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Nutritive value and in-vitro digestibility of peels and pomaces of different citrus species

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ABSTRACT: The chemical composition and in-vitro digestibility of bitter orange (*Citrus aurantium*), Grapefruit (*Citrus paradisi*), mandarin (*Citrus reticulata)*, lemon (*Citrus limon)* and *orange (Citrus sinensis)* peels and pomaces were assessed for their ability as alternative feed. Chemical composition assessment was done through chemical analysis, and ANKOM Daisy II incubator was used to determine the in-vitro digestibility. There was no statistical significance between citrus byproducts in terms of chemical composition ($p>0.05$). The citrus by-products had high amounts of total phenolic content ($p \leq 0.01$). The in-vitro digestibility of the peels was significantly higher than the pomaces ($p<0.001$). The five citrus species in this study showed high metabolizable energy and are viable as non-forage energy sources in ruminant feed. Lemon byproducts had higher protein compared to others but not sufficient as a single protein source while bitter orange pomace had higher total phenolic content and digestibility. The abundance of citrus agro-industrial byproducts, their chemical composition and their digestibility makes it a good consideration for ruminant feed modification.

***Keywords*:** Chemical composition; Digestibility; Citrus; By-product; Peel; Pomace

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INTRODUCTION

The environmental advantage of reducing the huge waste from citrus agro-industrial byproducts, reduction in the cost of production of citrus fruits, and the capacity for degradation and utilization of the byproducts by ruminal microorganisms (Yeganehpour et al., 2021) gives them the potential to be utilized as livestock feed alternatives (Woyengo, 2016; Spranghers et al., 2016). These factors have led to studies on the utilization strategies of citrus agro-industrial byproducts for improved production of beef, dairy and cheese quality (Chiofalo et al., 2004; Polyorach and Wanapat, 2015; Liotta et al., 2019).

Citrus byproducts are considered to be cheap, highly fermentable carbohydrates in the rumen, which are rich in energy, low in crude protein having a potential as a non-conventional replacement for energy sources in ruminant feed (Lashkari and Taghizadeh 2015; Tayengwa et al., 2021; Fegeros et al., 1995). Fegeros et al (1995) and Alnaimy et al (2017) confirmed the safe inclusion of dried citrus peels and pomaces as a non-forage replacement of energy sources in rations of beef, growing cattle, and lactating ruminant with limited risks and effects on the animals. Türkiye is a top citrus producer with a likelihood of high citrus byproduct generation although available reports are limited (FAO, 2016; Uzun and Yesiloğlu, 2012). Three years (2018/2019, 2019/2020, 2020/2021) average citrus distribution in Türkiye reported by USDA/GAIN 2020 showed total distribution to utilization (fresh consumption + processed) ratio of 1.2:1 for orange, 2:1 for mandarin, 2.7:1 for lemon and 1:0.9 for grapefruit leaving excess to exports and waste generated from the agro-industries and consumption.

Although the quantities of citrus agro-industrial byproducts generated, and their utilization was not clearly reported, Özkan et al (2017) suggested that there was a sizable use of these byproducts in ruminant ration. In addition, citrus peels and pomaces are rich in phenolic compounds which have various bioactive properties which include antimicrobial, antioxidant and anti-inflammatory properties (Paula et al., 2016). Natural and plant based phenolic compounds such as in citrus byproducts is preferred to synthetic phenolic compounds as feed additives in ruminant rations due to safety concerns and general perceptions. Also, the use of phenolic compounds as feed additive in ruminant diet is common because of their potentials for ruminal ecosystem modification, improvement of rumen fermentation conditions (Kalantar, 2018; Ehsan et al., 2013) and enhancement of nutrient utilization in the rumen. (Lee et al., 2017; Paula et al., 2016).

