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Research Article

The Effects of Fumaric Acid on *In Vitro* True Digestibility of Tea Wastes Produced with Different Cultivation Methods

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ABSTRACT

This study aims to determine the effects of adding different fumaric acid (FA) levels to tea factory wastes (TFW) produced by different cultivation methods on *in vitro* true digestibility. *In vitro* true digestibility of feed ($IVTD_{As\ feed}$), dry matter ($IVTD_{DM}$), organic matter ($IVTD_{OM}$), and neutral detergent fibre ($IVTD_{NDF}$) were performed with a Daisy Incubator. Fumaric acid did not add to the control group and added 0.1%, 0.2%, or 0.3% FA to the experimental groups. When the cultivation methods were compared (conventional and organic tea wastes), it was seen that FA made a significant difference ($P<0.05$) in conventional tea wastes at all levels. However, when comparing organic and conventional tea wastes themselves, there was no significant effect of FA level on digestibility parameters ($P>0.05$). There was a significant difference ($P<0.05$) for $IVTD_{As\ feed}$, $IVTD_{DM}$ and $IVTD_{OM}$ values in interaction between treatment and cultivation methods. The research results showed that the digestibility values of the conventional tea wastes were higher for ruminants than the tea waste produced by the organic method, and the use of 0.3% FA in organic tea wastes had a positive effect on IVTD values for ruminants.

Keywords: *By-product, feed, organic acid, ruminant, tea waste.*

Farklı Yetiştirme Yöntemleri İle Üretilen Çay Atıklarının *In Vitro* Gerçek Sindirilebilirliği Üzerine Fumarik Asitin Etkileri

ÖZET

Bu çalışma, farklı yetiştirme yöntemleri ile üretilen çay fabrikası atıklarına (TFW) farklı düzeylerde fumarik asit (FA) eklenmesinin *in vitro* gerçek sindirilebilirlik üzerindeki etkilerini belirlemeyi amaçlamaktadır. Çay fabrika atıklarının gerçek sindirilebilirlik (IVTDyem), kuru madde ($IVTD_{KM}$), organik madde ($IVTD_{OM}$) ve nötr deterjan lif ($IVTD_{NDF}$) in vitro gerçek sindirilebilirliği Daisy inkubatör sistemi ile gerçekleştirilmiştir. Kontrol grubuna FA ilave edilmezken deney gruplarına %0.1, %0.2 veya %0.3 FA eklenmiştir. Yetiştirme yöntemleri karşılaştırıldığında (konvansiyonel ve organik çay atıkları), FA'nın konvansiyonel çay atıklarında tüm seviyelerde önemli bir fark oluşturduğu ($P<0.05$) görülmüştür. Bununla birlikte, organik ve konvansiyonel çay atıklarının kendileri karşılaştırıldığında, FA seviyesinin sindirilebilirlik parametreleri üzerinde anlamlı bir etkisi olmamıştır(P>;0.05). Fumarik asit düzeyleri ve yetiştirme yöntemleri arasındaki etkileşimde $IVTD_{yem}$, $IVTD_{KM}$ ve IVT_{DOM} değerleri için önemli fark ($P<0.05$) görülmüştür. Araştırma sonuçları konvansiyonel çay atıklarının sindirilebilirlik değerlerinin ruminantlar için organik yöntemle üretilen çay atıklarına göre daha yüksek olduğunu ve organik çay atıklarında %0.3 FA kullanımının ruminantlar için IVTD değerleri üzerinde olumlu etkisi olduğunu göstermiştir.

Anahtar Kelimeler: *Çay atığı, organik asit, ruminant, yan ürün, yem.*

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Introduction

The increase in feed prices in the field of animal nutrition adversely affects the economic livestock movements. Based on this situation, the interest in food wastes, plant wastes, and by-products used in different fields within alternative feed materials is increasing (Akram and Fırıncıoğlu, 2019). Tea Factory wastes obtained during the production phase of harvested tea are also evaluated within the scope of these alternative searches (Ozyılmaz and Genc, 2019). With the prohibition of antibiotics as growth factors with the European Union decision (EC Regulation No. 1831/20031) in 2006, the search for alternatives have been started in this field. Since this decision, many new natural and chemical substances have been used in this field. Organic acids, one of these substances, are classified as “generally recognized safe” by the USA Food and Drug Administration in animal nutrition. The effects of organic acids as growth promoters in ruminants occur by more than one mechanism (Carro and Ungerfeld, 2015). Their effects, such as being a very rapidly digestible substrate, providing rumen pH stabilization (Martin, 2004), decreasing methane emission (Bharathidhasan et al., 2016), and affecting NH_4 (Zhou et al., 2012) concentration, are taken into account within different metabolic mechanisms.

