Effects of Black Grape Pomace Silage on Dairy Goat Performance, Milk Fatty Acids, and Environmental impact

Running title: Black grape pomace silage in the diet of dairy goats

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Abstract

One of the problems facing the wine production industry is the enormous amount of by-product that is generated in the manufacturing process, since it can, through poor management, constitute serious environmental damage. In 2023, Spain produced 1,398.7 thousand tonnes of winemaking by-product, so this work addresses the study of the productive, environmental and economic effects of its inclusion at different levels in the diet of dairy goats to replace part of the alfalfa hay, as a formula for circular economy and zero waste. Thirty-two Murciano-Granadina breed goats (43.1 ± 1.7 kg; 2.1 ± 0.2 births) were used in a cage study on the digestibility of four diets containing 0%, 6%, 12% or 18% dry matter of black grape pomace. The best results were obtained with the incorporation of 12% dry matter, as, on one hand, compared to the control group, consumption was not affected, and neither were the balance of digestible and metabolizable energies nor the milk production and its gross composition, nor, in general, the fatty acid profile of milk, but carbon dioxide emissions were significantly diminished (27%; $p < 0.001$) and methane was reduced very close to the level of statistical significance (17%; $p < 0.097$). On the other hand, it is important to replace as much alfalfa as possible to improve production costs. From an economic standpoint, this experiment indicates that this substitution means, compared to the control group, the saving of 340 g of alfalfa hay, although 530 g of fresh black grape pomace silage was needed, all of this per goat per day. So, the interest in its use will depend on the prices of the variables mentioned and on the appreciation of the environmental and health benefits. In this case, the reduction in diet cost would be 0.05€ per goat per day.

Keywords: Diet, black grape pomace, dairy goats, Murciano-Granadina breed.

INTRODUCTION

In 2003 [1], Spain was the country with the largest area worldwide dedicated to grape cultivation, with 913 thousand ha, and the third largest producer of grapes with 4,823 thousand tonnes, which generated 1,398.7 thousand tonnes of by-products. Grape pomace (GP) is the main winemaking by-product obtained after pressing and/or fermentation processes, and its components are the remaining skin, stems or stalks and seeds [2] which, with poor management, can cause serious damage to the environment [3, 4] or, for example, reduce root growth when applied to the soil without prior composting [5].

The valorization of by-products for animal feed contributes to the circular economy and zero waste [6], and may reduce the costs of animal feed. In this sense, Spain was, also in 2003, the seventh largest producer of goat milk worldwide and the second in the EU with 470.2 thousand tonnes. The dairy goat production system most used in this country is intensive, which depends on cereal grains and legumes, as well as cultivated forage such as alfalfa. These foods have become roughly 65% more expensive since 2020 [7], which can cause a serious loss of competitiveness for farms.

In the case of GP, several authors [8, 9] claim that it is necessary to determine its composition, digestibility and acceptance by the animals. This is especially important when using black GP (BGP), as it usually contains a high proportion of tannins (33.4-202.6 mg Tannin Acid Equivalents/g Dry Matter; DM) [10], which can affect the degradability of crude protein, although it also has functional components, such as polyunsaturated fatty acids (PUFA) [11] which could be transferred to the products obtained. Other benefits were stated by [12] who, when introducing GP into the diet of cows, observed lower levels of Somatic Cell Count (SCC) in milk and methane emissions than those in the control group, although they should be administered at levels of 10-15% DM, at most, in their diet [13]. In the same sense, Hagerman and Butler [14] also stated that tannins can be beneficial or harmful depending on the type and quantity consumed, the structure of their components and molecular weight, and the physiology of the consuming species.

Therefore, the aim of this work was to study the effects of BGP incorporated at different levels (6%, 12%, or 18% DM) in the diet of dairy goats, replacing part of the alfalfa hay of the control diet, on productive, environmental and economic results. We hypothesized that the optimal experimental food will not significantly affect the digestibility of the diet, maintaining the efficiency of milk production, improving the functional and nutritional quality of the milk by increasing the unsaturated fraction of fatty acids and reducing greenhouse gas emissions and feeding costs.

MATERIALS AND METHODS

Goats and general procedures

A total of thirty-two multiparous Murciano-Granadina breed goats were used in this experiment at the Universitat Politècnica de València (Spain). At parturition, goats remained with their kids for a period of 30 d post-kidding under a mixed rearing system. One month after rearing (Figure 1), goats were divided into four homogeneous groups (8 females each), based on similar births (2.1 ± 0.2; mean ± SD), live weight (43.1 ± 1.7 kg; mean ± SD) and daily milk production (2,240 ± 123; ml ± SD), and randomly assigned to the four experimental groups fed with a similar level of concentrate content but a different BGP content (0%, 6%, 12% or 18% DM) and alfalfa hay, for their adaptation to the diets (14 days). After the adaptation period, four goats per group, with similar number of births, live weight and milk production, were selected for transfer to the digestibility cages for 11 days (seven days of adaptation to the cages and four days of sampling). Since only eight cages were available, the experiment was carried out in two periods so that in each period two goats from each of the diets were placed in the cages. For each period, the animals spent seven days adapting to the cages and four days of sampling, consuming their corresponding diet. Inside the cages, the goats were able to communicate with each other and were given “ad libitum” water. The floor of the cages allowed for selective and individual separation of faeces and urine. Throughout lactation, goats were milked once per day (8 a.m.) with 40 kPa of vacuum measured at vacuum gauge (G-50, GEA, Albacete, Spain), 90 cycles per minute of pulsator rate and 60% of pulsator ratio. The feed was supplied to the animals twice a day, in the morning after milking (9:00 a.m.) and in the afternoon (5:00 p.m.).

