TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2020) 44: 201-213
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doi:10.3906/vet-1908-47

The forage quality and the in vitro ruminal digestibility, gas production, organic acids, and some estimated digestion parameters of tomato herbage silage with molasses and barley

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Received: 16.08.2019 • **Accepted/Published Online:** 28.01.2020 • **Final Version:** 06.04.2020

Abstract: In this study, nutrient composition and use of silage after harvest of waste green parts (herbage) of tomato (*Licopersicon esculentum* L.) were researched as a wet forage source for ruminants. Three different silages of tomato herbage: without additive (TS), with 5% sugar beet molasses (TSm), or 5% crushed barley grain (TSb) were prepared. The forage quality and the in vitro ruminal digestibility, gas production, organic acids, and some estimated digestion parameters for different silages of tomato herbage were determined. Besides, the in vitro digestion values of using at 25%, 50%, 75%, and 100% rates instead of corn silage in the dairy cattle total mix ration (TMR) were researched. The lactic acid content of TSb silage was higher than those of the TS and TSm silages ($P < 0.001$). The ensiling of tomato herbage with barley decreased the levels of acetic, butyric, and propionic acids in the silage ($P < 0.01$). The use limits of tomato herbage silages instead of corn silage in dairy cattle TMR were determined as 25% for TS, 75% for TSb, and 100% for TSm in terms of in vitro gas production and other digestion parameters during 24-h incubation. The volume of methane produced for mL/g DM at 24 h was decreased by increasing TS in dairy cattle TMR ($P < 0.001$) but was not changed by increasing TSm and TSb in dairy cattle TMR ($P > 0.05$). Consequently, results have shown that tomato herbage should be ensiled with 5% molasses. However, it has been concluded that using tomato herbage silage with barley up to 75% or sugar beet molasses up to 100% instead of corn silage in dairy cattle ration did not have a negative effect in vitro.

Key words: Forage, tomato plant waste, in vitro gas production, nutrient, silage acidity

1. Introduction

The tomato plant (*Lycopersicon esculentum* L.) belongs to the family Solanacae and is an annual or perennial plant [1,2]. Among greenhouse plants, tomato is the predominant crop [3]. The green fruits and leaves of tomato plant contain many antinutritional substances such as solanine and tannins. These substances may have a toxic effect on human and animal health [4,5]. Green forage/plant material ensiling is also an important method to detoxify many toxic substances [4]. According to the United Nations Food and Agriculture Organization, World tomato production is around 170 million tons/year [6]. After harvesting tomato fruits, huge amounts of biomass residues are left on the greenhouse or field, namely tomato harvest stalks (or tomato herbage) which include plant residues and plant wastes. These greenhouse wastes are mainly composed of 19% acid detergent lignin (ADL), 14% hemicellulose (HemC), about 50% cellulose and 5% pectin and are therefore valuable resources for further applications [7]. The crude protein (CP) contents of tomato leaf for different varieties by previous researchers were determined as about 18–24% [2]. Indeed, greenhouse tomato crops produce the greatest amount of plant waste, around 24–49 t per greenhouse hectare [3,7]. Plant wastes in greenhouse are often eliminated by dumping them in dry ravines or empty areas, originating uncontrolled burning, blocking of riverbeds, poisoning of cattle and sheep, and causing visual blight on the landscape [3,5]. In terms of global tomato production, tomato herbage, which is a by-product, will now reach very high levels. Despite that greenhouse tomatoes are agriculturally profitable, such intensive cultivation causes environmental impact due to the enormous volume of postharvest refuse [8]. In addition, the disposal of greenhouse plant waste in landfills is an environmental problem as that waste is a potential source of methane, a gas which negatively contributes to global warming [9]. The wastes of different agricultural process can be utilized as feed source [10,11]. However, to be able to utilize agricultural waste as a source of alternative livestock ruminant feed, the potential of nutrition of each kind of waste needs to be known. There are limited studies in the literature on nutrient matter content [7] and energy value for ruminants of plant waste products (tomato herbage) in greenhouse tomato production.

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Climate change is characterized by increased atmospheric carbon dioxide and rising temperatures. Rising temperatures and shifting precipitation patterns will alter the ability to meet crop water requirements, water availability, crop productivity, and costs of water access across forage production lands. Climatic conditions are an important problem for the production of feed plants and the sustainability for forage production [12-15]. In recent years, animal nutritionists have studied drought-resistant plants, agro-industrial wastes, and other by-products as alternative forage sources [16-18]. Searching for alternative forages is important for animal production sustainability. Some agricultural by-products, which include antinutritional factors or are tasteless, cannot be used in animal feeding because of their contents; and in addition, by-products with high water content can decompose rapidly depending on environmental conditions. The research potential of the forage characteristics of tomato herbage, which is greenhouse waste product, is important for both use for herbivorous diet and prevention of environmental pollution. In the literature, there was no study investigating tomato leaves or tomato herbage potential as forage. The by-products can be used as forage in animal nutrition by ensiling process [2,3,7,19]. Silage is produced when grass or other material of sufficiently low dry matter (DM) content, susceptible to spoilage by aerobic microorganisms and oxidative plant enzymes is stored anaerobically. The optimum quality values of corn silage, which is the most common silage for livestock animals, may be different from the silage values of agro-industrial by-products [20]. The solubility and easy-digestiblity of carbohydrates are essential for ideal anaerobic lactic acid fermentation. Ensiling of the feed/by-product materials with low levels of easy digestible carbohydrates may require additives with rich digestible carbohydrates. The determination of silage quality and digestion parameters for tomato herbage silage will demonstrate the availability of this waste product (by-product) in the ruminant diet. The aim of the present study was to determine silage quality and in vitro ruminal digestion potential of ensiling with molasses and barley grain of tomato herbage (waste or by-product), which can be alternative forage for ruminants due to fiber, carbohydrates, water, and other nutrients

2. Materials and methods

Scientific procedure of the study was conducted according to a research protocol approved (Date: August 12, 2015; Decision number: 15/105) by the Local Ethics Committee for Animal Experiments at Erciyes University.

