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Chemical Composition and *in vitro* and *in vivo* Digestibility of Tea Waste in Sheep

Research Article

M. Zahedifar[1*], H. Fazaeli[1], A.R. Safaei[1] and S.M. Alavi[2]

[1] Department of Animal Nutrition, Animal Science Research Institute of Iran (ASRI), Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran
[2] Emam Khomeini Higher Education Center, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

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*Correspondence E-mail: zahedifar44@gmail.com

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ABSTRACT

Tea processing includes several stages and in three of them namely drying, fiber-mat and screening some of the product is wasted. In this study, 25 percent of the tea processing plants in Iran were selected. Three samples were taken from each plant and analyzed for chemical composition and *in vitro* and *in vivo* digestibility and voluntary feed intake. Results showed that crude protein (CP) content of different tea by-products including drying, fiber-mat and screening were 198, 193 and 194 g/kg dry matter (DM), respectively, without significant difference among them. Concentration of ash were 64.0, 62.0 and 56.0 g/kg DM for drying, fiber-mat and screening respectively which was significantly (P<0.05) the lowest in Screening. The content of K, Ca, P, Mg and Na were 16.2, 4.2, 2.5, 2.2 and 0.73 g/kg DM, respectively, without significant difference among the samples. The mean values for *in vitro* digestibility coefficient of DM, organic matter (OM), OM/DM and gross energy of the tea wastes were 0.48, 0.44, 0.42 and 0.42, respectively. Inclusion of tea waste in the diet significantly (P<0.05) reduced *in vivo* digestibility coefficient of CP and neutral detergent fiber (NDF). The digestible energy was 10.0 MJ/kg which were not significantly different among the three types of tea waste. Inclusion of 80 g/kg DM tea waste in the diet significantly (P<0.05) reduced voluntary feed intake. Tea waste can be included in the diet of ruminants but its level should be limited due to presence of anti-nutritional factors.

KEY WORDS chemical composition, *in vitro* digestibility, *in vivo* digestibility, sheep, tea waste.

INTRODUCTION

The shortage of good quality feed has long been a serious constraint to the optimum efficiency of animal production in many parts of the world. Such a problem has been identified in Iran since the last four decades. While the shortage of feeds and fodder is well recognized, it is also equally important to understand how the available unconventional feed resources and by-products can be efficiently utilized. Over the two past decades, considerable emphasis has been placed on the beneficial role of agricultural wastes in livestock production in the country. By-products from tea *(Camellia sinensis)* processing are available in northern area of Iran and may be a potential roughage source in an amount of about 4000 metric ton annually, would be a valuable feed for livestock in that part of the country (Ananthasubramainam *et al.* 1978; Nikkhah and Hosseini, 1985).

Tea processing involves several stages; namely drying, fiber-mat and screening, during which some by-product is generated. The protein content of this by-product is relatively high but its nutritive value is not well known. Tea waste could be considered as roughage, which is relatively rich in crude protein (Konwar *et al*. 1986; Sutrdhar, 1990) that may be comparable to several legume forages like alfalfa and clover, at least with respect to its crude protein content. However, its digestibility may be affected by the tannin-polyphenolic compound (Jayasuriya *et al*. 1978) that may reduce the nutritive value compared to many forage legumes. Considering the available technology of tea leaves harvesting and the processing systems, the chemical composition and nutritive value of tea wastes may be variable. Its protein content has been reported from 179 to 280 g/kg DM. It could be also a rich resource of some micro nutrients such as magnesium and copper (Fazaeli *et al*. 2000).

The nutritive potential of tea waste in the diets of non-ruminants has been reviewed by some workers (Daimary *et al*. 1988; Das and Singh, 2004). However, few attempts have been made to determine the nutritive value of different types of such wastes, therefore its optimum utilization for ruminants is not well defined because of unclear understanding about potentiality of tea waste. When using new feed resources in feeding of animals, it is necessary to get sufficient information about its nutritive value. The aim of this study was to compare the chemical composition and *in vitro* digestibility of three types of tea waste and assess the effect of tea waste on *in vitro* and *in vivo* digestibility and voluntary feed intake in sheep.

