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A study to investigate the anti-methanogenic properties of black tea waste in dairy cows in order to achieve a cleaner environment

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ABSTRACT: In this study, the authors substituted alfalfa hay with factory black tea waste (FBTW) in dairy cattle total mixed ration (TMR) and subsequently tested its effects on in-vitro carbon dioxide and methane production, metabolizable energy (ME), organic matter digestibility (OMD), net energy lactation (NEL), microbial protein production (MP) and Daisy incubator digestibility parame-ters. Experimental diets included 0% (TW0), 25% (TW1), 50% (TW2) and 75% (TW3) FBTW sub-stituted alfalfa hay in dairy cattle TMR rations. Substitution of alfalfa hay with FBTW in TMR linearly decreased in-vitro GP, methane, ME, NEL and OMD values (P<0.05), and linearly in-creased true dry matter digestibility (TDMD), partition factor (PF) and microbial protein pro-duction efficiency (MPSE) values (p<0.05). Organic matter (IVOMD), in-vitro true dry matter (IVTDMD), and NDF digestibility (IVNDFD) values, determined in the Daisy II incubator, were decreased by substituting FBTW for alfalfa hay in TMR (p<0.05). Substitution of alfalfa hay by FBTW up to 50% in TMR as an alternative roughage source in ruminant animal nutrition can be beneficial yet in-vivo studies are required for a solid conclusion.

Keywords: black tea waste; methane production; cattle; in-vitro gas production

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INTRODUCTION

Ruminants and rumen flora hold a very important position in sustainably managed agriculture by using roughage and agricultural by-products as sources of energy and nutrition thus lowering the cost of production (Wright et al., 2011; Gerber et al., 2013; Galmessa et al., 2019). However, in recent years, the increasing population, climate change and drought have resulted in scarcity of arable land (Özkan, 2020; Yavuz et al., 2020). In Turkey, quality roughage is obtained from forage crops grown in field agriculture and meadow pastures. However, it is reported that the yield and quality of the pastures have decreased due to excessive and untimely grazing along with late maintenance works make pastures prone to severe erosion (Gençkan, 1992; Yolcu and Tan, 2008; Sürmen et al., 2008). For these reasons, forage crops and mead-ow-pastures produced in the field cannot adequately meet the nutritional needs of ruminant animals. Nonetheless, feed deficit of ruminants is met with low nutritional value roughage (straw, husk, etc.) or from concentrated mixed feed sources (Açıkgöz et al., 2005). The high inputs of concentrated mixed feed sources increase the prices of animal products. Almost 60-70% of the animal protein production expenses are related to feedstuff. Sustainable animal production is possible by providing quality roughage at a lower cost (Alçiçek et al., 2010; Kuşvuran and Veyis Tansı, 2011; Bıçakçı and Açıkbaş, 2018). For this reason, there is a need for more affordable alternative feed sources with beneficial health effects including higher digestive efficiency of animals (Yeganehpour et al., 2021; Palangi, 2021). Black tea waste, discarded after processing the wet tea leaves into black tea, has been suggested as an alternative to quality roughage in the nutrition of ruminants (Gheshlagh et al., 2021). It is reported that black tea waste contains 18.2% crude protein, 1.6% crude oil, 19.8% crude fiber, 5.7 g/kg condensed tannin and 10.2 ME (MJ/kg DM; ME = metabolic energy; DM = dry matter) (Kaya et al., 2014). Turkey being the 5th largest producer of dry tea, globally ranks 7th in the world in terms of tea farming lands (TÜİK, 2021). It is reported that the tea leaves have 20-30% dry matter depending on the harvesting conditions, and the black tea produced contains 2-4% moisture (Kacar, 1987). In the year 2021, Turkey produced 1,400,000 tons of fresh tea and the production of the commodity according to previous reports could result in 87,500 tons of waste (TÜİK, 2021). Processing discards cause environmental pollution, namely ecological and economic damage, since mostly the materials are discarded in landfills (Kaya et al., 2014). Tannin rich black tea waste when used in ruminants nutrition (as a direct feed source or added to the TMRs of ruminants) increased the amount of bypass protein (Barry and Blaney, 1987; Leng and Fujita, 1997) (TMR = total mixed ration). However, feeding animals with large amounts of tannin-containing feeds has been reported to have a toxic effect (Kamalak et al., 2005; Njidda and Nasiru, 2010). The condensed tannin content in feeds can be reduced at different rates by using different methods such as treatment with polyethylene glycol (PEG) (Kamalak et al., 2005; Njidda and Nasiru, 2010).