2.1. Tomato herbage and its ensiling

Tomato herbage, which is a waste of tomato fruit, was provided from material at the end of tomato production in greenhouse (high tunnel type) conditions in the Silifke district of Mersin Province, Turkey. The samples were cut 5 cm above the ground and included leaf, branches, and stem parts of the plant. They were randomly taken from 8 different parts of the greenhouse. The samples were manually cut into 2–3 cm lengths with a knife. The cut tomato herbage was ensilaged without additive (100% tomato herbage, as wet) (TS), and with 5% molasses (5% sugar beet molasses + 95% tomato herbage, as wet) (TSm), or 5% barley (5% crushed barley grain + 95% tomato herbage) (TSb) additives as about 450 g in five replicates. The cut tomato herbage with crushed barley or with sugar beet molasses were mixed in polyethylene (25 cm × 35 cm) silage bags. These polyethylene size bags were vacuumed using a vacuum machine (Caso VC100, Germany). The silage bags were stored in laboratory conditions in a sun-free environment. They were opened after 45 days and then the nutrient and in vitro analysis of silage samples were carried out. The volumes of silage gases were determined based on 50 mL of gas taken from silage bags using plastic syringes (TS, TSm, and TSb) at 45 days before the silage bags were opened.

2.2. The determination of chemical compositions in herbage and silages

The dry matter (DM) levels of the harvested tomato herbage and tomato silage samples were determined after waiting at 60 °C for 24 h and then at 105 °C for 24 h. Dried samples were mill in a grinder mill (IKA Werke, Germany) to a maximum particle size of 1 mm. The crude ash (CA), crude protein (CP) (nitrogen × 6.25) and diethyl ether extract (EE) levels were determined according to the method reported by the AOAC [21]. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were analyzed according to Van-Soest et al. [22]. NDF was determined using sodium sulfite (0.5 g) and heat stable amylase (200 µL) (aNDF). The NDF, ADF, and ADL were not inclusive of residual ash (aNDFom, ADFom, and ADL). The level of nonstructural carbohydrate (NSC) was calculated using the equation in NRC [23]; NSC = 100 − (NDF % + CP % + EE % + CA %). The hemicellulose (HemC) contents were calculated using the following formula; HemC % = NDF % − ADF %. The total condensed tannin (TCT) contents were determined by the butanol-HCl method by Makkar et al. [24].

2.3. The determination of acidity values of tomato herbage silages

The pH values of opened silages were immediately determined. A wet tomato silage sample of 25 g was shredded for 15 s with a laboratory type blender (Waring, USA) and then shredded for 10 s with 100 mL distilled water. The pH value of the mixture was measured with a digital pH meter (Mettler Toledo, S220 pH/ion meter, Ohio, USA) [25]. The lactic acid (LA) content in silage fluid was determined according to a modified method

by Barnett [26]. Silage fluid of 1.5 mL was put into microcentrifuge tubes and centrifuged at 21,206 × *g* for 15 min in microcentrifuge (Gyrozen 1524, Gyrozen Co. Ltd., Daejeon, Korea). The supernatant was diluted at 1:100 with distilled water. One milliliter of diluted silage fluid was put into falcon tubes and mixed with 0.1 mL of $CuSO_4$ (5%, w/v, in distilled water) and 6 mL of H_2SO_4 (98%) using a vortex. The tubes were cooled using ice bags; 0.1 mL of para hydroxy biphenol (1.5%, in 0.5% aqueous NaOH) was added to the cooled tubes and mixed using a vortex for 15 s and then the tubes were incubated at room temperature for 10 min. The absorbances of purple-violet color in the tubes were read at 565 nm using a UV-spectrophotometer (UviLine 9100, SI Analytics–Xylem Analytics Germany Sales GmbH&Co.KG, Mainz, Germany). The amount of LA in the sample fluid was calculated as lactate equivalent from the calibration curve ($R^2 = 0.95$) of standard lithium lactate (0.312–160 µg/ mL). The % LA content in silage DM was calculated using the following equations: LA, % in DM = ((Absorbance value × 10^{-2} × (100 – DM) / DM).

Silage fluid of 1.5 mL, which was centrifuged at 21,206 × *g* for 15 min in a microcentrifuge, was mixed with 0.1 mL of metaphosphoric acid (25%, w/v) in a gas chromatograph vial. The analysis of organic acids (or volatile fatty acids - VFA's) (acetic (AA), butyric (BA), propionic (PA), iso-butyric (IBA), valeric (VA), and iso-valeric (IVA) acids) in silage fluid was measured by using a gas chromatograph device with flame ionization detector (GC-FID, Thermo Trace 1300, Thermo Scientific, USA) with an autosampler (Thermo AI-1310, Thermo Scientific, USA) [27]. The concentrations (mmol/L) of organic acids were identified using the Xcalibur software program, according to the retention time and peak area in chromatograms. The % concentrations of organic acids in DM of silage were calculated.

2.4. The determination of in vitro ruminal digestion potential and rumen fluid collection

The dairy cattle total mix ration (TMR), which included corn silage, wheat straw, alfalfa herbage, and concentrated mix feed, was used as control. The dairy cattle TMR (control) was prepared in a composition to meet the energy and other nutrient requirements of dairy cattle, which has 25 L/day of milk yield and is on the 120th day of lactation [23].

The three different tomato herbage silages (TS, TSm, and TSb) were used at 25%, 50%, 75%, or 100% rates (in DM basis) instead of corn silage in the control TMR. All dairy cattle TMRs prepared as 1 kg mixtures using dried feedstuffs (corn silage, wheat straw, alfalfa herbage, concentrate mix feed, or tomato herbage silages) in laboratory. Dairy cattle TMR with tomato herbage silage was prepared as 12 different samples (3 × 4). The ingredients and compositions (NSC, aNDFom, and CP) of dairy cattle TMR samples are given in Tables 1–4. The in vitro digestion potential of the TMR and tomato herbage was analyzed using an in vitro gas production technique.

