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Feeding broccoli and cauliflower to dairy sheep: influence on feed intake, metabolic health status and milk production and composition

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ABSTRACT

The production of broccoli and cauliflower generates large amounts of vegetable by-products that often accumulate in the environment; however, these residues have nutritional value for ruminants. This study evaluated the effects of including industrial broccoli and cauliflower waste in the diet of dairy ewes on feed intake, metabolic health and milk production and composition. Thirty Assaf dairy ewes in mid-lactation were divided into three groups, with 10 animals per group. The control group (**CON**) received a total mixed ratio (**TMR**) *ad libitum*, the broccoli group (**BRO**) received the same TMR plus 1.5 kg FM/d of fresh chopped broccoli, and the cauliflower (**CAU**) group received the TMR plus 1.5 kg/d of fresh chopped cauliflower. The trial lasted 42 d, divided into 21 d for adaptation and 21 d of measurements, where feed intake and milk yield were recorded daily. Milk samples were taken once a week. At the end of the experiment, fresh cheese was prepared, using the milk from each feeding group. A sensory analysis was carried out, to detect eventual differences in flavour among treatments. Blood samples were taken to analyse the biochemical profile and acid-base status. The animals of the BRO and the CAU groups ate all the vegetal offered without decreasing the daily intake of TMR or affecting milk production. No differences were observed between groups in the biochemical profile or the blood acid-base status, with the exception of the urea concentration, which was higher in the BRO group compared to the control (55.2, 61.8, 57.2 mg/dL for CON, BRO and CAU; $P = 0.022$). Regarding milk composition, the most relevant differences were observed in protein content, being higher in the BRO group ($P = 0.006$) than in the CON and CAU groups, and in the fatty acids profile, which showed an increase in the saturated fatty acid content ($P = 0.012$) and a decrease in the monounsaturated fatty acid content ($P = 0.005$) in the groups supplemented with broccoli and cauliflower. In conclusion, supplementation with 1.5 kg of broccoli or cauliflower in lactating sheep did not affect feed intake, milk production or health status of the animals and had little impact on the quality of milk.

Implications

The use of food not marketable for humans, such as waste and surpluses of broccoli and cauliflower, for animal feeding has economic and environmental relevance. For growers, it offers a way to profit from current losses. Environmentally, feeding ruminants with these by-products would help reduce agro-industrial waste, which could otherwise contribute to environmental pollution. The inclusion of 1.5 kg of broccoli or cauliflower in the diet did not adversely affect the health status or milk production of lactating ewes. These findings suggest that such agricultural by-products can be considered as sustainable feed resources for dairy sheep.

Introduction

Worldwide broccoli and cauliflower production have considerably increased in last decades, reaching 27 million t in 2020 (FAO, 2020). This increase is likely due to their proven health-promoting effects as human food (Aghajanpour et al., 2017; Jeffery and Araya, 2009; Owis, 2015), combined with the fact that broccoli and cauliflower are relatively easy to grow, prepare, and are usually competitively priced compared to other legumes. Spain is one of the main producing countries of broccoli and cauliflower,

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with a production of 746 510 t in 2020 (Conversa et al., 2019; and FAO, 2020). Only between 61 and 80% of the above-ground biomass of broccoli and cauliflower, respectively, is comestible for humans (Rodríguez-Palleres and Rojas-González, 2022). Added to that, a significant proportion of comestible broccoli and cauliflower that do not meet commercial standards is discarded.

Brassica species appear to have a high nutritive value for ruminants, with high–protein content, although low in fibre and relatively high in the calcium-phosphorous ratio (Wiedenhoeft et al., 1994). Broccoli and cauliflower contain high amounts of secondary plant compounds, such as phenols, glucosinolates, sulforaphane; as well as vitamin C, E and A and a range of essential minerals (Nagraj et al., 2020; Picchi et al., 2020). However, despite the nutritive potential of these vegetables, not many studies have been carried out to evaluate their use for livestock feeding. To the best of our knowledge, no *in vivo* studies have been published yet regarding the use of cauliflower waste in dairy livestock, although *in vitro* studies suggest that it could be used without negative effects on ruminal fermentation (de Evan et al., 2020b).

In contrast, the inclusion of broccoli waste has been evaluated in dairy cows, goats and sheep, with contradictory effects on feed intake, milk yield and composition (Aziz, 2021; Monllor et al., 2020a, 2020b; Yi et al., 2015), which could be related to level of inclusion and the type of broccoli waste (dry, fresh or silage, or with leaves...). To our knowledge, no research has been conducted to study their effects on micelle properties or on sensory attributes of dairy products, as cheese.

This study was therefore aimed to evaluate the influence of supplementing lactating ewes with a limited amount of flowers and stems of broccoli or cauliflower on feed intake and efficiency, metabolic-health status and milk yield and composition.

Material and methods

Experimental design

The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Universidad de León (approval number ULE_014_2016) and the Junta de Castilla y León (Spain), following proceedings described in Spanish and European Union legislation (Royal Decree 53/2013 and Council Directive 2010/63/EU).

