Postbiotic yeast fermentation product supplementation to lactating goats increases efficiency of milk production by enhancing fiber digestibility and ruminal propionate, and reducing energy losses in methane

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List of Abbreviations:

C: carbon

CH_4: methane

CON: control diet

E: energy

FCM: fat corrected milk

HP: heat production

HPf: heat of fermentation

HPx: heat production from oxidation

kl: efficiency of use of metabolizable energy to milk production

kls: efficiency of use of metabolizable energy to milk and maintenance

N: nitrogen

OXCHO: oxidation of carbohydrate

OXF: oxidation of fat

OXP: oxidation of protein

POS: posbiotic diet

RE: energy retention

LAY SUMMARY:

Although in vitro data with mixed ruminal fluid demonstrated positive effects of postbiotics from lactobacilli on measures of fermentation and microbial profiles, there is a paucity of in vivo data with lactating ruminants. We evaluated the effects of incorporating a postbiotic yeast fermentation product in diets of lactating goats on energy partitioning,

carbon and nitrogen balance, and performance. The postbiotic led to greater ruminal propionate concentration and fiber digestibility, and decreased partitioning of energy to methane. Those changes were associated with greater milk production. Data suggested that postbiotics could enhance efficiency of nutrient use for milk production.

TEASER TEXT:

Our results demonstrate that feeding a postbiotic in late-lactation can increase energy efficiency for milk production in part by enhancing ruminal production of propionate and reducing methane emission.

ABSTRACT:

Although in vitro data with mixed ruminal fluid demonstrated positive effects of posbiotic diet (POS) from lactobacilli on measures of fermentation and microbial profiles, there is a paucity of in vivo data with lactating ruminants. The aim of the study was to evaluate the effects of incorporating POS into diets of lactating goats on energy (E) partitioning, carbon (C) and nitrogen (N) balance, and performance. Ten late-lactation Murciano-Granadina

goats were used in a cross-over design with 26-d periods. Goats in the control diet (CON) were fed daily at the rate of 1 kg alfalfa hay and 1.5 kg concentrate, and the treatment group (POS) was fed CON with the addition of 3.75 g/d of Probisan Ruminants™ (PENTABIOL S.L., Navarra, Spain). No differences in DMI were detected. However, ruminal fluid propionate and apparent total tract digestibilities of NDF and ADF were greater (18, 4.7 and 5.2%, respectively; $P < 0.05$) in POS compared with the CON diet. Daily partitioning of E to milk and efficiency of ME intake for milk production greater (11 and 3.0%, respectively; $P < 0.05$) in POS compared with CON. The non-protein RQ was greater in POS compared with CON due to greater ($P < 0.05$) oxidation of carbohydrate (213 vs. 115 kJ/kg of $BW^{0.75}$ per day) compared with fat (362 vs. 486 kJ/kg of $BW^{0.75}$ per day). Although no differences were found in C balance, goats in POS had lower ($P < 0.05$) amounts of C in CH_4 (1.1 vs. 1.3 g/kg $BW^{0.75}$ per day) compared with CON. There were no differences in N intake or N in feces or urine, but N in milk was greater ($P < 0.05$) in POS compared with the CON diet (0.8 vs. 0.7 g/kg $BW^{0.75}$ per day). Yield of fat corrected milk (FCM) (3.20 vs. 2.72 kg/d; $P < 0.05$) and concentration of true protein (3.4 vs. 3.3 kg/d; $P < 0.05$) and lactose (4.7 vs. 4.5 kg/d; $P < 0.05$) were greater in POS compared with CON. These responses were accompanied by lower ($P < 0.05$) urea (12.3 vs. 16.6 mM/L) and ammonia-N (6.6 vs. 8.8 mg/L) without changes in fat concentration (6.1 vs. 6.0%; $P > 0.05$) in POS compared with the CON diet. Daily amount of CH_4 emission did not differ $P > 0.05$ between diets. However, when expressed relative to unit of edible product, feeding POS reduced ($P < 0.05$) the amount of CH_4 by 46 g/kg of milk fat, 97 g/kg of milk protein and 3 g/kg of milk compared with CON. Overall, data indicated that feeding a postbiotic in late-lactation increased energy efficiency for milk production partly by reducing CH_4 emission.

Key words: postbiotic, milk performance, dairy goat, methane emission

INTRODUCTION

In the last decade, there has been increased interest in feeding bacterial and yeast fermentation products (i.e. probiotics) as feed additives to enhance ruminal fermentation and promote immune function and overall health (Seo et al., 2010). Probiotics are live non-pathogenic microorganisms that have the ability to improve the microbial balance in the gastrointestinal tract of the host. Besides the focus on digestion, there is interest in the use of these feed additives as preventive strategies that can potentially reduce the use of antibiotics in animal production (Signorini et al., 2012). In general, probiotics act through molecular and cellular mechanisms by disturbing the adhesion of pathogens, enhancing innate immunity, decreasing pathogen-induced inflammation, and promoting intestinal epithelial cell survival and barrier function (Williams, 2010).

Although beneficial effects of probiotics in livestock nutrition are clear, from a practical standpoint, these supplements require proper and careful handling when used in feeding of livestock, e.g. they are sensitive to environmental conditions such as sunlight and water pH. In addition to issues related to product handling, there is some concern about feeding probiotics because some may carry antibiotic resistant genes, particularly plasmid encoded bacteria, which could be transferred between organisms (Marteau et al., 2003; Shazali et al., 2014). The gene could transfer from probiotics to native microbes and potentially to pathogens. Thus, due to ease of handling and application postbiotics have been proposed as an alternative to probiotics. By definition, postbiotics are the metabolites of probiotic bacteria which elicit a probiotic effect in the absence of living microbial cells (Thanh et al., 2009). Thus, the mode of action of postbiotics is expected to be similar to probiotics.