Rumen fluid, which will be used in the in vitro gas production tecnique, was taken via esophageal tube from two Brown Swiss cattle. Approximately 1 L of rumen fluid was collected in a thermos, which included water, at 39 °C using CO_2 gas, and filtered with six layers of cheesecloth in the laboratory. The samples were incubated in rumen fluid and buffer mix in an anaerobic glass fermenter in 100 mL volumes (Model Fortuna, Germany) following the procedures by Menke et al. [28]. The 200 ± 10 mg of dried TMR sample was incubated with 20 mL of buffer mixture, (which included bidistilled water, macromineral solution, buffer solution, trace-mineral solution, resazurine solution, and reducing solution), and 10 mL of filtered rumen fluid. This mixture was incubated in a glass fermenter (Model Fortuna, Germany) at 39 ± 0.5 °C in an incubator (Yapar Stainless, Kahramanmaraş, Turkey) for 24 h. The initial volumes of in vitro glass fermenters were recorded. Each sample was studied in triplicate. In addition, three blank fermenters (no template; rumen fluid + buffer mixture) were used to calculate the total gas production.

2.5. The determination of in vitro total gas production, methane production, and estimated digestion values

The in vitro cumulative total gas production was recorded at 3, 6, 12, 18, and 24 h. After 24 h of incubation, the total gas volume was recorded from the calibrated scale in the in vitro glass fermenter. After reading the total gas volume, the methane volume in total gas was determined with the infrared methane analyzer (Sensor, Europe GmbH, Erkrath, Germany). The metobolizable energy (ME) and organic matter digestibility (OMd) contents of the tomato herbage and the dairy cattle TMRs with TS, TSm, and TSb were calculated using the equations by Menke and Steingass [29] as follows: (ME (MJ /kg DM) = 2.20 + 0.136 × Gas24h + 0.057 × CP) , (OMd (g/kg DM) = 14.88 + 0.889 × Gas24h + 0.45 × CP + 0.0651 × A). Short chain fatty acids (SCFA) were calculated using the equation: (SCFA (mmol/0.2g DM) = 0.0222 × Gas24h – 0.00425), Gas24h = 24 h net gas production (mL/200 mg), CP = Crude protein (g/kg DM), CA = Crude ash content (g/kg DM), EE=Ether extract (g/kg DM).

2.6. The determination of organic acids in the in vitro digestion fluid

The total gas volume at 24 h of in vitro incubation was recorded and 10 mL of digestion fluid in the glass fermenter was collected in Falcon tubes. The fluids were frozen at –20 °C until analysis, when the tubes were thawed at room temperature. Two milliliters of digestion fluid was placed into microcentrifuge tubes and centrifuged at 21,206 × *g* for 15 min in a microcentrifuge (Gyrozen 1524, Gyrozen Co. Ltd., Daejeon, Korea). For GC-FID, 1.25 mL of the

Table 1. The chemical compositions and in vitro cumulative gas production, methane production, and other estimated values of tomato herbage.

Tomato herbage			
Digestion values		Chemical composition,% in DM	
Gas 3h	16.31	DM %, feed basis	19.57
Gas 6h	24.11	CP	12.28
Gas 12h	29.69	CA	15.45
Gas 18h	35.27	EE	3.30
Gas 24h	35.68	aNDFom	26.62
ME	8.01	ADFom	25.53
OMd	58.35	ADL	14.39
SCFA	0.79	NSC	42.35
		HemC	1.09
		TCT	0.68

ME: Metabolisable energy as MJ/kg DM; OMd: Organic matter digestibility as %; Methane, mL = in vitro methane production as mL/g DM at 24 h; SCFA, mmol = Molarities of short chain fatty acids in fermentation fluid by 0.2 g DM at 24 h. ADFom: Acid detergent fiber, which is not inclusive of residual crude ash as % in DM; CP: Crude protein as % in DM; CA: Crude ash as % in DM; DM: Dry matter as %; aNDFom: NDF determined using heat stable amylase and not inclusive of residual crude ash as % in DM. NSC: Nonstructural carbohydrate as % in DM; TCT: Total condensed tannins as % in DM.

supernatant and 0.25 mL of metaphosphoric acid (25%, w/v) were mixed in a vial. Analysis of organic acids (or volatile fatty acids - VFA's) in the in vitro digestion fluid was determined by using a gas chromatograph device (Thermo Trace 1300, Thermo Scientific, USA) with an autosampler (Thermo AI-1310, Thermo Scientific, USA). The GC device was equipped with a Flame Ionization Detector (FID), with polyethylene glycol columns (length: 60 m, i.d: 0.25 mm × 0.25 µm, film thickness: 0.25 µm) (TG-WAXMS, Thermo Scientific, USA). The operation procedure of the device was according to the study of Ersahince and Kara [27]. A standard organic acid mixture was used for the determination of retention time and calibration curve (R^2 = 0.99). The concentrations (mmol/L) of acetic acid (AA), propionic acid (PA), butyric acid (BA), *iso* butyric acid (IBA), valeric acid (VA), and *iso* valeric acid (IVA) were identified using the Xcalibur software program according to retention time and peak area in the chromatograms. The concentration of total volatile fatty acids (TVFA) of the in vitro fermentation fluid was calculated with following formula using molarities of short chain- and branch short chain- fatty acids: TVFA (mmol/L) = AA (mmol/L) + PA (mmol/L) + BA (mmol/L) + IBA (mmol/L) + VA (mmol/L) + IVA (mmol/L)

2.7. Statistical analysis

Statistical significance among tomato herbage silages for chemical contents and in vitro digestion values were determined by one-way variance analysis. The multivariate analyses were implemented for homogeneous variances by General Linear Model procedures to test treatment differences for tomato herbage silages (TS, TSm, and TSb) at use levels (0%, 25%, 50%, 75%, and 100%) in TMR and silage type (TS, TSm, and TSb) in TMR. Data for in vitro digestion values were analyzed using a randomized complete design with supplement levels × tomato herbage silages.

The one-way variance analysis was conducted on chemical compositions of silages and in vitro digestion values tested in different tomato herbage silages. Data were analyzed using the following statistical model: $Yij = \mu ij + Si + ei$, where Yij is the general mean for each parameter investigated, μ is the mean of different silage for each parameter researched, S_i is the ith effect of different silages of tomato herbage on the observed parameters, and ei is the standard error value. The means were separated using Tukey's multiple range test at $P < 0.05$.

