

# A study on the feed value, *in vitro* digestibility and methane production of tobacco (*Nicotiana tabacum* L.) field waste

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**Summary.** This study aims to determine the nutrient contents, forage quality, *in vitro* true digestibility and methane production of the pellets prepared using straw and meal of tobacco (*Nicotiana tabacum* L.). Tobacco stalks (straws) and seeds collected from 25 different locations were used in this study. Daisy incubator was used to determine the *in vitro* true digestibility. *In vitro* gas production of the feed was determined using Hohenheim gas test while infrared methane analyzer was used to determine methane production. The study was designed in accordance with the completely randomized design. The results of the study showed that tobacco meal offers higher values in terms of crude protein and ether extract contents, while it offers lower ash, nitrogen free extract contents and cell wall fiber components when compared to tobacco straw ( $P < 0.001$ ). The crude protein content of the feed was found to be 4.69% for tobacco straw and 38.61% for tobacco meal. Tobacco meal outperformed tobacco straw with respect to methane production ( $P < 0.05$ ), organic matter digestibility, energy values and *in vitro* true digestibility ( $P < 0.001$ ). With respect to relative feed value, tobacco straw is classified under “5-reject” quality class. The condensed tannin contents of tobacco wastes were found to be below the risk limits for ruminants. The results suggest that tobacco straw can be used as an alternative to wheat straw and poor quality hay, while tobacco meal can be used as an alternative for some meals such as cottonseed meal and sunflower seed meal.

**Key words:** *In vitro* true digestibility, methane, pellet, tobacco straw, tobacco meal

## Introduction

Ruminants require the provision of quality and cheap forage on a regular basis. However, fodder crop cultivation in the world is not sufficiently able to meet the forage need which translates into significant amounts of forage deficit. Price hikes of the poor quality forage (straws, etc.) in some countries in times when there is a shortage of forage have a negative impact on the livestock production economy. Therefore, the importance of alternative resources to be used to close the forage gap comes to prominence. Today, several studies are investigating the potential use of tree leaves, prickly weeds and shrubs, industrial waste and

harvest waste as forage sources (1). With the use of a number of waste which are offering high nutritional values in animal nutrition, reintegration of such waste to the economy is of importance. Pehlevan and Ozdogan (2) reported that tobacco harvest waste of graze by small ruminants in a field after tobacco harvest. However, consumption of the animals was not at a satisfactory level. In this context, it can be seen that tobacco harvest waste cannot be used economically. Nevertheless, tobacco straws (TS) can be repurposed as forage source in animal nutrition while tobacco seeds can be used as concentrates feed source (tobacco seed meal = TM) after the oil is extracted for its use in biodiesel production.

Now the advantages of pelleting is becoming clear and straws, alfalfa, grape pomace, sugar beet leaves and straws are pelleted in order to be used in ruminant nutrition. In addition to physical benefits of pelleting, ease of transportation, homogeneity of the feed, longer shelf life, elimination of feed loss due to scatter, increased feed consumption, reduction in the pathogen microorganisms due to the heat component of the pelleting process are some of the other advantages of pelleting (1, 3, 4).

Ruminants on Earth produces 80 to 115 million ton of methane gas annually which leads to environmental issues and waste of valuable feed energy. Methane production in ruminants is more common when poor quality forages are consumed. Recent studies on rumen metabolism commonly focus on reduction of the methane production (5). *In vitro* gas production technique is often used in such studies. Wright et al. (6) reported that processed forage and pelleting reduce methane production while Gulecyuz (7) found that pelleting increases the digestibility. Current study aims to define the nutrient compositions, *in vitro* gas production, methane production and *in vitro* true digestibility (IVTD) in relation with the consumption of TS and TM, two tobacco harvest wastes.

## Materials and Methods

In the experiment we used stalks and seeds of tobacco (*Nicotiana tabacum L.*) plants collected from 25 different locations in 5 different geographical regions of Turkey. After being dried and ground to a size able to pass through 4mm sieve (tobacco straw=TS), TS was pelleted. However, TM was pelleted without any additives after the oil available in the seeds was extracted using a press for biodiesel production.

