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Research Article
Effect of Polyethylene Glycol Addition on Nutritive Value of Green and Black Tea Co-products in Ruminant Nutrition

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Abstract
Background and Objective: Efficient use of available unconventional feeds and co-products is very important in developing countries. Therefore, the present study aimed to investigate the chemical composition, *in vitro* gas production (GP), methane production (CH_4), dry matter digestibility, organic matter digestibility (OMD), metabolizable energy (ME), net energy lactation (NE_L) and short-chain fatty acid (SCFA) of green tea waste (GTW), black tea waste (BTW) and alfalfa hay in presence or absence of polyethylene glycol (PEG). **Materials and Methods:** The amount of dry matter digestibility, OMD, ME, NE_L, SCFA and CH_4 in experimental treatment was determined by *in vitro* gas production technique. Rumen fluid samples were collected from three Taleshi cows (live weight 335±2.5 kg) before the morning meal. Chemical composition data was analyzed by general linear models, using a completely randomized design (CRD); the means were compared with Duncan's test ($p<0.05$) procedures in SAS (version 9.1). Gas production, CH_4, dry matter digestibility, OMD, ME, NE_L and SCFA data were analyzed by general linear models, using a completely randomized design in a 2×3 factorial arrangement. The means were compared with Tukey's test ($p<0.05$) procedures in SAS (version 9.1). **Results:** The amount of phosphorus was not significantly affected by treatments but other chemical composition showed a wide variation ($p<0.05$) between treatments. The use of PEG increased ($p<0.05$) GP from GTW and BTW in all incubation times. The PEG significantly increased ($p<0.05$) the volume of GP from soluble fraction (a) The volume of GP from insoluble but fermentable fraction and (b) GP potential (a+b) in GTW and BTW. The CH_4 production significantly increased in BTW by PEG ($p<0.05$). The amount of dry matter digestibility, OMD, ME, NE_L and SCFA in GTW and BTW significantly increased by using PEG ($p<0.05$). **Conclusion:** The results of this study indicated that adding PEG to GTW and BTW can improve their nutritive value and could be considered as a potential feed for ruminants.

Key words: Chemical composition, co-product, digestibility, gas production, methane, rumen

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tea (*Camellia* spp.) co-products would be a potential feed for ruminant but there are studies showing the presence of anti-nutritional compounds (total phenolics compounds, condensed tannins and saponins) in this waste[1,2]. These compounds reduce the activity of rumen microorganisms, limit degradation of nutrients and reduce the production of volatile fatty acids[3,4]. High levels of condensed tannins can have harmful effects on palatability, feed intake and nutrient digestibility; therefore reduce animal performance[5,6]. Simple treatments or additives for such feed stuffs can improve their nutritive values as ruminant feeds [7].

The PEG is a non nutritive synthetic polymer having high affinity to form complexes, especially with the condensed tannins-proteins[3,8]. Accordingly, PEG can prevent the release of protein from tannin-protein complex and has been used to mitigate the adverse side effects of compounds on fermentation in rumen. However, an increased on *in vitro* gas production and degradation parameters with PEG addition was due to its effect on tannin in feedstuffs[8,9]. The use of PEG to neutralize condensed tannins has proved to be useful for protein-tannin complexes displaces due to the ability of condensed tannins to interact more strongly with PEG than they do with protein[8]. Thus, supplementation with PEG has been used to moderate the harmful effects of tannins on livestock[3,10]. However, their effects depend on the nature of the tannins and the nature of the formed tannin feedstuff complexes.

Kondo *et al.*[1] evaluated the influence of various molecular weights of PEG, polyvinyl pyrrolidone and polyvinyl polypyrrolidone as tannin-binding agents on nutritive value of tea waste. These researchers concluded that PEG (6000 and 20000 MW) improved GP, OMD and ME of tea by-products. Also, Yousef Elahi *et al.*[8] reported that addition of PEG has increased GP, OMD and ME in Prosopis cineraria leaves. *Prosopi spp.* has been reported to contain tannins.