Thirty Assaf dairy ewes in the middle of lactation (64 ± 0.6 d postpartum) were divided into three homogeneous groups (10 ewes per group) based on BW (73.6 ± 1.72 kg) and milk yield (1980 ± 102 g/d). All animals received the same total mixed ration (**TMR**) *ad libitum*. The control group (**CON**) only received the TMR, the broccoli group (**BRO**) received daily the TMR diet plus 1.5 kg of freshly chopped commercial broccoli florets and the cauliflower group (**CAU**) received daily the TMR diet plus 1.5 kg of freshly chopped commercial cauliflower florets. TMR and BRO or CAU supplements were given in different feeders. The chemical composition of the TMR, the broccoli and the cauliflower is summarised in Table 1.

Table 1
Ingredients of the total mixed ration (TMR), and chemical composition of TMR, broccoli and cauliflower used in the diet of lactating ewes.

Ingredients, g/kg	TMR		
Alfalfa hay	526		
Oat	76		
Corn	147		
Soy 44/47	134		
Sugar beet pulp	53		
Sugar beet molasses	42		
Calcium carbonate	4		
Salt	5		
Vitamin-mineral mixture	13		
Chemical composition (g/kg DM)	TMR	Broccoli	Cauliflower
DM (g/kg FM)	909	83.4	82.5
OM	884	887	889
CP	199	310	209
NDF	237	236	227
ADF	152	144	145
EE	9.01	37.0	22.0

Abbreviations: TMR = Total mixed ration, OM: Organic matter; EE: Ether extract.

Experimental procedures

Throughout the trial, the animals were housed in individual pens (2.25 m^2) allowing visual and tactile contact with other animals. Fresh water was offered *ad libitum*. The trial was divided into two periods: an adaptation (21 d) and a measurement period (21 d). During the adaptation period, the animals received the TMR *ad libitum* and broccoli and cauliflower were progressively introduced. During the experimental period, BRO and CAU sheep were given 1.5 kg of broccoli and cauliflower, respectively, immediately after milking (at 0900 h in the morning). After 2 h, they were offered the TMR *ad libitum*.

Individual feed intake was recorded during the measurement period. Daily, before milking, refusals from each animal were removed and weighed and a sample was taken for chemical analysis. In addition, two 250 g samples of TMR, chopped broccoli and cauliflower were taken, one at the beginning of the trial and one at the end for chemical analysis. The amount of TMR offered was calculated and weighed daily to allow 15% leftovers. The individual intake was calculated as the difference in DM of the offered feeds and the DM of the remains.

Animals were weighed at the beginning of the adaptation period (day 1), to balance the groups, and then on days 21 and 42 (beginning and end of the experimental period). All animals remained in good health during the trial, except one from the cauliflower group which had to be removed from the experiment due to health issues (mastitis) unrelated to experimental diets.

Blood samples were collected to assess the biochemical profile and health status of the animals on day 0 (just before the start of the trial), day 21 (before the experimental period), and day 42 (at the end of the experiment). Two samples were taken per animal, one with heparin for acid-base analysis and the other without anticoagulant for biochemical analysis.

During the experiment, ewes were milked once a day and milk yield was recorded using automatic meters (DeLaval® MM 25 SG, Germany). On days 1, 3, 7, 10, 14, 17, and 21 of the measurement period, milk samples were taken from each ewe to analyse chemical composition and somatic cell count. Additionally, a 12-mL milk sample was taken from each ewe on days 1, 7, 14 and 21, frozen, lyophilised and stored until fatty acid (**FA**) and liposoluble vitamin analysis. On the last day of the experiment, an additional milk sample (40-mL) was taken for casein micelle characterisation, and 1 L of milk was also taken from each ewe and used for making fresh cheese for sensory analysis. These samples were immediately refrigerated at 4 °C.

The daily milk yield and the daily DM intake were used to calculate the feed conversion ratio. The feed conversion ratio was calculated as the ratio between the average milk production and the average DM intake (milk production (kg) / total DMI) at weekly intervals during the 21-day experimental period.

Chemical analysis

The offered feed and the remains were analysed for DM (ISO 6496:1999; ISO, 1999), ash (ISO 5984:2002; ISO, 2002) and CP

(ISO 5983:2009; ISO, 2009). Broccoli florets and the cauliflower samples were dried at 40 °C for 24 h. Afterwards, samples were homogenised and frozen (−18 °C) until FA and liposoluble vitamin analysis, following the procedures described below for milk samples. Table 2 shows the FA percentage and liposoluble vitamins in the diet for the CON group (only TMR), the BRO group (TMR plus 1.5 kg of broccoli) and the CAU group (TMR plus 1.5 kg of cauliflower) calculated considering the average ingestion of TMR for each group.

Blood samples with heparin were immediately analysed after sampling in a VetStat blood gas and electrolytes analyzer (Idexx, Barcelona, Spain) to assess the acid-base status by determining pH, bicarbonate (HCO_3^-), CO_2 pressure (pCO_2), anion gap, total CO_2 (tCO_2), Na^+, K^+, and Cl^- concentrations.

Blood samples without anticoagulant were allowed to clot in a water bath at 37 °C for 30 min and then centrifuged at 3 520 × g for 16 min at 4 °C. The serum was stored at −80 °C until it was used to measure biochemical parameters [alanine aminotransferase (**ALT**), glucose, calcium, urea, creatinine, lactic acid, albumin, gamma-glutamyl transferase, β-hydroxybutyric acid (**BHB**) and non-esterified fatty acids (**NEFA**)] using the clinical chemistry and turbidimetry analyzer BA400 (Biosystems, Barcelona, Spain).