In this study, in-vitro gas (CO_2) and methane (CH_4) formation values, the digestibility parameters and the efficiency of microbial protein production were determined in experimental rations, prepared as iso-nitrogenic and iso-caloric by replacing the alfalfa hay in dairy cattle TMRs by replacing 0, 25, 50 and 75% factory black tea waste, and will be examined the usability of factory tea waste as an alternative roughage source instead of hay from alfalfa.

MATERIALS AND METHODS

Materials

In this work, the feed material consisted of TMRs used in the feeding of dairy cattle raised in Atatürk University Food and Livestock Application and Research Center. FBTW, which was used as substitute for alfalfa, was obtained from a private tea factory in Rize. For the experiments of in-vitro gas production, authors took rumen fluid from the freshly slaughtered cattle (sampled immediately after slaughtering of the animals) (Palangi et al., 2022), according to the method reported by Palangi et al. (2022) and transported to the laboratory under an-aerobic conditions. The rumen fluid was stored in a screw-capped bottle was taken to the laboratory in a temperature-controlled (insulated) box at approx. 39°C. The sampled rumen liquid was deployed for in-vitro gas production measurements after it had been filtered (four layers of cheesecloth in anaerobic environment under CO_2 blanketing).

The corn silage, alfalfa hay, dry meadow grass and factory feed and FBTW substitute for alfalfa in dairy cattle TMRs were ground in a feed mill to pass through a 0.5 mm diameter sieve after drying in an oven for in-vitro and chemical analysis. Trial rations, the chemical compositions of which are given in Table 1, were prepared by substituting 0% (FBTW0), 25 (FBTW1), 50 (FBTW2) and 75 (FBTW3) ratios of FBTW of alfalfa hay with tea waste in dairy cattle TMR. Approximate analysis of the experimental rations, including dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and crude ash (CA) were conducted according to AOAC (1998). Kjeldahl was utilized to determine the N content of samples (AOAC, 2005); Method 984.13). The method of van Soest et al. (1991) was used to determine the ADF and NDF of samples. The DM, CP, EE, NDF, ADF, ADL, CF and con-densed tannin (CT) contents of FBTW and alfalfa hay used in TMR were 92.19%-92.44%, 16.51-13.67%, 1.35-3.01%, 4.59-6.51%, 60.21-56.85%, 43.29-39.25%, 26.98- 27.5%, 27.58-30.1% 2.93-0 g/kg, respectively.

In-vitro gas, methane production and predicted parameters

The measurement values of the 24-hour carbon dioxide and methane production of the experimental rations were determined according to the in-vitro gas production technique reported by Menke et al. (1979). Five hundred milligrams of ground trial TMRs were placed in 100 ml glass syringes and 40 ml of buffered rumen fluid was added to it and incubated for 24 hours. At the end of the incubation, gas volume of feed samples was measured after 24h of incubation. The amount of formed gas was determined. It had to be corrected for the estimated gas production with 49.61 mL per 0.200 g on dry matter basis, which were reported for standard of Hohenheim University (44.16 ml/24 hours) (Palangi et al., 2022). To standardize samples and correct the gas production with the ruminal fluid origin, 6 syringes containing only ruminal fluid and buffer solution mixture without feed sample were considered as blank. The amount of methane gas at 24h of in-vitro incubation was measured by using an infrared CH_4 analyzer (Sensor Europe GmbH, 40699 Erkrath, Germany) (Goel et al., 2008) and the total volatile fatty acids (TVFA) and ammonia nitrogen (NH_3-N) values were determined according to the method reported by Markham (1942). Methane production (mL) was computed as follows:

CH_4 production (mL) = Total gas production (mL) x percentage of CH_4 (%) (Palangi and Macit, 2021).