The two-way variance analysis was conducted on the in vitro digestion values tested in different types (TS, TSb, and TSm) and different use levels (0%, 25%, 50%, 75%, and 100%) instead of corn silage in dairy cattle TMR of tomato herbage silages. Data were analyzed based on the statistical model: Yijk = µ + Ei + Dj + EDij + eijk, where Yijk is the dependent variable, µ is overall mean, E is effect of i – supplement levels on the observed parameters, D is effect of j – silage on the observed parameters, ED is the interaction between the i – use levels and j – silages, and eijk is the standard error term. The linear, quadratic and cubic effects (polynomial contrast) for the use levels of different tomato herbage silages were also determined. Statistical analysis of data was done with SPSS 15.0 software. Significance was defined at P-values of <0.05

3. Results

3.1. The chemical composition of tomato herbage and tomato herbage silages

The tomato herbage contained 19.57% DM, 12.28% CP, 15.45% CA, 3.30% EE, 26.62% aNDFom, 25.53% ADFom, 14.39% ADL, 42.35% NSC, 1.09% hemicellulose, and 0.68% TCT in the DM (Table 2). The nutrient contents of the tomato herbage silages (TS, TSm, and TSb) are given in Table 1. It was determined that the DM ($P \leq 0.001$) and NSC ($P = 0.006$) levels of the tomato herbage silages with molasses and barley silages were higher than those of the tomato herbage silages without additives. The CP content (13.75%) of the TSm silage was higher than that

Table 2. The chemical compositions and typical fermentation profiles of tomato herbage silage with molasses and barley

	TS	TSm	TSb	SD	SEM	P-value
DM	16.09[b]	19.45[a]	19.10[a]	1.69	0.43	≤0.001
CP	12.31[b]	13.75[a]	13.39[b]	1.72	0.49	0.002
CA	27.54[a]	20.73[b]	20.04[c]	3.45	1.09	≤0.001
EE	3.07	2.70	2.95	0.67	0.27	0.216
aNDFom	24.72	22.16	26.30	2.50	0.83	0.110
ADFom	23.23[a]	20.33[b]	25.00[a]	2.22	0.74	0.004
ADL	14.91	13.76	14.72	3.34	1.18	0.930
NSC	32.36[b]	38.66[a]	37.32[a]	1.08	3.25	0.006
TCT	0.66	0.84	0.65	0.04	0.13	0.111
pH	5.97[a]	5.76[b]	4.36[c]	0.20	0.81	≤0.001
LA	0.02[b]	0.02[b]	6.37[a]	3.10	0.80	<0.001
AA	0.57[a]	0.41[ab]	0.25[b]	0.16	0.04	<0.001
BA	0.28[b]	0.38[a]	0.02[c]	0.17	0.04	<0.001
PA	0.21[a]	0.10[ab]	0.01[b]	0.11	0.02	0.002
OA	0.05[a]	0.01[b]	0.01[b]	0.02	0.01	<0.001
TA	1.14[b]	0.93[b]	6.65[a]	2.75	0.71	<0.001
LA (in %TA)	1.42[b]	2.38[b]	95.76[a]	45.80	11.82	<0.001
Silage gases	520	15	130	-	-	-

ADFom: Acid detergent fiber, which is not inclusive of residual crude ash as % in DM; CP: Crude protein as % in DM; CA: Crude ash as % in DM; DM: Dry matter as %; aNDFom: NDF determined using heat stable amylase and not inclusive of residual crude ash as % in DM. NSC: Nonstructural carbohydrate as % in DM; TCT: Total condensed tannins as % in DM, LA: lactic acids as % in DM, AA : acetic acid as % in DM, BA: butyric acid as % in DM, PA : propionic acid as % in DM, OA: other acids (iso-valeric acid + iso-butyric acids + valeric acid + iso-caproic acid + hexanoic acid) as % in DM, TA: total acids. Silage gases: It is gas produced by silage bags, as mL. SEM: Standard error of means, SD: Standard deviation of means. [a,b:] Values within a column with different superscripts differ significantly at $P < 0.05$.

Table 3. The use of tomato herbage silages at different ratios instead of corn silage in dairy cattle TMR.

Ingredients, kg/day, as DM	TMR without TS	The TS instead of corn silage				The TSm instead of corn silage				The TSb instead of corn silage			
		25%	50%	75%	100%	25%	50%	75%	100%	25%	50%	75%	100%
TS	-	1.25	2.50	3.75	5.00	1.25	2.50	3.75	5.00	1.25	2.50	3.75	5.00
Corn silage	5.00	3.75	2.50	1.25	-	3.75	2.50	1.25	-	3.75	2.50	1.25	-
Wheat straw	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Alfalfa herbage	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Concentrate mix feed*	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Total, kg/day	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Chemical composition, %													
NSC	34.90	34.98	35.10	35.19	35.29	35.38	35.87	36.37	36.87	35.28	35.70	36.12	36.53
aNDFom	40.70	38.94	37.17	35.42	33.65	38.78	36.85	34.94	33.02	39.03	37.37	35.71	34.05
CP	14.50	14.94	15.27	15.59	15.91	15.02	15.40	15.79	16.18	15.16	15.70	16.23	16.77

CP: Crude protein; aNDFom: NDF determined using heat stable amylase (aNDF) and not inclusive of residual crude ash. NSC: Nonstructural carbohydrate; TS: tomato silage. *: Concentrate mix feed includes 2.80 Mcal/kg DM as ME and 19% as CP in DM.

Table 4. The in vitro cumulative gas production, methane production, and other estimated values of tomato herbage silages.

