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**THE EFFECTS OF SOME AGRICULTURAL BY-PRODUCTS ON RUMINAL  
FERMENTATION AND APPARENT DIGESTIBILITY OF HOLSTEIN DAIRY COW**

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**ABSTRACT**

This study was carried out to evaluate the effects of replacing alfalfa hay with ensiled mixed tomato and apple pomace (EMTAP) on ruminal fermentation and apparent digestibility of diets. Six multiparous Holstein dairy cows in mid lactation were used in 3×3 Latin square design and fed alfalfa hay plus concentrate mixture with three levels replacement with EMTAP (0, 15, 30%) during 63 days. Results showed that, differences between treatments were significant. Feeding EMTAP resulted in a significant higher acetic and propionic acids and total volatile fatty acids concentration and significant ( $P<0.01$ ) lower rumen pH. The substitution of EMTAP in dairy cow diets is associated with a better DM, OM, CP and NDF digestibility. It was concluded that, EMTAP can efficiently replace up to 30% alfalfa hay. The nutritive value of tomato and apple pomace could be improved when they are used together (50:50) in dairy cows diet.

**Keywords:** Agricultural By-Products, Tomato Pomace, Apple Pomace, Dairy Cow

**1. INTRODUCTION**

Shortage of inexpensive feed resources often impose major constrains on the promotion of animal production. Considerable quantity of agro-industrial by-products are generated every year in most developed and developing countries. Introduction of alternative feedstuffs is an interesting challenge for animal nutritionists and it could overcome the

problems of environmental and production costs. Ruminants which are able in converting waste items in to useful products such as meat, milk and skin, offers a feasible solution of using by-products and preventing pollution [Oni et al. 2008]. Tomato and apple pomace are two alternative by-products that obtained from tomato paste and apple juice industrial production, respectively. These by-products are produced in huge amount annually. The chemical composition of final pomace is linked to the morphology of the original feed stock and the extraction technique used. Although tomato and apple pomace are varying from nutrient density, effective processing can improve their nutritive value. According to NRC [2001] apple pomace (AP) is very low in protein (6.4% protein on DM basis), it also serves as a useful energy source because of high content of soluble carbohydrate for ruminants. Researches conducted on AP [Rumsey et al. 1978; Fontenot et al. 1977], showed that AP supplemented with natural proteins was comparable to protein enriched corn silage. In contrast, [Elloitt et al. 1981] demonstrated that, tomato pomace (TP) have the potential to be a good source of protein, however its energy source may be limited due to the high fiber content.

Previous researches reported different results from feeding TP and AP. The complementary composition of AP (low protein concentration) [Alibes et al. 1984; NRC 2001; Pirmohammadiet al. 2006] and TP (high protein content) (Fondevila et al. 1994; Del Valle et al. 2006; Weiss et al. 1997) suggest to use those by-products together. Our previous observations (unpublished data) showed that processed TP with AP (ratio of 50:50) had more palatability and digestibility than processing with urea, wheat straw, NaCl and NaOH for sheep. The aim of the present study is to evaluate the effect of ensiled mixed tomato and apple pomace (EMTAP) on ruminal fermentation and diet digestibility in Holstein dairy cows.

## **2. MATERIAL AND METHODS**

### **2.1. Tomato and apple pomace and silage preparation**

Fresh experimental samples of tomato pomace (TP) and apple pomace (AP) were collected from several factories in Urmia city (Iran). TP and AP were mixed together (50:50 on DM basis) and ensiled without any additive in a trench silo on a concrete floor. The mixed TP and AP silage (EMTAP) was sealed for 55 days, next fed as TMR diets in three levels replacement of alfalfa hay. Chemical composition of TP, AP and

EMTAP was determined using the method suggested by AOAC [2000]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using method of Van Soest et al. [1991].

