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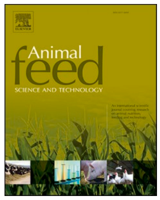

Use of cucumber waste silage as an alternative forage source for sheep

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A B S T R A C T

Horticultural production in the Mediterranean basin generates considerable quantities of by-products, which, if not valorised, can pose environmental problems. This study evaluated the potential of cucumber–straw silage as an alternative forage for sheep. Eighteen Segureña sheep (10 months old; 30 ± 0.4 kg BW) were allocated to two experimental forages: oats hay (CTL, 985 g/kg DM) or cucumber–straw silage (SIL, 15 g/kg DM). The SIL forage contained more NDF (780 vs. 516 g/kg DM) and less CP (64.9 vs. 82.9 g/kg DM) than CTL. The trial included one week of adaptation with *ad libitum* feeding followed by two weeks of restricted intake. Measurements during the last week included digestibility, N balance, rumen fermentation, and blood metabolites. Results indicated that health status remained unaffected, and DMI was similar under *ad libitum* feeding. Under restricted intake, SIL-fed sheep displayed slightly higher DMI (+12 %), greater digestible energy intake (+33 %), and higher ruminal acetate (+10 %) and butyrate (+40 %), accompanied by increased blood β-hydroxybutyrate concentrations (+89 %). Despite lower CP intake, SIL-fed animals exhibited higher ruminal iso-acid proportions and blood urea N, suggesting enhanced proteolysis and urea recycling. These compensatory mechanisms resulted in similar ruminal NH_3-N levels and microbial protein synthesis across treatments, although N retention and efficiency were significantly lower in the SIL group. In conclusion, SIL represents a feasible energy source for sheep, although its limited protein content may require further N supply in high-yielding ruminants. This silage valorisation could reduce food waste and improve circular agriculture and sustainability in livestock production.

1. Introduction

Ruminant production systems face increasing pressure to enhance productivity and meet the growing demand for nutritious and safe food, while addressing sustainability and climate change associated challenges (Halmemies-Beauchet-Filleau et al., 2018). At the same time, food waste has become a critical global issue, significantly contributing to environmental degradation and resource inefficiency (Tonini et al., 2018). In the EU, over 59 million tonnes of food are wasted annually, with Spain alone accounting for about 2.9 million tonnes, much of which is avoidable (Eurostat, 2024; Faostat, 2024). In Mediterranean countries, the expansion of intensive horticulture has generated substantial quantities of vegetable by-products, particularly from greenhouse crops such as cucumber, tomato, pepper, aubergine, and melon (Duque-Acevedo et al., 2020; Jones et al., 2021). Spain produces approximately 770,000 tonnes

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of cucumber annually, of which nearly one-third is discarded (FAO, 2014). Reducing these losses and valorising surplus produce are global priorities, with the United Nations identifying "zero waste" as a key Sustainable Development Goal. Although composting remains the most common recycling strategy, using horticultural waste as animal feed may represent an additional resource-efficient solution (Bakshi et al., 2016).

Fresh vegetable residues, however, present logistical and nutritional challenges due to their high-water content (76–93.5 %), which accelerates spoilage, complicates transportation, and limits their usability (Bakshi et al., 2016). Ensiling offers a low-cost preservation method that produces a stable feedstuff for ruminants (Dunière et al., 2013; Muck et al., 2020). Moreover, silage can be stored during prolonged periods to be used during episodes of feed scarcity. This is particularly relevant in the semi-arid regions where the scarcity of available pasture and forage often forces farmers to purchase expensive feeds (Dong et al., 2023). Numerous studies have examined silages made from fruits or vegetables, either alone or combined with forages, as ruminant feeds showing nutritional values comparable to conventional forages (Arco-Pérez et al., 2017; García-Rodríguez et al., 2019; Hassan et al., 2023; Romero-Huelva et al., 2012; Seok et al., 2016). However, there is a notable lack of information regarding the nutritional value of ensiled vegetables under *in vivo* conditions. Recent studies have explored optimal inclusion levels of these silages in ruminant diets using *in vitro* approaches (García-Rodríguez et al., 2019; Hassan et al., 2023). Among these, cucumber–straw silage has shown promising results *in vitro*, with studies indicating that it can fully replace the dietary forage (representing up to 70 % of the total diet) without negatively affecting rumen fermentation pattern (Hassan et al., 2023).

Building on this background, an *in vivo* experiment was conducted to test the hypothesis that cucumber–straw silage could provide sheep with nutritional value comparable to that of conventional forages in the Mediterranean basin, such as oats hay. Specifically, this experiment evaluated the effects of feeding cucumber–straw silage on rumen fermentation pattern, nutrient digestibility, N balance, and blood health indicators, while also assessing potential limitations for animal performance and health.