After recording gas and methane measurements, remaining contents in the syringes were transferred to a 250 mL glass beaker and 75 mL of NDF solution (prepared as re-ported by van Soest et al. (1991) was added to them and boiled for 1 hour to determine the true dry matter digestion (TDMD) amount. Contents were then filtered through Gooch crucibles with a pore value of 1 (Blümmel et al., 1997; Başer and Kamalak, 2020).

Metabolizable energy (ME), Net energy lactation (NEL) and organic matter digestibility (OMD) of feed raw materials were determined by the following equations reported by Menke and Steingass (1988).

OMD % = 11.03+0.9860 x GP + 0.0606 x HP

ME, MJ/kg DM = 1.68+0.1418 x GP+0.0073 x

Table 1. Composition of ingredients and chemical analysis of the experimental diets (g/kg DM)

Ingredient (g/kg DM)	FBTW0	FBTW1	FBTW2	FBTW3
Corn silage	240	240	240	240
Alfalfa hay	160	120	80	40
Dry meadow grass	360	360	360	360
Factory feed (CP 21%)	240	240	240	240
FBTW	-	40	80	120
Total	1000	1000	1000	1000
Chemical composition of the diets (g/kg DM)				
DM	922.4	922.3	922.2	922.0
CA	77.1	76.4	75.7	74.8
CP	121.4	122.4	123.3	124.1
EE	45.1	44.5	43.8	43.2
NDF	591.5	592.7	593.9	595.7
ADF	308.5	310.1	310.6	312.5
ADL	140.9	140.6	140.3	139.9
CF	209.5	208.6	207.6	206.4
CT	-	0.73	1.47	2.20

DM: Dry matter; CA: Crude ash; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; CF: Crude fiber; CT: Condensed tannin

CP+0.0217 x EE-0.0028 x CA

NEL (MJ/kg KM) = 0.06+0.1047 x GP+0.0049 x CP+0.0130 x EE-0.0010 x CA

(GP: 200 mg gas production of 24h, CP: crude protein (%) and EE: Ether extract (%))

True digestion dry matter (TDDM), partition factor (PF), microbial protein production (MP), microbial protein synthesis efficiency (MPSE) and true digestion degree (TDD) values of trial diets were determined by the following equations reported by Blümmel et al. (1997) and Vercoe et al. (2010).

TDDM (mg) = Incubated dry matter (mg) - residual dry matter (mg)

PF (mg/ mL) = TDDM (mg) / Gas production (mL)

MPP = TDDM (mg) - 2,2 x GP (ml)

MPSE (%)= TDDM (mg) - 2,2 x GP (mL) / TDDM x 100

TDD (%) = Incubated dry matter (mg) - residual dry matter (mg) / Incubated dry matter (mg) x 100

Determination of in-vitro true dry matter, NDF and organic matter digestibility

An Ankom Daisy II incubator D 220 (daisy-incubator, 2022) device was used to determine in-vitro true dry matter (IVTDMD), organic matter (IVTOMD) and in-vitro true NDF (IVTNDFD) digestibility values of trial rations by filter bag method (Van Soest et al., 1991; ANKOM, 2005). A total of 1966 ml of buffered rumen fluid (1566 ml of buffer solution + 400 ml of rumen fluid) was added to each glass jar in the Daisy II incubator in the presence of CO_2 gas, compare (Gürsoy et al., 2022). After a period of 48 hours of incubation in the Daisy II incubator, all the bags were removed from the glass jars and kept under tap water until the flow of clear water, then oven dried at 105°C for 2-3 hours. After weighing, the bags removed from the oven (D3), IVTDMD values were deter-mined by applying the following formula. To determine the INTNDFD content of the trial rations, the dried bags were treated with NDF solution at 105°C for 75 minutes in the ANKOM2000 Fiber Analyzer device and oven dried for 2-4 hours. The weights of the dried bags were recorded (D4) and the IVTNDFD values were determined with the help of the following formula. Then, the bags were burned at 550°C for 3-4 hours, and at the end of the incineration process, they were weighed on a precision scale and (D5) IVTOMD values were calculated.