Automated IR (Miko-Scan 225, A/S Foss Electric, Denmark) was used to determine the content of crude fat, CP, total solids, urea and BHB in milk samples. The lactose content was calculated by subtracting the amount of fat and CP from the total solids. For the analysis of somatic cells, a Fossomatic 90 analyzer (Foss Electric, Denmark) was used.

For FA analysis, duplicate samples of 0.15 g lyophilised ewe milk were submitted to *in situ* transesterification (Carrapiso et al., 2000). Gas chromatography was performed using the method of Liu et al. (2007) with minor modifications. Helium was used at 3 mL/min, injector, detector and transfer line temperatures were 200, 300, and 230 °C, respectively. Two μL of extract was injected with a 20:1 split ratio. The oven temperature started at 170 °C (held for 24 min) and then increased to 220 °C at 7.5 °C/min, and to 230 °C at 10 °C/min (held for 5 min). Detection, identification and quantification were carried out according to Andrés et al. (2019). Results were expressed as a percentage of total FA.

Retinol and tocopherols were determined, in duplicate, in the milk samples. For the analysis of retinol, an aliquot (0.25 g) of lyophilised ewe milk was subjected to a saponification reaction and then solvent extraction with hexane (Mestre Prates et al., 2006). The filtered (0.45 μm) hexane solution was analysed by high-performance liquid chromatography following Jin et al. (2015). Tocopherols were extracted, from 0.2 g of lyophilised milk, and analysed as described by Humada et al. (2014), with the exception that no internal standard was added. The external standard method, with retinol, α-tocopherol, γ-tocopherol and δ-tocopherol standards (Sigma Aldrich Química, S.L., Madrid, Spain) was used for identification and quantification.

Samples for micelle characterisation were centrifuged for 20 min at 3 000 × g to separate the fat content from the milk. An aliquot of 4 mL of centrifuged-skimmed ewe milk (in the bottom layer of the tubes) was diluted with 4 mL of water and then filtered through a 0.45 μm-pore-size membrane with the help of a syringe and afterwards refrigerated in plugged glass tubes for up to 24 h (Glantz et al., 2010).

Micelle characteristics (particle size, distribution, and surface charge) were measured by dynamic light scattering (DLS) using a Zetasizer Advance - Pro (Red) instrument (Malvern Panalytical Ltd, Malvern, UK) equipped with a 663 nm and 10 mW He–Ne laser operating, at an output power of 148 V and a scattering angle of 173°. The measurements were carried out in triplicate at 25 °C with a refractive index of 1.345 and an equilibrating time of 2 min per sample. The D (0.5) value (median), Z-average, and polydispersity index (PDI) data were collected.

Triangle tests were performed by an untrained 30 member-panel. Analyses were carried out with fresh cheese prepared from the ewe's milk from each experimental group. After milking, a mixture of one L of milk per ewe (10 L in total) was sieved and pasteurised at 65 °C for 30 min, the milk was then tempered to 36 °C and curdled with commercial microbial rennet in 40 min. The coagulum was cut crossways into c.a. 1 cm^3 cubes, stirred for 30 min, the whey was then drawn off and the curd was manually salted with 1.5% common salt. The salted curd was placed into 1-kg plastic moulds, was left to drain for 30 min and then stored under refrigeration until the next day. Two triangular tests were carried out with the cheeses in the same session. One to compare CON vs BRO cheeses and the other to compare CON vs CAU cheeses. In each test, three 20-g samples of ground cheese were given to the panellists; two samples came from the milk of one feeding treatment, and the other sample was from the other treatment. Each panellist was asked to identify the sample that did not match the other two. Physical facilities and sample preparation followed the recommendations described by Poste et al. (1991).

Table 2
Fatty acid (FA) percentage and liposoluble vitamins in the experimental diets for lactating ewes [CON group (only TMR), BRO group (TMR plus 1.5 kg of broccoli) and CAU group (TMR plus 1.5 kg of cauliflower)].

Item	CON	BRO	CAU
FA (mg/g total FA)			
SFA	11.63	12.07	12.16
14:0	0.83	0.81	0.81
15:0	0.35	0.34	0.35
16:0	0	0.57	0.65
18:0	4.80	4.74	4.76
20:0	1.92	1.90	1.91
22:0	1.47	1.44	1.44
24:0	2.25	2.24	2.24
MUFA	18.45	18.31	18.31
16:1	0.76	0.74	0.74
18:1	16.76	16.57	16.62
PUFA	69.92	69.62	69.53
16:2	0	0.06	0.01
16:3	0	0.02	0.01
18:2	46.08	45.42	45.41
18:3	23.84	24.12	24.10
20:1	0.94	1.01	0.92
18:2/18:3	1.93	1.90	1.90
PUFA/SFA	6.02	5.93	5.92
Liposoluble vitamins (mg/100 g fresh matter)			
β-carotene	1.12	1.10	1.10
α-tocopherol	2.81	2.80	2.82
γ-tocopherol	0.44	0.44	0.45

The values were estimated considering a 1.5 kg daily intake of fresh broccoli or cauliflower with 0.45% lipid content each and a TMR mean daily intake of 3.19 and 3.21 kg (DM) for BRO and CAU groups, respectively, with 9.01% lipid content on a DM basis.
Abbreviations: TMR = total mixed ration, SFA = total saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Statistical analysis

LBW changes and micelle properties were analysed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with the three experimental diets (CON, BRO and CAU) as the only source of variation. In all cases, the individual ewe was considered the experimental unit. All other data (feed intake, feed conversion ratio, LBW, blood analysis, milk production and composition and casein properties) were analysed by repeated measures using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included fixed effects of diet, time and their interaction. The effect of animals nested in the diet was used as the error term to test the effect

of diet. Significance was declared at $P < 0.05$, and means were separated using the least significant difference procedure. Results of sensory tests were analysed by statistical significance tables for triangle tests (ISO, 2021; norm 4120).

