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Article

Grape pomace silage (*Vitis labrusca* L. cv. Isabel) on the intake and digestibility of nutrients, nitrogen balance and ingestive behavior of lambs

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Abstract:

This study investigated the inclusion of grape pomace silage (GPS; 0, 10, 20 and 30%) were evaluated in diets of lambs on nutrient intake and digestibility, nitrogen balance and ingestive behavior. Four lambs of the Santa Inês breed with weight of 21.93 ± 0.87 kg and approximately seven months old, were housed in metabolic cages and distributed in a 4x4 latin square design. The treatments consisted of four diets with the inclusion of 0, 10, 20 and 30% GPS in diets. The nutrient intake was observed an increasing linear behavior for ether extract (EE) intake (P<0.05) according to the increase of EE in the diets, caused by content of EE of seeds in GPS. The diets did not differ in the digestibility coefficients of nutrients and nitrogen balance (*P*>0.05), with average digestibility of dry matter digestibility (DDM) of 678.6 ± 0.62 g kg^{-1} DM and average retention of 239.78 g kg^{-1} N ingested of N. The ingestive behavior the diets were influenced (*P*<0.05) by only the length of time that the animals remained idle in standing. This parameter showed a quadratic behavior with a maximum point estimated at 17.73 % of GPS (*P*=0.041). In conclusion, the use of GPS can be used until inclusion level of 30 % without negatively affecting the parameters evaluated.

Key words: Byproducts of fruits, Behavior, Intake, Digestibility, Lambs, Silage.

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Introduction

The use of alternative feeds as byproducts that help supply ruminant animal demand for nutrients in times of low pasture supply, mainly during winter or drought periods, arouses the interest of researcher's different areas, including feeds conserved. The primary sector annually generates tons of organic byproducts with excellent nutrient composition(1) that could be transformed into meat, milk, skin and wool by ruminants(2) and consequently can reduce threats of environmental pollution, since part of this byproduct is improperly stored or discarded in environment. Recent research has suggested partially replacing cereals grains by agricultural byproducts in feed animal(2,3,4), in order to promote more sustainable production. In addition, the use of byproducts of different fonts of raw material may contribute to meet consumer demand, regarding the sustainability of animal production systems and maintaining the integrity of the environment.

The use of agricultural and industrial byproducts is present from the production of chemical products to animal feed(1,5). The grape destined for wine industry and juices, for example, generation quantities of byproducts, as pomace and seeds, which offer risks economic and environmental(6). However, this byproduct is an alternative source of fiber, have low commercial cost, chemical composition of quality(7) and has traditionally been incorporated in ewe diets and lambs(8,9,10). Recent study showed the viability for storage in the form of silage, with satisfactory amounts of residual sugars and fibers, which meet the desirable characteristics of feed conserved(11). It is also an alternative for ensure silage throughout the year and proper destination of this byproduct. The use of byproducts evens can contribute with small farms which haven't areas of lands available for crops intended for the production of traditional silage, as whole corn and forages.

The use of grape pomace in diets for lambs had showed results considerable on nutritional composition, performance, nutrient consumption and acceptability by animals[10,12,13]. Although there are results in the performance of lambs with the inclusion of only grape pomace, the supply of this byproduct in the form of silage and the limitations of the respective levels of inclusion, related to the fiber content and ether extract of the seeds deserve to be investigated, since there is variety in the grape cultivars that can offer different effects qualitative on the silages and performance of animals. Based on this hypothesis, this work was carried out with the objective of evaluating the inclusion 0, 10, 20, 30 % of grape pomace silage (*Vitis labrusca L.* cv. Isabel) in diets of lamb and its effects on nutrient intake and digestibility, nitrogen balance and behavior ingestive.

Material and methods

Experimental animal, handling and diets

The study was carried in the sheep metabolism shed the school farm and Laboratory Animal Nutrition of the State University of Londrina, Paraná, Brazil in the July of 2012**.** All procedures in this study were conducted according the Ethics Committee on Animal Experiments of this University and approved under the identification number (Protocol nº 78/10).

Four lambs of the Santa Inês breed, male, castrated, with an average weight of 21.93 ± 0.87 kg and approximately seven months old with urine collecting fund, individual troughs for food and mineral supplement, as well as drinking fountain. The experimental design was a 4x4 Latin square, with four periods and four treatments. The animals underwent initial adaptation to the diets of 21 d, followed by 4 d for sample collection of feces, urine, and of the feed provided and leftovers in each period, and 1-d for behavioral data. The following collection periods were preceded by 10 d of adaptation for subsequent diets. The animals were weighed at the beginning and end of each period to adjust the intake and quantify the voluntary consumption of dry matter. The feed was given in two meals a day, and at 0730 h and 1630 h, adjusted daily in such a way that there was 15 % of the dry matter supplied, in order not to restrict consumption.