The *in vitro* gas production technique, have been used to evaluate the nutritional value of feed resources. Increased interest in use of non-conventional feed resources has led to an increase in use of this technique, since gas production can provide useful data on digestion kinetics of both the soluble and insoluble fractions of feedstuffs[11]. The ease of measuring fermentation end products makes this method more efficient than other *in vitro* methods for studies on feedstuffs, plant secondary metabolites and feed additives[12].

More research about tea waste focused on the waste that produced by beverage companies manufacturing various tea drinks (tea spent leaf) and there is little information about the waste that produced by tea drying factories. Therefore, the aim of this study was to evaluate the chemical composition of green tea waste (GTW), black tea waste (BTW) that was produced by tea drying factories and alfalfa hay and the effect of adding PEG during incubation on their *in vitro* gas production (GP), methane production (CH_4), dry matter digestibility, OMD, ME, NE_L and SCFA.

MATERIALS AND METHODS

This study was performed in November 2016 in the Nutrition and Physiology laboratory of Animal Science Research Institute of Iran, Karaj, Alborz, Iran. Green and black tea waste provided from tea drying factories in North of Iran with tea research institute of Iran cooperation.

Chemical composition: Dry matter (DM), organic matter (OM), ether extract (EE) and nitrogen (N) contents were calculated according to AOAC method[13]. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were measured by Fiber-Tec system[14]. Gross energy content was calculated by using Bomb calorimeter[13]. Minerals including Ca and P were measured by atomic absorption spectrometer[13]. Total phenols, total tannins, condensed tannins and hydrolysable tannins were determined according to Hagerman method[15]. The procedure of Yosioka *et al.*[16] was used for total saponin (TS) determination.

***In vitro* gas production:** Rumen fluid samples were collected from three Taleshi cows (live weight 335±2.5 kg) before the morning meal. The cows were fed with a TMR diet that was consisted of alfalfa hay, wheat straw and a concentrate supplement in order to meet maintenance requirements according to NRC[17] recommendation. The cows had free access to the diet delivered twice a day at 8:00 am and 6:00 pm. The rumen fluid collected from each cow was mixed and then passed through three layers of cheese cloth for use in the *in vitro* gas production. The *in vitro* gas production was carried out in 150 mL serum bottles. Three replicates were used for each treatment. Ten milliliters of particle-free rumen fluid and 200 mg of ground feed substrate was added to each bottle and 40 mL of the buffer solution (pH = 6.8-7) of Goering and Van Soest [18], without Trypticase was immediately added in 1:4 (v/v) ratio. The solution contained micro-minerals ($CaCl_2 \cdot 2H_2O$ (Reagent plus grade), $MnCl_2 \cdot 4H_2O$ (Reagent plus grade), $CoCl_2 \cdot 6H_2O$ (ACS reagent grade), $FeCl_3 \cdot 6H_2O$ (ACS reagent grade), macro minerals (Na_2HPO_4 anhydrous, KH_2PO_4 anhydrous, $MgSO_4 \cdot 7H_2O$ (ACS reagent grade) and reducing solution (1N NaOH (Analytical grade), $Na_2S \cdot 9H_2O$ (ACS reagent grade), in addition to resazurin (Bio reagent grade) and buffer reagent of NH_4HCO_3 (Reagent plus grade) and $NaHCO_3$ (Bio reagent grade)[19,20]. Four hundred milligram of PEG (Analytical grade) was added to each bottle in PEG treatment group. These serum bottles each were sealed with a butyl rubber stoppers and then incubated at 39°C for 96 h in an incubator. Volume of produced gas was recorded at 3, 6, 12, 24, 48, 72 and 96 h after incubation[20].

Measurements: Kinetic parameters of gas production were estimated by fitting GP results (mL g^{-1} DM) according to the model described by Orskov and McDonald[21].

$$P = a+b\,(1-e^{-ct})$$

where, P is the volume of GP at time t (mL g^{-1} DM), a is volume of GP from soluble fraction (mL g^{-1} DM), b represents the volume of GP from insoluble but fermentable fraction (mL g^{-1} DM), c is the rate of GP h^{-1} from the fraction b, t is the time (h) and e: 2.7182.