The effects of organic acids on rumen microecology and the tricarboxylic acid cycle in rumen fermentation have been focused on fumaric and malic acids (Castillo et al., 2004; Kolver and Aspin, 2006; Genc et al., 2020). Fumaric acid is an intermediate in the propionate formation mechanism formed in the rumen (Yang et al., 2012; Patra et al., 2017). With the increase of ruminal propionate, both the amount of hydrogen required for methanogenesis is reduced and the propionate absorbed from the rumen is transported to the liver to a large extent and becomes the main source of glucose for ruminants (Carro and Ungerfeld, 2015; Li and Guan, 2017).

Fumaric acid (FA) and its salts are prominent with *in vivo* and *in vitro* digestion trials as a growth factor for their bacteriostatic and bactericidal effects and also increase the population of beneficial microorganisms in the intestinal system (Angga et al., 2018).

This study is to determine the impact of different levels of fumaric acid on the *in vitro* digestion values of tea factory wastes produced by organic and conventional farming methods.

Materials and Methods

Since the rumen samples were only taken from slaughtered animals, no ethics committee approval was needed for this study. The samples (TFW) were obtained from several tea factories located in the Black Sea region in Turkey. The samples taken were obtained from the teas belonging to the first tea harvest in May. The FA (≥99% purity) was purchased from Sigma-Aldrich (St Louis, MO, USA).

In the study, tea factory wastes obtained from tea plants produced with conventional and organic techniques were used. Four different groups were formed, one of which was the control group, from the material representing each production technique. While no treatment was applied to the control groups, fumaric acid was added to the in vitro Daisy II incubator system jars of experimental groups at the level of 0.1%, 0.2% and 0.3% respectively. Tea factory waste samples were weighed and then dried at 65°C for 48h. The dried samples were ground in a mill with a 1 mm sieve to prepare them for chemical analysis. Each group sample’s dry matter (DM) content was evaluated in an air circulation drying oven at 105°C for 4 hours. The dry material was burned in an ash oven at 550°C for 4 hours to determine the content of ash. The proportion of crude protein was calculated using the Kjeldahl technique (CP). AOAC (2006) methods were used for ether extract (EE) determination. Metabolizable energy determination was made according to Krichgessner et al. (1977). The neutral detergent fiber (NDF) and the acid detergent fiber (ADF) contents were analyzed in an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., USA) by the method reported by Van Soest et al. (1991). The methods offered in ANKOM Daisy Incubator (ANKOM Technology Corp., USA) performed all parameters of the *in vitro* true digestibility (IVTD) analysis with the ANKOM Daisy Incubator (ANKOM Technology Corp., USA) (2002). The rumen fluid was collected post-mortem from the rumen of three-year-old three Holstein cows slaughtered at a commercial slaughterhouse in Samsun Province, Turkey. Animals were fed with a diet containing grass hay and maize silage (60%) and concentrate feeds (40%). The rumen fluid was taken from each animal’s rumen along with two handfuls of ruminal contents and carried in a thermos previously heated to 39°C and supplemented with CO_2. Samples were brought to the laboratory within 10 minutes. The rumen fluid was filtered through 4 layers of gauze. The F57 bags used in the study were soaked in 99.5% acetone for 3 minutes. All of the bags were dried for 2 hours at 105°C in a drying cabinet. The tare of the bags was weighed and 0.5 g of each samples (TFW) were transferred to separate F57 bags. According to ANKOM (2002) the buffer solution to be used in the analysis was prepared. In this experiment, four digestion units with a total volume of 2 L were used. The buffer solution was heated to 39°C and 1.6 L was given to each unit. Test units were closed by adding 400 ml of rumen fluid into the buffer solutions. Each digestion unit contained a total of 24 TFW samples, with six replicates prepared at the same time for each sample. The experimental groups received 0.1%, 0.2%, and 0.3% FA, respectively, whereas the control group received no FA. All samples were incubated for 48 hours. All liquids from the digestive units were removed after the incubation period, and the bags were rinsed under running distilled water. Washed bags were placed in the ANKOM Fiber Analyzer and were performed NDF analysis according to the method specified in ANKOM (2002). After NDF analysis, all bags were kept in an air-drying cabinet at 105°C until they reached a constant weight. The IVTD values of all samples were calculated by the formula reported in ANKOM (2002).

