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# Determination of Nutrient Content and *In Vitro* Digestibility Values of Organic and Conventional Tea (*Camellia sinensis*) Factory Wastes

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**Abstract**

The purpose of this study is determining the *in vitro* true digestibility values and nutritional content for tea (*Camelia Sinensis*) factory waste (TFW) produced by organic and conventional methods. Fresh tea plants and the TFW are collected from tea factories in the Blacksea Region of Turkey on the seasons of tea production which are the beginning and end of May (1st season), July (2nd season) and August (3rd season). In this study the nutrient composition of TFW, digestible organic material and metabolic energy values were determined in 3rd, 6th, 12th, 24th, 48th, 72nd and 96th hours by in *vitro* gas production technique. The nutrient values of TFW varied with seasons and periods. Values found are: dry matter (DM) 92-95%, ash values 3.56-4.59%, neutral detergant fiber (NDF) 51.10- 57.23%, acid detergant fiber (ADF) 40.93 – 49.27%, digestible organic material (DOM) 25.47 – 44.79% and metabolic energy (ME) 4.65 – 8.79 MJ/kgDM. DOM and ME values were found highest at the 3rd season followed by the 2nd season and 1st season ($P<0.05$). There is no statistical significant difference between TFW produced by organic and conventional methods for DOM and ME ($P>0.05$). It was concluded that, after the determination of the percentage of tannin and polyphenol contents in TFW; the 3rd season TFW can be added to ruminant rations which have high feed efficiency.

**Keywords:** Tea factory waste, *In vitro* digestibilty, nutrients, organic, ruminant.

## INTRODUCTION

In the field of animal feeding, the provision of quality roughage is an important element in terms of economy, animal health and yield. In recent years, tea plant products have been used in alternative forage research. Tea (*Camellia sinensis*), that belongs to theaceae family, was discovered in 2700 BC (FAO, 2015) and it is the most consumed beverage in the world. The flavonoids it contains are flavone, flavanone, isoflavone, flavonole, flavanole and antacyanins. These substances are often evaluated for their antioxidant, anticancerogenic and antiatherosclerotic properties (Balentine et al., 1997). Tea plant can be considered as a good alternative ruminant feed with its content of carbohydrate 56.68–59.84 g / 100 g, and protein 19.31–19.86 g / 100 g in its unprocessed natural state. However, it is defined as highly antinutritional due to its high phenolic and tannic acid values (Alasalvar et al., 2013).

During tea production, the remainder of the fresh tea is considered to be tea factory waste (TFW). This waste product is rich in leaf clippings and plant stem content. Each 100 kg of tea plants consists of 2 kg of waste. These wastes, which cannot be used effectively by factories, also have the potential to cause environmental pollution. There are reports indicating that this product, which may be harmful in waste form, can be used in the caffeine industry (Konwar, 1988), pig and poultry feeds (Chutia, 1983; Uuganbayar, 2006; Ko and Yang, 2008), fish feeds, fertilizer (Aşık and Kütük, 2012) and beverage industry (Chowdhury, 2016). The avail- ability of TFW for pellet production (Bilgin et al. 2016) may facilitate its use in animal feeding. When the studies on the use of all forms of tea in animal nutrition are examined, it is seen that the remaining pulp after beverage production is used more than TFW (Nasehi et al. 2017). Goats can benefit from high tannin containing plants (Ahmed et al., 2015) due to their proline synthesis capabilities (Narjisse et al. 1995). Imik et al. (1999) reported that the performance of goats was negatively affected when fed with conventional TFW supplemented ration containing 13.62% HP and 7.89% tannins. However, in another study of the same researchers (Imik et al. 2002), it was reported that, the performance data were not adversely affected in Akkaraman lambs fed with ration containing 10% TFW. Begovic et al. (1978) reported that, goats could tolerate tannin at 8-10% with tannase enzyme activity; while this rate becomes 3-5% in cattle and 1% in poultry. Kaya et al (2014) reported that 5.7% tannin containing TFW enhanced the antioxidant efficiency in poultry without any negative effect on performance when added to ration at 2-4% level. Zahedifar et al. (2019) reported that the growth performance of animals consuming 40 g / kg TFW instead of alfalfa was similar to other groups. Baruah (1997) reported that, adding 20% wheat bran and 15% decaffeinated TFW to ration instead of 35% wheat bran did not adversely affect the performance and health in dairy cows. Kondo et al. (2004) showed that TFW added to silage reduces serum cholesterol levels in dairy cows. In some studies (Ko et al. 2008; Hos- sain et al. 2012; Ahmed et al. 2015), TFW has been reported to have a positive effect on concanavanalin A and lipopoly- saccharide which proliferate T and B cells.