Results

Feed intake, milk yield and feed efficiency

Table 3 shows the mean values of feed intake and protein intake and BW gain during the measurement period (21 days). No significant differences were observed in the dry matter intake (DMI) of TMR between the different experimental groups (3.14, 3.21 and 3.14 kg DM/ewe per day for CON, BRO and CAU; $P = 1.00$). The DMI of broccoli and cauliflower was 0.125 and 0.124 kg/ewe per day, respectively. Thus, the total DMI by the broccoli and cauliflower group was 3.33 and 3.28 kg DM/ewe per day, respectively, being significantly ($P < 0.05$) higher in BRO than in CON group. Protein intake was also higher in BRO than in the other groups (643, 690, 660 g/ewe per day in CON, BRO and CAU; $P = 0.006$).

No differences were observed either in milk yield, weight gain or live weight between experimental groups. Feed conversion ratio (kg DMI/kg milk yield) was unaffected by dietary treatment ($P > 0.05$).

Biochemical blood analysis

Table 4 shows the biochemical profile and acid-base parameters of the three experimental groups and the values at the three sampling times (days 1, 21 and 42). Regarding biochemical profile, only urea showed significant differences ($P < 0.05$), with higher values in BRO group than in CON group. CAU animals showed intermediate values. Glucose, urea, BHB and NEFA varied between days of sampling ($P < 0.05$), increasing glucose and BHB concentration, and decreasing urea and NEFA concentration through the trial. There was no diet × day interaction in any biochemical parameter ($P > 0.05$).

Dietary treatment did not affect acid-base parameters, although the diet × day interaction was significant ($P < 0.05$) for anion gap and Na concentration. Anion gap decreased with time in CON group ($P = 0.001$), and increased in BRO group, with no changes on CAU group. Na concentration decreased with time in CON group and increased in both BRO and CAU groups ($P = 0.016$).

Milk quality trails

The mean values of milk quality parameters are shown in Table 5. There were no differences ($P > 0.05$) in lactose, total solids, fat, urea and BHB concentrations. However, the protein content was affected ($P = 0.003$) by the diet, being higher in the milk from animals supplemented with broccoli compared to CON group (49.7, 52.3, and 49.8 g/ kg for CON, BRO and CAU).

Table 6 shows the FA profile and the liposoluble vitamin content of the ewes' milk as a function of the dietary treatment. The mean values for each group include the results from the milk sampled during all the experimental periods (on days 1, 7, 14 and 21). The intake of brassicas by the ewes showed a modest but significant effect ($P < 0.05$) on several FA percentages. Dietary brassica supplementation increased the saturated fatty acid (**SFA**) content by more than two percent units while decreased that of monounsaturated fatty acids (**MUFA**). Neither polyunsaturated fatty acids (PUFA) nor total *n*-6 or *n*-3 percentages were affected, although the n-6/n-3 ratio decreased in the supplemented groups. Regarding individual FA, significant differences ($P < 0.05$) were observed between CON and both BRO and CAU ewe milk. The supplementation with either of the two brassicas decreased the percentage of *c9*-18:1 and increased that of c9,t11-18:2. The supplementation with BRO further increased 14:0 and 16:0 FA percentages and decreased those of iso-17:0 and 18:0 FAs compared to CON group.

Regarding the casein properties, the experimental diets appeared to affect micelle size and size distribution properties in ewe milk (Table 7). There was a statistical trend ($P = 0.066$) towards higher Z-average size in BRO milk. Moreover, the polydispersity index (indicating the variation in casein micelle sizes) was higher in CAU and in BRO than in CON ($P < 0.05$ and $P < 0.10$, respectively). Fig. 1 shows the mean particle size distribution of the micelles in the ewe milk from each dietary treatment. It can be observed a tendency towards a narrower bell-shaped curve for the CON milk and a tendency to larger particle size, i.e. a curve placed more to the right in the abscissa axis, for the BRO group milk.

The triangle test showed that fresh cheese from the BRO or CAU treatments could not be discriminated by the panelists ($P > 0.05$; only a total of 7 or 10 of the 30 panellists were right in their response when comparing the BRO or CAU with CON, respectively).

Discussion

Feed intake, milk yield and feed efficiency

The mean values of DMI (Table 3) agree with those observed by other authors in sheep of the same breed, in a similar physiological state and consuming diets of similar characteristics (Prieto et al., 2013; Pulido et al., 2012).

