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## Quebranta grape pomace from the Ica Valley improves milk lipid profile in Saanen goats

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### ABSTRACT

**Background:** Quebranta grape pomace (*Vitis vinifera*, Quebranta variety), a by-product of winemaking and pisco production, contains a distinct metabolite profile of phenolic compounds shaped by the arid agroecological conditions of the Ica valley. Its incorporation into ruminant diets may enhance milk quality while contributing to the valorization of agro-industrial waste; however, its effects on dairy goat milk composition have not been evaluated in Peru.

**Aim:** To assess the effects of partial substitution of dietary forage with Quebranta grape pomace powder on the physicochemical properties, lipid profile, phenolic compounds, and antioxidant activity of Saanen goat milk.

**Methods:** Twenty primiparous Saanen goats were assigned to two dietary treatments ( $n = 10$  each) for 7 weeks: a control diet (T0) and a diet with 8% grape pomace powder (dry matter basis) replacing maize stover (T1). Milk was sampled weekly for physicochemical analysis. Pooled samples from three time points were used for lipid profile, polyphenol, anthocyanin, and antioxidant capacity analyses; these results were therefore considered descriptive given pseudoreplication. Treatment effects were evaluated using linear models including day in milk as a covariate.

**Results:** Grape pomace inclusion increased milk fat content by 16.96% without affecting milk yield, protein, lactose, or total solids. Lipid profiling indicated a potential enrichment of polyunsaturated fatty acids (PUFAs), particularly linoleic acid, based on the magnitude of changes observed. Milk phenolic content and antioxidant capacity ( $>6,000 \mu\text{mol Trolox}/100 \text{ g}$ ) were not detectably altered, although further validation with increased replication is warranted.

**Conclusion:** Quebranta grape pomace improved milk lipid content in Saanen goats, with evidence suggestive of PUFA enrichment, while preserving milk yield, physicochemical characteristics, and antioxidant capacity. These findings support grape pomace as a functional and sustainable feed ingredient for dairy goat production.

**Keywords:** Agro-industrial residues, Goat milk, Phytochemicals, PUFA, Tannins.

### Introduction

The Ica Valley is one of Peru's most important agricultural regions. Despite its extreme aridity—marked by water scarcity, intense solar radiation, saline soils, and poor structure—the area has a long tradition of grape cultivation for wine and *pisco*, the country's national spirit (Rivera Chavez *et al.*, 2025). These harsh conditions influence grape physiology,

resulting in distinct metabolite profiles that shape the sensory and chemical characteristics of the final products (Palma *et al.*, 2025). For example, Quebranta seed extracts exhibit higher total phenolic content and antioxidant capacity than Borgoña (Barriga-Sánchez *et al.*, 2022). Based on national production of wine (19.1 million l) and pisco (8 million l), the required inputs of 1.4 kg of grapes per liter of wine and 6.5 kg per liter of pisco (MINAGRI, 2019), and an average pomace yield

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of 15% from fresh grapes (Dávila *et al.*, 2017), the Peruvian vitivinicultural sector is estimated to generate approximately 11,800 tons of grape pomace per year. Concomitantly, the Ica region supports a large goat population, as goats are particularly well adapted to arid and semi-arid conditions where other livestock are less viable (Assan and N, 2014; Martins Vieira *et al.*, 2016). They are a key source of meat and milk in these challenging systems (Bhimte and A, 2024; Temoche *et al.*, 2025). While hardy Creole breeds remain prevalent, higher-yielding Saanen goats have been adopted to improve milk production (Wilkinson and Stark, 1989; Cofré, 2001). Goat milk has gained prominence as a functional food due to its greater digestibility and lower allergenicity compared with the milk of other livestock species (Wang *et al.*, 2025), as well as its lipid profile enriched in polyunsaturated fatty acids (PUFAs) (Lopez *et al.*, 2019), which are associated with anti-inflammatory, cardioprotective and anti-obesity effects (Kapoor *et al.*, 2021). This lipid profile can be further enhanced through dietary bioactives such as phenolic compounds, which are abundant in grape pomace and have been reported to modulate ruminal biohydrogenating bacteria (Kholif and Olafadehan, 2022). Beyond nutritional benefits, milk production in goats relies heavily on concentrate feeds and supplements, which elevate production costs and expose producers to price volatility, undermining the sustainability of the system (Paraskevopoulou *et al.*, 2020).

In this context, grape pomace presents an opportunity as a locally available, low-cost alternative feed. Its use could reduce dependence on commercial feeds, lower production costs, and contribute to waste reduction—important in a region where agro-industrial residues can pose environmental risks such as soil and water contamination and pest proliferation vectors (Bustamante *et al.*, 2008; Singh Nee Nigam and Pandey, 2009; Correddu *et al.*, 2023). Grape pomace, composed of skins, seeds, pulp, and stems (Bordiga *et al.*, 2019), represents 13.5%–20% of the processed grape mass, depending on variety and vinification method (Rockenbach *et al.*, 2008; Ahmadi and Siahshar, 2011). It is rich in polyphenols, flavonoids, and other bioactive compounds that have been associated with changes in rumen fermentation and improvements in the lipid profile of animal products (Balasundram *et al.*, 2006; Buccioni *et al.*, 2015; Vasta *et al.*, 2019; Onache *et al.*, 2022).

While its benefits have been explored in ruminant diets in winemaking countries (Correddu *et al.*, 2020). No studies have evaluated the use of Quebranta grape pomace from the Ica valley, where unique climatic stressors shape its composition, in goat diets under Peruvian conditions. We hypothesize that Quebranta grape pomace can enhance goat milk quality, particularly its lipid profile, owing to the pomace's nutritional and bioactive attributes. Using this wine

and *pisco*-making by-product as a partial replacement for conventional forages aligns with circular-economy principles by valorizing vitivinicultural residues, reducing feed costs, and enhancing the sustainability of dairy goat production systems.

## Materials and Methods

### Study site

The study was conducted at AGROPECUARIA DUMAN S.A.C., located in the Huacán sector (11°06'55"S, 77°22'56"W; 395 m.a.s.l.) in the district of Santa María, Huaura Province, Lima, Peru. This site lies along the central coast of the country, approximately 22 km along the Huaura–Sayán highway. The experimental phase took place during the austral winter (July to September), under ambient temperatures ranging from 15°C to 22°C.

### Experimental animals and conditions

Twenty Saanen goats were selected, all primiparous females with singleton births and born within the same week. This sample size provided a statistical power of 0.91 ( $\alpha = 0.05$ ), assuming a standard error of 0.1 and an effect size corresponding to a 0.5% difference in milk fat content. At the onset of the experiment, goats had a mean age of  $22.1 \pm 1.0$  months and a mean body weight (BW) of  $46.2 \pm 2.2$  kg. BW was recorded in the early morning following an overnight fast throughout the experimental period (Supplementary Table 1). Animals were allocated to treatment groups in an alternating sequence according to the order of parturition, with a maximum interval of eight days between birth dates within groups. This approach ensured randomization and balanced distribution of animals by days postpartum across treatments. At the onset of lactation, BW, dry matter intake (DMI), and milk yield were comparable between groups (control: 41.4 kg BW, 2 kg DMI, and  $1.5 \text{ l goat}^{-1} \text{ day}^{-1}$ ; treated: 41.3 kg BW, 2 kg DMI, and  $1.6 \text{ l goat}^{-1} \text{ day}^{-1}$ ). Goats were managed under an intensive production system and housed in group pens of 10 animals per treatment, with a stocking density of  $5.6 \text{ m}^2$  per goat (Fig. 1). Milking was conducted daily between 06:00 and 07:00. The basal diet consisted of a formulated concentrate and dried maize stover, with *ad libitum* access to fresh water.

