Effect of rice bran as a replacement for oat grain in energy and nitrogen balance, methane emissions, and milk performance of Murciano-Granadina goats

P. Criscioni and C. Fernández¹

Animal Science Department, ACUMA Research Center, Polytechnic University of Valencia, 46022 Valencia, Spain

ABSTRACT

The objective of this experiment was to study the effects of substituting oat grain with rice bran on energy, nitrogen and carbon balance, methane emissions, and milk performance in dairy goats. Ten Murciano-Granadina dairy goats in late lactation (46.1 ± 3.07 kg) were assigned to 2 treatments in a crossover design, where each goat received both treatments in 2 periods. One group of 5 goats was fed a mixed ration with 379 g of oat grain/kg of dry matter (O diet) and the other group of 5 goats was fed a diet that replaced oat grain with 379 g/kg dry matter of rice bran (RB diet). Diets were formulated to be isoenergetic and isoproteic, so bypass fat was added to reach the same amount of energy in both diets. The goats were allocated to individual metabolism cages. After 14 d of adaptation, feed intake, total fecal and urine outputs, and milk yield were recorded daily over a 5-d period. Then, gas exchange measurements were recorded individually by a mobile open-circuit indirect calorimetry system using a head box. Dry matter intake was different for both diets [1.83 ± 0.11 vs. 1.61 ± 0.08 (means $\pm$ SD), for O and RB, respectively]. Metabolizable energy intake and heat production were not significantly different between diets, with average values of 1,254 [standard error of the mean (SEM) = 110.0] and 640 (SEM = 21.0) kJ/kg of BW^{0.75}, respectively. Significant differences were found in milk fat content (5.3 and 6.9%, SEM = 0.36; for O and RB, respectively) and milk fatty acids: medium-chain fatty acids (17.17 vs. 12.90 g/100 g, SEM = 0.969; for O and RB, respectively) and monounsaturated fatty acids (20.63 vs. 28.29 g/100 g, SEM = 1.973; for O and RB, respectively). Enteric CH₄ emission was lower for the RB diet (23.2 vs. 30.1 g/d, SEM = 2.14; for O and RB, respectively), probably because of the higher lipid content in RB diets than O diets (11.7 vs. 4.1%, respectively). Lactating goats utilized RB without detrimental effects on energy me-

tabolism. Higher milk fat and lower CH₄ emissions were observed with the RB diet compared with the O diet.

Key words: lactating goat, rice bran, energy balance, methane emissions

INTRODUCTION

Most of the concentrates for dairy ruminants are based on cereals. Cereal grains are the most common sources of readily available energy for livestock and comprise up to 60% of the total diet for high-yielding dairy ruminants. On the other hand, given the ability of ruminal microorganism to degrade fiber, some byproducts of other agricultural and industrial process are used to replace cereal (starch is replaced with highly digestible fibers as a main source of energy). One such byproduct is rice bran, with an estimated world production of 50 million tonnes (FAOSTAT, 2014).

Rice bran is obtained from the grain milling process, representing 5 to 8% of the total grain. Chemical analysis of rice bran varies widely, containing 11 to 14% CP, 16 to 21% NDF, and 21 to 28% of starch and minerals such as iron (0.015%), phosphorus (1.35%), and magnesium (0.80%). Rice bran differs from other byproducts in having higher levels of ether extract (**EE**), at 12 to 18%. The main fatty acids in rice bran oil are palmitic (21–26%), linoleic (31–33%), and oleic (37–42%) acids (Warren and Farrell, 1990; Oliveira et al., 2011).

Rice byproducts (rice straw, rice bran, heat rice bran, defatted rice bran) have been studied in cattle, sheep, and steers (Forster et al., 1994; Cao et al., 2010; Zhao et al., 1996). The effects of including rice bran in mixed diets upon intake, digestibility, energy, N and C balance, and milk performance of lactating goats have not been well investigated. Therefore, our aim was compare 2 mixed diets containing the same forage and replacing oat grain with rice bran in the concentrate.

MATERIALS AND METHODS

The experimental procedures were approved by the Committee on Animal Use and Care at the Polytechnic University of Valencia (Valencia, Spain) and follow

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¹Corresponding author: cjfernandez@dca.upv.es

Table 1. Ingredients and chemical composition (means $\pm$ SD) of the mixed diets

Item ¹	Oat (O)	Rice bran (RB)	Diet ²	
			O	RB
Ingredients, g/kg of DM				
Alfalfa hay			350	350
Oats	1,000		379	
Rice bran		1,000		379
Barley			182	197
Soy meal (44% CP)			64	29
Bypass fat			15	33
Calcium carbonate			3.3	3.3
Sodium chloride			1.2	4.1
Premix			5.4	5.4
Chemical composition, % of DM				
DM	90.0 ± 5.4	89.7 ± 3.6	88.8	89.1
Ash	2.9 ± 0.2	8.2 ± 0.4	7.9	9.7
OM			92.1	90.3
CP	8.4 ± 0.4	13.8 ± 0.8	15.3	16.1
Ether extract	4.9 ± 0.3	13.9 ± 0.8	4.1	11.7
NDF	31.2 ± 1.9	17.9 ± 0.9	27.2	22.8
ADF	17.1 ± 0.5	9.1 ± 0.4	12.4	10.4
NFC ²			45.5	39.8
Starch	37.0 ± 1.9	27.1 ± 1.4	28.2	21.6
Carbon			43.5	44.6
Gross energy, MJ/kg of DM			17.9	19.4

¹Bypass fat of palm fatty acid distillate (Norel Animal Nutrition, Norel S.A., Spain). Premix (NACCOOP S.A., Madrid, Spain) composition (per kg of premix): Se, 40 mg; I, 250 mg; Co, 80 mg; Cu, 3,000 mg; Fe, 6,000 mg; Zn, 23,400 mg; Mn, 29,000 mg; S, 60,000 mg; Mg, 60,000 mg; vitamin A, 2,000,000 IU; vitamin D₃, 400,000 IU; vitamin E, 2,000 mg; nicotinic acid, 10,000 mg; choline, 20,300 mg.