The planting of the sorghum (*Sorghum bicolor* L., cv. AG 2002) was carried out on the school farm of the State University (FAZESC-UEL) located in Londrina, Paraná (23º20'10" south latitude and 51º09'15" west longitude, 610 m high). The sorghum used for silage production was cultivated under a no-tillage system with planting in the October of 2011. The cut whole plant occurred with 28 % DM in the month of May 2012 with second cut of the plant, after cutting the sorghum was stored in a bunker silo compacted with tractor in layers and covered with plastic canvas protected by a 15 cm layer of soil.

The grape pomace byproduct cultivar Isabel (*Vitis labrusca* L.*)* collected from a homogeneous lot, directly from the juice industry (COROL, Rolândia, Paraná) after processing. The byproduct of Isabel grape (*Vitis labrusca* L.) and was largely composed of seeds (610 g kg^{-1} dry matter (DM)) peels and pulp residue (390 g kg^{-1} DM). At the time of collection in industry, the byproduct was 11% DM and was dehydrated outdoors, being turned three times a day, until reaching approximately 30% DM. After dehydration of byproduct was added 5 g kg^{-1} as fresh matter (FM) of urea as a chemical additive using manual mixing equipment. The ensiled mass of byproduct (grape pomace) was stored in February of 2012 in silos of the type plastic drums with a capacity of 100 to 200 liters with sealing lids. The storage time was five months in a covered shed until the date of opening the silos for beginning of the experiment. The chemical characteristics of grape pomace silage are represented in Table 1 and in this work about fermentative quality of grape pomace silage cv. Isabel (*Vitis labrusca L.*)[11].

Four isoproteic (160.46 ± 0.21 g kg^{-1} DM of CP) and isoenergetic (674.85 ± 5.23 g kg^{-1} DM of total digestible nutrients (TDN)) diets were employed, and grape pomace silage (GPS) was included at 0, 10, 20, and 30% of the DM base maintaining the bulk concentrated ration of 55:45 (Table 1). Initially, a standard diet was formulated (treatment without the inclusion of GPS, 0 %) and from this diet the others were made, removing 10, 20 and 30 % of sorghum silage and including 10, 20 and 30% of GPS. Due to the differences in the composition of sorghum silage and GPS, the levels of corn and soybean meal were changed to obtain less variation in the protein and TDN contents of the diets. For each 10 % inclusion of GPS, the corn content was increased by 1 % and the soybean meal content reduced by 1 %.

Table 1: Levels of ingredients and chemical composition of diets and ingredients (g/kg DM^{-1})

	Levels of GPS (%)			
Ingredients, g kg^{-1}	**0**	**10**	**20**	**30**
Sorghum silage	550.0	495.0	440.0	385.0
Grape Pomace silage	0.0	55.0	110.0	165.0
Corn grain	240.0	250.0	260.0	270.0
Soybean meal	210.0	200.0	190.0	180.0
Total	1000.0	1000.0	1000.0	1000.0
Chemical composition of diets				
DM	537.3	538.8	540.3	541.8
OM	940.9	943.4	945.8	948.3
CP	160.0	160.3	160.6	161.0
EE	21.2	25.1	29.0	32.9
NDF	454.4	451.6	448.8	446.0
ADF	264.3	269.2	274.1	279.0
TDN	662.7	670.8	678.9	687.0
Chemical composition of ingredients	Corn	Soybean meal	SS[3]	GPS
DM	885.6	897.9	278.6	305.9
OM	984.8	935.0	924.0	959.9
CP	90.1	505.9	58.4	139.8
EE	37.5	14.8	16.5	83.4
NDF	163.6	166.4	691.4	640.7
ADF	37.0	68.5	438.3	533.1
TDN	823.5	818.2	533.2	679.3
DIVDM	-	-	-	461.2

DM (Dry matter), OM (Organic matter), CP (Crude protein), EE (Ether extract), NDF (Fiber insoluble in neutral detergent), ADF (Fiber insoluble acid detergent), TDN (Total digestible nutrients), DIVDM (*In vitro* dry matter digestibility, GPS (Grape pomace silage), SS (Sorghum silage).