MATERIALS AND METHODS

Sampling method

Twenty of the national total of 80 tea processing plants facilities were selected as sampling units throughout the northern area of the Caspian Sea according to the systematic randomized sampling method (Bajpai, 2010). Each selected plant was visited during the working season (from May to August). Tea waste was obtained from the drying, fiber-mat and screening stages of processing at each processing plant were sampled and 60 samples (20×3=60) in total, were obtained. Therefore, one sample per type of waste-tea was obtained per factory. Samples were packed in 2-litre plastic containers and then were labelled. At the arrival at the lab, the plastic containers were opened and each sample was mixed. A sub-sample (about 20 g) was taken and milled, using laboratory 1-mm sieves mill, and used for chemical analysis and digestibility measurements.

Chemical analysis and energy determination

Samples were analyzed for dry matter (DM, ID number 934.01), organic matter (OM, ID number 967.05), ash (ID number 942.05), crude protein (CP=N×6.25, ID number 976.05) and ether extract (EE, ID number 920.39) according to AOAC (2000).

Neutral detergent fiber (NDF) assayed and expressed inclusive of residual ash (Van Soest *et al*. 1991). Sodium sulphite and α-amylase were not used. The gross energy (GE) was determined by using Parr adiabatic calorimeter (model 1261, Parr instrument, USA).

Measurement of minerals

Phosphorous was determined by wet digestion method using ammonium molybdate method followed by spectrophotometer measurement (ID number 965.17) (AOAC, 2000). A wet digestion method, with 15 mL of concentrated HNO_3 and 7.5 mL of concentrated $HClO_4$, was carried out for sample preparation for Ca, K, Na, Mg, Fe, Mn, Zn and Cu followed by atomic absorption spectrophotometric measurement (AOAC, 2000).

Measurement of total extractable phenolics and total tannins

Determination of total extractable phenolic compounds and total tannins was accomplished according to the method described by Makkar *et al*. (1993).

In vitro digestibility

The digestibility of samples for DM, OM and GE were determined by the *in vitro* system, using acid pepsin two-stage method (Tilley and Terry, 1963) and rumen liquor was collected from two fistulated mature cattle fed wheat straw-alfalfa based diet.

In vitro gas production technique

Samples (200 mg) were incubated in triplicate in 100 ml calibrated glass syringes according to the method described by Menke and Steingass (1988). Rumen liquor was collected from the three fistulated local breeds (Taleshi breed) of cattle fed at maintenance (8.65 MJ/kg ME and 102 g CP/kg DM).

Diet contained 300 g/kg alfalfa, 350 g/kg wheat straw, 200 g/kg wheat bran, 145 g/kg barley grain, 2.0 g/kg vitamin and mineral supplement, 3.0 g/kg salt on dry matter basis. Gas production was recored at 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours after incubation. The adverse effect of tannins on *in vitro* gas production of tea waste was assessed by adding of polyethylene glycol (PEG) to feed and gas production was measured.

Cumulative gas production data were fitted to the exponential equation: $Y= b(1-e^{-ct})$ of Orskov and McDonald (1979) modified by Blummel and Becker (1997). The ME content was calculated using equations of Menke and Steingass (1988) as:

$$ME\ (MJ/kg\ DM)= 2.20 + 0.136\ Gp + 0.0057\ CP + 0.00029\ CP^2$$

Where:

Gp: net gas production (mL) from 200 mg after 24 h of incubation and CP is crude protein in g/100 g DM.

Determination of voluntary feed intake and *in vivo* digestibility

Since the chemical composition of the three types of tea wastes were more or less similar and the little differences in concentrations of ash and minerals was unlikely to affect digestibility and feed intake, and on the other hand, in the processing plants the three types of residues are mix, a mixture was prepared by mixing equal proportions of drying, fiber-mat and screening tea waste. Tea waste was added at different levels to alfalfa to obtain five diets containing 0, 40, 80, 120 and 160 g/kg DM tea waste.