The proposed roles of postbiotics in the gastrointestinal tract are to prevent the colonization of pathogens by improving the environment of the gut for beneficial commensal bacteria to survive and propagate (Aguilar-Toalá et al., 2018). The presence of antimicrobial

metabolites such as organic acids and bacteriocins in postbiotics can reduce gut pH and inhibit the proliferation of opportunistic pathogens in the feed and gut of animals. This will encourage the production of organic acids that lead to lower pH and produce more antimicrobial compounds to inhibit the proliferation of pathogenic bacteria, promote beneficial bacteria growth which modulates microbial balance, induce immune cells and immune function, and helps maintain gut health (Seo et al., 2010).

There is a paucity of research on postbiotics in ruminants, particularly in vivo studies. One of the most studied types of postbiotics is from *Lactobacilli* strains (Cicenia et al., 2014; Kareem et al., 2014). These products contain killed, whole lactic acid bacterial bodies, lactic acid and lactic acid salts, and it is suggested to work as a biofilm coating the intestinal surface facing the gut lumen, thereby, preventing adhesion of pathogens (Kareem et al., 2014). Other studies have fed postbiotics from *Lactobacilli plantarum* in post-wean lambs and reported improvements in growth performance, nutrient intake and digestibility (Izzudin et al., 2018; 2019a). Thorsteinsson and Vestergaard (2020) reported no effect of a combination of a probiotic and postbiotic (from *Lactobacilli acidophilus*) in the milk replacer and the concentrate of veal calves on the overall health (no differences in IgG), and a positive effect on growth performance was detected. There are few reports of postbiotic feeding in lactating ruminants (e.g. Chida et al., 2021), but none addressing aspects of nutrient digestion and efficiency of energy (E) utilization. Thus, the aim of the current study was to investigate the effects of a postbiotic product from yeast fermentation on total tract digestibility, E utilization, carbon (C) and nitrogen (N) balance, methane (CH_4) emissions, and milk production and composition in dairy goats.

MATERIAL AND METHODS

Ethics Statement

Experimental procedures were approved (2017/VSC/PEA/00182) by the Committee on Animal Use and Care at the Polytechnic University of Valencia (UPV; Valencia, Spain), and followed the codes of practice for animals used in experimental work proposed by the European Union (EU, 2003). Authors declare that this manuscript does not involve ethical issues or affect any endangered or protected species.

Animals and Diets

The experiment was conducted at the Institute for Animal Science and Technology (UPV, Valencia, Spain). Ten multiparous mature Murciano-Granadina dairy goats in late-lactation (7^{th} month) were selected and divided into two homogenous groups of five goats based on similar body weight (BW; 48.0 ± 1.3 kg of BW) and milk production in the previous lactation (669 ± 82 kg of milk per 210 ± 30 days of lactation). Forage used was alfalfa hay and the concentrate a pelleted compound feed. Nutrient requirements followed published recommendations for lactating goats weighting 48 kg of BW and producing 2.5 kg milk per day (Calsamiglia et al., 2009). Ingredients and chemical composition of the diet are reported in **Table 1**. Treatments were applied in a crossover design (2 treatments crossed with 2 period) with the diet fed as a total mixed ration. The CON diet was fed at 1 kg alfalfa hay and 1.5 kg concentrate (40:60 forage to concentrate ratio) daily. The treatment group (POS) was the CON diet supplemented with the postbiotic at 3.75 g/d of Probisan Ruminants™ (PENTABIOL S.L., Navarra, Spain). Probisan Ruminants™ contains 19.6% CP, 4.6% EE, 0.82% lysine and 0.29% methionine. Half the daily ration was offered at 0800 h and half at 1600 h. The postbiotic was fed as a topdress, with half the daily dose at 0800 h and half at 1600 h.

Experimental Design and Measurements

The experiment had two 26-d periods divided as follows: during a 14-d adaptation period, goats were fed the experimental diets in pens and then allocated to individual metabolism cages (1.5 m length × 0.53 m width × 1.65 m height) at thermoneutrality (20-23

ºC determined by a Hobo probe, ONSET data loggers, Cape Cod, MA, USA) for another 7-d. Subsequently, during a 5-d period feed offered and refused, and total fecal, urine and milk output were recorded daily for each goat for calculation of nutrient balance. In addition, BW at the beginning and end of the experimental period (after 26-d) were recorded. Total feces were collected in wire-screen baskets placed under the floor of the metabolism crates and total urine was collected through a funnel into plastic buckets containing 100 mL 10% (vol/vol) of H_2SO_4 to prevent microbial degradation and loss of volatile ammonium. Then, all collected feces and 20 mL urine were dried in a forced-air oven at 55ºC for 48h and, representative samples (10%) of diets, feces and urine collected, stored at -20 ºC and later pooled for chemical analysis.

Goats were milked once daily at 0800 h with a portable milking machine (Flaco, model DL-170, J. Delgado S.A., Ciudad Real, Spain). Immediately after milking, individual milk yield was measured and a sub-sample of 250 mL per goat placed in a bottle and frozen until analysis. In addition, samples were collected into plastic vials (50 mL per animal) that contained 20 mg of potassium dichromate as a preservative and taken to the Interprofessional Dairy Laboratory of the Valencia Community Region (LICOVAL, Valencia, Spain) for composition analysis (total solids, total protein, true protein, fat and lactose). Prior to gas exchange determinations, goats were moved from metabolism cages to pens for 2-d during which ruminal fluid samples were collected by stomach tube (50 mL) before the morning feeding. Ruminal fluid was strained through 4 layers of cheesecloth and pH determined immediately using a portable pH meter (Model 265A, Orion Research Inc., Beverly, MA, USA). A sub-sample of ruminal fluid (4 mL) was acidified with 50% H_2SO_4 and frozen until later determination of ammonium. Samples (0.9 mL) for analysis of VFA were mixed with H_3PO_4 (0.1 mL) and kept frozen until analysis.