A1= Weight of bag tare, A2= Weight of sample, A3= Final bag weight after *in vitro* process and sequential treatment, B1= Blank bag correction (final oven-dried weight/original blank bag weight)

Data were analyzed according to the factorial experimental design by the Completely Randomized Experimental Design (one-way classification), and the results were interpreted. The sample distribution of the data obtained in the study was determined by calculating the group of means and their standard error. In addition, the differences between the cases in which the differences between the means of the main effects were significant as a result of the statistical analysis were investigated with the Duncan multiple comparison test. The differences among the group combinations (interactions) were shown by the Tukey multiple comparison test. In the study, calculation of the sample distribution statistics of the data, descriptive statistics, and all statistical analyses were carried out in the (2009, SAS Institute) statistical program (P<0.05).

Results

It was determined that TFW's had similar values of nutrient (g/100g DM) and metabolisable energy (MJ/kg DM) (Table 1).Conventional wastes had a higher value in terms of crude protein (CP) and a lower value in terms of ether extract (EE). It was seen that different FA levels have a significant (P<0.05) effect on *in vitro* digestion parameters (C) of tea factory wastes according to the cultivation method (Table 2). Accordingly, all *in vitro* digestion parameters were found to be higher (*P*<0.05) in conventional tea wastes treated with 0.1% and 0.2% FA. In addition, when FA was used at the 0.3% level, $IVTD_{As\ feed}$, $IVTD_{DM}$, and $IVTD_{OM}$ values were higher (P<0.05), while $IVTD_{NDF}$ values were lower (P<0.05) in organic tea wastes. The *in vitro* digestion parameters evaluated within the groups were not significantly affected by the different FA levels (P>0.05).

It was determined that the addition of 0.3% FA level in organic tea wastes caused a numerically higher value (*P*>0.05) for all digestion parameters compared to the control and other experimental groups. It was observed that the increasing FA level in conventional tea wastes caused a numerical decrease (P>0.05) in all *in vitro* digestion parameters.

Regardless of the effect of FA levels applied to examine the interaction between the group and the cultivation method, $IVTD_{As\ feed}$, $IVTD_{DM}$, and $IVTD_{OM}$ values were found to be significantly higher (P<0.05) in the conventional production method when *in vitro* digestion parameters were examined in conventional and organic tea wastes.

$$\%IVTD\ (as\ received\ basis) = \frac{100-[\mathrm{A3}-(\mathrm{A1xB1})]\mathrm{x}100}{\mathrm{A2}}$$

$$\%IVTD\ (DM\ basis) = \frac{100-[\mathrm{A3}-(\mathrm{A1xB1})]\mathrm{x}100}{(\mathrm{A2xDM})}$$

Table 1. Nutrient composition (g/100g DM) and metabolizable energy (MJ/kg DM) of conventional and organic tea factory wastes for ruminants.

Cultivation	DM	Ash	CP	EE	NDF	ADF	OM	ME
Conventional	95.95	4.06	12.71	0.50	55.12	48.55	91.89	6.94
Organic	95.67	3.97	10.44	1.03	54.90	48.18	91.70	7.00

DM: Dry matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, OM: Organic matter, ME: Metabolizable energy (MJ/kg DM)

