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EFFECT OF THE USE OF ENSILED APPLE BAGASSE ON PERFORMANCE AND RUMINAL FERMENTATION IN LAMBS

I. Mejía-Haro*,[1], M. De Alba-González, J.M. Martínez-Mireles[1], C.F. Aréchiga-Flores[2] and J. M. Silva-Ramos[2].

[1]Instituto Tecnológico El Llano, TecNM. Km 18, carretera Ags. - S.L.P., El Llano, Ags. C.P. 20330. México
[2]Unidad académica de Medicina Veterinaria y Zootecnia de la Universidad Autónoma de Zacatecas, México.
arechiga@uaz.edu.mx
*Corresponding author's E mail: ignacio.mh@llano.tecnm.mx

ABSTRACT

This study evaluates the effects of ensiled apple bagasse (EAB) in lamb diets on their performance and ruminal fermentation. Eighteen Dorper-Kathadin weaned, intact, male lambs (initial body weight 23.070 ± 2.207 kg) were randomly assigned to receive one of three treatments for 77 days: T1 (control diet without EAB); T2 (diet with 20% EAB); and T3 (diet with 40% EAB). The diets were formulated to be isonitrogenous, and the diets' nutrient compositions was determined. Daily feed intake was measured, lambs were weighed monthly, and the feed conversion ratio was calculated at the end of the experiment. VFAs and N-NH_3 production and the pH of ruminal fluid were determined. The data were analyzed by analysis of variance using SAS, and the means were compared using the Tukey test ($P < 0.05$). Daily weight gain was not different among treatments ($P > 0.05$). The feed conversion ratio was lower for T1 and T2 than T3 in the growing period ($P < 0.05$) but was not different in the finishing period. VFA production was higher in lambs fed T3 than those fed T1 and T2 ($P < 0.05$). No differences were observed in the proportion of acetic acid and N-NH_3 concentration. The propionic acid production was higher for T1 compared to T2 and T3, and butyric acid was higher for T3 than T1. The results show that EAB can be used as an alternative for lamb diets to reduce production costs.

Key words: Sheep, apple pomace, weight gain.

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INTRODUCTION

In animal production, it is important to formulate economical and efficient diets to maintain the profitability of livestock farms. One way to achieve this is through the use of low-cost feed ingredients, such as agro-industrial by-products and crop residues. In Aguascalientes, Mexico, apple pomace from the production of apple juices and nectars is available from August to October. It can be kept fermented in silos and used to feed sheep all year round. At the same time, environmental pollution is reduced by integrating it as a fertilizer in crops, particularly the acidification of agricultural soils.

Both waste apples and by-products provided by the industrialization of this fruit are a potential feed for livestock and contain highly fermentable metabolic substrates (Islam *et al*., 2018). Apple bagasse is known to be highly acceptable by several livestock species but has a low protein content. However, it has nutritional potential in its energy content derived from low-digestibility fiber and soluble carbohydrates (Ajila *et al.,* 2015; Xia *et al*., 2021).

In Mexico, apple bagasse is generally considered a by-product of low interest, although it is utilized in dairy cattle (Steyn *et al*., 2017), protein blocks (Bustamante, 2015), and fattening lambs (Mejia-Haro *et al*., 2018; Alarcon-Rojo *et al*., 2019). The most important nutritional component of apple bagasse is energy derived from low-digestibility carbohydrates (Bustamante, 2015; Mejia *et al*., 2018¸Thomas *et al.,* 2020). Its other components, such as dry matter and crude protein, show a certain degree of variation depending on the variety of apple, fruit maturity, and differences in processing (Lyu *et al*.,2020). The objective of this study was to evaluate the performance and concentrations of the main products of ruminal fermentation in lambs fed mixed diets based on ensiled apple bagasse (EAB).