### Preparation and characterization of grape pomace powder

Fresh grape pomace (*Vitis vinifera*, Quebranta variety) was sourced from wineries in the district of Los Aquijes, Ica, Peru. Processing was performed following a published protocol (Hernández-Salinas *et al.*, 2015). The pomace was pressed and dehydrated using a tunnel-type solar dryer (Fig. 2), reaching temperatures of up to 40°C during peak solar radiation hours (11:00–15:00). The material was manually turned once daily during the first week of drying. Dehydration proceeded for 14 days until a final moisture content of

**Table 1.** Ingredient proportions and nutritional composition of the experimental diets without substitution (T0) and with 8% substitution of dried maize stover with Quebranta grape pomace (T1).

| Ingredients (g/100 g DM)       | T0    | T1    |
|--------------------------------|-------|-------|
| Premix Vitamins/minerals       | 0.28  | 0.28  |
| Cotton seed meal 35            | 5.22  | 5.22  |
| Sugar cane molasses            | 2.61  | 2.61  |
| Salt                           | 0.36  | 0.36  |
| Dried maize stover             | 48.16 | 40.13 |
| Soy meal                       | 13.04 | 13.04 |
| Calcium carbonate              | 0.4   | 0.4   |
| Urea                           | 0.36  | 0.36  |
| Corn                           | 29.57 | 29.57 |
| Grape pomace                   | -     | 8.03  |
| <b>Nutritional composition</b> |       |       |
| DM (%)                         | 87.60 | 87.77 |
| TDN (%)                        | 62.43 | 62.33 |
| CP (%)                         | 15.81 | 16.21 |
| CF (%)                         | 18.31 | 17.04 |
| Ca (%)                         | 0.55  | 0.5   |
| P (%)                          | 0.30  | 0.29  |
| NDF (%)                        | 38.73 | 36.01 |
| ADF (%)                        | 22.21 | 21.35 |
| NE (Mcal/kg)                   | 1.5   | 1.42  |
| Fat (%)                        | 2.26  | 2.72  |
| Ash (%)                        | 5.43  | 5.22  |
| RDP (%)                        | 69.39 | 63.3  |
| RUP (%)                        | 29.95 | 27.9  |
| NFC (%)                        | 37.28 | 35.74 |

DM, dry matter; TDN, total digestible nutrients; CP, crude protein; CF, crude fiber; Ca, calcium; P, phosphorus; NDF, neutral-detergent fiber; ADF, acid-detergent fiber; EN, net energy; RDP, rumen degradable protein; RUP, rumen undegradable protein; NFC, non-fiber carbohydrates.

12% was achieved. The dried pomace was then milled into powder.

The chemical composition of grape pomace was analyzed by triplicate ( $n = 3$ ). Proximate composition was assessed using standard analytical procedures, such as AOAC 950.46, 984.13, 2003.05, 962.09, and 942.05 (AOAC International, 2005), with analyses conducted at Laboratorio de Evaluacion Nutricional de Alimentos, Universidad Nacional Agraria La Molina. Total polyphenols were quantified by UV-visible spectrophotometry using the Folin-Ciocalteu assay, following AOAC method 2017.13 (Kupina *et al.*, 2018). Samples were extracted in 50% acetone, reacted with Folin-Ciocalteu reagent and sodium carbonate, and absorbance was measured at 760–765 nm (limit of quantification: 1.00 mg gallic acid equivalents  $\text{kg}^{-1}$ ). Tannin content was measured from the total

phenolic extract. Tannin content was determined from the same extracts by selective precipitation with polyvinylpyrrolidone (100  $\text{mg ml}^{-1}$  extract), incubation at 4°C for 15 minutes, and centrifugation at 3,000 g for 10 minutes; non-tannin phenolics in the supernatant were quantified by the Folin-Ciocalteu assay, and tannins were calculated by difference on a dry-matter basis (FAO and IAEA, 2000). Total anthocyanins were quantified using the pH differential method, based on absorbance differences at 520 and 700 nm after dilution in pH 1.0 and 4.5 buffers (Barragán-Condori *et al.*, 2021; Giusti and Wrolstad, 2001), and expressed in mg cyanidin-3-glucoside per kg.

Fatty acid profiling was conducted on lipid extracts (obtained as described below for milk samples) using Gas Chromatography (GC) following the conditions described (Li and Watkins, 2001). Extracts were



**Fig. 1.** Animal pens housing Saanen goats for the experimental phase. Animals were housed in groups of 10 per treatment with partial shadow. Feed was supplied in concrete feeders and with *ad libitum* access to water.

injected (1 µl) into a GC system equipped with a flame ionization detector and a Restek SP2560 capillary column (100 m × 250 µm i.d., 0.20 µm film thickness) operating at a split ratio of 35:1. The detector was maintained at 200°C, and carrier gases included hydrogen (40 ml/min), air (450 ml/min), and nitrogen (30 ml/min). Fatty acid methyl esters (FAMES) were identified by comparison with certified standards, and quantification was based on peak area normalization. The analysis quantified major saturated and unsaturated fatty acids (SFA and USFA), from which standard fatty-acid ratios were subsequently calculated.

#### **Experimental diets and feeding**

Two experimental diets were formulated and evaluated as treatments in this study: a control diet (T0), which contained no grape pomace powder, and a test diet (T1), in which 8% grape pomace powder replaced dried maize stover. The 8% inclusion level was selected

based on a pre-experimental trial to preserve feed intake and maintain the integrity of the basal diet, ensuring comparable nutritional profiles across treatments. The composition and nutritional characteristics of each diet are presented in Table 1, with the detailed chemical composition of individual ingredients provided in Supplementary Table 2. Diets were offered at 2.5 kg per animal per day and administered in two equal portions, at 07:00 and 14:00. No dietary adaptation period was included, given the low level of grape pomace incorporation. Consumption records are presented in Supplementary Figure 1 and Table 3.

#### **Milk sample collection and analysis**

Daily milk production was recorded to estimate individual milk yield. Goats were milked using a mechanical milking system following standard hygiene procedures, including visual inspection of udders, cleaning of teats, pre-milking teat disinfection, and



**Fig. 2.** Facilities for Quebranta grape pomace drying. Top: tunnel-type solar dryer tunnel; bottom left: trays for pomace drying; bottom right: dried grape pomace collected after 14 days in the trays.

thorough drying with disposable paper towels prior to cluster attachment. Individual milk samples (80 ml per animal) were collected weekly from each goat in both treatment groups ( $n = 10$ ) during routine milking over the 7-week experimental period and transferred into sterile containers for subsequent analyses. Physicochemical traits, including titratable acidity ( $^{\circ}\text{D}$ ), fat (%), lactose (%), protein (%), salts (%), and total solids (%), were analyzed immediately after milk collection using a Lactoscan Milk analyzer, in accordance with the manufacturer's instructions as applied in previous studies (Gharibi *et al.*, 2020). Calibration of the analyzer was set for goat's milk.