The TND contents of the ingredients used in the formulation of the diets were estimated according to the equations proposed by Kearl[14]. For sorghum silage (SS) and grape pomace silage (GPS) the equation used was: %TND= - 21.9391 + (1.0538 x CP) + (0.9738 x NNE) + (3.0016 x EE) + (0.4590 x CF); where CP= crude protein, NNE= non-nitrogen extractives, EE= ether extract. For soybean meal: %TND = 40.3217 + (0.5398 x CP) + (0.4448 x NNE) + (1.4223 x EE) - (0.7007 x CF), where CF= crude fiber. Finally, for corn grain: %TND = 40.2625 + (0.1969 x CP) + (0.4028 x NNE) + (1.903 x EE) - (0.1379 x CF).

Intake, digestibility of nutrient, and nitrogen balance

Weighed the supplies and leftovers daily to adjust consumption, at the end of the adaptation period, for four consecutive days, samples of supplies were collected, leftovers directly in the trough, feces and urine were collected with the aid of a bag and bucket collector. The nutrient intake was estimated by subtracting nutrients from leftover nutrients. The percentage apparent digestibility was estimated to according Coelho and Leão[15] where: Apparent digestibility = ((Nutrients supplied (g) - Nutrients in leftovers (g)) / (Stool nutrients (g))) * 100. To determine the nitrogen balance the urine was collected and measured second Schneider and Flat[16]. The samples of feces, urine and feed supplied and rejected were analyzed for the nitrogen contents and calculated nitrogen retention according to Decandia *et al*[17] being: N retained = N ingested - (fecal N + urinary N); N ingested = (N supplied - N left over).

The samples of diets, ingredients, feed leftovers, feces and urine were collected and analyzed for dry matter (DM), organic matter (OM), crude protein (CP), nitrogen (N), ether extract (EE) according to the methodology of AOAC[18] described by Mizubuti *et al*[19], neutral detergent insoluble fiber (NDF), acid detergent insoluble fiber (ADF) assayed with a heat stable alpha amylase and corrected for ash according to the methodology of Van Soest[20] described by Detmann *et al*[21]. Total carbohydrates (TCHO) and non-fibrous carbohydrates (NFC) were calculated according to the proposed equations[22]. To determine the percentage of seeds, 500 g of the grape pomace was separated using a sieve and tweezers, in seeds and seedless portion. Subsequently, the portions were pre-dried for 72 h at 55 ºC in an oven with forced air circulation, crushed and analyzed for the final DM contents[18]. The *in vitro* digestibility of DM was estimated using the two-stage digestion technique according to the technique proposed by Tilley and Terry[23] and adapted by Mizubuti *et al*[19].

Ingestive behavior

The ingestive behavior was evaluated during 24 consecutive hours by means of direct observations at 5 min intervals performed on the fifth day of each of the four periods of data collection of the experiment, totaling 288 observations per period according to the method of Martin and Bateson[24].

A total of six trained observers made direct observations in pairs, during a period of 6 h of observation. One of the pairs took turns the observation period at dawn with rest during the day to complete the 24 h of observation. The observers were positioned strategically near the cages not to interfere with the behavior of animals. The artificial lighting was made of low incidence luminous flux lamps and fixed to the shed structure for the night observations. The ingestive behavior was observed after 7 d of adaptation of lambs to the cages, observers, artificial lighting in the night, and environment. The time spent in feeding, rumination lying down, rumination on foot, lying down and standing idle were observed according to the methodology by Johnson and Combs[25]. The chewing and rumination parameters were measured in terms of the number of chewing and the chewing time of five ruminal bolus in each of the four periods evaluated during the 24 h of observation.

The results concerning eating and rumination efficiency expressed as g DM h^{-1} and g NDF h^{-1}, respectively, were calculated by dividing the DM and NDF intake by the total time spent eating or ruminating within a 24-h period and were obtained by means of the equations[26]:

IEDM = CDM / FCON, where IEDM= Dry matter intake efficiency (g/h), CDM= consumption of dry matter (g/d), FCON= Feed consumption time (hours); IENDF= CNDF/FCON, where IENDF = Intake efficiency of neutral detergent insoluble fiber (g/h), CFDN= Consumption of neutral detergent insoluble fiber (g/d); DMRE= CMS / (TRP + TRD), where DMRE= Dry matter rumination efficiency (g/h), SRT= Standing rumination time (hours/day), RTLD= Ruminating time lying down (h/d); RENDF = CDM / (SRT + RTLD), where ERNDF= Efficiency of rumination of neutral detergent insoluble fiber (g/h); TCT= FCON + SRT + RTLD, where TCT= Total chewing time (min/day).

