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Short communication

Whole tomato plant in ruminant nutrition: effects on *in vitro* gas production, fermentation parameters and nutrient digestibility

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Abstract The aim of the present study was to determine the chemical composition and nutritive value of whole tomato plant (WTP) as well as the effects of different inclusion levels on diet digestibility and *in vitro* rumen fermentation. The chemical composition and nutritive value of WTP were initially compared with wheat straw (WS) and alfalfa hay (AH). Thereafter, the effect of substituting incremental levels of WTP (0, 5, 10, 15 and 20%, dry matter (DM) basis) for the dietary forage component on *in vitro* fermentation parameters was assessed. Crude protein (CP) content in WTP was higher than in WS, but a comparable to AH. Contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in WTP were lower but lignin was higher than other experimental feeds. The higher ash content of WTP was due to its lower organic matter (OM) content compared to WS and AH. Compared to WS, WTP yielded greater gas production (GP) at 16, 24 and 48 h of incubation, total GP, potential GP (b), *in vitro* DM (IVDMD) and OM (IVOMD) digestibility, estimated metabolizable energy (ME), microbial protein production (MP) and ammonia-nitrogen (NH_3-N) concentration. However, all of these parameters were lower in WTP than AH (P<0.05). Short-chain fatty acid (SCFA) concentration in WTP treatment was reduced compared to AH but was similar to the WS treatment (P>0.05). The highest and lowest GP at different incubation time, b, IVDMD, IVOMD, estimated ME, SCFA and MP production were observed in the diet supplemented with 10 and 20% WTP respectively (P<0.05). However, rate of GP (c), pH and ammonia-N were similar among WTP-supplemented diets (P>0.05). Overall, results of the present study indicated that WTP improved *in vitro* gas production, fermentation parameters and nutrient digestibility compared to WS while some of its parameters were comparable to AH.

Keywords: chemical composition, fermentation, *in vitro*, nutrition, whole tomato plant

Introduction

Animal feed deficiency in many developing countries is often exacerbated by dry climatic conditions. Consequently, the judicious use of agro-industrial by-products can often decrease feed costs and improve profitability (Molina-Alcaide et al., 2010; Salem, 2010) while reducing environmental pollution risks (Vasta et al., 2008).

Tomato crop by-products could represent a potential alternative feed source. The tomato plant (*Lycopersicum esculentum*) which belongs to the *Solanaceae* family (Friedman, 2002) yields two by-products- tomato pomace and whole tomato plants (WTP). Tomato pomace is a mixture of tomato peel, crushed seeds and pulp residues which remain after processing for juice, paste and/or ketchup (Ventura et al., 2009). Tomato pomace has been evaluated for use in different ruminant rations (Gasa et al., 1989; Fondevila et al., 1994; Denek and Can, 2006; Ben Salem and Znaidi, 2008).

Whole tomato plant (WTP) is another tomato by-product which contains tomato leaves and stems. After harvesting the tomato fruit, WTP are largely unused and few studies have been carried out to evaluate their nutritive value for ruminants. In Iran, cultivated vegetables account for approximately 530,000 hectares of which 28% relates to tomato cultivation (Khodaverdi et al., 2014). Whole tomato fodder production exceeds 900,000 t/year on a dry matter (DM) basis (Khodaverdi et al., 2014). In terms of chemical composition, WTP is comparable to other feedstuffs such as alfalfa hay (AH) and straws. In the study of Khodaverdi et al. (2014), dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ash contents in WTP were 20.1, 15.1, 39.9, 28.7 and 22.9%, respectively. Other studies with WTP have shown an *in vivo* CP digestibility of 64.8% (Fonolla et al., 1988), and up to 15% increase in DM digestibility when supplemented with molasses (Kamali, 2009).

The objective of this experiment was to determine the chemical composition, *in vitro* gas production (GP), fermentation parameters and nutrient digestibility of WTP compared to AH and wheat straw (WS). In addition, the effects of inclusion levels of WTP on diet digestibility and *in vitro* rumen fermentation were determined.