Gas exchange was measured for each goat during a 24-h period with an indirect calorimetry system based on two ventilated head-boxes designed for small ruminants (5 d period) described previously by Fernández et al. (2012, 2015, 2019). The whole system was calibrated by injecting pure nitrogen (N_2) and CO_2 into the head box (McLean and Tobin, 1987) determined gravimetrically using a precision scale (MOBBA mini-SP 0.2–30 kg, Industrial Weighing System, Barcelona, Spain). Calibration factors were calculated as described previously (Brockway et al., 1971). Production of CH_4 and CO_2 and oxygen (O_2) consumption were calculated as described previously (Aguilera and Prieto, 1986). An atmospheric air sample was collected and the gas concentrations were used as reference for calculations.

Chemical Analyses

Feed, feed refusals and fecal samples were first dried in a forced-air oven at 55 ºC for 48 h then ground to pass a 1 mm screen before analysis. Urine and milk were lyophilized prior to analyses. Chemical analyses of the diet, refusals and feces were conducted according to AOAC (2000) for DM (934.01), ash (942.05) and ether extract (920.39). The DM of diets and feces was determined by oven-drying at 102 ± 2 ºC for 24 h. Ash concentration was measured by incineration in an electric muffle furnace at 550 ºC for 6 h. The ether extract was determined with petroleum ether after acid hydrolysis to recover saponified fat (Soxhlet System HT Tecator, Hillerød, Denmark; 1047 Hydrolyzing Unit and 1043 Extraction Unit). The NDF and ADF were measured in an ANKOM Fiber Analyzer (A220, ANKOM Technologies, Fairport, NY, USA) according to a published protocol (Mertens, 2002) and AOAC (2000), respectively. The NDF was determined using sodium sulfite and alpha amylase. The NFC content of diets was calculated by difference based on chemical analysis of individual feeds according to NRC (2001; NFC = 100 – NDF – ash – CP – ether extract). GE content of the dry samples (feed, feces, urine and milk) was analyzed by combustion in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, UK). Starch content was determined with the α-

amylase method (Batey, 1982; Sigma-Aldrich, Steinheim, Germany). The C and N were analyzed by the Dumas principle (TruSpec CN; LECO Corporation, St. Joseph, MI, USA). Multiplying N by a factor of 6.25 converted the results to CP.

Determination of ruminal VFA was based on a method described previously (Jouany, 1982) using a gas chromatograph (Fisons 8000 series; Fisons Instruments SpA, Milan, Italy) equipped with a split/splitless injector and flame ionization detector. Milk composition (fat, total protein, true protein, lactose and total milk solids content) was analyzed with an infrared analyzer (MilkoScan FT120 Foss Electric, Hillerød, Denmark). Urea in ruminal fluid and milk were analyzed by flow injection analyses and enzymatic degradation (urease; EC 3.5.1.5), and application notes given by the manufacturer were followed (Foss Tecator AB, Höganäs, Sweden). The NH_3-N content was analyzed by direct distillation using the Kjeldahl method (2300 Kjeltec Analyzer Unit Foss Tecator, Hillerød, Denmark).

Calculations

Fat-corrected milk (FCM) at 4% was calculated according to a published equation for goats (Mavrogenis and Papachristoforou, 1988).

FCM (4%) = kg of milk × [0.411 + (0.147 × fat (%))].

The ME intake was calculated as the difference between GE intake and E losses in feces, urine and CH_4 (with an energy equivalent value of 39.5 kJ/L CH_4; Brouwer, 1965).

Heat production (HP) was determined from measurements of O_2 consumption, CO_2 and CH_4 production, and urine N (N_{urine}) using the equation of Brouwer (1965):

$$HP\ (kJ) = 16.18 \times O_2 + 5.02 \times CO_2 - 2.17 \times CH_4 - 5.99 \times N_{urine}$$

where gases were expressed in L/d and N_{urine} in g/d.

Recovered E was the difference between ME intake and HP.

Recovered E = ME intake – HP

249 Energy retention (RE) in the body was calculated as the difference between recovered
250 E and milk E (E_{milk}).

251 RE_{body} = Recovered E – E_{milk} = MEI – HP – E_{milk}

252 Energy associated with the oxidation of macronutrients as protein, carbohydrates and
253 fat (OXP, OXCHO and OXF, respectively) as follows:

254 $OXP = 6.25 \times N_{urine} \times 18.42$ (kJ/g),

255 $OXCHO = (-2.968 \times O_2 + 4.174 \times CO_{2x} - 2.446 \times N_{urine}) \times 17.58$ (kJ/g),

256 $OXF = (1.719 \times O_2 - 1.719 \times CO_{2x} - 1.963 \times N_{urine}) \times 39.76$ (kJ/g).

257 Where the CO_{2x} was calculated as $CO_2 - (2.4 \times CH_4)$, according to Fahey and Berger
258 (1988).

259 Then, the HP from oxidation of macronutrients (HPx) was:

260 $HPx\ (kJ) = 16.18 \times O_2 + 5.02 \times CO_{2x} - 5.99 \times N_{urine}$.