MATERIALS AND METHODS

This study was carried out in the animal production facilities of the Technological Institute El Llano in El Llano, Aguascalientes, Mexico. The facilities are located at 21° 55 'N and 101° 58' W at an altitude of 2020 m above sea level in an area with an annual rainfall

of 500 to 600 mm. Apple bagasse silage was prepared using 7 tons of fresh bagasse (Table 1) obtained from an apple juice factory (Valle Redondo) and 58 kg/ton of a concentrate mixture consisting of 30.36% soybean hulls, 30.67% soybean meal, 31.8% chicken manure, 6.5% urea, 0.67% microminerals, and 0.1% vitamins. The prepared mixture was pressed and covered with black plastic, left under the sun, and allowed to ferment for 60 days. It was then used in mixed diets in a performance trial with 18 intact weaned Dorper-Kathadin lambs (23.070 ± 2.207 kg, initial BW). The trial lasted for 77 days and had two periods: a growing period (from 23 to 30 kg of lamb live weight (LW)) and a finishing period (from 30 to 46 kg).

One week before the end of the experiment, ruminal fluid samples (about 150 mL) were collected from each lamb 6 hours after feeding at 09:00 AM by suction with a vacuum pump and a ½-inch plastic probe with blunt edges and one end with holes. Immediately after extracting the ruminal fluid, the pH was measured in all samples. Then, the ruminal fluid was used for laboratory analysis of volatile fatty acids (VFA) and ammonia nitrogen (N-NH_3). *In situ* degradability of DM was determined using four rumen-fistulated Holstein cows with a fermentation period of 72 hours (Orskov *et al*., 1980). Cows were used as a model for lambs since lambs were not available.

At the start of the experimental period, lambs were housed in individual elevated crates provided with feeder and automatic drinker. They were dewormed internally with ivermectin and levamisole (1 mL/20 kg^{-1} LW), vaccinated (*Pasteurella* and *Clostridium*), ear-tagged, and injected with vitamins (ADE, 1 mL $lamb^{-1}$). Lambs were provided with feed *ad libitum* at 09:00 and 15:00 every day. After a 15-day period for adaptation to the diets, they were assigned to one of three dietary treatments in a completely randomized design: T1, (a mixed control diet with 21% crude protein, without EAB); T2 (mixed diet with 20% EAB (DM)); and T3 (mixed diet with 40% of EAB), as shown in Table 2. The diets were formulated to be isonitrogenous, and their nutritional composition was analyzed. The feed intake and orts were measured every day for each lamb, and the lambs were weighed using a digital scale for two consecutive days at the beginning and end of the growing and finishing periods. Lambs in the finishing period were fed a diet containing 18% crude protein (% CP= %N x 6.25), as shown in Table 3.

The feed conversion ratio (kg of dry matter used per kg of LW gain) was calculated from feed intake and body weight-gain data in each period. Proximate analysis (AOAC, 2012) and neutral and acid detergent fiber analysis (Van Soest *et al*., 1991) were performed on samples of the diets. The response variables for the growing and finishing periods were feed intake (FI) in the period per kg LW and per $kg^{0.75}$; daily weight gain (ADG); feed conversion ratio (FC); final LW; ruminal pH; molar production of VFA; molar proportions of the ruminal concentrations of acetic, propionic, and butyric acids; the ratio of the concentration of acetic acid to propionic acid; the NH_3-N concentration in ruminal fluid; and *in situ* degradability of diets.

A solution of 20% ruminal fluid and metaphosphoric acid at a ratio of 4:1 v/v was prepared to determine VFA, and samples were kept refrigerated at 4°C, until analysis by gas chromatography (Perkin Elmer® Co., Clarus 560 D Gas Chromatograph, Erwin *et al*., 1961). NH_3-N was measured using a spectrophotometer (VARIAN CARY-1E, McCullough, 1967). Ruminal pH was measured using a Hanna HI 98130 portable potentiometer when the individual ruminal fluid samples were obtained. *In situ* degradability of the diets was tested in four ruminal fistulated cows using the technique reported by Orskov *et al*. (1980).

The data were subjected to an analysis of variance using SAS (2008), and the means were compared using a Tukey test ($P < 0.05$). The statistical model was as follows:

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$

where Y_{ij} is the response variable, μ is the general mean, T_i is the effect of the i^{th} treatment, and ε_{ij} is the experimental error.