A review of the chemical composition of five citrus byproducts used in this study (Table 1) showed that few studies had been done on bitter oranges (Gorinstein et al., 2001; Ersus and Cam, 2007; El-aal and Halaweish, 2010; Guimarães et al., 2010; Hegazy and Ibrahim, 2012; Sir Elkhatim et al., 2018; Rehman et al., 2020). Furthermore, the in vitro dry matter digestibility studies carried out by Lashkari and Tagizadeh (2013) on bitter orange peels and pomace gave IVTDM values of 87% and 84.8% respectively. Also, several studies carried on the IVTDM of lemon pom-

Table 1: Review of nutritive value for peels and pomace/pulp byproducts of bitter orange, grapefruit, mandarin, lemon and sweet orange

Citrus species	Dry matter (%)	Crude ash (%)	Crude protein (%)	Crude fibre (%)	Neutral detergent fibre (%)	Acid detergent fibre (%)
Peels						
Bitter orange	-	8.90	6.90	-	-	-
Grapefruit	87.66	3.29	5.78	10.68	-	-
Mandarin	96.21	3.96-10.03	2.16-8.55	7.14-27.89	-	-
Lemon	90.42-96.99	2.52-6.26	5.87-9.42	12.47-15.18	-	-
Orange	88.90-96.86	2.56-4.24	1.79-9.06	6.30-13.90	10	7.60-18.32
Pomace/ pulp						
Bitter orange	93.10	6.44	8.25	-	21.23	17.04
Grapefruit	87.34-90.91	4-5.87	8.01-9.14	-	16.66-20.9	13.08-17.6
Mandarin	88.72-91.02	4.8-6.78	6.64-6.99	-	11.38-22	8.48-17.9
Lemon	87.1-90.69	4.15-6.9	7.27-9.54	-	16.68-21.97	15.1-19.45
Orange	88.73-93.2	1.2-5.5	4.26-8.68	-	14.74-21.4	11.95-18.3

(Gorinstein et al., 2001; Figuerola et al., 2005; Marín et al., 2007; Magda et al., 2008; Hon et al., 2009; Bejar et al., 2011; Atta and El shenawi, 2012; Ghanem et al., 2012; Lashkari and Tagizadeh, 2013; Palangi et al., 2013; Lashkari and Tagizadeh, 2015; M'hiri et al., 2015; El-ghfar et al., 2016; Nagarajaiah and Prakash, 2016; Özkan et al., 2017; Beyzi et al., 2018; Castrica et al., 2019) ace resulted in high digestibility values that ranged from 85.76%-91.2% (Trıpodo et al., 2004, Lashkari and Tagizadeh, 2013). In addition, high IVTDM values within the range of 89.0%-90.6% were obtained for orange pomace from studies by Aregheore (2000), Tripodo et al (2004), Lashkari and Tagizadeh (2013), Alnaımy et al (2017). However, there seemed to be on a general basis limited research and available reports on the in vitro digestibility of citrus species of focus in this study. In addition, there are limited comparative studies on the chemical composition of the five citrus species used in this research although studies had been done on different citrus species.

The study assessed and compared the chemical composition and in vitro digestibility of agro-industrial byproducts of Citrus grown in Türkiye. The chemical composition assessment and in-vitro true digestibility studies in this work aimed to provide depth understanding of the difference of effects between the species and samples as well as their nutritive potentials as an alternative ruminant feed. The novelty included the identification of less consumed citrus species (bitter orange), mainly used as ornamentals in the Mediterranean as having high nutritive value and digestibility. Also, the comparisons of citrus byproducts present clarity on the difference of their nutritive value and digestibility which is important for choice and decision making on ruminant feed composition.

MATERIALS AND METHODS

Bitter orange (*Citrus aurantium*), grapefruit (*Citrus paradisi*), mandarin (*Citrus reticulata*), lemon (*Citrus limon*), and sweet orange were obtained from the commercial markets in Niğde and Adana provinces of Türkiye. Fruit washing and juice extraction were done after which 2.5 kg of each citrus fruit purchased was used for peels and the other half (2.5 kg) was prepared for pomace. The peels and pomaces were cut into pieces of 1-2 cm, weighed, and dried in the oven at 50°C for 48 h to obtain air-dry matter (AOAC, 2005). Grinding of the peels and pomace was done using Retsch ZM 200 mill with a 1 mm sieve. The samples were assessed for their chemical composition and in vitro digestibility at the Niğde Ömer Halisdemir University's animal nutrition research laboratory.