To evaluate lipid profile, total polyphenols content, and anthocyanin levels, composite milk samples were prepared by pooling daily milk production from each treatment group. In weeks 1, 4, and 7, three aliquots of 550 ml (pseudoreplicates) were collected from each treatment pool in each week (repeated measurements). All samples were stored in sterile containers and maintained at  $4^{\circ}\text{C}$ – $8^{\circ}\text{C}$  during transport to the Laboratorio de Análisis de Alimentos.

Fatty acids were extracted following the AOAC Official Method 996.06 (Latimer, 2023), employing a two-step procedure comprising sample preparation and methylation. Briefly, weighed milk samples containing 100–200 mg of fat were transferred into Mojonnier flasks and combined with 100 mg of pyrogallol, 2 ml of a triglyceride internal standard solution, 2 ml of ethanol, and 4 ml of distilled water. After gentle mixing, ammonium hydroxide was added, and the samples were incubated in a water bath at  $70^{\circ}\text{C}$ – $80^{\circ}\text{C}$  for 10 minutes with intermittent agitation. Lipids were extracted using 2–3 ml each of chloroform and diethyl ether, and the solvent phase was evaporated to dryness under a nitrogen stream. Methylation was achieved by adding 2 ml of 7% boron trifluoride ( $\text{BF}_3$ ) in methanol and 1 ml of toluene, followed by heating at  $100^{\circ}\text{C}$  for 45 minutes. Subsequently, water, hexane, and sodium sulphite were added to isolate the upper phase containing the FAMES, which were analyzed by GC under the same instrumental conditions used for grape pomace samples.

Total polyphenols and anthocyanins in milk were quantified using the same analytical protocols applied

**Table 2.** Composition, lipid profile, and total polyphenols content of grape pomace.

| Parameter                                    | Mean      | SEM    | IC 95%                |
|----------------------------------------------|-----------|--------|-----------------------|
| Water (%)                                    | 10.92     | 0.02   | (10.88–10.96)         |
| Total protein ( $N \times 6.25$ ) (%)        | 11.00     | 0.06   | (10.88–11.12)         |
| Fat (%)                                      | 6.95      | 0.04   | (6.87–7.03)           |
| Crude fiber (%)                              | 18.49     | 0.43   | (17.65–19.33)         |
| Ash (%)                                      | 4.56      | 0.05   | (4.46–4.66)           |
| Nitrogen-free extract (%)                    | 48.08     | 0.27   | (47.55–48.61)         |
| NDF (%)                                      | 33.03     | 0.04   | (32.95–33.11)         |
| ADF (%)                                      | 28.08     | 0.07   | (27.94–28.22)         |
| Decanoic acid (C10:0) (g/100 g)              | 0.25      | 0.01   | (0.23–0.27)           |
| Lauric acid (C12:0) (g/100 g)                | 0.49      | 0.01   | (0.47–0.51)           |
| Myristic acid (C14:0) (g/100 g)              | 0.59      | 0.03   | (0.53–0.65)           |
| Palmitic acid (C16:0) (g/100 g)              | 18.44     | 1.31   | (15.87–21.01)         |
| Palmitoleic acid (C16:1) (g/100 g)           | 0.81      | 0.05   | (0.71–0.91)           |
| Stearic (C18:0) (g/100 g)                    | 6.41      | 0.09   | (6.23–6.59)           |
| Cis-9-oleic acid (C18:1n-9) (g/100 g)        | 17.91     | 0.18   | (17.56–18.26)         |
| Linoleic acid (C18:2) (Omega 6) (g/100 g)    | 49.86     | 1.23   | (47.45–52.27)         |
| Arachidic acid (C20:0) (Omega 6) (g/100 g)   | 1.55      | 0.03   | (1.49–1.61)           |
| Cis-11-eicosenoic acid (C20:1) (g/100 g)     | 0.32      | 0.02   | (0.28–0.36)           |
| Linolenic acid (C18:3n3) (Omega 3) (g/100 g) | 1.77      | 0.04   | (1.69–1.85)           |
| Heneicosanoic acid (C21:0) (g/100 g)         | 0.30      | 0.02   | (0.26–0.34)           |
| Behenic acid (C22:0) (g/100 g)               | 1.29      | 0.01   | (1.27–1.31)           |
| SFA (g/100 g)                                | 29.32     | 1.40   | (26.58–32.06)         |
| USFA (g/100 g)                               | 71.97     | 1.41   | (69.21–74.73)         |
| Monounsaturated fatty acids (MUFA) (g/100 g) | 20.34     | 0.22   | (19.91–20.77)         |
| PUFA (g/100 g)                               | 51.63     | 1.27   | (49.14–54.12)         |
| Anthocyanin mg (Cyanidin-3-glucoside)/kg     | 36.35     | 0.04   | (36.27–36.43)         |
| Total polyphenols mg (Gallic acid-eq) /kg    | 67,200.80 | 689.31 | (65,849.35–68,552.25) |
| Tannins (%)                                  | 12.53     | 0.04   | (12.45–12.61)         |

NDF, neutral-detergent fiber; ADF, acid-detergent fiber. Butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), undecanoic acid (C11:0), tridecanoic acid (C13:0), myristoleic acid (C14:1), pentadecanoic (C15:0), cis-10-pentadecanoic acid (C15:1), margaric acid (C17:0), cis-10-heptadecanoic (C17:1), elaidic (trans) acid (C18:1 (w 9 trans)), linoelaidic (trans) acid (C18:2 (w 6 trans)),  $\gamma$ -linoleic acid (C18:3 (w 6)), linolenic acid (C18:3 (w 3)), cis-11,14- eicosadienoic acid (C20:2), cis-11, 14, 17 - eicosatrienoic acid (C20:3 (w 3)), arachidonic acid (C20:4 (w 6), cis-8, 11, 14- eicosatrienoic acid (C20:2 (w 6)) and erucic acid (C22:1 (w 9)) were also tested, but they did not exceed detection limits.

to grape pomace. Antioxidant capacity was assessed, accounting for both hydrophilic and lipophilic contributions to overall antioxidant activity (Arnao *et al.*, 2001).

#### Statistical analysis

Physicochemical parameters and fatty acid composition of the grape pomace powder were first evaluated using exploratory data analysis, including summary descriptive statistics, followed by assessment of data distribution and normality assumptions.