Table 1. Chemical composition (g/kg^{-1} DM) and energy content of ensiled apple bagasse.

Item	(g/kg)
DM	212
CP	55
EE	35
CF	273
ASH	19
NFE	593
NDF	409
ADF	379
ME (Mcal/kg)*	2.510
TDN*	678
NFC*	482

*Calculated, DM: Dry Matter; CP: Crude Protein, EE: Ether Extract; CF: Crude Fiber; NFE: Nitrogen
Free Extract, NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; ME: Metabolizable Energy,
TDN: Total Digestible Nutrients; NFC: Non-fiber Carbohydrates.
NFC= 100 - (%NDF+%CP+%EE+%ASH), NRC (2001).

Table 2. Feed ingredients and chemical composition (DM) of the growing lamb diet

INGREDIENT	CONTROL (T1)	20% EAB (T2)	40% EAB (T3)
Alfalfa hay	30	25	19.7
Soy bean meal	15	5.5	0
Sorghum grain	13.3	8.2	4.7
Molasses	8	8	8
Tallow	3	5.6	6.5
Rolled corn	16	6	0
Meat and bone meal	5	5	4
Microminerals	0.1	0.1	0.1
Salt	0.3	0.3	0.3
Sodium bicarbonate	1	1	1
Aluminosilicates	0.3	0.3	0.3
Urea	0	0	0.4
Soy plus	8	9	9
Ensiled apple bagasse	0	20	40
Cotton seed meal	0	6	6
Total	100	100	100
	Chemical Composition* (g/kg)		
DM	871.2	593.0	448.6
CP	210.9	210.9	209.8
EE	57.9	74.0	77.8
ASH	98.5	126.8	155.6
NFC	456.1	363	337
NDF	176.6	224.9	267.4
ADF	134.0	180.7	213.5
ME (Mcal/kg)**	2.57	2.57	2.57
Ca **	9.4	9.3	8.3
P**	5.3	5.6	5.1
% *in situ* DM *"*Degradability	62	60	60

*Determined in laboratory, **Calculated from NRC (2007). "Incubation of diets for 72 hours in 4 ruminally fistulated cows. NFC= 100-(%NDF+%CP+%EE+%ASH), NRC (2001).

Table 3. Feed ingredients and chemical composition (DM) of the finishing lamb diet.

INGREDIENT	CONTROL (T1)	20% EAB (T2)	40% EAB (T3)
Alfalfa hay	20	20	19.5
Soy bean meal	10.5	8	4.5
Sorghum grain	26.35	7.2	4.8
Molasses	8	8	8
Tallow	2.5	5.6	6.5
Rolled corn	23	13.5	4.9
Meat and bone meal	5	5	4
Microminerals	0.1	0.1	0.1
Salt	0.3	0.3	0.3
Sodium bicarbonate	1	1	1
Aluminosilicates	0.3	0.3	0.3
Urea	0.95	0	0.1
Soy plus	0	0	0
Ensiled apple bagasse	0	20	40
Cotton seed meal	0	6	6
Soy hulls	2	5	0
Total	100	100	100
	Chemical Composition* (g/kg)		
DM	872.1	592.6	448.4

CP	180.0	180.2	180.9
EE	51.3	74.1	78.0
ASH	73.9	124.0	155.0
NFC	534.4	372	310.4
NDF	160.4	249.7	275.7
ADF	108.8	176.7	204.6
ME (Mcal/kg)**	2.582	2.579	2.563
Ca **	8.4	9.1	8.5
P**	5.1	5.6	5.3

*Determined in laboratory, **Calculated from NRC (2007).
NFC= 100-(%NDF+%CP+%EE+%ASH), NRC (2001).

