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Condensed tannins fed to dairy goats: effects on digestibility, milk production, blood parameters, methane emission, and energy and nitrogen balances

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ABSTRACT

Condensed tannins (CT) are plant polyphenols that can affect feed digestibility and are potentially able to reduce enteric methane emissions in ruminants. In this in vivo trial with 8 lactating goats, we investigated the effects of 4 levels of inclusion of a commercial CT extract from quebracho (0, 2, 4, 6% on DM basis; C, Q2, Q4, Q6, respectively). The experimental design was a repeated 4 × 4 Latin square with 28-d periods (24 d of diet adaptation and 4 d of sample collection) using metabolic cages and 4 open circuit respiration chambers. The inclusion of CT in the diets did not affect the dry matter intake (DMI) but caused a linear decrease in diet digestibility, with reductions up to −11% for dry matter (DM), −21% for crude protein (CP), −23% for neutral detergent fiber (aNDFom), and −13% for gross energy, when comparing the Q6 and C diets. However, ruminal total volatile fatty acids (VFA) concentration was not affected by CT, although there were changes in VFA proportions. Milk yield (g/d) was highest for Q4 (3371) and lowest for Q6 (3066). In terms of milk composition, CT induced a linear reduction of fat and CP concentrations. The reduction in CP digestibility resulted in a linear reduction in the milk urea level, up to −37% with Q6. Positively, CT linearly reduced the somatic cells count expressed as linear score. The feed efficiency was linearly decreased by CT inclusion. Furthermore, a shift from urinary to fecal nitrogen excretion was observed with CT. The retained nitrogen was always negative (on average −1.93 g/d). The methane yield (on average 19.2 g CH_4/kg DMI) was linearly reduced by CT inclusion, up to −18% with Q6. Regarding the CH_4 intensity, CT induced a linear reduction when expressed per kg of milk, but not per kg of fat and protein corrected milk. Moreover, the CH_4 production per kg of digestible aNDFom was linearly increased by CT. The metabolizable energy intake (MEI) was not affected by the treatments, but the metabolizability (q = MEI/gross energy intake) was reduced as CT inclusion increased. From the results of the present study, it turned out that CT have a negative impact on feed digestibility and feed use efficiency. Condensed tannins can lower CH_4 emissions from ruminants; however, the main mechanism of action is likely the decrease in feed digestibility. Furthermore, CT did not improve the N use efficiency. According to these findings, the positive environmental impacts of CT are only related to the shift from urinary to fecal N excretion.
Key Words: dairy goats, condensed tannins, methane emission, nitrogen balance

INTRODUCTION

Tannins are plant polyphenol compounds mainly classified into hydrolyzable tannins (**HT**) and condensed tannins (**CT**) (Naumann et al., 2017; Aboagye and Beauchemin, 2019).

Tannins can have positive, negative, or no effect to animal nutrition, depending on their chemical structure and molecular weight, concentration in the diet, plant origin, and other factors, such as composition of the diet, animal species and physiological state (Makkar, 2003).

Tannins are known for their ability to bind proteins, forming insoluble complexes that subsequently precipitate; however, the details and mechanism are not fully understood (Lorenz et al., 2014; Mueller-Harvey et al., 2019). This characteristic is the cause of the oral perception of astringency that can lead to a reduction in feed palatability (Jayanegara et al., 2012; Zeller, 2019). The tannin-protein complex is pH dependent: it is stable in the rumen, where the pH ranges from 5.0 to 7.0, thus reducing the possibility of rumen microorganisms degrading proteins, but the tannin-protein

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complex is dissociated in the abomasum, where the pH is lower (Grazziotin et al., 2020). This causes a higher RUP in the diet, lower NH_3 concentration in the rumen, lower MUN, lower amount of N excreted with urine and higher fecal N excretion and can lead to a greater N utilization efficiency and milk protein content by improving the supply of AAs to the small intestine (Aguerre et al., 2020; Grazziotin et al., 2020; Herremans et al., 2020). The shift in N excretion from urine to feces has positive repercussions for the environment because fecal N is more stable, thus reducing the release of NH_3 and N_2O into the atmosphere (Castillo et al., 2000; Mueller-Harvey et al., 2019; Hristov et al., 2022).

Moreover, to a lesser extent, tannins also form complexes with carbohydrates, nucleic acids and metal ions, reducing DM, OM, and NDF digestibility (Makkar, 2003; Waghorn, 2008; Aboagye and Beauchemin, 2019).

Tannins have shown the potential to reduce CH_4 emission from enteric fermentations, with variable results depending on the type and concentration of tannin added, animal species and interaction with the diet (Bhatta et al., 2013; Hristov et al., 2013; Beauchemin et al., 2020, 2022). There are still knowledge gaps regarding the mechanisms of action of tannins in reducing methanogenesis; among these are the reduction of nutrient digestibility, the direct control action of methanogens, the reduction of protozoa and the hydrogen sink activity in the rumen environment (Bhatta et al., 2009; Becker et al., 2014; Díaz Carrasco et al., 2017).

In case of an effect, both positive or negative, this also depends on the ruminant species. In an in vitro study with ruminal fluids taken from dairy cattle (*Bos taurus taurus*), zebu beef cattle (*Bos taurus indicus*), water buffaloes (*Bubalus bubalis*), sheep (*Ovis aries*) and goats (*Capra hircus*), Bueno et al. (2015) found that small ruminants were less affected by the detrimental effects of tannin-rich diets than large ruminants. Goats are intermediate selectors with a great tolerance to tannins due to tannin-binding saliva (e.g., proline and other proteins), which counteracts the negative effects of tannins on ruminal digestibility and stimulates the proliferation of tannin-tolerant bacteria (e.g., *Streptococcus caprinus*), an adaptation that is lacking or poorly developed in sheep and cattle (Muir, 2011; Min and Solaiman, 2018; Schmitt et al., 2020).

However, to the best of our knowledge, only 2 studies have investigated the effect of tannins administered to dairy goats on milk production (Nascimento et al., 2021; Jerónimo et al., 2023), while 4 studies were conducted on nonlactating goats to evaluate the effect of tannins on digestibility, methane production, energy and nitrogen balance (Puchala et al., 2005; Animut et al., 2008a; b; Bhatta et al., 2013). In a previous in vitro study simulating ruminal fermentation (Battelli et al., 2023), we tested the effects of quebracho CT and chestnut HT at different levels of inclusion, finding a more pronounced reduction in CH_4 production and less negative effects on ruminal fermentation parameters with CT. Therefore, the aim of the present study was to test different levels of inclusion of quebracho CT on DMI, total-tract digestibility, rumen fermentation, milk production, gas exchange, plasma metabolites and enzymatic activities, and energy and nitrogen balances in lactating goats.

MATERIALS AND METHODS

The experiment was conducted at the "Cascina Baciocca" Research Centre of the University of Milan in Cornaredo (Milan, Italy). All animal procedures were conducted with the approval of the University of Milan Ethics Committee for Animal Use and Care and in accordance with the European Directive 2010/63 on the protection of animals used for scientific purposes and with the guidelines of the Italian law on animal welfare for experimental animals (Italian Ministry of Health, authorization no. 20/2021-PR). Animal procedures are reported in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines 2.0 (du Sert et al., 2020).

Goats, Diets, and Experimental Design

Eight multiparous (2.6 ± 1.2) lactating Alpine goats, similar in DIM (42 ± 5 d), milk yield and composition (3.42 ± 0.55 kg/d), and BW (67.1 ± 4.8 kg), were selected from the flock and subsequently divided into 2 groups of 4 animals each. Within each group, the animals were randomly allocated to one of the 4 following dietary treatments: a control diet (**C**) and 3 diets with 2, 4, and 6% (**Q2**, **Q4**, and **Q6**, respectively) inclusion of a commercial purified quebracho (*Schinopsis balansae*) CT extract (Silvafeed® Q powder, by SILVATEAM, San Michele Mondovì, Cuneo, Italy) with a total CT concentration of 68.3%, corresponding to 74.1% on DM. A more detailed description of the metabolomic profile of this quebracho CT extract is reported by Gazzonis et al. (2023).

The goats were fed the experimental diets for ad libitum intake twice daily. The animals had free access to drinking water. Orts were recorded daily, and the feeding rate was adjusted to obtain at least 5% of the supplied amount as orts (on an as-fed basis).