It seems that, the studies with TFW in animal nutrition have contradictory results. The aim of this study was to investigate the in vitro digestibility values of TFWs which have high storage cost and cause environmental pollution during the production process of conventional and organic tea.

## MATERIAL and METHODS

### Feed Material

Fresh tea and TFW (organic and conventional) were obtained from tea factories in the Black Sea Region of Turkey during the production seasons. The research groups consisted of materials taken in May ( 1st season ), July ( 2nd season ) and

August (3rd season) and in 2 periods as the beginning and end of each month. The rumen content required for *in vitro* digestibility determination was obtained from cattle fed *ad-libitum* mixed feed and straw in Samsun Florya Integrated Meat Facilities' Abattoir. Rumen content was taken immediately after slaughtering and brought to Ondokuz Mayis University Faculty of Veterinary Medicine Ruminant Feed Evaluation and Training Unit with 39 $^{o}$C thermos containing $CO_2$. Dry matter (DM), crude ash (CA), crude protein (CP) and ether extract (EE) analyzes of tea and TFW samples were performed with the methods reported in AOAC (2006). Crude protein (CP) was determined with the Kjeldahl method. Fat content was determined with ether extraction in the Soxhlet extraction system (Extraction system B-811, Switzerland). The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) levels of each feed were determined with the ANKOM 200 Fiber Analyzer by using methods of Van Soest et al. (1991). For in vitro digestibility detection, the gas production method which was reported in Menke and Steingass (1988) was used. Organic matter digestibility (OMD) values were calculated by using 24-hour gas production (GP), CP and CA values.

Metabolic energy gas production (MEGP) values were calculated according to the formula reported in Menke and Steingass (1988).

All chemical analyzes were performed in Ondokuz Mayıs University Faculty of Veterinary Medicine Department of Animal Nutrition and Nutritional Diseases Laboratories

**Statistical Analysis**

In this study, TFW nutrient contents and gas production values produced from two different types of tea (conventional and organic), and observational values for digestible organic matter (DOM) and metabolic energy (ME) data are summarized as arithmetic mean and standard error of mean. In order to determine the seasonal and periodic differences of two types of TFW, a statistical model was used in which 3 different seasons and tea waste origin were evaluated. In order to evaluate the periodic differences, the model was an alyzed according to the least squares method in factorial order. The Duncan multiple comparison test was used in cases where the differences between the averages were found to be significant in the investigated factors. SPSSv21 (2007) statistical package program was used to summarize and analyze the observation values obtained through the study.

## RESULTS and DISCUSSION

The values of DM%, CA%, NDF%, ADF%, CP%, EE%, OM% and ME (MJ/kg DM) contents of tea and TFW samples were determined by chemical analyzes and respectively given in Table 1 and Table 2.

**Table 1.** Nutrients (%), cell wall elements (%) and metabolic energy (MJ/kg DM) values of conventional (C) and organic (O) tea () according to production season (S).

| S | | **DM** | **CA** | **NDF** | **ADF** | **ME** | **CP** | **EE** | **OM** |
|---|---|---|---|---|---|---|---|---|---|
| 1 | C* | 89,86±0,50 | 5,21±0,26 | 33,89±2,53 | 29,20±3,25 | 10,15±0,54 | 16,88±1,54 | 1,40±0,25 | 84,65±0,24 |
| | O* | 89,48±0,23 | 5,30±0,31 | 31,01±0,01 | 24,75±0,72 | 10,89±0,12 | 18,56±0,29 | 1,38±0,42 | 84,18±0,08 |
| 2 | C* | 91,22±0,07 | 4,91±0,00 | 38,55±0,81 | 34,73±0,23 | 9,23±0,04 | 16,65±1,95 | 1,16±0,18 | 86,31±0,07 |
| | O* | 90,75±0,27 | 5,38±0,00 | 36,89±1,83 | 32,37±1,24 | 9,62±0,21 | 16,03±1,15 | 1,39±0,16 | 85,36±0,27 |
| 3 | C* | 91,67±0,30 | 5,12±0,05 | 38,77±1,13 | 34,00±0,90 | 9,35±0,15 | 17,79±0,61 | 1,69±0,25 | 86,55±0,35 |
| | O* | 90,64±0,22 | 5,85±0,04 | 33,21±3,40 | 30,82±3,94 | 9,87±0,65 | 15,46±1,09 | 1,55±0,45 | 84,79±0,18 |