Two groups of Afshari male sheep (n=5/group) were used for determination of voluntary feed intake and *in vivo* digestibility. Animals were housed in individual metabolic cages and fed the experimental diets containing 5 levels of tea waste in 5 successive periods. In the voluntary intake trial, animals (53±2.5 kg) received the diet 10% above appetite and the amount of feed and refused were recorded daily. In the digestibility trial, animals (51±1.7 kg) were fed at maintenance requirements and the amounts of feed offered and refused were recorded daily. Feces were collected, weighed and a sample (10% of total) was dried. Animals in both trials were fed twice daily at 8.00 and 15.00. Animals had free access to multi-mineral salt lick and water. Animals had one week break out of the metabolic cage before starting the next period.

Statistical analysis

A completely randomized design with 20 replications was used to assess the differences in chemical composition and *in vitro* digestibility of tea waste. A completely randomized design with 5 replications was used to assess the differences in *in vivo* digestibility and voluntary feed intake. Analysis of all data was carried out using general linear model procedure of SAS (1996). The difference between the means was determined using Duncan's multiple range test at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical composition

The only significant difference (P<0.05) in chemical composition including CP, NDF, EE and Ash among the three tea wastes (Drying, Fiber-mat and Screening) was observed in ash concentration (Table 1). No significant difference was observed in concentration of macro-minerals except for mg among the three tea wastes investigated in this study.

Among the four measured micro-minerals (Table 1), manganese was not different among the three types of tea wastes while a significant difference (P<0.05) was observed between the concentration of iron, magnesium, zinc and copper among them.

***In vitro and in vivo* digestibility**

No significant difference was observed among the three types of tea wastes for *in vitro* digestibility, organic matter (OM), organic matter in dry matter (OM/DM) and gross energy (GE). No significant change was observed in *in vivo* digestibility coefficient of dry matter, organic matter and organic matter in dry matter of alfalfa with increasing tea waste inclusion levels while, digestibility of NDF and especially crude protein was significantly reduced (P<0.05) at 160 g/kg DM substitution of tea waste (Table 4).

Results of chemical composition and *in vitro* gas production from alfalfa and tea waste are presented in Table 3. *In vitro* gas production of tea waste after 96 hours incubation was less compared to alfalfa despite containing more CP and less NDF. On the other hand, total extractable phenolics and total tannin in tea waste was higher than that in alfalfa (Table 3). Potential gas production from alfalfa, tea waste and tea waste + polyethylene glycol (PEG) revealed significant difference in bioavailability of the three samples for rumen microorganisms (Figure 1).

Voluntary feed intake

The highest level of dry matter and organic matter intake was from alfalfa hay (P<0.05). No effect was observed by inclusion of 40 g/kg DM tea waste in diet but all other levels negatively affected intake as compared with control and the lowest intake was observed when alfalfa was substituted for tea waste at the level of 160 g/kg DM (Table 5).

Chemical composition

The three types of tea waste contained high dry matter (see Table 1). The advantage of such dry matter is the ease of separation of wastes from tea and its preservation. The range of crude protein content in the three tea wastes confirmed their high protein content. The values of CP for tea waste in this study were lower than 283 and 276 g/kg DM reported by Kondo *et al.* (2014) and Makoto *et al.* (2014), respectively.

The protein content of tea wastes in this study was in agreement with data reported by Nikkhah and Hosseini (1985) (183.9 g/kg DM) and Kadirvel (1979) (192.0 g/kg DM). The values for ash obtained in this study was lower than those reported by Nikkhah and Hosseini (1985) and Konwar *et al.* (1986) who mentioned 76.0 and 91.0 g/kg DM, respectively, but were higher than the value (43.0 g/kg DM) reported by Kadirvel (1979).