Materials and methods

Collection of whole tomato plant and experimental diets

This study was conducted in May 2018 at Lorestan University (Khorramabad, Iran). After harvesting the tomato fruit, four WTP samples were obtained from a nearby farm (Fajre Safa Company, Khorramabad, Lorestan) and transported to the laboratory. Initially, the chemical composition and nutritive value of WTP samples were measured using the *in vitro* GP technique and compared to WS and AH. Based on these results, WTP was then substituted the dietary forage component at incremental levels of 0 (control), 5, 10, 15 and 20% (DM basis; Table 1).

Collecting ruminal fluid

Ruminal fluid was obtained from two male Lori sheep (live weight 45 ± 0.3 kg) fitted with permanent rumen cannulae. Sheep were housed at the Agricultural Campus of the Faculty of Agriculture, Lorestan University and handled in strict accordance with good animal practice. All experimental procedures were conducted according to The Care and Use of Agricultural Animals in Research and Teaching guidelines (FASS, 2010). Sheep were fed a total mixed ration (TMR) consisting of WS, 400; AH, 100; corn silage, 100; corn grain, 270; wheat bran, 110; urea, 9; calcium carbonate, 5.5; salt 2.5 and mineral/vitamin premix, 2.5 (g/kg DM). The TMR was formulated to meet all animal nutrient requirements (NRC, 2007). Sheep were fed twice daily (7am and 5pm) in equal amounts and had continuous access to fresh drinking water. After a 2-week adaptation period to the diet, ruminal contents were collected from all quadrants of the rumen of each sheep 3 h after the morning feed, and placed in a pre-warmed (39°C) thermal container (Azizi et al., 2018). Ruminal contents were transported to the laboratory, individually homogenized and strained through 3 layers of linen cloth under O_2-free CO_2 (Azizi et al., 2018). The interval between collection of ruminal contents and incubation never exceeded 30 min.

In vitro sample incubation in the ruminal fluid

A total of 99 bottles [(3 treatments × 10 replicates + 3 blanks = 33) in 3 separate runs (33 × 3= 99)] were incubated; 36 bottles for *in vitro* GP and a further 54 for fermentation parameter estimation as well as 9 blanks bottles. Kinetics of IVGP were measured for 96 h as described by Marten and Barnes (1980). Samples (250 mg on a DM basis and particle size 1 mm) were accurately weighed into 100 mL-serum bottles. Each bottle was filled with 5 mL strained rumen fluid and 20 mL

Table 1. Feed ingredients and chemical composition (% of DM) of the experimental diets containing different levels of whole tomato plant (WTP)

	Level of WTP in the diet (%DM)				
	Control	5	10	15	20
Ingredients					
Alfalfa hay	15	13	11	9.0	5.0
Wheat straw	10	7.0	4.0	1.0	0
Whole tomato plant (WTP)	0	5.0	10	15	20
Corn, ground	14	14	14	14	14
Barley, ground	35	35	35	36	36
Soybean meal	8.0	8.0	8.0	7.0	7.0
Wheat bran	15	15	15	15	15
Vitamin-mineral premix[1]	1.0	1.0	1.0	1.0	1.0
White salt	0.6	0.6	0.6	0.6	0.6
$NaHCO_3$	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	0.4	0.4	0.4	0.4	0.4
Chemical composition					
Dry matter	92.1	88.6	85.1	81.6	78.2
Organic matter	92.0	91.4	90.8	90.1	89.6
Crude protein	13.7	13.8	14	13.9	14.0
Neutral detergent fiber	33.7	32.7	31.6	30.6	30.1
Acid detergent fiber	16.2	15.3	14.5	13.6	13.0
Lignin	3.31	3.52	3.73	4.04	4.23
Metabolizable energy (Mcal/kg DM)	2.54	2.55	2.56	2.56	2.56

[1]Contained (per kg): 99.2 mg Mn, 50 mg Fe, 84.7 mg Zn, 1 mg Cu, 1 mg I, 0.2 mg Se2, 9000 IU vitamin A, 2000 IU vitamin D and 18 IU vitamin E (Roshd-Daneh, Karaj, Iran).