To assess the effects of partial substitution of dietary forage with grape pomace powder on the

physicochemical properties of milk, a linear mixed-effects model was employed, defined as follows:

$$Y_{ij} = \mu + \tau_j + \beta\delta_{ij} + \alpha_i + e_{ij} \quad (\text{Equation 1})$$

where  $Y_{ij}$  represents the physicochemical parameter of the milk sample,  $\mu$  is the overall mean,  $\tau_j$  denotes the fixed effect of the  $j$ -th treatment (T0 and T1), and  $\beta\delta_{ij}$  is the fixed effect of time included as a continuous covariate and defined as the day of milk production when sampling,  $\alpha_i \sim N(0, \sigma_\alpha^2)$  is the random effect of the  $i$ -th subject (goat), and  $e_{ij} \sim N(0, \sigma^2)$  is the residual error. Normality of residuals was evaluated using diagnostic plots presented in Supplementary Figure 2.

**Table 3.** Physicochemical parameters of goat milk ( $n = 10$ , per treatment) after dietary intervention without grape pomace (T0) and with 8% substitution of forage with grape pomace (T1).

| Parameters        | Treatments               |                          | Days            | <i>p</i> -values |
|-------------------|--------------------------|--------------------------|-----------------|------------------|
|                   | T0                       | T1                       |                 |                  |
| Acidity (°Dornic) | 15.6 ± 0.33              | 16.4 ± 0.33              | -0.016 ± 0.007* | 0.0620           |
| Fat (%)           | 3.36 <sup>a</sup> ± 0.12 | 3.93 <sup>b</sup> ± 0.12 | -0.021 ± 0.002* | 0.0045           |
| Lactose (%)       | 4.26 ± 0.07              | 4.21 ± 0.07              | -0.055 ± 0.003* | 0.6158           |
| Protein (%)       | 3.64 ± 0.04              | 3.56 ± 0.04              | 0.005 ± 0.001*  | 0.1211           |
| Salts (%)         | 0.67 ± 0.01              | 0.66 ± 0.01              | -0.005 ± 0.001* | 0.2306           |
| Total solids (%)  | 11.9 ± 0.18              | 12.4 ± 0.18              | -0.083 ± 0.004* | 0.2187           |
| Production (l)    | 1.66 ± 0.09              | 1.65 ± 0.09              | 0.001 ± 0.001   | 0.9856           |

Mean ± standard error. Different letters within a row indicate significant differences between treatments by Tukey's test ( $p < 0.05$ ). (\*): indicates significant effect ( $p < 0.05$ ) of time (sampling point).

Given that lipid profile, total polyphenols, and anthocyanin data were obtained from pooled rather than individual samples, these variables were analyzed using a standard linear model specified as:

$$Y_{ij} = \mu + \tau_i + B_j + e_{ij} \quad (\text{Equation 2})$$

Here,  $Y_{ij}$  corresponds to the variable of interest,  $\mu$  is the overall mean,  $\tau_i$  is the fixed effect of the  $i$ -th treatment (T0 and T1),  $B_j$  represents the fixed effect of the  $j$ -th sampling week (weeks 1, 4, and 7), and  $e_{ij} \sim N(0, \sigma^2)$  is the random error term.

Outcomes derived from pooled milk samples were not statistically replicated at the animal level (pseudoreplicates); therefore, formal population-level inference is not applicable. Accordingly,  $p$ -values reported for these variables should be interpreted with caution and considered hypothesis-generating, warranting confirmation in future studies with appropriate replication.

Post hoc comparisons between treatment means were conducted using Tukey's test at a 95% confidence level, implemented through the *emmeans* (Searle et al., 1980) function in R statistical software, version 4.4.2 (R Core Team, 2025).

#### Ethical approval

Ethical approval was not required for this study, as no invasive procedures or experimental interventions affecting animal welfare were conducted. Animals were managed in accordance with routine farm practices, and all procedures complied with the Peruvian National Animal Protection and Welfare Law No. 30407.

## Results

### Nutritional composition of quebranta grape pomace

The compositional analysis of Quebranta grape pomace (Table 2) revealed a high proportion of nitrogen-free extract (48.08%) and crude fiber (18.49%). Total protein content was relatively elevated for a plant-derived by-product, while the lipid fraction was dominated by PUFAs, with linoleic acid (omega-6) as the principal

component. SFAs comprised a smaller fraction of the total lipid profile. The calculated PUFA/SFA and USFA/SFA ratios were 1.76 and 2.45, respectively. As typically observed in plant matrices, trans fatty acids, eicosapentaenoic acid, and docosahexaenoic acid were below detection limits. Additionally, the pomace was a rich source of bioactive compounds, including anthocyanins (6.35 Cyanidin-3-glucoside), polyphenols (67,200.80 gallic acid-eq/ kg DM), and a substantial concentration of tannins (12.53%).

### Physicochemical properties of milk

Dietary inclusion of grape pomace powder (T1) induced changes in the physicochemical properties of goat milk compared to the control group (T0) (Table 3, Supplementary Fig. 3). The most noticeable change was a 16.96% rise in fat content ( $p < 0.05$ ). In contrast, non-significant reductions were detected in lactose concentration, milk yield, protein content, and salt levels.

Regardless of dietary treatment, the time covariate exerted a significant effect ( $p < 0.05$ ) on all physicochemical parameters, with the exception of milk yield (Table 3). Throughout the experimental period, acidity, fat, lactose, salt content, and total solids exhibited a declining trend, whereas protein concentration increased progressively.

### Fatty acid profile, polyphenols, and antioxidant activity of milk

Observations of fatty acid composition in goat milk after partial substitution of dietary forage with grape pomace powder were recorded (Table 4, Fig. 3). Given that observations come from pseudoreplication accounted differences found are descriptive, and we will only discuss changes higher than 20%. The most relevant potential differences were observed in omega-6 and PUFA concentrations, higher in the T1 group relative to the control (T0). Omega-6 fatty acids increase seems to be driven primarily by linoleic acid (C18:2 n-6 cis), which increased in a 22% (Table 4).

**Table 4.** Lipid profile, polyphenols and anthocyanin concentration in goat milk after dietary intervention without grape pomace (T0) and with 8% substitution of forage with grape pomace (T1).

