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Determination of *In Vitro* Digestion Values of Alfalfa Hay, Dried Tomato Pomace and their Combinations

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ABSTRACT Tomato pomace, a by-product of the processing of tomoto for paste, ketchup, juice, is made up of tomato skin, pulp and crushed seeds after the process. Evaluating of tomato pomace as an ingredient of a diet may occur a potential for ruminant nutrition. For this reason, the aim of this study was to determine *in vitro* true digestibility values of alfalfa hay and dried tomato pomace mixtures formed with different levels of them for ruminants. The mixtures were prepared as alfalfa hay (100%), dried tomato pomace (100%), alfalfa hay (25%) + dried tomato pomace (75%), alfalfa hay (50%) + dried tomato pomace (50%), alfalfa hay (75%) + dried tomato pomace (25%). The filter bags containing feed mixtures, buffer solutions and rumen fluid were prepared as described for Ankom Daisy^{II} *in vitro* fermentation system. After 48 h incubation, filter bags gently washed until they were completely clean. Thereafter, neutral detergent fiber procedure was performed. Whereas *in vitro* true organic matter and neutral detergent fiber digestibilities of alfalfa hay was the lowest, *in vitro* true organic matter and neutral detergent fiber digestibilities of dried tomato pomace was the highest in the study. There were increases in *in vitro* true organic matter and neutral detergent fiber digestibility values of the mixtures with the increasing levels of dried tomato pomace ($P<0.05$). The higher digestibility values of the mixtures formed by alfalfa hay and dried tomato pomace may be due to a positive interaction between alfalfa hay and dried tomato pomace.

Keywords: *Alfalfa hay, Tomato pomace, In vitro true organic matter digestibility, In vitro NDF digestibility*

ÖZ

Yonca Kuru Otu, Kurutulmuş Domates Posası ve Kombinasyonlarının *in vitro* Sindirim Değerlerinin Belirlenmesi

Domatesin salça, ketçap, meyve suyu üretimi aşamasının bir yan ürünü olan domates posası, domatesin kabuğu, meyve eti ve ezilmiş çekirdeğinden oluşur. Domates posasının diyetin bir bileşeni olarak değerlendirilmesi ruminant beslenme için potansiyel oluşturabilir. Bu nedenle, bu çalışmanın amacı, ruminantlar için farklı düzeylerde oluşturulan yonca kuru otu ve kurutulmuş domates posası karışımlarının *in vitro* gerçek sindirilebilirlik değerlerini belirlemekti. Araştırmada karışımlar yonca kuru otu (%100), kurutulmuş domates posası (%100), yonca kuru otu (%25) + kurutulmuş domates posası (%75), yonca kuru otu (%50) + kurutulmuş domates posası (%50), yonca kuru otu (%75) + kurutulmuş domates posası (%25) olacak şekilde hazırlandı. Araştırmada yem karışımlarını içeren filtre torbalar, tampon çözeltiler ve rumen sıvısı Ankom Daisy^{II} *in vitro* fermentasyon sistemi için tanımlanan şekilde hazırlandı. 48 saat inkübasyon sonrasında, filtre torbalar tamamen temizleninceye kadar yıkandı ve nötral deterjan lif prosedürü uygulandı. Çalışmada yonca kuru otunun *in vitro* organik madde ve nötral deterjan lif sindirilebilirlikleri en düşük, kurutulmuş domates posasının *in vitro* organik madde ve nötral deterjan lif sindirilebilirlikleri en yüksek bulundu. Yonca kuru otunun artan düzeylerde kurutulmuş domates posasıyla oluşturulan karışımlarının *in vitro* gerçek organik madde ve nötral deterjan lif sindirilebilirlik değerlerinde artış saptandı ($P<0.05$). Yonca kuru otu ve kurutulmuş domates posası ile oluşturulan karışımların daha yüksek sindirilebilirlik değerleri, yonca kuru otu ve kurutulmuş domates posası arasındaki pozitif bir etkileşimden kaynaklanmış olabilir.

Anahtar Kelimeler: *Yonca kuru otu, Domates posası, İn vitro gerçek organik madde sindirilebilirliği, İn vitro NDF sindirilebilirliği*

INTRODUCTION

Shortage of inexpensive feed resources, shortage in the quantity and quality of feed resources consistent throughout the year often cause major constrains on the enhancement of animal production. It is known that conventional forages used in diets meet nutrients and energy requirements of ruminants. However, rumen microbial population can utilize food processing by-products rich in structural fibre. Using food processing by-products in ruminant nutrition may be a practical alternative because of rumen ecosystem. In otherwords,

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non-forage fiber sources such as tomato pomace, grape pomace, soybean hulls which are obtained during processing of a commodity for human nutrition can be used in ruminant diets to supplement conventional forages (Mirzaei-Aghsaghali and Maheri-Sis 2008).

