

Effect of Species on Nutritive Value and Methane Production of Citrus Pulp for Ruminants

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Abstract

The experiment was carried out to determine the effect of species on the nutritive value and methane production of citrus pulps. Species had a significant effects ($P<0.001$) on the chemical composition, gas production, methane production, metabolisable energy (ME) and organic matter digestibility (OMD) of citrus pulps. Crude protein (CP) content of *Citrus paradise* was significantly ($P<0.001$) higher than those of the other citrus species. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of *Citrus sinensis* and *Citrus reticulata* were significantly ($P<0.001$) lower than the others. Methane production from *Citrus reticulata* was significantly ($P<0.001$) higher than those of *Citrus limon* and *Citrus paradise*. The ME of pulp from *Citrus reticulata* was significantly ($P<0.001$) higher than that of *Citrus limon* pulp whereas OMD of pulp from *Citrus reticulata* was significantly ($P<0.001$) higher than those of pulps from *Citrus limon* and *Citrus paradise*. As conclusion, citrus pulps from different species have a high potential as forage for ruminant animals since citrus pulps have high ME and OMD. However, the CP contents of citrus pulps should be taken into consideration and diets should be fortified with additional CP sources to compensate for the low crude protein when citrus pulps are included into ruminant diets.

Keywords: Citrus pulp, nutritive value, metabolisable energy, organic matter digestibility, methane production

Türün Narenciye Posalarının Besin Değerine ve Metan Üretimine Etkisi

Öz

Bu çalışma, türün narenciye posalarının besin değerine ve metan üretimine etkisini belirlemek için yürütülmüştür. Türün narenciye posalarının kompozisyonu, gaz üretimi, metan üretimi, metabolik enerji içeriği ve organik madde sindirim derecesi üzerinde önemli derecede etkisi vardır. Greyfurt posasının ham protein içeriği diğer narenciye posalarının ham protein içeriğinden önemli derecede yüksektir. Portakal ve mandalina posalarının NDF ve ADF içerikleri diğer posalardan daha düşük bulunmuştur. Mandalina posasının metan üretimi limon ve greyfurt posasının metan üretiminden daha yüksek bulunmuştur. Mandalina posasının metabolik enerji içeriği limon posasının metabolik enerji içeriğinden daha yüksek olmasına rağmen mandalina posasının organik madde sindirim derecesi limon ve greyfurt posalarının sindirim derecelerinden daha yüksek bulunmuştur. Sonuç olarak, yüksek enerji ve organik madde sindirim derecesine sahip farklı türlerden elde edilen narenciye posaları kaba yem olarak ruminant hayvanların beslenmesinde önemli bir potansiyele sahiptir. Bununla birlikte narenciye posaları ruminant rasyonlarına katılırken ham protein içeriklerinin göz önüne alınması ve düşük protein içeriğinin telafi edilmesi için rasyonun protein kaynakları ile zenginleştirilmesi gereklidir.

Anahtar Kelimeler: Narenciye posası, besin değeri, metabolik enerji, organik madde sindirim derecesi, metan üretimi

Introduction

It is well known that feed processing produces considerable amounts of byproducts which are widely used to meet the livestock requirements. The success of integration of byproducts in livestock diets depends on the available information about the chemical composition and nutritive value of byproducts. In Turkey, total citrus fruit

production averaged 3 480 505 tonnes/year from 2008 through 2013 (TUIK, 2014). The citrus fruit such as *Citrus sinensis*, *Citrus limon*, *Citrus paradise* and *Citrus reticulata* are consumed by humans as fresh fruit and processed juices. Although the considerable amounts of citrus pulp becomes available after extraction of juice, the exact amount of pulps from citrus produced in Turkey is

not known. Citrus pulps consists of peels, inside portions, seeds and culled fruit which account for approximately 50-65 % of whole fruit, depending on variety of fruit, the processing methods and environmental factors (Ashbell and Donehaye, 1984; Grasser et al., 1995). However fruit from *Citrus sinensis* accounts for %48.38 % of annual citrus production (TUIK, 2014). About 2-4 % of fruit from *Citrus sinensis* is processed into juice (Sancak and Aygoren, 2012). Recently there are considerable amount of citrus pulps productions in Turkey, however the information about nutritive value of pulps from different species is limited.