Table 1 Chemical composition and gross energy content of tea processing residues (mean±Sd)

Type of Residue	Drying	Fiber-mat	Screening	Mean of the three types	SEM	P-value
	Chemical composition (g/kg DM)					
Dry matter (DM)	933±8.1[b]	940±6.5[a]	937±9.2[ab]	936±8.3	1.79	0.052
Crude protein (CP)	198±19.3	193±22.9	194±29.6	195±23.9	5.41	0.796
Neutral detergent fiber (NDF)	478±15.0	476±8.6	465±15.0	473±13.0	5.20	0.053
Ether extract (EE)	10.4±4.1	8.4±4.0	9.2±3.8	9.3±4.0	0.89	0.283
Ash	65.9±8.8[a]	61.8±6.8[a]	55.6±6.1[b]	61.2±8.4	1.64	0.000
Ca	4.2±1.1	4.0±1.2	4.4±1.0	4.2±1.1	0.25	0.497
P	2.5±0.4	2.6±0.4	2.5±0.3	2.5±0.3	0.08	0.248
K	16.5±4.4	16.4±3.3	15.5±3.3	16.1±3.7	0.83	0.694
Na	0.7±0.2	0.7±0.4	0.8±0.4	0.7±0.3	0.08	0.723
Mg	2.4±0.2[a]	2.2±0.3[ab]	2.1±0.3[b]	2.2±0.3	0.06	0.046
Gross energy (GE) (MJ/kg)	19.5±0.68	19.6±0.46	19.6±0.45	19.5±0.5	0.12	0.716
	Micro mineral (mg/kg DM)					
Fe	726±207.7[a]	602±196.2[ab]	491±189.4[b]	606±217.1	44.26	0.003
Mn	1211±235.6	1112±261.1	1093±238.6	1140±0.246.5	54.83	0.279
Zn	56.2±12.0[a]	48.9±10.5[b]	41.6±9.8[c]	49.0±12.2	2.42	0.001
Cu	56.2±12.0[a]	45.9±9.6[b]	36.4±9.3[c]	46.3±13.1	2.32	0.000

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SD: standard deviation.
SEM: standard error of the means.

Table 2 The *in vitro* digestibility coefficient of tea processing residues (mean±Sd)

Parameters[2]	Type of residue			Mean of the three types	SEM	P-value
	Drying	Fiber-mat	Screening			
DM	0.48±0.048	0.48±0.057	0.47±0.059	0.48±0.056	0.013	0.766
OM	0.45±0.063	0.44±0.054	0.44±0.048	0.44±0.045	0.011	0.832
OM/DM	0.42±0.060	0.42±0.040	0.41±0.043	0.42±0.041	0.009	0.914
GE	0.42±0.047	0.42±0.031	0.41±0.017	0.42±0.039	0.009	0.616

DM: dry matter; OM: organic matter and GE: gross energy.
SD: standard deviation.
SEM: standard error of the means.

Table 3 Chemical composition, *in vitro* gas production and metabolizable energy (ME) of alfalfa and tea waste used in *in vivo* digestibility trial (DM basis)

Feeds	Parameters (g/kg DM)								GP (mL)[1]	ME (MJ/kg)[2]
	CP	NDF	EE	Ash	Ca	P	TEP	TT		
Alfalfa hay	145.0	568.0	12.0	90.0	12.0	1.7	5.9	0.4	49.67	8.00
Tea waste	195.0	473.0	9.3	61.0	4.2	2.5	58.7	45.5	31.99	5.77
Tea waste + polyethylene glycol	-	-	-	-	-	-	-	-	37.17	6.35

[1] GP: *in vitro* gas production after 96h of incubation.
[2] ME: metabolizable energy calculated using the equation: $ME= 2.2 + 0.136\ G + 0.0057\ CP + 0.0002589\ CP^2$ (Menke and Steingass, 1988).
CP: crude protein; NDF: neutral detergent fiber; EE: Ether extract; TEP: total extractable phenolics and TT: total tannins.

The ash content of tea wastes in this study was in the line with the finding of Ramdani *et al.* (2013) (61.4 g/kg DM). The values of Mn, Fe, Cu and Zn in this study were higher than those reported by Ramdani *et al.* (2013) (527, 116, 23.8 and 21.7 mg/kg DM, respectively). Some factors can affect the chemical composition of tea waste including: Tea cultivar, region, harvesting time and method of tea processing (Fazaeli *et al*. 2000), which may be some of the reasons for the findings of this study.