	TS	TSm	TSb	SD	SEM	P value
Gas 3h	3.50[b]	9.04[a]	6.90[a]	2.72	0.78	0.002
Gas 6h	6.35[b]	17.69[a]	16.56[a]	6.08	1.75	0.001
Gas 12h	10.83[b]	24.56[a]	20.90[a]	6.64	1.91	<0.001
Gas 18h	15.31[b]	31.42[a]	25.25[a]	7.57	2.18	<0.001
Gas 24h	17.12[b]	36.15[a]	28.01[a]	9.21	2.66	0.001
Methane	4.32[b]	7.99[a]	6.39[ab]	2.21	0.76	0.037
ME	5.49[b]	8.27[a]	7.03[a]	1.32	0.38	0.001
OMd	51.51[b]	62.52[a]	55.65[a]	5.98	1.72	0.012
SCFA	1.87[b]	3.99[a]	3.08[a]	1.02	0.29	0.001

ME: Metabolisable energy as MJ/kg DM; OMd: Organic matter digestibility as %; Methane, mL = in vitro methane production as mL/g DM at 24 h. SCFA, mmol = Molarities of short chain fatty acids for fermentation fluid by one g DM at 24 h SEM: Standard error of means; SD: Standard deviation of means. [a,b:] Values within a column with different superscripts differ significantly at $P < 0.05$.

of the TSb (13.39%) and TS (12.31%) silage ($P = 0.002$). The CA contents of tomato herbage silage with molasses or barley were lower than of tomato herbage silage without additive ($P < 0.001$). The ADFom content of the tomato herbage silage decreased significantly by ensiling with 5% molasses ($P < 0.01$). The ADFom content (25.00%) of the TSb was similar to that of the TS (23.23%). There were no significant effects on the aNDFom, ADL, TCT, and EE contents of ensiling tomato herbage with different additives ($P > 0.05$). The silage gases produced in the TS, TSm, and TSb silages at 45 days of ensiling are given in Figure 1. The pH values of tomato herbage silage were low: 5.76 and 4.36 at ensilaging with molasses and barley. The silage pH values of TSm and TSb were lower than that of TS ($P \leq 0.001$). The total acids (TA) in silage DM was increased by barley supplementation at ensiling of tomato herbage ($P < 0.001$) The LA content (%) in DM and LA % in TA of TSb silage were higher than those of the TS and TSm silages ($P < 0.001$). The ensiling of tomato herbage with barley decreased the levels of AA, BA, PA, and OA in silage DM ($P < 0.01$) (Table 2). Silage gases produced in silage bags at 45 days were average 520 mL for TS, average 15 mL for TSm and average 130 mL for TSb (Figure).

3.2. The in vitro digestion values of tomato herbage and tomato herbage silages

The silage gas produced by tomato herbage was 16.31 mL at 3h, 24.11 mL at 6h, 29.69 mL at 12 h, 35.27 mL at 18 h, and 35.68 mL at 24 h by 0.2 g DM. The ME, OMd, and SCFA values of tomato herbage were calculated as 8.01 MJ/kg DM, 58.35%, and 0.79 mmol/0.2 g DM, respectively (Table 1).

The in vitro cumulative total gas production at 3 ($P = 0.002$), 6 ($P = 0.001$), 12 ($P < 0.001$), 18 ($P < 0.001$), and 24 ($P = 0.001$) h of TSm and TSb were found to be higher than those of TS (Table 4). In the present study, it was determined that the methane amount (%) in the total gas produced at 24 h of incubation was similar in all three tomato herbage silages ($P > 0.05$). However, the volume (mL) of methane produced by one g of DM for TSm was higher than that of TS at 24 h of incubation and similar to that of TSb ($P < 0.05$; Table 3). The estimated ruminal ME ($P = 0.001$), OMd ($P = 0.012$), and SCFA ($P = 0.001$) values of the TS silage were lower than those of TSm and TSb (Table 4).

3.3. The in vitro digestion values of tomato herbage silages in dairy cattle TMR

The use of TS, up to 25% instead of corn silage in the dairy cattle TMR did not change the cumulative gas production during 24-h incubation ($P > 0.05$). The use of TS up to 75% instead of corn silage in the dairy cattle TMR did not affect total gas production in the first 12 h ($P > 0.05$) and linearly decreased total gas production in the 18th and 24th h ($P < 0.001$). The use of TS up to 100% instead of corn silage in TMR linearly reduced total gas production for all incubation hours ($P < 0.001$) (Table 4). The use of TSm up to 100% instead of corn silage in TMR did not change the in vitro cumulative gas production of TMR (Linear contrast $P > 0.05$; Quadratic contrast $P > 0.05$; cubic contrast $P > 0.05$). Using TSm up to 100% instead of corn silage in TMR linearly increased only at 3 h of incubation ($P = 0.020$) (Table 4). In the study, use

The silage gases produced by TS at 45 days	The silage gases produced by TSm at 45 days	The silage gases produced by TSb at 45 days

Figure. The silages and silage gases in tomato herbage silage at 45 days of ensiling.

of TSb up to 100% instead of corn silage in TMR linearly decreased in vitro gas production ($P < 0.05$). This decrease was not significant up to the 75% supplementation level ($P > 0.05$), but was significant at the 100% supplementation level ($P < 0.05$) (Table 5).

In the two-way ANOVA analysis of the study data, the tomato silage type (without additive, with molasses, and with barley), the use level of the tomato silage (25%, 50%, 75%, and 100%), and both interactions were found to be significant in the in vitro cumulative gas production ($P < 0.001$) (Table 5). The use of TS silage up to 100% instead of corn silage linearly decreased the volume (mL) of methane produced by the unit DM ($P < 0.001$). The use of TS silage instead of corn silage linearly reduced ME and OMd levels in relation to the supplementation level ($P < 0.001$; Table 6). This decrease was not statistically significant in the use of 25% of TS silage. However, the use of TS at 50% and above instead of corn silage significantly reduced the ME, OMd, and SCFA levels ($P < 0.001$). At the 24th h of in vitro incubation, the pH value of the fermentation liquid did not linearly change with the supplementation level of TS ($P = 0.119$). There was only a quadratic difference among the supplementation level of TS ($P = 0.005$) (Table 6).