Discussion

In ruminant nutrition, the nutrient content of tea wastes as an alternative feed source varies. Ozyılmaz and Genc (2019) stated that the conventional tea factory wastes (CTFW) obtained in the July harvest had 95.80% DM, 12.67% CP, 4.1% ash, 0.30% EE, 52.52% NDF, and 47.98% ADF, while organic tea factory wastes (OTFW) had 95.93% DM, 10.20% CP, 3.92% ash, 0.76% EE, 57.23% NDF and 49.27% ADF levels, and these values vary depending on the organic or conventional growing and harvest periods of tea plants. Similar to these results, the effect of the cultivation method on the CP value coincides with the findings obtained in our research, and it is seen that the CP value is 17.8% higher for CTFW than OTFW. In an *in vitro* digestibility experiment (Genc et al., 2020) performed previously using a Daisy incubator, it was seen that CTFW nutrient values were 93.5% DM, 4.8% ash, 18.2% CP, 1.16% EE, 34.6% ADF, and 40.5% NDF. Nasehi et al. (2017) reported that in their study with CTFW, the values of DM, CP, CA, OM, EE, NDF, ADF and ME were 92.72%, 15.66%, 5.75%, 94.24%, 1.16%, 38.47%, 25.87% and 7.7 MJ/kg DM respectively. Another study (Angga et al., 2018) performed with Sumatra TFW reported that DM, OM, and CP values of CTFWs were 93.59%, 88.08%, and 19.63%, respectively. These results agree with the DM and OM values in our study.

Ozyilmaz and Genc (2019) reported that there was no difference (P<0.05) between gas volumes, $IVTD_{OM}$, and ME values in CTFW and OTFW. They emphasized that cultivation methods significantly affect these parameters.

Table 2. Effects of the addition of different concentrations (%) of fumaric acid to tea factory wastes (n=6) on *in vitro* true digestibility values (Mean±SEM).

Parameters	Cultivation Control		Treatment				Total
			FA 0.1%	FA 0.2%	FA 0.3%		
$IVTD_{As\ feed}$	Conventional		54.07±0.46^{bc}	53.35±1.26^{c}	53.32±0.58^{c}	54.3±0.28^{bc}	53.76±0.28^{B}
	Organic		56.07±0.36^{a}	56.02±0.5^{a}	55.26±0.58^{ab}	53.63±0.15^{c}	55.24±0.28^{A}
	Total		55.07±0.39	54.69±0.39	54.29±0.39	53.96±0.39	54.5±0.25
	P value	C			0.0013		
		T			0.3087		
		CxT			0.0463		
$IVTD_{DM}$	Conventional		51.99±0.48^{bc}	51.24±1.32^{c}	51.21±0.61^{c}	52.23±0.3^{bc}	51.67±0.29^{B}
	Organic		54.21±0.37^{a}	54.16±0.52^{a}	53.37±0.61^{ab}	51.68±0.15^{c}	53.36±0.29^{A}
	Total		53.1±0.44	52.7±0.81	52.29±0.52	51.95±0.18	52.51±0.27
	P value	C			0.0006		
		T			0.3107		
		CxT			0.0468		
$IVTD_{OM}$	Conventional		53.15±0.46^{cd}	52.4±1.26^{d}	52.33±0.63^{d}	53.61±0.34^{bcd}	52.87±0.37^{B}
	Organic		55.48±0.4^{a}	55.17±0.54^{ab}	54.34±0.63^{ac}	52.66±0.17^{d}	54.41±0.31^{A}
	Total		54.31±0.45	53.79±0.78	53.33±0.52	53.14±0.23	53.64±0.27
	P value	C			0.0013		
		T			0.2635		
		CxT			0.0219		
$IVTD_{NDF}$	Conventional		12.55±0.87^{c}	12.86±1.16^{c}	11.12±1.11^{c}	12.98±0.54^{c}	12.38±0.47^{B}
	Organic		16.93±0.67^{a}	16.84±0.95^{a}	15.4±1.1^{ab}	13.27±0.99^{bc}	15.61±0.54^{A}
	Total		14.74±0.84	14.85±0.93	13.26±0.99	13.12±0.54	13.99±0.42
	P value	C			0.0001		
		T			0.1425		
		CxT			0.0500		

$^{a-d}$: There is no difference between cultivation treatment interactions with the same letter,$^{A-B}$: There is no difference between the main effects of cultivation and treatment with the same letter. $IVTD_{As\ feed}$: *In vitro* true digestibility as feed, $IVTD_{DM}$: *In vitro* true digestibility of dry matter; $IVTD_{OM}$: *In vitro* true digestibility of organic matter; $IVTD_{NDF}$: *In vitro* true digestibility of neutral detergent fiber; C: Cultivation; T: Treatment

In our current study, it was seen that the value of $IVTD_{OM}$ is significantly (P<0.05) different for CTFW compared to OTFW. These differences can be thought to be due to the difference in the cultivation method.