## 2.2 Dairy cow management

Six multiparous dairy Holstein cows were used in a 3×3 Latin square experimental design with three 3-wk periods. They were kept in individual concrete tie-stalls and had free access to drinking water at all times. The daily TMR diets were given in two equal feeds at 08:00 and 20:00 h to provide approximately 10% feed refusal each day (as-fed basis). Feed refusal were removed and weighed before feed offered at 08:00 h. Body weight was recorded prior to morning feeding on 2 consecutive days at the beginning and at finish of each period. The experimental periods lasted 21 d, including 14 d of adaptation and 7th d of sampling and data collection. During the last 7th d of the experimental period collection and sampling of TMR diets, feed refusal, rumen fluid, blood, feces and urine were performed. Normal herd management practices were followed during the experiment. Daily feed intakes and milk production were recorded for individual cows throughout the experiment.

## 2.3. Treatments and digestibility study

The dietary treatments consisted of three levels replacement of alfalfa with EMTAP and include diet 1 (control or 0% EMTAP), 2(15% EMTAP) and 3(30% EMTAP) on DM basis. The diets were formulated according to the NRC (2001) guidelines. Ingredients and chemical composition of the diets are reported in Table 1. To evaluate the diet's nutritive value, the apparent digestibility was determined at 4th, 5th and 6th -day of each sampling period. Total feces were collected individually from each animal, sub sampled and stored (-20°C) for the subsequent laboratory analysis.

## 2.4 Data collection, analytical methods and laboratory analyses

Samples of rumen contents were collected on 6th day of each sampling period, 3 h post feeding by esophageal tube connected to vacuum pump. The first 20-30 ml of ruminal fluid obtained was discarded prior to collecting samples to minimize the saliva contamination. Rumen contents were squeezed through two layers of cheesecloth and immediately analyzed for pH using a Schott Titrator Titroline easypH-meter. To inhibit microbial growth and NH<sub>3</sub> volatilization the pH value was maintained less than 2.0. Five milliliters of strained rumen fluid were preserved by adding 1 ml of

25% H<sub>2</sub>PO<sub>4</sub> to determine volatile fatty acids (VFA), and 10 ml of filtrate were preserved by adding 1 ml of 1% H<sub>2</sub>SO<sub>4</sub> to determine ammonia-N. Samples were frozen at 20°C and subsequently analyzed for ammonia-N using micro Kjeldahl method, (according to AOAC 2000 recommendations) and VFA concentrations (acetic, propionic, butyric, Valeric, Isovaleric) by gas chromatography (mark Philips, Varian 3700, serial number pu4410, Varian Specialties Ltd., Brockville, Ontario, Canada) with a 15-m (0.53 mm i.d.) fused silica column (DBFFAP column; J and W Scientific, Folsom, CA) in Animal Nutrition Laboratory in Tehran University.

Feces were collected and extracted through cheesecloth into a clean beaker; the urine samples were taken via vulva stimulation. Feces and urine pH was measured using a Schott Titrator Titroline easypH-meter. For the estimation of DM intake and apparent digestibility, sample of feed offered, rejected feeds and feces were dried (60°C for 48 h), weighed and sub sampled to be later analyzed for chemical composition. The dried samples were grounded through a 1-mm screen and were assayed for DM, OM, (AOAC, 2000; #930.15) and CP (AOAC, 2000; #990.03). The organic matter (OM) was calculated as weight loss upon ash. Neutral detergent fiber

(NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest et al. [1991]. Dry matter intake was calculated as the difference between DM offered and refused.