2. Material and methods

2.1. Cucumber-straw silage preparation and experimental forages

Two experimental forages were evaluated, and their chemical composition are presented in Table 1. The first forage was used as control, consisting of oats hay (CTL), which is commonly used in intensive and semi-intensive sheep production systems in the Mediterranean. The forage tested against the control was a commercial silage (SIL) made from cucumber fruit and wheat straw. The cucumber–straw silage was obtained from a horticulture by-products factory (Frutilados del Poniente S.L., El Ejido, Almeria, Spain). This factory collects vegetable residues from local production, which are ensiled and subsequently distributed to farms in south-east Spain. Briefly, cucumber surplus or unmarketable pieces were chopped and mixed with wheat straw to reach an average particle size of approximately 10–15 cm in a ratio of 20 % and 80 % (on a fresh matter (FM) basis), which correspond to 1.50 % and 98.5 % DM basis, respectively. This mixture was homogenized using a modified mixer (Tatoma MBS 30, Spain), pressed, and wrapped with multiple layers of bale wrap plastic (25 µm stretch film) using a bale wrapper machine (Lisbach MVA 750, Switzerland). The resultant cylindrical bales measured 1.2 m in hight, 0.8 m in diameter, and weighted approximately 550 kg. Silage quality was evaluated upon opening after around 70 days of ensiling by assessing colour, aroma, and the absence of mould, together with pH measurement. The SIL was considered acceptable as it displayed a golden-brown colour, a firm texture, and emitted a mild, slightly acidic odour, with no effluent or visible spoilage (Kung et al., 2018), and the measured pH was 4.01. Representative samples were collected from the top, middle, and bottom layers of the bale for further nutrient analysis, resulting in an average DM content of 265 ± 15 g/kg FM. In addition, oats were harvested at the dough stage (90–110 days old), dried on the field for approximately one week and stored as hay until the experiment commenced.

Table 1
Chemical composition of the experimental forages (g/kg DM unless stated).

Item	CTL	SIL	SEM	*P*-value
DM (g/kg FM)	922	262	5.84	< 0.001
OM	898	933	5.69	0.012
CP	82.9	64.9	0.661	< 0.001
EE	16.5	20.8	0.279	< 0.001
NDF	516	780	3.32	< 0.001
ADF	240	475	2.54	< 0.001
ADL	23.5	45.5	0.287	< 0.001
NSC	282	67.1	6.45	< 0.001
GE (MJ/kg DM)	16.3	18.2	0.330	0.016
ME (MJ/kg DM)	8.66	9.84	0.184	0.011

CTL = oats hay; SIL = cucumber–straw silage made of cucumber fruit (200 g/kg FM) and wheat straw (800 g/kg FM), equivalent to 15 and 985 g/kg DM, respectively. FM = fresh matter; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = natural detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; Non-structural carbohydrates were calculated as 1000 - (Ash + CP + EE + NDF); GE = gross energy (MJ/kg DM); ME = metabolizable energy (MJ/kg/DM) calculated as: GE x 0.82; (Sauvant et al., 2004).

2.2. Animals and experimental procedures

The experiment was conducted at Estación Experimental del Zaidín (EEZ-CSIC), Granada, Spain. Care of animals was conducted according to Spanish Royal Decree 53/2013 for experimental animal protection, and all the experimental protocols of this study were approved by the Committee for the Care and Use of Experimental Animals of the Andalusian Regional Government (approval number A/18/03/2019/042). All animals remained healthy during the entire duration of the trial, as confirmed by veterinary inspection. Eighteen 10-month-old Segureña sheep with an average BW of 30.0 ± 0.40 kg were randomly assigned to two experimental forages ($n = 9$) following a completely randomized design. Sheep were housed in individual pens (1.2 m x 1.2 m) provided with a slatted floor and individual feeders. All animals had continuous access to fresh water, and the room temperature was maintained between 20 and 22°C during the entire experiment.

The experiment consisted of three consecutive periods: week 1 for adaptation with *ad libitum* feed intake to determine voluntary DM intake (DMI), week 2 was used for adaptation under restricted intake, and week 3 was used for measurements in metabolism crates under restricted intake. After the *ad libitum* phase, the feed allowance was fixed at 0.5 kg DM/day, representing approximately 85 % of the mean voluntary DMI measured in week 1, to standardize DMI across animals and minimize variability. During the *ad libitum* phase, forage provision was expected to slightly exceed maintenance requirements, whereas under restricted feeding the allowance was slightly below maintenance requirements (NRC, 2007). Animals were housed individually, received two equal meals daily (08:30 and 15:00 h), had free access to water, were inspected several times daily, and weighed weekly to monitor BW changes.

2.3. Samples collection

Forage digestibility and N balance were measured during the last week using individual metabolism crates (1.5 × 0.65 × 1.7 m) equipped with separate faeces and urine collection systems, individual feeders, and drinking devices. All sheep had been accustomed to the crates prior to the trial. To avoid potential carry-over effects of crate adaptation, data collection was conducted over three consecutive days after an adaptation during two extra days. During the three-day sampling, daily faeces and urine production were recorded. Faecal samples were weighed, and a subsample (20 % of daily excretion) was dried in a forced-air oven at 60°C for 72 h, pooled per animal, ground at 1 mm diameter, and stored at −4°C for further analysis. Urine samples were collected daily in buckets containing 50 mL HCL (6 N) to maintain a pH below 3 to prevent ammonia (NH_3-N) volatilization. The total urine volume was recorded, and aliquots (10 % of the daily excretion) were pooled per animal and stored at −20 °C for further quantification of N, creatinine, and purine derivative (PD) excretion. Feed refusals were also collected daily, pooled per animal, and analysed to calculate the real nutrient intake.

On the last day of the experiment, blood samples (5 mL) were collected from each animal 2 h after the morning feeding. Blood samples were placed in tubes without anticoagulant, kept 20 min at room temperature, centrifuged at 2000 × g for 15 min and the serum was immediately frozen at −20ºC. Rumen content (50 mL) was collected 3 h after the morning feeding from each animal using a stomach tube connected to a vacuum pump following the method previously described by Ramos-Morales et al. (2014). Rumen samples were filtered through two layers of cheesecloth, and the solids were discarded, the pH level was immediately measured using a laboratory pH probe, and several aliquots were collected from the rumen liquid: One sample (0.8 mL) was diluted with 0.8 mL of acid solution (metaphosphoric acid solution 2 % and crotonic acid 0.4 % in 0.5 M HCL) for volatile fatty acid (VFA) determination, whereas other samples (0.8 mL) were diluted with 0.5 mL of trichloroacetic acid solution (25 %) to determine NH_3-N, and lactic acid concentrations. All samples were frozen at −20 °C until further analysis.