261 Gases were expressed in L per day and N_{urine} in g/d.

262 The heat of fermentation (HPf) was estimated subtracting HP from HPx.

263 The non-protein RQ from oxidation of nutrients was determined as:

264 Non-protein RQ = $(CO_{2x} – (N_{urine} \times 6.25 \times 0.774)) / (O_2 – (N_{urine} \times 6.25 \times 0.957))$.

265 The efficiency of use of ME for lactation (kl) in the absence of change in body E stores
266 was calculated according to ARC (1980). Energy lost from the body, indicating mobilization
267 of body fat reserves in support of milk secretion, was assumed to be used for milk synthesis
268 with an efficiency of 0.84 and the concomitant E storage during lactation was taken to be 0.95
269 times the milk secretion efficiency. Consequently, the corrected milk E was estimated as E_{milk}
270 + (0.84 × negative E retention) + (1.05 × positive E retention). The kl was calculated as:

271 $$k_l = \frac{\text{corrected milk E}}{(\text{ME intake} - \text{MEm})}$$

Where MEm was the ME for for Granadina goats (401 kJ/kg of $BW^{0.75}$ and day; Aguilera et al., 1990). Furthermore, the efficiency of ME for milk and maintenance (kls) was calculated according to INRA (2018);

$$kls = 0.65 + 0.247 \times (q - 0.63)$$

where q was the metabolisability (ME/GE).

For C and N balance, we followed the equations and values proposed previously (McLean and Tobin, 1987). Briefly it was calculated as follow:

The C balance gives the total amount of C retained in the body and the amount of C retained in fat can be calculated by subtracting the amount of C retained in protein determined by N balance. Assuming that fat has an energy equivalent of 39.76 kJ/g and contains 76.7%C, and that protein has an energy equivalent of 23.86 kJ/g and contains 16%N and 52%C. The RE in protein and fat can be calculated as:

$$RE_{protein} = N_{balance} \times 6.25 \times 23.86$$

$$RE_{fat} = [C_{balance} - (N_{balance} \times 6.25 \times 0.52)] \times 1.304 \times 39.76$$

Where RE was expressed in kJ and CN balance in g. If the equations are not multiplied by the energy equivalent, we obtain protein and fat retention in g.

Statistical Analysis

The experiment was conducted as a crossover design with each goat receiving both treatments in 2 periods. Effects of diet on intake, digestibility, ruminal fermentation, milk performance, E and C and N balances, and oxidation of nutrients were analyzed using a mixed model (lme function from the nlme library) in R (2016). The following statistical model was used:

$$Y = \mu + D + T + D \times T + goat + \varepsilon$$

Where: Y is the dependent variable, μ is the overall mean, and D and T are the fixed effects of diet and period of time, respectively, and their interaction; goat is the random effect

297 of goat; and ε is the random error. Least squares means were reported throughout and
298 differences were considered significant at $P < 0.05$.

299 **RESULTS AND DISCUSSION**

300 The average value for the calibration factor of O_2, CO_2 and CH_4 was 1.0015 ± 0.00230
301 (n = 4), 1.0014 ± 0.00931 (n = 4) and 0.9898 ± 0.00681 (n = 4), respectively. The consistent
302 values confirmed the absence of leaks and good functioning of the entire indirect calorimetry
303 system. No significant effect was observed for period and their interaction in the crossover
304 design (tables report only the effect of diet).

305 ***Feed Intake, Digestibility and Ruminal Fermentation***

306 No difference in total DMI ($P > 0.05$) was observed between diets (1.97 kg/d, on
307 average) indicating that POS had no negative impact (**Table 2**). Apparent total tract
308 digestibility coefficients of DM, OM, CP, ether extract and E also did not differ ($P > 0.05$).
309 Thus, values obtained for DM digestibility (72%, on average) were similar to those reported
310 previously in lactating goats, i.e., Bava et al. (2001) with late-lactation Saanen goats obtained
311 a value of 74% and Tovar-Luna et al. (2010) with late-lactation Alpine goats consuming 60%
312 of concentrate obtained an average value of 72%. Izzudin et al. (2019a) reported greater DMI
313 and fiber degradability and overall improvements in DM, CP, and NDF digestibility in post-
314 wean lambs supplemented with a postbiotic. Thus, in our study, increases of 6 and 5%
315 (respectively) in NDF and ADF digestibility ($P < 0.05$) with POS compared with the CON diet
316 confirmed the beneficial effects reported previously. Although we did not assess ruminal
317 microbiota profiles, previous data indicated that probiotics may contribute to beneficial effects
318 in terms of enhancing populations of ruminal cellulolytic bacteria (Dawson et al., 1990) leading
319 to greater fiber digestibility and contributing to better growth performance (Oyetayo and
320 Oyetayo, 2005) including in young lambs (Izuddin et al. 2018; 2019a; 2019b).

Average ruminal pH never fell below 6.5 (**Table 3**) and was within a range sufficiently high to maintain normal ruminal fermentation (Ørskov and Fraser, 1975). Izuddin et al. (2018) reported that postbiotic inclusion had no effect on ruminal fluid pH in vitro. A lack of change in ruminal pH might have been indicative of proper adaptation of the ruminal environment to the presence of lactic acid from POS. With exception of propionic acid ($P < 0.05$), no differences due to POS were observed for NH_3-N, urea and VFA. Previous studies feeding *Lactobacilus plantarum* RG14 in lambs reported greater ruminal NH_3-N (Izuddin et al., 2019a) and production of VFA in the rumen, particularly butyric acid (Izuddin et al. 2019b). Such an effect was also associated with greater papillae length and width. The greater concentration of propionic acid with POS might have been due to increases in numbers of *Propionibacterium spp.* Seo et al. (2010) proposed that a greater proportion of lactic acid in postbiotics can enhance numbers of these microorganisms through the provision of a constant supply of lactic acid, which can then be used to produce propionic acid.