The experimental diets, formulated to meet the protein and energy requirements of lactating goats producing 3.5 kg of milk/day, according to the French INRA model (Sauvant et al., 2007), consisted of the same

basal ration added with one of 4 experimental feedstuffs mix characterized by a different concentration of quebracho CT extract depending on the treatment. The basal ration was composed (on a DM basis) of 3rd cut meadow hay (65.5%), corn grain steam-flaked (10.5%), corn grain fine-ground (7.5%), soybean seeds whole extruded (4.6%), wheat bran (4.3%), sugarcane molasses (2.3), flax seeds extruded (2.7%), and mineral and vitamin supplement (2.4%) and was prepared by means of a mixer wagon (Triolet Solomix 2–4000 STB series 09, Oldenzaal, The Netherlands), with a reduction in the forage particle size. The experimental feedstuffs mixes were prepared by a feed compounder firm, were composed of barley meal, corn meal, cane molasses, and the commercial quebracho CT extract, the latter included in the mixes at different levels (0, 11.1, 20.2, and 28.0% on DM, for C, Q2, Q4, and Q6 treatments, respectively). Each mix was included to the respective diet at different doses (16.4, 18.1, 19.8, and 21.5% on DM, for C, Q2, Q4, and Q6 diets, respectively) to achieve the desired quebracho CT extract concentrations in the diets, corresponding to 0, 1.5, 3.0, and 4.5% of CT on DM.

Before administration to animals, the basal ration and the 4 experimental feedstuffs were manually mixed with 15% of water (wt/wt), reaching a DM content of about 78%, to make the ration homogeneous and limit selection by the animal. The composition of the 4 experimental diets is reported in Table 1.

The experimental design of the trial was a repeated Latin square (2 squares x 4 goats x 4 dietary treatments), balanced for the carry-over effect. The first square was complete, while the second one was an uncompleted square because one of the 4 goats was removed from the trial due to a bad adaptation to the metabolic cage and 2 other goats were removed in 2 different periods, one due to mastitis in the last period, and the other one for problem with the catheter. Therefore, the dietary treatments had the following observations: C = 6, Q2 = 7, Q4 = 7, and Q6 = 6. Two weeks before the beginning of the trial, the goats were gradually adapted to the metabolic cages and then to the respiratory chambers. Each period of 4 weeks was composed of 24 d of adaptation to the diet followed by 4 d of data collection. During the first 21 d of adaptation, the goats were housed in free stalls of 3 animals each (1 goat per experimental square and 1 reserve goat). Then, the goats were placed into individual metabolic cages and moved into individual open-circuit respiration chambers. Finally, the last 4 d of the experimental period were used for data and sample collection. Having 4 respiration chambers, for each period, the experiment was conducted on the 2 squares of 4 animals in 2 consecutive weeks, one for each square.

Gas Exchange Determination and Sample Collection

Inside the individual open circuit respiration chamber, 4 24 h cycles of respiratory exchange were measured for each goat in each period. The chambers measured 3.6 m (length) × 2.4 m (width) × 2.3 m (height). The chambers were equipped with a small prechamber for personnel entrance and wide glass walls to allow the animals to see each other and outside. The individual metabolic cages were equipped with removable mangers, and they were wide enough to allow the goats to lie down but not to turn around, thus facilitating a precise collection of feed leftovers, urine, and feces.

The air temperature in the chambers was maintained at 18 ± 1°C, and a low negative pressure was maintained inside the chambers to prevent losses of air. The air flow through the chambers was measured using a diaphragm flowmeter (PH 20/335 G 25, 40 m^3/h, Sacofgas, Città di Castello, Perugia, Italy). The air flux was, on average, maintained at 15 ± 1 m^3/h. The daily O_2 consumption and the CO_2 and CH_4 production were determined by calculating the volume of air entering the chamber in 24 h (referring to the standard temperature and pressure conditions) and multiplying this volume by the difference between the relative concentrations of the gases measured every 10 min in the incoming and outgoing air for a total of 144 observations/d for each gas and each goat. The CH_4 and CO_2 concentrations were measured using a URAS 4 analyzer (Hartmann and Braun AG, Frankfurt am Main, Germany). The O_2 concentration was measured using a Magnos 6G analyzer (Hartmann and Braun AG). Each cycle of gas measurements (one for the incoming air and 4 for the outgoing air) lasted 120 s, 110 s for air change and the last 10 s for O_2, CO_2, and CH_4 determination. Corrections were applied to account for the entrance of personnel based on the volume of the prechamber and the time spent inside the chamber.

The total heat production was determined using the Brouwer equation (1965): heat production (kJ/d) = 16.18 O_2 + 5.16 CO_2 – 5.90 N – 2.42 CH_4, where gas volumes (L/d) are expressed under standard conditions and N (g/d) is the urinary N.

The goats were fed 2 times per day (0800 and 1600 h) and were milked once daily (0730 h). At each milking, milk production was recorded, and during the collection period, samples of milk (10%) were taken for further analysis. Daily, one milk sample was stored at 4°C with the addition of 2-bromo-2-nitropropan-1,3-diol as a preservative for milk quality analysis. A second sample (raw milk without preservative) was kept at −20°C from the time of collection until used for biochemical measurements.

During the collection period, total urine and feces collection was performed. The goats were fitted with Foley urinary catheters (Model 1851H18, C. R. Bard Inc., Covington, GA, USA) to avoid contact between urine and feces and the loss of urine by evaporation that occurs when urine is collected by mechanical separation in the metabolic cage. Additionally, urine was collected in plastic bins containing sulfuric acid (20% vol/vol) to maintain the pH below 2.5, preventing NH_3 losses. Due to the structure of the metabolic cages, the feces were conveyed by gravity into a container. Feces and urine were weighed daily, sampled (20 and 10% of the total weight, respectively, for feces and urine), pooled per goat during each collection period, and stored at −20°C.

Daily, the orts were recorded and sampled, pooled per goat during each collection period, and stored at −20°C.

Ruminal fluid was taken at the end of each experimental period. The ruminal fluid was collected 5 h after the morning feeding with an esophageal semirigid probe with an apical ogive with small holes. To avoid saliva contamination, the first collected rumen sample (about 70–100 mL) was discarded. The pH was measured immediately after sampling, and aliquots were stored at −20°C for subsequent VFA analysis.

Blood was collected once from each goat on the last day of each experimental period by jugular venipuncture into heparinized evacuated tubes in the morning, after milking and before distribution of the treatment diet (0750 h). Blood samples (9 mL) were immediately centrifuged for 20 min at a speed of 3000 g at 4°C. Plasma was harvested and stored in 2 aliquots (1.0 mL tubes) at −20°C until being used for analysis.

Frozen blood plasma and raw milk samples were shipped with ice packs at the Laboratory of Biological and Clinical Chemistry of the CREA Research Center for Animal Production and Aquaculture of Lodi.

At the beginning and end of each experimental period, the goats were weighed.

Table 1: Composition (% on DM) and chemical analysis (% on DM, unless otherwise noted) of the experimental diets

	Diet[1]			
Ingredient	C	Q2	Q4	Q6
Meadow hay, third cut	54.8	53.7	52.6	51.5
Corn grain fine-ground	13.9	13.7	13.4	13.1
Corn grain steam-flaked	8.75	8.58	8.40	8.23
Barley grain ground	7.69	7.53	7.38	7.23
Soybean seeds, whole extruded	3.88	3.80	3.73	3.65
Sugarcane molasses	2.99	2.93	2.87	2.81
Wheat bran	3.68	3.61	3.54	3.47
Flax seeds, extruded	2.28	2.24	2.19	2.14
Salts[2]	1.86	1.82	1.79	1.75
Vitamin-mineral mix[3]	0.162	0.158	0.156	0.152
Vitamin E[4]	0.016	0.016	0.015	0.015
Commercial quebracho CT extract[5]	0.00	2.01	4.00	6.01
Chemical analysis				
DM (%)	89.1	89.2	89.2	89.3
OM	92.0	92.1	92.2	92.3
Ash	8.05	7.95	7.81	7.66
CP	15.2	15.0	14.8	14.5
Ether extract	2.80	2.74	2.66	2.52
aNDFom[6]	37.1	36.4	35.6	34.9
ADFom[7]	22.1	21.7	21.4	20.9
ADL	5.42	5.33	5.26	5.21
Starch	23.3	22.5	22.1	21.9
GE (MJ/kg DM)[8]	17.81	17.88	17.95	18.05

[1]Diets with different levels of quebracho condensed tannin (CT) extract: C = no CT; Q2 = 2% of CT; Q4 = 4% of CT; Q6 = 6% of CT.

[2]Salts: 42.0% calcium carbonate, 34.6% sodium bicarbonate, 8.9% magnesium oxide, 8.3% sodium chloride, 6.2% dicalcium phosphate.

[3]Provided (per kg): 5,000 mg of Fe, 40,000 mg of Zn, 6,500 mg of Cu, 28,000 mg of Mn, 120 mg of Se, 90 mg of Co, 800 mg of I, 4,800 kIU of vitamin A, 1,200 kIU of vitamin D, and 23,000 IU of vitamin E.