2.4. Chemical analyses

Chemical composition of the forages, refusals, and faeces was assessed following the standard methodology outlined by the Association of Official Analytical Chemists (AOAC, 2005), including DM (method 934.01), OM (method 942.05), and N content (method 990.03). Additionally, urine N was determined to calculate total N excretion (method 993.13), apparent N absorption, and apparent N retention. The consecutive assessment of the neutral detergent fibre (aNDFom-NDF), acid detergent fiber (aADFom-ADF) and acid detergent lignin (aADLom-ADL) were conducted by the Ankom^{220} model fibre analyser (ANKOM Technology) following the protocol established by Van Soest et al. (1991). The NDF analysis incorporated the use of the α-amylase enzyme, and the values were expressed exclusive of residual ash. The ADL content was measured through cellulose solubilization in the ADF residue using 72 % sulphuric acid. Ether extract (EE) content in feed ingredients and faecal samples was determined through extraction with petroleum ether (method 920.39). Furthermore, the gross energy (GE) content of feed, refusal, faecal, and urinary samples were determined using an oxygen bomb calorimeter (Model 6100 compensated jacket, Parr Instruments Co., Moline, IL), with all samples being subjected to oven-drying except for the freeze-dried urine samples.

The quantification of individual VFA concentrations in the rumen samples was carried out using a gas chromatography system equipped with a flame ionization detector (Auto-System, Perkin Elmer, Waltham, MA) following the method described by Isac et al. (1994). Rumen NH_3-N and lactic acid concentrations were assessed through a colorimetric technique outlined by Barker and Summerson, (1941) and Weatherburn, (1967) respectively. The creatinine and PD concentrations in urine were performed according to Balcells et al. (1992) using an HPLC system in our facilities, the total excretion of total PD was calculated by the sum of the quantities of hypoxanthine, xanthine, uric acid, and allantoin excreted in the urine. Serum samples were send to the Laboratorio de Tecnicas Instrumentales, Universidad de León (León, Spain) to quantify the concentrations of parameters related to energy metabolism [β-hydroxybutyrate (BHB), glucose, triglycerides, and cholesterol], protein metabolism (total protein, albumin, creatinine, and urea

N), and hepatic function enzymes (Alkaline Phosphatase "ALP", alanine aminotransferase "ALT", and aspartate aminotransferase "AST") using an auto biochemistry analyzer device (Biosystems BA-400).

2.5. Calculations and statistical analyses

Apparent digestibility of nutrients was calculated as the difference between the amount of nutrients ingested and the amount excreted in the faeces. Energy and N balance were estimated during the measurement period. The energy balance was calculated as the difference between gross energy intake and energy losses in faeces and urine. Nitrogen balance was calculated as the difference between the amount of N ingested and the amount excreted in faeces and urine. Metabolizable energy (ME) content (MJ/kg DM) of the experimental forages was calculated as digestible energy (DE) multiplied by 0.82, which assumes that typical losses of methane and urinary energy are about 18 % of DE (Sauvant et al., 2004).

Data were analysed by one-way ANOVA test using the statistical software package InfoStat general linear model (GLM) procedure as follows:

$$Y_{ij} = \mu + T_i + A_j + e_{ij}$$

Where Y_{ij} is the observations, μ represents the overall mean of the population, T_i is the fixed effect of the treatment (i = CTL *vs* SIL), A_j is the random effect of the animal (j = 1–9), and e_{ij} is the residual error. Once detecting significant effects, comparisons of means were conducted through Fisher's Least Significant Difference LSD test (Fisher, 1935). *P*-values less than 0.05 and 0.1 were considered statistically significant and a tendency, respectively.

3. Results

3.1. Feed composition

The chemical composition of the experimental forages differed markedly (Table 1). Compared to oats hay, cucumber–straw silage had (in g/kg DM) higher OM, EE, and fibre content in terms of NDF (780 vs. 516), ADF (475 vs. 240), and ADL (45.5 vs 23.5), resulting in a higher GE content (18.2 vs 16.3 MJ/kg DM). On the contrary, oats hay had a higher concentration of CP (82.9 vs 64.9 g/kg DM) and non-structural carbohydrates (282 vs 67 g/kg DM).

Table 2
Nutrient intake, rumen fermentation, and apparent digestibility in sheep fed oats hay (CTL) or cucumber–straw silage (SIL).

Items	CTL	SIL	SEM	*P*-value
Ad-libitum phase[1]				
Voluntary DMI, g/day	605	565	22.0	0.215
Restricted phase[2]				
Nutrients intake, g/day				
DM	441	495	8.34	< 0.001
OM	396	462	7.74	< 0.001
CP	36.6	32.1	0.57	< 0.001
NDF	228	386	6.20	< 0.001
ADF	106	235	3.72	< 0.001
EE	7.27	10.3	0.17	< 0.001
Apparent digestibility, %				
DM	63.9	63.3	1.52	0.776
OM	64.7	65.9	1.48	0.583
CP	55.0	43.9	2.07	0.002
NDF	52.3	70.1	1.72	< 0.001
ADF	44.6	70.0	1.76	< 0.001
EE	65.5	67.4	2.87	0.639
Rumen fermentation				
pH	6.44	6.65	0.062	0.027
NH_3-N, mg/dl	8.34	8.90	0.85	0.693
Lactate, mg/dl	0.963	0.966	0.106	0.986
Total VFA, mmol/L	58.6	57.7	4.76	0.894
Acetate, %	60.0	65.9	1.26	0.004
Propionate, %	26.2	12.0	1.26	< 0.001
Butyrate, %	12.1	17.0	1.39	0.023
Isobutyrate, %	0.29	1.32	0.09	< 0.001
Valerate, %	0.85	1.25	0.08	0.002
Isovalerate, %	0.56	2.53	0.33	< 0.001
Ace/Pro	2.38	5.52	0.26	< 0.001

DMI= dry matter intake; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = natural detergent fiber; ADF = acid detergent fiber; SEM = standard error of the mean (n = 9).
[1]Forages provided *ad-libitum* for one week.
[2]Forage allowance restricted to 0.5 kg DM/day for two weeks (equivalent to 85 % of voluntary intake).