The use of TSb silage instead of corn silage did not change the in vitro methane production and estimated ME, OMd, SCFA, and ruminal pH levels ($P > 0.05$, Table 6). The use of TSb silage at 100% ratio instead of corn silage added did not change in vitro methane production of TMR ($P > 0.05$). The estimated ME, OMS, and SCFA levels of TMR were linearly decreased by increasing the supplementation ratio of TSb silage ($P < 0.05$). The decrease in these parameters was not significant ($P > 0.05$) up to 75% use of TSb, but was statistically significant at 100% use ($P > 0.05$; Table 6). The molarities of TVFA, AA, BA, PA, BA, IVA, VA, and IBA in digestion fluid of fermentation used TS in TMR decreased linearly with the supplementation ratio ($P < 0.001$) (Table 7). The molarities of TVFA in digestion fluid for TS use at 0%, 25%, 50%, 75%, and 100% instead of corn silage in dairy cattle TMR changed to 126, 109, 111, 101, and 97 mmol/L, respectively ($P < 0.001$). The molarities of AA and PA in digestion fluid for TSm and TSb use at a 25%, 50%, and 75% ratio instead of corn silage in TMR did not differ from those of TSb use at 0% (non-TSb use in TMR) (Table 7). The molarities of BA in digestion fluid, which fermented of TMR, linearly decreased at all supplementation ratios in TMR with TS, decreased at 75% and 100% supplementation ratios in TMR with TSm and TSb (linearly and quadratic) ($P < 0.05$). The IBA, VA, and IVA molarities in digestion fluid for TSb and TSm (up to 100% instead of corn silage) did not change ($P > 0.05$) (Table 7).

4. Discussion

4.1. Chemical compositions of tomato herbage and tomato herbage silages

In the present study, tomato herbage is a rich-moderate source in terms of CP as forage, but appears to be inadequate in terms of fibrous compounds (aNDFom). Tomato greenhouse wastes were rich in inorganic substances by the previous researchers stated, especially in terms of nitrogen and salt [3]. In the current study, high ash content in greenhouse tomato wastes may be related to this result of researchers [3,8]. According to NRC [23], CP content of alfalfa herbage has been reported to change

Table 5. The in vitro cumulative gas production values of using different tomato herbage silages instead of corn silage in dairy cattle TMR.

Silage	Using level	In vitro cumulative gas production				
		Gas3h	Gas6h	Gas12h	Gas18h	Gas24h
TS	0%	11.44	19.26	27.48	35.72	40.01
	25%	12.37	18.94	25.30	31.66	35.94
	50%	11.36	17.79	23.52	29.24	31.97
	75%	10.62	16.66	21.99	27.34	31.85
	100%	5.40	9.92	13.39	16.86	19.36
P-value	L	0.001	0.001	0.001	0.001	<0.001
	Q	0.001	0.002	0.020	0.084	0.066
	C	0.243	0.120	0.088	0.090	0.040
TSm	0%	11.50	19.12	27.64	36.17	40.73
	25%	12.57	19.85	26.54	33.24	37.98
	50%	12.50	18.51	25.41	32.31	37.75
	75%	14.38	21.69	29.54	37.39	41.91
	100%	13.09	20.24	27.31	34.37	38.31
P-value	L	0.020	0.194	0.609	0.930	0.903
	Q	0.239	0.966	0.578	0.428	0.760
	C	0.306	0.404	0.175	0.115	0.179
TSb	0%	11.50	19.12	27.64	36.17	40.73
	25%	12.66	20.23	27.47	34.71	39.74
	50%	11.29	18.29	25.08	31.87	36.59
	75%	10.39	18.41	25.72	33.05	36.57
	100%	8.62	15.63	21.58	27.53	31.03
P-value	L	0.001	0.012	0.007	0.005	0.003
	Q	0.017	0.139	0.361	0.558	0.431
	C	0.339	0.958	0.569	0.374	0.598
Two-way ANOVA	TS	10.24^{b}	16.51^{c}	22.34^{b}	28.16^{b}	31.82^{b}
	TSb	12.79^{a}	19.91^{a}	27.26^{a}	34.60^{a}	39.34^{a}
	TSm	10.88^{b}	18.36^{b}	25.47^{a}	32.58^{a}	36.93^{a}
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001
Silage		<0.001	<0.001	<0.001	<0.001	<0.001
Use level		<0.001	<0.001	<0.001	<0.001	<0.001
Silage*Use level		<0.001	<0.001	<0.001	<0.001	0.004

L: Linear, Q: Quadratic, C: Cubic.
[a,b,c:] Values within a column with different superscripts differ significantly at $P < 0.05$.

from 18.7% to 22.2% compared to the phenological period of the plant. The CP contents of tomato leaf for different varieties by previous researchers were determined as about 18–24% [2]. Therefore, according to results of Kulcu [8], greenhouse tomato plant wastes contained about 10% CP (nitrogen × 6.25). About 12–13% CP content of tomato herbage or tomato herbage silage, which contain leaf and stem, demonstrate that greenhouse tomato wastes are alternative forage with moderate CP. The stems of different tomato varieties included an average of 33–62% insoluble dietary fiber, which consists of mainly ADF, and 1.5–12 % soluble fiber on a DM basis [2]. The 27% aNDFom and

Table 6. The in vitro methane production and estimated digestion values of using different tomato herbage silages instead of corn silage in dairy cattle TMR.

Silage	Using level	Methane	ME	OMd	SCFA	pH
TS	0%	46.99	8.87	58.94	4.50	6.91
	25%	33.46	8.27	55.97	4.01^{ab}	6.90
	50%	32.68	7.68	53.08	3.53^{b}	6.86
	75%	30.62	7.67	53.68	3.51^{b}	6.86
	100%	21.96	5.98	44.03	2.13^{c}	6.91
P-value	L	<0.001	<0.001	<0.001	<0.001	0.191
	Q	0.320	0.072	0.075	0.071	0.005
	C	0.031	0.028	0.031	0.042	0.029
TSm	0%	46.99	8.87	58.94	4.50	6.91
	25%	40.40	8.50	57.43	4.19	6.90
	50%	36.56	8.47	57.97	4.16	6.86
	75%	47.99	9.03	62.19	4.63	6.86
	100%	40.60	8.55	60.05	4.23	6.91
P-value	L	0.519	0.904	0.275	0.903	0.191
	Q	0.184	0.761	0.746	0.760	0.005
	C	0.035	0.179	0.192	0.748	0.029
TSb	0%	46.99	8.87	58.94	4.50^{a}	6.91
	25%	40.89	8.74	58.91	4.39^{a}	6.85
	50%	39.60	8.31	58.58	4.04^{ab}	6.86
	75%	34.04	8.31	56.99	4.03^{ab}	6.90
	100%	39.70	7.56	53.00	3.42^{b}	6.87
P-value	L	0.034	0.003	0.035	0.003	0.648
	Q	0.844	0.431	0.242	0.432	0.124
	C	0.310	0.599	0.334	0.512	0.022
Two-way ANOVA	TS	33.14^{b}	7.69^{b}	53.14^{b}	3.53^{b}	6.89
	TSb	42.51^{a}	8.69^{a}	59.32^{a}	4.07^{a}	6.88
	TSm	40.24^{a}	8.36^{a}	57.28^{a}	4.34^{a}	6.89
	P-value	<0.001	0.001	<0.001	0.001	0.359
Silage		<0.001	<0.001	<0.001	<0.001	0.359
Use level		<0.001	<0.001	<0.001	<0.001	<0.001
Silage*Use level		<0.001	0.002	0.002	0.002	<0.001