Statistical analyses

The data were submitted to the Shapiro-Wilk and Bartlett tests, in order to verify the assumptions of normality test for distribution of errors and homogeneity of variance, respectively. Once these assumptions were met, the data were submitted to analysis of variance for digestibility of nutrient and nitrogen balance. The regression analysis (α= 0.05) was applicable for nutrient intake and ingestive behavior. The statistical package ExpDes of the statistical program R (Version 2013) was used to study the mean values by regression analysis, using "F" test (α= 0.05), following the model:

$$\text{Yijk} = \mu + Ti + \alpha j + \beta k + ijk$$

where:

Yijk = is the value observed in the i^{th} row and k^{th} column for the j^{th} treatment;

$\boldsymbol{\mu}$= is the general average;

$\boldsymbol{Ti}$= is the effect of the i^{th} treatment; αj= is the effect of the j^{th} line;

$\boldsymbol{\beta k}$= is the effect of the k^{th} column;

$\boldsymbol{ijk}$= is a component of random error, associated with the i^{th} row, k^{th} column and j^{th} treatment.

Results and discussion

In order to evaluate the DM and nutrient intake of the diets, it was observed that the inclusion of grape pomace silage influenced (P<0.05) in linearly increasing only the consumption of EE (Table 2). The EE in GPS was due to the higher density of the seeds (610 g kg^{-1} DM) in comparison to the bark and pulp (390 g kg^{-1} DM) constituting the grape pomace(11) and seeds, in turn, have a high oil concentration(27). Therefore, this behavior can be explained by the higher concentration of EE in the GPS and providing an increase in the concentration this nutrient in the diet, according to the increase of GPS levels inclusion.

The increase in EE in diets and intake is often observed according to the increased level of inclusion of grape residues of up to 15 %, respectively(10,12,13). The maximum level of EE of 32.9 g kg^{-1} DM offered in the diet with 30 % of inclusion in this work, does not exceed the maximum limit 50 g kg^{-1} DM proposed by Palmquist and Mattos(28).

The mean intake of DM (3.80 of live weight percentage), met the requirements of the animals and presented value higher than recommended in the NRC(29) is 3.51 % for the animal category analyzed with 30 kg and daily weight gain of 300 g/d. The FDNI recommended by Mertens(30) for ruminant animals should maintain an intake of NDF of around 1.2 % of their live weight, thus in this study the NDFI was 1.43 %, that could be related to the amount of fibrous fractions of diets of each treatment.

Table 2: Nutrient intake (g kg^{-1}) in diets for lambs containing grape pomace silage (GPS)

Intake	Levels of GPS (%)				Mean	R^2	CV	*P*-value
	0	**10**	**20**	**30**				
	g d^{-1}							
DM	1226.3±54.78	1246.4±118.32	1242.6±83.63	1226.7±121.96	1235.5±10.50		5.12	0.951
OM	1176.9±55.57	1197.5±114.50	1199.1±84.59	1183.4±114.21	1189.2±55.57		5.16	0.942
CP	220.3±16.13	222.4±34.35	224.9±18.91	218.1±23.27	221.4±2.88		6.61	0.923
EE	38.6±11.45	41.3±12.88	50.8±17.21	52.5±12.53	$\hat{Y}$=38.1+0.513x	0.92	11.22	0.021
NDF	468.8±28.97	463.5±35.23	462.1±51.56	451.9±62.72	461.6±6.13		7.34	0.937
TCHO	918.0±34.93	933.8±87.90	923.5±71.42	912.9±102.95	922.0±8.95		4.98	0.925
NFC	477.9±44.25	470.3±63.03	461.3±32.72	460.9±43.95	467.6±8.11		7.08	0.865
TDN	864.4±69.13	855.6±69.38	876.0±76.31	854.6±70.62	862.7±9.93		4.85	0.876
	g kg^{-1} of live weight							
DM	37.80±5.64	37.98±4.09	38.44±7.12	37.84±5.51	38.02±0.29		7.08	0.985
OM	36.27±5.39	36.48±3.83	37.08±6.81	36.49±5.09	36.58±0.35		7.10	0.972
CP	6.78±0.93	6.74±0.75	6.91±0.90	6.68±0.47	6.78±0.10		8.07	0.940
EE	1.18±0.36	1.25±0.33	1.55±0.47	1.59±0.21	$\hat{Y}$=1.16+0.015x	0.9	9.96	0.013
NDF	14.39±2.56	14.19±1.94	14.39±3.59	14.01±2.91	15.25±0.18		9.02	0.968
TCHO	28.31±4.34	28.50±3.51	28.62±5.88	28.22±4.84	28.41±0.18		7.13	0.991
NFC	14.59±0.90	14.31±1.88	14.24±2.37	14.21±1.96	14.34±0.18		8.07	0.962
TDN	26.61±	26.07±	27.09±	26.33±	26.53±0.44		6.80	0.869