²NFC = 100 - (NDF + ash + CP + ether extract).

the codes of practice for animals used in experimental works proposed by the European Union (2003).

Animals and Diets

The experiment was conducted at the Animal Science Department Experimental Farm (ACUMA Research Center, Valencia, Spain). Ten multiparous mature Murciano-Granadina dairy goats in late lactation were selected and divided into 2 homogeneous groups of 5 goats based on similar BW (46.1 ± 3.07 kg of BW), milk production in previous lactations (661.5 ± 44 kg of milk per 210 $\pm$ 30 d of lactation, on average), and milk yield at the beginning of the experiment ($2,375 \pm 375.1$ g of milk per day, on average), in a crossover design (2 treatments crossed with 2 periods). Treatments consisted of 2 different mixed rations (Table 1). Goats were fed daily with 0.800 kg of alfalfa hay and 1.5 kg of concentrate (the forage:concentrate ratio was 35:65, expressed as percentage). The concentrate and premix were mixed and pelleted. One group was fed concentrate with 379 g/kg of DM of oat grain (**O** diet) and the other with rice bran (**RB** diet). Nutrient requirements followed the recommendations of Lachica and Aguilera (2003) and Calsamiglia et al. (2009) for goats in lactation. In an attempt to feed isoenergetic diets, bypass fat was added to each diet; that is, diets

were formulated on an energy basis using book values for chemical composition of ingredients (FEDNA, 2010). The chemical composition of oats, rice bran, and the whole mixed diet (forage and pelleted concentrate) is reported in Table 1. After feed manufacturing and chemical analyses of diets, we found that the diets were not isoenergetic and that the RB diet had greater gross energy value. Half of the daily ration was offered at 0800 h and half at 1600 h. Goats had free access to water.

Experimental Schedule and Measurements

Apparent total-tract digestibility, gas exchange, energy partitioning, C and N balance, oxidation of nutrients, and milk composition and yield were determined. The experiment was conducted in a crossover design in two 30-d periods. During the adaptation period, goats were fed the experimental diets in pens for 7 d and then allocated to individual metabolism cages at thermoneutrality (20–23°C determined by Hobo data loggers, Onset Corp., Cape Cod, MA) for another 7 d. Next, data on amounts of feed offered and refused and total fecal, urine, and milk outputs were recorded daily for each goat during a 5-d period; BW was recorded at the beginning and end of the period. Feces were collected in wire-screen baskets placed under the floor of

the metabolism crates, and urine was collected through a funnel into plastic buckets containing 100 mL of 10% (vol/vol) H_2SO_4 to acidify the urine of each goat. The acidification of urine was necessary to prevent microbial degradation and loss of volatile ammonia-N ($\text{NH}_3\text{-N}$). Representative samples (10%) of diet, feces, and urine were collected over 5 consecutive days, stored at -20°C , and pooled for chemical analysis. The 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, the individual milk yield was measured and a 10% sample was placed in a bottle and frozen until analysis. In addition, samples were collected into plastic vials 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 compositional analysis (DM, CP, fat, and lactose). Ruminal fluid samples were collected by stomach tube before the morning feeding on the last day of the apparent digestibility trial. Ruminal fluid pH was immediately determined using a portable pH meter (model 265A, Orion Research Inc., Beverly, MA). A ruminal fluid sample was acidified with 50% H_2SO_4 and frozen until later determination of $\text{NH}_3\text{-N}$. Samples for analysis of VFA were mixed with H_3PO_4 and kept frozen until analysis.

Gas exchange was measured for each goat during 24 h (5 goats/treatment) by an indirect calorimetric system based on a ventilated head-box designed for small ruminants. To this end, therefore, each digestibility-balance period include 10 days of gas exchange determinations in the incomplete crossover design. The respirometry system was equipped with a head hood, a flow meter (Thermal Mass Flowmeter Sensyflow VT-S, ABB, Alzenau, Germany), and air suction provided by a centrifugal fan (CST60 Soler Palau Inc., Parets del Vallès, Barcelona, Spain). The concentrations of CH_4 and CO_2 were measured using the infrared principle, and O_2 was measured by the paramagnetic principle (Easyflow Gas Analyzer, model 3020, ABB, Alzenau, Germany). Although the unit was an autocalibrated model, the analyzers were calibrated with reference gases before each test. Fernández et al. (2012, 2015) described the mobile open-circuit respirometry system used for these measurements. The whole system was calibrated by injecting pure N_2 and CO_2 into the head box (McLean and Tobin, 1987), and the calibration factor of N_2 and CO_2 was determined gravimetrically using a precision scale (mini-SP 0.2–30 kg, Industrial Weighing System, Mobba, Barcelona, Spain). Calibration factors were calculated according to Brockway et al. (1971). Production of CH_4 and CO_2 and consumption of O_2 were calculated as described by Aguilera and

Prieto (1986). An initial atmospheric air sample was collected and the gas concentrations were used as reference for calculations.