buffer solution, and immediately flushed with O_2-free CO_2 before being closed with a butyl rubber stopper, sealed with aluminum crimp, shaken and placed in a water-bath at 39ºC. The buffer (Marten and Barnes, 1980) contained 1 L of solution A [(per liter): 10.0 g KH_2PO_4, 0.5 g $Mg_2SO_4.7H_2O$, 0.5 g NaCl, and 0.1 g $CaCl_2.2H_2O$] and 20 mL of solution B [(per 100 mL): 15.0 g Na_2CO_3, and 1.0 g $Na_2S.9H_2O$] and the pH was adjusted to 6.8 by gradually adding solution B to solution A. The gas volume produced was recorded at 3, 6, 8, 12, 16, 24, 48, 72 and 96 h using a digital pressure transducer (Tracker 200, Baley and Mackey, Ltd., Birmingham, UK). Based on the linear equation obtained from the calibration curve, gas pressure of different points was converted to its corresponding volume (Theodorou et al., 1994).

Fermentation parameters were determined after 24 h of incubation. Bottles were placed in an ice-bath to stop fermentation and gradually warmed up to 25ºC. The GP volume was recorded and pH determined (GLP22+ pH meter; Crison Instruments SA, Spain). From each bottle, a sub-sample of supernatant (5 mL) was immediately preserved with 5 mL of HCl 0.1 N and stored at -20ºC for ammonia-nitrogen (NH_3-N) analysis. Concentration of NH_3-N was determined as described by Broderick and Kang (1980). Fermentation residues were oven-dried at 60ºC for 48 h to estimate the DM disappearance (DMD).

Calculations

Kinetic parameters throughout the *in vitro* fermentation period were estimated using the exponential equation of Ørskov and McDonald (1979) as:

$$Y = a + b(1-e^{-ct})$$

where, *Y* is the volume of gas produced at time t; *a* is the gas produced from soluble fractions (mL); *b* is the asymptotic gas production (mL); *c* is the rate of gas production (/h) and *t* is the incubation time (h).
Metabolizable energy (ME, MJ/kg DM) and *in vitro* organic matter (OM) disappearance (IVOMD) were estimated according to Menke et al. (1979) as:

ME = 2.20+ 0.136 GP + 0.0057 CP (g/kg DM) IVOMD = 148.8 + 8.89 GP + 0.45 CP (g/kg DM) + 0.65 ash (g/kg DM)

where, GP is net gas production in mL from 200 mg dry sample after 24 h of incubation.
Concentration of short-chain fatty acids (SCFA) was calculated according to Getachew et al. (2002) as:

SCFA (mmol/g DM) = 0.0222 GP – 0.00425

where, GP is the 24 h net gas production (mL/g DM). Microbial production (MP) was calculated according to Blümmel et al. (1997) as:

MP (mg/g DM) = mg ADS − (mL gas × 2.2 mg/mL)

where, 2.2 mg/mL is a stoichiometric factor which expresses mg of C, H and O required for the SCFA gas associated with production of one mL of gas.
Using to the *in vitro* incubation data from the first part of the experiment, we substituted the forage component of the diet with WTP at incremental levels of 0 (control), 5, 10, 15 and 20% (DM basis). All *in vitro* incubation procedures used at this stage (stage 2) were similar to stage 1, except for the number of bottles [total of 159 bottles (5 treatments × 10 replicates + 3 blanks = 53) in 3 separate runs (53 × 3= 159)]. The feed ingredients used and chemical composition of the diets containing WTP are presented in Table 1.