3.2. Nutrient intake, rumen fermentation and digestibility

No differences were noted in the voluntary DMI when forages were offered *ad libitum* during the first week of the adaptation period. Throughout the measurement period, despite all animals being offered the same restricted amount of feed, results indicated that sheep fed SIL had higher DMI than those offered the same quantity of CTL forage ($P < 0.001$). These differences were also higher in terms of OM, NDF, ADF, and EE intake ($P < 0.001$). On the contrary, animals fed SIL resulted in a lower CP intake than those fed the CTL forage ($P < 0.001$).

Experimental forages promoted notable changes in ruminal fermentation pattern (Table 2). Rumen pH was higher with the SIL than with the CTL forage ($P = 0.027$). The concentration of NH_3-N remained low and similar between groups (8.62 ± 0.85 mg/dL). Likewise, ruminal total VFA concentration was low (58.1 ± 4.76 mmol) and unaffected by the experimental forages. Notably, the rumen molar proportions of acetate (P= 0.004), butyrate ($P = 0.023$), valerate ($P = 0.002$), iso-butyrate, and iso-valerate (P < 0.001) were significantly higher for animals fed the SIL than CTL forage. In contrast, the CTL forage promoted a significantly higher proportion of propionate in the rumen compared to the SIL forage ($P < 0.001$). Similar apparent digestibility values were observed between groups for DM, OM, and EE. Sheep fed SIL had higher values of NDF and ADF digestibility ($P < 0.001$), whereas those fed CTL had higher CP digestibility (P= 0.002).

3.3. Nutrients use efficiency

All sheep exhibited a tendency to lose weight throughout the experiment (Table 3). Sheep fed the SIL forage had higher GE intake ($P < 0.001$), and digestible energy intake ($P < 0.001$) compared to those on the CTL forage, though they also showed slightly higher faecal energy excretion (P= 0.042). Despite these differences, no significant variations were found between forages in urine energy excretion or energy use efficiency. Both forages resulted in a similar small negative energy balance, leading to comparable BW loss over the experimental period.

Regarding N metabolism, sheep on the SIL forage had lower total N and digestible N intakes than those in the CTL forage ($P < 0.001$). As a result, N retention ($P < 0.001$) and N use efficiency ($P = 0.002$) were lower in the SIL than in the CTL animals. However, no differences were observed between groups in terms of faecal N, urinary N, and total N excretions. Despite the large differences in the water content between both forages, a similar urinary production was observed across animals fed the experimental

Table 3
Body weigh change, energy and N utilization and microbial protein synthesis in sheep fed oats hay (CTL) or cucumber–straw silage (SIL).

Items	CTL	SIL	SEM	*P*-value
Initial BW, kg	31.8	31.9	0.391	0.3501
Final BW, kg	30.3	30.1	0.394	0.7835
BW change, kg/day	−0.061	−0.075	0.011	0.3715
Energy utilization, MJ/day				
GE intake	7.17	9.09	0.135	< 0.001
Digestible energy intake	4.46	5.94	0.137	< 0.001
Faecal Energy excretion	2.71	3.15	0.142	0.042
Urine Energy excretion	5.29	6.01	0.503	0.328
Energy use efficiency, %	62.5	65.4	1.430	0.136
Energy balance	−0.843	−0.865	0.418	0.984
N utilization, g/day				
N intake	5.85	5.14	0.091	< 0.001
Digestible N intake	3.24	2.26	0.112	< 0.001
Faecal N	2.62	2.88	0.133	0.175
Urine N	0.309	0.308	0.044	0.985
Total N excretion	2.93	3.19	0.145	0.212
Retained N	2.93	1.95	0.125	< 0.001
Faecal N / Urine N ratio	8.86	9.43	0.834	0.665
N use efficiency, %	50.0	38.0	2.300	0.002
Urinary excretion				
Total urine, mL/day	881	794	126	0.634
Total PD, µmol/day	3100	3222	306	0.779
Hypoxanthine, µmol/day	538	564	88.3	0.836
Xanthine, µmol/day	177	223	33.8	0.343
Uric acid, µmol/day	279	274	22.5	0.891
Allantoin, µmol/day	2044	2180	260	0.715
Creatinine, µmol/day	2409	2508	271	0.800
PD / Creatinine ratio	1.34	1.21	0.07	0.200
PD / dOM ratio	3.75	3.76	0.36	0.980
PD / N intake ratio	38.6	40.2	4.32	0.803

BW= body weight; Digestible N = intake N – fecal N; Total N excretion = fecal N + urine N; Retained N = N intake – total N excretion; Utilization of N efficiency= retained N / N intake x 100; DE = GEI – FE; Energy utilization efficiency = digestible energy/energy intake x 100; Energy balance = energy intake – (faecal energy + urine energy); PD = purine derivatives; dOM = digestible organic matter; SEM = standard error of the mean ($n = 9$). Total energy excretion doesn't include CH_4 emissions.

forages. As for microbial protein synthesis, no significant differences were detected between experimental groups in the urinary excretion of total PD and specific PD (e.g., hypoxanthine, xanthine, uric acid, and allantoin). Likewise, creatinine excretion and in the ratios of PD to creatinine, PD to digestible OM, and PD to N intake remained unaffected by the type of forage.