The CH_4 production was estimated according to Fievez *et al.*[22].

The dry matter digestibility (DMD %) was determined according to the formula given in Khazaal *et al.*[23]:

$$\text{DMD (\%)} = 69+74(a+b)+2264(c)+0\text{-}36(CP)$$

where, CP is expressed in percent.

ME (MJ kg^{-1} DM) and OMD (%) were estimated according to the formula given in Menke *et al.*[24]:

$$\text{ME (MJ kg}^{-1}\text{ DM)} = 2.20+0.136\text{ GP}+0.057\text{ CP}$$

$$\text{OMD (\%)} = 14.88+0.889\text{ GP}+0.45\text{ CP}+0.0651\text{ XA}$$

where, DM and CP are expressed in percent; XA: Ash in percent and GP: The net gas production in milliliters from 200 mg dry sample after 24 h of incubation.

NE_L was calculated according to Menke and Steingass[25] as:

$$NE_L\text{ (MJ kg}^{-1}\text{ DM)} = 0.096\text{ GP}+0.0038\text{ CP}+0.000173\text{ EE}^2+0.54$$

where, GP is net gas production in milliliters from 200 mg dry sample after 24 h of incubation, CP and EE in percent.

The SCFA was calculated was determined according to Getachew *et al.*[26] as:

$$\text{SCFA (m mol mg}^{-1}\text{ DM)} = 0.0222\text{GP}-0.00425$$

where, GP is the net gas production in milliliters from 200 mg dry sample after 24 h of incubation.

Statistical analysis: Chemical composition data were analyzed by general linear models, using a completely randomized design; the means were compared with Duncan's test ($p<0.05$) procedures in SAS (version 9.1)[27]. Gas production, methane production (CH_4), DMD, OMD, ME, NE_L and SCFA data were analyzed by general linear models, using a completely randomized design in an 2×3 factorial arrangement. The experimental factors included PEG application (PEG (-) and PEG (+)) and feedstuffs (alfalfa hay, green tea waste and black tea waste). Means were compared with Tukey's test ($p<0.05$), procedures in SAS (version 9.1)[27].

RESULTS AND DISCUSSION

Chemical composition: The chemical composition of experimental treatments is shown in Table 1. The DM content in GTW was significantly greater than other treatments ($p<0.05$). The amount of Ash in alfalfa hay was higher than other feedstuffs ($p<0.05$) but no significant difference exist between GTW and BTW about that. There was no significant difference between GTW and BTW in OM content but it was significantly lower in alfalfa hay related to GTW and BTW ($p<0.05$). Crude protein (CP) and EE content in alfalfa hay were greater than other treatments ($p<0.05$). The amount of NDF was lower in BTW ($p<0.05$) than other feeds but there was no significant difference between alfalfa hay and GTW about NDF content. The ADF content was significantly lower in BTW than other treatments ($p<0.05$). The amount of ADL was not significantly different between GTW and BTW but it was higher in alfalfa hay than others. The GE value in BTW was greater than other treatments ($p<0.05$). The Ca content in alfalfa hay was significantly higher than other treatments ($p<0.05$) but there was no difference between GTW and BTW about that. Total phenols, total tannins, condensed tannins and total saponin in GTW were greater than other treatments ($p<0.05$). There was no difference about hydrolysable tannins between GTW and BTW but it was significantly lower in alfalfa hay than other treatments ($p<0.05$).