| Evaluated compounds                                          | Treatments                     |                                |                      |
|--------------------------------------------------------------|--------------------------------|--------------------------------|----------------------|
|                                                              | T0                             | T1                             | Difference T1–T0 (%) |
| Caproic acid (C6:0) (g/100 g fat)                            | 0.08                           | 0.09                           | 12.50                |
| Caprylic acid (C8:0) (g/100 g fat)                           | 0.090                          | 0.093                          | 3.33                 |
| Capric acid (C10:0) (g/100 g fat)                            | 0.28                           | 0.29                           | 3.57                 |
| Lauric acid (C12:0) (g/100 g fat)                            | 0.12                           | 0.13                           | 8.33                 |
| Myristic acid (C14:0) (g/100 g fat)                          | 0.28                           | 0.29                           | 3.57                 |
| Pentadecanoic acid (C15:0) (g/100 g fat)                     | 0.024                          | 0.022                          | -8.33                |
| Palmitic acid (C16:0) (g/100 g fat)                          | 0.66                           | 0.69                           | 4.55                 |
| Palmitoleic acid (C16:1) (g/100 g fat)                       | 0.011                          | 0.012                          | 9.09                 |
| Margaric acid (C17:0) (g/100 g fat)                          | 0.020                          | 0.022                          | 10.00                |
| Stearic acid (C18:0) (g/100 g fat)                           | 0.33                           | 0.37                           | 12.12                |
| Oleic acid (C18:1 (w 9 cis)) (g/100 g fat)                   | 0.61                           | 0.65                           | 6.56                 |
| Linoleic acid (C18:2 (w 6 cis)) (g/100 g fat)                | 0.09                           | 0.11                           | 22.22                |
| SFA (g/100 g fat)                                            | 1.93                           | 2.04                           | 5.70                 |
| Monounsaturated fatty acids (MUFA) (g/100 g fat)             | 0.62                           | 0.67                           | 8.06                 |
| PUFA (g/100 g fat)                                           | 0.09                           | 0.12                           | 33.33                |
| Omega-6 (n-6) (g/100 g fat)                                  | 0.09                           | 0.12                           | 33.33                |
| Omega-9 (n-9) (g/100 g fat)                                  | 0.61                           | 0.65                           | 6.56                 |
| Fat (g/100 g DM)                                             | 2.72                           | 2.90                           | 6.62                 |
| Total anthocyanins [mg/100 g sample, (IC 95%)]               | 1.89<br>(1.54–2.24)            | 1.56<br>(1.21–1.91)            | -17.46               |
| Total polyphenols (mg gallic acid-eq/100 g sample), [IC 95%] | 42.00<br>(38.35–45.65)         | 37.58<br>(33.93–41.23)         | -10.52               |
| Antioxidant capacity (μ Mol trolox/100 g sample, [IC 95%])   | 6351.71<br>(4,735.42–7,967.99) | 6421.49<br>(4,805.20–8,037.77) | 1.10                 |

Gallic acid-eq: gallic acid equivalent. Butyric acid (C4:0), undecanoic acid (C11:0), tridecanoic acid (C13:0), myristoleic acid (C14:1), cis-10-pentadecanoic acid (C15:1), cis-10-heptadecanoic (C17:1), elaidic (trans) acid (C18:1 (w 9 trans)), linoelaidic (trans) acid (C18:2 (w 6 trans)), γ-linoleic acid (C18:3 (w 6)), linolenic acid (C18:3 (w 3)), arachidic acid (C20:0), cis-11-eicosenoic acid (C20:1), heneicosanoic acid (C21:0), cis-11,14- eicosadienoic acid (C20:2), cis-11, 14, 17 - eicosatrienoic acid (C20:3 (w 3)), arachidonic acid (C20:4 (w 6), cis-8, 11, 14- eicosatrienoic acid (C20:3 (w 6)), Behenic acid (C22:0) and erucic acid (C22:1 (w 9)) were also tested, but they did not exceed detection limits.

Total polyphenols content, anthocyanin concentration, or antioxidant capacity of milk did not show such high increments or reductions, plus high variability among experimental units was observed (Table 4).

### Discussion

Grape pomace represents a valuable source of nutrients and bioactive compounds, supporting its use as a functional feed ingredient in animal nutrition. In the present study, Quebranta grape pomace contained 11.0% crude protein and 6.95% fat, values consistent with those reported for other grape varieties (Frincu *et al.*, 2023; Garay-Dominguez *et al.*, 2024). Relative to dried maize stover, the higher protein content of grape pomace is more conducive to ruminal ammonia production, thereby supporting microbial growth and fiber fermentation. Its crude fiber (18.49%)

and neutral detergent fiber (NDF; 33.03%) contents indicate an intermediate fibrous profile that can sustain ruminal function without substantially compromising digestibility, according to practical classifications (Shi *et al.*, 2023). However, the acid detergent fiber (ADF) fraction (28.08%), while promoting chewing activity and digesta passage (Lu *et al.*, 2005), may also reflect a greater proportion of less digestible fiber, considering the NDF content. In addition, the fatty acid profile of grape pomace was notably enriched in PUFAs (51.6% of total fat). Carmona-Jiménez *et al.* (2022) documented markedly higher PUFA levels in oils obtained from the pomace of five grape varieties, suggesting that discrepancies in fatty acid profiles could reflect differences in extraction efficiency or cultivar-specific genetic factors.



**Fig. 3.** Major fatty acids concentration in milk by dietary treatment. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. T0: basal diet; T1: basal diet with 8% substitution of forage with grape pomace, in replacement of dried maize stover.

Milk fat content is a key determinant of dairy product quality and processing efficiency, including cheese yield (Mohan *et al.*, 2020), and changes in fat concentration may therefore have important technological implications. Our results indicate that dietary inclusion of grape pomace significantly increased milk lipid concentration in primiparous Saanen goats ( $p < 0.05$ ), with potential contributions from nearly all fatty acids in the lipid profile (Table 2). Previous studies have shown that dietary fat supplementation is reflected in milk fat content (Chilliard *et al.*, 2003), consistent with the slightly higher lipid content of the pomace diet. Increases in milk fat derived from dietary lipid sources are typically driven by long-chain fatty acids rather than short- or medium-chain fractions (Shingfield *et al.*, 2013), suggesting that *de novo* fatty acid synthesis may also be modulated, although confirmation requires targeted metabolic analyses.

Lipid profiling further indicated that PUFAs, particularly linoleic acid, were the most responsive fraction, although validation in larger biological cohorts is warranted. Beyond processing considerations, enrichment of milk with PUFAs is nutritionally relevant, as these fatty acids have been associated with improved human health outcomes; notably, linoleic acid and its conjugated isomers have been linked to diverse bioactivities, including anti-obesity, antioxidant, immunomodulatory, and anticarcinogenic effects (Badawy *et al.*, 2023). As a rich source of

unsaturated fats, Quebranta grape pomace was expected to favourably modulate milk fatty acid composition, consistent with reports using other USFA-rich feed ingredients (Moya *et al.*, 2023; Pajor *et al.*, 2023). Interestingly, results in goats have been inconsistent. Renna *et al.* (2023) found no significant changes in the milk lipid profile of Camosciata delle Alpi goats supplemented with 6% DM of Barbera grape pomace (dried at 60°C). Similarly, Antunović *et al.* (2024) observed no alterations in milk fat with up to 10% inclusion of grape seed meal but did report enhanced antioxidant activity in the blood of multiparous Alpine goats. In contrast, positive responses have been reported in other ruminant species. In Churra sheep, supplementation with 10% grape pomace increased milk linoleic acid without affecting total PUFA or milk fat content (Manso *et al.*, 2016), while studies in dairy cows have documented improvements in milk PUFA profiles (Ianni and Martino, 2020). For example, Akter *et al.* (2025) reported increased PUFA and linoleic acid concentrations following 30-day supplementation with 15% grape pomace, whereas Chedea *et al.* (2017) found no such effects at the same inclusion level. These inter- and intra-species differences likely reflect variation in pomace characteristics, animal genotype, physiological status, and feeding systems.