3.4. *Blood metabolites*

Sheep on the SIL forage had significantly higher blood concentrations of BHB ($P < 0.001$), albumin ($P = 0.029$), and urea ($P = 0.049$), compared to those on the CTL forage (Table 4). However, no significant differences were observed between groups in other blood parameters related to energy metabolism (glucose, triglycerides, and cholesterol), N metabolism (total proteins, globulins, and creatinine), and liver function enzymes (ALP, ALT, and AST).

4. Discussion

4.1. *Feed composition*

Silages made from straw combined with horticultural by-products offer a promising option for forage preservation (Arco-Pérez et al., 2017). However, their chemical composition and nutritional value vary depending on the plant species, ensiling conditions, and straw-to-waste ratios (Arco-Pérez et al., 2017). Previous studies on tomato- or mixed cucumber–straw silages reported low CP (36–80 g/kg DM) and high NDF (700–800 g/kg DM) contents (Denek and Can, 2006; Hassan et al., 2023). In our study, cucumber–straw silage had comparable CP, EE, NDF, and ADF concentrations, aligning with previously reported ranges for similar straw-based silages (Hassan et al., 2023). Furthermore, a recent multivariate cluster analysis of chemical composition demonstrated that cucumber–straw silage shares over 95 % similarity with other horticultural silages (e.g., tomato, watermelon, pepper-cucumber, and aubergine-tomato) (Hassan et al., 2023), suggesting that our findings may be broadly applicable to a range of similar silages, regardless of the specific fruits or vegetables included. In this study, both experimental forages were low in CP and high in fibre content. However, compared to CTL, SIL contained significantly higher NDF (+264 g/kg DM) and ADF (+235 g/kg DM) primarily due to the inclusion of wheat straw in its formulation, which typically contains about 850 g/kg DM of NDF (Oosting and Waanders, 1993). In contrast, SIL contained 18 g/kg DM less CP and up to four times less NSC than CTL forage, likely due to protein and starch degradation during the ensiling process (Messman et al., 1994; Gordo et al., 2023). Additionally, it is important to consider that vegetables and fruits often contain large proportions of non-protein N, which can further limit the amount of rumen-available protein (Imafidon and Sosulski, 1990).

4.2. *Nutrients intake and digestibility*

The moisture content of forage is a key determinant of preservation quality and animal acceptance. In general, silages achieve optimal fermentation and intake at 60–75 % moisture (depending on the silage type; maize, grass, or horticultor by-products mixed with straw); values above 75 % often impair fermentation and reduce palatability, while those below 55–60 % hinder compaction and increase aerobic spoilage risk (Fahey, 1994; Kim et al., 2015; Liu et al., 2025). Similarly, the recommended range for oats hay is 10–15 % moisture (Kung et al., 2018; An et al., 2020). In the present study, the SIL had a moisture content within the optimal range, making it highly palatable to sheep, and providing sufficient water for the animals. Given that daily water consumption in maintenance-level sheep typically ranges from 140 to 170 mL/kg of metabolic weight (Al-Ramamneh et al., 2012), the water supplied by the SIL forage (when fed *ad libitum*) could meet 70–80 % of their daily water requirements. This is particularly relevant in arid and semi-arid climates, where water availability can be a limiting factor for semi-extensive sheep production. Moreover, under *ad libitum* feeding, individual sheep showed high variability in voluntary DMI. This variation been shown to significantly impact digestibility, rumen fermentation, and the efficiency of nutrient utilization (Huhtanen et al., 2009). Despite offering the same restricted DM

Table 4
Blood parameters related to energy and protein metabolism and liver function in sheep fed oats hay (CTL) or cucumber–straw silage (SIL).

Items	CTL	SIL	SEM	*P*-value
Total protein, g/L	73.2	77.9	2.8	0.251
Albumin, g/L	38.2	42.1	1.14	0.029
Globulin, g/L	34.9	35.8	2.55	0.818
BUN, mg/dL	12.6	20.4	2.60	0.049
Creatinine, mg/dL	1.04	0.97	0.05	0.362
BHB, mg/dL	4.13	7.79	0.66	< 0.001
Glucose, mg/dL	60.9	64.9	2.12	0.209
Triglycerides, mg/dL	18.8	23.0	4.59	0.527
Cholesterol, mg/dL	88.7	81.8	4.81	0.317
ALP, U/L	117	115	13.32	0.910
ALT, U/L	20.3	16.8	1.86	0.204
AST, U/L	95.4	77.3	9.52	0.186

BUN = blood urea nitrogen; BHB= beta-hydroxybutyrate; ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate aminotransferase; Globulin was calculated as the difference between total protein and albumin. SEM = standard error of the mean ($n = 9$).

allowance, SIL-fed sheep achieved slightly higher DMI, which was mainly due to lower refusals, suggesting that SIL was more palatable and better accepted than the CTL forage. Greater consumption of SIL also resulted in higher intakes of NDF and ADF, which have important nutritional consequences, such as stimulating chewing activity and saliva production, supporting rumen buffering and fibre digestion (Allen, 1997).