RESULTS AND DISCUSSION

Feed intake: The feed intake of lambs in the growing period was higher ($P < 0.05$) for T3 (40% EAB) than either T1 or T2 in terms of kg, g kg^{-1} LW, and g / $kg^{0.75}$. Lambs receiving T2 (20% EAB) had a greater feed intake than those with T1 (0% EAB) in terms of g kg^{-1} LW (Table 4). The palatability of apple bagasse silage could have increased the feed intake of T3 lambs, but this increase was no longer observed in lambs in the finishing period. This probably occurred because lambs and microbes of the rumen were already well adapted to the respective diets (Mahgob *et al*., 2000). Chemical changes in the EAB could also affect its palatability. In addition, the small particle size of the T3 diet achieved by the greater inclusion of EAB could influence feed intake by increasing the consumption of finely chopped feed, which could minimize the effect of the physical filling of the rumen (Fimbres *et al*., 2002).

Taasoli and Kafilzadeh (2008) also reported increases in feed intake in growing lambs fed diets containing 30% EAB in intensive systems, which did not occur in the fattening period. In contrast, Rumsey and Lindahl (1982) fed gestating ewes a diet containing 60% (DM) fresh apple bagasse until parturition and observed that energy intake was deficient. This was especially apparent in the last third of pregnancy, when nutrient intake was limited by the high moisture and low nutrient content of apple bagasse. In our study, lambs' energy and protein requirements were met, the EAB content was limited to 20 and 40%, and the EAB contained less moisture because it was mixed with other by-products in the silage process.

Pedraza and Pacheco (2000) obtained average feed intakes between 55 and 60 g DM $kg^{0.75}$ in 24-kg male sheep fed *Digitaria decumbens* hay. These values are below those found in our study (98 and 120 g DM $kg^{0.75}$ for growing lambs fed diets containing 20 and 40% EAB, respectively). In part, this could have occurred because of the higher fiber content and lower energy diets, which increase the ruminal retention time. Consequently, the intake of dry matter decreases (Paulina *et al*. 2013).

Table 4. Performance of lambs in growing period fed diets containing different concentrations of EAB.

Variable	T 1	T 2	T3	MSE
Initial LW (kg)	23.23	22.56	23.20	2.207
Final LW (kg)	32.05 [a]	31.98 [a]	32.07 [a]	3.395
Feed Intake, (kg)	31.707 [b]	35.255 [b]	43.502 [a]	4.950
Feed Intake/kg LW, (g)	36.95 [c]	41.66 [b]	50.87 [a]	4.279
FI (g/$kg^{0.75}$)	87.9[b]	98.4[b]	120.3[a]	9.8
ADG (g d^{-1})	287 [a]	303 [a]	287 [a]	53
TGP (kg)	8.821 [a]	9.417 [a]	8.877 [a]	1.621
FC	3.63 [b]	3.79 [b]	4.92 [a]	0.392

[abc]Different letters in the same row indicate differences among treatments, $P < 0.05$.
MSE= Mean Standard Error; Initial LW= Initial Body Weight; Final LW= Final BW; FI= Feed Intake (DM); ADG= Average Daily Gain; TGP= Total Weight Gain in the Period; FC= Feed Conversion Ratio

Weight gain: No differences ($P \geq 0.05$) were observed in daily weight gain of lambs (Tables 4 and 5) among treatments in both growing and finishing periods. This is partly because the diets of the three treatments were formulated to be isocaloric and isonitrogenous, and the degradability of DM was not different since the EAB carbohydrates have high-digestibility components (Castillo *et al*., 2011). It is suggested that EAB contained similar digestibility to the control diet, which results in no changes in ADG of lambs (Taasoli and Kafilzadeh, 2008). When protein exceeds the requirements, only differences in energy below 2.6 Mcal/kg are reflected in ADG (Rios-Rincon *et al*., 2014; Saro *et al*., 2020). In our experiment, protein exceeded the requirements and the metabolizable energy was in the limit of 2.6 Mcal/kg and was not different among treatments.

Mejía-Haro *et al*. (2018) obtained weight gains of 183 and 148 g d^{-1} in lambs using 30% EAB in diets with and without poultry excreta, respectively. These values are lower than those obtained in this study, partly because female lambs were used, which have lower weight gain than male lambs (Bores *et al*., 2002). They also used low-true protein feeds such as poultry excreta and urea. Furthermore, alcoholic fermentation of apple

bagasse was achieved in the silage process, which influences the feed intake (Castillo *et al*., 2011).