High contents of CT could negatively affect the digestion processes in the rumen, impact the growth of rumen bacteria and enzyme activity[10,12], even depress consumption or reduce nutrient digestibility[10]. Dey *et al.*[6] reported that moderate levels (1-4%) of condensed tannins produce positive effects, when coupled with dietary protein forms complexes, preventing their degradation in the rumen. On the other hand,

Table 1: Chemical composition (% DM) of alfalfa hay, green tea waste and black tea waste

Chemical composition	Alfalfa hay	Green tea waste	Black tea waste	p-value	SEM
DM	93.71[b]	94.84[a]	92.72[c]	0.0001	0.06
Ash	10.77[a]	5.58[b]	5.75[b]	0.0001	0.09
OM	89.22[b]	94.41[a]	94.24[a]	0.0001	0.09
CP	17.29[a]	16.31[b]	15.66[c]	0.0001	0.20
EE	1.32[a]	1[b]	1.16[ab]	0.008	0.08
NDF	41.40[a]	42.15[a]	38.47[b]	0.0001	0.46
ADF	31.21[b]	32.95[a]	25.87[c]	0.0001	0.41
ADL	9.38[a]	8.61[b]	8.36[b]	0.01	0.29
GE (Kcal kg^{-1} DM)	4218.29[c]	4551.79[b]	4621.27[a]	0.0001	18.47
Ca	1.06[a]	0.35[b]	0.40[b]	0.0001	0.03
P	0.23[a]	0.21[a]	0.27[a]	0.12	0.02
TP	1.58[c]	16.26[a]	12.18[b]	0.0001	0.44
TT	0.75[c]	12.28[a]	10.35[b]	0.0001	0.39
CT	0.60[c]	8.35[a]	6.46[b]	0.0001	0.52
HT	0.14[b]	3.93[a]	3.89[a]	0.0001	0.45
TS	1.40[c]	14.64[a]	9.68[b]	0.0001	0.38

DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, GE: Gross energy, Ca: Calcium, P: Phosphorus, TP: Total phenols, TT: Total tannins, CT: Condensed tannins, HT: Hydrolysable tannins, TS: Total saponins, the means within the same row with at least one common letter, do not have significant difference ($p>0.05$)

Table 2: *In vitro* gas production (mL g^{-1} DM) of alfalfa hay, green tea waste and black tea waste, without (-) PEG or with (+) PEG

		Incubation time						
Treatments		3	6	12	24	48	72	96
Alfalfa hay	-	17.50[a]	26.83[a]	38.36[a]	44.50[a]	45.66[a]	48.00[a]	49.12[a]
	+	16.36[ab]	26.53[a]	36.40[a]	42.33[ab]	44.75[a]	47.21[a]	49.04[a]
Green tea waste	-	9.650[d]	14.33[c]	21.03[c]	33.59[c]	36.09[b]	38.79[b]	39.76[b]
	+	13.18[c]	19.31[b]	25.16[b]	39.03[b]	43.28[a]	45.80[a]	47.13[a]
Black tea waste	-	9.180[d]	13.68[c]	20.56[c]	32.20[c]	35.00[b]	37.92[b]	39.06[b]
	+	13.86[bc]	18.58[b]	25.94[b]	38.75[b]	43.30[a]	46.31[a]	47.46[a]
p-value		0.020	0.040	0.020	0.005	0.002	0.004	0.008
SEM		1.280	1.520	1.690	1.650	1.530	1.650	1.72

PEG: Polyethylene glycol (Merck, MW = 6000), the means within the same column with at least one common letter, do not have significant difference ($p>0.05$)

Table 3: Kinetic parameters of gas production of alfalfa hay, green tea waste and black tea waste, without (-) PEG[1] or with (+) PEG

		Items				
Treatments		a	b	a+b	c	CH_4
Alfalfa hay	-	0.48[b]	46.79[a]	47.28[a]	0.14[a]	20.00[a]
	+	0.74[b]	45.67[a]	46.41[a]	0.13[a]	19.03[ab]
Green tea waste	-	0.83[b]	38.11[b]	38.94[b]	0.07[b]	11.63[d]
	+	2.00[a]	43.94[a]	45.95[a]	0.07[b]	14.26[cd]
Black tea waste	-	0.81[b]	37.26[b]	38.10[b]	0.06[b]	12.20[d]
	+	2.17[a]	44.08[a]	46.25[a]	0.07[b]	16.40[bc]
p-value		0.04	0.001	0.002	0.19	0.060
SEM		0.29	1.240	1.500	0.07	1.410

