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Ruminant Nutrition

RUMINANT NUTRITION

Changes in nutrient balance, methane emissions, physiologic biomarkers, and production performance in goats fed different forage-to-concentrate ratios during lactation

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Abstract

The objective was to determine the effect forage-to-concentrate (**F:C**) ratio and stage of lactation on methane emissions, digestibility, nutrient balance, lactation performance, and metabolic responses in lactating goats. Twenty Murciano-Granadina dairy goats were used in an experiment divided into 3 periods: early (30 d), mid (100 d), and late (170 d) lactation. All goats were fed a diet with 35:65 F:C (**FCL**) during early-lactation. Then, 1 group (n = 10 goats) remained on FCL through mid- and late-lactation while the other group (n = 10 goats) was fed a diet with 50:50 F:C at mid-lactation (**FCM**) and 65:35 (**FCH**) at late lactation. A greater proportion of concentrate in the diet was associated with greater overall intake and digestibility ($P < 0.05$). Energy balance was negative in early-lactation (–77 kJ/kg of $BW^{0.75}$, on average) and positive for FCL at mid- and late-lactation (13 and 35 kJ/kg of $BW^{0.75}$, respectively). Goats fed FCM and FCH maintained negative energy balance throughout lactation. Plasma concentrations of non-esterified fatty acids at mid-lactation were greater for FCM than FCL (680 mEq/L), and at late-lactation concentrations were greater for FCH and FCL (856 mEq/L). A similar response was detected for plasma β-hydroxybutyrate. Methane emission was greater ($P < 0.05$) for FCM than FCH (1.7 g CH_4/d). This study demonstrated that differences in F:C across stages of lactation lead to distinct metabolic responses at the level of the rumen and tissues.

Key words: forage-to-concentrate ratio, lactating goat, milk metabolites, stage of lactation

Introduction

The dairy goat industry in Mediterranean countries is undergoing rapid structural developments, with herd size and number of animals per person being managed continuing to increase (Silanikove, 2000). Such expansion unavoidably places strain on the farm system, including a degree of stress on the animal. Factors associated with the natural cycle of physiological changes during the lactation cycle such as depletion and accretion of body reserves in combination with plane of nutrition, drought, and global warming can have a strong impact on animal efficiency

Abbreviations

BHB	β -hydroxybutyrate
EE	ether extract
F:C	forage-to-concentrate ratio
HP	heat production
GE	gross energy
GEI	GE intake
MEI	metabolizable energy intake
NEB	negative energy balance
NEFA	non-esterified fatty acids
UPV	University of Valencia
Ym	methane conversion factor
TE	tissue energy

(Moyes et al., 2013; Guinard-Flament et al., 2007; Larsen et al., 2016; Pragna et al., 2018). Managing nutrient density, i.e., by altering forage-to-concentrate (**F:C**) ratio in the diet is an effective strategy to prevent excessive and extended periods of negative energy balance (**NEB**) that could impact negatively efficiency (Bjerre-HarpØth et al., 2012). Although body fat (and likely muscle) mobilization during NEB helps goats cover partially their lactation requirements, in situations such as thermal stress extended periods of NEB due to improper nutritional management can lead to substantial physiological imbalance, compromising efficiency and health (Hamzaoui et al., 2013).

In large and small ruminant systems, the most commonly used approach to monitor physiological and nutritional status of animals is through the analyses of biomarkers in blood. Glucose, β -hydroxybutyrate **(BHB),** and non-esterified fatty acids (**NEFA**) are the most common indicators of the rate and extent of adipose tissue mobilization and hepatic metabolism, hence, are useful in both evaluating energetic status and generating indices of physiological imbalance (Bjerre-HarpØth et al., 2012; Larsen et al., 2016). However, blood sampling is invasive, time-consuming, and cannot be automated. Estimation of the nutritional status of an animal via variations in milk components is attractive as milk samples can be collected automatically. In contrast to cows (Piccioli-Cappelli et al., 2014), to our knowledge, there are no published data on the role of nutrient supply across stages of lactation on ruminal function, performance, and efficiency in dairy goats.

Our objective was to use different F:C ratios across stages of lactation to vary the nutrient supply and examine animal responses in a more holistic fashion. Thus, we focused on how F:C impacts DMI, total tract digestibility, methane emissions, energy, nitrogen and carbon balance, milk yield, and metabolic responses in lactating goats. Plasma biomarkers and milk composition were used to examine their potential to describe nutritional status of the goats during "spontaneous" NEB in early-lactation and "extended" NEB (by increasing the F:C ratio) at mid- and late-lactation.