Acetic and butyric acids are considered lipogenic substrates and propionic acid is considered a glucogenic substrate (van Knegsel et al., 2007). Differences ($P < 0.05$) were detected when the ratio of acetic to propionic acid was determined, being lower with POS compared with CON. Thus, based on van Knegsel et al. (2007), we speculate that the POS diet had a tendency to induce a glucogenic effect, whereas the CON diet induced a lipogenic effect.

Energy Balance

Due to similar daily DMI, no differences ($P > 0.05$) in GE intake (1,800 kJ/kg of $BW^{0.75}$, on average) were observed (**Table 4**). As no differences in digestibility were detected, digestible E was also similar (1,318 kJ/kg of $BW^{0.75}$, on average). Urine E losses were greater (19%; $P < 0.05$) with POS, and lower (9.7%; $P < 0.05$) losses in E losses in CH_4 were detected with the POS compared with CON. Despite the differences in urine E between diets, the daily

346 ME intake was similar (1,190 kJ/kg of $BW^{0.75}$, on average). Izzudin et al. (2019a) reported
347 greater ME intake in post-wean lambs supplemented with a postbiotic (*L. plantarum* RG14)
348 due to greater responses in intake and digestibility. No differences were observed in HP (679
349 kJ/kg of $BW^{0.75}$, on average), and values were in the range of previous work with goats, i.e.
350 637 kJ/kg of $BW^{0.75}$ for late-lactation Saanen goats (Bava et al., 2001) and 680 kJ/kg of
351 $BW^{0.75}$ in late-lactation Alpine goats fed diets with 60% concentrate (Tovar-Luna et al., 2010).
352 The E_{milk} was greater with POS (11%; $P < 0.05$) compared with the CON diet, E balance
353 was positive with both diets, and no differences in RE_{body} were detected (35 kJ/kg of $BW^{0.75}$,
354 on average). The kls, as defined by INRA (2018), was the same in both diets and the kl was
355 greater (3.0%; $P < 0.05$) in POS compared with CON. Similar values were reported previously
356 for Granadina (0.67; Aguilera et al., 1990) and Alpine goats (0.63; Tovar-Luna et al., 2010).
357 When expressed as % GE intake, E_{milk} was greater (11%; $P < 0.05$) and RE_{body} lower (67%; P
358 < 0.05) with POS compared with CON.

359 ***Oxidation of Nutrients***

360 Production of CO_2 is derived from nutrient oxidation and ruminal fermentation. Thus,
361 separation between these two components is necessary to calculate substrate oxidation and the
362 proportion that supports total HP associated with oxidative processes. Diet had no effect on
363 HPx and HPf, but differences ($P < 0.05$) were observed in OXCHO and OXF (**Table 5**). When
364 expressed relative to HPx, the OXCHO was greater (17% vs. 33%) and OXF lower (73% vs.
365 56%) with POS than CON diet. The greater OXCHO in POS compared with the CON diet
366 suggested a preference for the use of dietary carbohydrate as a source of fuel, and the opposite
367 for lipids. Because the gas exchange method does not discriminate between oxidation of
368 exogenous and endogenous glucose, the data more closely represented net catabolism of
369 glucose. The low dietary fat content suggested that the greater contribution of OXF with the
370 CON diet likely originated from lipid mobilization (Chwalibog et al., 1997; Derno et al., 2013).

Few studies in ruminants have reported data on nutrient oxidation. Because the basal diet fed to both CON and POS was the same, the available data do not allow for a thorough understanding of the causes for the differences observed in OXCHO and OXF with POS. A significant difference ($P < 0.05$) was observed for non-protein RQ, with POS resulting in greater (6.2%) values than CON likely due to the greater OXF in CON animals as mentioned above.

Carbon and Nitrogen Balance

No differences ($P > 0.05$) were observed in C intake or C in feces and urine (**Table 6**). Compared with CON, losses in C from CH_4 were lower (15%; $P < 0.05$) and C in milk was greater (11%; $P < 0.05$) when POS was fed. The efficiency of milk C output relative to C ingested was 24% and 21% for POS and the CON diet, respectively. Goats ingested and excreted similar ($P > 0.05$) amount of N. Milk N was greater (13%; $P < 0.05$) and N retained in the body lower (18%; $P < 0.05$) in POS compared with CON diet. The ratio between milk N output and N ingested was greater with POS than CON (23 vs. 19%).

From the C and N balance (**Table 6**), retention of protein and fat expressed in kJ or g were calculated according to McLean and Tobin (1987). There was no difference in RE_{fat} between diets (which was negative indicating lipid mobilization in both groups; RQ <1). These results seem contradictory because, although the RQ was 6.2% lower in CON compared with POS, there was no difference in fat mobilization between the diets. An RQ lower than 1 indicated fat mobilization and predominance of OXF compared with OXCHO (Chwalibog et al., 1997), as we observed in our study being lower in POS compared with CON. Furthermore, $RE_{protein}$ was positive and greater (17%; $P < 0.05$) in CON than in POS diet, without any clear explanation. In this regard, indirect calorimetry only estimates the total net loss of substrates (carbohydrates and lipids), but does not consider any metabolic transformation, exchange or cycling that the substrate itself or its intermediates undergo along the biochemical pathways to

396 complete oxidation (Derno et al., 2013). Because indirect calorimetry does not "see"
397 intermediate metabolic pathways, without the help of internal metabolic biomarkers, it is
398 difficult to explain the lack of differences in RE_{fat} and the differences detected in the $ER_{protein}$.
399 Probably the different approaches could be partly responsible for the discrepancies observed;
400 RE_{body} by the RQ method and REf_{at} and $RE_{protein}$ by the CN method. It is important to keep in
401 kind that the total energy balance (RE_{body}) was positive with both diets (**Table 4**), and to study
402 it, body retention was separated into fat and protein following the CN method.