PEG: Polyethylene glycol (Merck, MW = 6000), a: The volume of GP from soluble fraction (mL g^{-1} DM), b: The volume of GP from insoluble but fermentable fraction (mL g^{-1} DM), a+b: GP potential (mL g^{-1} DM), c: The rate of GP (/h) from the fraction b, CH4: Methane production volume (mL/200 mg DM), The means within the same column with at least one common letter, do not have significant difference ($p>0.05$)

high levels of CT in feedstuff have been reported to restrict the nutrient utilization and reduce voluntary food intake, nutrient digestibility and N retention[28,29]. The use of PEG to neutralize condensed tannins has proved to be useful for protein-tannin complexes displaces due to the ability of condensed tannins to interact more strongly with PEG than they do with protein[8]. Therefore, supplementation of PEG can be recommended to reduce the detrimental effect of tannin in some feedstuff such as tea co-products which contain a lot of tannin[8].

***In vitro* gas production:** The *in vitro* gas production (mL/200 mg DM) of alfalfa hay, GTW and BTW, without or with PEG is shown in Table 2. Inclusion of PEG, increased the amount of gas produced from GTW and BTW after all incubation time ($p<0.05$). The PEG supplementation did not significantly change gas produced from alfalfa hay.

The kinetic parameters of gas production of alfalfa hay, green tea waste and black tea waste without (-)PEG and with (+)PEG is presented in Table 3. The volume of GP from soluble

Table 4: Contents of DMD, OMD, ME, NE_L and SCFA in alfalfa hay, green tea waste and black tea waste, without (-) PEG[1] or with (+) PEG

		Items				
Treatments		DMD	OMD	ME	NE_L	SCFA
Alfalfa hay	-	58.84^{a}	62.92^{a}	9.24^{a}	4.88^{a}	0.98^{a}
	+	57.02^{ab}	60.99^{a}	8.94^{a}	4.67^{ab}	0.93^{ab}
Green tea waste	-	46.11^{d}	52.44^{c}	7.69^{c}	3.83^{c}	0.74^{c}
	+	52.20^{c}	57.28^{b}	8.44^{b}	4.35^{b}	0.86^{b}
Black tea waste	-	45.09^{d}	50.93^{c}	7.47^{c}	3.69^{c}	0.71^{c}
	+	53.35^{bc}	56.75^{b}	8.36^{b}	4.32^{b}	0.85^{b}
p-value		0.004	0.005	0.005	0.005	0.006
SEM		1.770	1.470	0.220	0.160	0.030

DMD: Dry matter digestibility, DMD (%): 69+74(a+b)+2264(c)+0-36 (CP), OMD: Organic matter digestibility, OMD (%): 14.88+0.889 GP+0.45 CP+0.0651 XA, ME: Metabolizable energy, ME (MJ kg^{-1} DM) = 2.20+0.136 GP+0.057 CP, NEL: Net energy lactation, NEL (MJ kg^{-1} DM) = 0.096 GP+0.0038 CP+0.000173 EE2+0.54, SCFA: Short-chain fatty acid, SCFA (m moL mg^{-1} DM) = 0.0222 GP-0.00425. The means within the same column with at least one common letter, do not have significant difference (p>0.05)

fraction (a) of alfalfa hay did not get affected by PEG addition, but this fraction is significantly increased in GTW and BTW by using PEG ($p<0.05$). The volume of GP from insoluble but fermentable fraction (b) of gas production has significantly improved in GTW and BTW by PEG treatment ($p<0.05$). The amount of alfalfa hay b fraction of gas production did not significantly change by PEG addition. The PEG inclusion significantly increased potential extent of gas production (a+b) in GTW and BTW ($p<0.05$) but it did not change in alfalfa hay after PEG addition. The rate of GP from the fraction b (c) did not change by PEG in all treatments. The CH_4 production from alfalfa hay and GTW did not significantly change by PEG supplementation but PEG addition significantly increased CH_4 production from BTW ($p<0.05$).

