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Effects of partial substitution of alfalfa hay with green tea waste on growth performance and *in vitro* methane emission of fat-tailed lambs

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ABSTRACT

In this study, the effects of partial substitution of alfalfa hay (AH) with green tea waste (GTW) on feed intake, growth performance, digestibility, nitrogen (N) retention, ruminal fermentation, serum metabolites and methane emission of fattening fat-tailed lambs were evaluated. Methane emission was determined in *in vitro* condition using gas production technique. Twenty Chall male lambs with an initial body weight of 34.36 ± 3.35 kg were randomly assigned into four isoenergetic and isonitrogenous diets in which AH was replaced with different levels [(0 (control), 20, 40 or 60 g/kg dietary dry matter (DM))] of GTW for a period of 84 days. The replacement of AH by GTW did not affect dry matter intake (DMI). The replacement of AH up to 60 g/kg GTW significantly decreased average daily gain (ADG) (linear (L) and quadratic (Q), $P < 0.05$) and increased feed conversion ratio (FCR) (L, $P < 0.05$). The highest crude protein (CP) digestibility was observed in lambs receiving 20 g/kg GTW in the diet (L and Q, $P < 0.05$). N retention in the 20 g/kg GTW recipient group was similar to the control, but it was significantly more than other treatments (L and Q, $P < 0.05$). Increase in the proportion of GTW in the diets decreased ammonia-N in the rumen fluid (L, $P = 0.003$). When the levels of GTW in the diets were increased, serum concentration of urea-N decreased (L, $P < 0.05$). Dietary treatments decrease the amount of methane emission (L and Q, $P < 0.05$). According to the results, substitution of AH with GTW at 20 g/kg DM level in the diet of fat-tailed lambs can improve growth performance by improving digestibility, N retention and reducing methane emission.

1. Introduction

Tea is one of the most popular beverages in the world and is also very useful for health. Tea is rich in secondary metabolites such as tannins, saponins and proteins, amino acids, lipids, sugars, vitamins, fiber and minerals (Ramdani et al., 2013). In ruminants, some plant secondary metabolites such as tannins, may increase the amount of protein passing through the rumen and non-ammonia nitrogen and increase their absorption in the small intestine (Ramdani et al., 2013). Tannins also have the ability to reduce methane production in the rumen. Furthermore, saponins can play a role in reducing methane and ammonia production (Ramdani et al., 2013). The use of tea waste as a feedstuff in ruminant nutrition can be beneficial both economically and for environmental reasons. In order to evaluate the possibility of using tea waste as a feedstuff in the diet of ruminant, the amount of tannins in it should be noted (Kondo et al., 2014). Kondo et al. (2007) reported that the addition of ensiled black tea waste to more than 10 g/kg of dry matter in the diet reduced the amount of ammonia nitrogen in *in vitro* condition. The researchers also observed that the addition of ensiled black tea waste at 50 g/kg of dry matter in the diet reduced the digestibility of crude protein compared to the control treatment. Addition of tea catechins in the diet of goat improved daily weight gain, increased protein content and dry matter in goat meat (Tan et al., 2011b). Zhou et al. (2012) stated that the addition of tea saponins in goat diet did not change the degradability of dry matter, nitrogen, NDF and ADF in the rumen. Also, tea saponins did not change the pH, ammonia nitrogen and volatile fatty acids of rumen fluid (Zhou et al., 2012). Most of the studies on tea waste are related to black tea or tea beverage waste, and there is no information on the chemical composition of green tea waste from tea drying factories and its use in ruminant nutrition. Therefore, the present study was conducted to investigate the effect of using different levels of green tea waste from tea drying factories on nutrient intakes, growth performance, nutrients digestibility, nitrogen balance, rumen fermentation parameters, serum parameters

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Table 1
The chemical analyses of green tea waste (g/kg DM).

Chemical analyses	Green tea waste
Dry matter (g/kg)	948
Organic matter	944
Crude protein	163
Ether extract	10
Natural detergent fiber	421
Acid detergent fiber	329
Acid detergent lignin	86.1
Calcium	3.5
Phosphorous	2.1
Total phenols	162
Total tannins	122
Condensed tannins	83.5
Hydrolysable tannins	39.3
Saponins	146
Metabolizable energy (MJ/kg DM)[a]	7.6

[a] The metabolizable energy value was measured using a gas production technique, as described by Menke and Steingass (1988).

and methane emission of Chall lambs.

2. Materials and methods

2.1. Green tea waste preparation and experimental diets

The green tea waste used in this research was prepared in co-operation with the Tea Research Institute of Iran from tea drying factories located in the city of Lahijan in Gilan province of Iran. Chemical composition of green tea waste is presented in Table 1. Four iso-energetic and isonitrogenous diets were formulated according to NRC (2007), where alfalfa hay was replaced by green tea waste (Table 2).

2.2. Animals and management

Experiment on animals was performed in accordance with the Care and Use of Agricultural Animals in Research and Teaching guidelines (Federation of Animal Science Societies, 2010).