Tomato pomace, a by-product of the processing of tomoto for paste, ketchup, juice, is made up of tomato skin, pulp and crushed seeds after the process (Nobakht and Safamehr 2007). Tomato pomace contains many nutrients such as crude protein, fat and fiber that are important in nutrition of animal species (Selcuk et al. 2013). It is reported (Aghajanzadeh-Golshani et al. 2010; Rahbarpur et al. 2013) that tomato pomace is rich in crude protein (22.6-24.1%) and crude fiber (20.8-30.5%). Selcuk et al. (2013) state that tomato pomace contains 18.4, 10.51, 32.85 and 29.17% crude protein, ether extract, crude fiber and nitrogen free substance, respectively.

In vivo, in situ and *in vitro* methods are generally used to estimate the nutritive value of feedstuffs. *In vitro* digestibility experiments are less expensive and the results of them are produced more rapidly than those of *in vivo* studies. Preparing rumen conditions makes the estimation of *in vitro* digestibility of feeds possible. The technique is easily applicable and commonly used for the evaluations of conventional ruminant feeds. *In vitro* Daisy fermentation technique is a useful technique for feed evaluation, as it is capable of measuring digestibility. Evaluating of tomato pomace as an ingredient of a diet may occur a potential for ruminant nutrition. For this reason, the aim of this study was to determine in vitro true digestibility values as feed ($IVTD_{FEED}$), *in vitro* true organic matter digestibility in DM basis ($IVTOMD_{DM}$) and *in vitro* NDF digestibility in DM basis ($IVNDFD_{DM}$) of alfalfa hay and dried tomato pomace mixtures formed with different levels of them for ruminants.

MATERIALS and METHODS

Chemical analyses and *in vitro* digestibility of alfalfa hay, tomato pomace and their mixtures were conducted in the Ruminant Feed Evaluation Laboratory of Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine of Ondokuz Mayis University, Samsun, Turkey.

Feed material

Alfalfa hay (AH) and dried tomato pomace (DTP) used as feed material were ground approximately 2 cm lenght. The mixtures prepared from ground AH and DTP were presented in Table 1.

Table 1. Feed mixtures

Mixtures	Alfalfa hay %	Dried tomato pomace %
Alfalfa hay	100	0
Dried tomato pomace	0	100
25% Alfalfa hay + 75% Dried tomato pomace	25	75
50% Alfalfa hay + 50% Dried tomato pomace	50	50
75% Alfalfa hay + 25% Dried tomato pomace	75	25

Chemical analysis

Dry matter, ash, crude protein levels and NDFcontents of AH, DTP and their mixtures were analyzed according to AOAC (2006) methods and determined with the Ankom Fiber Analyzer, respectively.

Preparation of filter bags

The bags (F57) used in Ankom Daisy^{II} *in vitro* fermentation system were firstly rinsed in acetone for 3 min and they were allowed to dry and marked. After the bags were dried, at a mass of 0.5 g of AH, DTP and their mixtures passed through a 1 mm sieve were weighed into each filter bag (8 bags for AH, DTP and their each mixture) and then the bags were sealed by an impulse bag sealer. A bag without feed was also used for a blank.

Rumen content

Rumen content obtained from cows freshly slaughtered at a local slaughterhouse was collected into a prewarmed thermos (39 °C under a CO_2 atmosphere) and immediately transferred to the laboratory within 15 min, and then rumen content was filtered through four layers of cheese cloth. Anaerobic conditions were maintained throughout the preparation stages of rumen fluid and conduct of the experiment.

In vitro digestion method

The Ankom Daisy^{II} *in vitro* fermentation system was used for determination of $IVTD_{FEED}$, $IVTOMD_{DM}$ and $IVNDFD_{DM}$ of the mixtures. The procedure for *in vitro* fermentation system was conducted according to the operating instructions supplied by Ankom. Buffer solutions required *in vitro* fermentation system were prepared and poured into each digestion jar of incubator described by Ankom. Each digestion jar contained 1600 ml buffer solution. After the temperature of buffers was set 39 °C, 400 mL rumen fluid and prepared bags were added to each digestion jar. Each digestion jar was aerated with CO_2 gas immediately before activating incubator for 48 h. At the end of 48 h incubation time, the digestion jars were removed, the incubation medium inside of digestion jars was discharged and the bags were gently washed under running water until they were completely clean. Thereafter, the bags were placed in the Ankom Fiber Analyzer and NDF procedure was performed. The percentage of $IVTD_{FEED}$, $IVTOMD_{DM}$ and $IVNDFD_{DM}$ values of the mixtures were calculated with equations consisting of difference between the amounts of incubated and the residue after NDF procedure.