IVTDMD=100 - ((D3-D1)/(D2-D1)*100)

IVTNDFD=100 - ((D4-D1)/(D2-D1)*100)

IVTOMD=100 - ((D5-D2)/(D7-D6)*100)

(D1: Tare of F57 bags, D2: Sample weighed in bags, D3: Amount of sample remaining at the end of incubation, D4: Weight after treatment with NDF, D5: Remaining amount after burning at 550 °C, D6: DM content of samples, D7: Organic matter content of samples)

Statistical analysis

The experimental data were analyzed in the SPSS 17.0 (SPSS, 2004) package program, and the differences among the means were established with the Duncan multiple comparison test (Snedecor and Cochran, 1976). The authors used linear, quadratic, and cubic polynomial contrasts to assess the effects of different levels of FBTW, compare (Kaya et al., 2018).

RESULTS

In-vitro CO2 and CH4 production, ME, NEL and OMD contents

In-vitro gas (ml), methane (ml and %), ME (MJ/kg DM), NEL (MJ/kg DM) and organic matter digestion (OMD) contents of the experimental rations are summarized in Figure 1 (ME = metabolic energy, NEL = Net energy lactation).

The 24-hour in-vitro gas production amounts of experimental rations varied between 89.83 and 78.24 ml. The highest gas production values were obtained from FBTW 0 and FBTW 2 groups. FBTW substitution at higher levels in experimental rations linearly de-creased in-vitro gas production (P<0.001). Differences between the experimental groups in terms of in-vitro methane production were significant. The lowest methane production amount was obtained from the FBTW 3 group with 10.27 ml. The differences between organic matter digestion, metabolic energy, and net energy lactation values, calculated by considering the nutrient composition and 24-hour in vitro gas production values of TMRs, were established to be significant (P<0.001). The order in terms of ME, NEL and OMD values in experimental rations was FBTW0=FBTW1>FBTW2=FBTW3. These parameters de-creased linearly with increasing FBTW in TMRs.

True Digestible Dry Matter and predicted parameters

The true digestible dry matter (TDDM), partition factor (PF), microbial protein production (MP), microbial protein synthesis efficiency (MPSE) and true digestion degree (TDD) values of the trial TMRs are given in Figure 2, compare (Gürsoy et al., 2022) for the methodology. The differences between TMRs in terms of PF and MPSE values were significant (P<0.05). The highest PF and MP values were determined in the FBTW2 and FBTW3 groups. In-creasing FBTW in experimental rations increased PF and MPU values linearly. Ranking between groups in terms of PF and MPSE values was determined as FBTW3=FBTW2> FBTW1=FBTW0.

Daisy incubator digestibility parameters

The mean in-vitro true dry matter (IVTDMD), NDF (IVTNDFD) and organic matter (IVTOMD) digestibility values and variance analysis results of the experimental group TMRs are given in Figure 3. Differences in IVTDMD, IVTNDFD and IVTOMD values of the experimental rations were statistically significant (P<0.001). After 48 hours of fermentation in Daisy incubator, the highest and lowest IVTDMD values were recorded for FBTW0 (54.25%) and FBTW3 (52.73%) groups, respectively. The highest and lowest IVTNDFD values were recorded for FBTW0 (68.52%) and FBTW3 (62.66%) groups, respectively. The highest and lowest IVTOMD values were recorded for FBTW0 (93.92%) and FBTW3 (93.71%) groups, respectively. It was determined that increasing levels of FBTW in the trial TMRs linearly decreased the Daisy incubator parameters (P<0.001).