Taasoli and Kafilzadeh (2008) fed male lambs diets containing ensiled (30% DM) or dried (20%) apple bagasse and reported daily weight gains of 200 and 192 g, respectively. These values are lower than those obtained in our study, in part because they used diets containing higher fiber contents. Furthermore, they did not include molasses as a flavoring for apple bagasse silage, which stimulates feed intake (Mordenti *et al*., 2021). The breed used could also have possibly influenced the response of the lambs in weight gain.

Feed conversion ratio: Values of feed conversion ratio in growing lambs (Table 4) were lower for T1 and T2 than T3 ($P \leq 0.05$). This could be due to the high moisture content of apple bagasse and higher NDF and ADF content in the T3 diet, which had the largest amount of EAB. This large amount could have filled the capacity of the rumen, producing an increase in the rate of passage (Allen, 1996). This could result in greater intake and decreased DM digestibility with respect to T1 and T2.

The feed conversion ratio in lambs in the finishing period (Table 5) did not show statistical differences ($P \geq 0.05$) among treatments, which could be due to the fact that the rumen volume of the T3 lambs increased with respect to lambs of T1 and T2 due to the filling stimulus (Owens and Goetsch, 1993). Therefore, no differences in feed intake and daily weight gain were observed among treatments. The FC values obtained are similar to those reported by Taasoli and Kafilzadeh (2008) in male sheep fed diets containing ensiled apple bagasse. Higher feed conversion ratios were reported by Mejía-Haro *et al*. (2018) in female lambs fed a diet containing 30% apple bagasse with 0 and 20% poultry excreta (6.24 and 5.72 kg of feed/kg of body weight gain, respectively).

Table 5. Performance of lambs in finishing period fed diets with different concentrations of EAB.

Variable	T1	T2	T3	MSE
Initial LW (kg)	32.054	30.983	32.075	3.390
Final LW (kg)	46.813[a]	44.942[a]	46.258[a]	4.194
Feed Intake, (kg)	68.235[a]	63.315[a]	68.888[a]	5.548
Feed Intake/kg LW, (g)	37.70[a]	35.86[a]	38.30[a]	4.950
FI ($g/kg^{0.75}$)	144.5[a]	136.6[a]	146.7[a]	5.4
ADG ($g\ d^{-1}$)	320[a]	303[a]	310[a]	55
TGP (kg)	14.758[a]	13.958[a]	14.183[a]	2.562
FC	4.78[a]	4.57[a]	4.93[a]	0.687

[abc]Different letters in the same row indicate differences among treatments, $P < 0.05$.

MSE= Mean Standard Error; Initial LW= Initial Body Weight; Final LW= Final Body Weight; FI= Feed Intake (DM);

ADG= Average Daily Gain; TGP= Total Weight Gain in the Period; FC= Feed Conversion Ratio

Ruminal Fermentation Products: The total VFA concentration (Table 6) was higher ($P < 0.05$) for T3 than T1 and T2, and no differences between T1 and T2 were found. The reason is unclear, and differences in the degradability of NDF among treatments could have influenced the results. Although the chemically determined NDF in the T3 diet was higher than in T1 and T2, the reduced particle size, specific gravity, and high content of pectin and other components of the EAB could favor the degradability and consequently increase molar production of VFA (Allen and Grant, 2000; Del Valle *et al*., 2006).

All three diets contained 8% molasses, which provides rapidly available carbohydrates in the rumen. In addition, T3 included urea, which was also a component of EAB and provided rapidly available N. Along with molasses, it is a source of easily degradable carbohydrates that benefit the microbial synthesis of the rumen and its fibrolytic activity (Gozho and Mutsvangwa, 2008). A higher production of VFA was associated with lower ruminal pH values for T3, which had lower pH than T2 and T1. Abdollahzadeh and Abdulkarimi (2012) also found higher VFA production and lower ruminal pH in dairy cows fed diets where 15 or 30% of alfalfa was replaced with a mixture of apple and tomato pomace silage.