[4]Provided (per kg): 485,000 IU of vitamin E.

[5]Containing 71% of condensed tannins.

[6]aNDFom: α-amylase- and sodium sulfite-treated NDF corrected for insoluble ash.

[7]Values corrected for insoluble ash.

[8]GE: gross energy.

Chemical Analyses

The administered feeds, orts, and feces were analyzed for chemical composition. Before analysis, samples of feeds, orts and feces were thawed, oven-dried at 55°C until constant weight, and ground through a 1-mm screen (Pulverisette 19, Fritsch, Idar-Oberstein, Germany). Analytical DM was determined by drying in a ventilated oven at 100°C overnight (AOAC, 1995; method 945.15). The ash content was determined by incineration at 550°C overnight in a muffle furnace (AOAC, 1995; method 942.05). The fiber content, expressed exclusive of residual insoluble ash (aNDFom), was determined using an Ankom200 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY, USA) following the procedure reported by Mertens (2002), with the inclusion of heat-stable α-amylase and sodium sulfite. Acid detergent fiber, expressed exclusive of residual insoluble ash (ADFom), was determined following ANKOM procedures in an ANKOM200 Fiber Analyzer (Ankom Technology Corporation). Acid detergent lignin (ADL) was determined by solubilization of cellulose with sulfuric acid (Van Soest et al., 1991). The ether extract (EE) was determined according to AOAC (1995), method 920.29. The administered feeds, orts, composite milk, fresh feces, acidified urine, and condensed water collected in the chambers were analyzed for N content according to the Dumas method (AOAC, 1995; method 990.03) using a Rapid MAX N Exceed Elementar (Elementar Analysensysteme GmbH, Frankfurt Main, Germany). Condensed tannins concentration of the commercial quebracho CT extract was determined by using acetone butanol−HCl assay following the procedure reported by Grabber et al. (2013). The milk fat, lactose and MUN concentrations were determined using a Fourier transform infrared analyzer (MilkoScan FT6000; Foss Analytical A/S, Hillerod, Denmark). The MUN concentration was determined using a differential pH technique (ISO, 2004; method 14637). The fat and protein corrected milk (**FPCM**) was calculated with the following equation: FPCM = milk yield × (0.26 + 0.1352 × Fat% + 0.079 × CP%). The value calculated with this equation is referred to as a standardized milk with 3.5% fat, 3.4% crude protein and 4.5% lactose. The equation coefficients are derived from the following heat combustion values (kJ/g): fat = 38.9, true protein = 23.9, NPN = 9.25, lactose = 16.5. Considering an average NPN content of goat milk equal to 8.5% of total N, the combustion heat of milk crude protein will be 22.6 kJ/g. The heat of combustion of the administered feeds, orts, feces, urine, and milk was determined using an adiabatic calorimeter (IKA 6000; IKA Werke GmbH and Co. KG, Staufen, Germany). The total SCC was analyzed according to UNI EN ISO 13366–2:2007 by differential count (Fossomatic^{TM} FC; Foss A/S, Hillerod, Denmark). The total SCC was expressed as the linear score (**LS**) = $\log_2$ (SCC/12,500).

For the VFA analyses, 1 mL of 25% meta-phosphoric acid was added to 5 mL of rumen fluid. After 30 min, the mixture was centrifuged at 3,500 × g for 10 min. Two μL of the supernatant were injected into a Varian CP-3800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, United States) operated with a split/splitless injector at 220°C and a flame ionization detector at 250°C. A Nukol fused silica capillary column (30 m length; 0.25 mm diameter; 0.25 μm film thickness; Supelco) was used with helium as the carrier gas at 4 mL/min. The oven was programmed to increase from 80 to 200°C at 8°C/min.

Blood plasma samples were analyzed using an automated benchtop clinical chemistry analyzer (ILAB Aries; Instrumentation Laboratory, Lexington, MA) by colorimetric and enzymatic methods at 37°C. The following biochemical analytes were measured: glucose (**P-GLU**), total cholesterol (**P-TChol**), triglycerides (**P-TG**), albumin (**P-ALB**), total protein (**P-TP**), creatinine (**P-CREA**), urea (**P-urea**), total bilirubin (**P-TBIL**), calcium (**P-Ca**), inorganic phosphorous (**P-Pi**), magnesium (**P-Mg**), and iron (**P-iron**) concentrations and activities of the enzymes amylase (**P-AMY**), aspartate aminotransferase (**P-AST**), alanine aminotransferase (**P-ALT**), alkaline phosphatase (**P-ALP**), gamma-glutamyl transferase (**P-GGT**), and lactate dehydrogenase (**P-LDH**), using commercial kits (QuantiLab, Werfen). Concentrations of sodium (**P-Na^+**), potassium (**P-K^+**), and chloride (**P-Cl^-**) ions in plasma were determined using the integrated Ilab Aries ISE module (with 3 specific ion-selective electrodes and a reference electrode), utilizing quantitative and indirect potentiometric measurements. Reagents, calibration sera and control sera (SeraChem Control Level 1 and Level 2) were purchased from Werfen Italia (Instrumentation Laboratory Spa, Milano, Italy).

Plasma nonesterified fatty acids (**P-NEFA**) and β-hydroxybutyrate (**P-BHB**) were measured using standardized commercial kits supplied by Randox (Kit NEFA no. FA115 colorimetric method and Kit Ranbut, no. Rb 1007, enzymatic method, Randox Laboratories Ltd., UK) and a chemical autoanalyzer (ILab Aries). Reagents, standards, and control sera (Bovine Chemistry Assayed Level 1 and Level 2 Control) were purchased from Randox Laboratories Ltd. (Randox Laboratories Ltd., Roma – Italia).

The biochemical composition of milk was determined as described above for blood plasma analytes.

Before analysis, frozen raw milk samples (100 mL) were heated in a water bath at 35.0°C for 1.5 h; after thawing, a composite milk sample (40 mL) was created

(proportionally to milk yield of each of the 4 d of sample collection) for each animal at each period, centrifuged 2 times at 1620 g for 10 min and upon fat separation and elimination, defatted milk was transferred to new tubes for analysis.

Defatted milk samples were subjected to biochemical tests for the determination of the following parameters: free glucose (**M-GLU**), creatinine (**M-CREA**), urea (**M-urea**), total bilirubin (**M-TBIL**), iron (**M-Iron**), nonesterified fatty acids (**M-NEFA**), and β-hydroxybutyrate (**M-BHB**) concentrations and activities of the enzymes: alkaline phosphatase (**M-ALP**), alanine aminotransferase (**M-ALT**), aspartate aminotransferase (**M-AST**), gamma-glutamyl transferase (**M-GGT**), and lactate dehydrogenase (**M-LDH**).

Energy Metabolism Calculations

Milk yield energy (kJ/BW$^{0.75}$) was corrected as a function of the retained energy (kJ/BW$^{0.75}$) as follows: corrected milk yield energy (**MYEc**) = milk yield energy + (1.20 × positive retained energy) or MYEc = milk yield energy + (0.84 × negative retained energy). The coefficient used with the positive values of retained energy (1.20) was calculated by regression between the retained energy and the milk energy, both expressed as percentage of the metabolizable energy for production. The coefficient used with the negative values of retained energy (0.84) was obtained from the literature (ARC, 1980). The efficiency of use of the metabolizable energy for lactation ($\mathbf{k_l}$) was calculated with the following equation: $k_l = MYEc/(MEI - ME_m)$, where **MEI** (kJ/BW$^{0.75}$) is the metabolizable energy intake and $\mathbf{ME_m}$ (kJ/BW$^{0.75}$) is the metabolizable energy for maintenance. The ME_m was determined by means of regression analysis between milk yield energy plus retained energy versus MEI. The metabolizability (**q**) was calculated by the ratio of MEI/GEI, where GEI is the gross energy intake, both expressed as kJ/BW$^{0.75}$. Net energy for lactation ($\mathbf{NE_l}$) of the diet, expressed in MJ/kg DM, was computed as ME × k_l, where ME is the metabolizable energy concentration of the diet (MJ/kg DM).

Statistical Analysis

Statistical analysis was performed using the mixed procedure of SAS, version 9.4 (SAS Institute Inc.).

