Journal of Animal Science, 2022, **101**, 1–11
https://doi.org/10.1093/jas/skac370
Advance access publication 7 November 2022
Ruminant Nutrition

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

Carlos Fernández,[†,1] Tamara Romero,[†] Ignacio Badiola,[‡] Jesús Díaz-Cano,[‖] Gregorio Sanzol,[‖] and Juan J. Loor[§,]

[†]Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, 46022 Valencia, España
[‡]IRTA-Centre de Recerca en Sanitat Animal, Campus de Bellaterra, Bellaterra, 08193 Barcelona, España
[‖]PentaBiol Animal Health, Polígono Noáin-Esquiroz, Esquiroz, 31191 Navarra, España
[§]Department of Animal Sciences, Division of Nutritional Sciences, University of Illinois, Urbana, IL 61801, USA

[1]Corresponding author: cjfernandez@dca.upv.es

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 crossover 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 nonprotein 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.

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.

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

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; k_l, 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

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 nonpathogenic 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

Received April 8, 2022 Accepted November 4, 2022.

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, for example, 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 postwean 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.

Materials 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 (seventh 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 d 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 d (Calsamiglia et al., 2009). Ingredients and chemical composition of the diet are reported in Table 1. Treatments were applied in a crossover design (two treatments crossed with two 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 hours and half at 1600 hours. The postbiotic was fed as a topdress, with half the daily dose at 0800 hours and half at 1600 hours.

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 to 23 °C 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 55 °C for 48 h and, representative samples (10%) of diets, feces and urine collected, stored at –20 °C and later pooled for chemical analysis.

Goats were milked once daily at 0800 hours 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

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, nonfibrous carbohydrate content = 100 – (NDF + ash + CP + ether extract).

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 four 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 to 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 °C 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 °C for 24 h. Ash concentration was measured by incineration in an electric muffle furnace at 550 °C 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).

$$\text{FCM (4\%)} = \text{kg of milk} \times [0.411 + (0.147 \times \text{fat (\%)})].$$

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)

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

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

Recovered E was the difference between ME intake and HP.

$$\text{Recovered } E = \text{ME intakeHP}$$

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

$$RE_{body} = \text{Recovered E} E_{milk} = \text{MEIHP} E_{milk}.$$

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

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

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

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

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

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

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

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

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

The nonprotein RQ from oxidation of nutrients was determined as

$$\text{Nonprotein RQ} = (CO_{2x} (N_{urine} \times 6.25 \times 0.774)) / (O_2 (N_{urine} \times 6.25 \times 0.957)).$$

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

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

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)

$$\text{kls} = 0.65 + 0.247 \times (q0.63)$$

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 two 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

$$Y = \mu + D + T + D \times T + \text{goat} + \varepsilon$$

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

Results and Discussion

The average value for the calibration factor of O_2, CO_2, and CH_4 was 1.0015 ± 0.00230 (n = 4), 1.0014 ± 0.00931 (n = 4), and 0.9898 ± 0.00681 (n = 4), respectively. The

consistent values confirmed the absence of leaks and good functioning of the entire indirect calorimetry system. No significant effect was observed for period and their interaction in the crossover design (tables report only the effect of diet).