Rumen fluid used in *in vitro* studies with approximately 150-200 grams rumen content collected from three Simmental X Holstein breed bulls (Average 24 months age and 650-750 kg Live weight) slaughtered at a local slaughterhouse. Then it was brought to the laboratory within 15-20 minutes in thermos (39 °C). Rumen content mixed and it was taken under CO<sub>2</sub> and were filtered through two layers of cheesecloth.

### *Determining Chemical Composition of Samples*

All the TS and TM were dried. Then, dried TS and TM's were milled in a hammer mill through a 1 mm sieve for determining chemical compositions, *in vitro* gas production parameters and IVTD's assays. The samples were analysed for ash, dry matter (DM) and crude protein (CP) contents were analysed according to AOAC (8). Kjeldahl N and CP was calculated by multiplying N by 6.25. The crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) analysis were done according to Van Soest *et al.* (9) using Ankom 2000 semi-automated fiber analyser. Besides, ether extract (EE) content was determined using Ankom <sup>XT15</sup> analyzer (10). The contents of organic matters (OM=DM-ash), nitrogen free extract (NFE=DM-(CP+ash+EE+CF)), cellulose (Cel= ADF-ADL) and hemicellulose (Hcel = NDF-ADF), were determined by calculation. Condensed tannin contents were determined according to Makkar et al. (11). All chemical analyses of samples were carried out in triplicate.

### *Determining rumen fluid pH, volatile fatty acids (VFA) and ammonia-N (NH<sub>3</sub>-N) contents*

The pH value of rumen fluid before using *in vitro* studies was determined using digital pH meter (Hanna I. 1332) with 3 replications. The volatile fatty acids analysis of rumen fluid were done using a gas chromatography (Agilent Technologies 6890N gas chromatography, Cat. 11023, Stabilwax-DA, 30 m, 0.25 mm ID, 0.25 µm df. Maximum temperature: 260°C) according to Wiedmeier et al. (12). Rumen fluid ammonia-N analysis were done using Kjeldahl methods according to Blümmel et al. (13) in 3 replicates.

### *Determining in vitro gas production of TS and TM*

In this study, approximately 200 mg dry weight of samples (TS and TM) were weighed into 100 ml calibrated glass syringes following Hohenheim gas test procedures of Menke and Steingass (14). The syringes were warmed at 39 °C before the injection of 30 ml rumen fluid-buffer mixture (1:2) into each syringe and incubated in a water bath at 39 °C. 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 produc-

tions of TS and TM were determined at 24 h after incubation and corrected for blank and hay standard (University of Hohenheim, Germany). Cumulative gas production data were fitted to the model of Ørskov and McDonald (15) by the NEWAY computer package programme:  $y = a + b(1 - \exp^{-ct})$

where: a, gas production from the immediately soluble fraction (ml), b, gas production from the insoluble fraction (ml), a + b, potential gas production (ml), c, gas production rate constant for the insoluble fraction (ml/h), t, incubation time (h), y, gas produced at time 't'.

Organic matter digestibility (OMD), metabolic energy (ME) and net energy lactation (NE<sub>L</sub>) contents of TS (1) and TM (2) were estimated using equations given below:

- OMD, % = 14.88 + 0.8893GP + 0.448CP + 0.651 ash (16)....(1)
- OMD, % = 0.7602GP + 0.6365CP + 22.53 (17)....(2)
- ME, MJ/kg DM = 2.20 + 0.136GP + 0.057CP + 0.002859 EE<sup>2</sup> (16)....(1)
- ME, (MJ/kg KM) = 1.06 + 0.157GP + 0.084CP + 0.22 EE - 0.081 ash (14)....(2)
- NE<sub>L</sub>, MJ/kg DM = 0.101GP + 0.051CP + 0.11EE (14)....(1)
- NE<sub>L</sub>, (MJ/kg DM) = 0.075GP + 0.087CP + 0.161EE + 0.056 NFE - 2.422 (14)....(2)