The data were analyzed with the following model:

$$Y_{ij(k)m} = \mu + S_m + G_{im} + P_{jm} + T_{(k)} + e_{ijm},$$

where $Y_{ij(k)m}$ represents the dependent variable, calculated as the mean of the daily measurements during each sampling period; μ is the overall mean; S_m represents the fixed effect of the experimental square, with $m = 1, 2$; G_{im} represents the random effect of goat i within the square, with $i = 1, \ldots, 4$; P_{jm} represents the fixed effect of period j, with $j = 1, \ldots, 4$ within the experimental square; $T_{(k)}$ represents the fixed effect of treatment k, with $k = 1, \ldots, 4$; and e_{ijm} represents the residual error. Estimates of the least squares means are reported. Differences between treatments were determined by PDIFF. The same mixed model was used to obtain linear and quadratic polynomial contrasts. The data were tested for normality of the residuals by using the Shapiro–Wilk test. Homogeneity of the variance was tested by using Bartlett's test. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$ for all the statistical analyses.

RESULTS

Diet Composition, Intake, Fecal Output and Digestibility

The chemical composition of the 4 diets is reported in Table 1.

The inclusion of CT in the treated diets caused a reduction in ash, CP, aNDFom, EE, and starch contents compared with the C. In contrast, due to the high GE content of the quebracho extract (19.0 MJ/kg DM), the GE value of the diets increased with the level of inclusion of the extract, from 17.81 with the C to 18.05 MJ/kg DM with the Q6 diet.

The DMI, fecal output, and digestibility values are shown in Table 2.

The DMI was not affected by the treatment, but the Q4 diet numerically had the highest ingestion (2488 g/d). However, a tendency ($P = 0.072$) of a positive linear effect was registered for DMI with increasing CT inclusion in the diet. The inclusion of quebracho CT extract induced a linear increase ($P < 0.001$) in fecal excretion (g DM/d) without affecting the fecal DM content, which was on average 35.1%. The DM and OM digestibility were affected by the presence of quebracho CT extract ($P < 0.001$ for both variables), which caused a linear reduction in both DM and OM digestibility, reaching a reduction of 10.7% and 11.5% for DM and OM digestibility, respectively, between the Q6 and C diets. Moreover, the inclusion of quebracho CT extract induced a significant and linear reduction in CP, aNDFom, and starch digestibility ($P < 0.001$ for all 3 variables). Consequently, the GE digestibility was also significantly reduced, up to 12.9% with the

Table 2: Dry matter intake, fecal excretion, and digestibility of the experimental diets

	Diets[1]					*P*-value[2]		
	C	Q2	Q4	Q6	SEM	T	Lin	Quadr
DMI (g/d)	2159	2253	2488	2371	170	0.111	0.072	0.259
Fecal excretion (g DM/d)	673[c]	755[bc]	855[ab]	915[a]	65.2	0.007	<0.001	0.772
Fecal DM (%)	34.3	36.1	34.7	35.4	1.92	0.472	0.680	0.506
Digestibility (%)								
DM	68.9[a]	66.4[b]	65.5[b]	61.5[c]	0.847	<0.001	<0.001	0.149
OM	71.4[a]	68.6[b]	67.5[b]	63.2[c]	0.846	<0.001	<0.001	0.156
Ash	39.7	40.3	42.4	40.1	1.34	0.355	0.543	0.238
CP	63.6[a]	57.7[b]	56.7[b]	50.2[c]	1.47	<0.001	<0.001	0.797
aNDFom[3]	58.0[a]	53.2[b]	51.4[b]	44.6[c]	1.61	<0.001	<0.001	0.356
Starch	90.2[a]	89.0[ab]	88.2[b]	86.5[c]	0.574	0.002	<0.001	0.627
GE[4]	68.3[a]	65.2[b]	64.5[b]	59.5[c]	0.850	<0.001	<0.001	0.089

[1]Diets with different levels of quebracho condensed tannin (CT) extract: C = no CT; Q2 = 2% of CT; Q4 = 4% of CT; Q6 = 6% of CT.
[2]*P*-value of: T = treatment; Lin = linear effect of CT inclusion; Quadr = quadratic effect of CT inclusion.
[3]aNDFom: α-amylase- and sodium sulfite-treated NDF corrected for insoluble ash.
[4]GE: gross energy.
[a-c]Mean values in the same row with different superscripts differ ($P < 0.05$).

Q6 diet, by the inclusion of quebracho CT extract ($P < 0.001$).

Ruminal Fermentation Characteristics

The ruminal fermentation characteristics are reported in Table 3.

The rumen fluid pH was on average 6.70, without differences between treatments. The total VFA concentration was not affected by the treatments and was on average 77.7 mmol/L. Regarding the proportion of the individual VFA (mol/100 mol), acetate tended to linearly decrease ($P = 0.053$) as the level of quebracho CT extract inclusion increased. Propionate was quadratically affected by the level of quebracho CT extract inclusion ($P = 0.005$), and its proportion was higher for Q6 and C (21.4%) and lower for C, Q2 and Q4 (18.9, 18.3 and 17.4%). Isobutyrate and isovalerate were both linearly reduced by the presence of quebracho CT extract ($P = 0.016$ and $P = 0.011$, respectively). In contrast, butyrate and valerate contents linearly increased ($P = 0.092$ and $P = 0.035$, respectively) in the presence of quebracho CT extract.

Milk Production and Composition

The milk production and composition data are presented in Table 4.

Milk production (g/d) was quadratically affected by the level of quebracho CT extract ($P = 0.027$), showing a significant difference ($P < 0.05$) between the Q4 (highest yield) and C and Q6 treatments. The FPCM was not affected by the treatments.

Regarding the milk composition, the fat and CP contents were linearly reduced by the CT level of inclusion ($P = 0.021$ and $P = 0.017$); however, only for the fat

Table 3: Ruminal pH, total VFA, and VFA molar proportion of the ruminal fluid of the lactating dairy goats fed the four experimental diets

	Diets[1]					*P*-value[2]		
	C	Q2	Q4	Q6	SEM	T	Lin	Quadr
pH	6.67	6.73	6.81	6.58	0.098	0.268	0.594	0.097
VFA mmol/L	76.6	72.3	79.3	82.7	6.30	0.649	0.319	0.492
VFA mol/100 mol								
Acetate	64.2	63.9	63.9	59.7	1.55	0.135	0.053	0.195
Propionate	18.9[b]	18.3[b]	17.4[b]	21.4[a]	0.690	0.008	0.045	0.005
Isobutyrate	1.64	1.40	1.36	1.15	0.123	0.077	0.016	0.886
Butyrate	15.2	17.1	16.8	17.2	1.02	0.146	0.092	0.289
Isovalerate	1.97[a]	1.53[ab]	1.63[ab]	0.911[b]	0.228	0.042	0.011	0.526
Valerate	1.37	1.28	1.49	1.79	0.142	0.130	0.035	0.191

[1]Diets with different levels of quebracho condensed tannin (CT) extract: C = no CT; Q2 = 2% of CT; Q4 = 4% of CT; Q6 = 6% of CT.
[2]*P*-value of: T = treatment; Lin = linear effect of CT inclusion; Quadr = quadratic effect of CT inclusion.
[a,b]Mean values in the same row with different superscripts differ ($P < 0.05$).

Table 4: Milk yield, composition, and dairy efficiency of the dairy goats fed the four experimental diets

	Diets[1]					*P*-value[2]		
	C	Q2	Q4	Q6	SEM	T	Lin	Quadr
Production (g/d)								
Milk	3134[b]	3186[ab]	3371[a]	3066[b]	191	0.050	0.954	0.027
FPCM[3]	3543	3529	3597	3306	184	0.097	0.104	0.097
Composition (%)								
Fat	4.48[a]	4.25[ab]	4.01[b]	4.13[b]	0.133	0.043	0.021	0.107
CP	3.45	3.39	3.38	3.28	0.062	0.089	0.017	0.562
Casein	2.65	2.61	2.61	2.55	0.047	0.321	0.097	0.698
Lactose	4.39	4.36	4.39	4.41	0.076	0.293	0.348	0.147
Yield (g/d)								
Fat	139	136	135	127	6.60	0.149	0.038	0.363
CP	108	108	113	100	6.63	0.061	0.235	0.041
Casein	82.7	83.2	87.8	78.1	5.23	0.066	0.400	0.041
Lactose	137	140	148	136	9.34	0.054	0.785	0.030
LS[4]	6.33	5.35	5.10	4.68	0.664	0.105	0.021	0.495
MUL[5] (mg/dL)	25.8[a]	22.6[ab]	20.3[b]	16.3[c]	1.89	0.002	<0.001	0.711
Feed efficiency								
Milk/DMI	1.46	1.43	1.37	1.31	0.075	0.101	0.018	0.708
FPCM/DMI	1.66[a]	1.57[ab]	1.46[bc]	1.41[c]	0.075	0.020	0.003	0.673

[1]Diets with different levels of quebracho condensed tannin (CT) extract: C = no CT; Q2 = 2% of CT; Q4 = 4% of CT; Q6 = 6% of CT.
[2]*P*-value of: T = treatment; Lin = linear effect of CT inclusion; Quadr = quadratic effect of CT inclusion.
[3]FPCM: fat and protein corrected milk = milk yield × (0.26 + 0.1352 × Fat% + 0.079 × CP%).
[4]LS: linear score = $\log_2$ (SCC/12,500).
[5]MUL: milk urea level.
[a-c]Mean values in the same row with different superscripts differ ($P < 0.05$).

concentration did we observe a significant difference between the treatments, with the C having the highest value (4.48%) and the Q4 and Q6 diets having the lowest fat concentration (4.01 and 4.13%, respectively).