Diets

Four isoenergetic and isoproteic diets were developed for an average production of 2.5 l of milk per day, according to [15], whose composition is shown in Table 1. The control diet (D0) consisted of a concentrated feed and alfalfa hay, while the other three diets incorporated BGP in increasing amounts of 6%, 12% or 18% DM (D6, D12, D18), respectively, as a substitute for alfalfa hay. The discrepancy between the percentage of BGP silage incorporation and the actual percentage of BGP incorporation that appears in Table 1 (6.7%, 13.3% and 20.0%) was due to fact that it was necessary to incorporate a small fraction of

Table 1. Ingredients and chemical composition of diets

Items	Diet[1)], %			
	0	**6**	**12**	**18**
Ingredients, kg fresh/% of dry matter				
Concentrate	1.51/61.2	1.50/60.3	1.57/63.7	1.46/58.3
Alfalfa hay	0.90/36.8	0.73/33.0	0.56/22.9	0.49/19.6
Barley straw	0.05/2.0	0/0	0/0	0/0
Grape pomace silage	0/0	0.27/6.7	0.53/13.3	0.81/20.0
Soybean	0/0	0/0	0/0	0.05/2.0
Chemical composition, % of dry matter, unless otherwise stated				
Dry Matter (DM; %)	91.5	90.2	89.3	89.2
Organic Matter	92.8	92.0	91.8	92.0
Ash	7.2	8.0	8.2	8.0
Ether Extract (EE)	3.7	3.6	3.9	4.2
Crude Protein (CP)	17.2	18.2	18.2	18.2
Neutral Detergent Fibre (NDF)	42.8	37.1	37.7	35.4
Acid Detergent Fibre (ADF)	20.7	21.7	22.2	20.3
Acid Detergent Lignin (ADL)	1.3	3.9	4.8	4.1
Hemicellulose	22.1	16.4	15.5	15.1
Cellulose	19.4	17.8	17.4	16.2
Non-Fibrous Carbohydrates (NFC)	29.1	33.1	32.6	34.5
Gross Energy (MJ/kg DM)	16.5	16.5	16.4	16.1
Condensed Tannins (g/kg DM)	0	3.2	4.8	6.3

[1)]Level of incorporation of black grape pomace dry matter in the diet; Hemicellulose = NDF - ADF; Cellulose = ADF - ADL; NFC = 100 - (NDF + ash + CP + EE).

straw to the fresh BGP for the manufacture of the ball silages. The forage components of the diet (alfalfa hay, BGP silage and straw) were chopped into fragments up to 2 cm in length, to avoid the possibility of selection by the goats, mixed with the concentrate and offered to the dams in an amount 10% higher than the voluntary feed intake, according to the consumption observed during the 14 days of adaptation to the diets. The concentrate (6.54 MJ net energy/kg DM) was supplied by NANTA S.A.U. (Torre Pacheco, Murcia, Spain) and the BGP silage, whose composition is presented in Table 2, by the Instituto de Investigación Agroalimentaria y Agroambiental, Universidad Miguel Hernández de Elche (UMH), Spain.

Table 2. Chemical composition from black grape pomace (BGP)

Items	Items, % of DM, unless otherwise stated											
	DM	**Ash**	**OM**	**EE**	**CP**	**NDF**	**ADF**	**ADL**	**NFC**	**HE[1)]**	**CE[2)]**	**CT**
BGP	92.0	7.3	92.7	8.6	13.7	51.3	43.3	25.9	19.1	8.0	17.4	27.5

DM, Dry Matter (%); OM, Organic Matter; EE, Ether Extract; CP, Crude Protein; NDF, Neutral Detergent Fibre; ADF, Acid Detergent Fibre; ADL, Acid Detergent Lignin; NFC, Non-Fibrous Carbohydrates = 100 - (NDF + ash + CP + EE); [1)] HE, Hemicellulose = NDF – ADF; [2)]CE, Cellulose = ADF – ADL; CT, condensed tannins (g/kg DM).

The fatty acid composition of the diets is presented in Table 3.

Table 3. Fatty acid composition of experimental diets

Items (g/100 g Fatty Acid)	Diet[1)], %			
	0	**6**	**12**	**18**
Main individual Fatty Acids				
Palmitic Acid Methyl Ester (C16:0)	23.562	22.126	22.354	21.891
Stearic Acid Methyl Ester (C18:0)	3.541	3.779	3.910	4.224
Oleic Acid Methyl Ester (C18:1n9c)	26.787	23.938	23.958	24.245
Linoleic Acid Methyl Ester (C18:2n6c)	36.490	39.363	39.721	40.130
Linolenic Acid Methyl Ester (C18:3n3)	3.790	5.273	4.911	4.266
Grouping results				
Saturated Fatty Acids (SFA)	30,10	28,85	28,85	28,74
Monounsaturated Fatty Acids (MUFA)	29,57	26,44	26,47	26,82
Polyunsaturated Fatty Acids (PUFA)	40,33	44,70	44,68	44,44
Conjugated Linoleic Acid	36.49	39.36	39.72	40.13
n3	3,79	5,27	4,91	4,27
n6	36,54	39,43	39,77	40,17
n6/n3	9,64	7,48	8,10	9,42
Short Chain Fatty acids	0,02	0,03	0,04	0,03
Medium Chain Fatty Acids	27,80	26,19	25,91	25,46
Long-Chain Fatty Acids	75,01	76,53	76,32	76,91
Thrombogenicity Index (TI)	0,51	0,47	0,48	0,48
Atherogenicity Index (AI)	0.37	0.34	0.34	0.33

[1)]Level of incorporation of black grape pomace dry matter in the diet; Short Chain Fatty acids, C6-C10; Medium Chain Fatty Acids, C11-C17; Long-Chain Fatty Acids, C18-C22; TI = (C14:0 + C16:0 + C18:0)/[(0.5 × ∑MUFA) + (0.5 × ∑n-6PUFA) + (3 × ∑n-3PUFA) + (n-3 / n-6)]; AI = [C12:0 + (4 × C14:0) + C16:0]/∑Unsaturated Fatty Acids.