Given the prominence of linoleic acid as the predominant PUFA in grape pomace and one of the most abundant in ruminant feeds (Lanier and Corl, 2025),

together with our observations suggesting potential milk lipid enrichment, the mechanisms governing linoleic acid transfer to milk warrant consideration. Because ruminants are unable to synthesize PUFAs *de novo*, their abundance in milk reflects both dietary supply and the extent to which these fatty acids escape ruminal biohydrogenation (Jenkins, 1993; Doreau *et al.*, 2016). In the rumen, biohydrogenation is mediated primarily by *Butyrivibrio* spp. and related microbial consortia, which sequentially convert dietary USFA into more saturated forms (Lourenço *et al.*, 2010). This process involves multiple enzymatic steps carried out by distinct bacterial populations and can be modulated by PUFA concentration; at sufficiently high levels, PUFAs can constrain microbial activity, resulting in incomplete biohydrogenation and the accumulation of intermediate fatty acids (Lanier and Corl, 2015). One such intermediate, vaccenic acid, can escape the rumen and be transported to the mammary gland (Doreau *et al.*, 2016), where it is desaturated by mammary  $\Delta 9$ -desaturase to form conjugated linoleic acid (CLA) (Lock *et al.*, 2008). An increased supply of vaccenic acid may enhance  $\Delta 9$ -desaturase-mediated CLA synthesis in the mammary gland, as previously suggested for lipid deposition in ruminant tissues (Bessa *et al.*, 2015), thereby contributing to higher linoleic acid-derived lipid fractions in milk. In addition, linoleic acid may reach milk through direct ruminal bypass, reaching the small intestine for absorption, facilitated by partial inhibition of biohydrogenation or by PUFA-induced microbial toxicity (Wąsowska *et al.*, 2006; Maia *et al.*, 2010).

Another dietary component of grape pomace that may influence ruminal lipid metabolism is tannins. In the grape pomace used in this study, total tannins accounted for 12.53% of dry matter. Tannins can occur as hydrolysable (HT) or condensed (CT) forms, which differ in degradability and microbial effects, with CT generally showing greater resistance to ruminal breakdown. Although tannins are often associated with a reduction in the overall abundance of biohydrogenating bacteria, previous studies in goats indicate that CT can shift microbial populations toward *Butyrivibrio fibrisolvens* (group A) at the expense of *Butyrivibrio proteoclasticus* (group B). Such microbial shifts have been proposed to favour CLA formation by limiting complete biohydrogenation or promoting the accumulation of CLA-related intermediates in *B. fibrisolvens*. However, CT and HT effects are highly dose- and context-dependent, varying with animal species, diet composition, and tannin source (Purba *et al.*, 2020; Silva De Sant'ana *et al.*, 2022). Supplementation with quebracho tannins (1.5%–3% DM) has increased linoleic acid concentrations in Holstein milk (Henke *et al.*, 2017), although possible negative effects on digestibility and milk yield have been noted. Grape-derived tannins, in particular, have been reported to inhibit ruminal biohydrogenation,

thereby facilitating the transfer of USFA to milk (Makmur *et al.*, 2022). However, excessive inclusion may negatively affect intake, nutrient digestibility, microbial activity, and overall performance (Makkar, 2003; Patra and Saxena, 2011).

Although observations of our study could not suggest grape pomace inclusion effects on milk antioxidant capacity, values exceeded 6,000  $\mu\text{mol Trolox}/100\text{ g}$ , levels comparable to those found in antioxidant-rich foods such as hazelnuts and walnuts (3,500–9,000  $\mu\text{mol Trolox}/100\text{ g}$ ) (Arcan and Yemenicioğlu, 2009). This activity likely stems from both endogenous antioxidants, such as peptides, vitamins, enzymes, and carotenoids (Lindmark-Månsson and Åkesson, 2000; Stobiecka *et al.*, 2022), and other dietary phytochemicals (O'Connell and Fox, 2001; Kontodimos *et al.*, 2023). Lack of clues derived from our observations about anthocyanin transfer to milk, may reflects insufficient concentration in Quebranta grape pomace (36 mg/kg), which is markedly lower than levels reported for wine grapes (270–7,510 mg/kg; Mazza and Francis, 1995), together with the low inclusion of pomace in the diet. In contrast, increases in milk anthocyanins and antioxidant capacity have been observed when anthocyanin-rich forages, such as purple Napier or purple corn silage (813.7–1,180.6 mg/kg DM and 861 mg/kg DM, respectively), replace a substantial proportion (>50%) of the ration (Tian *et al.*, 2019; Onjai-uea *et al.*, 2024). Beyond dosage, the transfer of dietary phenolics into milk is constrained by compound structure, interactions with the food matrix and other nutrients, botanical origin, and host metabolism (Tian *et al.*, 2025). Absorbed phenolics may undergo hepatic biotransformation and preferential urinary excretion too, so partial protection from ruminal degradation does not ensure intestinal absorption (Chedea *et al.*, 2017; Leparmarai *et al.*, 2019).

Overall, the observed increase in milk fat likely reflects modulation of ruminal lipid metabolism by grape pomace components in primiparous Saanen goats. The patterns of milk lipid enrichment, while requiring further replication, are consistent with potential effects of key grape pomace constituents, such as PUFAs, phenolic compounds, and tannins, on the ruminal biohydrogenation process. Definitive confirmation of these mechanisms will require targeted mechanistic studies capable of disentangling microbial, metabolic, and host-level contributions.

#### Limitations

This study evaluated Quebranta grape pomace as a partial substitute of dried maize stover in primiparous Saanen goats during early lactation under an intensive system therefore, extrapolation to other physiological stages, sexes, or production systems is limited. Pseudoreplication further constrains inference to the animal group studied. Nonetheless, the observed effects on milk lipid profile and polyphenol-related traits align with previous evidence on grape pomace functionality.

Future studies with greater replication should incorporate ruminal biohydrogenation intermediates, milk somatic cell count, and blood metabolomics to clarify nutrient transformation mechanisms, assess dose–response effects, and disentangle the specific roles of tannins and other phenolic compounds.

### Conclusion

Replacing 8% of maize stover (DM basis) with Quebranta grape pomace meal from the Ica Valley improved milk lipid content in Saanen goats without affecting milk yield. Descriptive lipid profiling suggests potential enrichments of PUFAs, particularly linoleic acid. These findings support grape pomace as a sustainable feed ingredient with potential to enhance the nutritional value of milk, while advancing circular economy strategies through the valorisation of agro-industrial by-products in a major viticultural region. Future research should explore alternative inclusion levels, particularly in Creole goats, and address issues of standardisation, pricing, and on-farm integration to facilitate commercial adoption.

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### Conflict of interest

The authors declare no conflicts of interest.

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### Authors' contributions

Conceptualization: E.A.S., J.S.-J.; Data Curation:; Formal Analysis: A.V., J.C., J.H.-R., D.J.C.; Funding Acquisition: J.C.; Investigation: J.C., J.C.-G., N.R.-C., J.T., G.C.-L., E.A.S., J.S.-J.; Methodology: A.V., E.A.S., J.S.-J., J.H.-R., D.J.C.-F.; Project Administration: E.A.S., J.C.; Resources: J.C.-G., J.C.; Supervision: E.A.S., J.C.; Validation: D.J.C.; Visualization: D.J.C., J.A.H.-R.; Writing – Original Draft Preparation: E.A.S., J.S.-J., J.A.H.-R., D.J.C.; Writing – Review & Editing: D.J.C., J.A.H.-R.