In this experiment, Chall male lambs (20 heads with an average weight of 34.36 ± 3.35 kg and 4–5 months of age) which are Iranian heavy and meat breeds, were used. The lambs were randomly assigned into four dietary treatments (5 replicates per treatment). The lambs were housed in individual boxes (1.5 m × 1.5 m) in a closed building and had free access to feed and water through the trial. At the beginning of the experiment and before the transfer of lambs, all individual boxes and equipment were completely cleaned and disinfected. All of the animals were vaccinated against enterotoxemia and foot and mouth disease. In order to eliminate parasitic infections, these lambs received internal anti-parasite drug (Albendazole, Zagros pharmed, Iran). The feeding trial lasted 70 days preceded by 14-day adaptation period to the individual pens, diets and the experimental conditions (total of 84 days). Lambs were fed two times a day at 08.00 and 18.00 h with total mixed ration during this time. All the lambs were individually weighed on days 0, 14, 28, 42, 56 and 70 at 08.00 h after 16 h of feed deprivation. The live weight changes were measured at 2 weeks intervals and the ADG was estimated by fitting a simple linear regression model of weight over time (Rattanaronchart et al., 1983). Individual feed intake was recorded daily and FCR for each lamb was calculated as the ratio of daily DMI to ADG (Papi et al., 2017).

2.3. Laboratory analyses

Samples of green tea waste, feed offered, orts and faeces were oven-dried at 60 °C until a constant weight to determine DM content, then ground to pass 1 mm sieve (Wiley mill, Swedesboro, USA) and stored until analysis. Samples were analyzed for ADF (method 983.18), ash (method 924.05), N (method 984.13), ether extract (EE; method 954.02) calcium (method 927.02) and phosphorus (method 965.17) by AOAC (1990) procedures. The aNDF was measured using heat-resistant ά -amylase without sodium sulfite (Van Soest et al., 1991). The aNDF and ADF fractions include residual ash. Lignin (sa) was measured using the sulfuric acid method (Robertson and Van Soest, 1981). Total extractable phenolic compounds, total tannins, condensed tannins and hydrolysable tannins of green tea waste were measured based on Hagerman (2002) method. The procedure of Yosioka et al. (1974) was employed for determination of green tea waste saponins. The metabolizable energy value of green tea waste was determined using a gas production technique, as described by Menke and Steingass (1988).

Table 2
Ingredients and chemical composition of the experimental diets (g/kg DM).

Item	Level of green tea waste in diet (g/kg DM)			
	0	20	40	60
Ingredients				
Alfalfa hay	250	230	210	190
Green tea waste	0	20	40	60
Wheat straw	50	50	50	50
Corn grain	400	400	400	400
Soybean meal	100	100	100	100
Wheat bran	175	175	175	175
$CaCO_3$	5	5	5	5
Urea	5	5	5	5
Salt	5	5	5	5
Sodium bicarbonate	5	5	5	5
Vitamin-mineral premix[a]	5	5	5	5
Chemical composition (g/kg DM)				
Dry matter (g/kg Fresh)	893.7	894.1	894.5	894.9
Organic matter	936.4	932.5	934.4	936.0
Crude protein	153.6	155.0	156.4	157.8
Ether extract	17.8	20.0	21.4	23.8
Natural detergent fiber	314.2	311.0	307.8	304.6
Acid detergent fiber	182.2	180.0	178.8	175.6
Calcium	5.5	5.3	5.1	4.9
Phosphorous	5.0	4.9	4.9	4.9
Total tannins	1.8	4.1	6.4	8.7
Condensed tannins	1.5	2.9	4.5	6.1
Hydrolysable tannins	0.3	1.0	1.7	2.5
Saponins	3.5	6.1	8.7	11.3
Metabolizable energy (MJ/kg DM)	10.8	10.9	10.9	11.00

DM, dry matter;
[a] premix contained (per kg): vitamin A, 500,000 IU; vitamin D3, 100,000 IU; vitamin E, 100 mg; Ca, 190,000 mg; P, 90,000 mg; Na, 50,000 mg; Mg, 19,000 mg; Fe, 3000 mg; Cu, 300 mg; Mn, 2000 mg; Zn, 3000 mg; Co, 100 mg; Se, 1 mg; Antioxidant, 3000 mg.

2.4. In vivo digestibility and N balance

Whole tract apparent digestibility coefficients of DM, organic matter (OM), CP, NDFom, ADFom and EE were estimated using the total faecal collection method (Givens et al., 2000). On day 72 of the experiment, 4 animals per treatments (*i,e.,* 16 lambs in total) were selected on the basis of BW as representing the average BW in each experimental treatments. Animals were placed into individual metabolism crates equipped with faeces and urine collectors, in a complete randomized design. The digestibility trial lasted for 10 days, with 3 days for adaptation to metabolism crates and 7 days for the collection period. During the 7 days of collection period, the amount of feed offered, orts and faeces from each lamb were recorded daily and then 10% representative samples were taken. At the end of the period, daily samples were pooled for each lamb within the treatment, thoroughly mixed and stored at −20 °C for later analysis.

At the same time, total urine produced daily was collected in plastic vessels containing 100 ml of sulphuric acid solution (10%, v/v), to keep