Feed intake, digestibility, and ruminal fermentation

No difference in total DMI ($P > 0.05$) was observed between diets (1.97 kg/d, on average) indicating that POS had no negative impact (Table 2). Apparent total tract digestibility coefficients of DM, OM, CP, ether extract, and E also did not differ ($P > 0.05$). Thus, values obtained for DM digestibility (72%, on average) were similar to those reported previously in lactating goats, that is, Bava et al. (2001) with late-lactation Saanen goats obtained a value of 74% and Tovar-Luna et al. (2010) with late-lactation Alpine goats consuming 60% of concentrate obtained an average value of 72%. Izzudin et al. (2019a) reported greater DMI and fiber degradability and overall improvements in DM, CP, and NDF digestibility in postwean lambs supplemented with a postbiotic. Thus, in our study, increases of 6% and 5% (respectively) in NDF and ADF digestibility ($P < 0.05$) with POS compared with the CON diet confirmed the beneficial effects reported previously. Although we did not assess ruminal microbiota profiles, previous data indicated that probiotics may contribute to beneficial effects in terms of enhancing populations of ruminal cellulolytic bacteria (Dawson et al., 1990) leading to greater fiber digestibility and contributing to better growth performance (Oyetayo and 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) and 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 ME intake was similar (1,190 kJ/kg of $BW^{0.75}$, on average). Izzudin et al. (2019a) reported greater ME intake in postwean lambs supplemented with a postbiotic (*L. plantarum* RG14) due to greater responses in intake and digestibility. No differences were observed in HP (679 kJ/kg of $BW^{0.75}$, on average), and values were in the range of previous work with goats, that is, 637 kJ/kg of $BW^{0.75}$ for late-lactation Saanen goats (Bava et al., 2001) and 680 kJ/kg of $BW^{0.75}$ in late-lactation Alpine goats fed diets with 60% concentrate (Tovar-Luna et al., 2010).

The E_{milk} was greater with POS (11%; $P < 0.05$) compared with the CON diet, E balance was positive with both diets, and no differences in RE_{body} were detected (35 kJ/kg of $BW^{0.75}$, on average). The kls, as defined by INRA (2018), was the same in both diets and the kl was greater (3.0%;

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 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
k_l	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); k_l, ME efficiency for milk production; DE, digestible energy.
[2]CON, control; POS, postbiotic.

$P < 0.05$) in POS compared with CON. Similar values were reported previously for Granadina (0.67; Aguilera et al., 1990) and Alpine goats (0.63; Tovar-Luna et al., 2010). When expressed as % GE intake, E_{milk} was greater (11%; $P < 0.05$) and RE_{body} lower (67%; $P < 0.05$) with POS compared with CON.

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} × 6.25 × 0.774)]/[O_2 – (N_{urine} × 6.25 × 0.957)], where CO_2, CO_2 production from oxidation, and N_{urine}, N in urine}.
[2]CON, control; POS, postbiotic.

Oxidation of nutrients

Production of CO_2 is derived from nutrient oxidation and ruminal fermentation. Thus, separation between these two components is necessary to calculate substrate oxidation and the proportion that supports total HP associated with oxidative processes. Diet had no effect on HPx and HPf, but differences ($P < 0.05$) were observed in OXCHO and OXF (Table 5). When expressed relative to HPx, the OXCHO was greater (17% vs. 33%) and OXF lower (73% vs. 56%) with POS than CON diet. The greater OXCHO in POS compared with the CON diet suggested a preference for the use of dietary carbohydrate as a source of fuel, and the opposite for lipids. Because the gas exchange method does not discriminate between oxidation of exogenous and endogenous glucose, the data more closely represented net catabolism of glucose. The low dietary fat content suggested that the greater contribution of OXF with the 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 nonprotein 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 andlipids), but does not consider any metabolic transformation, exchange, or cycling that the substrate itself or its intermediates undergo along the biochemical pathways to complete oxidation (Derno et al., 2013). Because indirect calorimetry does not "see" intermediate metabolic pathways, without the help of internal metabolic biomarkers, it is difficult to explain the lack of differences in RE_{fat} and the differences detected in the $ER_{protein}$. Probably the different approaches could be partly responsible for the discrepancies observed; RE_{body} by the RQ method and REf_{at} and $RE_{protein}$ by the CN method. It is important to keep in mind that the total energy balance (RE_{body}) was positive with both diets (Table 4), and to study it, body retention was separated into fat and protein following the CN method.

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

Table 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/d	2.09	2.49	0.061	<0.001
FCM (4%), kg/d	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
nonfat 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–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.