Experimental data and sample collection

Through a screening process, the seeds present in the BGP were manually selected for counting.

After the goats had been in the digestibility cages for seven days, daily data collection began for each animal for four consecutive days on the amount of feed offered and rejected, faeces and milk production. A representative sample fraction (10%) of them was frozen (-20 ºC) for subsequent analysis. Another fraction of milk was immediately sent to the milk analysis laboratory of the Universitat Politècnica de València for gross analysis (fat, protein, lactose, urea and SCC).

Chemical analysis

Official analysis methods of [16] for DM (934.01), ash (942.05) and ether extract (EE; 920.39) of the diets, refusals, BGP silage and faeces were followed. According to [17] with the ANKOM filter bag technique, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined. For crude protein (CP; Official method 990.03), total N was calculated using a LECO CN628 elemental Analyzer (Leco Corporation, St. Joseph Mi., USA). For the gross energy (GE) content, preserved samples of diets, rejected food and faeces were sent to the SERIDA laboratories (Villaviciosa, Asturias, Spain) and analysed by combustion in an adiabatic bomb calorimetric. The measurements were carried out in an IKA Werke 5003C bomb calorimeter (IKA Gmbh, Staufen, Germany); 0.5 g of sample were weighed and placed in the quartz crucible to carry out combustion; the correct operation of the pump is verified by analysing a benzoic acid control tablet. Gross milk composition (fat, protein, lactose and urea) was analysed with a medium infrared analyser (Milkoscan® FT6000; Foss Iberia, Barcelona, Spain) and SCC was determined by the fluoro-opto-electronic method (ISO, 2008; Fossomatic® 5000, Foss Iberia, Barcelona, Spain).

The Fatty Acid Methyl Esters (FAME) content of diets was prepared according to [18] and analysed on a Focus Gas Chromatograph (Termo Fisher Scientific, Milan, Italy). Separation of methyl esters was performed in a capillary column (SP-2560, Supelco, Bellefonte, PA; 100 m x 0.25 mm x 0.2 µm thickness). Analysis of the milk fatty acid (FA) profile was carried out in duplicate, with an extraction using the Folch et al. [19] method with some variations reported in [20] and a subsequent methylation according to the method of Nudda et al. [21], similar to [22]. The chromatograph, column and FAME mix for identification of the milk FAs were the same as those used in the diets.

Polyphenolic compounds in the grape pomace were analysed at the UMH using an Acquity UPLC system with PDA detector (Waters Corp., Milford, MA, USA). The extraction procedure proposed by [23] was used, with slight modifications. Briefly, 5 ml of methanol (30 %) with ascorbic acid was added to 0.7 g of sample in a test tube. The mixture was sonicated for 30 min and left for 20-24 h in the fridge. Then, it was sonicated again and the resultant extract centrifuged at 4°C to collect the supernatant. For identification and quantification of the polyphenols, samples were separated in an Acquity UPLC BEH C18 column and eluted with a gradient system of formic acid in water and acetonitrile. The polyphenols content was calculated using the Empower 3.0 program based on the peak areas in the chromatograms and expressed as mg per 100 g of product.

Dry Matter degradability and gas production "in vitro"

The DM degradability (g/kg DM) was obtained following the "in vitro" incubation method described by [24], with some variations recommended by [25]. The culture medium used for these incubations was prepared according to [26] from rumen fluid extracted by oesophageal cannula from goats fed standard diet at the UMH farm. A 0.5 g sample was incubated in a 100 ml glass vial (digestion unit) prewarmed to 39°C before infusion with CO_2 and injection of 60 ml of rumen culture medium. The incubation was maintained in an orbital bath at 39°C. After 48 h, the contents of the digestion unit were filtered with Whatman No. 2 paper and the residue was dehydrated in an oven at 105°C for 24 h. The NDF degradability (g/kg DM) was determined from the digested material according to [17]. For the determination of enteric CH_4 and CO_2 produced during in vitro digestion, gas samples were taken from the headspace of the digestion unit. The gas was manually extracted using 50 ml syringes at times 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 24 h, 36 h and 48 h. These samples were processed as described by [25]. The results correspond to the gas emission obtained in a complete 48-h cycle and were expressed as ml/g DM of substrate degraded [27]. Each extraction was collected in a sealed vial and under negative pressure. The gas concentrations in the samples were established using a gas chromatograph (HP 6890, Agilent Technologies) equipped with a flame ionization detector (FID). A standard corresponding to a mixture of CH_4 and CO_2 was used to establish the standard curve (R2 = 0.99) for each gas. The concentrations of these gases were obtained by interpolation of the measurements within the standard curve.