Interestingly, sheep fed on the SIL forage exhibited greater apparent digestibility of NDF and ADF, despite its higher fibre and lignin concentrations compared with CTL. This result contrasts with the general negative relationship between fibre content and digestibility but can be explained by several interacting factors. Ensiling is known to modify plant cell walls, partly solubilizing hemicellulose and reducing fibre crystallinity, thereby improving microbial access (Muck et al., 2020). Additionally, the smaller particle size of silage compared to hay in this trial may have increased the surface area exposed for microbial colonization and therefore accelerating ruminal fermentation (McAllister et al., 1994). The higher voluntary fibre intake of SIL-fed sheep may also have stimulated fibrolytic microbial activity (Belanche et al., 2017). Similar effects were reported in dairy goats, where replacing oats hay with tomato waste silage resulted in a 16 % increase in NDF digestibility (Arco-Pérez et al., 2017), supporting our interpretation that ensiling and feed structure can compensate for higher fibre concentrations. However, the magnitude of improvement in this study was smaller than in ours, possibly due to the inclusion of sunflower oil in the diet, which is known to linearly decrease fibre digestion as a result of its negative impact on the rumen fibrolytic microbiota (Patra, 2014). Despite the improvements in fibre digestibility, overall DM digestibility remained comparable between the experimental forages. This likely reflects a balance between opposing effects: the greater NDF and ADF digestibility in SIL versus the reduced digestibility of CP and NSC, both of which are limited by the high lignocellulosic fraction of wheat straw (Oosting, 1993). In particular, the NSC concentration of SIL was almost four times lower than that of CTL, reducing the contribution of readily fermentable substrates. As a result, SIL sustained similar DM digestibility to CTL but shifted the digestion profile toward structural carbohydrates rather than NSC or protein. This trade-off underscores the importance of considering nutrient composition, not just overall digestibility, when evaluating alternative forages. Data on the intake and digestibility of cucumber-straw silage is scarce. Research using the rumen simulation technique (RUSITEC) suggests that the forage preservation methods influence feed microbial colonization (Belanche et al., 2017). Specifically, fibre digestibility is higher in fresh forage than in the same forage preserved as hay (Belanche et al., 2016), largely due to an accelerated feed colonization process by rumen bacteria, protozoa, and anaerobic fungi. This mechanism described for fresh forages may also contribute to the improved fibre digestion in silages due to their high-water content as silage stands in between fresh forage and hay. In contrast, although, oats hay typically contains moderate levels of NDF and lignin, which make it reasonably digestible while supplying higher levels of CP and soluble carbohydrates than wheat straw-based silages (Van Soest et al., 1984), its digestion is a slower process that requires more complex symbiotic microbial interactions to occur, as drying forage to hay is known to reduce cell wall degradability compared to ensiling or feeding fresh forage, partly due to decreased microbial colonization and slower particle breakdown (Belanche et al., 2017). Therefore, the lower fibre digestibility in CTL compared with SIL is consistent with previous observations that hay preservation limits the accessibility of structural carbohydrates to rumen microbes. Moreover, our findings align with previous studies on various silages and forages (oats hay, alfalfa hay, and grass hay), which also reported increased fiber intake and a strong correlation between dietary fibre content and digestibility, under different concentrate-to-forage ratios feeding regimes (Romero-Huelva et al., 2012; Carro et al., 2012; An et al., 2020). These results suggest that SIL, despite its higher NDF and lignin content, enhanced fibre digestion relative to CTL forage, while CTL provided superior protein digestibility. This complementarity indicates that SIL could be strategically combined with higher-protein forages or supplemented with additional protein sources to balance nutrient supply and improve overall diet quality for high yielding ruminants.

4.3. Rumen microbial fermentation

The higher rumen pH in sheep fed the SIL forage, compared to those on the CTL forage, even though nature of the SIL is acidic, both were within the normal range and this can be attributed to several factors: 1) the SIL matrix may have contained buffering components (e.g., residual minerals or organic salts) that moderated acid load in the rumen that enhanced rumen buffering capacity (Playne and McDonald, 1966; Cui et al., 2022), which is supported by the linear increase in pH recorded in batch cultures incubated with increasing proportions of cucumber feed blocks (Romero-Huelva and Molina-Alcaide, 2013), 2) increased saliva secretion, likely stimulated by greater chewing and rumination due to the higher fibre intake (Jiang et al., 2017), 3) even though total ruminal VFA concentrations were similar between groups, enhanced VFA removal might be occurred, either through greater absorption across the rumen wall or increased rumen passage rate (Dijkstra et al., 1993, 2012), and 4) potential shifts in rumen microbial populations, such as stimulation of cellulolytic (e.g., *Ruminococcus albus*, *Fibrobacter succinogenes*) and lactate-utilizing bacteria (e.g., M*egasphaera elsdenii, Selenomonas ruminantium*), which ferment more slowly or consume lactic acid, thereby contributing to stabilization of rumen pH (Piknova et al., 2004; Calsamiglia et al., 2008; Cui et al., 2022). Interestingly, although total VFA production did not differ between forages, their molar proportions showed clear shifts. Sheep fed SIL showed a higher acetate and butyrate proportion and lower propionate proportion, consistent with the greater NDF and lower NSC content of SIL, since fibrous diets favour acetate and butyrate production, whereas starch- and sugar-rich diets promote propionate synthesis (Van Soest et al., 1984; Bergman, 1990). These findings align with our previous *in vitro* study (Hassan et al., 2023), in which oats hay, was completely replaced with SIL, resulting in higher acetate and lower propionate proportions, without affecting total VFA concentrations. Similar increments in acetate molar proportions have also been attributed to the higher fibre content when batch cultures were supplemented with cucumber or cucumber plus tomato feed blocks (Romero-Huelva and Molina-Alcaide, 2013). A higher acetate: propionate ratio generally supports fibre digestion and milk fat synthesis but provides less glucogenic energy, which may limit performance in growing or lactating animals that rely on propionate as the main precursor for gluconeogenesis (Bergman, 1990).