Milk production and chemical composition

Milk yield was grater (16%; $P < 0.001$) with POS compared with the CON diet (Table 7). When milk yield was expressed as FCM, the response was greater with POS (15%; $P < 0.001$). Feed efficiency expressed as milk yield over DMI was greater (16%; $P < 0.001$) with POS compared with CON. These differences were also observed for FCM/DMI (14%; $P < 0.001$). According to Miettinen and Huhtanen (1996), moderate levels

Table 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.

of concentrates in the diet of dairy cows increase the ratio of ruminal propionic to butyric acid, often increases milk yield, protein, lactose, and decreases milk fat content. The same observation was reported by van Knegsel et al. (2007) when glucogenic and ketogenic diets were compared. Accordingly, in this study, the POS diet increased the ratio propionic to butyric acid (0.77 vs. 0.51 for POS and CON, respectively), milk yield, true protein, and lactose without effects on milk fat. This simple measure of efficiency determines the relative ability of goats to turn feed nutrients into milk because it affects both economic and environmental efficiency; feeding POS increased the milk from every kg of DM consumed and fewer nutrients were excreted in manure.

No differences were observed in milk composition with exception of greater true protein (2.9%; $P < 0.05$) and lactose (4.3%; $P < 0.05$) in POS compared with the CON diet. As Seo et al. (2010) reported, higher populations of *Propionibacterium* spp. in the rumen favored the conversion of lactic acid into propionic acid. Thus, the POS diet might have been associated with greater production and absorption of propionic acid followed by greater production of glucose via gluconeogenesis to support lactose synthesis and greater milk volume. Milk urea and $N–NH_3$ were lower (26% and 25%, respectively; $P < 0.05$) in POS compared with CON. Together with the greater true protein percentage in POS compared with CON, this effect suggests a positive effect on N partitioning to milk due to POS.

In the Mediterranean countries, goat's milk production has traditionally been destined for cheese manufacture. Thus, the physicochemical characteristics and composition of raw milk are essential for the successful development of the dairy goat industry and also, for the marketing of the final products. In Spain, farmers are paid based on two components in the milk; protein plus fat (cheese extract). The cheese extract is the main parameters for farmers, because the price of milk depends on it (milk price per cheese extract was 0.0937 €; consulted 20 August 2022 at Lonja de Albacete, Castilla-La Mancha, www.oviespana.com). No differences in cheese extract were observed in this study (9.4%), and the same price per kg of milk was obtained; 0.92 $/kg of milk. Because greater milk yield was obtained with POS compared with CON, the estimated farmers income would amount to 2.28 or 1.91 $/d per goat, respectively.

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% and 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 nonnutritive 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.

Acknowledgments

This study was supported by PYME-H2020 Project, Spain (ref. 733627 HEALTHSTOCK-2-16), funded by the EU Commission (Brussels, Belgium).

Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

Literature Cited

Aguilar-Toalá, J., R. Garcia-Varela, H. Garcia, V. Mata-Haro, A. González-Córdova, B. Vallejo-Cordoba, and A. Hernández-Mendoza. 2018. Postbiotics: An evolving term within the functional foods field. *Trends Food Sci. Technol*. 75:105–114. doi:10.1016/j.tifs.2018.03.009

Aguilera, J. F., and C. Prieto. 1986. Description and function of an open-circuit respiration plant for pigs and small ruminants and the techniques used to measure energy metabolism. *Arch. Anim. Nutr*. 11:1009–1018. doi:10.1080/17450398609429522

Aguilera, J. F., C. Prieto, and J. Fonollá. 1990. Protein and energy metabolism of lactating Granadina goats. *Br. J. Nutr*. 63:165–175. doi:10.1079/bjn19900104

AOAC International. 2000. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 18th ed. Arlington (VA): Association of Official Analytical Chemists.