Chemical Analysis

Samples of feed, feed refusals, and feces were first dried in a forced-air oven at 55°C for 48 h and then ground to pass a 1-mm screen before analysis. Urine and milk were dried by lyophilization. Chemical analyses of the diet, refusals, and feces were conducted according to AOAC International (2000) for DM (method 934.01), ash (method 942.05), and EE (method 920.39). The DM of diets and feces was determined by oven-drying at $102 \pm 2^\circ\text{C}$ for 24 h. Ash concentration was measured following incineration in an electric muffle furnace at 550°C for 6 h. Ether extract was extracted with petroleum ether after acid hydrolysis to recover saponified fat (Soxtec 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) according to Mertens (2002) and AOAC International (2000), respectively. The NDF was determined using sodium sulfite and α amylase. The NFC content of diets was calculated by difference method based on chemical analysis of individual feeds according to NRC (2001): $\text{NFC} = 100 - \text{NDF} - \text{ash} - \text{CP} - \text{EE}$. The gross energy (GE) content of the dried samples (feed, feces, urine, and milk) was analyzed by combustion in an adiabatic bomb calorimeter (Autobomb; Gallenkamp, Loughborough, UK). Starch content was determined by enzymatic method (α -amylase obtained from Sigma-Aldrich, Steinheim, Germany) according to Batey (1982). Carbon and nitrogen were analyzed according to the Dumas principle (TruSpec CN; Leco Corporation, St. Joseph, MI). Multiplying N by a factor of 6.25 converted the results to CP.

Milk composition (fat, protein, lactose, citrate, and total milk solids content) was analyzed with an infrared analyzer (MilkoScan FT120, Foss Electric, Hillerød, Denmark). Fatty acid methyl esters of total milk lipids were prepared directly as previously described O'Fallon et al. (2007). The FAME were analyzed in a Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless injector and a flame-ionization detector. Separation of FAME was performed in a fused silica capillary column SP 2560 (Supelco, Bellefonte, PA; $100 \text{ m} \times 0.25 \text{ mm} \times 0.2 \mu\text{m}$ film thickness). The carrier gas was helium at a linear velocity of 20 cm/s. The samples were injected with a split ratio of 1/100. The initial oven temperature was set at 140°C held for 5 min, increased to 240°C at $4^\circ\text{C}/\text{min}$, and finally maintained at that temperature for 30 min. Both detector and injector

temperatures were set at 260°C. A subset of milk samples was collected and analyzed for BHB, glucose, uric acid, and milk urea. Milk BHB was analyzed using the enzymatic oxidation of the metabolite, and a coupled reaction was determined by fluorometry (Larsen and Nielsen, 2005). Milk urea acid was analyzed following procedures described by Larsen and Moyes (2010). Milk urea was analyzed using flow injection analyses following the manufacturer instructions (Foss Tecator AB, Höganäs, Sweden). Glucose was determined according to standard procedures (Siemens Diagnostics Clinical Methods for Advia 1650) using an autoanalyzer (Advia 1650 Chemistry system, Siemens Medical Solutions, Tarrytown, NY).

The $\text{NH}_3\text{-N}$ content of ruminal fluid samples was analyzed by the Kjeldahl procedure (2300 Kjeltac Analyzer Unit, Foss Tecator, Hillerød, Denmark). Determination of ruminal VFA was based on the method described by Jouany (1982) using a gas chromatograph (Fisons 8000 series; Fisons Instruments SpA, Milan, Italy) equipped with a split/splitless injector and flame-ionization detector.

Calculations

Metabolizable energy intake (**MEI**) was calculated as the difference between GE intake and energy losses in feces, urine and CH_4 (with an energy equivalent value of 39.5 kJ/L of 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):

$$\text{HP (kJ)} = 16.18 \times \text{O}_2 + 5.02 \times \text{CO}_2 - 2.17 \times \text{CH}_4 - 5.99 \times \text{N}_{\text{urine}}$$

where gases were expressed in liters per day and N_{urine} in grams per day. Body tissue energy (**RE_{body}**) was calculated as $\text{MEI} - \text{HP} - \text{milk energy (E}_{\text{milk}})$.

The energy associated with the oxidation of protein (**OXp**), carbohydrate (**OXCHO**), and fat (**OXF**) was calculated by the method of Brouwer (1958) and Chwalibog et al. (1997) for ruminants. The CO_2 production from oxidation (**CO_{2x}**) was calculated as $\text{CO}_2 - (2 \times \text{CH}_4)$, according to Fahey and Berger (1988). The calculations were carried out as follows:

$$\text{OXp} = 6.25 \times \text{N}_{\text{urine}} \times 18.42 \text{ (kJ/g)},$$

$$\text{OXCHO} = (-2.968 \times \text{O}_2 + 4.174 \times \text{CO}_{2x} - 2.446 \times \text{N}_{\text{urine}}) \times 17.58 \text{ (kJ/g)},$$

$$\text{OXF} = (1.719 \times \text{O}_2 - 1.719 \times \text{CO}_{2x} - 1.963 \times \text{N}_{\text{urine}}) \times 39.76 \text{ (kJ/g)}.$$

Then, the HP from oxidation (**HP_x**) was

$$\text{HP}_x \text{ (kJ)} = 16.18 \times \text{O}_2 + 5.02 \times \text{CO}_{2x} - 5.99 \times \text{N}_{\text{urine}}.$$

Again, gases were expressed in liters per day and N_{urine} in grams per day. Heat of fermentation (**HP_f**) was estimated by subtracting HP from HP_x . The nonprotein respiratory quotient from oxidation of nutrients (**RQ_{npx}**) was determined as follows: $\text{RQ}_{\text{npx}} = [\text{CO}_{2x} - (\text{N}_{\text{urine}} \times 6.25 \times 0.774)] / [\text{O}_2 - (\text{N}_{\text{urine}} \times 6.25 \times 0.957)]$. For C and N balance, we followed the equations and values proposed by McLean and Tobin (1987), and the grams retained in protein (**R_{protein}**) and fat (**R_{fat}**) were calculated.