Moreover, ruminal iso-butyrate and iso-valerate proportions were three folds higher in sheep on the SIL compared to the CTL group, suggesting an increased degradation of valine and leucine, respectively (Zhang et al., 2013; Wang et al., 2019). This finding suggests increased ruminal proteolysis in animals fed the SIL forage, which could partially explain why rumen NH_3-N concentration remained unchanged despite the 12 % lower N intake relative to the CTL forage. As a result of this, rumen NH_3-N concentrations met the minimum requirement of 5 mg/dL NH_3-N for optimal microbial growth (Firkins et al., 2007). Thus, while SIL promoted a fermentation profile compatible with maintenance diets, the reduced propionate yield highlights a limitation for production-oriented systems unless combined with additional easily degraded carbohydrates sources.

4.4. Nutrient use efficiency

This study showed that SIL represents a promising alternative energy source for ruminants, leading to higher GE intake (+37 %) and digestible energy (+33 %), while faecal energy excretion was only 16 % higher than in the CTL forage. Despite its higher fibre content, the SIL resulted in similar energy use efficiency and energy balance compared to the CTL forage. This could be attributed to the adaptive capacity of the rumen microbiota, which enhances its ability to digest fibrous by increasing microbial abundance of bacteria, methanogens, and protozoa, as well as promoting greater microbial diversity and network complexity (Belanche et al., 2019). A further description of the rumen microbiota could help to support this hypothesis under our experimental conditions. In line with our results, (Arco-Pérez et al., 2017) observed an increased supply of digestible energy when dairy goats were supplemented with tomato silage, although this did not significantly affect feed efficiency and milk production. As our sheep were fed a restricted amount of forages, all sheep were in negative energy balance which resulted in a small BW loss (average −68 g/day). This was expected and aimed to have comparable intake conditions for a net nutritional assessment but did not intend to simulate farm feeding conditions.

Although SIL had a much higher water content than CTL forage, urinary output did not differ between the two groups. This may be explained by compensatory drinking behaviour, whereby animals consuming wetter diets such as silages reduce their voluntary water intake, leading to a similar overall water balance (Kim et al., 2015). Since water intake was not measured in this study, this remains a limitation that should be addressed in future trials.

Nitrogen digestion and utilization in ruminants depend to a large extent on rumen microbial degradation and subsequent faecal and urinary N excretion (Calsamiglia et al., 2010; Tolkamp, 2010). In this study, sheep fed the SIL forage consumed less N but excreted similar amounts of N in faeces and urine compared with CTL, which reduced both retention and efficiency of use. Nevertheless, N balance remained positive across animals. Interestingly, despite these reductions and the small BW losses observed in both groups, N balance remained positive, with the N use efficiency being lower. This apparent discrepancy can be explained by the different dynamics of energy and protein metabolism: when nutritional requirements are not fully met (expected in both experimental forages), leading to a negative energy balance, animals may mobilize body fat to compensate for the deficit while still retaining absorbed N in body tissues, particularly in short-term feeding trials (Hristov et al., 2019; Orquera-Arguero et al., 2023, 2024). Thus, N retention can occur despite modest BW loss, as BW change reflects both fat and protein turnover. In SIL-fed sheep, the lower N intake and efficiency likely limited the extent of retention compared with CTL. Several factors may explain this reduced N use efficiency: 1) increased protein breakdown, supported by the higher ruminal proportions of iso-butryrate and iso-valerate, 2) insufficient or poorly synchronized dietary energy and N degradation in the rumen, as the rapid availability of N combined with slowly degradable energy may limit optimal microbial activity, leading to elevated blood urea concentrations as noted in this study (Firkins et al., 2007; Zhang et al., 2020), and 3) presence of N bound to lignin in the wheat straw from the SIL forage may limit intestinal N digestion and reducing N use efficiency as escribed for similar agro-industrial by-products (Gasa et al., 1989; Vastolo et al., 2022). These findings align with previous research indicating that wheat straw and unconventional silages, particularly those containing fruits and vegetables, often result in lower N digestibility and reduced N and energy efficiency compared to traditional forages such as alfalfa or oats hay (Van Soest, 1994; Poppi and McLennan, 1995; Romero-Huelva and Molina-Alcaide, 2013; Seok et al., 2016; Arco-Pérez et al., 2017). Therefore, to enhance N utilization efficiency, supplementing straw-based silages with additional protein sources is recommended, particularly for ruminants with higher nutritional requirements (e.g., fattening, pregnancy, or lactation).

Urinary purine derivatives, including allantoin, uric acid, xanthine, and hypoxanthine, have been broadly used as indicators of microbial protein synthesis, as they originate from microbial nucleic acids reaching the duodenum, ultimately determining microbial N flow (Carro et al., 2012; Zhou et al., 2017). Our results showed no significant differences in PD excretion, PD/creatinine ratio, or microbial protein supply between sheep fed the SIL and CTL forages. This suggests that replacing oats hay with SIL did not negatively impact microbial protein synthesis or protein metabolism. These findings are consistent with previous studies showing that diets incorporating low-quality forages such as wheat straw (up to 25 % in lambs and 7.5 % DM in calves) had no adverse effects on PD excretion or microbial protein synthesis, regardless of the dietary protein level (Dorri et al., 2021; Mahboobi et al., 2023; Jalayerinejad et al., 2024). Furthermore, creatinine excretion remained stable, indicating that muscle metabolism and protein turnover remained unchanged (Wyss and Kaddurah-Daouk, 2000).