Chemical analysis

The chemical analysis carried out towards assessing the chemical composition of the samples included the determination of dry matter (DM) after oven drying at 105°C for 24 h, crude ash (XA) with the use of a muffle`s furnace at 600°C for 4 h and crude protein estimation with the use of kjelroc digestion unit and OPSIS liquid line analyzer according to AOAC, 2005. In addition, acid detergent fibre, crude fibre (CF), and neutral detergent fibre (NDF) were analyzed using the Van Soest et al (1991) method. Total phenolic content was also analyzed using the Folin-Ciocalteu's reagent method (Waterhouse, 2001). Absorbance readings were measured at 765 nm and a gallic calibration curve was determined (R^2, 0.9959). Total phenolic content was expressed as mg GAE/g. All assessments were done in replicates for the peels and pomaces of the citrus species. The ME values for the citrus peels and pomaces were calculated using equations by Dugmore (1995), ME (MJ/KgDM): 13.50+0.263%EE-0.133%XA-0.13%ADF (ME- metabolizable energy; EE- ether extract; XA-crude ash (%DM); ADF-acid detergent fibre).

Citrus digestibility

Peel and pomace samples of the five species were assessed for in vitro digestibility using the ANKOM technology, DAISY II incubator (Robinson et al 1999). Combined buffer A and B solutions were prepared and adjusted to pH of 6.8 at 39°C. Fresh rumen liquor and content of two postmortem mature Holstein cattle was used for in vitro digestibility. The animals were fed a total mixed ration containing 20 % forage and 80 % concentrate prior to slaughter. The rumen liquor was collected, blended and purged with CO_2 at 39°C. Thereafter, the rumen digesta was filtered using a four-layered cheese cloth. Subsequently, 1600 ml of combined buffer A and B solution with 400 ml of the rumen inoculum were transferred into each of the four digestion jars of the Daisy II incubator. The samples were incubated in triplicates using F57 bags with blanks included for correction factor all at 39°C±0.5 for 48 h. The bags were rinsed under tap water after incubation. Following this, NDF was determined using an ANKOM 200 fiber analyzer and the percentage of IVTDM was then calculated on a dry matter basis using the equation: IVTDM =100-(W3-(W1*C1)/W2*DM)*100, where W1= weight of the bag, W2= Weight of sample, W3= bag weight following in vitro digestion and ND treatment, C1=Blank bag correction.

Analysis of variance

A non-parametric analysis of variance, Kruskal-Wallis was done for all the chemical compositions assessed. In addition, the posthoc test was done to understand the effects of species and samples on the total phenolic content and IVTDM. All analyses were achieved using Jamovi, an R-based statistical software (The Jamovi project, 2021).

RESULTS

The chemical composition of the peels and pomaces of bitter orange, grapefruit, mandarin, lemon, and sweet orange did not significantly differ, p> 0.05 while the total phenolic content of citrus peels differed significantly from their pomaces (p<0.01). The in vitro true digestibility of the peels and pomaces was significantly different at p<0.001.

Chemical composition assessment

Table 2a showed that among the peel samples bitter orange had the highest DM while mandarin had the highest DM for pomace samples. Conversely, lemon peel and pomace had the lowest DM. In addition, the highest XA was obtained in the peel and pomace samples of bitter orange while the lowest XA was found in the peel and pomace of grapefruit. The highest CP, NDF and ADF was found in the peel and pomace samples of lemon while the bitter orange peels and pomaces had the lowest CP. The peels of sweet orange and bitter orange had low CF of the peel samples while, grapefruit pomace had the lowest CF amongst the citrus pomaces assessed. The highest NDF and ADF was found in the peel and pomace samples of lemon while the grape fruit pomaces had the lowest NDF and ADF amongst the pomaces and sweet orange had the lowest NDF and ADF amongst the peels. Table 2b showed that Bitter orange pomace had the highest ME amongst all the citrus peels and pomaces. In contrast, Lemon pomace had the lowest ME of all the citrus peels and pomaces. In addition, the pomaces of the citrus species had higher ME compared to their peels except for lemon pomace which had lower ME compared to its peels (Figure 1). No statistical significance (p >0.05) was established for the aforementioned parameters, rather all values had been ranked based on numerical difference (high and low) as provided by the Kruskal-Wallis non-parametric test conducted. Furthermore, the overall model of

Table 2a: Chemical composition and phenolic of citrus species and their byproducts