The animals showed a high degree of acceptance for the broccoli and cauliflower, as they ate the whole amount offered. Due to the high moisture content of broccoli and cauliflower (92%), their daily DMI was around 125 g/d, accounting for less than 4% of the total DMI. This is probably the reason why supplementation with broccoli or cauliflower did not decrease TMR consumption.

Table 3
Effect of the different experimental diets [CON group (only TMR), BRO group (TMR plus 1.5 kg of broccoli) and CAU group (TMR plus 1.5 kg of cauliflower)] on feed intake, protein intake (g), live BW (kg), BW gain (kg), milk yield and feed efficiency in lactating ewes.

	Diet				*P*-value		
Parameter	CON	BRO	CAU	SED[1]	Diet	Day	Diet × Day
TMR intake (kg DM/d)	3.14	3.21	3.14	0.071	0.581	0.553	1.000
Total DMI (kg/d)	3.14[a]	3.33[b]	3.28[ab]	0.071	0.032	0.553	1.000
Protein intake (g/d)	643[a]	690[b]	660[a]	13.9	0.006	0.555	1.000
Live BW (kg)	74.6	77.6	80.0	5.36	0.594	0.002	0.927
BW gain (kg)	4.65	4.69	7.03	–	0.544	–	–
Milk yield (L)	1.90	1.82	1.92	0.053	0.147	0.991	1.000
FCR	1.68	1.91	1.78	0.230	0.314	0.067	0.902

Abbreviations: TMR = Total mixed ration, DMI = DM intake, FCR = feed conversion ratio (kg DMI/kg milk).
Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] SED when comparing experimental groups.

Table 4
Effect of the different experimental diets [CON group (only TMR), BRO group (TMR plus 1.5 kg of broccoli) and CAU group (TMR plus 1.5 kg of cauliflower)] and sampling days (day 1, day 21 and day 42) on blood biochemical and acid-base parameters in lactating ewes.

	Diet				Day				*P*-value		
Item	CON	BRO	CAU	SED[1]	1	21	42	SED[2]	Diet	Day	Day × diet
Albumin (g/L)	39.7	40.2	40.7	1.04	40.6	39.9	40.1	1.03	0.618	0.832	0.984
ALT (U/L)	21.6	19.7	22.3	1.18	21.3	21.8	20.4	1.17	0.092	0.498	0.984
Calcium (mg/dl)	10.6	10.8	10.8	0.13	10.6	10.7	10.8	0.13	0.225	0.263	0.994
Creatinine (mg/dL)	0.972	0.962	0.929	0.019	0.969	0.928	0.966	0.019	0.097	0.074	0.979
GGT (U/L)	59.6	58.6	63.3	2.63	63.3	59.0	59.1	2.61	0.183	0.179	0.951
Glucose (mg/dL)	68.8	69.4	69.4	1.09	67.1^{a}	68.9^{a}	71.6^{b}	1.09	0.828	0.001	0.182
Urea (mg/dL)	55.5^{a}	61.8^{b}	57.2^{ab}	2.03	64.0^{b}	56.4^{ab}	54.1^{b}	2.01	0.010	<0.001	0.852
BHB (mmol/L)	0.669	0.649	0.643	0.0478	0.574^{a}	0.658^{b}	0.702^{b}	0.0806	0.841	0.018	0.731
Lactate (µg/dL)	10.9	8.50	8.9	1.33	10.5	8.7	9.2	1.32	0.157	0.381	0.593
NEFA (mmol/L)	0.158	0.131	0.109	0.025	0.239^{b}	0.073^{a}	0.086^{a}	0.025	0.189	0.001	0.795
pH	7.47	7.46	7.48	0.012	7.47	7.47	7.49	0.012	0.647	0.268	0.762
pCO_2 (mmHg)	37.9	36.3	37.3	1.15	37.6^{b}	38.6^{b}	35.3^{a}	1.19	0.367	0.018	0.885
HCO_3 (mmol/L)	25.2	23.7	24.6	0.70	24.3	25.1	24.0	0.70	0.102	0.252	0.639
AnGap (mmol/L)	15.3	16.2	15.3	0.79	16.5^{b}	14.5^{a}	16.0^{ab}	0.79	0.463	0.044	0.001
tCO_2 (mmol/L)	26.3	24.8	25.8	0.73	25.5	26.3	25.1	0.72	0.108	0.224	0.648
Na (mmol/L)	147.5	147.2	147.7	0.38	146.9^{a}	148.0^{b}	147.4^{ab}	0.38	0.406	0.026	0.016
K (mmol/L)	4.68	4.77	4.63	0.107	4.94^{b}	4.59^{a}	4.54^{a}	0.106	0.447	0.006	0.389
Cl (mmol/L)	111.6	111.6	112.5	0.59	111.2^{a}	113.0^{b}	111.9^{ab}	0.89	0.390	0.009	0.089

Abbreviations: TMR = total mixed ration, ALT = alanine transferase; GGT = gamma glutamyltransferase; BHB = β-hydroxybutyric acid; NEFA = non-esterified fatty acids; pCO2: CO_2 pressure; tCO2: CO_2 tension; AnGap = Anion gap.
Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] SED when comparing experimental groups.
[2] SED when comparing the effect of time.

Table 5
Effect of the different experimental diets [CON group (only TMR), BRO group (TMR plus 1.5 kg of broccoli) and CAU group (TMR plus 1.5 kg of cauliflower)] on milk quality parameters in lactating ewes.