ARC. 1980. *Agricultural research Council. The Nutrient Requirements of Ruminants Livestock*. Technical review. Farnham Royal, Wallingford (UK): CAB.

Batey, I. L. 1982. Starch analysis using thermostable alpha-amylases. *Stach/Stärke*. 34:125–128. doi:10.1002/star.19820340407

Bava, L., L. Rapetti, G. M. Crovetto, A. Tamburini, A. Sandrucci, G. Galassi, and G. Succi. 2001. Effects of a NonforageDiet on Milk Production, Energy, and Nitrogen Metabolism in Dairy Goats throughout Lactation J. *Dairy Sci*. 84:2450–2459. doi:10.3168/jds.S0022-0302(01)74695-4

Brockway, J. M., A. W. Boyne, and J. G. Gordon. 1971. Simultaneous calibration of gas analyzers and meters. *J. Appl. Physiol*. 31:296–297. doi:10.1152/jappl.1971.31.2.296

Brouwer, E. 1958. On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat metabolized in ruminants, from data on gaseous exchange and urine N. In: Proceedings of the 1st Symposium on Energy Metabolism. London (UK): EAAP. Publ. 8. Academic Press; p. 182–194.

Brouwer, E. 1965. Report of sub-committee on constants and factors. In: Blaxter, K.L. , editor. Proceedings of the of the 3rd Symposium on Energy Metabolism. London (UK): EAAP. Publ. 11. Academic Press; p. 441–443.

Calsamiglia, S., A. Bach, C. de Blas, C. Fernández, and P. García-Rebollar. 2009. *Nutritional requirements for dairy ruminants*. Madrid (Spain): Fundación Española para el Desarrollo de la Nutrición Animal (FEDNA).

Chida, S., M. Sakamoto, T. Takino, S. Kawamoto, and K. Hagiwara. 2021. Changes in immune system and intestinal bacteria of cows during the transition period. *Vet. Anim. Sci* 14:100222. doi:10.1016/j.vas.2021.100222

Chwalibog, A., A. H. Taunso, and G. Thorbek. 1997. Quantitative oxidation of nutrients in growing calves. *Z. Ernährungswiss*. 36:313–316. doi:10.1007/BF01617806

Cicenia, A., A. Scirocco, M. Carabotti, L. Pallotta, M. Marignani, and C. Severi. 2014. Postbiotic activities of lactobacilli-derived factors. *J. Clin. Gastroenterol*. 48:18–22. doi:10.1097/MCG.0000000000000231

Dawson, K., K. Newman, and J. Boling. 1990. Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. *J Anim. Sci*. 68:3392–3398. doi:10.2527/1990.68103392x

Derno, M., G. Nürnberg, P. Schön, A. Schwarm, H. M. Hammon, C. C. Metges, R. M. Bruckmaier, and B. Kuhla. 2013. Short-term feed intake is regulated by macronutrients oxidation in lactating Holstein cows. *J. Dairy Sci*. 96:971–980. doi: 10.3168/jds.2012-5727

European Union. 2003. *Protection of animals used for experimental purposes. Council Directive 86/609/EEC of 24 November 1986, amended 16.9.2003*. Brussels (Belgium): European Council.

Fahey, G. C., and L. L. Berger. 1988. Carbohydrate nutrition of ruminants. In: Church, D.C., editor. *The Ruminant Animal. Digestive Nutrition and Physiology*. Englewood Cliffs (NJ): Prentice-Hall; p. 269–297.