## 2.5 Experimental design and statistical analysis

Data collected from DM, OM and CP digestibility and pH (rumen, urine and feces) were statistically analyzed using the GLM procedure [SAS. 1998, Inst. Inc., Cary, NC]. Level of significance was  $\alpha = 0.05$ , and the Tukey test was used to test for all pairwise comparisons among means. The model used for this analyze was:

$$Y_{ijk} = \mu + T_i + C_j + P_k + \epsilon_{ijk}$$

Where Y is dependent variable,  $\mu$  is the overall mean, T is treatment effect (i = 1, 2, 3 EMTPA levels), C is cow effect (j= 1 to 6), P is period effect (k= 1, 2, 3) and  $\epsilon$  is random residual error term. Remaining data from VFA concentration and ammonia-N were analyzed as unbalanced completely randomized design. GLM procedure and Duncan test were used to compare differences between treatments.

## 3. RESULTS AND DISCUSSION

### 3.1 Ruminal fermentations

Concentration of total VFA profile, ammonia-N and pH in ruminal fluid at 3 h post feeding were used to observe the ruminal

fermentation pattern (Table 2). Results from analysis of rumen fluids indicated that ruminal pH and VFA concentrations were different ( $p < 0.05$ ) among diets but ammonia-N was not affected. Data obtained showed that both levels of EMTAP resulted in higher acetic, propionic, acetic: propionic ratio and total VFA concentrations, but in lower ruminal pH ( $P < 0.01$ ). These trends are similar to those described by Rumsey et al. [1978]; who worked on supplementation of AP with non-protein nitrogen (NPN) in gestating beef cows and fistulated steers, and Chumpawadee and Pimpa [2009], who worked on effect of some non-forage fiber sources such as TP in beef cattle nutrition. The rate and extent of carbohydrate degradation in the rumen affected VFA production. Rumsey et al. [1978] reported that feeding AP was associated with a slightly reduction of ruminal pH, a higher acetic to propionic acid ratio and lower proportions of ruminal branched-chain

fatty acids. Same results were also observed by Oltjen et al. [1977] when compared AP with corn silage. Due to the high content of acids components of EMTAP (pH: 3.5), especially malic and citric acid [NRC, 2001] a decrease of rumen pH was observed. At the same time its high amount of pectin (AP 15 and TP 7.55 %) can represents a substrate for rumen bacteria to produce acetate [Del Valle et al. 2006]. Concerning TP, present results are comparing with findings reported by Chumpawadee and Pimpa [2009], those authors observed that total VFA concentrations and ruminal acetate of animal fed TP were higher ( $P < 0.05$ ) compared with other (rice straw, palm meal, dried brawler gain, soybean hull) diets. The authors conclude that these results **Table 2. Volatile fatty acids, ammonia-N and pH of rumen content, feces and urine pH in cows fed diets with different content of EMTAP** are due to high carbohydrate fraction in TP.

	Diets (EMTAP levels)		
	1	2	3
<b>Ingredients</b>	0% EMTAP	15% EMTAP	30% EMTAP
Alfalfa hay	45.67	33.35	18.41
EMTAP‡	0	15	30
Soy bean meal	10.25	10.23	9.92
Barley	37.96	37.99	38.2
Fat (Oil plant)	0	0.57	0.99
Wheat bran	5.4	2.09	1.54
Caco3	0.22	0.27	0.44
Premix†	0.5	0.5	0.5
<b>Nutrient compositions</b>	<b>(% based DM)</b>		
DM	98	78.3	63.1
NEL (mcal/kg)	1.54	1.58	1.62
CP	15.4	15.5	15.5
NDF	35.4	35.2	35.1
ADF	21.4	23.1	24.3
Calcium	0.6	0.6	0.5
Phosphorus	0.4	0.4	0.4
Concentrate	54.33	51.65	51.59
Forage	45.67	48.35	48.41

‡EMTAP, ensiled mixed tomato and apple pomace; DM, dry matter; NEL, net energy for lactation; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber. †Premix supplied (on a concentrate DM basis): 400.000 IU of vitamin A/kg, 100.000 IU of vitamin D3/kg, 100 mg of vitamin E/kg, 219 mg/kg of Mn, 69 mg/kg of Zn, 116 mg/kg of Fe, 23 mg/kg of Cu, 1.8 mg/kg of I, 0.6 mg/kg of Co, and 0.46 mg/kg of Se.