Where; GP: 24 h net gas production (ml/200mg DM), CP: Crude protein (%), EE: Ether extract (%), NFE: Nitrogen free extract (%)

#### Determination of *in vitro* true digestibilities

Daisy incubator makes *in vitro* NDF disappearance study easy and efficient because it use an equipment which was designed with four rotating digestion jar and maintains constant, uniform heat and agitation within a controlled (39.5 °C) chamber (9; 18). Daisy incubator instrument contains 4 cylinder incubators which 1 cylinder need 1600 ml buffer solution and 400 ml rumen fluid as inoculums and bag filter (25 pieces). Filter bags F57 could be placed inside the each other cylinder with solution. The cylinder was bubbled with CO<sub>2</sub> immediately before closed with lid of cylinder well and placed into incubator for 48 h. After incubation, filter bags was cleaned under water flow and dried. The bags was analyzed for NDF digestibility

with fibre analyzer. *In vitro* true digestibilities of TS and TM samples were estimated as follows;

$$\text{IVTD, \%} = 100 - ((W3 - (W1 \times C1)) * 100) / W2$$

Where : W1: Weight of filter bag, W2: Weight of sample, W3: Final weight after NDF analysis, C1: The bag without sample was prepared also for correction.

#### Determining methane production of TS and TM

Methane contents (%) of total gas produced at 24 h fermentation of TS and TM were measured using an infrared methane analyzer (Sensor Europe GmbH, Erkrath Germany) (19). After measuring gas produced at 24 h incubation, gas samples was transferred into inlet of the infrared methane analyzer with the syringe. Methane production (mL) was calculated as follows.

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

#### Determination of relative feed values (RFV)

Because of, the chemical contents of TM were similar concentrates, it was not calculated the RFV for TM. However, RFV of TS were calculated as follows (20).

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

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

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

#### Statistical Analysis

The data obtained from the experiments is analyzed using SPSS 20.0 software package program. Nutrient content, *in vitro* gas production and IVTD data of the feeds investigated in this study were analyzed in accordance with the completely randomized design controlling for normality and variance homogeneity. Permutational multivariate analysis of variance method was used to analyze condensed tannin content of the feeds using the NPMANOVA software (21).

## Results

Nutrient contents of the TS and TM tested in the experiment are shown in Table 1. It was found that the OM content of TS was 91.09% while the ash content of TS was 8.91%. The OM content of TM was

determined to have an average of 93.86%, while the ash content of TM was found 6.14%. According to these results, it can be said that TM is a forage source with high OM content. With respect to the CP content which is one of the most important nutrient when assessing forages, TS was found to have an average of 4.49% while TM was found to have an average of 38.61%. It was also seen that TS has a higher CF content (49.88%) when compared to TM (27.73%).

According to Table 1, some of the important contents of TS are as follows: NDF content was 70.02%; ADF content was 51.13%; ADL content was 17.82%; HCell content was 18.89% and Cell content was 33.31%. For tobacco meals, on the other hand, NDF content was 43.44%, ADF content was 32.41%, ADL content was 17.82%, HCell content was 11.03% and Cell content was 17.39%. Results showed that TS has higher cell wall fiber components when compared to TM ( $P < 0.001$ ).

The pH,  $\text{NH}_3\text{-N}$  and VFA contents of the rumen fluid used for *in vitro* studies were found as pH: 5.70 (5.65-5.80), total volatile fatty acid (TVFA):  $95.35 \pm 0.85$  mmol/L, acetic acid:  $49.7 \pm 0.28$  mmol/L, propionic acid:  $24.15 \pm 0.76$  mmol/L, butyric acid:  $18.12 \pm 0.61$  mmol/L, isobutyric acid:  $2.09 \pm 0.34$  mmol/L, isovaleric acid:  $1.07 \pm 0.04$  mmol/L and valeric acid:  $1.21 \pm 0.08$  mmol/L and rumen fluid  $\text{NH}_3\text{-N}$  contents:  $29.17 \pm 1.16$  mg/100 ml.