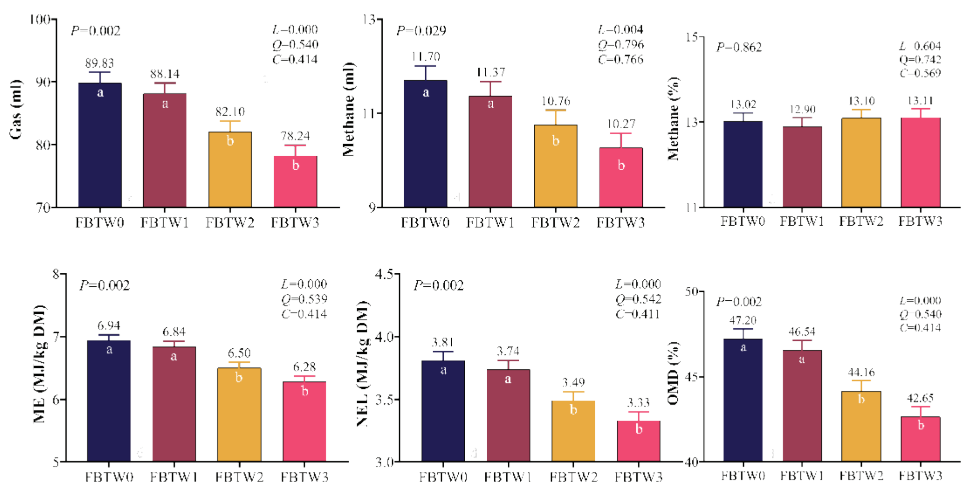

Figure 1. CO_2 production, methane production, ME, NEL and OMD values of trial TMRs measured in vitro. OMD: organic matter digestibility, NEL: Net energy lactation, ME: metabolic energy.

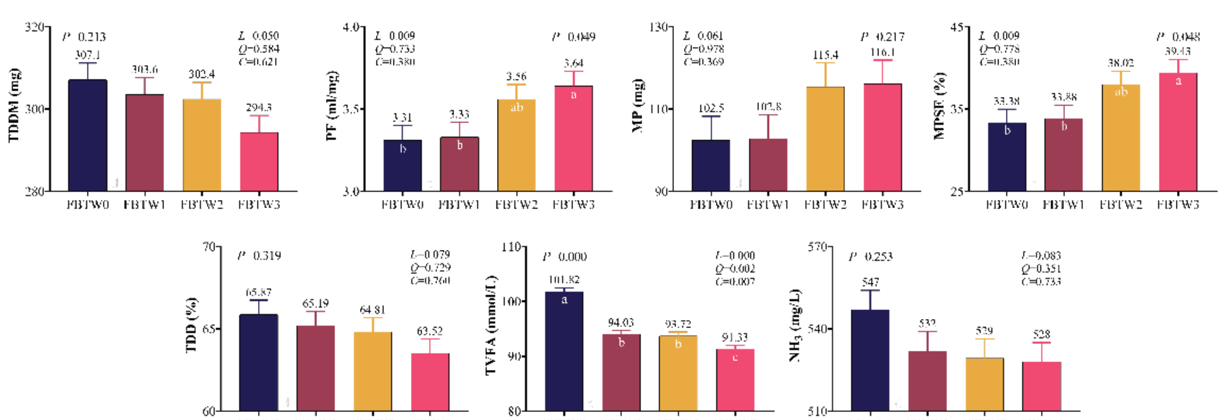

Figure 2. TDDM, PF, MP, MPSE and TDD values of trial TMRs.