	Diet					*P*-value		
Item	CON	BRO	CAU	SED[1]	SED[2]	Diet	Day	Diet × day
Total solids (g/kg)	164	165	161	2.2	3.4	0.143	0.439	0.970
Fat (g/kg)	57.1	55.4	54.1	1.70	2.60	0.235	0.241	0.954
Protein (g/kg)	49.7^{a}	52.3^{b}	49.8^{a}	0.85	1.29	0.006	0.850	0.999
Lactose (g/kg)	47.9	47.6	47.4	0.43	0.66	0.556	0.783	0.995
Urea (mg/kg)	552	577	598	15.1	24.1	0.144	0.615	0.999
BHB (mmol/L)	0.025	0.012	0.013	0.009	0.014	0.291	0.356	0.765
Log10 somatic cells	2.00	1.96	1.98	0.251	0.980	0.258	0.299	0.409

Abbreviations: TMR = total mixed ration, BHB = β-hydroxybutyric acid.
Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] SED when comparing experimental groups.
[2] SED when comparing the effect of time.

Due to the high moisture content of these materials, the water consumption associated with the feed was much higher in the BRO and CAU groups compared to the CON ones (283, 1 662, 1 664 g/ewe per day for CON, BRO and CAU). Some authors, such as Felton and DeVries (2010), observed a linear reduction in DMI as DM content of the ration was reduced. This effect is associated with distension of the rumen and the response of ruminal mechanoreceptors, which send signals to the brain's satiety centres (Allen, 1996). However, in the present study, increased moisture in the ration did not result in lower DMI. This may be due to the animals of the broccoli and cauliflower group compensating for the greater intake of water from the ration with a lower intake of drinking water (INRA, 2019). Monllor et al. (2020b) observed a decrease in feed intake when goats were supplemented with broccoli silage, however, it is important to highlight that the proportion of broccoli in the ration was much higher than in our study (40–60 vs 4%).

The milk yield agreed with those reported by Pulido et al. (2012) and Prieto et al. (2013) for Assaf ewes milked once a day. Milk yield was unaffected by dietary treatments, which is in concordance with the small magnitude of the differences observed on feed intake. In fact, there were no differences between the experiment groups in live BW change during the experimental period. These results are in agreement with those of Yi et al. (2015) in dairy cows and Monllor et al. (2020a) in goats, who, despite the higher inclusion of broccoli (40%), also did not observe differences in milk production.

Biochemical blood profile

It should be noted that all animals (ewes in mid-lactation) showed a positive BW change, which suggest that they were in positive energy balance. In fact, blood glucose values increased and NEFA decreased throughout the experimental period, suggesting a reduction in glucose oxidation and lipolysis (Lisuzzo et al., 2022), associated with a reduction in the daily milk production and a positive energy balance. It should also be noted that the ewes did not become pregnant after the peak of lactation, so the energy requirements for milk production decreased throughout the experiment.

During negative energy balance, fat mobilisation increases the flux of NEFA to the liver, which is partially transformed into ketone bodies, such as BHB (Lisuzzo et al., 2022). On this physiological basis, a reduction in blood BHB would be expected as NEFAs

Table 6
Effect of the different experimental diets [CON group (only TMR), BRO group (TMR plus 1.5 kg of broccoli) and CAU group (TMR plus 1.5 kg of cauliflower)] in lactating ewes on milk FA profile (% FA) and liposoluble vitamin content.

	Diet				*P*-value		
Item	CON	BRO	CAU	SED	Diet	Week	Diet × Week
Fatty acids (%)							
SFA	73.7[a]	76.0[b]	75.4[b]	0.71	0.012	0.458	0.641
8:0	2.34	2.32	2.44	0.073	0.225	<0.001	0.119
10:0	9.92	10.5	10.3	0.31	0.190	0.265	0.633
12:0	6.69	6.95	6.95	0.252	0.500	0.428	0.998
14:0	12.4[a]	13.16[b]	12.81[ab]	0.300	0.038	0.030	0.945
15:0	1.03	1.03	1.07	0.038	0.443	0.409	1.000
16:0	30.1[a]	32.5[b]	30.5[a]	0.85	0.022	0.419	0.934
17:0	0.714	0.648	0.699	0.027	0.053	0.746	0.979
18:0	7.23[b]	5.68[a]	6.98[b]	0.358	<0.001	0.401	0.921
BCFA	2.10	1.94	2.07	0.078	0.114	0.258	0.985
iso-17:0	0.405[b]	0.366[a]	0.404[b]	0.016	0.030	0.307	0.736
anteiso-C17:0	0.731	0.684	0.704	0.040	0.507	0.433	0.998
MUFA	20.41[b]	19.26[a]	19.19[a]	0.611	0.003	0.492	0.680
c7-16:1	0.347	0.346	0.348	0.012	0.992	0.156	0.575
c9-16:1	0.599	0.657	0.597	0.054	0.457	0.614	0.985
t11-18:1	1.48	1.63	1.48	0.117	0.343	0.009	0.742
c9-18:1	15.7[b]	13.0[a]	14.0[a]	0.601	0.001	0.161	0.379
c11-18:1	0.417[a]	0.518[b]	0.442[a]	0.023	<0.001	<0.001	0.461
PUFA	5.86	5.96	5.86	0.171	0.798	<0.001	0.957
18:2n-6	3.30	3.18	3.16	0.095	0.291	0.571	0.984
18:3n-3	0.994	1.07	1.02	0.034	0.103	<0.001	0.921
c9,t11-18:2[&]	0.358[a]	0.439[b]	0.424[b]	0.027	0.012	<0.001	0.802
n-6	3.76	3.61	3.61	0.107	0.303	0.658	0.981
n-3	1.07	1.15	1.11	0.035	0.095	<0.001	0.932
n-6/n-3	3.57[c]	3.18[a]	3.33[b]	0.058	<0.001	<0.001	0.853
PUFA/SFA	0.08	0.08	0.08	0.003	0.919	<0.001	0.997
Liposoluble vitamins (mg/100 g)							
α-tocopherol	106	104	90	13.1	0.442	0.097	0.858
Retinol	130	127	121	7.3	0.497	0.103	0.049