Calculations

Apparent digestibility (AD) of each substance was calculated as:

$$AD = [((\text{ingested fraction} - \text{faeces content})/\text{ingested fraction}) \times 100]$$

Digestible Energy (DE) was calculated as:

$$DE\ (MJ/kg\ DM) = GE/kg\ DM \times [(GE_{\text{ingested fraction}} - GE_{\text{faeces content}}) / GE_{\text{ingested fraction}}]$$

Metabolizable Energy $(ME)_{\text{in vivo}}$ will be calculated from the DE using the equation developed by [28]:

$ME_{in\ vivo}$ (MJ / Kg DM) = [(0.9611 x DE – 0.299) x 4.184]

Chen and Liu [29] proposed a series of indices to improve knowledge of the effect of milk FA composition on human health, that were calculated as follows:

Thrombogenicity index (TI) was calculated as:

$$TI = (C14:0 + C16:0 + C18:0)/[(0.5 \times \sum MUFA) + (0.5 \times \sum n\text{-}6PUFA) + (3 \times \sum n\text{-}3PUFA) + (n\text{-}3 / n\text{-}6)]$$

Atherogenicity index (AI) was calculated as:

$$AI = [C12:0 + (4 \times C14:0) + C16:0]/\sum UFA,$$

where MUFA = monounsaturated fatty acids; UFA = unsaturated fatty acids.

Statistical analysis

Statistical analysis was performed using the SAS package [30]. Dry Matter Intake, faeces and “in vivo” digestibility variables were analysed with PROC MIXED, according to [31], considering the fixed effects of Diet (0%, 6%, 12% or 18% DM of BGP), Period (1,2), Day (1,4), the Diet x Period and the Diet x Day interactions, and the random effect of Goat within the Diet and Period. Variables of milk production, gross composition and SCC were analysed with the previous model, also incorporating the mean of the values in the pre-experimental period as a covariate. SCC was analysed in log, in order to normalize its distribution [32]. When an interaction or covariate was not significant, it was removed from the model.

The “in vitro” digestibility variables were analysed with the PROC GLM, considering the effects of Diet (0%, 6%, 12% or18% DM BGP) and day (1 to 3). Fatty acid composition of milk was analysed with PROC GLM, considering the fixed effect of Diet.

In all statistical analyses, when an effect was significant, pairwise comparison of means was performed applying Student t-test.

RESULTS AND DISCUSSION

Since the Diet x Period and the Diet x Day interactions were not statistically significant, they were removed from the model, allowing the presentation of the tables as shown in this manuscript.

Diet characteristics and “in vitro” degradability

Grape pomace silage had a chemical composition (Table 2) similar to that described by [33]. It can be seen that, compared to forages and straw by [34], BGP has a higher EE content than forages and straw (2-4.4% DM), a lower CP content than alfalfa (15.9-17% DM), similar to other hays (vetch, ray grass = 11.1-14.7% DM) and higher than cereal silages and straw (3.7-12.7% DM). The NDF content of the cell walls is of the same order as that of hays and silages (49-60% DM) and much lower than that of cereal straw (72% DM). However, a differential component for BGP is observed in these walls, which is its high proportion of ADL, since it represents 50.5% of the NDF, compared to 5.95% for wheat silage or 18% for alfalfa hay. The ADL fraction includes lignin and significant amounts of tannins and cutin, which hinder the digestibility of the silage [33].

Another characteristic of GP is that it is composed of two main components: seeds and pulp, which includes part of the pulp and skins. In this study, the seed content was 300 g/kg DM of BGP, lower than the results of [10] (329 to 470 g/kg DM) and [35] (406 to 574 g/kg DM). In addition to the differences in seed content between grape varieties, the addition of straw initially in the ensiling process has contributed to reducing these values in this experiment. It has been verified [10] that the lignin content of BGP (31.4% DM) is concentrated mainly in the seeds (64-70.3% DM) as a structural constituent of the seed coat, which encloses the seed's nutrients within, highlighting its high fat (9.5-11.7% DM) and protein (12.2% DM) contents. For digestion, the shell must be destroyed (crushed) by chewing, otherwise the seeds can pass through the digestive tract without being digested.

The tannin content of the BGP obtained in this experiment (27.5 g/kg DM) is higher than that found by [10] in BGP from different grape varieties (15.3 g/kg DM). These authors observed a significant reduction in tannin content during the ensiling process, which decreased (42.8%) from 35.7 g/kg DM in fresh BGP to 15.3 g/kg DM in silage. They also observed that 67.3% of the tannins in BGP are concentrated in the seeds.

Those particularities of the seed walls, with their high concentration of lignin and tannins, makes them a difficult-to-digest material, but at the same time it is an advantage for the digestibility of the rest of the pomace components, which will be less affected by the high levels of LAD and tannins present in the total composition of the pomace. Thus, Guerra-Rivas et al. [35] found that the true "in vitro" digestibility of DM was 51% in seeds, while it reached a value of 81% in the pulp.