Methane: The volume of methane produced for mL/g DM at 24 h; ME: Metabolic energy as MJ/kg DM; OMd: Organic matter digestibility as %; pH: pH values at 24 h of incubation.

26% ADFom values of tomato herbage were similar to the NDF (30.9%) and ADF (24%) values reported for alfalfa herbage in the vegetation period by the NRC [23]. In the present study, since the greenhouse tomato plant wastes have contained leaf and stem parts of tomato plant, it was expected that fiber content would be close to fibrous feedstuffs. The fiber contents of the greenhouse tomato plant wastes in present study were lower than those of Ercolano et al. [7], which can depend on the plant leaf/stem ratio, plant species, and plant height.

The amount of silage gases produced in laboratory type plastic (polyethylene) silage bags, which were closed by vacuum, were very high for TS silage, moderate for TSb silage, and too little for TSm. The most important silage gases are nitrogenous gases (N_2, NO_2) and carbon dioxide. Besides, a portion of the DM loss from clostridial

Table 7. The molarities of volatile fatty acids in digestion fluid for tomato herbage silage using in dairy cattle TMR.

Supplementations of TS to dairy cattle TMR, mmol/L						P-value		
	0%	25%	50%	75%	100%	L	Q	C
TVFA	126.50	109.02	111.99	101.46	97.30	0.001	0.178	0.103
AA	80.54	70.40	72.58	67.50	63.41	0.001	0.405	0.053
PA	24.61	20.36	21.25	17.71	18.51	0.001	0.020	0.604
BA	13.76	11.91	12.16	11.03	10.37	0.001	0.480	0.204
IVA	3.90	3.23	2.98	2.61	2.47	0.001	0.213	0.741
VA	2.08	1.77	1.71	1.45	1.44	0.001	0.158	0.894
IBA	1.60	1.34	1.30	1.17	1.09	0.002	0.307	0.440
Supplementations of TSm to dairy cattle TMR, mmol/L						P-value		
	0%	25%	50%	75%	100%	L	Q	C
TVFA	126.50	133.12	125.78	115.88	109.02	0.009	0.102	0.231
AA	80.54	84.36	79.57	74.24	69.34	0.009	0.107	0.296
PA	24.61	25.78	24.03	22.54	20.81	0.025	0.232	0.472
BA	13.76	14.76	14.51	12.47	12.21	0.020	0.071	0.117
IVA	3.90	4.21	3.95	3.35	3.43	0.066	0.414	0.165
VA	2.08	2.29	2.13	1.88	1.85	0.034	0.160	0.102
IBA	1.60	1.72	1.59	1.40	1.39	0.032	0.351	0.156
Supplementations of TSb to dairy cattle TMR, mmol/L						P-value		
	0%	25%	50%	75%	100%	L	Q	C
TVFA	126.50	126.33	128.03	118.05	105.65	0.001	0.010	0.594
AA	80.54	80.05	80.86	75.64	65.81	0.001	0.006	0.246
PA	24.61	23.70	24.95	23.08	19.49	0.001	0.005	0.046
BA	13.76	14.56	15.86	12.90	11.84	0.031	0.015	0.487
IVA	3.90	4.11	3.25	3.22	4.40	0.876	0.018	0.019
VA	2.08	2.23	1.77	1.84	2.32	0.762	0.023	0.022
IBA	1.60	1.68	1.34	1.38	1.80	0.733	0.016	0.024

AA: Acetic acid, PA: Propionic acid, BA: Butyric acid, IBA: *iso* butyric acid. VA: valeric acid, IVA: *iso* valeric acid, TVFA: total volatile fatty acids.
TVFA (mmol/L) = AA (mmol/L) + PA (mmol/L) + BA (mmol/L) + IBA (mmol/L) + VA (mmol/L) + IVA (mmol/L).
[a,b,c:] Values within an acid with different superscripts differ significantly at $P < 0.001$.

fermentation in silage environment of glucose or lactate is the production of hydrogen gas, the primary reason for higher gross energy losses from clostridial activity [30]. In the present study, the addition of molasses and barley grain, which are easily digestible and soluble carbohydrate sources, to tomato herbage at ensiling increased the NSC content in silage material and provided ideal silage environment for silage bacteria. However, the fermentation in TS silage was not stopped (stable phase) since the ideal acidity was not achieved and then the NSC and CP fermentations continued. The CA value of TS was higher than those of TSm and TSb during the fermentation in TS silage and was not stopped (stable phase) since the ideal acidity was not achieved and then organic matter may have been lost as silage gasses. The NSC (32.36%) content of tomato herbage silage without additive, which has the lowest soluble/easy digestible carbohydrate content (NSC content) in silage, decreased by about 10% compared to the NSC content (42.35%) of tomato herbage. As previously mentioned, the silage gases produced in TS silage were thought to cause the decrease in NSC content. The NSC contents of the TSb (37.32%) and TSm (38.66%) silages were higher than that of TS silage, and similar to that of tomato herbage. According to this result, high NSC

contents in silage with barley and molasses have shown that additives preserve NSC content in tomato herbage silage, especially with molasses.