Due to quick, cheap, less time consuming, the in vitro gas production technique with chemical composition has been used to determine the metabolisable energy and organic matter digestibility of previously uninvestigated feedstuffs (Canbolat, 2012; Guven, 2012; Kamalak et al., 2011; Maheri-Sis et al., 2011; Kaplan et al., 2014; Wencelova et al., 2014). In addition, in vitro gas production technique has been also used to determine the methane reduction potential of feedstuffs (Jayanegara et al., 2009, Jayanegara et al., 2011, Lin et al., 2013, Thang et al., 2012) since enteric methane produced during fermentation is the major contributors to global warming (Lassey, 2007). Recently there are considerable amount of citrus pulps productions and used in ruminant diets to some extent in Turkey, the information about chemical composition and nutritive value of pulps from different citrus species is limited. Therefore the current experiment was carried out to determine the effect of species on the nutritive value and methane production of citrus pulps.

Materials and Methods

Citrus pulp sample

The experiment was carried out in University of Kahramanmaraş Sutcu Imam, Faculty of Agriculture, and Department of Animal Science. The experimental protocols were approved by the Animal Experimentation Ethics Committee of University of Kahramanmaraş Sutcu Imam, Faculty of Agriculture (Protocol No: 2013/ 09-5). Pulps from four different citrus species namely, *Citrus sinensis*, *Citrus limon*, *Citrus paradise* and *Citrus reticulata* were obtained by using a manual citrus juicer. Pulps samples were dried at 50 °C and milled in a hammer mill through a 1 mm sieve for subsequent analysis.

Chemical analysis

Dry matter (DM) content of citrus pulps was determined by oven drying at 105°C 24 hours. Ash content was analyzed by igniting the samples of citrus pulps in muffle furnace at 525°C for 8 h. Nitrogen (N) content of citrus pulps was determined by the Kjeldahl method (AOAC, 1990). Crude protein of citrus pulps was estimated as N X 6.25. Neutral detergent fiber (NDF) of citrus pulps was analyzed by the method of Van Soest and Wine (1967) and ADF content of citrus pulps were analyzed by the method of Van Soest (1963). All chemical analyses were carried out in triplicate.

Determination of gas and methane production

Citrus pulps milled through a 1 mm sieve were incubated in vitro rumen fluid in 100 ml calibrated glass syringes according to the method described by Menke et al. (1979). Rumen fluid was collected from three fistulated Awassi sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%) with a free access to water and mineral block. Before mixing the rumen fluid with buffered solution, rumen fluid was filtered through four layers of cheesecloth under flushing with CO₂ and combined with buffered solution in the ratio of 1:2, respectively. Approximately 0.200 gram citrus pulp samples were incubated with 30 mL rumen fluid-buffer mixture in calibrated glass syringes in a water bath at 39°C. Gas production was determined at 24 h after incubation and corrected for blank and hay standard (University of Hohenheim, Germany).

ME (MJ/kg DM) content and organic matter digestibility of citrus pulp samples was estimated by the equation described by Menke et al. (1979) as follows:

$$ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP + 0.002859 EE$$

$$OMD (\%) = 14.88 + 0.889GP + 0.45CP + 0.651 XA$$

Where GP = 24 h net gas production (ml/200 mg);

CP = Crude protein (%), EE: Ether extract (%), XA: ash content (%).

