was the molasses added group (pH 5.30), while the pH value of the other silages ranged between 7.58-8.43, that is an undesirable pH value in terms of silage quality. Although molasses addition was expected to show a lower pH value than the other groups, it was concluded that the amount of molasses required to decrease the pH value of tobacco silage should be higher than the dose used in this study.

It was determined that the required pH (RpH) value in silages, the lowest was the molasses added group (5.64) and generally ranged between 5.64-5.73. It was found that the measured pH (MpH) value of other groups were quite high except the value observed in the molasses treatment (5.30). Therefore, in order to ensure the proper pH value of the tobacco silages in this study, it is recommended to use additives that reduce the pH (enhancing lactic acid production) of other groups except the molasses treated group.

According to the organoleptic analysis results shown in Table 3, all silages were in the good quality silage class except urea only treated group, which was evaluated in the medium quality silage. According to the sensory evaluation made, it is thought that making tobacco residue silage with urea alone may have some negativities compared to other treatments and may be a problem with the animal feed intake. However, it should not be forgotten that the quality assessment based on the organoleptic analysis results of silages alone will not be healthy. In determining the quality of silages, nutrient content, voluntary feed intake and its effect on animal performance should be taken into account.

The highest lactic acid content in silages was determined in molasses addition as expected, while the lowest found in urea treated groups. This is due to the use of easily soluble carbohydrates by lactic acid bacteria. In terms of acetic acid content, silages with molasses + urea added had the highest values, and the lowest value was determined in the control groups. In isobutyric acid content, molasses added group showed the highest value, followed by molasses + urea added groups. On the other hand, the lowest isobutyric acid content was determined in the control groups. Butyric acid was not found in the control and molasses-added silage groups, while the highest value was observed in molasses + urea treated groups. According to this, it can be said that LA, which is desired to be high in quality silages, found a higher value in the addition of molasses, the other silages need LA content enhancing additives in order to improve the silage quality, especially in groups with high acetic acid content; urea and molasses + urea added groups.

Table 2: DMD, DMI and RFV values of the feeds used in the study

Samples	**DMD, %**	**DMI, %LW**	**RFV**
		Silages	
Control	54.22±1.33b	1.98±0.06ab	83.20±4.65ab
Molasses	51.41±0.25c	1.92±0.02bc	76.49±1.07c
Urea	47.60±0.06e	1.79±0.01d	65.94±0.58e
Molasses+Urea	49.33±0.96d	1.91±0.00c	72.90±1.31cd
		Straws	
Control	55.65±0.63a	2.03±0.04a	87.44±2.71a
Molasses	54.08±0.80b	1.99±0.03ab	83.29±2.39ab
Urea	49.03±0.40d	1.83±0.03d	69.42±1.40de
Molasses+Urea	53.30±0.48b	1.99±0.05ab	82.03±2.89b
Significantly	0.000	0.000	0.000

DMD: Dry matter digestibity, DMI: Dry matter intake, RFV: Relative feed value, $P<0.001$; a,b,c..: Means with different superscripts in the same column are significantly different. According to the Quality Grading Standard assigned by The Hay Marketing Task Force of the American Forage and Grassland Council, the RFV were assessed as roughages based on prime >151, 1 (premium) 151-125, 2 (good). 124-103, 3 (fair). 102-87, 4 (poor). 86-75, 5(reject)< 75.

In terms of Flieg score in tobacco silages, the best results were observed in the groups with molasses alone, while the lowest found in treatments with urea. When silages were classified in terms of ammonia nitrogen content according to Wilkinson [27], the silages control group had good quality fermentation with an average of 3.92, while groups with molasses was determined 5.54 and it was placed in the class of good fermented silages. In the silages treated with urea and urea + molasses these values were determined as 37.02 and 37.68 respectively, and since the values found are above 15, they were classified as poor fermented silages. This may be attributed to the negative effect of urea supplementation on lactic acid fermentation. Therefore, the NH_3-N content in the silages above 10-15 is a sign of a significant amount of protein breakdown and may shorten the storage time of silages.

Table 3: Silage pH, volatile fatty acid contents, other parameters and silage qualities

	Control	Molasses	Urea	Molasses+Urea
Measured pH	7.58±0.18	5.30±0.46	8.43±0.11	8.01±0.08
Required pH	5.70±0.06	5.64±0.13	5.65±0.03	5.73±0.02
Lactic acid %	0.48±0.25	6.81±0.58	0.00±0.00	1.00±0.93
Acetic acid %	1.00±0.40	1.70±0.25	3.27±2.21	28.58±5.26
Isobutyric acid %	1.17±0.53	4.83±0.35	3.09±0.25	4.06±0.07
Butyric acid %	0.00±0.00	0.00±0.00	0.31±0.01	3.13±0.05
Gas loss* %	1.35±0.34	3.00±1.24	0.93±0.27	1.45±0.43
NH_3-N	3.62±0.89	5.54±1.13	37.02±2.44	37.68±4.89
Smell	10.9	9	8.2	8.5
Structure	3.7	3.25	3.3	3.6
Color	1.95	1.85	1.95	1.9
Total Point	16.55	14.1	13.45	14
Quality Class	Good	Good	Moderate	Good
Flieg Point	27.60±4.90	115.39±18.08	-9.07±4.94	11.84±3.00
Quality Class	Poor	Very Good	Very Poor	Very Poor

*: Gas loss at the end of fermentation.

4. Conclusion

According to the findings obtained in the study, among treatments it was observed that the highest feed intake (on DM) will be the straw and silage of control group and that the CP content will increase in the groups added with urea. It was determined that the pH value of the silage added with molasses was lower than the other treatments. However, in future studies, it is recommended to use more molasses than the amount used in this study in order to achieve the ideal pH value (3.8-4.2). In terms of relative feed value (RFV), silages were listed as Control> Molasses = Molasses + Urea> Urea, and straws as Control = Molasses> Molasses + Urea> Urea. As a result, it is thought that the tobacco stalks (straw) and its silage, which are left in the field after harvest and cannot be used economically, can be used as alternative roughage in ruminant feeding. Finally, it is recommended to study the effects of silages of tobacco straw (molasses and urea additions) on animals under *in vivo* conditions and directly determine their effects on feed intake.

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