For brevity, only the fatty acids showing percentages higher than 0.4 in any of the treatments were included.
Abbreviations: TMR = total mixed ration, FA = fatty acid, SFA = total saturated fatty acids, BCFA = branched-chain fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids (including conjugated linoleic acids).
SED when comparing experimental groups.
Values within a row with different superscripts differ significantly at $P < 0.05$.
[&] coelutes with *t*7,*c*9-18:2.

Table 7
Effect of the different experimental diets [CON group (only TMR), BRO group (TMR plus 1.5 kg of broccoli) and CAU group (TMR plus 1.5 kg of cauliflower)] on micelle size and size distribution properties in ewe milk.

	Diet				
Item	CON	BRO	CAU	SED[1]	*P*-value
Z-Average (nm)	192	203	189	6.1	0.066
Size for the 50 the percentile (nm)	154	154	143	10.7	0.489
Polydispersity index	0.092[a]	0.105[b]	0.106[b]	0.0058	0.040
Z-Potential (mV)	−22.94	−23.38	−23.78	0.932	0.667

Abbreviations: TMR = total mixed ration.
Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] SED when comparing experimental groups.

decreased and glucose increased. However, in the present study, an opposite evolution was observed, although it was probably not biologically relevant since the values were below the minimum (>0.8 mmol/L) considered to suggest animals in subclinical ketosis (Araujo et al., 2020).

Although blood urea was higher in the BRO group than in the CON group, values are within the normal range for sheep (21–75 mg/dL; Jackson and Cockcroft, 2002). This parameter is closely related to the ingestion of degradable protein and in this case, protein intake (643 and 690 g/ewe per day for CON and BRO) was higher in the BRO group, and *in vitro* studies (de Evan et al., 2020a, 2020b) have determined high rumen degradability of protein in cauliflower (88.5%) and in broccoli (85.3%), which may lead to increase both production of ammonia in the rumen and blood urea levels. In contrast, Monllor et al. (2020b) observed a lower concentration of urea in the blood in goats fed diets including 25–40% of broccoli silage (on DM basis), although in this study, feed intake was depressed in supplemented animals, reducing also protein intake. Although, in our experiment, the broccoli supplement was given fresh and not ensiled, it should be noted that it represented only 10% of the total DM consumed. Therefore, a negative effect when using higher levels of broccoli cannot be ruled out.

There was also a trend in ALT to decrease in the BRO group (Table 4). ALT is used to assess liver status, with high values consistent with liver damage, while low values are associated with vitamin B6 deficiency (Ramati et al., 2015) and other factors. In broccoli, 70–90% of glucosinolates are glucoraphanin, known for

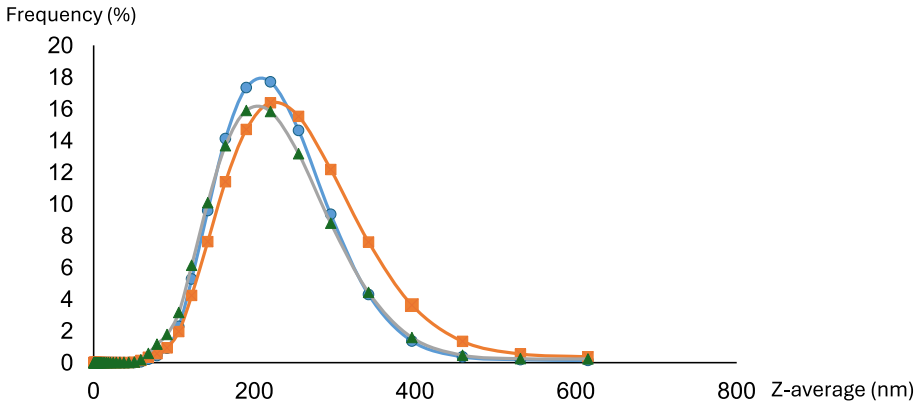

Fig. 1. Distribution of micelle by size (frequency in % vs Z-average in nm) in the ewe milk from the control, broccoli and cauliflower groups. Blue: control; orange: broccoli; grey: cauliflower.

its antioxidant properties and association with liver protection. Human studies, such as the one conducted by Satomi et al. (2022), have demonstrated a reduction in ALT values after a 24-week ingestion of 137.1 μg of glucoraphanin. Notably, broccoli flowers contain approximately 246 μg/g of DM glucoraphanin and could be the reason for the reduction of ALT in BRO group. Nevertheless, all values are within the normal range (Lisuzzo et al., 2022) and the observed differences could not have biological significance.