Fernández, C., M. C. López, and M. Lachica. 2012. Description and function of a mobile open-circuit respirometry system to measure gas exchange in small ruminants. *Anim. Feed Sci. Technol*. 172:242–246. doi:10.1016/j.anifeedsci.2012.01.006

Fernández, C., M. C. López, and M. Lachica. 2015. Low cost open-circuit hood system for measuring gs exchange in small ruminants: from manual to automatic recording. *J. Agri. Sci*. 153:1302–1309. doi:10.1017/S0021859615000416

Fernández, C., J. Gomis-Tena, A. Hernández, and J. Saiz. 2019. An open circuit indirect calorimetry head hood system for measuring methane emissions and energy metabolism in small ruminants. *Animals* 9:380. doi:10.3390/ani9060380

Fernández, C., A. Hernández, J. Gomis-Tena, and J. J. Loor. 2021. Changes in nutrient balance, methane emissions, physiologic biomarkers, and production performance in goats fed different forage to concentrate ratios during lactation. *J. Anim. Sci*. 99:1–13. doi:10.1093/jas/skab114

INRA feeding system for ruminants. 2018. Wageningen (The Netherlands): Wageningen Academic Publishers.

Izuddin, W. I., T. C. Loh, A. A. Samsudin, and H. L. Foo. 2018. In vitro study of postbiotics from Lactobacillus plantarum RG14 on rumen fermentation and microbial population. *Rev. Bras. Zootec*. 47:1–7. doi:10.1590/rbz4720170255

Izuddin, W. I., T. C. Loh, A. A. Samsudin, H. L. Foo, A. M. Humam, and N. Shazali. 2019a. Effect of postbiotic supplementation on growth performance, ruminal fermentation and microbial profile, blood metabolite and GHR, IGF-1 and MCT-1 gene expression in post-weaning lambs. *BMC Vet. Res*. 15:315. doi:10.1186/s12917-019-2064-9

Izuddin, W. I., T. C. Loh, H. L. Foo, A. A. Samsudin, and A. M. Humam. 2019b. Postbiotic Lactobacillus plantarum RG14 improves ruminal epithelium growth, immune status and upregulates the intestinal barrier function in post-weaning lambs. *Sci. Rep*. 9:9938. doi:10.1038/s41598-019-46076-0

Johnson, K. A., and D. E. Johnson. 1995. Methane emissions in cattle. *J. Anim. Sci*. 73:2483–2492. doi:10.2527/1995.7382483x

Jouany, J. P. 1982. Volatile fatty acid and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermentor contents. *Sci. Aliments* 2:131–144.

Judy, J. V., G. C. Bachman, T. M. Brown-Brandl, S. C. Fernando, K. E. Hales, P. S. Miller, R. R. Stowell, and P. J. Knonoff. 2018. Energy balance and diurnal variation in methane production as affected by feeding frequency in Jersey cows in late lactation. *J. Dairy Sci*. 101:10899–10910. doi:10.3168/jds.2018-14596

Kareem, K. Y., F. H. Ling, L. T. Chwen, O. M. Foong, and S. A. Asmara. 2014. Inhibitory activity of postbiotic produced by strains of Lactobacillus plantarum using reconstituted media supplemented with inulin. *Gut Pathog*. 6:23. doi:10.1186/1757-4749-6-23

Knapp, J. R., G. L. Laur, P. A. Vadas, W. P. Weis, and J. M. Tricarico. 2014. *Invited review*: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. *J. Dairy Sci*. 97:3231–3261. doi:10.3168/jds.2013-7234

van Knegsel, A. T. M., H. van den Brand, J. Dijkstra, W. M. Van Straalen, M. J. Heetkamp, S. Tamminga, and B. Kemp. 2007. Dietary energy source in dairy cows in early lactation: Energy partitioning and milk composition. *J. Dairy Sci*. 90:1467–1476. doi:10.3168/jds.S0022-0302(07)71632-6

Lonja de Albacete, Castilla-La Mancha, 2022 www.oviespana.com. Accessed August 20, 2022.