No.	Citrus	Byproduct samples	DM (%)	XA (%)	CP (%)	CF (%)	NDF (%)	ADF (%)
1	Orange	Pomace	91.68±0.02	4.33±0.01	5.48±0.02	10.60±0.50	14.54±0.15	10.85±0.00
2	Orange	Peel	91.67±0.09	4.51±0.02	4.98±0.05	11.80±0.10	16.33±0.25	12.13±0.20
3	Lemon	Pomace	91.11±0.16	5.10±0.02	7.78±0.03	15.25±1.25	20.21±0.15	14.97±0.06
4	Lemon	Peel	91.46±0.00	5.01±0.01	7.38±0.14	14.65±0.55	20.51±0.01	14.63±0.02
5	Mandarin	Pomace	93.08±0.01	3.91±0.00	5.71±0.03	12.10±1.70	14.6±0.08	11.33±0.09
6	Mandarin	Peel	93.52±0.02	4.29±0.01	4.59±0.13	12.55±1.85	17.23±0.90	12.63±0.17
7	Grapefruit	Pomace	91.56±0.04	3.57±0.04	4.62±0.04	10.15±0.55	13.58±0.10	10.77±0.07
8	Grapefruit	Peel	92.09±0.03	3.72±0.03	4.76±0.12	12.65±0.85	17.45±0.04	12.93±0.05
9	Bitter Orange	Pomace	91.58±0.02	5.52±0.04	3.65±0.09	11.7±0.10	15.78±0.10	12.41±0.06
10	Bitter orange	Peel	93.58±0.04	5.28±0.00	2.70±0.61	13.50±0.20	18.05±0.05	14.09±0.02

Values presented in the table are mean ± SEM.

Table 2b: Metabolic energy and Total phenolic of citrus species and their byproducts

No.	Citrus	Byproduct samples	EE (%)⸸	ME (MJ/Kg DM)	Total Phenolic (mg GAE/g)*
1	Orange	Pomace	0.86	11.65±0.000	462.8±15.95
2	Orange	Peel	0.74	11.42±0.031	277.2±1.46
3	Lemon	Pomace	0.81	10.97±0.006	488.5±12.06
4	Lemon	Peel	1.20	11.12±0.005	506.2±5.67
5	Mandarin	Pomace	1.52	11.79±0.012	538.1±19.21
6	Mandarin	Peel	1.34	11.52±0.022	529.7±8.69
7	Grapefruit	Pomace	0.85	11.76±0.004	780.2±19.51
8	Grapefruit	Peel	1.13	11.51±0.012	660±5.67
9	Bitter Orange	Pomace	4.76	12.17±0.014	902.3±15.95
10	Bitter orange	Peel	1.23	11.17±0.003	326.3±1.46

* Phenolic concentrations presented in the table are mean ± SEM and the overall model of all five citrus species against byproduct samples is indicated with * given the p ≤0.01.

⸸ The ether extract (EE) is only presented in this table as one of the parameters of metabolizable energy estimation.

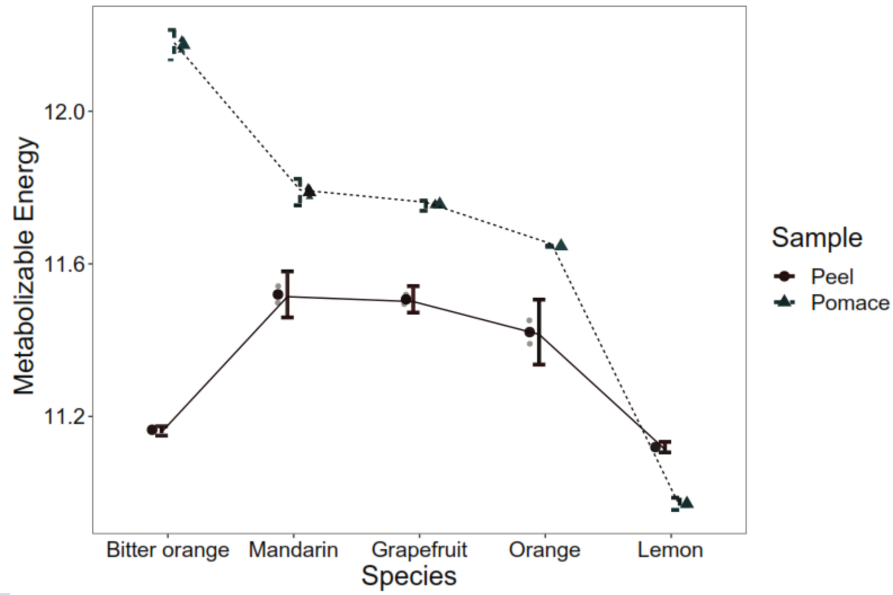

Figure 1: Metabolizable energy for citrus species and byproducts. The Flex plot shows that the metabolizable energy of the pomace of the citrus species is numerically higher than the peels except for the Lemon pomace which has lower ME than Lemon peels.