*P>0,05

**DM:** Dry matter, **CA**: Crude ash, C**P:** Crude protein, **EE:** Ether extract, **OM:** Organic matter, **NDF:** Neutral detergent fiber, **ADF:** Acid detergent fiber, **ME:** Metabolic energy.

**Table 2.** Nutrients (%), cell wall elements (%) and metabolic energy (MJ/kg DM) values of conventional (C) and organic (O) tea factory waste () according to production season (S).

| **S** | | **DM** | **CA** | **NDF** | **ADF** | **ME** | **CP** | **EE** | **OM** |
|---|---|---|---|---|---|---|---|---|---|
| 1 | C* | 94.11±1.51 | 3,56±0,25 | 57,21±2,96 | 48,85±2,59 | 6,89±0,43 | 8,03±1,92 | 0,28±0,03 | 90,55±1,7 |
| | O* | 92,69±0,09 | 4,01±0,35 | 51,10±1,61 | 45,38±2,78 | 7,46±0,46 | 7,21±2,29 | 0,65±0,05 | 88,67±0,44 |
| **2** | C* | 95,80±,14 | 4,21±0,15 | 52,52±2,59 | 47,98±0,56 | 7,03±0,09 | 12,67±0,04 | 0,30±0,19 | 91,59±0,3 |
| | O* | 95,93±0,26 | 3,92±0,04 | 57,23±2,33 | 49,27±1,09 | 6,82±0,18 | 10,20±0,24 | 0,76±0,27 | 92,00±0,30 |
| **3** | C* | 93,42±0,63 | 4,69±0,09 | 47,76±2,83 | 40,93±2,92 | 8,20±0,48 | 14,07±1,23 | 1,07±0,11 | 88,65±0,4 |
| | O* | 93,79±0,35 | 4,27±0,06 | 49,34±6,64 | 43,16±6,54 | 7,83±1,09 | 10,97±1,07 | 0,89±0,23 | 89,29±0,62 |

*P>0,05

**DM:** Dry matter, **CA**: Crude ash, C**P:** Crude protein, **EE:** Ether extract, **OM:** Organic matter, **NDF:** Neutral detergent fiber, **ADF:** Acid detergent fiber, **ME**: Metabolic energy.

*In vitro* digestibility values (gas volume ml/h, DOM% and ME Mj/kg DM) of TFW materials are given in Table 3.

**Table 3.** Period (PE) and production type (T), *in vitro* gas volume (ml), digestible organic matter (DOM, %) and metabolic energy (ME, Mj/kg DM) values () of conventional (C) and organic (O) tea factory wastes (n=3) in different hours (h) according to production season (S),

| | | 3h | 6h | 12h | 24h | 48h | 72h | 96h | DOM | ME |
|---|---|---|---|---|---|---|---|---|---|---|
| S ** | 1 | 31,63±3,69a | 38,25±3,65c | 51,01±3,59c | 70,66±3,86c | 77,60±4,90c | 72,55±7,47c | 73,72±7,20a | 25,47±2,64c | 4,65±0,12c |
| | 2 | 31,09±10,73a | 55,79±10,75b | 89,55±11,01b | 132,65±12,17b | 166,20±12,79b | 167,14±13,12b | 168,33±13,27b | 32,03±1,96b | 6,70±0,32b |
| | 3 | 37,44±1,19a | 77,36±2,76a | 137,70±4,65a | 205,97±4,76a | 236,32±12,93a | 240,83±15,86a | 242,59±16,39a | 44,79±2,81a | 8,79±0,18a |
| PE* | 1 | 29,79±2,51a | 51,42±3,74b | 84,57±7,42b | 129,58±11,98b | 147,40±14,18b | 147,03±15,12b | 148,53±15,35b | 32,29±1,07a | 6,49±0,34a |
| | 2 | 38,09±7,52a | 66,60±9,04a | 108,36±13,34a | 157,33±18,33a | 185,78±22,47a | 187,16±24,73a | 188,42±24,71a | 37,56±4,18a | 7,26±0,57a |
| T | C | 37,15±7,06a | 61,90±8,25a | 99,87±11,08a | 147,38±16,83a | 173,15±20,64a | 173,69±22,56a | 175,77±22,57a | 35,35±3,37a | 7,02±0,51a |
| NS | O | 30,19±2,72a | 54,95±4,93a | 91,16±9,55a | 137,31±13,83a | 157,03±16,63a | 157,35±17,91a | 158,09±18,21a | 34,06±2,35a | 6,67±0,41a |