	g kg^{-1} of live weight$^{0.75}$							
DM	90.10±10.78	90.83±8.70	91.53±13.77	90.18±11.10	90.66±0.66		6.50	0.983
OM	86.46±10.30	87.25±8.18	88.30±13.20	86.97±10.19	87.25±0.87		6.53	0.972
CP	16.16±1.82	16.13±1.89	16.48±1.70	15.95±0.92	16.18±0.22		7.62	0.940
EE	2.82±0.83	2.99±0.83	2.70±1.14	2.80±0.59	$\hat{Y}$=2.77+0.037x	0.85	10.16	0.014
NDF	34.28±5.04	33.89±3.86	34.20±7.29	33.35±6.13	33.93±0.42		8.51	0.965
TCHO	67.48±8.28	68.13±7.38	68.12±11.57	67.22±9.97	67.74±0.46		6.52	0.987
NFC	34.87±1.44	34.24±4.27	33.92±4.54	33.87±3.92	34.23±0.46		7.7	0.944
TDN	63.46±8.16	62.35±4.89	64.51±10.16	62.77±6.29	63.27±0.94		6.22	0.872
	% live weight							
DM	3.78±0.56	3.80±0.41	3.84±0.71	3.78±0.55	3.80±0.03		7.08	0.985
OM	3.63±0.54	3.65±0.38	3.71±0.68	3.65±0.51	3.66±0.04		7.10	0.972
CP	0.68±0.09	0.67±0.08	0.69±0.09	0.67±0.05	0.68±0.01		8.07	0.940
EE	0.12±0.04	0.12±0.03	0.15±0.05	0.16±0.02	$\hat{Y}$=0.116+0.002x	0.9	9.96	0.013
NDF	1.44±0.26	1.42±0.19	1.44±0.36	1.40±0.29	1.43±0.02		9.02	0.968
TCHO	2.83±0.43	2.85±0.35	2.86±0.59	2.82±0.48	2.84±0.02		7.13	0.991
NFC	1.46±0.09	1.43±0.19	1.42±0.24	1.42±1.18	1.43±0.02		8.07	0.962
TDN	2.66±0.41	2.61±0.24	2.71±0.51	2.63±0.33	2.65±0.04		6.80	0.869

DM= (Dry matter), OM (Organic matter), CP (Protein crude), EE (Ether Extract) NDF (Neutral detergent Fiber), TCHO (Total carbohydrate), NFC (Non-fibrous carbohydrates), TDN (Total digestible nutrients), CV (Coefficient of variation) , R^2 (Coefficient of determination).

These values of nutrient intake (Table 2) agrees with a study[10] that found values for DMI of 1,192, 1,144 and 1,127 g kg^{-1} for levels of 0, 10 and 20 % respectively, of inclusion of grape marc silage in diets for lambs, as well as other nutrients that are in the range for the interval observed by these authors on nutrient consumption. Some authors[12] found DMI of 1,445.8, 1,379 and 1,482.4 g d^{-1} of lambs feed with levels of 0, 5 and 10 % of wine grape pomace. Lambs fed with 10 % of wine grape pomace did not increase their DMI and had greater average daily gain that lambs in the both supplementation 0 and 5 %. Despite value DMI was higher than this study, the nutrient intake not was influenced for inclusion of grape pomace.

No variations ($P>0.05$) were observed in the apparent digestibility of nutrients as a function of the increase in the GPS contents (Table 3). Factors such as the similarity in the NDF contents of the diets (Table 1), the absence of differences in DM consumption and the association between grape pomace silage and other foods, may be associated with the similarity between the digestibility of nutrients in the diets.

Reduction in the digestibility of DM and nutrients was observed in sheep diets[31] by associating 50 % dehydrated grape residue to different energy sources, that according to the authors, the digestibility of the diets was affected by the low digestibility of the dehydrated grape residue of 30 % determined *in vitro*. It was also observed a significant reduction in the digestibility for the diets of red grape marc[32]. These authors related the decrease of digestibility with the presence of tannins and the high lignin content in grape marc. However, the values found for nutrient digestibility of the present study (Table 3) are superior to the study by Zalikarenab *et al*[32]. For DDM the average value was of 678.6 ± 0.62 g kg^{-1} DM also observed as higher as the value found by others[33] with DDM of 285 g kg^{-1} DM of when evaluating the digestibility of silage bagasse for ruminants.