Methane contents (%) of total gas produced at 24 h fermentation of citrus pulps were measured using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel et al., 2008). After measuring gas produced at 24 h incubation, gas samples was transferred into inlet of the infrared methane analyzer using the plastics syringe. The infrared methane analyzer displays methane as percent of total gas. Methane production (mL) was calculated as follows.

$$\text{Methane production (mL)} = \text{Total gas production (mL)} \times \text{Percentage of Methane (\%)}$$

Statistical analysis

One-way analysis of variance (ANOVA) was carried out to determine the effect of species on chemical composition metabolisable energy (ME), organic matter digestibility (OMD) and methane production of citrus pulps. Significance between individual means was identified using the Duncan multiple range test. Mean differences were considered significant at $P < 0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

Results

The effect of species on the chemical composition on the citrus pulp is given in Table 1. Dry matter content, CP and ash content ranged from 19.24 to 23.90 %, 6.29 to 8.51% and 4.43 to 6.87%, respectively. On the other hand NDF, ADF and EE contents ranged from 17.05 to 23.91%, 14.59 to 20.57% and 3.51 to 11.44%, respectively. Crude protein of *Citrus paradise* was significantly ($P < 0.001$) higher than those of the other citrus species, whereas the ash content of *Citrus reticulata* was significantly ($P < 0.001$) higher than those of the other citrus species. NDF and ADF contents of *Citrus sinensis* and *Citrus reticulata* were significantly

($P < 0.001$) lower than those of *Citrus limon* and *Citrus paradise*. On the other hand, EE content of *Citrus limon* was significantly ($P < 0.001$) higher than those of *Citrus sinensis*, *Citrus paradisi* and *Citrus reticulata*.

The effect of species on the gas production, methane production, ME and OMD of citrus pulps were given in Table 2. The gas and methane production of citrus pulps at 24 h incubation ranged from 68.70 to 77.64 ml and 10.15 to 13.62 ml, respectively. The gas production of pulp from *Citrus reticulata* was significantly higher than that of *Citrus limon*, whereas methane production pulp from *Citrus reticulata*, was significantly ($P < 0.001$) higher than those of *Citrus limon* and *Citrus paradise*. On the other hand, the percentages of methane from *Citrus reticulata* and *Citrus sinensis* were significantly ($P < 0.001$) higher than those of *Citrus limon* and *Citrus paradise*. The ME and OMD of citrus pulps ranged from 12.02 to 13.16 MJ/kg DM and 82.72 to 91.53%, respectively. The ME of pulp from *Citrus reticulata* was significantly ($P < 0.001$) higher than that pulp from *Citrus limon*, whereas OMD of pulp from *Citrus reticulata* was significantly higher than those of pulps from *Citrus limon* and *Citrus paradise*.

Table 1. The effect of species on the chemical composition of citrus pulp

Nutrients (%)	Species				SEM	Significance
	<i>C.sinensis</i>	<i>C.limon</i>	<i>C.paradisi</i>	<i>C.reticulata</i>		
DM	23.90 ^a	19.24 ^b	21.50 ^{ab}	21.12 ^{ab}	1.402	***
CP	6.29 ^d	7.86 ^b	8.51 ^a	6.99 ^c	0.085	***
CA	4.43 ^b	4.96 ^b	4.80 ^b	6.87 ^a	0.336	***
NDF	17.05 ^b	21.97 ^a	23.91 ^a	17.28 ^b	0.765	***
ADF	15.86 ^c	17.60 ^b	20.57 ^a	14.59 ^c	0.468	***
EE	3.26 ^b	11.44 ^a	4.17 ^b	3.51 ^b	0.515	***

^{a b c} Row means with common superscripts do not differ ($P > 0.05$); SEM: standard error mean; DM : Dry matter CP : Crude protein, CA: Crude ash, NDF :Neutral detergent fiber, ADF : Acid detergent fiber, EE: Ether extract, *** $P < 0.001$

Table 2. The effect of species on the gas production, methane production, metabolisable energy and organic matter digestibility of citrus pulp

Parameters	Species				SEM	Significance
	<i>C.sinensis</i>	<i>C.limon</i>	<i>C.paradisi</i>	<i>C.reticulata</i>		
TG(mL)	74.00 ^{ab}	68.70 ^b	71.79 ^{ab}	77.64 ^a	2.154	***
CH ₄ (mL)	12.55 ^{ab}	10.15 ^c	11.06 ^{bc}	13.62 ^a	0.581	***
CH ₄ (%)	16.95 ^a	15.12 ^b	15.40 ^b	17.53 ^a	0.474	***
ME	12.63 ^{ab}	12.02 ^b	12.46 ^{ab}	13.16 ^a	0.293	***
OMD	86.38 ^{ab}	82.72 ^b	85.66 ^b	91.53 ^a	1.915	***

^{abc} Row means with common superscripts do not differ ($P > 0.05$); SEM: Standard error mean;

TG: total gas production(ml), ME :Metabolisable energy MJ /Kg DM, OMD :Organic matter digestibility (%) *** $P < 0.001$.