**NS**: Not significant statistically; *,a,b: P<0.05; **,a,b,c: P<0.01 significant statistically
**a,b,c:** There is no statistical difference between the sample means with the same letter on the basis of columns (P>0.05).

In our study, *in vitro* gas production amounts of both conventional and organic TFW wastes increased in direct proportion with the incubation time. Both conventional and organic tea and TFW had mathematical differences in nutritional values in all seasons, but these differences were statistically insignificant (P>0.05). It was observed that the NDF and ADF values of TFW produced by conventional methods decreased (P>0.05) as the season progressed, while CP, EE and ME values increased (P>0.05). It was determined that, the NDF and ADF values of TFW, produced by organic method, had the highest value in the second season. At the same season, CP, EE and ME values of organic TFW were similar to conventional TFW's. Produced gas volume (ml), DOM (%) and ME (MJ / kg KM) values were the highest in the third season and the difference was statistically significant (P<0.05). The gas volumes for all seasons were higher in the second period products (P<0.05). The difference between the periods was not significant in terms of DOM and ME (P>0.05). It was determined that tea cultivation methods (conventional-organic) did not make a statistically significant difference (P<0.05) on gas volumes, DOM and ME values.

In our study, although they were not practically used in animal nutrition, the nutrient contents of the fresh tea leaves were determined to make a comment on the nutrient contents of TFW. Sarıca et al. (2008) reported that CP, CA and EE values of the fresh tea leaves were between 15-19%, 5% and 2-3%, respectively. Nas et al. (1991) reported that CA values in fresh tea ranged between 3.31-5.65% according to cultivation periods. In our study, the nutrient values of fresh tea leaves are consistent with these findings. In the study of Imik et al. (2002), the values of DM, CP, EE, CA of TFWs were reported respectively as 93.02%, 14.38%, 1.06% and 4.38%. When these values are compared with the results of our study, it is seen that CP and EE values are similar to those of conventional TFW group in the 3rd season. In the same study, it was reported that the addition of 10% TFW to ruminant diets did not affect health and yield performance. Angga et al. (2018) reported that DM, OM, and CP values of Sumatra TFWs were 93.59%, 88.08%, and 19.63% respectively, and they reported that after decreasing the tannin amount, the CP value was not adversely affected and could be used in broiler rations. These results are in agreement with DM and OM values in our study and CP values were higher in Sumatra TFW. Fazaeli et al. (2000) reported that such differences may occur depending on the variety of tea. Nasehi et al. (2017), in their study with green and black TFW, the values of DM, CP, CA, OM, EE, NDF, ADF and ME in black TFW were 92.72%, 15.66%, 5.75%, 94.24%, 1.16%, 38.47%, 25.87% and 7.7 Mj/kg DM respetively. In a similar study, Imik and Şeker (1999) found that TFW had 94.67% OM, 5.33% CA, 13.62% CP, 1.21%EE, 59.68% NDF and 48.98% ADF. Among the studies conducted with TFW, it is seen that Imik and Şeker (1999)'s study is the most consistent study with the results of our research. It is evaluated that, this harmony was observed because the samples were taken and processed from the same geographical region in a similar period and with similar techniques. Konwar et al. (1985), reported that CP, EE and CA values of TFWs were determined as 19.8%, 1.37% and 7.58%, respectively. Zahedifar et al. (2019) found that DM and NDF values of waste products were 94% and 47.6%, respectively. Ramdani et al. (2013) determined the CA value as 6.14%. In a study of Ahmed et al. (2015) reported that DM, CP, EE and CA values of green TFW were 80.88%, 20.1%, %2.1, and 4.88% respectively. In Ahmed et al (2015)'s study, live weight gain and feed consumption in goats, consuming diets formed by adding TFW at 0%, 0.5%, 1% and 2% respectively in the basal diet, showed a linear increase in proportion to the amount of waste (P<0.05) and feed efficiency rate was not significantly affected. It was observed that, the blood serum glucose and total cholesterol levels decreased significantly (P<0.05) with increasing TFW ratio. It was emphasized that, TFW increased feed consumption and live weight gain in goats due to the evaluable CP level varying between 22-35%. As reported by Tan et al. (2001) the increase in live weight and feed consumption are linked to the efficacy of tea catechins that support the development of intestinal and ruminal microorganism growth. Great differences of nutrients and metabolic energy values of TFW in different studies are attributed to differences of tea species, geographical region, harvest season and tea processing techniques (Fazaeli et al. 2000). Landau et al. (2000) reported that stem and leaf fraction ratios in tea wastes affect nutrient values, flavors, and nutritional contents and digestibility. Because of the use of different tea processing techniques; (Çaykur, Rotervan, Orthodox and crushing-tearing-curling) nutrients, minerals and ash values have been reported (Nas et al. 1991) to be affected by different degrees and durations of heat and pressure applications.