It is likely that the digestibility values of the diets observed in the present experiment are due to the better utilization of grape pomace silage by the animal, considering 461.2 g kg^{-1} DIVDM of the byproduct used (Table 1). However, it is worth mentioning that the digestibility values of the nutrients found in this work, refer to the grape pomace of the Isabel *Vitis Labrusca L.* variety[11] and because there are not yet studies with this variety in the lamb feed, it is not possible to draw comparisons between the results obtained. In addition, differences in digestibility may be related to variations between the byproducts used in the diets, in addition to the type of processing and additive used for conservation. According to Rogério *et al*[34] processing in the fruit agro industries results in a great variation in the chemical composition of the generated residues, being observed variations even between lots that have undergone the same type of processing.

Table 3: Apparent digestibility of nutrients (g kg DM^{-1}) in diets containing levels-of GPS

Variables	Levels of GPS (%)				Mean	CV (%)	*P*-value
	0	10	20	30			
DDM	675.5±4.62	671.9±2.08	686.2±1.26	680.7±3.72	678.6±0.62	4.25	0.901
DOM	696.1±4.20	691.0±1.79	704.5±1.23	699.4±3.58	697.8±0.57	3.71	0.897
DCP	696.4±4.67	695.9±4.69	701.8±4.08	684.5±3.03	694.7±0.73	4.63	0.890
DEE	870.7±4.34	861.8±3.69	909.2±2.43	901.0±3.28	885.7±2.30	2.46	0.057
DNDF	621.9±5.98	559.6±4.96	590.0±4.54	559.0±5.43	582.6±2.99	5.89	0.114
DTCHO	658.3±8.76	681.4±1.55	693.2±1.64	690.8±3.82	680.9±1.59	6.06	0.116
DNFC	747.3±4.63	800.5±3.54	794.3±2.30	818.8±3.80	790.2±3.04	4.03	0.083
DTDN	700.6±2.66	690.3±1.94	705.8±2.33	701.1±2.26	699.5±0.65	1.08	0.640

DDM (Digestibility of dry matter), DOM (Digestibility of organic matter), DCP (Digestibility of crude protein), DEE (Digestibility of ether extract), DNDF (Digestibility of neutral detergent insoluble fiber), DTCHO (Digestibility of total carbohydrates) DNFC (Digestibility of non-fibrous carbohydrates), DTDN (Digestibility of total digestible nutrients). GPS (Grape pomace silage), CV (Coefficient of variation).

Table 4: Absorption, excretion and nitrogen in lambs fed diets with inclusion of grape pomace silage (GPS)

	Inclusion levels of GPS (%)						
Variable	**0**	**10**	**20**	**30**	**Mean**	***P*-value**	**CV**
Nitrogen ingested							
g d^{-1}	35.25±2.58	35.58±5.50	35.98±3.03	34.89±3.72	35.43±0.46	0.922	6.61
Nitrogen fecal							
g d^{-1}	10.53±1.74	10.86±2.61	10.71±1.65	10.95±0.93	10.76±0.18	0.977	13.72
g $kg^{-1}$of N ingested	298.81±46.46	304.08±46.86	298.15±40.83	315.42±30.27	304.12±7.99	0.837	9.91
Nitrogen urine							
g d^{-1}	18.48±0.78	14.82±3.57	17.14±4.50	14.11±2.18	16.14±2.03	0.452	25.07
g $kg^{-1}$of N ingested	525.45±26.91	417.72±79.18	473.27±101.3	407.98±74.14	456.11±54.4	0.352	20.79
Nitrogen retain							
g d^{-1}	6.24±2.40	9.90±4.78	8.13±2.04	9.84±4.10	8.53±1.73	0.546	45.87
g $kg^{-1}$of N ingested	175.74±58.95	278.20±120.2	228.58±67.47	276.60±89.44	239.78±48.5	0.556	46.37

CV (Coefficient of variation), N (Nitrogen).