**Table 2: Volatile fatty acids, ammonia-N and pH of rumen content, feces and urine pH in cows fed diets with different content of EMTAP**

	Diets (EMTAP Levels) ‡			S.E.M	P value
	1 0% EMTAP	2 15% EMTAP	3 30% EMTAP		
<b>concentrations</b>					
Total VFA, mmol/L	68.15 <sup>c</sup>	83.7 <sup>b</sup>	91.24 <sup>a</sup>	0.56	<0.01
Acetic, mmol/L	40.1 <sup>b</sup>	51.75 <sup>a</sup>	53.15 <sup>a</sup>	0.29	<0.01
Propionic, mmol/L	18.55 <sup>b</sup>	20.2 <sup>b</sup>	26.2 <sup>a</sup>	0.35	0.04
Butyric, mmol/L	8.6	9	9.55	0.18	0.25
Valeric, mmol/L.	0.7	1.55	2.55	0.36	0.25
Isovaleric, mmol/L	0.2	0.3	0.65	0.08	0.21
Acetic: Propionic, mmol/L	2.03 <sup>b</sup>	2.16 <sup>b</sup>	2.57 <sup>a</sup>	0.05	0.04
Ammonia-N, mmol/L	6.98	7.3	7.45	0.11	0.35
<b>pH variations</b>					
Rumen	6.55 <sup>a</sup>	6.31 <sup>b</sup>	6.01 <sup>c</sup>	0.04	<0.01
Faeces	7.3 <sup>a</sup>	6.65 <sup>b</sup>	6.59 <sup>b</sup>	0.12	<0.01
Urine.	8.5	8.28	8.23	0.09	0.19

Table 3: Nutrient digestibility and dry matter intake of cows fed diets differing in ratio of EMTAP.

Item	Diets (EMTAP Levels) ‡			S.E.M	P value
	1	2	3		
	0% EMTAP	15% EMTAP	30% EMTAP		
DM intake( kg/d)	21.3 <sup>b</sup>	23.7 <sup>ab</sup>	24.5 <sup>a</sup>	0.68	0.02
DM	64.24 <sup>b</sup>	66.59 <sup>a</sup>	66.84 <sup>a</sup>	0.6	0.03
OM	68.30 <sup>b</sup>	70.36 <sup>a</sup>	70.21 <sup>ab</sup>	0.5	0.04
CP	66.19	66.25	66.24	0.14	0.94
NDF	58.02	59.01	58.60	0.27	0.08

‡Diets; 1= (control or 0% EMTAP); 2= (15% EMTAP); 3= (30% EMTAP); DM intake = dry matter intake (kg/d); S.E.M = standard error of means

‡Diets, 1= (control or 0% EMTAP); 2= (15% EMTAP); 3= (30% EMTAP); S.E.M = standard error of means; a,b,c Means in the rows with different superscripts are significantly different ( $P < 0.05$ ).

### 3.2 Digestibility study

Mean values of diets digestibility are shown in Table 3. The results showed that DM and OM digestibility tended significantly ( $P < 0.05$ ) to increase with add EMTAP in the diet. It was showed by several studies [Ibrahim and Alwash 1983; Ojeda and Torrealba 2001] that TP can improve the nutritional value of the diet, due to its content in more digestible protein (61.2 %) and ether extract (86.3 %). Rumsey et al [1978] reported that AP is similar to corn silage for total digestible nutrients content as pectin, pentosans and ether extract. (Already reported before) Generally, the presence of more nitrogen free extract (NFE), appreciable quantities of soluble carbohydrates [NRC 2001; Hang and Woodams 1986, Rumsey et al. 1978] and

pectin [Del Valle et al. 2006] in AP and TP, may lead to higher digestibility of DM and OM in diets containing EMTAP respect to the control diet.

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