The LS was linearly reduced ($P = 0.021$) as the level of quebracho CT extract in the diet increased.

The milk urea level (**MUL**, mg/dL) was statistically affected by the treatment ($P = 0.002$) with a linear reduction ($P < 0.001$).

The treatment quadratically affected the CP, casein, and lactose yields ($P = 0.041$, $P = 0.041$, and $P = 0.030$, respectively), reaching the highest yields with the Q4 diet and the lowest with the Q6 diet for all 3 variables.

Feed efficiency was linearly reduced by the increasing inclusion of quebracho CT extract ($P = 0.018$ and $P = 0.003$ for milk/DMI and FPCM/DMI, respectively).

Blood plasma and milk biochemical parameters

Plasma parameters determined in dairy goats fed the 4 diets are summarized in Table 5.

No significant differences between diets were observed for metabolic markers of nutritional and health status. A trend for a treatment effect was observed on P-ALB ($P = 0.089$) and P-iron ($P = 0.085$), accompanied by a significant linear effect only for P-ALB ($P = 0.030$).

Dietary supplementation with quebracho CT extract significantly affected P-urea ($P < 0.006$) in a linear way ($P < 0.001$), with lower values observed in Q6 compared with C and Q2 diets.

Biochemical analytes measured in defatted milk samples in the 4 dietary treatments are summarized in Table 6.

No significant variations were observed between treatments, except for M-urea, which also showed a negative linear effect ($P < 0.001$).

Gas Exchange and Enteric Methane Production

The gas exchange and enteric CH_4 production are shown in Table 7.

The inclusion of quebracho CT extract at different levels in the diet did not affect O_2 consumption, CO_2 production, or the respiratory quotient (**RQ**).

The CH_4 yield (g CH_4/kg DMI) was significantly reduced ($P < 0.001$) by the inclusion of quebracho CT extract with a linear effect ($P < 0.001$) as the level of quebracho CT extract increased, reaching a reduction of 17.8% with the Q6 diet compared with C. Moreover, the CH_4 production expressed in relation to the kg of both digested DM and digested OM was linearly reduced by the treatment ($P = 0.006$ for both variables). The CH_4 production per kg of digested NDF was significantly ($P = 0.043$) and linearly increased ($P = 0.014$),

Table 5: Blood plasma biochemical analytes of the lactating dairy goats fed the four experimental diets

	Diets[1]					*P*-value[2]		
	C	Q2	Q4	Q6	SEM	T	Lin	Quadr
Total protein (g/L)	81.6	81.3	84.7	88.1	2.52	0.689	0.046	0.418
Albumin (g/L)	40.7	40.7	40.9	41.8	0.525	0.089	0.030	0.163
Alkaline phosphatase (U/L)	42.3	46.5	52.1	46.4	6.01	0.382	0.352	0.225
Alanine aminotransferase (U/L)	11.0	11.2	12.5	13.9	2.46	0.110	0.025	0.475
Amylase (U/L)	45.9	44.1	42.7	45.3	5.66	0.917	0.854	0.545
Aspartate aminotransferase (U/L)	69.5	70.0	67.3	76.5	3.95	0.113	0.147	0.096
Gamma-glutamyl transpeptidase (U/L)	44.5	44.6	44.9	45.3	2.95	0.979	0.695	0.899
Lactate dehydrogenase (U/L)	426	440	445	448	33.9	0.943	0.577	0.839
Total bilirubin (μmol/L)	2.84	2.73	2.77	2.58	0.258	0.916	0.549	0.862
Creatinine (μmol/L)	70.7	66.2	69.7	71.4	2.63	0.293	0.573	0.149
Urea (mmol/L)	6.09[a]	5.68[ab]	4.86[bc]	3.78[c]	0.423	0.006	<0.001	0.371
Glucose (mmol/L)	3.16	3.14	3.22	3.36	0.100	0.277	0.101	0.306
Triglyceride (mmol/L)	0.206	0.270	0.189	0.254	0.041	0.411	0.734	0.986
Total cholesterol (mmol/L)	3.96	4.12	4.02	3.87	0.248	0.501	0.544	0.203
B-hydroxybutyrate (mmol/L)	0.766	0.974	0.852	0.807	0.078	0.193	0.998	0.086
Nonesterified fatty acids (mmol/L)	0.423	0.490	0.340	0.414	0.080	0.115	0.399	0.924
Calcium (mmol/L)	2.34	2.31	2.45	2.39	0.049	0.106	0.157	0.653
Inorganic phosphate (mmol/L)	1.93	2.08	2.10	2.10	0.290	0.953	0.649	0.758
Magnesium (mmol/L)	1.16	1.11	1.14	1.13	0.088	0.971	0.887	0.791
Iron (μmol/L)	24.5	21.6	24.5	20.4	1.72	0.085	0.127	0.642
Chloride (mmol/L)	108	107	106	107	1.20	0.516	0.531	0.191
Potassium (mmol/L)	4.54	4.32	4.43	4.30	0.152	0.625	0.362	0.751
Sodium (mmol/L)	151	150	150	151	0.611	0.741	0.715	0.314

[1]Diets with different levels of quebracho condensed tannin (CT) extract: C = no CT; Q2 = 2% of CT; Q4 = 4% of CT; Q6 = 6% of CT.
[2]*P*-value of: T = treatment; Lin = linear effect of CT inclusion; Quadr = quadratic effect of CT inclusion.
[a-c]Mean values in the same row with different superscripts differ ($P < 0.05$).

up to 18.7% with the Q6 diet compared with C, as the level of quebracho CT extract increased. Moreover, the CH_4 energy loss as a percentage of GEI was affected by the treatment ($P < 0.001$), with a linear reduction as the level of quebracho CT extract increased ($P < 0.001$). The CH_4 intensity (g CH_4/kg milk) was linearly reduced as the level of quebracho CT extract increased ($P = 0.047$), while the CH_4 intensity expressed per kg of FPCM or per kg of milk fat or milk protein was not influenced by the dietary treatment.

Energy Balance

The energy balance results are reported in Table 8.

The daily gross energy intake (**GEI,** kJ/$BW^{0.75}$) was not affected by the diets. The fecal energy, expressed

Table 6: Biochemical analytes measured in the defatted milk samples of the lactating dairy goats fed the four experimental diets

	Diets[1]					*P*-value[2]		
	C	Q2	Q4	Q6	SEM	T	Lin	Quadr
Free glucose (mmol/L)	0.096	0.090	0.095	0.121	0.022	0.428	0.240	0.253
B-hydroxybutyrate (mmol/L)	0.420	0.441	0.443	0.530	0.057	0.233	0.076	0.365
Nonesterified fatty acids (mmol/L)	0.231	0.244	0.250	0.318	0.061	0.502	0.214	0.529
Total bilirubin (μmol/L)	7.06	6.48	6.02	8.32	1.68	0.690	0.624	0.321
Creatinine (μmol/L)	134	128	128	118	5.46	0.195	0.050	0.647
Urea (mmol/L)	5.10[a]	5.06[a]	4.17[b]	3.44[c]	0.327	0.001	<0.001	0.148
Alkaline phosphatase (U/L)	51.5	27.1	32.3	50.0	13.4	0.087	0.984	0.018
Alanine aminotransferase (U/L)	6.07	5.31	4.58	5.36	0.616	0.284	0.263	0.157
Aspartate aminotransferase (U/L)	48.1	44.9	35.2	40.4	7.28	0.144	0.105	0.289
Gamma-glutamyl transpeptidase (U/L)	201	179	146	171	48.9	0.566	0.457	0.514
Lactate dehydrogenase (U/L)	395	379	333	321	60.0	0.553	0.193	0.961
Iron (μmol/L)	5.43	7.58	6.20	8.76	1.49	0.196	0.106	0.844

[1]Diets with different levels of quebracho condensed tannin (CT) extract: C = no CT; Q2 = 2% of CT; Q4 = 4% of CT; Q6 = 6% of CT.
[2]*P*-value of: T = treatment; Lin = linear effect of CT inclusion; Quadr = quadratic effect of CT inclusion.
[a-c]Mean values in the same row with different superscripts differ ($P < 0.05$).