The results of the present study showed a significant difference (P<0.05) for $IVTD_{As\ feed}$, $IVTD_{DM,}$ and $IVTD_{OM}$ values in terms of interaction (CxT) between treatment and cultivation methods (conventional and organic). Organic acids improved the digestion and absorption of nutrients by affecting the rumen microbiota. As reported in the European Commission 2003 report, FA is one of the naturally occurring compounds involved in metabolism. Genc et al. (2020) reported that the statistically highest $IVTD_{OM}$ value was in the group with 0.1% FA in their *in vitro* digestion trial with conventional tea wastes and that $IVTD_{As\ feed}$, $IVTD_{DM}$, and $IVTD_{NDF}$ values increased mathematically with 0.1% and 0.2% FA application. In these study conducted with alternative roughage raw materials, it was observed that a 0.3% FA level in *Q. cerris* leaves significantly increased the values of $IVTD_{As\ feed}$, $IVTD_{DM}$, $IVTD_{OM}$, and $IVTD_{NDF}$. It seems that these findings are in harmony with our research results. Since both acids are organic dicarboxylic acids, it has been observed that they do not have a positive synergistic effect on IVTD parameters when used together, and similar findings were mentioned in an *in vivo* study (Ebrahimi et al., 2015). Li et al. (2018) reported that the rumen bacterial population and $IVTD_{DM}$ values were not affected by the FA level (P>0.05) in an *in vitro* digestion experiment in which they used alfalfa hay with better quality nutrient content than tea wastes. Lopez et al. (1999) reported that sodium fumarate did not show a positive effect on rumen bacteria, and the bacterial population decreased ($P<0.01$) after prolonged incubation for more than 48 hours. Genc et al. (2020) reported that FA treatment had a positive effect on IVTD, but high-level (10 mM and 0.3%) FA supplementation caused a negative effect on the same parameter. Consistent with these data, it is seen that the digestion values were at the lowest level ($P>0.05$) in the CTFW group using 0.3% FA in our study. Sahoo and Jena (2014) explained this situation in relation to the anionic effects of organic acids that may adversely affect ruminal microbial life. In digestibility studies (Burner et al., 2008; Chen et al., 2011; Genc et al., 2020) on alternative feed properties of high tannin-containing vegetable raw materials, the effect of tannin and polyphenol fractions to prevent fermentation was mentioned, and it was reported that rumen digestibility of these raw materials could be higher if the tannin level was lowered. Yu et al. (2010) have also reported that organic acids do not show the expected effect in *in vitro* digestion trials in the presence of factors that adversely affect the degree of digestion.

Variable results in *in vitro* digestion studies using organic acids can be attributed to the different effects of these acids on rumen microbial life. It can be thought that situations such as Gram (+) and Gram (-) bacteria having different atomic numbers, being affected differently by different organic acids (Partanen, 2001), and being affected by the practices of microbial (bacteria-protozoa-archaea) life order can cause different effects on *in vitro* digestion values. In *in vitro* digestion experiments, how these microbial forms are affected by the practices should also be examined. The digestive properties of substrates in digestion experiments are affected by many factors (Kara et al., 2015). Condensed tannin density, organic acids, easy soluble nutrient profile, and microbiota population are the prominent factors (Kara et al., 2018). Kara et al. (2018), in a study on the *in vitro* digestive valence of organic acids, reported that formic acid reduced digestive values and pointed out that this may be due to the reduction of the total number of ruminal bacteria and Entodiniinae and Diplodiniinae ciliate protozoa due to the antimicrobial properties of organic acids. Li et al. (2009) reported that while fumarate does not affect $IVTD_{NDF}$ value, it affects $IVTD_{DM}$ value negatively. The similar effects of the increasing fumaric acid dose are also seen for the CTFW in the present study.