Chemical analyses

Samples of WTP, AH, WS and diets containing different levels of WTP were analyzed for DM (method 930.15), ash (method 924.05) and N (method 954.01) and measured using standard methods as described in AOAC (1995). The NDF (inclusive of residual ash) was measured without the use of sodium sulfite or amylase (Van Soest et al., 1991). Acid detergent fiber (ADF, method 973.18) was determined and expressed inclusive of residual ash (AOAC, 1995). Acid detergent lignin (ADL) was determined using the sulfuric acid method and expressed inclusive of residual ash (Robertson and Van Soest, 1981). Non-fiber carbohydrate (NFC) was calculated according to Hall (Hall, 2000):

NFC (g/kg DM) = 1 – [NDF (g/kg DM) + CP (g/kg DM) + EE (g/kg DM) + ash (g/kg DM)]

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (2001) according to the model:

$Y_{ijk} = \mu + T_i + R_j + e_{ijk}$

where, Y_{ijk} is the measured value, μ the general mean, T_i the effect of treatment on measured parameters, R_j the random effect of run and e_{ijk} the residual error. Means were compared by the Duncan's multiple range test. Significance was declared at (P<0.05).

Results

Chemical composition

WTP contained CP levels that were higher than WS and comparable to those in AH (see Table 2). NDF and ADF contents in WTP were also lower but ADL was higher than WS and AH. The higher ash content in WTP resulted in a lower OM content compared to WS and AH.

In vitro gas production and fermentation parameters

Compared to WS, WTP yielded greater gas production (GP) after 16, 24 and 48 h of incubation as well as total GP and GP potential (b) but all of its GP parameters were lower than AH (P<0.05; Table 3).

In addition, WTP fermentation parameters (Table 3), IVDMD, IVOMD, estimated ME, MP and NH_3-N concentration after 24 h incubation were higher and lower than WS and AH, respectively (P<0.05). However, pH values were similar among all feeds (P>0.05). Concentration of SCFA in WTP was reduced compared to AH while being similar to WS.

Table 4 summarizes the effects of different levels of dietary WTP on GP parameters. The highest and lowest GP volumes after 16, 24, 48, 72 and 96 (total GP) h of

Table 2. Chemical composition (% of DM) of the dietary ingredients (n = 4)

Composition	Experimental feed		
	WTP	Alfalfa hay	Wheat straw
Dry matter	23.1	93.6	94.6
Organic matter	78.1	90.2	90.4
Crude protein	13.2	14.6	3.22
Neutral detergent fiber	38.1	40.8	71.7
Acid detergent fiber	26.1	33.4	46.3
Non-fiber carbohydrates	23.5	31.2	10.1
Lignin	12.2	7.86	8.65
Ash	21.4	9.80	9.61

WTP, whole tomato plant.

Table 3. *In vitro* gas production and fermentation parameters of the dietary ingredients

Parameters	Feed ingredients			SEM	P-value
	WTP	Alfalfa	Wheat straw		
GP parameters					
GP_{16}	30.9[b]	52.8[a]	24.1[c]	1.71	<0.01
GP_{24}	41.6[b]	60.9[a]	34.3[c]	1.005	<0.01
GP_{48}	51.1[b]	73.5[a]	44.4[c]	1.75	<0.01
GP_{72}	53.3[b]	74.9[a]	48.5[b]	2.03	<0.01
TGP	56.9[b]	75.3[a]	48.9[c]	2.01	<0.01
b	59.5[b]	75.5[a]	50.2[c]	2.11	<0.01
c	0.06[ab]	0.075[a]	0.044[b]	0.005	0.02
Fermentation parameters					
IVDMD	55.7[b]	69.8[a]	45.4[c]	2.22	<0.01
IVOMD	54.9[b]	68.6[a]	44.7[c]	2.15	<0.01
ME	7.96[b]	10.6[a]	6.91[c]	0.24	<0.01
SCFA	4.55[b]	6.73[a]	3.88[b]	0.35	<0.01
MP	465[b]	564[a]	379[c]	11.4	<0.01
pH	6.30	6.23	6.34	0.038	0.21
Ammonia-N (mg/dL)	17.6 [a]	18.5[a]	11.4[b]	0.95	<0.01

WTP, whole tomato plant; GP_{16}, gas production after 16 h incubation; GP_{24}, gas production after 24 h incubation; GP_{48}, gas production after 48 h incubation; GP_{72}, gas production after 72 h incubation; TGP, volume of gas production (GP) after 96 h of incubation (mL/250 mg sample); b, GP from the fermentable fraction (mL); c, rate constant of GP (mL/h); IVDMD, *in vitro* dry matter (DM) disappearance (%); IVOMD, *in vitro* organic matter disappearance (%); ME, estimated metabolizable energy (MJ/kg DM); SCFA, short chain fatty acid (mmol/g DM); MP, microbial protein (mg/g DM); SEM, standard error of the mean.