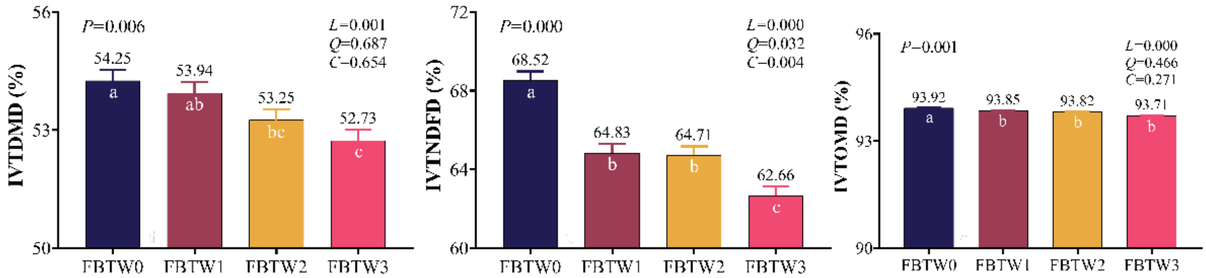

Figure 3. IVTDMD, IVTNDFD, and IVTOMD values of trial TMRs.

IVTDMD: in vitro true dry matter digestibility, IVGNDFD: in vitro true NDF digestibility, IVTOMD: in vitro true organic matter digestibility.

DISCUSSION

Danielsson et al., (2017) reported a positive correlation (R=0.96) between the in-vivo method and the in-vitro method. According to Algan et al. (2018), the in-vitro method developed by Menke et al. (1979) has made it possible to predict the digestibility of alternative ruminant feedstuff and the final fermentation products (NH_3, VFA, etc.) as well as the digestion rates of the feed. It has been reported that the differences between in-vitro gas, me-thane, ME, NEL, OMD values of TMRs may be due to the fermentation of rumen microorganisms, the amount of degradable substances and secondary metabolites in the feed (Alexander et al., 2008; Olomonchı et al., 2022). Chou et al. (1999) showed that catechin rich tea extracts inhibited the growth and proliferation of rumen microorganisms, and even negatively affected the rate of microorganisms to break down the feed, compared to the control group.

While Wiseman et al. (1997) stated that black tea plant may contain up to 9% catechin, Kaya et al. (2014) reported that black tea waste contains 5.7 g/kg DM condensed tannins, and it has a negative effect on rumen microorganisms to break down the feed. In the current study, the in-vitro gas production of FBTW3 group decreased by 14.8% compared to FBTW0. It has been reported that an increase in in-vitro gas production occurs when feed sources such as FBTW, which contains condensed tannin, are treated with polyethylene glycol (PEG) (Baba et al., 2002; Singh et al., 2005). In-vitro gas production values obtained from the experimental groups were higher than the values reported by Kaya and Kaya (2021). Methane gas, re-leased as a product of carbohydrate ruminal fermentation, has a greenhouse gas effect 23-25 times more than CO_2 gas and constitutes 2-15% of the total energy taken into the body. In addition, ruminants cannot benefit from the energy of methane gas (Flachowsky, 2011; Meale et al., 2012). Bhatta et al. (2015) stated that plant secondary metabolite compounds such as tannins are generally used in food production systems as they are environmentally friendly and safe natural products. These bioactive have been successfully used by animal breeders to reduce enteric methane production (Getachew et al., 2005; Bhatta et al., 2015). Wang et al. (2009) reported that tannins have antiprotozoal effects, thus reducing methane production due to methanogens related to protozoa. The decrease in production of methane in the experimental groups is due to the tannins of FBTW. The class of feedstuff, in terms of antimetanogenic effects, shall be taken into consideration when preparing the ration to increase the energy efficiency in ruminants and reduce the methane gas production (López et al., 2010). Enteric methane, released during fermentation, is not desired by both environmentalists and feeders because it causes global warming and energy loss of feed. In the current study, when we looked at the in-vitro methane (%) values of the experimental groups, it was determined that they showed low anti-methanogenic effects. TMRs are produced by mixing forages, by-products, compound feeds, minerals, vitamins, and additives. Ruminants meet the energy (ME, NEL) required for their survival and productivity needs from this nutrient mixture (Schingoethe, 2017). Our ME and NEL values of the experimental groups were lower than those reported by Boguhn et al. (2003), Kaya and Kaya (2021) and Boğa et al. (2020). These differences may be due to different formulas used to calculate energy.