The CP values of the samples show a mathematical increase according to the progress of the production season. Consistent with this increase, the amount of the gas production was higher in latter seasons. The *in vitro* gas production depends on fermentation of carbohydrates and proteins found in feeds, volatile fatty acids (Getachew et al. 1998; Getachew et al. 2004) and microbial activity. The quantity and quality of protein have a great importance in this activity (Cone and Van Gelder, 1999; Blummel et al. 2003). Norton et al. (1994) reported that, feeds should contain 10% of CP at least for the maintenance of rumen microbial activities. The reports of Parissi et al. (2005) and Kamalak et al. (2005) which explain that there is a positive correlation between CP level and gas production level, are consistent with our research. In the calculation of DOM and ME values, 24 hour gas production value, CP and EE values are used. It can be concluded that, depending on the season and the period, increase in CP values affect the DOM and ME values. Feedstuffs, which are rich in ADF and NDF, have lower gas production values (Canbolat et al. 2013). In our study, although the ADF and NDF values did not show statistical significance, they showed a mathematical decrease depending on the season (P>0.05). It can be thought that, the decrease in ADF and NDF values has a supportive effect on the increase in gas production, DOM and ME values depending on the season. As a matter of fact, the increase in cell wall elements and the decrease in microbial activity are also remarkable in another research (Karabulut et al. 2007). In tea production, N and tannin compounds may differ due to soil quality differences, constantly changing tannin, phenol and nitrogen load and uncontrolled fertilization (Nas et al. 1991; Seyis et al. 2018). Thus, the nutrient profile of tea plants changes according to each production season. In our study, the occurrence of periodical differences in gas production DOM and ME values during each production season may be the results of these reasons. Kondo et al. (2014) reported that, the gas production value of the $24^{th}$ hour was 29.9 ml in their study with extracted tea waste. In our study, the values measured at this hour were higher in all groups. The differences may be attributed to the decrease in the amount of digestible nutrients in the wastes after tea extraction and lower fermentation in the gas production system. In a study (Nasehi et al. 2017) performed with TFW, it was found that, in vitro gas production increased significantly (P<0.05) when TFW was treated with polyethylene glycol. They explained the increase in values by increasing the utilization of polyethylene glycol by rumen microorganisms from nutrients. Hernandez et al. (2015) attributed this effect to the more efficient use of the substrate by rumen microorganisms and complexation of tannins with polyethylene. A similar finding and explanation can be seen in a study of Bakhshizadeh and Taghizadeh (2013). In contrast, with these results, Zahedifar et al. (2019) reported that polyethylene glycol did not significantly affect the digestibility of organic matter (P>0.05). The fact that, usage of different feed evaluation system devices in *in vitro* gas production technique (Cone and Van Gelder, 1999) is also effective in producing different results in researches.

It is seen that, the nutrient content and metabolic energy values of tea factory wastes may have differences depending on the geographical region, species, cultivation methods and processing methods. It was concluded that, the tea factory wastes are richer in nutrients than quality cereal straw. It is considered that, the factory wastes of the tea harvested in August ($3^{rd}$ season) are better in terms of DOM and ME, and can be added at appropriate level to the ruminant rations after determining the tannin and polyphenol contents.

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**CONFLICT of INTEREST**

The authors are declared that there is no conflict of interest.

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