Table 3: In-vitro digestibility of Peels and Pomaces of five Citrus Species (%)

No.	Citrus	Byproduct samples	IVTD-DM (%) *	NDFD (%) *
1	Orange	Pomace	94.8±0.38	63.8±2.39
2	Orange	Peel	94.3±0.38	64.6±2.39
3	Lemon	Pomace	92.1±0.38	59.7±2.39
4	Lemon	Peel	90.1±0.38	54.6±2.39
5	Mandarin	Pomace	93.2±0.38	52.9±2.39
6	Mandarin	Peel	92.7±0.38	57.3±2.39
7	Grapefruit	Pomace	94.7±0.38	60.0±2.39
8	Grapefruit	Peel	92.0±0.38	52.7±2.39
9	Bitter Orange	Pomace	96.4±0.38	76.3±2.39
10	Bitter Orange	Peel	91.6±0.38	52.8±2.39

Values presented in the table are mean ± SEM of in vitro digestibility parameters of the citrus species. The overall model of IVTD-DM, in vitro true digestibility dry matter and NDF, neutral detergent fibre disappearance of citrus species against the byproduct samples was significant (*) at p-value, <0.001.

the total phenolic content showed statistical significance ($p \leq 0.01$) and grapefruit had the highest total phenolic content among the peel samples while bitter orange pomace had the highest among the pomaces. Conversely, the peels and pomaces of sweet orange had the lowest total phenolic content (Table 2b).

In-vitro true digestibility

Citrus peels had higher IVTD-DM than the pomaces across the five species (Figure 2). Bitter orange, grapefruit, and lemon pomaces had higher NDFD than their corresponding peels. In contrast, Mandarin and sweet orange pomace had lower NDFD compared to their corresponding peels (Figure 3). There were species and sample effect shown by the significant difference ($p < 0.001$) in the results obtained for in-vitro true digestibility between *Citrus sinensis* (sweet orange), *Citrus limon* (lemon), *Citrus reticulata* (mandarin), *Citrus paradisi* (grapefruit) and *Citrus aurantium* (bitter orange).

DISCUSSION

The chemical composition assessment of the citrus species examined were mostly consistent with previous research of the individual citrus species. Within the scope of evaluation, no significance was established in the chemical composition assessed except in the total phenolic content. The in vitro digestibility results in this study were novel owing to the limited comparative reports on these citrus peels and pomaces.

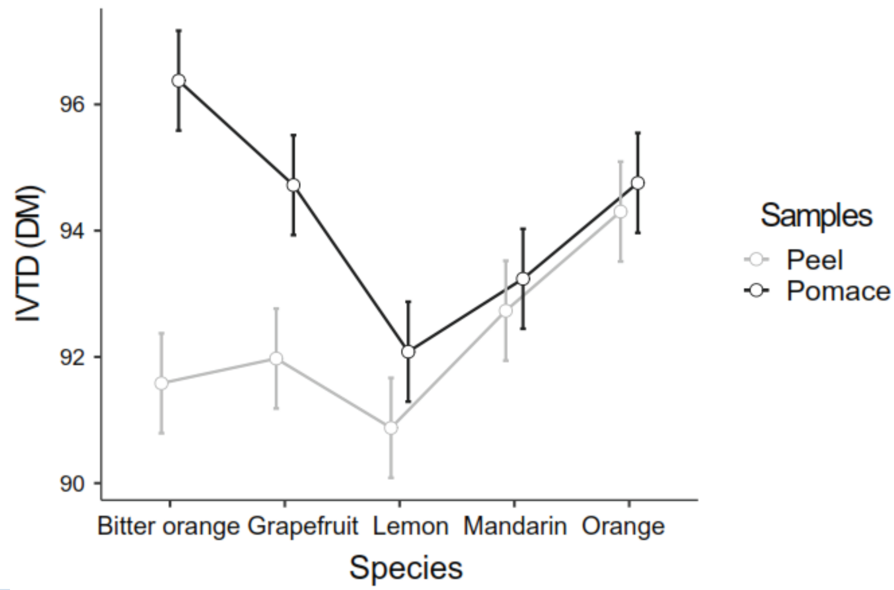

Figure 2: IVDT-DM for citrus species and byproducts. The analysis of variance (ANOVA) showed pomaces of the citrus species had higher IVTD-DM and these significantly differed from peels of the same species at P<0.001.