Table 2 shows the *in vitro* gas production and pH value after 96 h for both TS and TM. According to the Table 2, however there are differences between TS and TM in terms of *in vitro* gas production at the onset, no significant difference was found between the two after 12 hours incubation. In terms of pH value at the end of 96 h incubation, lower values were found for TM when compared to TS ( $P < 0.05$ ).

Table 3 shows the *in vitro* gas production parameters, methane production, OMD, ME,  $\text{NE}_L$  and IVTD for both TS and TM. Tobacco meal outperformed TS with respect to OMD, ME,  $\text{NE}_L$  and IVTD ( $P < 0.001$ ). In addition, results showed that TM has significantly higher IVTD values when compared to TS ( $P < 0.001$ ). A significant difference was found between the TS and TM analyzed in this study with respect to the gas production rate constant "c value" ( $P < 0.001$ ) TM was found to have a higher gas production rate constant.

Nevertheless, there were no significant differences between TS and TM in terms of total gas production, "a+b value". However, TM outperformed TS with respect to methane production ( $P < 0.05$ ) OMD, ME,  $\text{NE}_L$  and IVTD contents ( $P < 0.001$ ).

Table 4 shows the RFV, DMD, DMI forage quality class and condensed tannin content of TS and TM. Adopted in many developed countries in the marketing and pricing of forages, RFV was found at an average of 65.88 for TS tested in this study. According to the RFV results TS was classified under "5-reject" quality class. It was also found that the CT contents of TS and TM were below 5% and there was no significant difference between CT results.

## Discussion

According to Table 1, it can be seen that tobacco straws have a DM content similar to straws and hay commonly used in animal nutrition while tobacco meals have a DM content similar to grains and meals commonly used in animal nutrition (22). As there is no research in the literature on the assessment of tobacco as forage, TS was compared to straws and poor quality hay with similar nutrient content, while TM was compared to sunflower seed meal and cotton seed meal. In this context, the ash content of wheat straws was found in the range of 7.0-12.3% in a number of studies (7, 22-24). It can be said that the ash content of TS found in this study is partially similar to the ash content of wheat straws. Feeds with higher OM content and lower ash content are considered to have a higher nutritional value (22). Nevertheless, it is expected that TS with high ash content will also have a higher mineral content while offering lower energy content and digestibility. Another reason behind the high ash content available in a feed is material mixed in the feed such as soil, sand, clay, etc. In this respect, attention must be paid in order to avoid impurities in tobacco waste.

It can be seen that TS has a similar CP content when compared to wheat straws (7, 22, 24-26). Crude protein content of wheat straws was reported by a number of authors (22, 23, 27) in the range of 2.9 - 4.1%. Looking into the CP content of TM, it can

be said that TM is an alternative to some expensive concentrates sources such as cottonseed meal (26.0-41.0% CP) and sunflower seed meal (30.0-36.0% CP). Moreover, it was suggested that the CP content depends on the oil extraction process applied to the seeds and that the extraction process leads to reduced oil content of the meal while the CP content is increased (22). Kara (28) reported the nitrogen content of the tobacco waste, a mix of TS and tobacco seed, in a range of 1-3%. This N content is the equivalent of CP content in the range of 6.25-18.75% which is between the values of TS and TM in this study. This can be accounted for by the use of mix of straws and seeds. In addition, it is reported that factors such as feed type, soil structure, fertilization, harvest time, ratio of straws and seeds in the hay, etc. can account for the differences in the CP content of samples collected from different locations (24).

It was found that the EE content of TS is average 1.44% and this result is similar to the CP content of wheat straws (1.3-2.5%) (22, 29). On the other hand, EE content of TM was found as average 10.64%. In this study, meal was obtained after tobacco seeds were crushed under the press and the EE content of the meal remained at a higher level. It can be said that TM is a nutrition source with high nutritional value in terms of its CP and EE contents. However, it can be seen that TS is poor in energy due to its low EE content.