Materials and Methods

The 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 (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 Experimental Farm from the Institute for Animal Science and Technology (UPV, Valencia, Spain). Twenty multiparous mature Murciano-Granadina dairy goats were selected and divided into 2 homogenous groups of 10 goats based on similar body weight (**BW**) at the beginning of gestation (45.6 ± 1.3 kg of BW) and milk production in the previous lactation (689.3 ± 55.7 kg of milk per 210 ± 30 d of lactation, on average). Animals were free of clinical disease prior to start of the experiment. Goats were housed in pens as 2 independent groups, fed twice daily (0800 hours and half at 1600 hours) and milked once (0700 hours) in a high-line Casse type milking parlor (2 platforms; 12 goats per platform; 6 milking units) with established milking machine parameters (vacuum = 40 kPa, pulsation rate = 90 ppm and pulsation ratio = 60%; De Laval Agri, Tumba, Sweden). Concrete pens were bedded with sawdust, and goats had free access to clean water at all times.

Ingredients and chemical composition of the mixed diet (alfalfa and pelleted concentrate) are reported in Table 1. Daily control of the quantity of concentrate and forage offered and refused was carried out in order to calculate daily feed intake. Nutrient requirements followed recommendations by Calsamiglia et al., (2009) for lactating goats fed mixed rations. Experimental diets used alfalfa hay as forage and a pelleted concentrate as compound feed (Table 1). Previous to the start of the experiment, goats from both groups received a diet with F:C ratio at 35:65 (**FCL**). Subsequently, the experimental periods were divided into early (30 ± 8 DIM), mid (100 ± 12 DIM), and late (170 ± 11 DIM) lactation. During early-lactation, although groups were separated, both were fed FCL. At mid-lactation, 1 group remained on FCL and the other was fed a diet with an F:C ratio of 50:50 (**FCM**). At late-lactation, 1 group continued on FCL and the other was fed a diet with an F:C of 65:35 (**FCH**). Thus, 1 group was continuously fed a diet with low an F:C ratio throughout lactation, while the other received a diet with progressive increases in F:C during mid- and late-lactation. Schematic representation of the experimental design is shown in Figure 1.

Experimental schedule and measurements

In summary, each experimental period in metabolic cages comprised a total of 24 d (7 d adaptation + 5 d digestibility + 2 rumen liquor and blood + 10 gas exchange). Prior to this, goats were housed as a group in pens. A visual description is depicted in Figure 1. Below is a more detailed description of activities performed with the animals during the experimental periods.

During their time in metabolic cages, goats were at thermoneutrality (20 to 23 °C determined by a Hobo probe, ONSET data loggers, Cape Cod, MA) and fed the experimental diets in individual metabolism cages. First during 7 d for adaptation and then 5 more days for measuring apparent total tract digestibility and nutrient balance. Thus, feed offered and refused and total fecal, urine, and milk outputs were recorded daily for each goat during the last 5 d. In addition, BW at the beginning and end was recorded. 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 10% (v/v) of H_2SO_4. Representative samples (10%) of diet, feces, and urine were collected over 5 consecutive days, stored at –20 °C and pooled prior to 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 subsample placed in a bottle and frozen until

Table 1. Ingredients and chemical composition of the diets

Item			Diet[1]		
Forage-to-concentrate ratio			35:65	50:50	65:35
	Alfalfa	Concentrate	FCL	FCM	FCH
Ingredients, g/kg DM					
Alfalfa hay	1000		350	500	650
Barley		350	228	175	123
Corn		309	201	155	108
Wheat bran		150	98	75	53
Soybean meal, 44% CP		148	96	74	52
Bypass fat		5	3	3	2
Calcium carbonate		22	15	11	8
Sodium chloride		11	7	5	4
Premix[3]		5	3	3	2
Chemical composition, % of DM					
Dry matter	86	89	88	88	87
Organic matter	91	93	93	92	92
Ash	9	7	7	8	8
Crude protein	16	17	16	16	16
Ether extract	1	2	2	2	2
Neutral detergent fiber	52	18	30	35	40
Acid detergent fiber	33	3	14	18	22
Acid detergent lignin	12	0	4	6	8
NFC[4]	22	56	44	39	34
Starch	1	27	18	14	10
Carbon	46	44	45	45	45
Nitrogen	3	3	3	3	3
Carbon:nitrogen	18	16	17	17	17
Gross energy, MJ/kg DM	16	17	17	17	16