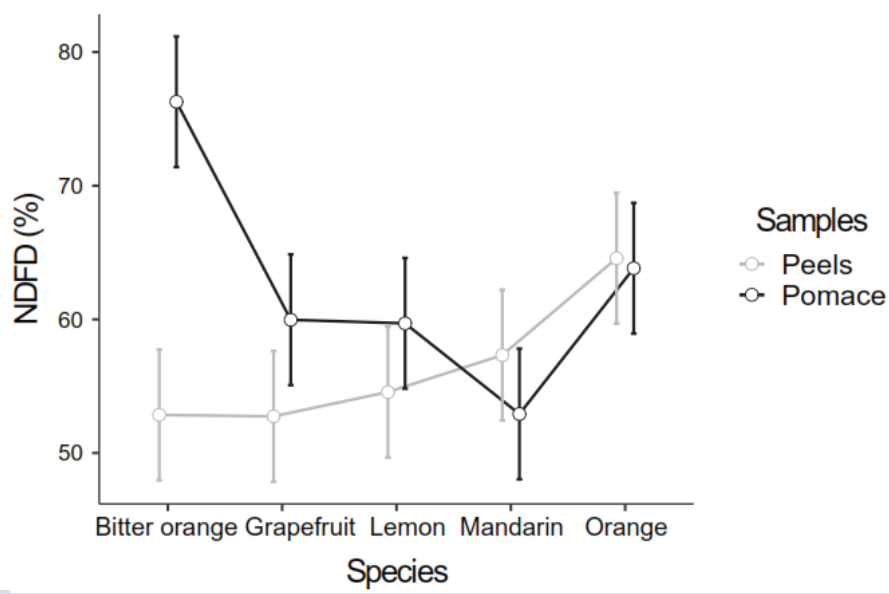

Figure 3: The neutral detergent fibre disappearance (NDFD) of peel and pomace by-products for five citrus species. The analysis of variance (ANOVA) showed pomace samples of bitter orange, and grapefruit, had higher NDFD compared to peels of the same species while mandarin and orange peels had higher NDFD compared to pomaces of the same species. All these were significantly different at P<0.001.

Chemical composition of citrus agro-industrial by-products

Bitter orange is a less utilized citrus species in Türkiye perhaps due to the sour taste, but the peels had the highest DM among similar samples and the pomace sample had a DM comparable to the reports of Lashkari and Taghizadeh (2013). Similarly, the DM for bitter orange byproducts is comparable to grapefruit, mandarin, lemon and sweet orange (Ghanem et al., 2012; Palangi et al., 2013). Although the crude ash for bitter orange peels and pomaces were higher than in the other species, they were lower than previous reports (Atta and El shenawi, 2012; Lashkari and Taghizadeh, 2013). Fruit maturity, sourcing and the method of assessments may account for the difference in the chemical composition obtained in this study similar to the suggestions of Ammerman and Henry (1991); Garcia-Rodriguez et al (2019).

Crude fibre is a common approach of fibre measurement in feed given its negative correlation to energy value and digestibility, however the inaccuracy with the approach makes its use less desired (Fahey et al., 2019). NDF may be useful to predict the voluntary feed intake given its relationship with dry matter intake (Harper and McNiell, 2015) while ADF is an indicator for digestibility (Olowu and Yaman Fırıncıoğlu, 2019). High fibre content of lemon peels samples compared to bitter orange, grapefruit, mandarin and sweet orange in this study agrees with prior studies. Agro-industrial byproducts of lemon may be attractive to the ruminant considering that the NDF is within the limit of increased dry matter intake (Harper and McNiell, 2015), but the high ADF is indicative of possibly low digestibility compared to bitter orange pomace, grapefruit (peel and pomace), mandarin (peel and pomaces) and sweet orange (peel and pomace). In addition, lemon (peel and pomace) may provide higher protein for ruminant feed compared to the other citrus species in the study as asserted by prior studies (Marín et al., 2007; Janati et al., 2012; Ghanem et al., 2012; El-ghfar et al., 2016), however, the protein value is still low compared to the dietary requirement for dairy cattle (Wachirapakorn et al., 2014).