In a study on the digestibility effects of organic acids (Newbold et al., 2005), it was reported that disodium fumarate increased $IVTD_{DM}$ activity, but the same effect was not seen in the use of fumaric acid. In an *in vivo* study in which the acid form of fumaric acid was used instead of the salt form (Molano et al., 2008), it was reported that the feed consumption values of lambs decreased with the increasing fumaric acid dose. This data shows that the use of fumaric acid in different forms may have different results. In some studies (Bayaru et al., 2001; McGinn et al., 2004; Beauchemin and McGinn, 2006; Kolver and Aspin, 2006) where fumaric acid was used at doses of 12 to 50 g/kg DM, it was reported that it had no positive effect on DM digestion, energy, and fiber digestibility. Yu et al. (2010) associated the inability to see the expected effect of fumaric acid in *in vitro* digestibility trials with the lack of sufficient fermentable carbohydrates in the feed raw materials used.

In a study (Nasehi et al., 2017) on the use of black and green tea wastes in ruminant feeding, it was reported that tea wastes treated with polyethylene glycol (PEG) had a significant effect ($P<0.05$) on $IVTD_{DM}$, $IVTD_{OM}$, $IVTD_{ME}$, and $IVTD_{NEL}$ values. The researchers attributed this effect, as Kumar and Vaithiyanatha (1990) and Silanikove et al. (1996) reported, to the strong binding of PEG and the prevention of the binding of tannins to proteins, especially with the high level of condensed tannins possessed by tea wastes. Bryant (1973) reported that cellulolytic rumen bacteria could not benefit enough from ammonia that cannot release in this case. Based on this interpretation, future studies might consider using organic acids together with tannin binders such as PEG to demonstrate the effect of organic acids on digestive parameters of tannin-containing feeds. Even though the effects of organic acids on rumen metabolism are expressed by very complex mechanisms, they can be simply characterized by their effect of accelerating fermentation. However, according to the calculations in *in vitro* studies, it is seen that a significant part of the fumarate added to the system does not participate in fermentation (Carro and Ungerfeld, 2015). As can be seen in our research results, a possible explanation might be that although the dose used is increased, digestibility does not increase in direct proportion. In the light of this information, it should be considered whether the amount of organic acid used in *in vitro* digestibility tests is effective or not. As a matter of fact, as Li et al. (2018) also reported in their research, it is known that the metabolism of fumaric acid is not fully understood in terms of bacterial population activity, especially fumarate-utilizing bacteria.

Conclusion

It is observed that the *in vitro* digestion parameters of tea factory wastes produced by the conventional method are higher with the application of fumaric acid compared to the wastes produced by the organic method. However, considering the levels of fumaric acid used, it suggests that the use of organic acids in tea factory wastes with additives such as tannin binders may yield more meaningful results due to higher *in vitro* digestion values in control groups. It was concluded that there is a need for different *in vivo* and *in vitro* studies considering the effects of antinutritional factors contained in tea factory wastes and their effects on ruminal microbiota.

Conflict of interest

The authors declare that they have no conflict of interest in this study.

References

Akram, M. Z., & Fırıncıoğlu S. Y. (2019). The use of agricultural crop residues as alternatives to conventional feedstuffs for ruminants. *Eurasian Journal of Agricultural Research.* 3(2), 58-66. https://dergipark.org.tr/tr/pub/ejar/issue/50318/632177

Angga, W. A., Rizal, Y., Mahata, M. E., Ahadiya, Y., & Mayerni, R. (2018). Potential of waste tea leaves (*Camellia sinensis*) in West Sumatra to be processed into poultry feed. *Pakistan Journal of Nutrition*, (17), 287-293. https://doi.org/10.3923/pjn.2018.287.293

ANKOM (2002). Operator's manual. Ankom 200/220 fiber analyzer. Ankom Technology Corp., Fairport, USA.

AOAC (2006). Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Inc., Arlington, VA. pp. 354-361.

Bayaru, E., Kanda, S., Kamada, T., Itabashı, H., Andoh, S., Nishida, T., Ishida, M., Itoh, T., Nagara, K., & Isobe, Y. (2001). Effect of fumaric acid on methane production, rumen fermentation and digestibility of cattle fed roughage alone. *Animal Science Journal*, 72(2), 139–46.

Beauchemin, K.A., & McGinn, S.M. (2006). Methane emissions from beef cattle: effects of fumaric acid, essential oil, and canola oil. *Journal of Animal Science*, 84(6), 1489–96. https://doi.org/10.2527/2006.8461489x

Bharathidhasan, A., Karunakaran, A., Pugazhenthi, T.R., & Ezhilvalavan, S. (2016). The effect of supplemental organic acid on methane reduction to decrease the global warming from dairy cattle. *Interational Journal of Advanced Chemical Science and Applications*, 3(4), 60-64.