The efficiency of use of ME for lactation was calculated according to AFRC (1993). 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 energy storage during lactation was taken to be 0.95 times the milk secretion efficiency. Consequently, the corrected milk energy was estimated as $\text{E}_{\text{milk}} + (0.84 \times \text{negative energy retention}) + (1.05 \times \text{positive energy retention})$. The efficiency of use of ME for milk production (**k_l**) was calculated as corrected milk energy / ($\text{ME} - \text{ME}_m$), with ME_m being the metabolizable energy for maintenance, which was obtained from the estimation of Aguilera et al. (1990) for Granadina goats from both positive and negative energy retentions (401 kJ/kg of $\text{BW}^{0.75}$). Net energy for lactation (**NE_L**) was computed as $\text{MEI} \times k_l$.

Statistical Analysis

The effects of substituting oat with rice bran on intake, digestibility, ruminal fermentation, milk performance, energy and C and N balances, and oxidation of nutrients were analyzed using the PROC MIXED of SAS Institute (2001). The experiment was conducted as a crossover design: each goat received both treatments in 2 periods; goat served as the experimental unit for all data. The model for the dependent variables included the fixed effect of diet and period, with goat as random effect. The following statistical model was used: $Y = \mu + D + T + \text{goat} + \varepsilon$, where Y is the dependent variable; μ is the overall mean; D and T are the fixed effects of diet and period of time, respectively; goat is the random effect of goat; and ε is the random error.

Least squares means are reported throughout and differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

No significant effect was observed for period in the crossover design. The average value for the calibration factor was 1.0055 ± 0.00139 ($n = 5$) and 0.9936 ± 0.00953 ($n = 5$) for O_2 and CO_2 , respectively.

Feed Intake, Digestibility, and Rumen Fermentation

The mixed diets were formulated based on book values of FEDNA (2010) to reach isoenergetic and isoproteic balance (average values of 15.7% of CP on DM basis). However, after feed manufacturing, samples of each diet were analyzed in the laboratory and we determined that the diets were not isoenergetic and the RB diet presented greater gross energy values (17.9 ± 0.89 vs. 19.4 ± 1.17 MJ/kg of DM in O and RB diets, respectively). The main difference among diets was the source of carbohydrate in oat grain and rice bran, although RB diet had twice the amount of bypass fat as the O diet. Rice bran has a large proportion of broken rice (starch), oil, and protected protein (Elliot et al., 1978). The chemical composition (Table 1) was not equal, and NDF, ADF, NFC, and starch were lower in RB than in O; ether extract was greater in RB than O (11.7% vs. 4.1%, respectively).

Intake and total-tract apparent digestibility of nutrients by dairy goats during late lactation are shown in Table 2. Dry matter intake differed ($P < 0.05$) between diets (1.83 ± 0.11 vs. 1.61 ± 0.08 kg/d, for O and RB respectively), possibly because the animals' intake was regulated by energy requirements, so less of the richest diet was eaten. Also, the high content of fat on diet RB reduced DMI, as has been indicated by other authors supplementing rice bran oil in dairy cows because reduced gut motility (Lunsin et al., 2012). No differences were found for DM apparent digestibility with a value for the RB diet of 73.9% (SEM = 1.57), similar to the rice bran value of 70% found by Van Soest (2006). Apparent digestibility coefficients of fiber were higher ($P < 0.05$) in RB than in O (48.7 vs. 34.7%, SEM = 4.42; for NDF, and 48.1 vs. 35.0%, SEM = 4.16; for ADF, respectively). High fat content in the diet decreases OM and fiber degradability and reduces fermentable substrate. It is expected that high levels of fat would inhibit fiber digestion, possibly by coating food particles and preventing bacterial attachment (Palmquist and Jenkins, 1980). In the present work, the RB diet showed increased fiber digestibility even though it had 11.7% fat, and could be related to the lower amount of fiber and DMI in the RB diet than the

Table 2. Body weight, intake, and apparent digestibility coefficients of Murciano-Granadina goats ($n = 10$) during late lactation according to the type of diet

Item	Diet		<i>P</i> -value	
	Oats	Rice bran	SEM	Diet
BW, kg	45.8	46.4	1.35	0.84
DMI, kg/d	1.83	1.61	0.112	0.047
Digestibility, % of DM				
DM	70.4	73.9	1.57	0.30
OM	72.2	76.3	1.52	0.19
CP	71.3	72.6	1.45	0.70
Ether extract	67.2	79.8	3.68	0.09
NDF	34.7	48.7	4.42	0.12
ADF	35.0	48.1	4.16	0.12
NFC	95.0	94.3	0.31	0.36
Starch	98.1	97.1	0.26	0.06
Gross energy	72.8	77.1	1.53	0.19

O diet (see Tables 1 and 2, respectively). Zhao et al. (1996) reported, in steers, that the addition of fat to high-concentrate diets containing 12% ADF could have shifted the site of OM digestion from the rumen to the intestine. Higher digestibility ($P < 0.05$) was found in RB than in O for fat (79.8 vs. 67.2%, SEM = 3.68; respectively) and the digestibility of starch was almost complete.