### Data availability

The data of the experiments will be available from the authors if required.

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## Supplementary Material

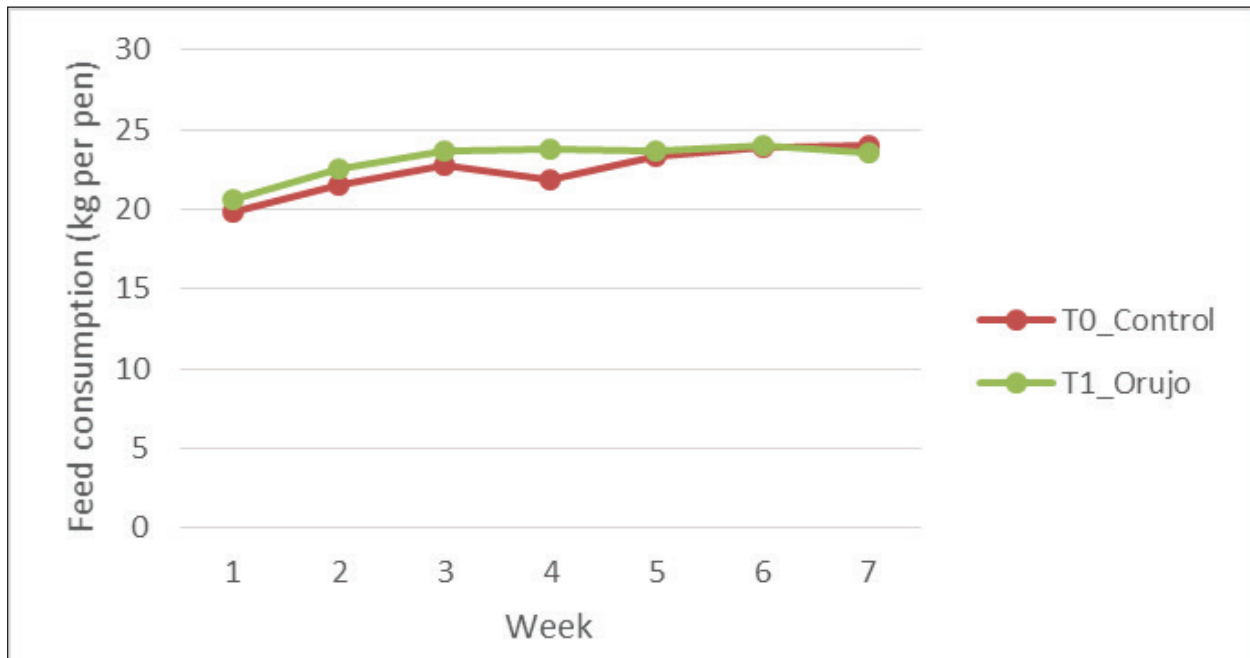
**Supplementary Table 1.** Initial and final BW of animals per treatment.

| Animal ID | Week 1     |            | Week 7     |            |
|-----------|------------|------------|------------|------------|
|           | T0 BW (kg) | T1 BW (kg) | T0 BW (kg) | T1 BW (kg) |
| 1         | 45.90      | 44.80      | 52.20      | 38.70      |
| 2         | 43.30      | 36.60      | 45.80      | 38.60      |
| 3         | 44.80      | 34.65      | 51.00      | 33.70      |
| 4         | 41.00      | 44.30      | 42.30      | 54.20      |
| 5         | 38.70      | 42.60      | 45.20      | 47.00      |
| 6         | 43.40      | 41.50      | 45.20      | 48.80      |
| 7         | 37.45      | 35.35      | 47.10      | 48.70      |
| 8         | 39.75      | 36.05      | 45.20      | 40.90      |
| 9         | 36.45      | 54.35      | 42.20      | 60.70      |
| 10        | 43.60      | 42.95      | 47.50      | 49.70      |
| Mean      | 41.44      | 41.32      | 46.37      | 46.10      |

**Supplementary Table 2.** Nutritional composition of the feed ingredients used in the formulation of basal diet (T0) and basal diet with 8% substitution of forage with grape pomace powder (T1).

| Ingredient               | Price (Soles) | DM (%) | TDN (%) | CP (%) | CF (%) | Ca (%) | P (%) | NDF (%) | ADF (%) | NE (Mcal/kg) | Fat (%) | Ash (%) |
|--------------------------|---------------|--------|---------|--------|--------|--------|-------|---------|---------|--------------|---------|---------|
| Premix Vitamins/minerals | 6.8           | 99.0   | -       | -      | -      | -      | -     | -       | -       | -            | -       | -       |
| Cotton seed meal 35      | 1.35          | 87.5   | 68.0    | 39.0   | 12.0   | 0.2    | 1.2   | 35.6    | 23.0    | 1.68         | 2.3     | 6.7     |
| Cane sugar molasses      | 0.72          | 75.0   | 74.3    | 5.0    | -      | 1.0    | 0.1   | 0.4     | 0.2     | 1.76         | 0.2     | 13.3    |
| Salt                     | 0.50          | 99.0   | -       | -      | -      | -      | -     | -       | -       | -            | -       | -       |
| Dried maize stover       | 0.30          | 87.0   | 50.0    | 5.9    | 34.4   | 0.57   | 0.10  | 67.0    | 39.0    | 1.11         | 1.3     | 7.2     |
| Soy meal                 | 2.33          | 90.0   | 81.6    | 51.0   | 3.4.0  | 0.35   | 0.7   | 14.9    | 10.0    | 1.9          | 2.0     | 6.7     |
| Calcium carbonate        | 0.14          | 99.0   | -       | -      | -      | 39.0   | -     | -       | -       | -            | -       | -       |
| Urea                     | 1.40          | 100.0  | -       | 280.0  | -      | -      | -     | -       | -       | -            | -       | -       |
| Corn                     | 1.28          | 88.1   | 88.7    | 9.4    | 2.6    | 0.04   | 0.3   | 9.5     | 3.4     | 2.01         | 4.2     | 1.5     |
| Grape pomace powder      | -             | 89.0   | 61.4    | 11.0   | 18.4   | -      | -     | 33.0    | 28.0    | 1.38         | 6.9     | 4.5     |