[a,b] Within rows, means with common letter(s) are not different (P>0.05).

incubation and b were observed in the diet supplemented with 10 and 20% WTP, respectively (P < 0.05). However, GP rate (c) was similar among WTP-supplemented diets.

Dietary supplementation with WTP up to 10% increased IVDMD, IVOMD, estimated ME, SCFA and MP production compared to the 20% level (P<0.05). However, adding WTP to the diet did not change the pH level or ammonia-N concentration.

Discussion

Results from chemical composition and nutritive value assessments (i.e. chemical composition, *in vitro* GP and fermentation parameters) showed that WTP does have the potential to be used as an alternative ruminant feedstuff. In particular, it has a higher nutritive value than WS due to its higher CP and NFC and lower NDF and ADF contents. However, in most parameters [except for CP and fiber contents and rate of GP (c coefficient)], WTP had a lower nutritive value than did AH. Also, its lower DM and OM contents compared to the other experimental feeds were mainly due to its higher moisture and ash contents, respectively (Ventura et al., 2009). These results agree with those of Khodaverdi et al. (2014) who reported respective DM, CP, NDF, ADF and ash contents for greenhouse WTP of 20.1, 15, 39.9, 28 and 22.9%, and field WTP of 26.3, 14, 40.2, 28.3 and 19.7%. Another study (Abbasi et al., 2015) reported DM, CP and ash contents for WTP of 18.2, 13.6 and 21.7%, respectively which are also consistent with our results. However, unlike our findings, Ventura et al. (2009) recorded DM, CP, NDF, ADF and ash contents for WTP of 17.7, 7.40, 45.7, 35.6 and 18.1%, respectively. Differences in chemical composition among feed ingredients may relate to the inherent characteristics of each feedstuff and its ability to accumulate nutrients from the soil, the atmosphere (N), and to other factors such as planting location, plant component, plant age and season (Salem, 2012).

Gas production deviations reflect differences in chemical composition between feed ingredients and have been used for prediction of their nutritive value. Additionally, it reflects SCFA and MP production (Getachew et al., 1998). Gas is produced when carbohydrates and proteins are fermented to acetate, butyrate and ammonia (Getachew et al., 1998). Gas production and fermentation parameters of AH were higher than other treatments. Increased GP and fermentation parameters of WTP compared to WS were probably due to differences in their chemical composition. The higher non-fiber carbohydrate (NFC) content in WTP may explain its increased GP compared to WS. The higher

Table 4. *In vitro* gas production and fermentation parameters of the experimental diets containing different levels of whole tomato plant (WTP)

Parameters	Level of WTP in the diet (%DM)					SEM	P-value
	0	5	10	15	20		
GP parameters							
GP_{16}	52.6[a]	50.6[a]	51.1[a]	40.6[ab]	33.1[b]	3.68	0.01
GP_{24}	63.1[a]	62.5[a]	61.3[a]	49.7[b]	42.4[b]	3.36	<0.01
GP_{48}	66.3[a]	66.6[a]	72.1[a]	54.1[b]	47.4[b]	3.05	<0.01
GP_{72}	68.1[a]	67.9[a]	73.9[a]	56.5[b]	50.1[b]	3.05	<0.01
TGP	68.9[b]	68.2[b]	76.4[a]	57.5[c]	51.1[c]	2.01	<0.01
b	69.1[a]	70.1[a]	77.2[a]	56.4[b]	50.6[b]	2.83	<0.01
c	0.118	0.109	0.088	0.103	0.082	0.014	0.44
Fermentation parameters							
IVDMD	62.5[a]	62.2[a]	62.4[a]	55.8[b]	52.5[b]	1.85	<0.01
IVOMD	61.8[a]	61.1[a]	61.5[a]	54.5[b]	50.6[b]	1.81	<0.01
ME	9.31[a]	9.22[a]	9.24[a]	8.24[b]	7.74[b]	0.18	<0.01
SCFA	5.81[a]	5.59[a]	5.65[a]	4.48[b]	3.65[c]	0.19	<0.01
MP	509[a]	511[a]	512[a]	468[b]	452[b]	9.58	<0.01
pH	6.01	6.06	6.07	6.07	6.09	0.054	0.18
Ammonia-N (mg/dL)	18.3	17.6	18.1	17.3	18.7	1.32	0.81