Results from rumen liquor samples are shown in Table 3. The average rumen pH never decreased below 6.2, so the values obtained can be considered sufficiently high to maintain normal rumen fermentation (Ørskov and Fraser, 1975). Goats fed O (slightly greater amount of structural and nonstructural carbohydrates and lower fat than RB) had a higher pH ($P < 0.05$) and a lower NH_3 -N ($P < 0.05$) concentration compared with goats fed RB (15.8 vs. 23.3 mg/dL, SEM = 2.00; for O and RB, respectively). No effect of treatment on VFA was observed, and differences in NH_3 -N concentration might suggest partial inhibition of microbial synthesis by the rice bran fat. The ruminal NH_3 -N and VFA values from our study are within the range of those found in other studies with goats fed byproducts (Romero-Huelva et al., 2012). Therefore, the lower NH_3 -N results for diet O compared with RB suggest more fermentation activity. We recognize that the starch in rice bran escapes rumen fermentation almost entirely and also drives fat and bypass protein to the duodenum (Elliot et al., 1978).

Energy Balance

Daily energy balance obtained with the 2 diets is listed in Table 4. No statistically significant differences were observed for GE intake ($1,833 \pm 137.1$ kJ/kg of $BW^{0.75}$, on average) and the rest of energy partition, with exception of feces and CH_4 . Energy losses in feces were higher ($P < 0.05$) for O than RB, probably associ-

Table 3. pH, ammonia-N (NH₃-N), and VFA of Murciano-Granadina goats (n = 10) during late lactation according to the type of diet

Item	Diet		SEM	P-value
	Oats	Rice bran		
pH	7.3	7.0	0.08	0.03
NH ₃ -N, mg/dL	15.8	23.3	2.00	0.05
Total VFA, mmol/L	21.5	19.6	1.83	0.64
Individual VFA, mol/100 mol				
Acetic acid	61.6	56.7	2.11	0.28
Propionic acid	15.0	19.1	1.47	0.18
Isobutyric acid	3.3	3.0	0.48	0.85
Butyric acid	13.0	14.0	0.85	0.63
Isovaleric acid	3.7	3.4	0.44	0.78
<i>n</i> -Valeric acid	2.3	2.5	0.39	0.84
<i>n</i> -Caproic acid	0.8	0.8	0.25	0.90
Heptanoic acid	0.5	0.5	0.18	0.92

ated with greater DMI. Urine energy losses were not significantly different between treatments. The O diet presented significant differences ($P < 0.05$) on energy losses in CH₄ (95 kJ/kg of BW^{0.75}) compared with the RB diet (73 kJ/kg of BW^{0.75}), indicating that increasing the level of lipids in diet reduced CH₄ production, as indicated by different authors and reviewed by Knapp et al. (2014). No differences between treatments were observed for MEI (1,254 kJ/kg of BW^{0.75}, on average; SEM = 110.0). The HP values from our study ($P = 0.63$; 640 kJ/kg of BW^{0.75}, on average; SEM = 21.0) were within the range of some literature values; for example, Bava et al. (2001) found an average value of 642 kJ/kg of BW^{0.75} for Saanen goats fed mixed diets in late lactation. Tovar-Luna et al. (2010) fed Alpine goats during late lactation with 60% concentrate and found values of 680 kJ/kg of BW^{0.75}. The tissue energy recovered in the body was positive and did not differ between diets (107 kJ of RE_{body}/kg of BW^{0.75}, SEM = 68.4).

No significant differences were observed between diets for k_l (0.72 on average). The value obtained in the present work was similar to that reported by Bava et al. (2001) in Saanen goats (0.73). Aguilera et al. (1990) and Tovar-Luna et al. (2010) found lower values (0.67 and 0.63, respectively). The increase in k_l with increasing dietary fat is well established in the literature and has traditionally been attributed to a decrease in the heat increment of feeding and the relatively low energy cost of the transfer of absorbed fatty acids to milk fat compared with the cost of novo synthesis of fatty acids (Moraes et al., 2015). Furthermore, in our study, we assumed a value of 401 kJ/kg of BW^{0.75} for MEm, but animals in late lactation have lower production and lower metabolic activity of visceral organs for maintenance (i.e., lower MEm) than animals in earlier lactation stages. These factors likely explain the high

k_l obtained in our experiment. Net energy for lactation was expressed as megajoules per kilogram of DM and significant values of 8.4 ± 0.42 and 10.2 ± 0.41 were found in O and RB mixed diets, respectively.

Oxidation of Nutrients

The proportional contribution to HP_x due to oxidation of nutrients is shown in Table 5. Diet had no significant effect on HP_x (623 kJ/kg of BW^{0.75}, on average; SEM = 20.2). Numerically lower HP_f was found in RB than O, probably linked to the higher fat content in the RB diet (11.7%), as we mentioned previously.

The oxidation of nutrients as OXCHO increased ($P < 0.05$) from 15 to 33% in diets RB and O, respectively (SEM = 4.5). Consequently, OXF decreased ($P < 0.05$) from 64 to 48% in RB and O, respectively (SEM = 4.3). A significant difference ($P < 0.05$) was observed for RQnpx, which was significantly lower for RB (0.77) than for O (0.83) with an SEM of 0.015. Chwalibog et al. (1997) reported that an RQnpx < 1 indicates predominance of OXF versus OXCHO, and under positive energy balance, part of the OXF should originate from ingested carbohydrate (mainly fiber) and diet fat content.