Bryant, M.P. (1973). Nutritional requirements of the predominant rumen cellulolytic bacteria. *Federation Proceedings.* Jul; 32(7):1809-13.

Burner, D.M., Carrier, D.J., Belesky, D.P., Pote, D.H., Ares, A., & Clausen, E.C. (2008). Yield components and nutritive value of *Robinia pseudoacacia* and *Albizia julibrissin* in Arkansas. *Agroforestry Systems,* 72(1), 51-62.https://doi.org/10.1007/s10457-007-9098-X

Carro, M.D., & Ungerfeld, E.D. (2015). Utulization of organic acids to manipulate ruminal fermentation and improve ruminant productivity. In: Puniya A., Singh R, & Kamra D. (Eds) Rumen Microbiology: From Evolution to Revolution. Springer, New Delhi.

Castillo, C., Benedito, J.L., Méndez, J., Pereira, V., Lopez-Alonso, M., Miranda, M., & Hernández, J. (2004). Organic acids as a substitute for monensin in diets for beef cattle. *Animal Feed Science and Technology*, 115(1-2), 101-16. https://doi.org/10.1016/j.anifeedsci.2004.02.001

Chen, Y., Zhao, Y., Fu, Z., Ma, Z., Qian, F., Aibibuli, F., Bin, Y., Abula, R., Xiaoli, X., & Aniwaer, A. (2011). Chemical composition and *in vitro* ruminal fermentation characteristics of tetraploid black locust (*Robinia pseudoacacia* L.). *Asian Journal of Animal and Veterinary Advances,* 6(7), 706-14. https://doi.org/10.3923/ajava.2011.706.714

Ebrahimi, S.H., Datta, M. M., Heidarian, V., Sirohi, S.K., & Tyagi, A.K. (2015). Effects of fumaric or malic acid and 9,10 anthraquinone on digestiblity, micobial protein synthesis, methane emission and performance of growing calves. *The Indian Journal of Animal Sciences,* 85(9), 1000-1005.

Genc, B., Salman, M., Bolukbas, B., Kaya, I., & Acıcı, M. (2020). The effects of fumaric and malic acids on the *in vitro* true digestibility of some alternative feedstuffs for ruminants. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 67(2), 185-192. https://doi.org/10.33988/auvfd.623821

Kara, K., Aktug, E., Cagrı, A., Guclu, B. K., & Baytok, E. (2015). Effect of formic acid on *in vitro* ruminal fermentation and methane emission. *Turkish Journal of Agriculture-Food Science and Technology,* 3(11), 856–860.https://doi.org/10.24925/turjaf.v3i11.856-860.491

Kara, K., Ozkaya, S., Erbas, S., & Baytok, E. (2018). Effect of dietary formic acid on the *in vitro* ruminal fermentation parameters of barley based concentrated mix feed of beef cattle. *Journal of Applied Animal Research*, 46(1), 178-183. https://doi.org/10.1080/09712119.2017.1284073

Kirchgessner, M., Kellner, R. J., Roth, F. X., & Ranfft, K. (1977). Zur Schatzung des Futterwertes mittels Rohfaser und der Zellwandfraktionen der Detergentien Analyse. Landwirtschaftliche Forschun

Kolver, E., & Aspin, P.W. (2006). Supplemental fumarate did not influence milk solids or methane production from dairy cows fed high quality pasture. *Proceedings of the New Zealand Society of Animal Production*, 66, 409–415.

Kumar, R., & Vaithiyanatha, S. (1990). Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. *Animal Feed Science and Technology*, 30(1-2), 21-38. https://doi.org/10.1016/0377-8401(90)90049-E

Li, X.Z., Yan, C., Choi, S.H., Long, R., Jin, G.L., & Song, M.K. (2009). Effects of addition level and chemical type of propionate precursors in dicarboxylic acid pathway on fermentation characteristics and methane production by rumen microbes *in vitro*. *Asian-Australasian Journal of Animal Sciences*, 22(1), 82–89. https://doi.org/10.5713/ajas.2009.80413