403 According to Judy et al. (2018), the $RE_{protein}$ accounts for energy used in tissue protein
404 synthesis, thus, a positive N balance along with positive RE balance suggested that goats in the
405 current study were accreting protein. In late lactation goats replenish tissue reserves for the
406 subsequent lactation, which probably occurred in the current study as in cattle (NRC, 2001)
407 and goats (Fernández et al., 2021), although the concomitant fat mobilization to maintain milk
408 production during spring time, as happening at the present study, was more pronounced in POS
409 diet. These theorical estimates indicated that feeding CON led to more tissue protein synthesis,
410 while feeding POS led to more milk protein synthesis. When protein and fat body retention
411 was expressed in g, no differences were detected between diets.

412 ***Milk Production and Chemical Composition***

413 Milk yield was grater (16%; $P < 0.001$) with POS compared with the CON diet (**Table**
414 **7**). When milk yield was expressed as FCM, the response was greater with POS (15%; $P <$
415 0.001). Feed efficiency expressed as milk yield over DMI was greater (16%; $P < 0.001$) with
416 POS compared with CON. These differences were also observed for FCM/DMI (14%; $P <$
417 0.001). According to Miettinen and Huhtanen (1996), moderate levels of concentrates in the
418 diet of dairy cows increases the ratio of ruminal propionic to butyric acid, often increases milk
419 yield, protein, lactose and decreases milk fat content. The same observation was reported by
420 van Knegsel et al. (2007) when glucogenic and ketogenic diets were compared. Accordingly,

421 in this study, the POS diet increased the ratio propionic to butyric acid (0.77 vs. 0.51 for POS
422 and CON, respectively), milk yield, true protein and lactose without effects on milk fat. This
423 simple measure of efficiency determines the relative ability of goats to turn feed nutrients into
424 milk because it affects both economic and environmental efficiency; feeding POS increased
425 the milk from every kg of DM consumed and, fewer nutrients were excreted in manure.
426 No differences were observed in milk composition with exception of greater true
427 protein (2.9%; $P < 0.05$) and lactose (4.3%; $P < 0.05$) in POS compared with the CON diet. As
428 Seo et al. (2010) reported, higher populations of *Propionibacterium spp.* in the rumen favored
429 the conversion of lactic acid into propionic acid. Thus, the POS diet might have been associated
430 with greater production and absorption of propionic acid followed by greater production of
431 glucose via gluconeogenesis to support lactose synthesis and greater milk volume. Milk urea
432 and N-NH_3 were lower (26 and 25%, respectively; $P < 0.05$) in POS compared with CON.
433 Together with the greater true protein percentage in POS compared with CON, this effect
434 suggests a positive effect on N partitioning to milk due to POS.
435 In the Mediterranean countries, goat's milk production has traditionally been destined
436 for cheese manufacture. Thus, the physicochemical characteristics and composition of raw milk
437 are essential for the successful development of the dairy goat industry and also, for the
438 marketing of the final products. In Spain, farmers are paid based on two components in the
439 milk; protein plus fat (cheese extract). The cheese extract is the main parameters for farmers,
440 because the price of milk depends on it (milk price per cheese extract was 0.0937€; consulted
441 08/20/22 at Lonja de Albacete, Castilla-La Mancha, www.oviespana.com). No differences in
442 cheese extract were observed in this study (9.4%), and the same price per kg of milk was
443 obtained; 0.88 €/kg of milk. Because greater milk yield was obtained with POS compared with
444 CON, the estimated farmers income would amount to 2.19 or 1.84 €/d per goat, respectively.
445 ***Methane Emission***

Although no differences were observed in rates of daily CH_4 emission or when CH_4 was expressed relative to DMI and OM intake, the production of CH_4 relative to NDF intake, fat in milk, protein in milk, cheese extract and milk yield was lower (11, 20, 23, 20 and 21%, respectively; $P < 0.05$) in POS compared with CON (**Table 8**). Ruminants lose between 2-12% of their dietary GEI as CH_4, and the average Ym (CH_4 conversion factor) of 4.9 obtained in this study was a typical value reported when mixed diets are fed to ruminants (Johnson and Johnson, 1995; Knapp et al., 2014). Together, the observed reduction of CH_4 relative to production of edible products along with the greater ruminal propionate when POS was fed are indicative of a ruminal effect. It is likely that postbiotic compounds in POS elicited changes in microbiota profiles associated with methanogenesis as has been demonstrated with other non-nutritive additives (Patra et al., 2017).

SUMMARY AND CONCLUSIONS

The inclusion of a postbiotic in lactating dairy goats improved ruminal fluid propionate, apparent total tract digestibility of NDF and ADF, and the efficiency of ME intake for milk production. Milk yield and concentration of true protein and lactose were greater in POS compared with the CON diet. When CH_4 was expressed relative to milk yield and chemical composition, feeding POS reduced the amount of CH_4 compared with the CON diet. Hence, data indicated that feeding a postbiotic in late-lactation increases energy efficiency for milk production and reduces CH_4 emission per unit of milk edible product.