Carbon and Nitrogen Balance

The daily C and N balance and the calculated tissue recovered as protein and fat are given in Table 6. No significant differences were observed in C intake, feces, or urine. Following the trend observed for methane energy, the C losses in CH₄ were significantly lower with RB than with O (1.3 vs. 1.7 g/kg of BW^{0.75}, respectively; SEM = 0.14) because of the lower CH₄ production

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

Item ¹	Diet		SEM	P-value
	Oats	Rice bran		
GEI	1,889	1,777	137.1	0.71
E _{feces}	504	396	35.8	0.04
E _{urine}	41	50	4.9	0.41
E _{methane}	95	73	7.6	0.04
MEI	1,248	1,259	110.0	0.97
HP	651	628	21.0	0.62
E _{milk}	491	524	36.0	0.67
RE _{body}	107	106	68.4	1.00

¹GEI = gross energy intake; E_{feces}, E_{urine}, and E_{methane} = energy losses in feces, urine, and methane, respectively; MEI = metabolizable energy intake; HP = heat production; RE_{total} = total recovered energy; E_{milk} = recovered energy in milk; RE_{body} = recovered energy in tissue (RE_{body} = MEI - HP - E_{milk}).

Table 5. Heat production (kJ/kg of BW^{0.75}) from oxidation and fermentation; daily oxidation (kJ/kg of BW^{0.75}) of protein, carbohydrate, and fat; and their contribution to the heat production from oxidation substrates (%) of Murciano-Granadina goats (n = 10) during late lactation according to the type of diet

Item ¹	Diet		SEM	P-value
	Oats	Rice bran		
HPx	632	614	20.2	0.68
HPf	19	15	1.5	0.16
OXp	119	126	6.2	0.62
OXCHO	211	100	29.6	0.05
OXF	302	388	24.1	0.07
OXp/HPx	19	21	1.2	0.49
OXCHO/HPx	33	15	4.5	0.04
OXF/HPx	48	64	4.3	0.06
RQnpx	0.83	0.77	0.015	0.04

¹HPx = 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 $CO_2x = CO_2$ production from oxidation and $N_{urine} = N$ in urine}.

with this diet. The C secreted into the milk was not significantly affected by treatment and the retention of C was positive (1.5 g/kg of BW^{0.75}, on average; SEM = 0.58). The efficiency of milk C output regards to C ingested was 25%, on average.

Goats of the 2 groups ingested similar amounts of N (2.6 g/kg of BW^{0.75}, on average; SEM = 0.18); Table 6 indicates a numerically lower intake in RB compared

Table 6. 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

Item ¹	Diet		SEM	P-value
	Oats	Rice bran		
C _{intake}	45.7	40.8	3.26	0.49
C _{feces}	14.0	10.6	1.09	0.13
C _{urine}	1.3	1.5	0.08	0.18
C _{CO2}	16.4	14.8	0.66	0.25
C _{CH4}	1.7	1.3	0.14	0.04
C _{milk}	10.6	11.2	0.76	0.73
C _{retained body}	1.7	1.4	0.58	0.93
N _{intake}	2.6	2.4	0.18	0.58
N _{feces}	0.7	0.7	0.06	0.61
N _{urine}	1.0	1.1	0.05	0.62
N _{milk}	0.8	0.8	0.04	0.75
N _{retained body}	0.0	–0.2	0.10	0.38
R _{protein}	0.2	–1.0	0.44	0.41
R _{fat}	2.0	2.4	0.33	0.88

¹C_{intake} = C intake; C_{feces} = C losses in feces; C_{urine} = C losses in urine; C_{CO2} = C losses in CO₂; C_{CH4} = C losses in methane; C_{milk} = recovered C in milk; C_{retained body} = recovered C in tissue; N_{intake} = N intake; N_{feces} = N losses in feces; N_{urine} = N in urine; N_{milk} = recovered N in milk; N_{retained body} = recovered N in tissue; R_{protein} = retained protein; R_{fat} = retained fat.

with O. No differences were found in N losses in feces, urine, milk, and N retention. The ratio between milk N output and N ingested had an average value of 0.31. We observed N retention around zero, and slightly negative N balance for RB (0.0 vs. –0.2 g/kg of BW^{0.75}; SEM = 0.10). Although some authors (Kebreab et al., 2010) report a reduction in urinary N output in dairy cows when ME intake increases, no differences were observed in our study in ME intake and urinary N, despite the lower DMI observed with the RB diet. The negative N balance detected with RB is probably linked to a larger amount of NH₃-N observed in the ruminal liquid, which would indicate an inefficient use of ruminal NH₃-N for ruminal proteosynthesis (Casper et al., 1999) due to fat interference in microbial activity (Palmquist and Jenkins, 1980).

The values of N retained in the body were converted to grams of protein, and from this and the C balance value, grams of retention of fat were calculated. No differences were found between diets. As shown in Table 4, energy balance was positive, so R_{fat} was also positive (2.0 vs. 2.4 g/kg of BW^{0.75}, for O and RB respectively; SEM = 0.33) and hence no fat mobilization was observed. The negative R_{protein} in diet RB (–1.0 g/kg of BW^{0.75}) was shown previously (Van Kneegsel et al., 2007).