[1] FCL, forage-to-concentrate ratio low; FCM, forage-to-concentrate ratio medium; FCH, forage-to-concentrate ratio high.
[2] Bypass fat of palm FA distillate. Provided by Norel Animal Nutrition (Norel S.A., Madrid, Spain).
[3]Provided by NACOOP S.A. España. Premix composition (ppm or IU per kilogram of premix): Se, 40 mg/kg; I, 250 mg/kg; Co, 80 mg/kg; Cu, 3,000 mg/kg; Fe, 6,000 mg/kg; Zn, 23,400 mg/kg; Mn, 29,000 mg/kg; S, 60,000 mg/kg; Mg, 60,000 mg/kg; vitamin A, 2,000,000 IU/kg; vitamin D3, 400,000 IU/kg; vitamin E, 2,000 ppm; nicotinic acid, 10,000 ppm; choline, 20,300 ppm.
[4]NFC = non-fibrous carbohydrate content: 100-(NDF+ash+CP+EE).

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 (Valencia, Spain) for composition analysis.

An additional 2 d were used for collection of blood samples and ruminal fluid. Jugular blood was sampled in 10 mL tubes containing EDTA and immediately centrifuged at 1,800 × *g* for 10 min at 4 °C, and the plasma stored at –20 °C until analyzed. Ruminal fluid was collected by stomach tube before the morning feeding following the procedure described by Ramos-Morales et al., (2014). Ruminal fluid was strained through 4 layers of cheesecloth, and pH determined immediately using a portable pH meter (Model 265A, Orion Research Inc., Beverly, MA). A subsample of ruminal fluid was acidified with 50% H_2SO_4 and frozen until later determination of ammonium. Samples for analysis of VFA were mixed with H_3PO_4 and kept frozen until analysis.

The last 10 d of each experimental period were used for the measurement of gas exchange in each goat during a 24-hr period using an indirect calorimetry system based on 2 ventilated head-box designed for small ruminants. Each goat was in the respiratory unit for 2 d, the first for adaptation and the second for recording gas exchange. The respirometry system was equipped with 2 head hoods, 2 flow meters (Thermal Mass Flowmeter Sensyflow VT-S, ABB, Alzenau, Germany) and 2 air suctions provided by centrifugal fans (CST60 Soler Palau Inc., Parets del Vallès, Barcelona, Spain). Concentrations of CH_4 and carbon dioxide (CO_2) were measured using the infrared principle, and O_2 measured with the paramagnetic principle (Easyflow Gas Analyzer, model 3020, ABB, Alzenau, Germany). Fernández et al., (2019) described the mobile open-circuit respirometry system used for these measurements. 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 according to Brockway et al., (1971). The CH_4 and CO_2 production and oxygen (O_2) consumption 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

Feed, feed refusals, and fecal samples were first dried in a forced-air oven at 55 °C for 48 hr 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 (2006) for DM (934.01), ash (942.05), and ether extract (**EE**; 920.39). The DM of diets and feces was determined by oven-drying at 102 ± 2 °C for 24 hr. Ash concentration was measured by incineration in an electric