High CF content has adverse effects on the forage quality. Therefore, TS should not be used as a single forage in ruminant nutrition. The CF content of wheat straws was found in the range of 37.1-42.5% in a number of studies (22-24). These results can be interpreted as TS (49.88% CF) offers a lower forage value when compared to wheat straws. In addition, Table 1 shows that the CF content of TM was found to have an average of 27.73% and these results were similar to the CF contents obtained from pressed cotton seed meal (19.0%-25.0%) and sunflower meal (19.0%-21.0%) (22).

Nitrogen free extract is an indicator of the easily fermented carbohydrate content available in the feed. It is considered that energy conversion of the feed with higher NFE content is faster. The NFE content of the TS tested in this study was found at an average of

**Table 1.** Nutrient compositions of tobacco straws (TS) and tobacco meals (TM), DM,%

	DM*	OM	Ash	CP	EE	CF	NFE	NDF	ADF	ADL	HCel	Cel
TS	93.66±0.05	91.09±0.18	8.91±0.18	4.69±0.25	1.44±0.07	49.88±0.48	35.08±0.56	70.02±0.64	51.13±0.44	17.82±0.31	18.89±0.25	33.31±0.31
TM	93.11±0.04	93.86±0.08	6.14±0.08	38.61±0.23	10.64±0.20	27.73±0.40	16.89±0.43	43.44±0.30	32.41±0.19	15.03±0.17	11.03±0.26	17.39±0.17
Significant	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

\*As feed, DM: Dry matter, 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, HCel: hemicellulose, Cel: cellulose

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

	3, ml	6, ml	9, ml	12, ml	24, ml	48, ml	72, ml	96, ml	pH*
TS	13.03±0.77	19.49±1.00	23.65±1.12	27.10±1.19	29.95±1.31	33.40±1.51	35.56±1.58	37.83±1.60	6.40±0.02
TM	17.79±0.94	24.71±1.24	28.81±1.39	31.26±1.49	33.73±1.67	35.97±1.89	37.86±1.98	39.58±2.04	6.47±0.03
Significant	0.000	0.001	0.004	0.030	0.077	0.290	0.365	0.502	0.019

\*Measured pH values after 96.hours incubation

35.08%. Nitrogen free extract content of wheat straws was reported in the range of 37.7-52.3% by Kutlu and Celik (22), Sehu et al (30) and Mohamoud Abdi (24) and the minimum value is similar to the one found for TS. But, TS having slightly lower NFE content from wheat straws. Moreover, the low NFE content found in TM (16.89%) is due to the high CP and EE contents.

It was reported that feed with high NDF content is less appetizing for the animals; and feed with high ADF content offers low digestibility (20). In this study, the NDF contents found for TS were similar to the ones found for the wheat straws (54.4-78.89%) (23, 24, 27). Furthermore, it can be seen that TM also has fiber components which is similar to many concentrates (22).

The ash (8.39%), CP (15.52%), CF (32.30%), NDF (50.36%) and ADF (42.53%) contents reported by Pehlevan and Ozdogan (2) for tobacco wastes with varying TS and tobacco seed ratios are in the ranges reported in the current study. However, the reason behind the high EE (15.29%) content reported by Pehlevan and Ozdogan (2) is that the tobacco waste contains tobacco seeds. On the other hand, we have used the TM remaining after the pressing process for biodiesel production in this study.

It was found that the gas production (66.69 ml/200 mg DM) as measured by Gulecyuz (7) for wheat straws at the end of 96 h incubation was higher than the average (37.83 ml/200 mg DM) found for TS in this study. Pelleting process may account for the lower gas production. The pH value measured at the end of 96 h incubation indicates the functionality of the tampon used in the injectors, if the pH is acidic then the tampon was consumed and could not perform its duty. However, the pH values obtained in this study do not indicate that the tampon is consumed (31). The gas production of TM in 96 hours were close to the ones reported by Kilic and Saricicek (32) for hazelnut kernel oil meal (49.07%)

and canola meal (45.83%) and similar to the ones reported by Kilic and Boga (33) for sunflower seed meal (30.21-43.30%). Kilic and Boga (33) used sunflower seed meal (32%-44%) with different CP contents and the one with the highest CP content was also the one with the highest gas production. In this study, TM had a CP content of 38.61% and its gas production was in the range between these two sunflower seed meals.