Milk Production, Fatty Acids, and Metabolites

Table 7 reports milk yield and milk metabolites of goats during the experiment. Diet had no effect on milk yield; average milk yield was 2,196.4 ± 125.0 g/d. Chilliard et al. (2003) observed that fat supplementation increases milk yield in dairy cows, but not in goats, and increases milk fat content in goats, but not always in dairy cows. The replacement of oat (cereal grain with high fiber content) with rice bran (fibrous byproduct with less fiber content than oat) made the O diet greater in fiber compared with the RB diet. Increased milk fat content is common when dietary fiber concentrations increase at the expense of starch, but in our study, milk fat content was high in RB compared with O. The added bypass fat might be responsible for the significant differences ($P < 0.01$) in milk DM (14.9 and 16.4%, for O and RB, respectively; SEM = 0.39) and milk fat content (5.3 and 6.9%, for O and RB, respectively; SEM = 0.36). Further, in all studies reviewed by Chilliard et al. (2003), milk fat content increased with almost all studied fat supplements in goats but not in cows because their lipolytic systems differ. Milk protein and lactose contents were not statistically different among treatments [4.0%, SEM = 0.12, and 4.8%, SEM = 0.08, on average, for protein and lactose contents, respectively]. Again, we observe that fat supplementa-

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

Item	Diet		SEM	<i>P</i> -value
	Oats	Rice bran		Diet
Milk production, g/d	2,244.5	2,148.2	125.03	0.729
Composition, %				
DM	14.9	16.4	0.39	0.04
Fat	5.3	6.9	0.36	0.01
Protein	4.0	4.1	0.12	0.91
Lactose	4.9	4.7	0.08	0.30
Milk metabolites				
BHB, $\mu\text{mol/L}$	55.3	54.0	2.92	0.83
Glucose, mmol/L	0.17	0.13	0.010	0.05
Glucose-6-phosphate, mmol/L	0.14	0.10	0.010	0.02
Isocitrate, mmol/L	0.08	0.08	0.004	0.38
Uric acid, $\mu\text{mol/L}$	40.5	58.4	7.64	0.27
Milk urea, mmol/L	2.8	2.4	0.12	0.06

tion decreases milk protein content in dairy cows but not in goats.

Analysis of milk metabolites indicated that the 2 diets provided goats with enough energy for their production levels; consequently, the values of the energy indicators [BHB and citrate, with average values of 54.7 mmol/L (SEM = 2.92) and 0.08 mmol/L (SEM = 0.004), respectively] were in the normal range, consistent with those obtained by others authors (Bjerre-Harpøth et al., 2012). Glucose is the main precursor for lactose synthesis in the mammary gland (Linzell, 1968), and a higher ($P < 0.05$) value was found for O than for RB (0.17 vs. 0.13 mmol/L , respectively; SEM = 0.010). Palmquist and Jenkins (1980) reported reductions in blood glucose and insulin when protected lipids replaced concentrate. No differences were observed in the potential biomarkers in milk or indicators of rumen N flow, such as uric acid and urea in milk [49.6 $\mu\text{mol/L}$ (SEM = 7.64) and 2.6 mmol/L (SEM = 0.12) on average, respectively].

Effects of diet on the fatty acid profile of milk fat are shown in Table 8. Compared with cow milk, goat milk is higher in medium-chain fatty acids (caprylic acid and, especially, capric acid). Medium-chain fatty acids contents were higher ($P < 0.05$) with the O diet compared with the RB diet: capric acid, undecanoic acid, myristic acid, myristoleic acid, and pentadecanoic acid. Undecanoic acid and pentadecanoic acid are potential biomarkers of rumen function because they are found in rumen bacterial lipids and might be partially synthesized endogenously from rumen substrates in the mammary gland (Vlaeminck et al., 2006, 2015; Fievez et al., 2012). The differences between treatments (lower contents of those fatty acids in the milk of RB goats) suggest a negative effect of RB oil on rumen bacterial metabolism, both de novo synthesis of bacterial lipids

and fermentative activity. Our ammonia-N results in rumen liquid were in accordance with the differences observed in these fatty acids. Higher values ($P < 0.05$) were found for oleic acid and arachidic acid in RB compared with O. Thus, significant differences were found in medium-chain fatty acids (17.17 vs. 12.90 g/100 g for O and RB, respectively; SEM = 0.969) and for MUFA (20.63 vs. 28.29 g/100 g for O and RB, respectively; SEM = 1.973). No significant differences were found in CLA between diets. Hence, milk from goats fed the RB diet was the highest in MUFA, and goats fed the O diet had milk with more medium-chain fatty acids. Fatty acids with 16 or fewer carbon atoms derive from de novo synthesis, whereas those with 18 or more carbons atoms come from the diet or from lipid mobilization (Chilliard et al., 2003). In the present experiment, under positive energy balance, recovered energy in tissue was positive (Table 4) and retained fat was positive (Table 6). The atherogenicity index (calculated as $\text{C12:0} + 4 \times \text{C14:0} + \text{C16:0}/\text{UFA}$) was calculated as according to Ulbricht and Southgate (1991). The milk of goats fed the RB diet had a lower atherogenicity index than that of goats fed the O diet (2.17 vs. 3.50 on average, respectively; SEM = 0.248).