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CONFLICT OF INTEREST STATEMENT

All authors have no conflicts of interest.

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Table 1. Ingredients and chemical composition of the diets

	Diet[1]
Item	CON
Ingredients, g/kg DM	
Alfalfa hay	400
Barley	170
Corn	60
Soybean meal (46% CP)	65
Corn gluten feed (21% CP)	90
Sunflower meal (28% CP)	10
DDGS maize	30
Rapeseed expeller	36
Wheat bran	97
Molasses beet	12
Fat hydrogen	3
Bypass fat[2]	11
Sodium bicarbonate	6
Sodium chloride	2
Limestone	5
Premix[3]	2
Chemical composition, % of DM	
DM	94
OM	89
Ash	11
CP	18
Ether extract	4
NDF	34
ADF	17
ADL	3
NFC[4]	33
Starch	21
Carbon	39
Nitrogen	3
Carbon : Nitrogen	13
Gross energy, MJ/kg DM	18

[1] Provided by de HEUS Nutrición Animal SAU. España. CON = control.

[2] Bypass fat of palm fatty acid distillate.

[3] Provided by NACOOP S.A. (Spain) to supply (ppm or IU/kg of premix): Se, 40 mg/kg; I, 250 mg/kg; Co, 80 mg/kg; Cu, 3,000 mg/kg; Fe, 6,000 mg/kg; Zn, 23,400 mg/kg; Mn, 29,000 mg/kg; S, 60,000 mg/kg; Mg, 60,000 mg/kg; vitamin A, 2,000,000 IU/kg; vitamin D3, 400,000 IU/kg; vitamin E, 2,000 ppm; nicotinic acid, 10,000 ppm; choline, 20,300 ppm.

[4]NFC, non-fibrous carbohydrate content = 100-(NDF+ash+CP+Ether Extract).

Table 2. Dry matter intake and apparent digestibility coefficients (% of DM) of Murciano-Granadina goats (n = 10) during late-lactation according to the type of diet

	Diet[2]			
Item[1]	CON	POS	SEM	*P*-value
DMI, kg/d	1.96	1.98	0.019	0.617
Apparent total-tract digestibility, %				
DM	71.1	72.0	1.62	0.792
OM	73.2	74.4	1.49	0.699
CP	78.1	78.5	1.22	0.858
Ether extract	45.9	50.6	2.19	0.467
NDF	65.1	68.7	1.08	0.039
ADF	57.3	60.1	0.94	0.049
Energy	72.6	74.0	1.52	0.651

[1] CON = Control; POS = postbiotic.

Table 3. pH, ammonia-N (NH_3-N) and VFA from rumen a of Murciano-Granadina goats (n = 10) during late-lactation according to the type of diet

	Diet[1]			
Item	CON	POS	SEM	*P*-value
pH	6.7	6.7	0.11	0.456
NH_3-N, mg/dL	40.4	41.2	3.39	0.912
Urea, mM/L	14.4	13.7	0.61	0.740
Total VFA, mM	41.9	37.6	3.07	0.516
Individual VFA, mM/L				
Acetic acid	24.00	20.70	1.846	0.346
Propionic acid	5.01	6.08	0.308	0.041
Isobutyric acid	0.66	0.73	0.054	0.522
Butyric acid	9.91	7.88	0.779	0.210
Isovaleric acid	1.05	1.20	0.089	0.434
n-Valeric acid	0.77	0.89	0.093	0.532
n-Caproic acid	0.11	0.11	0.006	0.750
Heptanoic acid	0	0.01	0.004	0.347
Acetic/Propionic ratio	4.79	3.41	0.101	0.048

[1] CON = Control; POS = postbiotic.

Table 4. Daily energy partitioning (kJ/kg of $BW^{0.75}$) of Murciano-Granadina goats (n = 10) during late-lactation according to the type of diet

	Diet[2]			
Item[1]	CON	POS	SEM	*P*-value
DMI, g/kg of $BW^{0.75}$	110	107	1.1	0.250
GEI	1,818	1,782	17.8	0.327
E_{feces}	503	462	26.5	0.455
DE	1,315	1,320	30.8	0.938
E_{urine}	35	43	2.4	0.042
E_{CH4}	93	84	2.1	0.035
MEI	1,187	1,193	30.3	0.928
HP	688	671	8.6	0.398
E_{milk}	449	503	14.0	0.045
RE_{body}	50	19	33.0	0.615
kls	0.66	0.66	0.0	0.856
kl	0.64	0.66	0.0	0.050
% GEI				
DE	72	74	0.7	0.727
ME	65	66	0.7	0.888
HP	38	37	0.4	0.116
E_{milk}	25	28	0.3	0.039
RE_{body}	3	1	0.0	0.045
MJ/kg of DM				
GE	16.6	16.7	0.17	0.683
DE	12.0	12.3	0.12	0.376
ME	10.8	11.1	0.11	0.112
NE_L	4.1	4.7	0.04	0.041

[1] GEI = gross energy intake; E_{feces} = energy losses in feces; E_{urine} = energy losses in urine; E_{CH4} = energy losses in methane; MEI = metabolizable energy intake; HP = heat production; E_{milk} = recovered energy in milk; RE_{body} = energy retention (RE_{body} = MEI − HP − E_{milk}); kls = ME efficiency for milk production according to INRA (2018); kl = ME efficiency for milk production; DE = digestible energy.

[2] CON = Control; POS = postbiotic.