According to Table 3, the results obtained for a+b value which is the total gas production were lower than the one reported by Gulecyuz (7) for wheat straws (77.54 ml). Methane production of TS was found to be 4.62 ml. Methane production of wheat straw, on the other hand, was reported to be 6.57 ml (7). As poor quality forage would lead to increased methane production, it is believed that it would be best to combine TS with quality forages. Furthermore, methane production is a function of digestible organic matter and it must be considered the fact that feed with lower digestibility will lead to higher methane production. Therefore, considering the fact that the OMD content of tobacco straws are found to have an average of 49.42% while OMD content of wheat straws was found at an average of 56.80% (7), it can be said that TS with lower digestibility will lead to increased methane production. However, it was found that wheat straws as a poor quality forage will give results similar to TS.

Table 3 shows that OMD, ME, NE<sub>L</sub> and IVTD contents of TM are similar to the other meals. Kilic and Boga (33) reported c value of 0.008 ml/h and a+b value in a range of 30.26-43.36 ml for sunflower seed meal. This report is in compliance with the results of this study. The ME and NE<sub>L</sub> contents of wheat straws are reported as 7.73 MJ/kg DM and 4.21 MJ/kg DM, respectively (7). Accordingly, it can be said that wheat straws have a slightly higher energy content when compared to tobacco straws. Moreover, it was also found that the IVTD content of tobacco straws (52.05%) is

**Table 3.** *In vitro* gas production parameters, methane productions, OMD, ME, NE<sub>L</sub> and IVTD values for TS and TM

	c, ml/h	a+b, ml	Methane, ml	OMD, %	ME, MJ/kg DM	NE <sub>L</sub> , MJ/kg DM	IVTD, %
TS	0.10±0.01	36.37±1.59	4.62±0.30	49.42±1.13	6.55±0.17	3.42±0.13	52.05±0.68
TM	0.13±0.00	37.54±1.98	5.75±0.44	66.17±1.48	9.32±0.22	6.55±0.17	74.79±0.31
Significant	0.000	0.647	0.034	0.000	0.000	0.000	0.000

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<sub>L</sub>: Net energy lactation, IVTD: *In vitro* true digestibility

higher than the digestibility values reported by Mohamoud Abdi (24) for wheat straws (39.06%), sorghum straws (49.02%) and soybean straws (46.06%). In addition, tobacco straws were found to be of poor quality with the values found for DMD at 49.07% and DMI at 1.72 % LW.

ME and NE<sub>L</sub> contents of Tobacco meals found in this study were higher than the ME (5.47-7.28 MJ/kg DM) and NE<sub>L</sub> (4.49-6.14 MJ/kg DM) reported by Kilic and Boga (33) for sunflower seed meal while OMD contents (53.6-68.4%) were similar in both studies. ME contents reported by Seven et al., (34) for sunflower seed meal and cotton seed meal (8.92 MJ/kg DM and 8.75 MJ/kg DM, respectively) were lower than the ME contents found in this study for TM. This was a result of the higher EE and CP contents (which are used in ME calculations) found for TM in this study along with the differences in species tested, soil structure, fertilization, harvest time, meal production methods, etc. (31).

The reason behind the higher “c value” for the gas production of TM when compared to TS is that TM is classified under concentrates. Therefore, higher methane production, OMD, ME and NE<sub>L</sub> contents found for TM when compared to TS may lead to the recommendation of using TM for animal nutrition as forages with lower methane production has also lower OMD and energy contents. That is why the most suitable forage combination must be achieved in animal nutrition.