The incorporation of BGP into the diets as a partial substitute for alfalfa hay resulted in some variations in their chemical composition compared to the control diet (Table 1). The three diets that incorporated BGP presented a fairly similar chemical composition, showing, compared to the control group, a reduction in NDF (13.3-17.3%), hemicellulose (25.8-32.0%) and cellulose (8.2-16.5%), as well as an increase in NFC content (12-18%) and ADL (3-3.7 times more). However, these compositional variations did not significantly affect the "in vitro" degradability of DM, OM or NDF of the ground samples (Table 4), but they did affect CO_2 emissions ($p < 0.01$) and were very close to significance ($p = 0.097$) for CH_4 emissions. These emissions are reduced with the incorporation of 12% BGP DM into the diet, but reach maximum values when incorporating 18% BGP DM. The increase in emissions between D12 and D18 seems surprising, as their chemical composition (EE, PC, cellulose, hemicellulose, ADL and CNF) is similar, with the difference that the tannin contents are low but increase from 4.8 g/kg DM for D12 to 6.3 for D18 g/kg DM (Table 1). This increase in the level of tannins should, where appropriate and according to the consulted references [36-38], tend to reduce and not increase gas emissions by reducing ruminal fermentation. However, Akter et al. [39] obtained similar results to those of this experiment when incorporating 10% and 15% of fresh GP into the diet of dairy cows, both for CH_4 production (not significant g/kg DMI) and CO_2 (greater for GP diets); the latter result seems to indicate a greater rate of microbial digestion compared to the control group [39]. Moate et al. [40] state that although the role of GP tannins in reducing methane emissions is widely discussed, the mechanism of CH_4 reduction is unclear, although, in the case of this experiment, the fact of the low levels of tannins in the diets and their lower effect on goats than on cows or sheep [41-43] may help explain the results obtained.

Table 4. "In vitro" degradability of dry and organic matter, and gas production of diets for goats fed with black grape pomace silage

Item	Diet[1)], % of DM, unless otherwise stated				SEM	*p* - value
	0	6	12	18		
Dry Matter (%)	72.6	71.5	71.4	72.5	0.392	0.218
Organic Matter	81.6	81.7	81.6	81.5	0.430	0.985
Neutral Detergent Fibre	56.2	54.0	53.1	49.8	1.716	0.247
Methane (ml/0.5 g DM)	19.2	20.1	16.0	22.6	1.153	0.097
Carbon Dioxide (ml/0.5 g DM)	71.0^{b}	49.9^{a}	51.8^{a}	87.3^{b}	1.111	< 0.001

[1)]Level of incorporation of black grape pomace dry matter in the diet; ^{a,b,c}Values with different letters, a, b and c, are significantly different.

Furthermore, among the different diets, little variation was observed in the degradability of DM (71.4–72.6% ± 0.39) and OM (81.5–81.7% ± 0.43), but greater variation in NDF, since its average value decreased from 56.2% to 49.8% ± 1.71 as the proportion of BGP in the diets increased. This evolution is probably due to a parallel increase in the amount of grape seed, reflecting the low degradability of their NDF.

Therefore, although from the point of view of "in vitro" degradability of the diet it is possible to incorporate BGP up to 18% of the DM, from an environmental point of view, a maximum incorporation of 12% of BGP would be the most correct option.

Regarding the composition of the diets in fatty acids, it was observed that, for the diets that incorporated BGP, the amount of linoleic acid increased between 7.9% and 10% for C18:2n6c, and between 12.7% and 39% for C18:3n3 (Table 3), compared to the control group. Linoleic acid is a long-chain FA that is considered beneficial to human health and, when a by-product of these characteristics is introduced into the diet of dairy cows, it can enrich the milk fat produced in this type of FA [44, 45]. The introduction of BGP in the diet, in this experiment, upwardly modified PUFAs (10-10.8%) but reduced SFA (4-5%) and MUFA (9.3-10.6%) (Table 3).

Diet intake and "in vivo" digestibility

The diets did not significantly affect feed intake (fresh or DM), the amount of feed rejected or faecal production (Table 5), which seems to indicate that the incorporation of BGP in the diets at levels of 6%, 12% or 18% DM, as a partial substitute for alfalfa hay, was well accepted by the goats.

The chemical composition of the rejected feed (Table 6) shows a certain increase in the fibrous parts of the diet compared to the diets supplied initially, highlighting the increase in the most indigestible fraction (ADL), which agrees with what was indicated by [46]. A great uniformity is also observed between the diets with BGP, except in the ADF and ADL fractions, which are higher in diets with a higher proportion of BGP (D12 and D18). Compared to the control group, the rejected diets with BGP presented less NDF and slightly more NFC and ADL (the latter in D12 and D18), as occurs in the original diets, which seems to indicate that the consumption of the diets was homogeneous, in terms of its main components, due to their minced preparation. However, a higher EE and CP content is observed in the rejection of diets with BGP, probably because these are nutrients due to the presence of seeds.

The digestibility coefficients in Table 5 are apparent, as the amount of food digested and absorbed is determined by subtracting from that amount of food ingested the amount excreted in the faeces, so that said digestibility is overestimated. This is because, in general in all animal species, there are substances secreted in the digestive tract, for example saliva, that are not absorbed and, particularly in ruminants, methane is also produced and emitted into the environment based on to the breakdown of carbohydrates. Furthermore, in the case of diets such as those in this experiment, the secretion of glycoproteins in saliva aims to neutralize the effects of tannins [47, 48].

Table 5. Dry matter intake and apparent total tract digestibility of experimental diets for goats fed with black grape pomace silage and housed in digestibility cages for 11 days

Item	Diet[1)], %				SEM	*p* -value
	0	6	12	18		
Food consumption, g / day						
Offered	2,460	2,530	2,580	2,650	0.156	-
Refused	590	762	507	519	125	0.479
Consumed	1,870	1,768	2,073	2,131	125	0.195
Dry Matter Intake	1,711	1,595	1,851	1,901	112	0.258
Faces, g/day						
Fresh faeces	1,148	1,027	1,475	1,498	172	0.191
Apparent total tract digestibility, %						
Dry Matter	77.5	77.5	71.3	74.3	2.30	0.225
Organic Matter	79.2	79.0	73.1	75.9	2.15	0.209
Crude Protein	79.5	82.2	76.7	80.2	2.11	0.365
Ether Extract	85.4	85.3	84.5	83.8	1.83	0.919
Neutral Detergent Fibre	68.4[a]	62.6[ab]	52.2[b]	53.7[b]	3.64	0.029
Acid Detergent Fibre	62.5[a]	60.7[ab]	49.0[bc]	47.9[c]	3.92	0.042
Non-Fibrous Carbohydrates	73.3	77.9	77.6	78.2	2.70	0.552
Gross Energy	77.0	76.6	69.9	72.9	2.38	0.170
Digestible and metabolizable energy of diets, MJ/kg DM						
Digestible	12.7	12.6	11.5	12.0	0.389	0.156
Metabolizable	10.9	10.9	9.8	10.3	0.372	0.156

[1)]Level of incorporation of black grape pomace dry matter in the diet; [a,b,c]Values with different letters, a, b and c, are significantly different.