The parameters of ingestion, fecal excretion, urinary excretion and nitrogen retention were not influenced (*P*>0.05) by the diets (Table 4), and possibly that the use of isoproteic diets and crude protein consumption were not influenced by diets, are the reasons for the similarity observed for the nitrogen balance between diets and similar CP levels. The positive balance of the nitrogen contents with mean values of 8.53 g d^{-1} of nitrogen retain and 239.78 g kg^{-1} nitrogen ingested may indicate, that there was retention of protein in the animal body, providing conditions so that no weight loss occurred and probably the protein requirements were met by the diets[(35)]. The results of the present study indicated that the experimental diets had a balanced supply of protein and energy, which in turn may have improved the use of dietary protein.

Evaluated diets with dehydrated grape residue and different levels of urea for lambs found average values of 22.62 g d^{-1}, for retention of N[(36)]. According to the authors, the high value can be explained by the fact that the animals are growing and required high amounts of protein for tissue formation. When replacing sorghum silage with dehydrated fruit coproducts, no difference was observed for nitrogen balance between diets[(37)]. According to these authors, this fact indicates that the animals retained protein from the diet and the objective of the study was reached, besides these products are good alternative for use during feed shortage and potentially reduce feed costs.

Despite the observed values for N retained in the present study, 8.53 g d^{-1}, are lower than those observed elsewhere[(36)] showed no damage to the development of the animals. However, the values are close to retained N and higher for ingested, fecal and urine N, to those found by others[(13)] when they included grape residue in diets with 11% CP to feed lambs. N retention is closely linked to the balance and timing of degradation between carbohydrates and dietary proteins. According to some authors[(15)], higher nitrogen retentions are a reflection of the better balance between energy and protein characteristic of each food, allowing greater efficiency in protein utilization.

The excretion of N via feces was less than the excretion described by Van Soest[(38)] for ruminants, 6 to 8 % of the ingested protein, since for consumption of CP 221.4 g d^{-1} obtained in this research, and losses fecal excretion of 13.3 g N d^{-1}. It can be inferred that the amount of tannin present in the grape pomace did not cause damage to protein degradation or that the maximum amount of GPS, 16.5 % present in the diet with a 30 % inclusion, was not sufficient to cause this effect undesirable. Min *et al*[(39)] reported that the tannins can affect the digestion process by means of complex formation with enzymes and mainly with proteins, which would cause lower degradation, absorption and consequently higher excretion of protein via feces.

Table 5: Ingestive behavior of lambs fed with diet containing different levels of grape pomace silage (GPS)

	Levels of GPS (%)				**Mean**	**R^2**	**CV**	***P*-value**
	0	**10**	**20**	**30**				
DMI, g d^{-1}	1226.3±54.78	1246.4±118.32	1242.6±83.63	1226.7±121.96	1235.5±10.50	-	5.12	0.951
CNDF, g d^{-1}	468.8±28.97	463.5±35.23	462.1±51.56	452.0±62.72	461.60±6.13	-	11.22	0.942
TCON, min d^{-1}	237.5±81.45	270.0±83.77	276.3±79.41	255.0±33.42	259.7±17.27	-	26.23	0.854
TIL, min d^{-1}	315.0±20.21	342.5±94.65	351.3±74.99	320.0±76.70	$\hat{Y}$=A	0.98	4.59	0.041
TLD, min d^{-1}	493.8±119.8	395.0±86.70	437.5±61.98	468.8±73.30	435.3±42.59	-	15.23	0.295
TRS min d^{-1}	30.0±26.46	21.2±15.48	22.5±23.27	16.3±8.54	22.5±5.68	-	113.5	0.894
TRD, min d^{-1}	363.8±40.72	411.3±36.83	352.5±38.62	380.0±32.40	376.9±25.55	-	12.85	0.414
EIDM, g h^{-1}	388.1±102.3	341.9±82.00	337.1±102.1	346.1±42.92	355.29±23.47	-	20.67	0.749
EINDF, g h^{-1}	181.2±47.78	159.6±38.28	157.4±47.66	161.6±20.04	164.92±10.96	-	20.67	0.751
EDMR, g h^{-1}	237.0±15.72	202.8±18.16	234.9±29.10	221.6±21.84	224.09±15.75	-	20.61	0.717
ERNDF, g h^{-1}	110.7±7.34	94.7±8.48	109.7±13.59	103.5±10.35	104.61±7.35	-	20.61	0.717
TCT, min d^{-1}	631.3±105.0	702.5±65.89	651.3±64.21	651.3±40.29	659.06±30.45	-	9.57	0.481

DMI, Dry matter intake; CNDF, Consumption of neutral detergent insoluble fiber; TCON, Consumption time; TIL, Standing idle time; TLD, Idle time lying down; TRS, Ruminating time standing; TRD, Ruminating time lying down; EIDM, Efficiency of dry matter intake, EINDF, Efficiency of ingestion of neutral detergent insoluble fiber; EDMR, Efficiency of dry matter rumination; ERNDF, Rumination efficiency of neutral detergent insoluble fiber; TCT, Total chewing time; CV, Coefficient of variation; A= $313.9+4.64x-0.1147x^2$.