Discussion

As represented in Table 1 the species had a significant effect on the chemical composition of citrus pulp. The CP contents of *Citrus sinensis* and *Citrus limon* were

consistent with findings of Nazem et al. (2008) who reported that CP contents of *Citrus sinensis* and *Citrus limon* were 6.3 and 6.8 %, respectively. The NDF and ADF contents of *Citrus limon* were consistent with findings of Nazem et al. (2008) who reported that NDF

and ADF contents of *Citrus limon* were 21.3 and 17.9 %, respectively. However the NDF and ADF contents of *Citrus sinensis* were considerably lower than those reported by Nazem et al. (2008) who found that the DF and ADF contents of *Citrus sinensis* were 26.1 and 20.3%, respectively. On the other hand Although NDF and ADF contents of *Citrus sinensis* and *Citrus limon* were considerably lower than those reported by Ibrahim et al. (2011), the EE contents of *Citrus sinensis* and *Citrus limon* were consistent with findings of Ibrahim et al. (2011). The differences among these studies were possibly associated with differences in variety of citrus, the processing methods and environmental factors.

As can be seen from Table 1, although it seems to be likely that CP contents of citrus pulp except for *Citrus sinensis* will meet the CP requirements of ewes for maintenance, it is unlikely that citrus pulp studied in this experiment will meet the CP requirement of ewes for lactation, since El-Shatnawi and Mohawesh (2000) reported that ewes require 7-9% CP for maintenance and 10-12% for lactation.

The species had a significant effect on the gas production, methane production, ME and OMD of citrus pulp (Table 2). The OMD values of *Citrus sinensis* and *Citrus limon* were considerably higher than those reported by Nazem et al. (2008) who reported that OMD values of *Citrus sinensis* and *Citrus limon* were 78.2 and 75.5 %, respectively.

As can be seen from Table 2, pulps from *Citrus limon* and *Citrus paradise* tends to give the lower gas production and methane production when compared with those of pulps from *Citrus sinensis* and *Citrus reticulata*. The reason why *Citrus limon* and *Citrus paradise* produced lower gas production is possibly associated with high NDF and ADF contents. As can be seen from Table 1, NDF and ADF contents of pulps from *Citrus limon* and *Citrus paradise* were higher than those of pulps from *Citrus sinensis* and *Citrus reticulata*. It is well known that there is negative correlation between gas production and NDF or ADF contents (Kamalak et al., 2005a; Kamalak et al., 2005b). In addition, essential oil in *Citrus limon* and *Citrus paradise* might have resulted in lower gas and methane production. Therefore essential oil from *Citrus limon* and *Citrus paradise* is deserved to be tested for their effect on the gas and methane production.

Lopez et al. (2010) suggested that methane percentage of total gas produced after 24 hour fermentation can be used to determine the methane reduction potential of any

feedstuffs and the feedstuffs can be classified in three groups, low potential (% methane in gas between >11% and ≤14%), moderate potential (% methane in gas between >6% and <11%), high potential (% methane in gas between >0% and <6%). Therefore it seems to be unlikely that citrus pulps studied in the current study had no methane reduction potential since the percentage of methane for citrus pulp is higher than %14.

Conclusion

As conclusion, citrus pulps from different species have a high potential as forage for ruminant animals, since citrus pulps have high ME and OMD. However, the CP contents of citrus pulps should be taken into consideration and diets should be fortified with additional crude protein sources to compensate for the low crude protein when citrus pulps are included into ruminant diets.

Kaynaklar

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