GP_{16}, gas production after 16 h incubation; GP_{24}, gas production after 24 h incubation; GP_{48}, gas production after 48 h incubation; GP_{72}, gas production after 72 h incubation; TGP, volume of gas production (GP) after 96 h of incubation (mL/250 mg sample); b, GP from the fermentable fraction (mL); c, rate constant of GP (mL/h); IVDMD, *in vitro* dry matter (DM) disappearance (%); IVOMD, *in vitro* organic matter disappearance (%); ME, estimated metabolisable energy (MJ/kg DM); SCFA, short chain fatty acid (mmol/g DM); MP, microbial protein (mg/g DM); SEM, standard error of the mean.

[a,b] Within rows, means with common letter(s) are not different (P>0.05).

IVDMD, IVOMD and SCFA production of WTP compared to WS were due to its greater fermentability as indicated by its higher GP volume. It has been demonstrated that increased asymptotic GP results in greater DMD, as gas is produced when carbohydrates are fermented to acetate and butyrate (Getachew et al., 1998). Greater GP, improved fermentation parameters and nutrient digestibility of AH compared to other experimental feeds (i.e. WTP and WS) were due to its higher OM, NFC and CP content. Increased NH_3-N concentration with incubation of WTP or AH compared to WS most likely reflects their higher CP contents, which are degraded by rumen proteolytic microbes. Indeed, it has been reported that both protein structure and level in the diet are key factors in determining its susceptibility to rumen microbial proteases and thus, its degradability (Bach et al., 2005). Ventura et al. (2009) also reported similar IVOMD (579 g kg^{-1}) and ME (7.96 MJ/kg^{-1} DM) contents in WTP. The digestible energy content of complete tomato plants have been estimated at 6.9–9.2 MJ/kg (INRA, 1988).

Increasing the level of WTP in the diet increased the ash and consequently decreased the OM contents (Table 1). The potential effects of using WTP in complete diets up to 10% (DM basis) on *in vitro* GP and fermentation parameters is shown in Table 4. The findings suggested that at low inclusion levels, this by-product has a potential value to be used in complete diets for small ruminants, and decrease the feed costs. Decreased GP and fermentation parameters at higher WTP levels (i.e. 15 and 20%) in the diet were probably due to the higher ash content, which decreased the dietary ME content. This is in line with the findings of Ventura et al. (2009) who concluded that tomato by-products can be fed to adult goats at up to 100 g/kg DM without any negative effects on digestive parameters. Similarly, Fondevila et al. (1994) showed that supplementing tomato pomace at 200 g/kg diet DM promoted similar nitrogen retention and growth performance as soybean protein when fed to growing lambs on a basal barley diet. Furthermore, no differences in dietary DM or OM intake were observed when incremental tomato pomace levels were fed to these lambs. The WTP may also have potential applications as a component of other products such as feed blocks (Ben Salem and Znaidi, 2008).

Conclusions

Results from the present study indicated that WTP yielded an increased *in vitro* GP, and improved the fermentation parameters and nutrient digestibility compared to WS while its CP content was comparable to AH. Data on animal performance are required to validate the benefits of utilizing WTP in the ruminant diets, and whether the alterations in GP observed are accompanied by changes in gas composition, with respect to reducing the enteric methane production.

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