Table 7: Gas exchange and methane production of the lactating dairy goats fed the four experimental diets

	Diets[1]					*P*-value[2]		
	C	Q2	Q4	Q6	SEM	T	Lin	Quadr
O_2 consumption (L/d)	626	640	636	608	27.6	0.278	0.319	0.097
CO_2 production (L/d)	698	707	714	669	35.8	0.358	0.381	0.149
RQ[3]	1.11	1.10	1.12	1.10	0.016	0.430	0.591	0.466
CH_4 production (g/d)	44.7	45.7	46.3	40.4	3.22	0.115	0.146	0.057
CH_4 (g/kg DMI)	20.8[a]	20.4[a]	18.5[b]	17.1[c]	0.701	<0.001	<0.001	0.244
CH_4 (g/kg dDM[4])	30.3[a]	30.8[a]	28.3[b]	27.9[b]	0.898	0.013	0.006	0.442
CH_4 (g/kg dOM[5])	31.7[a]	32.3[a]	29.8[b]	29.3[b]	0.923	0.013	0.006	0.392
CH_4 (g/kg dNDF[6])	95.2[b]	105[ab]	102[b]	113[a]	3.79	0.043	0.014	0.808
CH_4 (% GEI[7])	6.43[a]	6.29[a]	5.68[b]	5.24[c]	0.216	<0.001	<0.001	0.237
CH_4 (g/kg milk)	14.8	14.3	13.8	13.5	0.823	0.222	0.047	0.943
CH_4 (g/kg FPCM[8])	13.0	13.1	13.0	12.5	0.606	0.654	0.335	0.439
CH_4 (g/kg of milk fat)	333	342	349	326	15.7	0.576	0.800	0.212
CH_4 (g/kg of milk protein)	429	423	408	409	21.9	0.618	0.255	0.800

[1]Diets with different levels of quebracho condensed tannin (CT) extract: C = no CT; Q2 = 2% of CT; Q4 = 4% of CT; Q6 = 6% of CT.
[2]*P*-value of: T = treatment; Lin = linear effect of CT inclusion; Quadr = quadratic effect of CT inclusion.
[3]RQ: respiratory quotient (CO_2 production / O_2 consumption).
[4]dDM: digestible DM.
[5]dOM: digestible OM.
[6]dNDF: digestible NDF.
[7]GEI: gross energy intake.
[8]FPCM: fat and protein corrected milk = milk yield × (0.26 + 0.1352 × Fat% + 0.079 × CP%).
[a-c]Mean values in the same row with different superscripts differ ($P < 0.05$).

both as kJ/$BW^{0.75}$ and in %GEI, was significantly affected by the dietary treatment ($P = 0.005$ and $P < 0.001$, respectively) and positively linearly correlated ($P < 0.001$ for both) with the level of quebracho CT extract inclusion. Despite the difference in fecal energy between treatments, the digestible energy intake (**DEI**) was similar when expressed as kJ/$BW^{0.75}$, while when expressed as %GEI, the inclusion of quebracho CT extract caused a linear reduction in DEI up to 12.9% with Q6 ($P < 0.001$). Both urinary and CH_4 energy (%GEI) were linearly reduced ($P < 0.001$) as the level of quebracho CT extract increased, which, associated with the linear reduction of DEI (%GEI), led to a significant and linear reduction of MEI (%GEI, $P < 0.001$). Moreover, the CH_4 energy (%DEI) and the milk energy (%GEI) were linearly reduced as the level of quebracho CT extract increased ($P = 0.10$ and $P = 0.005$, respectively). In contrast, the retained energy (%GEI) was not affected by the diets and was positive only for Q4. The ME_m was found to be 393 (kJ/$BW^{0.75}$). The k_l was affected by the inclusion of quebracho CT extract, with Q4 reaching the highest efficiency (69.2%) and the Q2 diet the lowest (62.5%). The metabolizability was linearly reduced by the levels of quebracho CT extract from 58.7% with the C diet to 52.0% with the Q6 diet. The NE_l was not affected by the treatment and was on average equal to 6.60 kJ/g DM.

Nitrogen Balance

The results concerning the N balance are presented in Table 9.

No difference in N intake (**NI**, g/d) was observed between the diets. The fecal N excretion, expressed both as g/d and as percentage of NI, was linearly and positively affected by the level of quebracho CT extract included in the diet ($P = 0.001$ and $P < 0.001$, respectively). In contrast, the urinary N excretion, expressed both as g/d and as percentage of NI, was linearly reduced ($P < 0.001$) as the level of quebracho CT extract in the diet increased. With the Q6 diet, the N fecal excretion (%NI) increased by 36.8%, while the urinary excretion decreased by 38.8%, compared with the C diet. A quadratic effect ($P = 0.041$) was observed for the N excreted with the milk (g/d), with the Q4 diet having the highest and the Q6 the lowest milk N excretion (17.8 vs. 15.8 g/d). Conversely, when milk N excretion was expressed as a percentage of NI, the increasing level of quebracho CT extract caused a linear reduction ($P = 0.021$). The N balance (both as g/d and as %NI) was negative for all the diets, and the effect of the treatment was not significant.

DISCUSSION

This study aimed to test 3 levels of inclusion of quebracho CT extract compared with a control diet to

Table 8: Energy balance of the lactating dairy goats fed the four experimental diets

	Diets[1]					*P*-value[2]		
	C	Q2	Q4	Q6	SEM	T	Lin	Quadr
GEI[3], MJ/d	38.6	40.3	44.7	42.9	3.07	0.096	0.054	0.285
GEI, kJ/BW$^{0.75}$	1901	1995	2225	2067	142	0.102	0.107	0.166
Fecal energy, kJ/BW$^{0.75}$	604^{c}	693^{bc}	786^{ab}	838^{a}	56.4	0.005	<0.001	0.598
DEI[4], kJ/BW$^{0.75}$	1297	1302	1439	1229	92.0	0.109	0.806	0.081
Urinary energy, kJ/BW$^{0.75}$	26.1	25.3	22.9	21.2	2.07	0.076	0.015	0.712
CH_4 energy, kJ/BW$^{0.75}$	122	125	127	107	8.13	0.057	0.094	0.032
MEI[5], kJ/BW$^{0.75}$	1114	1121	1254	1074	82.8	0.116	0.959	0.096
Heat production, kJ/BW$^{0.75}$	661	676	680	628	27.5	0.102	0.188	0.038
Milk energy, kJ/BW$^{0.75}$	474^{a}	470^{a}	486^{a}	434^{b}	23.0	0.021	0.045	0.031
Retained energy, kJ/BW$^{0.75}$	−23.1	−24.7	87.9	−11.8	46.6	0.126	0.232	0.318
Methane energy (%DEI)	9.41^{a}	9.63^{a}	8.82^{b}	8.78^{b}	0.272	0.013	0.010	0.468
% of GEI								
Fecal energy	31.7^{c}	34.8^{b}	35.5^{b}	40.5^{a}	0.850	<0.001	<0.001	0.089
Digestible energy	68.3^{a}	65.2^{b}	64.5^{b}	59.5^{c}	0.850	<0.001	<0.001	0.089
Urinary energy	3.27^{a}	2.88^{ab}	2.61^{bc}	2.29^{c}	0.188	0.016	0.002	0.830
Methane energy	6.43^{a}	6.29^{a}	5.68^{b}	5.24^{c}	0.216	<0.001	<0.001	0.237
q[6]	58.7^{a}	56.1^{b}	56.2^{b}	52.0^{c}	0.744	<0.001	<0.001	0.138
Heat production	35.2^{a}	34.5^{a}	30.7^{b}	31.0^{b}	1.37	0.004	0.001	0.496
Milk energy	25.2^{a}	23.6^{ab}	22.1^{b}	21.4^{b}	1.10	0.029	0.005	0.553
Retained energy	−1.93	−2.03	3.46	−0.399	2.20	0.080	0.209	0.253
k_l[7]	64.4^{ab}	62.5^{b}	69.2^{a}	67.4^{ab}	2.59	0.050	0.073	0.990
NE_l[8], MJ/kg DM	6.74	6.28	7.02	6.34	0.298	0.064	0.647	0.607

[1]Diets with different levels of quebracho condensed tannin (CT) extract: C = no CT; Q2 = 2% of CT; Q4 = 4% of CT; Q6 = 6% of CT.
[2]*P*-value of: T = treatment; Lin = linear effect of CT inclusion; Quadr = quadratic effect of CT inclusion.
[3]GEI: gross energy intake.
[4]DEI: digestible energy intake.
[5]MEI: metabolizable energy intake.
[6]q: metabolizability = MEI/GEI.
[7]k_l = efficiency of MEI utilization for lactation.
[8]NE_l: net energy for lactation.
[a-c]Mean values in the same row with different superscripts differ ($P < 0.05$).