Statistic analysis

The data were summarized in the form of arithmetic means and standard errors. The statistical significance for each feed mixture used in the study was determined by one-way variance analysis. Tukey Posthoc test was used to determine statistical differences. Relationship between increasing levels of DTP in the mixtures and $IVTOMD_{DM}$ was determined by regression analysis. SPSS (2012) package program was used for statistical analysis.

RESULTS

Nutrient composition and $IVTD_{FEED}$, $IVTOMD_{DM}$, $IVNDFD_{DM}$ values of AH, DTP and their mixtures were presented in Table 2 and 3, respectively. Whereas $IVTD_{FEED}$ value of AH was the lowest (57.00%), $IVTD_{FEED}$ value of DTP was the highest (74.90%) in the study (Table 3). There was an increase in the same value of the mixtures combined with at least 25% DTP was higher ($P<0.05$) than that of AH. $IVTOMD_{DM}$ values of the mixtures formed with at least 50% DTP were higher ($P<0.05$) than that of AH and AH75%+DTP25%. $IVNDFD_{DM}$ value of the mixtures were

similar to that of DTP. A positive relationship (R^2=0,6923) between increasing levels of DTP in the mixtures and $IVTOMD_{DM}$ was presented in Figure 1.

Table 2. Nutrient composition of alfalfa hay (AH), dried tomato pomace (DTP) and their mixtures.

Mixtures	DM%	Ash%	CP%	NDF%
AH	93.20	10.10	16.50	49.70
DTP	92.30	4.50	19.50	57.20
AH25%+DTP75%	92.40	5.9	18.60	55.20
AH50%+DTP50%	92.60	7.4	17.90	53.50
AH75%+DTP25%	92.80	10.80	17.30	51.60

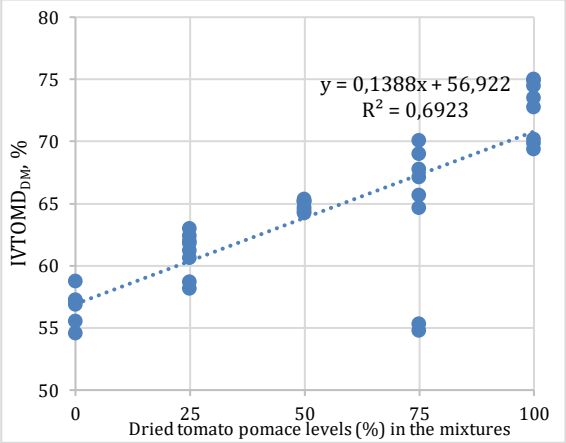

Figure 1. Regression between increasing levels of dried tomato pomace in the mixtures and *in vitro* true organic matter digestibility ($IVTOMD_{DM}$)

Table 3. $IVTD_{FEED}$, $IVTOMD_{DM}$ and $IVNDFD_{DM}$ values of alfalfa hay (AH), dried tomato pomace (DTP) and their mixtures

Mixtures	**$IVTD_{FEED}$% $x \pm Sx$**	**$IVTOMD_{DM}$% $x \pm Sx$**	**$IVNDFD_{DM}$% $x \pm Sx$**
AH	57.00±0.42^{d}	56.80±0.44^{c}	18.10±0.37^{b}
DTP	74.90±0.39^{a}	72.50±0.83^{a}	27.10±0.36^{a}
AH75%+DTP25%	61.60±0.55^{c}	61.00±0.61^{bc}	24.50±0.51^{a}
AH50%+DTP50%	65.90±0.20^{b}	64.80±0.15^{b}	27.90±0.13^{a}
AH25%+DTP75%	66.10±1.84^{b}	64.30±2.10^{b}	26.70±2.22^{a}

Means in the same column having different superscripts differ significantly (P<0.05); $IVTD_{FEED}$: *in vitro* true digestibility values as feed, $IVTOMD_{DM}$: *in vitro* true organic matter digestibility in DM basis, $IVNDFD_{DM}$: *in vitro* NDF digestibility in DM basis

DISCUSSION

The feeding value of feeds are associated with both their nutrients composition and their digestibility. The digestibility of feed mixtures may differ from their individual digestibility (Niderkorn and Baumont 2009). However, feed supply and quality are one of the fundamental problems in ruminant nutrition. Therefore, there are many researches (Aghajanzadeh-Golshani et al. 2010; Abdollahzadeh and Abdulkarimi 2012; Tuoxunjiang et al. 2017) on alternative feed sources and the use of food industry by-products in ruminant feeding.