Marteau, P., and F. Shanahan. 2003. Basic aspects and pharmacology of probiotics: an overview of pharmacokinetics, mechanisms of action and side-effects. *Best Pract. Res. Clin. Gastroenterol*. 17:725–740. doi:10.1016/s1521-6918(03)00055-6

Mavrogenis, A. P., and C. Papachristoforou. 1988. Estimation of the energy value of milk and prediction of fat-corrected milk yield in

sheep and goats. *Small Rum. Res*. 1:229–236. doi:10.1016/0921-4488(88)90051-X

McLean, J. A., and G. Tobin. 1987. *Animal and Human Calorimetry*. Cambridge: Cambridge University Press.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing beakers or crucibles: collaborative study. *J. AOAC Int*. 85:1217–1240.

Miettinen, H., and P. Huhtanen. 1996. Effects of the ratio of ruminal propionate to butyrate on milk yield and blood metabolites in dairy cows. *J. Dairy Sci*. 79:851–861. doi:10.3168/jds.S0022-0302(96)76434-2

NRC. 2001. *National Research Council. Nutrient requirements of dairy cattle*. 7th rev. ed. Washington, (DC): Natl. Acad. Press.

Ørskov, E. R., and C. Fraser. 1975. The effects of processing of barley-based supplements on rumen pH, rate of digestion and voluntary intake of dried grass in sheep. *Br. J. Nutr*. 34:493–500. doi:10.1017/s0007114575000530

Oyetayo, V., and F. Oyetayo. 2005. Potential of probiotics as biotherapeutic agents targeting the innate immune system. *Afr. J. Biotechnol*. 4:123–127.

Patra, A., T. Park, M. Kim, and Z. Yu. 2017. Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. *J. Anim. Sci. Biotechnol*. 26:8–13. doi:10.1186/s40104-017-0145-9

R Core Team (2016). *R: A language and environment for statistical computing*. Vienna (Austria): R Foundation for Statistical Computing. URL https://www.R-project.org/.

Seo, J. K., S. W. Kim, M. H. Kim, S. D. Upadhaya, D. K. Kam, and J. K. Ha. 2010. Direct-fed microbials for ruminant animals. *Asian-Australas. J. Anim. Sci*. 23:1657–1667. doi:10.5713/ajas.2010.r.08

Shazali, N., H. L. Foo, T. C. Loh, D. W. Choe, and R. A. Rahim. 2014. Prevalence of antibiotic resistance in lactic acid bacteria isolated from the faeces of broiler chicken in Malaysia. *Gut Pathog*. 6:1. doi:10.1186/1757-4749-6-1

Signorini, M. L., L. P. Soto, M. V. Zbrun, G. J. Sequeira, M. R. Rosmini, and L. S. Frizzo. 2012. Impact of probiotic administration on the health and fecal microbiota of young calves: A meta-analysis of randomized controlled trials of lactic acid bacteria. *Res. Vet. Sci*. 93:250–258. doi:10.1016/j.rvsc.2011.05.001

Thanh, N. T., T. C. Loh, H. L. Foo, M. Hair-Bejo, and B. K. Azhar. 2009. Effects of feeding metabolite combinations produced by Lactobacillus plantarum on growth performance, faecal microbial population, small intestine villus height and faecal volatile fatty acids in broilers. *Br. Poult. Sci*. 50:298–306. doi:10.1080/00071660902873947

Thorsteinsson, M., and M. Vestergaard. 2020. Performance and health of young rosé veal calves supplemented with yeast *(Saccharomyces cerevisiae)* and a postbiotic from *Lactobacillus acidophilus*. *J. Anim. Feed Sci*. 29:115–124. doi:10.22358/jafs/124040/2020

Tovar-Luna, I., R. Puchala, T. Sahlu, H. C. Freetly, and A. L. Goetsch. 2010. Effects of stage of lactation and dietary concentrate level on energy utilization by Alpine dairy goats. *J. Dairy Sci*. 93:4818–4828. doi:10.3168/jds.2010-3315

Williams, N. T. 2010. Probiotics. Adverse effects and safety. *Am. J. Health Syst. Pharm*. 67:449–458. doi:10.2146/ajhp090168