4.5. Blood metabolites and liver enzymes

Blood parameters and liver enzymes are key indicators of animal health, nutrition, and metabolic function, particularly in ruminants, where they reflect the dietary impact on physiological processes. In this study, all sheep remained healthy, with blood metabolites falling within the physiological range (Desco et al., 1989), confirming that both experimental forages adequately supported overall health during the trial.

However, animals fed the SIL forage exhibited a remarkably higher blood BHB concentration (+3.66 mg/dL) compared to those on the CTL forage. This increase may be partially attributed to two complementary mechanisms. First, the higher rumen butyrate molar proportion (+40 %), as butyrate is converted to BHB during absorption through the rumen wall (Holtenius and Holtenius, 1996). Second, SIL-fed sheep experienced greater but not statistically significant BW loss (14 g/day lower) due to high inter-individual variability, suggesting a more negative energy balance than CTL. Increased adipose mobilization under energy deficit would elevate circulating NEFA (not measured here) and thereby enhance hepatic ketogenesis, as reported in similar contexts (Mekuriaw, 2023). Thus, the BHB increase in SIL likely reflects both fermentation-derived precursors and enhanced fat mobilization.

Liver enzymes (ALP, ALT, AST) did not differ between groups and remained within physiological ranges (Braun et al., 2010), suggesting that SIL did not compromise liver function. Similarly, plasma glucose concentrations were maintained within physiological ranges, indicating that gluconeogenesis was sufficient to meet basal energy needs, with fat reserves serving as the main energy source. This adaptive response has been described previously, where higher ketone bodies did not necessarily indicate impaired health, but rather a shift in energy metabolism under restricted intake (Emmison et al., 1991; Guliński, 2021). Therefore, the elevated BHB in SIL-fed sheep is best interpreted as evidence of a more negative energy balance, consistent with their slightly greater BW loss, but within the scope of a short-term physiological adaptation rather than a pathological condition.

In terms of N metabolism, the SIL-fed sheep exhibited a 7.8 mg/dL increase in BUN concentrations, which was not expected as these sheep exhibited similar rumen NH_3-N concentrations, and they experienced lower N intake than the CTL-fed sheep. However, several mechanisms could explain this result: (i) ruminants consuming low-protein diets exhibit greater urea recycling efficiency, with up to 95 % of synthesized urea being returned to the rumen via saliva and the rumen wall when CP intake falls below 7 % (Carneiro de Souza et al., 2021), (ii) increased urea fluxes may therefore elevate circulating BUN while ruminal NH_3-N remains stable because recycled urea is rapidly utilized by microbes. In addition, higher iso-acid concentrations in SIL suggest greater ruminal proteolysis, which could increase hepatic urea synthesis (Getahun et al., 2019; Hailemariam et al., 2021; Nichols et al., 2022). Importantly, plasma creatinine and urinary creatinine excretion were not different between groups, and urinary PD were similar, arguing against extensive muscle proteolysis as the main source of higher BUN. These processes provide a crucial N source for microbial protein synthesis under dietary N-shortage (Oliveira et al., 2020). This physiological adaptation may explain why rumen NH_3-N concentrations and microbial protein synthesis remained similar between treatments, despite the lower N intake with the SIL forage. Furthermore, this compensatory mechanism appeared sufficient to mitigate N deficiency, as no differences were observed in total blood protein concentrations across forages. Notably, animals on the SIL forage exhibited higher albumin levels, may indicate adequate protein status despite low dietary CP. However, as total protein and creatinine concentrations were unaffected, and urinary output was similar between diets, dehydration is unlikely to be the main cause, further supporting the experimental forages adequacy in maintaining N balance for ruminants with low nutritional needs. However, studies under short-term balance may not fully capture these dynamics, and future work should include body composition measurements or isotope tracer studies of urea kinetics to disentangle recycling from proteolysis.

5. Conclusions

This study indicates that cucumber-straw silage can be considered a safe and feasible alternative to conventional forages (i.e. oats hay) for sheep fed at near maintenance level. This silage constitutes a valuable energy source, as led to higher fibre digestibility, rumen acetate and butyrate proportions and blood BHB concentration than oats hay. Although its low N content was partially offset by enhanced urea recycling, resulting in similar ruminal NH_3-N values and microbial protein synthesis to those observed with oats hay, this limitation, together with the reduced N efficiency, may constrain its use in high-yielding ruminants unless additional N supplementation is provided. Further on-farm trials, particularly involving growing or lactating ruminants, are warranted to validate these findings under practical conditions and to assess the contribution of cucumber-straw silage to circularity and sustainability in ruminant production.

CRediT authorship contribution statement

Mahmoud Hassan: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Rivelli Ines:** Methodology, Investigation, Writing – review & editing. **David R. Yáñez-Ruiz:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Alejandro Belanche:** Writing – review & editing, Validation, Supervision, Methodology, Data curation, Conceptualization.

Ethics statement

All sheep used in this study were cared for and managed by qualified personnel in accordance with the provisions of Spanish Royal Decree 53/2013, which governs the protection of animals used for scientific purposes. The experimental procedures were approved by the Andalusian Regional Government (approval number A/18/03/2019/042), the competent authority responsible for animal welfare oversight.

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Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use generative AI or AI-assisted technologies in the preparation of this work.

Declaration of Competing Interest

The authors declare no competing interests.

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Data availability

None of the data were deposited in an official repository. However, the data supporting the study's findings can be obtained upon request.

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