The results of "in vivo" digestibility showed that the diets did not significantly affect ($p > 0.05$) the digestibility of DM, OM, CP, EE and NFC, with the only significant effect ($p < 0.05$) being found in the NDF and ADF fractions. In both components, the diets with a higher proportion of BGP (D12 and D18) presented lower digestibility than the control diet, while D6 had intermediate values. These results are consistent with those found in the "in vitro" degradability. The most significant differences between diets, observed in the fibrous fractions, are justified because as the proportion of BGP increases, the quantity of seeds also increases. Both the similarity of the "in vitro" degradability (ground samples) for DM, OM and NDF with the respective "in vivo" digestibility values, as well as of digestibility between the control diet and the diets containing BGP (DM, OM, CP, EE, and NFC), indicate that the seeds were ground and digested, to a large extent, by the goats. The DE and ME of the diets were not affected by the incorporation of BGP as a substitute for alfalfa hay.

Table 6. Ingredients and chemical composition of refused diet for goats fed with black grape pomace silage and housed in digestibility cages for 11 days

Chemical composition, % of dry matter, unless otherwise stated	Diet[1)], %			
	0	**6**	**12**	**18**
Dry Matter (%)	91.7	90.4	90.8	90.1
Organic Matter	91.4	91.5	92.6	91.0
Ash	8.6	8.5	8.4	9.0
Ether Extract	0.7	2.5	2.6	3.2
Crude Protein	14.3	17.1	16.3	17.9
Neutral Detergent Fibre	53.4	38.9	40.2	42.4
Acid Detergent Fibre	38.0	24.9	28.9	29.6
Acid Detergent Lignin	8.7	5.6	11.4	10.2
Hemicellulose	15.4	14.0	11.3	12.8
Cellulose	29.3	19.3	17.5	19.5
Non-Fibrous Carbohydrates	23.0	33.0	32.5	27.5
Gross Energy, MJ/Kg of DM	16.5	16.5	16.5	16.7

[1)]Level of incorporation of black grape pomace dry matter in the diet; Hemicellulose = NDF – ADF; Cellulose = ADF – ADL.

Bibliographical results are not very abundant in goat livestock. On one hand, the works of Baghsiyah et al. [48] show that by incorporating 5%, 10% or 15% of DM GP, as a substitute for beet pulp in the diet of Saanen goats, neither the consumption nor the digestibility of DM, OM, CP, NDF or ADF was altered, which seems to confirm that the seeds were, at least largely, digested given their already proven important content in the composition of the GP. However, in cattle a linear reduction in the digestibility (p < 0.001) of the dietary components (DM, OM, CP, EE, NDF and ADF) was observed when increasing the amount of BGP (5%, 7,5% or 10% DM) supplied [45], which has been related to the fact that a large part of the seeds passes through the digestive tract without being digested. In sheep, the incorporation of GP in high proportions, from 76.2% to 30% DM [49-51], causes significant decreases in the digestibility and ME of the diets. However, when GP is incorporated in low proportions (2% DM) it increases the richness of the ruminal bacterial community [52] and improves the digestibility of OM, CP, CNF and NDF [53]. At 5-10% DM in lambs, GP improves intake, conversion rate and growth [54, 55], but higher GP proportions (15 and 20%) reduce intake and growth [48]. In dairy sheep, Nudda et al. [56] incorporated 3.9% DM into diets and observed a 5% drop in DM intake, while [57-59] observed that GP proportions of 5 to 12% DM did not affect DM intake. FEDNA [33] recommends the incorporation of GP in the diet of sheep at around 5% DM, but there is no recommendation for goats. In this work it has been verified that goats admitted up to 18% of BGP without negative effects on DM intake and diet digestibility. These results may be due to the fact that [42] observed that goats, unlike in cattle and sheep, possessed a salivary protein that blocked tannins, even in cases where the animals had not been subjected, for long periods, to diets containing CT, as occurs in this experiment.

Milk production and chemical composition

In this experiment, the diets did not significantly affect ($p > 0.05$) milk production or its gross composition: fat, protein, lactose, cheese extract, urea or SCC (Table 7). However, Baghsiyah et al. [48] found that such incorporation of GP at levels of 15% DM in goats led to a reduction in milk production by 12,5%, and the composition in protein and non-fat solids, by 3%, for all levels tested (5%, 10% and 15% DM), compared to the control group. This discrepancy may be due to the higher tannin content in the GP diets of these authors (7.4–19.0 g/kg DM) compared to the GP silage used in this work with lower values in the diets (3.2-6.3 g/kg DM). Tannin content of pomace at certain dietary levels can reduce milk production [37,40], since, among other negative effects, it can bind to dietary proteins, reducing their absorption and the ability to produce milk. In goats, Renna et al. [60] and Michelon et al. [61] observed that the incorporation of GP at levels between 6-21% did not affect milk production and composition, as did [62] at levels of 0.5-1% of live weight. On the other hand, in sheep, Nudda et al. [56], with 3.9% DM of GP in the diet, observed an increase in milk production (16.5%) and a decrease in fat (12.8%) and protein (7%) content compared to the control group. Manso et al. [58] and Bennato et al. [59] did not observe effects of the addition of pomace in the diet (5-10% DM) on milk production, although they reported a reduction by 5% in lactose content. Two of the factors that could have affected the disparity in results observed in the literature may be due, on the one hand, to the animal species used and, on the other, to the different levels of tannins contained in the diet.