The metabolizable energy contents of the citrus peels and pomaces were relatively high (except in lemon peels) and similar to the results obtained in the study done by Ozkan et al (2017) where ME for Citrus pulp was evaluated through the IVTD gas method. The low ME in Lemon peels and pomace compared to other citrus species could be attributed to their high ADF values (Figure 1). The high ME spotlights Citrus as a consideration for ruminant feed modification given its potential as non-forage energy source.

The total phenolic content of bitter orange pomaces, 902.3 mgGAE/g was significantly different from all other samples and higher than reported by Rehman et al (2020) reported, 158.9 mgGAE/g. The genetic variation of citrus species (Ghanem et al., 2012), agro-climatic conditions of the environment where they are produced (Hussain et al., 2017) as well as extraction solvent and analysis procedure (Singh et al., 2020) may account for the significant total phenolic content. Dry matter, crude ash, neutral detergent fiber, acid detergent fiber and total phenolic content are important to ration formulation for ruminants as they guide with nutrient intake and antioxidant benefits to the animal.

In-vitro digestibility

The IVTD-DM in pomace byproduct of bitter orange in this research was higher (Lashkari and Taghizadeh, 2013) while IVTD-DM value of other species were comparable to prior studies (Tripodo et al., 2004; Aregheore, 2000; Alnaimy, 2017; Olivo et al., 2017). Bitter orange pomace had higher NDFD compared to other citrus byproducts in this study. This indicates that bitter orange pomace is a potentially highly digestible citrus byproduct for consideration where the ruminant feed modification target is to increase digestibility. The byproducts of the five citrus species in this study indicate their potential for increased digestible neutral detergent fiber and pertinent for higher dry matter intake (DMI) (Van Soest, 1994; Harper and McNiell, 2015) hence citrus byproducts having low NDF such as bitter orange, grapefruit, mandarin, and sweet orange may be considered for energy. A clear limitation for use of citrus byproducts for ruminant feed medication is the high moisture content and low shelf life in moist form (Mamma and Christakopoulos, 2014). This limitation can be eliminated through silage or inclusion as dry feed ingredients in ruminant ration (Scerra et al., 2001; Zoiopoulos et al., 2008).

Citrus agro-industrial byproducts for ruminant feed modification

The three scenarios presented by this study are the considerations of citrus agro-industrial byproducts for ruminant feed modification as first, byproducts that can enhance feed intake with nutritive benefits such as energy and growth; second, byproducts with high antioxidant capacity; and third, byproducts that with high digestibility to ensure nutrient availability to the ruminant.

Diets rich in soluble sugar are recommended for ruminants (Aregheore, 2000; Bampidis and Robinson, 2006; Lashkari and Tagizadeh, 2013). In addition, the protein composition of lemon makes it a consideration for growth. Bitter orange pomace and grapefruit byproducts could be considerations for feed modification where the target is to enhance antioxidant capacity having established high total phenolic content in this study. At IVTD-DM range of 85-97 %, the citrus species examined in this research indicated high dry matter intake and the highly digestible NDF showed that citrus byproducts were highly digestible and fermentable (Garcia-Rodriguez et al., 2019). Furthermore, the high IVTD-DM and NDFD established in bitter orange pomace is a pointer that this citrus byproduct is useful for high digestibility. The similarly high IVTD-DM and total phenolic content of bitter orange pomace agrees with Paula et al (2016) on the possibility of non-alteration of total digestibility of nutrients and dry matter despite the change in patterns of rumen fermentation.

CONCLUSION

The availability of cheap, alternative ruminant feeds sources through citrus agro-industrial byproducts is clearly an important strategy for the improvement of ruminant nutrition, energy, growth, antioxidant capacity, and digestibility. Depending on the focus on ruminant feed modification, the byproducts of the five citrus species studied have their importance. Citrus byproducts showed viability as non-forage energy sources given their high metabolizable energy. In addition, bitter orange pomace stood out for its antioxidant capacity potential and digestibility. Further studies are recommended to establish the antioxidant capacity and likely antinutritive factors of Citrus byproducts that may limit growth. Also recommended are studies on the digestibility of mixed species of citrus byproducts which may provide insight into additive, antagonistic or synergistic effects.

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