Aghajanzadeh-Golshani et al. (2010) and Omer and Abdel-Magid (2015) reported that dry matter contents of DTP were 92.00 and 91.01%, respectively. In this study, dry matter content of DTP is similar to those of Aghajanzadeh-Golshani et al. (2010) and Omer and Abdel-Magid (2015).

Silva et al. (2016) stated that crude protein contents of tomato pomace containing six different rates of skin and seed were between 16.81 and 23.25%. Aghajanzadeh-Golshani et al. (2010) reported that crude protein value of tomato pomace was 22.17%. Savrunlu and Denek (2016) mentioned that crude protein level was 14.98%. In the present study, DTP crude protein level was higher than that of AH. Therefore, crude protein value was increased in the mixtures formed AH combined with increasing level of DTP.

Omer and Abdel-Magid (2015) and Gebeyew et al. (2015) stated that NDF contents of DTP were 65.24 and 48.9%, respectively. In this study, the result for NDF value of DTP was lower than Omer and Abdel-Magid (2015), higher than that of Gebeyew et al. (2015) and similar to that of Savrunlu and Denek (2016) who reported that NDF content of tomato pomace was 55.23%.

Dry matter, organik matter or NDF digestibility are the methods bywhich determining nutritive value of forages or alternative feed sources. Tahseen et al. (2014) reported that potential dry matter degradability of the mixture tomato and cucumber greenhouse residues were 72%. Chumpawadee (2009) stated that potential dry matter and organic matter degradabilities of tomato pomace 63.5% and 61.6%, respectively. The organic matter digestibility value of tomato pomace estimated by using *in vitro* gas production technique was 62% in a study conducted by Mirzaei-Aghsaghali et al. (2011). In the present study, $IVTOMD_{DM}$ value of DTP was the highest (72.50%) whereas $IVTOMD_{DM}$ value of AH the lowest (56.80%). Denek and Deniz (2004) reported that crude protein content and *in vitro* organic matter digestibility value of alfalfa hay were 15.19 and 57.93%, respectively. Deniz et al. (2000) and Nantoume et al. (2001) stated that *in vivo* organik matter digestibility of alfalfa hay was 58.56% and 59.20 %, respectively. The result of the present study for *in vitro* true organic matter digestibility of alfalfa hay was compatible with those of Denek and Deniz (2004), Deniz et al. (2000) and Nantoume et al. (2001). Omer and Abdel-Magis (2015) reported that OM digestibility of TMR increased when DTP included to TMR at the percentage of 10 or 15. It has been also revealed that incorporation DTP up to 15% may be useful in feeding lambs and dried tomato waste could be used as a substitute for good quality roughages, preferably in dried form in ration of lambs. Abdollahzadeh and Abdulkarimi (2012) mentioned that *in vivo* DM and OM digestibilities of alfalfa hay plus concentrate mixture with two levels of mixed tomato and apple pomace silage (15 and 30%) were better than that of alfalfa hay plus concentrate mixture without mixed tomato and apple pomace silage in dairy cows. In our study, $IVTOMD_{DM}$ value increased in the mixtures formed AH combined with increasing level of DTP. Namely, increasing level of DTP in the mixtures was result in improvement of $IVOMD_{DM}$ value of the mixtures. This result is consistent with that of Abdollahzadeh and Abdulkarimi (2012).

Keklikci and Selçuk (2018) reported that the average $IVNDFD_{DM}$ value of DTP for ruminants was 23.60%. In the current study, the same parameter was found higher than that of Keklikci and Selcuk (2018). Tuoxunjiang et al. (2017) stated that NDF digestibility of the diet 10% corn silage replaced by tomato pomace silage was not significantly affected by the amount of tomato pomace in the diet but this parameter tended to be higher than that of control diet without tomato pomace silage. In the present

study, $IVNDFD_{DM}$ values of DTP and the mixtures was similar to each other and higher ($P<0.05$) than that of AH. The higher digestibility values of the mixtures may be caused by interaction between DTP and AH.

Finally, it may be concluded that DTP could be incorporated at least 25% in alfalfa hay due to a positive relationship between increasing levels of DTP in the mixtures and $IVTOMD_{DM}$. However, further researches are needed to determine the effects of DTP inclusions in ruminant diets on *in vivo* digestion, ruminal fermentation and performance of ruminants.

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