In vitro and *in vivo* assays

Non significant difference between the *in vitro* digestibility coefficients (Table 3) was expectable because most of chemical compositions of the three tea wastes were not significantly different (Table 1). The diets used in *in vivo* trials contained various levels of alfalfa and tea waste. Regarding lower NDF and ash content and also higher concentration of CP in tea waste as compared to alfalfa (Table 3), it was expected that nutritive value of tea waste would be higher but *in vitro* gas production from tea waste was lower than that of alfalfa. This implies that this feed may contain anti-nutritional factors that negatively affected rumen microbes (or rumen microbes are less capable of degrading tea waste). Content of total extractable phenolics and total tannins in tea waste was almost ten times higher than that of alfalfa (Table 3).

Table 4 Effect of different levels of tea waste on *in vivo* digestibility coefficient of diet, digestible energy and metabolizable energy

Parameters	Inclusion of tea waste in diet (g/kg DM)					P-value		
	0	40	80	120	160	SEM	Linear	Quadratic
Dry matter	567	561	536	548	545	13.81	0.134	0.801
Organic matter	592	582	557	571	562	14.81	0.123	0.883
Organic matter in dry matter	536	524	505	517	514	14.32	0.150	0.900
Crude protein	741[a]	576[bc]	601[b]	567[bc]	541[c]	17.45	0.000	0.000
Neutral detergent fiber	426[a]	399[ab]	342[b]	338[b]	367[ab]	25.00	0.034	0.829
Gross energy	570	560	560	553	554	10.80	0.520	0.447
Digestible energy (MJ/kg)	10.24	7.99	9.86	9.94	10.07	1.16	0.824	0.126
ME (MJ/kg)[1]	8.40	6.55	8.09	8.15	8.26	0.95	0.824	0.126

[1] Calculated using the equation metabolizable energy (ME)= DE × 0.82 (Robbins, 1993).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

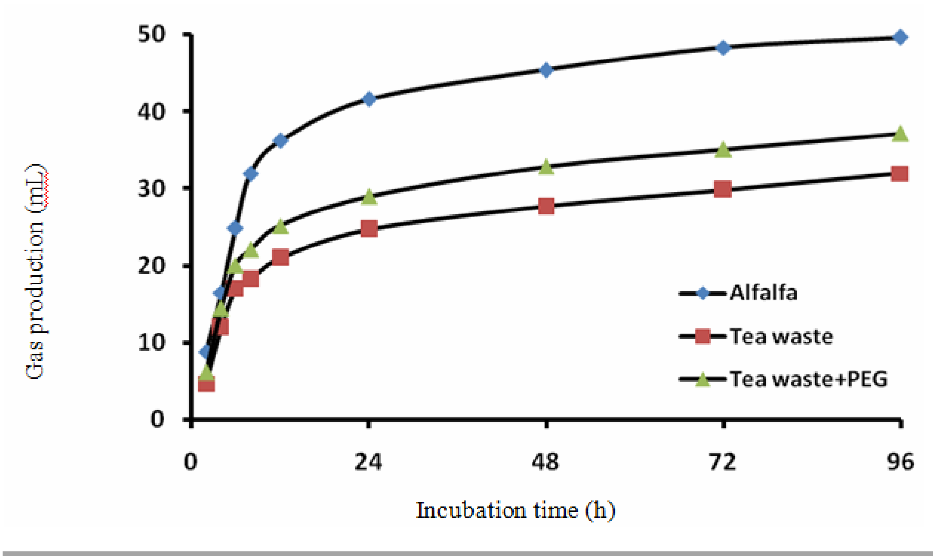

Figure 1 *In vitro* gas production from alfalfa, tea waste and tea waste + polyethylene glycol