Regarding SCC, Alba et al. [63] observed a decrease in SCC in sheep's milk when including grape residue flour in the diet, a result not found in this experiment; perhaps the short period of time (25 days) in which the goats received the pomace silage was insufficient for the manifestation of this effect, since Moate et al. [40] obtained similar SCC results to those in this experiment in cows fed with grape marc for only 18 days. Somatic cells in milk include leukocytes (of blood origin) and desquamation cells of the glandular epithelium [64], so the potential reduction of SCC would be based on the decrease in blood cells due to the anti-inflammatory capacity of the pomace. A longer period of consumption of diets with pomace could be interesting to delimit the results of this experiment in this sense.

As in the case of gas emissions, from a production point of view, the incorporation of 12% of pomace in the goats' diet seems to be the most favourable option, as it maintains the level of milk production and composition characteristics compared to the control group.

Table 7. Milk production and composition from goats fed with black grape pomace silage and housed in digestibility cages for 11 days

Item	Diet[1)], %				SEM	*p*-value
	0	6	12	18		
Milk production, g/day	2,227	2,046	2,221	2,119	103.8	0.570
Chemical composition, %, unless otherwise stated						
Dry Matter	13.87	13.20	13.32	13.52	0.329	0.530
Fat	4.90	4.53	4.54	4.68	0.285	0.783
Protein	3.33	3.34	3.38	3.38	0.075	0.966
Lactose	4.77	4.60	4.66	4.67	0.070	0.478
Cheese Extract	8.31	7.87	7.87	8.07	0.350	0.775
Urea (mg/kg)	606	626	613	614	27.80	0.967
Log SCC	5.62	5.80	5.64	5.49	0.152	0.653

[1)]Level of incorporation of black grape pomace dry matter in the diet.

Fatty acids composition of milk

Fatty acids, among other things, are used by the body's cells as energy for muscular contraction and general metabolism, but they also are associated with cardiovascular diseases [65], so the FA composition of milk should be estimated to determine their medicinal and nutritional interest.

A total of 87 FAs were identified in milk fat simples (Table 8). Capric (C10:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids were the predominant saturated FAs in milk samples from control and BGP diets, while oleic acid (C18:1 n9 cis) and linoleic acid (C18:2 n6 cis) were the major monounsaturated and polyunsaturated FAs, respectively. These results are very similar to those obtained by [12] using diets that also incorporated GP.

None of the FA grouping classes studied (Table 8) were found to be statistically significant, although a trend towards an increase in MUFAs (2.6-3.8%), PUFAs (5-10%) and long-chain FAs (1.2-10.9%) was observed when incorporating BGP into the goats' diet. MUFAs and PUFAs are long-chain unsaturated fatty acids that have important benefits for human health [66]. Among other things [67], PUFAs prevent inflammation caused by eicosanoids (prostaglandins, thromboxane and leukotrienes) or arthritis and delay ageing and cancer progression, although some of these effects are not fully proven. There must be a balance between the n-3 and n-6 series for the function of PUFAs to be optimal.

Regarding the n6/n3 ratio, the differences were significant between diets, such that when introducing 12% and 18% of BGP, this ratio increased by 19.5% and 22%, respectively. The value of the control diet is in line with the indications of [68] in Western populations (15.0-16.7), which would favour the pathogenesis of many diseases according to the French Agency for Food Safety [69], which sets the ideal proportion of n6/n3 for human health at values close to 5. However, EUFIC [70] reflects the opinion of several authors who state that more important than the n6/n3 ratio are the absolute levels of intake to achieve an increase in the body of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids.

Table 8. Fatty acid composition of milk from goats fed with black grape pomace silage and housed in digestibility cages for 11 days