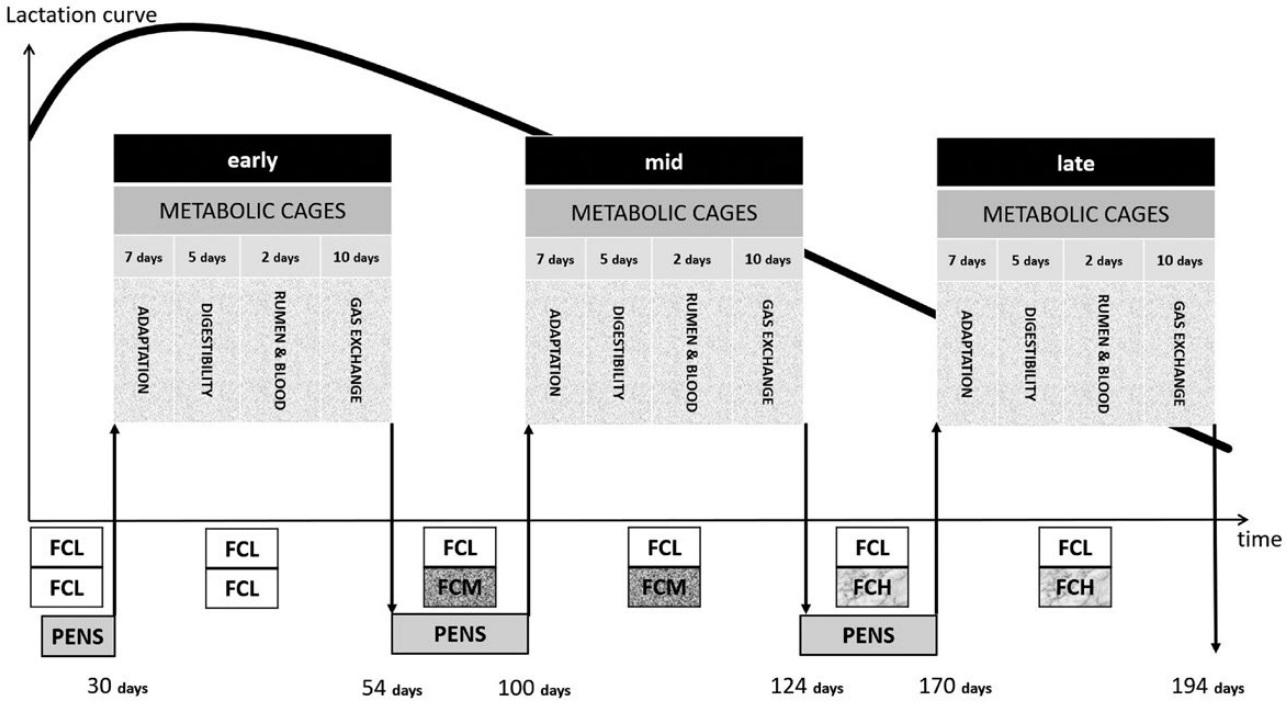

Figure 1. Description of the experimental design. Goats were housed in metabolic cages 3 times during the study. During early-lactation, although groups were separated, both were fed FCL. At mid-lactation, 1 group remained on FCL and the other was fed a diet with an F:C ratio of 50:50 (**FCM**). At late-lactation, 1 group continued on FCL and the other was fed a diet with an F:C of 65:35 (**FCH**). Thus, 1 group was continuously fed a diet with low an F:C ratio throughout lactation, while the other received a diet with progressive increases in F:C during mid- and late-lactation.

muffle furnace at 550 °C for 6 hr. The EE was extracted 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 content were measured in an ANKOM Fiber Analyzer (A220, ANKOM Technologies, Fairport, NY) according to Mertens (2002) and AOAC (2006), respectively. The NDF was determined using sodium sulfite and α 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 – EE. The gross energy (**GE**) content of the dried samples (feed, feces, urine, and milk) was determined by combustion in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, UK). Starch content was determined by an enzymatic method (α-amylase obtained from Sigma-Aldrich, Steinheim, Germany) according to Batey (1982). The C and N were analyzed by the Dumas principle (TruSpec CN; LECO Corporation, St. Joseph, MI). Multiplying N by a factor of 6.25 converted the results to CP.

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. Milk composition (fat, protein, lactose and total milk solids content) was analyzed with an infrared analyzer (MilkoScan FT120 Foss Electric, Hillerød, Denmark). Fatty acid (**FA**) methyl esters of milk and diet lipids were prepared directly as described previously by O´Fallon et al., (2007). The FA methyl esters were analyzed in a Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless injector and a flame ionization detector. Separation of methyl esters was performed in a fused silica capillary column SP 2560 (Supelco, PA; 100 m × 0.25 mm × 0.2 µm film thickness). The carrier gas was Helium at a linear velocity of 20 cm/s. Samples were injected with a split ratio of 1/100. The initial oven temperature was set at 140 °C held for 5 min and increased to 240 at 4 °C/min and finally maintained at that temperature for 30 min. Both detector and injector temperatures were set at 260 °C.

Urine and plasma ammonium, total protein, urea, uric acid, and glucose were analyzed according to standard procedures (Siemens Diagnostics Clinical Methods for ADVIA 1800). Plasma NEFA were determined using the NEFA C Acyl-CoA synthetase-Acyl-CoA oxidase assay method, and BHB was determined as described by Harano et al., (1985). Both NEFA and BHB analyses were performed on the ADVIA 1800 System. Minor milk constituents such as glucose and glucose-6-phosphate (Larsen, 2015), isocitrate (Larsen 2014), BHB (Larsen and Nielsen, 2005), and uric acid (Larsen and Moyes, 2010) were determined by enzymatic-fluorometric methods. Milk urea was analyzed by flow injection analyses and enzymatic degradation. Application notes given by the manufacturer were followed (Foss Tecator AB, Höganäs, Sweden).