Acetic acid production was similar among treatments ($P > 0.05$), and propionic acid content was higher for T1 ($P < 0.05$) than in treatments with EAB. This can be explained by the T1 diet containing a higher percentage of grains than T2 and T3, which favors the production of propionic acid and decreases the acetate-to-propionate ratio. In contrast, butyric acid content was higher ($P < 0.05$) for T3 than for T1. These values are influenced by the concentration of propionic acid, which in turn is favored by the level of grains in the diet (Agle *et al*., 2010; Ramos *et al*., 2021).

The acetate-to-propionate ratio was higher for T3 than for T1, and no differences were observed between T1 and T2 ($P > 0.05$). The ratio of acetate-to-propionate for T3 indicates that for every 2.6 units of acetate, one unit of propionate is obtained. However, in fattening cattle, a lower ratio is desirable, which is achieved with high-concentrate diets (Agle *et al*., 2010) when the amount of concentrate is greater than 70% and the VFA ratio (acetic:propionic:butyric acid) is 45:40:15. In our study, the average ratio was 56:27:16. Mejia-Haro *et al*. (2019) found a similar value (58:29:14) in lamb diets containing 30% forage.

The concentration of NH_3-N in rumen is a balance between its production and its use or absorption. NH_3-N is a critical nutrient for ruminal microorganisms in concentrations ranging from 1 to 29 mg / 100 mL^{-1} (Khalili and Sairanen, 2000). Several factors influence the concentration of NH_3-N in rumen fluid: degradability of the dietary protein, availability of carbohydrates,

sampling time after feeding, level and rate of intake, and inclusion of urea in the diet (Castillo-Lopez and Domínguez-Ordóñez, 2019). In our study, the average concentration of NH_3-N was not different ($P > 0.05$) among treatments (Table 6), which suggests similar protein degradability and utilization among treatments. T2 and T3 presented values of 5 to 8 mg/100 mL^{-1}, which has already been reported and considered optimal for an adequate production of microbial protein (Kim *et al*., 2010). Mata-Espinoza *et al.* (2006) also found no differences in ruminal NH_3-N concentration in lambs supplemented with meal from three tropical forage shrubs.

The degradability of proteins is directly related to their solubility within the rumen. When it is low, the release of ammonia decreases, so the synthesis of microbial protein is limited by the lack of this compound (Castillo-Lopez and Domínguez-Ordóñez, 2019). The average ruminal pH value was lower in T3 lambs than in T1 and T2 lambs and was reflected in a higher concentration of VFA. Abdollahzadeh and Abdulkarimi (2012) also found a reduction in pH while increasing the production of VFA as a result of an increase in the degradation rate of apple pomace and other feed by-products.

Table 6. Average values of ruminal pH, VFA production, and ammonia nitrogen in ruminal fluid of lambs.

Item		T1	T2	T3	SEM
VFAt	mM L^{-1}	54.81[b]	64.25[b]	92.66[a]	9.816
Acetic	%	54.66[a]	56.26[a]	58.27[a]	2.652
Propionic	%	32.50[a]	27.20[b]	22.68[b]	3.157
Butyric	%	12.81[b]	16.56[ab]	19.10[a]	2.835
A:P		1.70[b]	2.11[ab]	2.60[a]	0.331
NH_3- N	mg dL^{-1}	2.97[a]	7.13[a]	7.28[a]	3.291
Ruminal pH		6.63	7.06	6.37	0.55

A:P= Acetate-to-Propionate Ratio
VFA_t= Total Volatile Fatty Acids
[ab] Different letters in the same row indicate differences among treatments ($P < 0.05$); SEM= Standard Error of the Mean.

Conclusions: The results indicated that the grains in the lamb diets could be replaced with EAB at up to 20 or 40%. Comparable performance and no significant differences were observed in the ruminal fermentation products. These results suggest there was similar efficiency of feed utilization.

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