The effect of PEG addition on DMD, OMD, ME, NE_L and SCFA of alfalfa hay, GTW and BTW is presented in Table 4. The use of PEG increased DMD and OMD in GTW and BTW ($p<0.05$). The PEG inclusion increased the amount of ME and NE_L in GTW and BTW ($p<0.05$). The Production of SCFA in GTW and BTW improved by PEG addition ($p<0.05$).

In this study, inclusion of PEG, increased the amount of gas production characteristics in GTW and BTW (Table 2 and 3). The increase in the GP from GTW and BTW in the presence of PEG can be due to an increase in the availability of nutrients to rumen microorganisms, especially N[30]. Addition of PEG can caused different responses in GP between different feed stuffs. These variable responses of GP could be due to variations in tannin content between different feed resources[8]. Gas production is connected to volatile fatty acids production following fermentation of substrate; therefore, more fermentation of a substrate will result in a greater GP[31]. The GP improvement when feed resources were incubated with PEG was also reported by other authors. Arhab *et al.*[32] evaluated the influence of tannins present in arid zone forages from North Africa including *Aristida plumosa*, *Astragalus gombiformis*, *Genista saharae*, two date palm fractions (leaves and racemes) and vetch-oat hay taken as control on *in vitro* GP. They found that PEG inclusion resulted in an overall increase in GP (20.2%), with the exception of *Danthonia and Aristida*. The variations in methane production support the view that tannins decrease methane production. Other dietary components such as NDF also contributed to explain total variation in methane production. Higher NDF increases methane production by shifting short chain fatty acid proportion towards acetate which produces more hydrogen[33].

The use of PEG increased DMD and OMD in GTW and BTW ($p<0.05$) (Table 4). These findings are supported by Salem *et al.*[3] and Arhab *et al.*[32] studies. The CT interferes with attachment of rumen bacteria to feed particles, affecting the bacterial population and ruminal fermentation, in conclusion reducing DMD and OMD[8]. When the feedstuff is incubated with PEG, PEG-tannins complex was formed, which facilitated the substrate digestion by bacteria, improving DMD and OMD[7].

The PEG inclusion increased the amount of ME and NE_L in GTW and BTW ($p<0.05$) (Table 4). Some factors could be responsible for ME and NE_L improvement in PEG treated group such as high gas production in the treated substrate and complex formation between the PEG and tannins[7].

Production of SCFA in GTW and BTW improved by PEG addition ($p<0.05$) (Table 4). According to this research, Getachew *et al.*[9] reported an increased SCFA production due to PEG addition. The addition of PEG increased iso acid concentration, also in the absence of PEG; there was greater absorption of iso acids, because tannins protected protein from deamination[9]. Mohammadabadi *et al.*[34] reported that there is a positive correlation between SCFA and GP. The increase of *in vitro* GP and SCFA production due to the addition of PEG in the present study confirm the effect of tannins on DMD and OMD. The high tannin contents in the green tea waste and black tea waste may limit their utilization by ruminant livestock. The PEG inclusion improved *in vitro* gas production characteristics, DMD, OMD, ME, NE_L and SCFA in green tea waste and black tea waste.

CONCLUSION

In this study it is concluded that, addition of PEG is apparently suitable to deactivate the suppressive activity of the tannins in green tea waste and Black tea waste. Therefore, PEG addition can be used for reducing adverse effect of these feed stuffs tannin in ruminant diet.

SIGNIFICANCE STATEMENTS

This study has discovered that the use of polyethylene glycol can be beneficial for improving the nutritional value of green tea waste and black tea waste in ruminant nutrition. This study will help the researcher to uncover the critical areas of tea drying factories by-products use in ruminant nutrition that many researchers were not able to explore. Thus a new theory on how to use polyethylene glycol for improving the nutritional value of these co-products in ruminant nutrition was obtained.

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