As shown in Table 4, forage quality of TM was defined in order to be compared to TS. However, a classification under forages is not suitable for TM in terms of its nutrient content. The IVTD was found as average 74.79% for TM and better results obtained when compared to forages are marked findings. According to the RFV index, TM was classified under

“2-Good” quality. Significant differences ( $P < 0.001$ ) were reported for all the nutrient contents explored in this experiment for TS and TM. It can be said that it will be better to address TM under concentrates as it is rich in CP and EE content while being poor in cell wall fiber components.

Kilic and Mohamoud Abdi (4) reported that the lowest IVTD among grape pomace pellets were found for the ones with the highest condensed tannin content. No statistically significant differences were found between TS and TM with respect to their CT contents. However, TS was found to have a slightly lower CT content and a lower IVTD value. These findings are similar to the literature. It was suggested that a tannin content of 5-10% results in disgust, reduces forage consumption and live weight gain, decreases the digestibility level and absorption of nutrition, reduces performance and lead to toxicity related effects (35). As the CT contents of both TS and TM used in this study were below 5%, it is believe that this will not have an adverse effect on the feed consumption. Nevertheless, it is likely that pelleting of tobacco waste will reduce the adverse effects of CT.

The CT contents found in TS and TM were significantly lower than expected. Therefore, TS and TM can be used as forage. It is observed that small ruminants, especially goats, consume tobacco seeds as field waste while TS is not commonly preferred due to its stalk's thickness. However, it is possible to use TS after being ground into the size of straws and being pelleted as a portion of straws or poor quality hays in animal nutrition. Ruminants show difference tolerances against condensed tannin and the tolerance of goats are significantly higher than sheep. Thus, it is believed that goats can tolerate 8-10% tannin in ratio and that goats are the most suitable animals for the use of tobacco harvest waste (4). Furthermore, future research

**Table 4.** Rleative feed values, quality class and condensed tannin contents of TS and TM

	RFV	DMD, %	DMI, % LW	RFV Quality Class	CT, %
TS	65.88±1.04	19.07±0.34	1.72±0.02	5 (Reject)	4.62 ± 0.39
TM	136.86±1.15	63.65±0.15	2.77±0.02	2 (Good)	3.28 ± 0.53
Significant	<0.000	<0.000	<0.000		0.122

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 forages based on prime >151, 1 (premium) 151-125, 2 (good). 124-103. 3 (fair). 102-87, 4 (poor). 86-75, 5 (reject). < 75. RFV: Relative feed value, DMD: Dry matter digestibility, DMI; Dry matter intake, CT: Condensed tannin.

may focus on the use of additive in order to increase the pellet quality and to improve the nutritional value of tobacco waste and on the use of TM in a specific ratio with cottonseed meal or sunflower seed meal, which it can be an alternative to.

According to the findings of this study, it should be taken into consideration that TS has nutrient content similar to wheat straws while its consumption will be at a lower level due to its higher NDF and ADF contents. TM, on the other hand, proves to be advantageous when compared to TS as it has nutrient content similar to concentrates and has higher OMD, ME, NE<sub>L</sub> and IVTD values. It must be remembered that oil extraction process may alter the nutrient content of seed meals. It was found that the method used to obtain meals has a direct impact on the CP and EE contents among nutrient contents. Extraction method is recommended as it is the method to almost fully extract the oil content of oily seeds (22). Thus, TM can be considered as an alternative for a number of forages such as expensive sunflower seed meal and cotton seed meal.

## Conclusions

In conclusion, TS and TM are recommended to be pelleted as the methane production of pelleted forages is reduced. However, an economic analysis in which the costs of seed collection, seed processing, and their use as fertilizers in the field must be conducted. The use of additives such as molasses, soybean meal, clay minerals etc. is recommended in order to improve nutritional values and pellet quality in the pelleting of TS. In addition, it is possible to ensile TS after being ground to the size of straw with a suitable humidity content (60-70% humidity). It is believed that this process helps remove the possible phenolic components during fermentation which then be safely used in animal nutrition.

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