Methane Emissions

The effects of diet on CH_4 emissions are shown in Table 9. Goats fed the RB diet produced significantly ($P < 0.05$) less CH_4 (23.2 g/d) than those on the O diet (30.1 g/d; SEM = 2.14). The inhibition of CH_4 production is normally accompanied by an increase in propionate production, which uses hydrogen and lactic acid. Cao et al. (2010) fed sheep fermented (high in lactic acid) and nonfermented TMR containing whole-crop rice and rice bran and although the fermented mixed

Table 8. Fatty acid composition (g/100 g of identified fatty acids) of milk fat for Murciano-Granadina goats (n = 10) during late lactation according to the type of diet

Item	Diet		SEM	P-value
	Oats	Rice bran		
C4:0	0.33	0.34	0.023	0.893
C6:0	1.06	0.97	0.072	0.570
C8:0	1.99	1.68	0.139	0.297
C10:0	9.61	6.81	0.680	0.027
C11:0	0.30	0.21	0.021	0.034
C12:0	4.20	3.23	0.593	0.452
C14:0	10.57	7.76	0.597	0.006
C14:1	0.17	0.12	0.010	0.004
C15:0	0.11	0.08	0.008	0.002
C16:0	37.81	34.63	1.866	0.434
C16:1	0.92	0.91	0.050	0.948
C17:0	0.53	0.48	0.053	0.677
C17:1	0.21	0.23	0.035	0.738
C18:0	9.70	14.25	1.278	0.072
C18:1n-9 <i>trans</i>	1.40	0.27	0.316	0.070
C18:1n-9 <i>cis</i>	19.38	27.15	1.957	0.037
C18:1n-7	0.37	0.35	0.031	0.810
C18:2n-6 <i>cis</i>	3.21	3.30	0.189	0.815
C20:0	0.18	0.30	0.027	0.014
C18:3n-6	0.01	0.00	0.004	0.731
C20:1	0.15	0.11	0.017	0.176
C18:3n-3	0.32	0.28	0.017	0.284
CLA <i>cis</i> -9, <i>trans</i> -11 + <i>trans</i> -9, <i>cis</i> -11	0.70	1.19	0.156	0.124
CLA <i>trans</i> -10, <i>cis</i> -12	0.02	0.01	0.002	0.093
C20:4n-6	0.21	0.17	0.014	0.196
Medium-chain fatty acids	17.17	12.90	0.969	0.015
MUFA	20.63	28.29	1.973	0.043
PUFA	3.74	3.76	0.196	0.966
SFA	75.46	69.96	3.579	0.483
Atherogenicity index ¹	3.50	2.17	0.248	0.001

¹Atherogenicity index calculated as C12:0 + 4 × C14:0 + C16:0/UFA(Ulbricht and Southgate, 1991).

ration increased digestibility, it decreased ruminal CH₄ emission from 28.3 to 21.2 g/d as a result of the conversion from lactic acid to propionic acid in the rumen.

According to Johnson and Johnson (1995), fermentation of fibrous carbohydrates produces more CH₄ than fermentation of soluble sugars, which in turn produces more CH₄ than fermentation of starch. As discussed previously, structural carbohydrates, NFC, and starch were lower in the RB than the O diet (5.6 points lower

for NDF, NFC, and starch, on average), and the main difference was the higher lipid content in the RB diet (11.7 vs. 4.1% for RB and O diets, respectively). Fat content on the RB diet explains the CH₄ mitigation observed in this diet. Feeding a diet with added lipids is one way to lower enteric CH₄ emissions by ruminants (Knapp et al., 2014). Increasing the lipid content of the diet is acknowledged as a CH₄ mitigation strategy due to reduction of methanogenesis and biohydrogenation of UFA (alternative sink of H₂). Moreover, CH₄ output is positively correlated with milk fatty acids C6:0 to C16:0 (Fievez et al. 2012), which result mainly from mammary de novo synthesis, based primarily on the use of acetate produced in the rumen during fiber digestion, and fatty acids ranged from C6:0 to C16:0, lower in the RB diet due to lower fermentation activity (Table 8).

Methane conversion ratio (the Y_m factor) represents energy loss as CH₄ per unit of GE intake; for both diets, it averaged 4.7% (SEM = 0.47). Although in the literature CH₄ emissions are commonly expressed relative to GE intake, the most meaningful expression is relative to intake of OM. In the present work, CH₄ production

Table 9. Methane emission of Murciano-Granadina goats (n = 10) during late lactation according to the type of diet

Item ¹	Diet		SEM	P-value
	Oats	Rice bran		
CH ₄ , g/d	30.1	23.2	2.14	0.05
Y _m , %	5.2	4.3	0.47	0.39
CH ₄ /DMI, g/kg	16.7	15.0	1.52	0.61
CH ₄ /OMd, g/kg	25.6	21.8	2.59	0.50
CH ₄ /NDFd, g/kg	236.2	137.1	46.02	0.32
CH ₄ /milk, g/kg	13.6	11.1	1.16	0.31

¹Y_m = methane energy/gross energy intake; OMd = digested OM; NDFd = digested NDF.

expressed relative to DMI, digested OM, digested NDF, or kilograms of milk produced was not significant.

CONCLUSIONS

The total replacement of oat grain with rice bran (higher in lipids content than oat grain) reduced DMI by 12% without affecting milk yield (2.2 kg/d, on average) in Murciano-Granadina goats during late lactation. This was accompanied by increases in ammonia N concentration in the rumen liquor and might suggest partial inhibition of microbial synthesis by the rice bran fat. The differences found between treatments (lower contents of undecanoic acid and pentadecanoic acid in milk of goats fed rice bran) suggest a negative effect of rice bran oil on rumen bacterial metabolism; that is, on both de novo synthesis of bacterial lipids and fermentation activity. Enteric CH₄ emissions were reduced 6.9 g/d by the diet that incorporated rice bran. Milk fat content and MUFA increased when rice bran was incorporated in the diet, so lactating goats could utilize rice bran diets without detrimental effects on milk performance. Additional studies evaluating rice bran without any bypass fat added should be considered.

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