The presence of tannins causes nitrogen partition, causing a lower proportion to be excreted in the urine, directing their excretion into the feces(40). This behavior was not observed in the present experiment, the urinary excretion of N 16.14 g d^{-1}, was superior to the fecal excretion of N 10.76 g d^{-1}. When the rate of protein degradation exceeds that of carbohydrate fermentation, a large amount of nitrogen compounds can be eliminated via urine(38).

There were no differences for intake of DM and NDF (Table 5), what can indicate that palatability was not negatively affected by the inclusion of silage GPS of cultivar Isabel *Vitis Labrusca L.* cultivar Isabel (*P*>0.05). Gao *et al*(13) when evaluating the inclusion of up to 15 % of grape residue in diets of lambs, found that the values lower for DM intake and NDF intake were increased with higher inclusion levels.

The time spent with consumption, rumination, idle time lying down and total chewing were not influenced (*P*>0.05) by the inclusion of GPS (Table 5). The absence of effects of diets on these parameters may be due to the similarity between the roughage and concentrated levels of the diets, as well as the levels of fiber, consumption and digestibility of DM and NDF. In addition to the moisture content, caused by the use of silage, it has facilitated the consumption of diets by animals, making the time spent on feeding easier.

The time spent on rumination is proportional to the cell wall content, particle size and effectiveness of the food fiber, with a greater need to process the fiber, as well as more time for feed consumption(38). Also regarding the influence of the NDF content on the ingestive behavior, Cardoso *et al*(41) evaluated diets with different NDF levels (25, 31, 37 and 43 %), observed no change in ingestive behavior and reported that variations in intake were observed in diets with NDF contents higher than those observed in the present experiment, or when there is greater amplitude between the fiber contents in the evaluated diets.

The time spent with ingestive behavior the diets influenced only the length of time that the animals remained idle in standing (Table 5). This parameter showed a quadratic behavior with a maximum point estimated at 17.73 % of GPS (*P*=0.041). Although there were no great variations in the NDF content and the NDF consumption was not influenced by the levels of grape pomace silage inclusion. Considering fiber content as a parameter, it is expected that the idle time will decrease as the NDF content in the diet increases, that is, the greater the need to process dietary fiber, the shorter the permanence of idle animals(42,43). As observed in the present study for the level 0 % of inclusion with the highest content NDF of 454.4 g kg^{-1} DM with the lower idle time of 315 min^{-1}. This is due to the fiber characteristics of the sorghum silage(44), and in the present study it presented a lower ADF content and a higher NDF content than GPS and also in the diet (Table 1).

When considering the total fiber content through the sum of NDF and ADF (725 g kg^{-1} of fiber total) for level of inclusion of 30 % GPS, the value of 320 min d^{-1} of idle time may have been influenced by total content fiber. This is due to the fiber characteristics of the GPS(11), composed 610 g kg^{-1} of DM of seeds, 390 g kg^{-1} of DM pulp residue and husks. The seeds represented the highest proportion in silage and contributed to increase the levels of NDF and ADF in the diet at the level of 30% of inclusion (Table 1), which may have been demanded a greater need for rumination and less idle time between the levels of inclusion (Table 5). For confined lambs, the inclusion of GPS can keep them active and contribute to stress reduction and encourage rumination natural behavior.

The efficiency of ingestion and rumination of DM and NDF were not influenced (P>0.05) by the treatments with the different levels of inclusion. This behavior can be justified by the consumption of DM and NDF (1,235.5 and 461.6 g d^{-1}), respectively, which did not show significant variation (P>0.05) between treatments. According to several works(45,46) the ingestion and rumination efficiencies of DM and NDF are directly related to the consumption of DM and NDF, which may be influenced by particle size, quality and diet content.

Conclusions and implications

Grape pomace silage can be used to feed lambs up to 30 % inclusion in diets containing 55 % roughage, without causing changes in nutrient intake and digestibility, as well as nitrogen balance and ingestive behavior. The grape pomace silage has favorable characteristics for use in diets for lambs, with silage being a good alternative for its storage, in addition to offering the correct destination for this byproduct.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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