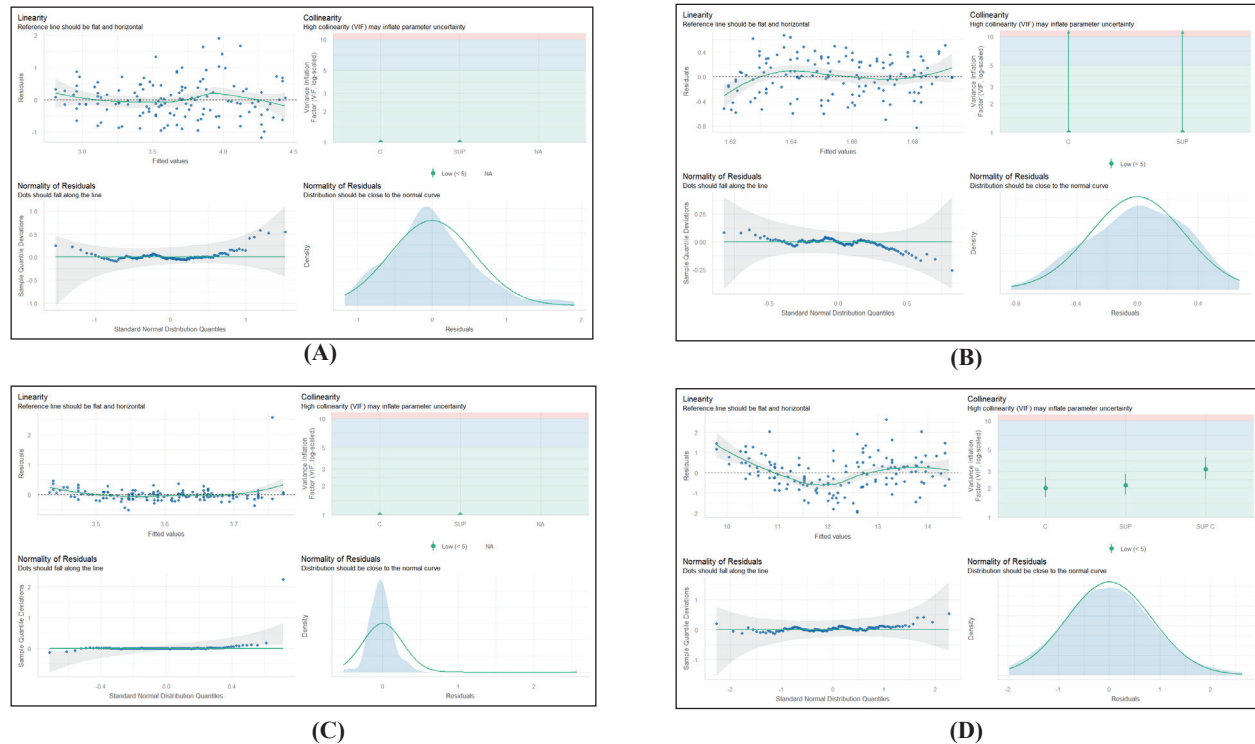
DM, dry matter; TDN, total digestible nutrients; CP, crude protein; CF, crude fiber; Ca, calcium; P, phosphorus; NDF, neutral-detergent fiber; ADF, acid-detergent fiber; NE, net energy. Soles are Peru national currency.



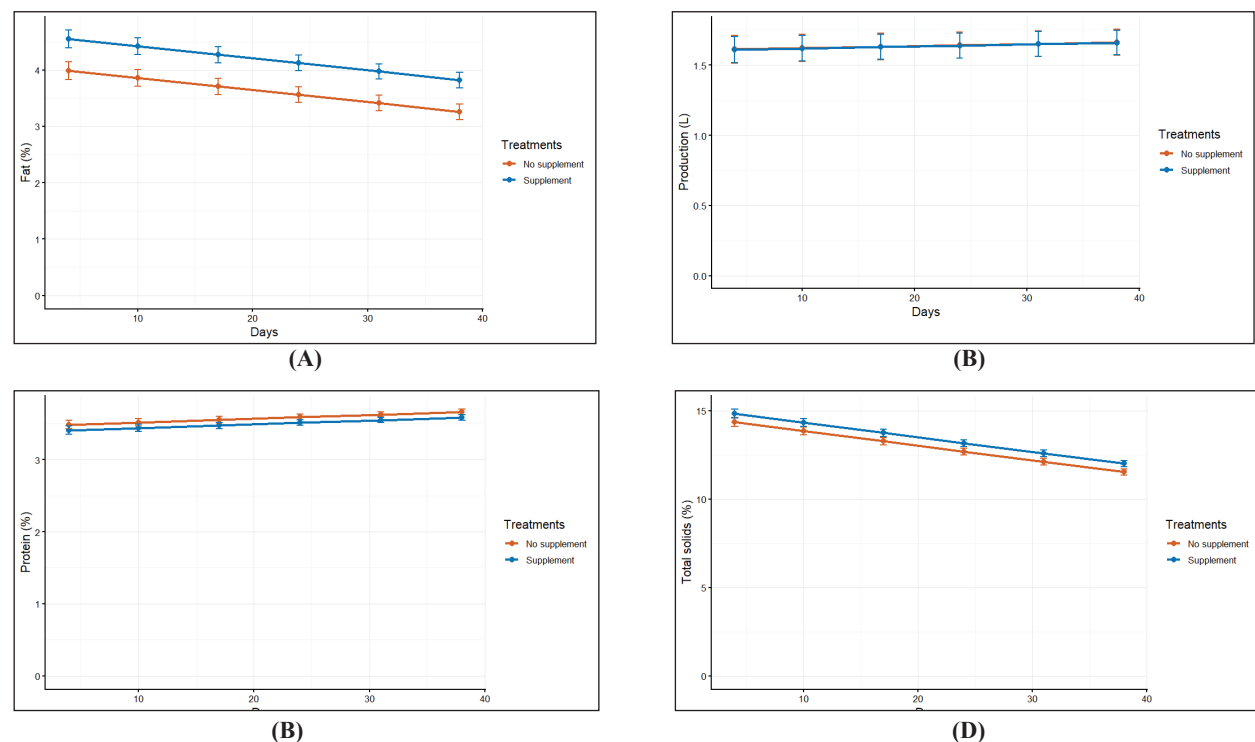
**Supplementary Fig. 1.** Average feed consumption per treatment pen.

**Supplementary Table 3.** Average feed consumption per treatment pen. Pens house 10 goats each.

| Semana | T0 control (kg) (n = 10) | T1 Orujo (kg) (n = 10) |
|--------|--------------------------|------------------------|
| 1      | 19.84                    | 20.66                  |
| 2      | 21.58                    | 22.54                  |
| 3      | 22.81                    | 23.65                  |
| 4      | 21.85                    | 23.78                  |
| 5      | 23.29                    | 23.63                  |
| 6      | 23.85                    | 23.99                  |
| 7      | 24.00                    | 23.60                  |



**Supplementary Fig. 2.** Diagnostic residual plots for milk composition variables. (A) Milk fat (%), (B) milk yield ( $\text{L animal}^{-1} \text{ day}^{-1}$ ), (C) milk protein (%), and (D) total solids (%). For each response variable, the diagnostic panels show: top left, assessment of linearity; top right, collinearity using the variance inflation factor (VIF); bottom left, normality of residuals assessed by sample quantile deviation; and bottom right, distribution of residuals.



**Supplementary Fig. 3.** Weekly trajectories of milk composition variables. (A) Milk fat (%), (B) milk yield ( $\text{L animal}^{-1} \text{ day}^{-1}$ ), (C) milk protein (%), and (D) total solids (%). Bars in every timepoint represent confidence intervals.