Min et al. (2005), determined that rumen bacteria slowed down the hydrolysis rate of the feed and inhibited the growth rate of the bacteria due to the increase in the condensed tannin level in the environment. Blummel et al. (1997) stated that the value of partition factor of TMR, being an important factor for the determination of microbial protein synthesis efficiency, should be between 2.75 and 4.41. Blummel et al. (1997) reported that the increase in PF value in tannin containing feedstuff was due to the fact that the condensed grain appeared to be fragmented during fermentation but did not contribute to gas production in any way. In the current study, higher concentrations of tannin in experimental diets with higher levels of FBTW led to greater PF values. Ruminants meet their protein needs from by-pass protein and microbial protein. It has been reported that the nutritional value of the feeds used in the nutrition of ruminants should be considered in the production and synthesis of microbial protein (Leng, 1993; Van Soest, 1994; Beever, 1996). It has been reported that gas production, short-chain fatty acid (SCFA) and microbial protein amounts are inversely related in in-vitro analyses and rations should be prepared according to the gas production values of the feeds alone as well as the microbial protein production amounts (Makkar et al., 1995). Blummel et al. (1997) reported that PF value is associated with microbial protein synthesis efficiency, and TMR with the highest PF value will also have the highest microbial protein synthesis efficiency. A large part of the energy needs of ruminants (55-70%) is provided by the volatile fatty acids released as a result of the fermentation of feeds in the rumen (Noziere et al., 2011). TVFA values of the experimental groups were higher than the values reported by Sun et al. (2016). It is stated that the amount of TVFA formed in the rumen is dependent on many factors such as the digestion of nutrients, absorption rate, rumen pH, the rate of passage of feed from the rumen, as well as the microbial population and activities in the rumen (Morsy et al., 2015).

The Dasiy incubator technique could help researchers in determining the digestion values of feed raw materials in a timely manner since in-vivo digestion analyses are labor-intensive, costly and time-consuming (Tassone et al., 2020). In the in-vitro studies, when determining the potential feed value and digestibility values of feed raw materials, the degree of estimation is low (R2=0.48) when only the cumulative gas production amounts are considered, but the degree of estimation is low when the other nutrient compositions of the feed (NDF, ADF and HP) are taken into account (R2=0.87) (Plaizier and Li, 2013). Our IVTDMD values were lower than the values reported by Rofiq and Görgülü (2014) and higher than the values reported by Kaya and Kaya (2021) and Yalchi and Hajieghrari (2010). According to Spanghero et al. (2010) this decrease is related to the amount of ADL in the structure of TMR, the amount of digestible substances and anti-nutritional substances. It is stated that the IVOMD values of feed raw materials or TMRs may vary depending on the amount of raw ash in its structure and the digestibility values in the Dasiy incubator (Yalchi and Hajieghrari, 2010). Our IVOMD values of TMRs were similar to those reported by Kaya and Kaya (2021) yet higher than the ones reported by Yalchi and Hajieghrari (2010).

CONCLUSION

Ruminants are animals that can transform low quality roughage into quality products by microorganisms in their digestive system. However, it is seen that the antimicrobial factors in the structure of the feed consumed by ruminants prevent the rumen micro-organisms from working and reduce the utilization of the feed. Correspondingly, for efficient use of black tea waste in the TMR as an alternative forage source for ruminants, catechin and condensed tannins with antimicrobial properties shall be eliminated by techniques such as polyethylene glycol. In our study, substitution of alfalfa hay with FBTW in TMR reduced in-vitro methane gas by 12% compared to the control group. Further in-vivo studies shall be performed to observe the effect of FBTW on feed consumption and efficiency, milk yield and organic matter removal in manure.

CONFLICT OF INTEREST

No competing interests are declared by the authors.

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