Table 5. Daily heat production (kJ/kg of $BW^{0.75}$) from oxidation of nutrients (kJ/kg of $BW^{0.75}$) and their contribution to the heat production of Murciano-Granadina goats (n = 10) during late-lactation according to the type of diet

	Diet[2]			
Item[1]	CON	POS	SEM	*P*-value
HPx	665	649	8.9	0.366
HPf	23	21	0.9	0.391
OXP	63	75	4.4	0.205
OXCHO	115	213	10.3	0.041
OXF	486	362	6.8	0.030
OXP/HPx	0.09	0.11	0.010	0.126
OXCHO/HPx	0.17	0.33	0.013	0.037
OXF/HPx	0.73	0.56	0.011	0.018
RQnpx	0.76	0.81	0.004	0.042

[1]HPx = heat production from oxidation of nutrients; HPf = heat production of fermentation [HPf = HP − HPx (Brouwer, 1958)]; OXP = heat production associated with the oxidation of protein; OXCHO = heat production associated with the oxidation of carbohydrates; OXF = heat production associated with the oxidation of fat; RQnpx = nonprotein respiratory quotient (unitless) from oxidation of nutrients $\{[CO_{2x} - (N_{urine} \times 6.25 \times 0.774)]/[O_2 - (N_{urine} \times 6.25 \times 0.957)]$, where CO_2 = CO_2 production from oxidation, and N_{urine} = N in urine$\}$.

[2] CON = Control; POS = postbiotic.

Tabla 6. Daily carbon and nitrogen balance (g/kg of $BW^{0.75}$) of Murciano-Granadina goats (n = 10) during late-lactation according to the type of diet

	Diet[2]			
Item[1]	CON	POS	SEM	*P*-value
C_{intake}	43.1	41.7	0.47	0.123
C_{feces}	13.6	12.6	0.72	0.472
C_{urine}	0.9	1.1	0.06	0.110
C excretion	28.6	28.0	0.92	0.782
C_{CO2}	15.3	14.7	0.23	0.220
C_{CH4}	1.3	1.1	0.03	0.023
C waste	31.1	29.5	0.78	0.324
C_{milk}	8.9	10.0	0.28	0.040
$C_{body\ retained}$	3.1	2.2	0.83	0.567
N_{intake}	3.6	3.5	0.04	0.252
N_{feces}	0.7	0.7	0.04	0.976
N_{urine}	0.5	0.6	0.04	0.204
N excretion	1.3	1.4	0.04	0.298
N_{milk}	0.7	0.8	0.02	0.017
$N_{body\ retained}$[3]	1.7	1.4	0.07	0.046
$RE_{protein}$, kJ/kg of $BW^{0.75}$	176	147	0.0	0.001
RE_{fat}, kJ/kg of $BW^{0.75}$	-115	-119	0.5	0.095
Retained body protein, g/d	187	164	7.8	0.118
Retained body fat, g/d	-51	-56	17.2	0.929

[1] C_{intake} = C intake; C_{feces} = C losses in feces; C_{urine} = C losses in urine; C_{CO2} = C losses in CO_2; C_{CH4} = C losses in methane; C_{milk} = recovered C in milk; $C_{body\ retained}$ = recovered C in tissue; N_{intake} = N intake; N_{feces} = N losses in feces; N_{urine} = N losses in urine; N_{milk} = recovered N in milk; $N_{body\ retained}$ = recovered N in tissue; RE = energy retention.

[2] CON = Control; POS = postbiotic.

[3] $N_{body\ retained}$ = is apparently retained.

Table 7. Daily milk production and composition of Murciano-Granadina goats (n = 10) during late-lactation according to the type of diet

	Diet[2]			
Item[1]	CON	POS	SEM	*P*-value
Milk yield, kg/day	2.09	2.49	0.061	<0.001
FCM (4%), kg/day	2.72	3.20	0.059	<0.001
Feed efficiency				
Milk yield/DMI	1.06	1.26	0.024	0.001
FCM/DMI	1.38	1.61	0.031	0.001
Chemical composition, %				
Total solids	15.2	15.0	0.10	0.322
Fat	6.1	6.0	0.09	0.527
Total protein	3.6	3.6	0.02	0.789
True protein	3.3	3.4	0.02	0.048
Lactose	4.5	4.7	0.03	0.001
Non-fat dry extract	9.1	9.0	0.03	0.127
Cheese extract	9.4	9.4	0.10	0.183
Urea, mM/L	16.6	12.3	0.16	0.001
$N\text{-}NH_3$, mg/L	8.8	6.6	0.50	0.014

[1] DMI = dry matter intake; Cheese extract = milk fat + milk protein.

[2] CON = Control; POS = postbiotic.

Tabla 8. Daily methane (CH_4) emission of Murciano-Granadina goats (n = 10) during late-lactation according to the type of diet

	Diet[2]			
Item[1]	CON	POS	SEM	*P*-value
CH_4, g	29.9	28.2	0.65	0.172
CH_4 /CO_2 in breath	0.08	0.07	0.002	0.155
Ym, %	5.1	4.7	0.11	0.059
CH_4/DMI, g/kg	15.3	14.2	0.33	0.105
CH_4/OMI, g/kg	17.2	15.9	0.37	0.097
CH_4/NDFI, g/kg	42.5	37.8	1.22	0.049
CH_4/fat in milk, g/kg	235	189	9.7	0.008
CH_4/protein in milk, g/kg	430	333	13.4	0.042
CH_4/cheese extract, g/kg	152	121	5.5	0.012
CH_4/milk, g/kg	14.3	11.3	0.54	0.001

[1]Ym = methane conversion factor (energy in methane/gross energy intake); DMI = dry matter intake; OMI = organic matter intake; NDFI = neutral detergent fiber intake.

[2] CON = Control; POS = postbiotic.

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