Table 9: Urine excretion and nitrogen balance of the lactating dairy goats fed the four experimental diets

	Diets[1]					*P*-value[2]		
	C	Q2	Q4	Q6	SEM	T	Lin	Quadr
Urine excretion (g/d)	2412	2264	2613	2314	234	0.335	0.941	0.609
Nitrogen intake (NI, g/d)	50.3	52.0	56.4	52.7	3.93	0.255	0.282	0.225
Nitrogen excretion (g/d)								
Fecal	18.4^{c}	21.9^{bc}	24.2^{ab}	26.4^{a}	2.03	0.010	0.001	0.620
Urinary	17.7^{a}	16.9^{ab}	15.0^{b}	11.1^{c}	1.20	0.002	<0.001	0.085
Milk	16.9	17.0	17.8	15.8	1.04	0.061	0.235	0.041
Nitrogen excretion (%NI)								
Fecal	36.4^{c}	42.3^{b}	43.3^{b}	49.8^{a}	1.47	<0.001	<0.001	0.797
Urinary	35.3^{a}	33.3^{a}	27.0^{b}	21.7^{b}	2.67	0.002	<0.001	0.383
Milk	34.0	32.8	32.1	30.1	1.61	0.115	0.021	0.685
Nitrogen balance								
Retained nitrogen (g/d)	−2.92	−3.77	−0.511	−0.524	1.51	0.241	0.126	0.759
Retained nitrogen (%NI)	−6.19	−8.42	−2.41	−1.56	3.01	0.216	0.120	0.545

[1]Diets with different levels of quebracho condensed tannin (CT) extract: C = no CT; Q2 = 2% of CT; Q4 = 4% of CT; Q6 = 6% of CT.
[2]*P*-value of: T = treatment; Lin = linear effect of CT inclusion; Quadr = quadratic effect of CT inclusion.
[a-c]Mean values in the same row with different superscripts differ ($P < 0.05$).

assess the effect on digestibility, milk production, CH_4 production, rumen fermentation parameters, plasma metabolites and enzymatic activities, nitrogen balance and energy balance of lactating goats.

To the best of our knowledge, this is the first study in which different levels of commercial quebracho CT extract were tested on lactating goats to evaluate the influence on CH_4 production, energy balance and nitrogen balance. A similar work was recently conducted by Nascimento et al. (2021) with the inclusion of mimosa CT extract, up to 7.5%, on lactating goats with a lower milk yield (with a mean of 910 g/d) and without analyzing CH_4 emissions and energy balance, characteristics that are particularly significant when tannins are studied.

In the present study, the Q2 diet did not induce major differences compared with C for many of the variables studied. This is further confirmation of the greater tolerance of goats to CT compared with cows, for which generally lower levels of CT are used. However, Q6 showed several detrimental effects, leading us to assume that 4% might be the threshold for quebracho CT inclusion in dairy goats.

Dry Matter Intake, Digestibility, and Milk Production

Tannins were once considered anti-nutritive agents. Among the negative effects attributed to CT fed to ruminants were the reduction in DMI and nutrient digestibility (Makkar, 2003; Aboagye and Beauchemin, 2019). However, goats were found to be more adaptable to CT than cows and sheep also due to the production of saliva rich in proteins that exhibit tannin-binding properties (Muir, 2011; Min and Solaiman, 2018; Schmitt et al., 2020), representing one of the primary adaptive strategies used by goats and other ruminants to counteract tannins (Alonso-Díaz et al., 2012; Gerlach et al., 2018a). In the present study, CT tended to linearly increase the DMI, consistent with the equation proposed by Min and Solaiman (2018).

In the rumen, tannins are known to form complexes with protein and other feed fractions, leading to a reduction in protein and fiber degradability (Muir, 2011), as observed in the present study, which showed that the reduction in digestibility caused by CT was linear for all feed fractions except ash. The significant decrease in digestibility of DM, OM, CP, aNDFom and GE of the CT diets in comparison with the C was already found with the Q2 diet, whereas for starch digestibility, the decrease toward C was significant only from the Q4 diet. The highest reduction in digestibility was observed for the Q6 diet (−21.1 and −23.1% for CP and aNDFom, respectively) compared with C. These values are greater than those of Nascimento et al. (2021), which had reductions of −7.30, −9.65, and −4.82% for DM, CP, and aNDFom, respectively, obtained with the highest level of inclusion of CT extracted from mimosa (7.5% of CT) fed to dairy goats. Moreover, Dschaak et al., (2011), in a trial on dairy cows fed diets with a maximum inclusion of 3% quebracho CT extract, did not observe changes in DM, CP, and NDF digestibility, while Norris et al. (2020) observed a reduction in DM, CP, and aNDF digestibility when quebracho CT were fed at concentrations up to 4.5% to steers. The inconsistency in the results could be explained by the different inclusion levels, the variability of the chemical structure and molecular weight of CT and by the interaction with the diets (Beauchemin et al., 2007; Naumann et al., 2017; Aboagye and Beauchemin, 2019).

The reduction in feed digestibility induced by CT did not affect the ruminal concentration of total VFA, although the percentage concentrations of individual VFA changed. Condensed tannins tended to decrease acetate concentrations linearly, which can be linked to decreased aNDFom degradability and lower CH_4 generation with rising CT levels. Moreover, the linear reduction, with increasing levels of CT, in isobutyrate and isovalerate contents, which are branched-chain VFA that are byproducts of amino acid deamination in the rumen, can be associated with the reduction in CP degradability and blood urea level. A similar trend was observed in vitro (Battelli et al., 2023) and in vivo (Bhatta et al., 2013).

The milk production was quadratically affected by the level of inclusion of CT and was consistent with the DMI trend; however, considering the FPCM, no differences between the diets were registered, consistent with what was found by Nascimento et al. (2021) with a maximum level of 7.5% of CT on DM. The inclusion of CT lowered fat concentration and fat yield linearly, possibly due to the decrease in aNDFom digestibility, which caused the decrease in ruminal acetate concentration. The reduction in CP digestibility probably caused the linear reduction in milk CP concentration and the quadratic effect of reduction in milk CP yield, which decreased with the Q6 diet. This can also be associated with the reduction of MUL: as reported by Rapetti et al. (2014), values of MUL lower than 23 mg/dL indicate a deficit of metabolizable protein. The decrease in milk protein content and yield can also be attributed to the lower MEI of the goats fed CT: a lower MEI likely indicates a lower growth of the microbial population in the rumen and, in turn, less energy and amino acids for the udder.

Interestingly, the LS was linearly reduced by CT inclusion, similar to what was observed by Min et al. (2005). Milk production in goats is apocrine, resulting in a larger quantity of cytoplasmic particles and epi-

thelial cells aside from leucocytes listed in milk somatic cells, compared with milk production in cows, which is merocrine (Min et al., 2005). The different milk secretion types could explain the absence of an effect of CT on the somatic cell count observed in dairy cows (Benchaar et al., 2008; Moate et al., 2014); however, the mode of action in goat milk is not explained (Min et al., 2005).

The inclusion of quebracho CT extract induced a significant reduction in feed efficiency (FPCM/DMI) with a linear pattern, similar to what Aguerre et al. (2020) observed in cows. This, combined with the absence of a difference in the FPCM yield and the reduction in diet digestibility, indicates the need for goats to utilize their body energy reserves for milk production.

Enteric Methane Production and Energy Balance

Tannins are listed in the dietary strategies to reduce CH_4 production in ruminants (Beauchemin et al., 2020, 2022), but the effects appear to depend on ruminant species, tannin source and concentration, as well as diet composition (Beauchemin et al., 2009; Mueller-Harvey et al., 2019). This is the first study on dairy goats in which the effect of different levels of commercial CT extract on CH_4 has been tested.

In our study, the inclusion of CT from quebracho linearly lowered the CH_4 yield, and the differences with the C were statistically significant starting from Q4. The reduction in CH_4 yield induced by CT is in line with the results of Animut et al. (2008a), who tested different levels of CT-containing forage on male goats. Moreover, similar linear patterns of reduction were found for CH_4 production expressed in relation to dDM and dOM. In contrast, the inclusion of CT linearly increased CH_4 production when expressed per kg of dNDF, due to the strong reduction in fiber digestibility. The findings of the present work support the hypothesis advanced by Carulla et al. (2005) that the reduction in CH_4 production associated with CT is mostly due to the decrease in fiber degradability, also confirming what was observed in vitro by Battelli et al. (2023). Condensed tannins linearly lowered the CH_4 intensity when expressed as per kg of milk but not when expressed as per kg of FPCM, confirming that CT had a stronger ruminal CP- and fiber degradability-lowering effect than an anti-methanogenic effect. In terms of overall CH_4 energy losses (% of GEI and % of DEI), the current findings are consistent with those of Rapetti et al. (2021), with enteric CH_4 production representing 5 to 6% of the GEI and 8 to 9% of the DEI in lactating goats.