Finally, a diet × day interaction ($P = 0.005$) was detected for the anion gap seeing that values increase over time in BRO and CAU group animals but not in CON. At the end of the experiment (day 42), the values of the three groups remained within the normal range (12–24 mmol/L, Jackson and Cockcroft, 2002). The anionic gap is calculated as the difference between the cations (Na^+, K^+) and the anions (Cl^- and $HCO3^-$) and the increase in the anion gap may be related to an increase in Na^+ values. In our case, Na^+ values increased slightly over time in the groups supplemented with broccoli and cauliflowers. This is in line with the study of Monllor et al. (2020b) in which they found an increase in Na^+ concentrations in the milk of goats fed 40% broccoli. This increase in Na^+ milk values was possibly caused by an increase in Na^+ in the plasma.

Milk production and quality

The mean values of milk chemical composition (total solids, fat, protein and lactose), as well as of milk production (Table 5), were into the ranges reported for the same breed of sheep in similar conditions (mid-lactation and milked once daily; (Prieto et al., 2013; Pulido et al., 2012)). In our trial, protein intake was higher in BRO ewes compared to CON group and this could explain the higher milk protein content in the animals supplemented with broccoli (Jaime and Purroy, 1995; Pulina et al., 2006).

Regarding the fatty acid profile in milk, the most important changes were an increase in SFA and a decrease in MUFA in the BRO and CAU groups compared to CON (Table 6). Few previous studies were found on the effect of brassica supplementation in dairy ruminants on the FA profile of milk (Monllor et al., 2020a, 2020c; Seguel et al., 2020). Seguel et al. (2020) fed lactating cows with a diet containing turnip or rape at an approximate level of 20% of the DMI reported an increase of SFA and PUFA percentages and a decrease of MUFA levels in supplemented animals respect to control ones. Similar effects were observed by Monllor et al. (2020a) who reported an increase in SFA percentages and a decrease in MUFA and PUFA percentages in the broccoli group versus the control group. This is in partial agreement with our results, since we observed a higher percentage of SFA and lower percentages of MUFA (a few percentage units each) and no effect on PUFA.

Regarding the effect on those milk fatty acids relevant for human health, we observed that brassica feeding decreased values in the n-6/n-3 ratio, with being BRO more effective than CAU. This difference is linked to the observed tendency to lower n-3 values in the milk from CON group, although there were no significant differences in the separated values of n-6 and n-3 percentages among treatments. The lower n-6/n-3 ratio in BRO milk is also in concordance with Monllor et al. (2020a) who observed a decrease in both n-3 and n-6 concentration in the milk of the animals that received broccoli.

Looking at casein properties, the experimental diets seemed to influence micelle size and size distribution properties in ewe milk, as illustrated in Table 7 and Fig. 1. It has been reported that micelle diameters may be directly correlated with the content of total protein and negatively with the content of some caseins, such as k-casein (Freitas et al., 2019; Li et al., 2022). Therefore, the greater z-average diameter observed in BRO group could be related to the greater protein content. Unfortunately, the casein profile was not evaluated so we cannot confirm any effect of dietary treatments on the casein profile.

Specific feedstuffs can contain low threshold odour active compounds or its direct metabolic precursors that can be transferred to the blood and then to milk. This is the case of S-methyl-cysteine sulfoxide or glucosinolates, which are present at relatively high amounts in brassica plants (Barry, 2013; Wiedenhoeft and Barton, 1995). In the event that these compounds passed into milk, their flavour could be detected. In this study, despite being fed on brassicas, it appeared that the potential presence odour–active compounds did not affect milk flavour. Other studies carried out in lactating cows also observed no effects on flavour characteristics of milk or cheeses when fed different brassica species, such as forage rape (Seguel et al., 2020), typon (a hybrid of a Chinese cabbage and turnip) forage (Wiedenhoeft and Barton, 1995) or forage turnip (Seguel et al., 2020). Likewise, the milk from cows fed with high concentrations of S-methyl-cysteine sulfoxide (Maiga et al., 2011) was also unaffected.

In conclusion, daily supplementation with 1.5 kg of fresh broccoli or cauliflower florets in lactating sheep fed a TMR *ad libitum* did not affect either feed intake, milk production or health status of the animal. Milk quality was also not negatively affected by the inclusion of the vegetables but a slight increase in protein content was observed in broccoli-supplemented ewes.

Remains to be explored the possibility of including these vegetables in the diet of dairy sheep to replace conventional dietary components, through longer-term studies and with higher inclusion rates. Furthermore, it would be worth investigating affordable methods for their conservation and use as feed, since the high-water content of these vegetables poses challenges in terms of

storage and environment, but it is also an opportunity to reuse water that would be lost without any benefit.

Ethics approval

The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Universidad de León (approval number ULE_014_2016) and the Junta de Castilla y León (Spain), following proceedings described in Spanish and European Union legislation (Royal Decree 53/2013 and Council Directive 2010/63/EU).

Data and model availability statement

None of the data or models are deposited in an official repository. The data presented in this article will be available upon reasonable request to the authors.

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

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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