Li, F., & Guan, L.L. (2017). Metatranscriptomic profiling reveals linkages between the active rumen microbiome and feed efficiency in beef cattle. *Applied and Environmental Microbiology*, 83(9), e00061–17. https://doi.org/10.1128/AEM.00061-17

Li, Z., Liu, N., Cao, Y., Jin, C., Cai, C., & Yao, Y. (2018). Effects of fumaric acid supplementation on methane production and rumen fermentation in goats fed diets varying in forage and concantrate particle size. *Journal of Animal Science and Biotechnology*, 9(1), 1-9. https://doi.org/10.1186/s40104-018-0235-3

Lopez, S., Valdés, C., Newbold, C., & Wallace, R. (1999). Influence of sodium fumarate addition on rumen fermentation *in vitro*. *British Journal of Nutrition*, 81(1), 59-64.

Martin, S.A. (2004). Effects of DL-malate on *in vitro* forage fiber digestion by mixed ruminal microorganisms. *Current Microbiology*, 48(1), 27–31. https://doi.org/10.1007/s00284-003-4081-x

McGinn, S.M., Beauchemin, K.A., Coates, T., & Colombatto, D. (2004). Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. *Journal of Animal Science*, 82(11), 3346–3356. https://doi.org/10.2527/2004.82113346x

Molano, G., Knight, T.W., & Clark, H. (2008). Fumaric acid supplements have no effect on methane emissions per unit of feed intake in wether lambs. *Australian Journal of Experimental Agriculture*, 48(2), 165-168. https://doi.org/10.1071/EA07280

Nasehi, M., Torbatinejad, N., Rezaie, M., & Ghoorchi, T. (2017). Effect of polyethylene glycol addition on nutritive value of green and black tea co-products in ruminant nutrition. *Asian Journal of Animal and Veterinary Advances*, 12, 254-260. https://doi.org/10.3923/ajava.2017.254.260

Newbold, C., López, S., Nelson, N., Ouda, J., Wallace, R., & Moss, A. (2005). Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal fermentation *in vitro*. *British Journal of Nutrition*, 94(1), 27-35. https://doi.org/10.1079/bjn20051445

Ozyilmaz, N., & Genc, B. (2019). Determination of nutrient content and *in vitro* digestibility values of organic and conventional tea (*Camellia sinensis*) factory wastes. *International Journal of Veterinary and Animal Research*, 2019;2 (2): 29-33.

Partanen, K. (2001). Organic acids- their efficacy and modes of action in pigs, In: Piva A, Bach Knudsen KE, Lindberg JE. (Eds.) Gut Environment in Pigs. Nottingham University Press. 2001; pp. 154-162.

Patra, A., Park, T., Kim, M., & Yu, Z. (2017). Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. *Journal of Animal Science and Biotechnology*, 8(1), 13. https://doi.org/10.1186/s40104-017-0145-9

Sahoo, A., & Jena, B. (2014). Organic acids as rumen modifiers. *International Journal of Science and Research*, 3, 2262-2266.

Silanikove, N., Gilboa, N., Perevolotsky, A., & Nitsan, Z. (1996). Goats fed tannin-containing leaves do not exhibit toxic syndromes. *Small Ruminant Research*, 21(3), 195-201. https://doi.org/10.1016/0921-4488(95)00833-0

Van Soest, P.J., Robertson, J.B., & Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74(10), 3583-97. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Yang, C.J., Mao, S.Y., Long, L.M., & Zhu, W.Y (2012). Effect of disodium fumarate on microbial abundance, ruminal fermentation and methane emission in goats under different forage: concentrate ratios. *Animal*, 6(11), 1788–94. https://doi.org/10.1017/S1751731112000857

Yu, C.W., Chen, Y.S., Cheng, Y.H., Cheng, Y.S., Yang, C.M.J., & Chang, C.T. (2010). Effects of fumarate on ruminal ammonia accumulation and fiber digestion *in vitro* and nutrient utilization in dairy does. *Journal of Dairy Science*, 93(2), 701–710. https://doi.org/10.3168/jds.2009-2494

Zhou, Y., Mcsweeney, C., Wang, J., & Liu, J. (2012). Effects of disodium fumarate on ruminal fermentation and microbial communities in sheep fed on high-forage diets. *Animal*, 6(5), 815-823. https://doi.org/10.1017/S1751731111002102