Considering the energy balance, the value found for MEm (393 kJ/$BW^{0.75}$) is similar to those previously found by Rapetti et al. (2005) and by Aguilera et al. (1990) (403 and 401 kJ/$BW^{0.75}$, respectively). The linear increase in energy excreted with the feces is further evidence of the decrease in the digestibility of the diet as its content of CT increases. Furthermore, the decreased urine energy content with greater levels of CT inclusion can be explained by the lower ruminal CP degradation and CP digestibility and is consistent with the trend of reduced blood and milk urea concentrations. A similar trend of increased fecal energy and decreased urinary energy with CT was observed by Bhatta et al. (2013) with male goats, while Norris et al. (2020) observed only an increase in fecal energy with cattle, even if the urinary energy of the control treatment was approximately 20% greater than the one with the inclusion of 3% on DM of quebracho CT extract. The heat production (% of GEI) was reduced when CT supplementation was equal to or higher than 4%. A reduction in heat energy was also observed by Puchala et al. (2012) with meat goats when fed CT. On average, the retained energy was −0.225% of GEI, and only the Q4 diet had a positive energy balance (3.46% of GEI) that can be explained by the numerically higher DMI and GEI. However, in terms of milk composition, it is remarkable that the inversion of fat and protein percentages syndrome has never occurred due to the appropriate energy content of the diets. This inversion is a common problem in high-yielding goats from 4 to 5 mo to 7–8 mo of lactation, especially during the spring and summer period (Morand-Fehr et al., 2000; Chilliard et al., 2003).

The average k_l found in this study (65.9) is consistent with those (on average 67.0) found by other studies on goats (AFRC, 1990; Aguilera et al., 1990; Tovar-Luna et al., 2010; Rapetti et al., 2021). Usually, q and k_l are positively associated, as reported by Vermorel (1978); however, in the present study, q decreased linearly as the level of CT increased, while k_l did not. This can be explained by 2 factors: first, the quebracho CT extract has a high GE content (19.0 MJ/kg DM), which the animal cannot use, resulting in an underestimation of q; second, the reduction of ruminal nutrient degradability, due to the formation of complexes with tannins, could lead to a greater quantity of intestinal digestible nutrients, especially, from an energy standpoint, starch, than can be at least partially digested with a higher efficiency that would occur at the rumen level (Nocek and Tamminga, 1991; Deckardt et al., 2013; Moharrery et al., 2014).

Blood Plasma and Milk Biochemical Analytes

Analysis of the biochemical profile in blood and milk in dairy goats is not routinely performed, and it has

been less investigated by authors compared with dairy cows to assess animal health and nutritional status or for early diagnosis and prevention of metabolic diseases (Overton et al., 2017; Andjelić et al., 2022).

No significant differences were determined when comparing the 4 dietary treatments (except for P-urea contents); our findings were within the reference values reported for goats by Kaneko et al. (1997) and Jackson and Cockcroft (2007) and consistent with those reported in previous studies in dairy goats during lactation (Guzmán et al., 2020, 2021) and fed with commercial tannins (de Lucena et al., 2018).

Plasma analytes related to energy and lipid metabolism showed that only TCHOL concentrations were high in all 4 diet treatments, while GLU, BHB, NEFA, and TRI were within the physiological range and sustained by reports of other studies (Jackson and Cockcroft, 2007; Guzmán et al., 2020; Zamuner et al., 2020). Increased blood NEFA and BHB levels have become accepted biomarkers of lipid mobilization and oxidation rates associated with negative energy balance conditions, just as increased activities of blood enzymes (ALT, AST, GGT, ALP and LDH) due to infiltration and damage of hepatocytes with fatty acids are considered good indicators of impaired liver function in cows (Bobe et al., 2004). In the present study, P-NEFA values ranged from 0.34 mmol/L in Q4 to 0.49 mmol/L in Q2 diet, which agree with reported observations in clinically healthy dairy goats of 0.45 $\pm$ 0.41 mmol/L (compared with 1.32 $\pm$ 0.26 mmol/L of goats with subclinical hyperketonemia) by Huang et al. (2023). On the other hand, goats fed diets with the addition of quebracho CT extract showed P-BHB concentrations (from 0.807 mmol/L in Q6 to 0.974 mmol/L in Q2 diet) above the threshold of 0.8 mmol/L proposed for predicting the risk of developing hyperketonemia during late pregnancy in dairy sheep and used in dairy goats because of the absence of validated goat thresholds (Pichler et al., 2014). However, in our experiment, the activities of ALT, AST, AMY, GGT, and ALP enzymes were low and in the range reported in the literature (Jackson and Cockcroft, 2007; Kaneko et al., 1997; Guzmán et al., 2020, 2021), except for P-LDH activities, which were slightly higher than the reported ranges (0–400 units/L) by Jackson and Cockcroft (2007).

Concentrations of urea in blood plasma and in defatted milk decreased in dairy goats fed higher quebracho CT extract supplementation percentages in the diet and were highly correlated ($r = 0.83$; $P < 0.001$). These results were in accordance with previous studies in dairy cows (Zhang et al., 2019) and goats (Bendelja Ljoljić et al., 2020).

Interestingly, although no significant differences between treatments were evidenced in this study, we found the highest (n.s.) concentrations of P-TRI, TCHOL, NEFA, and BHB with the Q2 diet; likewise, in the Q4 diet, we observed the lowest values of P-TRI and P-NEFA. These findings are consistent with the results of the calculated energy balances, suggesting higher lipomobilization in the Q2 treatment than in the Q4 treatment, the only diet showing a positive energy balance. However, since the values of P-TPRO, P-ALB, and P-TBIL, together with P-CRE, inorganic minerals, and electrolytes, in the present study were in the range reported by Guzmán et al. (2021), the results of the present study suggest that dairy goats did not have impaired hepatic or renal function affecting health status.

The results obtained in our study show that dietary supplementation with quebracho CT extract at 3 doses did not influence the milk biochemical profile (except urea). The activity of the M-GGT enzyme and the levels of some M-TBIL and M-CREA were higher in milk than in plasma; however, little information is available concerning biochemical metabolites and enzyme activities in milk and the possible relationships between them in milk and blood in ruminants (Djokovic et al., 2017; Andjelić et al., 2022).

Most likely, the activities of the enzymes, particularly lactate dehydrogenase (LDH), may be related to the mammary health status (Katsoulos et al., 2010).

Nitrogen Balance

Due to the formation of tannin-protein complexes, which probably determined a reduction in ruminal protein degradation, which would be consistent with the lower levels of blood urea, a shift from urinary to fecal N excretion was observed. This shift is consistent with observations in other experiments on goats (Bhatta et al., 2013; Nascimento et al., 2021) and has a positive repercussion for the environment because fecal N is more stable, thus reducing the release of NH_3 and N_2O in the atmosphere (Castillo et al., 2000; Mueller-Harvey et al., 2019; Hristov et al., 2022). In addition to the already discussed effects of CT on milk N, we observed a decrease in N efficiency (expressed as the percentage of NI recovered in milk N) due to reduced amino acid absorption and supply for milk protein, as also reported in cows (Grainger et al., 2009; Gerlach et al., 2018b; Aguerre et al., 2020). Moreover, the N balance was always negative and not affected by the treatments. These results led us to assume that quebracho CT caused the shift from urinary to fecal N excretion without improving the N use efficiency, in agreement with what was suggested by Herremans et al. (2020) in a recent meta-analysis on dairy cows.

CONCLUSION

The inclusion of quebracho CT extract at different levels, up to 6%, in the diets of dairy goats caused a reduction in nutrient digestibility. The FPCM yield was not affected by CT inclusion, however, CT linearly reduced the milk efficiency. Although CT lowered the CH_4 yield linearly, most likely due to the reducing effect on digestibility, CT addition had no influence on CH_4 emissions per kg of FPCM. In addition, CT did not affect the metabolic parameter levels in blood and milk, except for urea concentrations. Furthermore, CT did not improve N efficiency. According to these findings, the positive environmental impacts of CT are mainly related to the shift from urine to fecal N excretion.

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Data availability The data of this study are available from the corresponding author upon reasonable request.

Conflict of Interest The authors have not stated any conflicts of interest.

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