Items (g / 100 g fatty acid)	Diet[1)], %				SEM	*p*-value
	0	6	12	18		
Main and significant different individual Fatty Acids						
C8:0	2.56	2.36	2.43	2.52	0.140	n.s.
C10:0	9.06	8.12	8.89	8.91	0.370	n.s.
C12:0	3.89	3.33	4.03	3.90	0.220	n.s.
Iso C13:0	0.01^{b}	0.01^{a}	0.01^{b}	0.01^{b}	0.001	0.030
C14:0	8.16	7.90	8.25	7.46	0.205	n.s.
C16:0	26.34	25.72	25.18	25.18	0.814	n.s.
Iso C17:0	0.10^{a}	0.33^{b}	0.28^{b}	0.30^{b}	0.031	0.001
C17:0	0.81^{a}	0.79^{ab}	0.71^{b}	0.70^{b}	0.027	0.033
Iso C18:0	0.07^{a}	0.06^{a}	0.05^{b}	0.05^{b}	0.003	0.011
C18:0	11.00	12.39	10.46	11.54	1.132	n.s.
	3.22	3.47	4.24	4.14	0.644	n.s.
C18:1(n9 cis)	17.79	18.28	17.63	17.43	1.070	n.s.
C18:2 c9 t12	0.14^{a}	0.10^{ab}	0.06^{b}	0.05^{b}	0.020	0.038
C18:2(n6 cis)	3.21	3.30	3.57	3.29	0.213	n.s.
C18:2 t12 c15	0.07^{a}	0.07^{a}	0.06^{b}	0.05^{b}	0.003	0.015
CLA 11t 13t	0.01^{ab}	0.02^{a}	0.01^{c}	0.01^{bc}	0.001	0.005
C22:0	0.05^{b}	0.07^{a}	0.04^{b}	0.04^{b}	0.007	0.027
Grouping results						
Saturated Fatty Acids	67.5	66.5	65.6	66.2	1.40	0.803
Monounsaturated Fatty Acids (MUFA)	26.6	27.2	27.5	27.2	0.98	0.923
Polyunsaturated Fatty Acids (PUFA)	5.8	6.1	6.8	6.4	0.51	0.600
Conjugated Linoleic Acid	1.4	1.6	2.0	1.9	0.32	0.545
n3	0.2	0.2	0.2	0.2	0.01	0.439
n6	3.6	3.7	3.9	3.7	0.22	0.700
n6/n3	18.1^{b}	18.5^{b}	21.9^{a}	20.9^{a}	0.65	0.004
Short Chain Fatty acids	14.8	13.4	14.3	14.8	0.63	0.394
Medium Chain Fatty Acids	43.6	41.9	42.4	41.3	0.78	0.293
Long-Chain Fatty Acids	41.8	44.5	43.4	43.7	0.66	0.072
Thrombogenicity index (TI)	1.3	1.3	1.1	1.2	0.07	0.131
Atherogenicity index (AI)	1.9	1.8	1.8	1.8	0.09	0.650

[1)]Level of incorporation of black grape pomace dry matter in the diet; [a,b,c]Values with different letters, a, b and c, are significantly different; TI = (C14:0 + C16:0 + C18:0)/[(0.5 × ∑MUFA) + (0.5 × ∑n-6PUFA) + (3 × ∑n-3PUFA) + (n-3 / n-6)]; AI = [C12:0 + (4 × C14:0) + C16:0]/∑Unsaturated Fatty Acids.

The Atherogenicity Index (AI) is related to the ability to modify total cholesterol levels and low-density lipoprotein cholesterol (LDL-C), whereas the Thrombogenicity Index (TI) indicates the tendency to form clots in blood vessels and shows the contribution of different FAs [29]. In both cases, the lower its value, the more favourable it will be [65, 71]. In this experiment, although the results are not statistically significant, a trend towards a reduction in the value of these indices is observed when including BGP in the diet. Values of AI in this experiment (1.8-2) are among the lowest collected by [29] (1.89-2.91), in their literature review, for different goat breeds, while the values of TI (1.2-1.4) are lower (2.7-3.2) than those obtained in goat milk from the Sardinian market. These authors state that an FA composition with a lower AI and TI has a better nutritional quality. The Health-Promoting Index (HPI) is sometimes used to evaluate the FA profile in milk, since it is the inverse of the AI and is considered beneficial to assess the effects on human health [72].

According to the results obtained, the inclusion of BGP at a level of 12% dry matter in the diet of dairy goats seems to be the most valid option if the aim is to replace part of the alfalfa with this by-product, as the digestibility of the most important parameters (DM, OM and NDF) is similar to that in the rest of the groups. There are no losses in milk production compared to the control group, while in groups D6 and D18 they are between 102 and 175 ml/day, and methane and carbon dioxide emissions are reduced, especially compared to group D18. On the other hand, it is important that the replacement of alfalfa is carried out at the highest possible level in order to maximize benefits. From an economic standpoint, this experiment indicates that this substitution means, compared to the control group, the saving of 340 g of alfalfa hay, although 530 g of fresh silage are needed, all of this per goat per day. In this experiment, the cost of D0 was 0.20€, while the cost of D12 was 0.15€, meaning a saving of 0.05€ per goat per day. In other situations, the economic interest will depend on the prices of the variables mentioned and, on the other hand, on the appreciation of the health and environmental benefits.

Since this experiment was carried out in the short term, it would be interesting to carry out long-term experiments to confirm the productive, economic and environmental trends observed here, and, also, the quality of milk and milk derivatives (fresh and cured cheeses, yogurt and kefir), as well as consumer acceptance.

COMPETING INTEREST

No potential conflict of interest relevant to this article was reported.

FUNDING SOURCES

This publication is part of the project PID2021-1229620B-C32, funded by MCIN/AEI/10.13039/501100011033/FEDER, EU.

ACKNOWLEDGEMENTS

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

Upon reasonable request, the datasets of this study can be available from the corresponding author.

AUTHOR'S CONTRIBUTIONS

Conceptualization: NF, JRD, CP, MR.

Data curation: NF, CA, AMO, CP, MR.

Formal analysis: NF, AMO, CP, MR.

Methodology: NF, JLP, JRD, CP, MR.

Software: NF, CA, JLP, CP, MR.

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Investigation: NF, CA, AMO, JRD, CP, MR.

Writing – original draft: NF, AMO, JRD, CP, MR.

Writing – review &editing: NF, CA, JLP, AMO, JRD, CP, MR.

ETHICS APPROVAL AND CONSENTO TO PARTICIPATE

The experimental procedures were reviewed by the Ethics Committee of the Universitat Politècnica de València and by the Generalitat Valenciana and approved with code 2023-VSC-PEA-0120 type 2.

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Figure 1. Major steps in the experimental design; [1)]BGP, Black Grape Pomace