Table 5 Voluntary feed intake of experimental diets in sheep

Intake	Inclusion of tea waste in diet (g/kg)[1]					SEM
	0	40	80	120	160	
Dry matter g/d	1175[a]	985[a]	747[b]	379[c]	376[c]	101.0
Organic matter g/d	1068[a]	896[a]	683[b]	345[c]	348[c]	98.0
Dry matter g/kg live weight	68.0[a]	56.9[ab]	45.2[b]	23.9[c]	23.6[c]	6.8
Dry matter g/kg $W^{0.75}$	61.8[a]	51.8[ab]	41.4[b]	21.7[c]	21.9[c]	6.2
Digestible dry matter g/d	664[a]	584[a]	350[b]	210[c]	2.5[c]	59.0
Digestible organic matter g/d	630[a]	548[a]	385[b]	194[c]	198[c]	55.0
Digestible dry matter g/kg $W^{0.75}$	38.4[a]	33.8[a]	24.2[b]	13.2[c]	13.0[c]	3.9

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Concentration of total extractable phenolic compounds and total tannins in tea waste in this study were in agreement with the result of Torbati-Nejad (1988), Jayasuriya *et al.* (1978) and Konwar *et al.* (1992). Addition of PEG to tea waste significantly increased *in vitro* gas production. Affinity of PEG to tannins has been well documented (Makkar *et al.* 1995a). Improvement in *in vitro* gas production by addition of PEG indicates that PEG has inactivated some of anti-nutritional poly-phenolics compounds (Robbins *et al.* 1987; Reed, 1995; Silanikove *et al.* 2001).

Reduction in digestibility coefficient of NDF and CP (Table 4) is very likely due to presence of inhibitory compounds specially tannins in tea waste (Torbati-Nejad, 1988; Jayasuriya *et al.* 1978; Konwar *et al.* 1992). Tannins can bind to the cell wall and reduce cell wall digestibility and consequently reduce production of volatile fatty acids (Makkar *et al.* 1995b; Robbins *et al.* 1987; Silanikove *et al.* 2001). Tannins may have positive effect through binding to feed proteins and make insoluble complexes and reduce digestibility in the rumen (Giner-Chavez, 1996; Kumar and

Vaithiyanathan, 1990) and afterward, the low pH in the abomasums as well as in the small intestine can stimulate dissociation (Perez-Maldonado *et al.* 1995; Zahedifar *et al.* 2001).

In this way, tannins can improve the total availability of protein in forages by reducing the wasteful deamination of protein in the rumen in diets where rumen degradable protein is in excess of microbial requirements (Mangan, 1988; Perez-Maldonado and Norton, 1996). It has been reported that tannins can bind to bacterial enzymes in the rumen and reduce efficiency of hydrolytic enzymes (Makkar *et al.* 1995b; Bhatta *et al.* 2000; Imik and Seker, 1999; Barry and Manley, 1984). It seems that apart from tannins, other compounds like caffeine are also responsible in this respect (Giner-Chavez, 1996).

Voluntary feed intake

A declining trend was observed in dry matter and other feed intake characteristics by substitution of alfalfa with tea waste in the diet.

Torbati-Nejad (1988) reported that daily feed intake in sheep was 827 g when 250 g/kg DM of alfalfa was replaced with tea waste which was higher than the values observed in this study (Table 5). In another experiment inclusion of 100 g/kg DM tea waste in diet of fattening male calves did not affect intake (Nikkhah and Hosseini, 1985). Such difference is likely related to chemical composition and quality of tea waste.

Quality of some agricultural by products and residues of feed processing plants could be very heterogeneous and their composition is dependent on the method of harvesting and processing (Fazaeli *et al.* 2000). Variation in ratio of leaf to stem and thickness of stem in tea waste affect ratio of fibrous fraction and concentration of anti-nutritional factors and consequently affect digestibility and palatability of feed (Landau *et al.* 2000; Silanikove *et al.* 1994; Silanikove *et al.* 2001).

Astringency of feed which is related to content of condensed tannins and its protein precipitating capacity is an important factor affecting palatability of feed (Bajec and Pickering, 2008; Barahona *et al.* 1997).

CONCLUSION

Results of this study showed that chemical composition of residues of tea processing including drying, Fiber-mat and Screening was more or less similar. Tea by-product can be classified as low quality roughage. Presence of tannins in tea by-product limits availability of its nutrients especially protein therefore, it is suggested to include up to 4 percent of it in diet of sheep.

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