The desired fermentation of tomato herbage silage can be provided with barley (pH value 4.36), which is preferred as an easy fermentable carbohydrate source. However, the use of crushed barley grain did not bring the desired ranges to the pH value of tomato herbage silage. The optimum pH value for corn silage is between 3.80 and 4.20 [31]. The CP content (13.75%) of TSm was higher than those of the TSb (13.39%) or TS (12.31%) silages. The high CP level in the TSm silage may be associated with the low amount of silage gases produced (especially nitrogenous gases). According to NRC [20], the CP level for corn silage has been reported to vary between 8.5% and 9.7%. These results show that both tomato herbage and tomato herbage silage have higher CP content than that of corn silage.

The presence of 1–2% condensed tannins in feeds can provide by-pass properties to some proteins, can inhibit methanogenic protozoa (*Isotricha* spp) in the rumen, and then can reduce ruminal methane production [32]. However, more than 2% condensed tannins demonstrate a negative effect on the ruminal digestibility of nutrient matter [32,33]. In the current study, the 0.68 TCT content of tomato herbage and 0.66–0.84% TCT contents of tomato herbage silage did not negatively affect the digestion characteristics of feeds and assimilation of nutrient substances in ruminants. The aNDFom contents of tomato herbage and tomato herbage silages changed from 22.10% to 26.62% in DM. The aNDFom and ADFom contents of corn silage is 54.1% and 34.1% for immature herbage (<25% DM), 45.0% and 28.1% for seed bulking herbage (32–38% DM), and 44.5% and 27.5% for mature herbage (>40% DM) [23]. Tomato herbage silages have lower values according to the aforementioned values of corn silage. The NRC [23] has stated that the total mix ration of dairy cattle has 25–33% aNDFom content and the forage in dairy cattle rations have a minimum of 15% aNDFom. The tomato herbage silage might be affected negatively in terms of fiber content forage for a dairy cattle total mix ration.

4.2. Silage acidity values of tomato herbage silages

The silage pH values of TSb and TSm in the present study were lower than that of TS and could be related with the soluble and easy digestible carbohydrate contents of barley and molasses [33,34]. The pH value provided with 5% crushed barley grain supplementation to tomato herbage in the present study was similar to the ideal pH of legume silage; but was a little higher than that of ideal corn silage according to a previous research [33]. The high LA content and L value of TSb silage have demonstrated that crushed barley affected positively lactic acid fermentation in the ensiling of tomato herbage. During anaerobic fermentation, sugars and easy digestible carbohydrates are fermented into volatile fatty acids like lactic, acetic, propionic, and butyric acids by anaerobic microorganisms (mostly lactic acid producing bacteria and acetic acid producing bacteria) [33,34]. The decrease of AA, BA, PA, and OA in TSb silage may be related to high lactic acid fermentation in TSb silage. Preserved silage is desired to have a maximum of 2% AA, which provides the smell of vinegar in the material, for an antimicrobial effect [20]. The high BA content (>0.1%) in silage shows that there is no ideal lactic acid fermentation and that reach butyric acid fermentation by Clostridia microorganisms. In the butyric acid fermentation, LA converts to BA and carbon dioxide, resulting in a high pH value [20,33]. In the present study, the BA concentration of TS was higher than that of TSb can be in relation with increasing lactic acid fermentation and increasing soluble/digestible carbohydrate content by barley supplementation.

4.3. The in vitro gas production and estimated digestion parameters

In the present study, the in vitro cumulative gas production, ME, OMd, and SCFA values of tomato herbage silages with additive (TSb and TSm) were higher than those of TS. This may be optimum (close to optimum) silage pH (especially with barley) or provided by ideal silage fermentation. In addition, the NSC content in TSm and TSb silages were also higher than that of TS and can be related with the increase of in vitro digestion values and in vitro gas production. The in vitro gas production of TSm at 24 h of incubation was higher than those of other silage and can be connected with high NSC content and low ADFom and ADL contents of TSm. Although the easy-digestible fibers (hemicellulose) in NDF can be digested quickly, the cellulose in ADF can be digested slowly and lignin cannot be digested. The increase of soluble/digestible carbohydrate content in feed positively affected in vitro gas production and the two parameters were correlated positively as stated in previous studies [17,18,32].

In the present study, the use of TS up to 100% instead of corn silage in TMR did not change the NSC content (+ 0.39%), but decreased the aNDFom content by about 7%. The use of TS at 25% instead of corn silage in TMR did not change gas production in the first 24 h. However, the use of TS >25% instead of corn silage in TMR reduced linearly gas production at 24 h. The use of TS up to 100% instead of corn silage in TMR increased the NSC content by approximately 2%, which had a positive effect on in vitro gas production potential, but decreased the aNDFom, which had a negative effect on in vitro gas production potential. It did not negatively affect the in vitro gas production and estimated digestion parameters. This result can be related with silage ideal pH value and silage nutrient composition. The digestion values of the use of TSb up to 75% instead of corn silage in TMR was

similar to those of TMR with 100% corn silage and may be connected with the aforementioned carbohydrate and other nutrient compositions of TSm. Both TS and TSb can be used at 25% instead of corn silage in TMR.

In conclusion, waste-herbage parts of the tomato plant, which is mostly cultivated in the Mediterranean and Aegean Regions of Turkey, has the following potentials as conservative forage: I. The ensiling of tomato herbage with barley can increase lactic acid content and decrease levels of acetic, butyric, and propionic acids in silage. II. Tomato herbage silage can be prepared with 5% sugar beet molasses or barley grain. III. The silage gases produced in the ensiling of tomato herbage can be reduced with 5% sugar beet molasses. IV. The ensiling of tomato herbage with barley grain or molasses positively changed pH, NSC, and DM of the silages and reduced their organic matter loss. V. The use of tomato herbages silage with barley grain or molasses in dairy cattle rations did not affect negatively NSC and fiber contents of ration and digestion parameters. VI. In addition, tomato herbage or tomato herbage silage may be used as a nitrogen source in ruminant nutrition.

Acknowledgement
This study was based on the Master Thesis by Mahmut TEKİN and was supported by project No. TYL-2015-6310, granted by the Research Fund of Erciyes University, Kayseri (Turkey).

Conflict of interest
The authors declare that they have no conflict of interest.

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