Calculations

The ME intake (**MEI**) was calculated as the difference between GE intake (**GEI**) and energy losses in feces, urine, and CH_4 (with an energy equivalent value of 39.5 kJ/L CH_4; Brouwer, 1965). Heat production (**HP**) was determined from measurements of O_2 consumption, CO_2 and CH_4 production, and urine N (N_{urine}), using the equation of Brouwer (1965):

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

where gases were expressed in L per day and N_{urine} in g per day. Recovered energy was the difference between ME and HP. Tissue energy (**TE**) was calculated as the difference between recovered energy and milk energy (E_{milk}). Non-protein respiratory quotient

(RQnp) was determined as: RQnp = (CO_2 – (N_{urine} × 6.25 × 0.774))/(O_2 – (N_{urine} × 6.25 × 0.957)).

For C and N balance, we followed the equations and values proposed by McLean and Tobin (1987), and the retention in protein ($R_{protein}$, g) and fat (R_{fat}, g) were calculated.

Efficiency of ME to milk and maintenance (k_{ls}) was calculated according to INRA (2018); k_{ls} = 0.65 + 0.247 × (q – 0.63), being q the metabolisability (ME/GE).

Statistical analysis

Effects of diet on intake, digestibility, ruminal fermentation, milk performance, energy and C and N balances, methane emissions, and metabolites were analyzed using a mixed model (lme function from the nlme library) in R (2016). The following statistical model was used:

$$Y_{ij} = \mu + D_i + S_j + (D \times S)_{ij} + \text{goat} + \varepsilon_{ij}$$

where Y_{ij} is the dependent variable, μ is the overall mean, and D_i is the fixed effect of the treatment diet (F:C ratio), S_j is the fixed effects stage of lactation (early, mid, and late), $(D \times S)_{ij}$ is the interaction between treatment and stage of lactation, goat is the random effect, and ε is the random error. Multiple comparisons using Tukey–Kramer were used and were declared significant at $P < 0.05$.

Results and Discussion

Although dietary GE and CP content were similar among treatments, 17 MJ/kg DM and 16% (DM basis), respectively, as F:C ratio increased from 35:65 to 65:35, NDF increased from 30% to 40% (DM basis) and starch decreased 18% to 10% (DM basis). Thus, as desired, we were able to achieve differences in the profiles of nutrients available to the animal for maintenance and production.

Feed intake, digestibility, and ruminal fermentation

Stage of lactation and F:C ratio influenced BW change, DMI, and apparent total tract digestibility (Table 2). Dietary treatments did not influence overall BW (44.6 kg on average), but BW increased ($P < 0.05$) as lactation progressed from 42.5 to 44.5 and 47.0 kg at early-, mid-, and late-lactation, respectively.

Differences in DMI ($P < 0.05$) were observed between diets and stage of lactation, with values averaging 1.80, 1.63, and 1.28 kg/d, for FCL, FCM, and FCH, respectively. Average DMI for stage of lactation was 1.85, 1.73, and 1.47 kg DM/d at early-, mid-, and late-lactation, respectively. Multiple mechanisms regulate DMI in ruminants, but it generally decreases with increasing NDF (Allen, 2000), i.e., with bulk density of the diet. Thus, the lower DMI as F:C increased agrees with established knowledge.

Differences ($P < 0.05$) were detected for the effect of F:C ratio on apparent total tract digestibility of DM, OM, and energy, with lower values in FCM and FCH compared with FCL. The greater proportion of concentrate in the diet was associated with more extensive digestibility of nonstructural carbohydrates. Differences ($P < 0.05$) also were observed for stage of lactation; lower DM, OM, and energy digestibility as lactation advanced and, compared with early lactation, a reduction in CP digestibility in mid- and late-lactation, probably associate with protein excess. Digestibility of EE was greater at mid compared with early and late-lactation and fiber digestibility was lowest at mid-lactation. An interaction was observed for apparent total tract digestibility ($P < 0.05$) when forage increased and lactation progressed.

Average ruminal pH never fell below 6.2 (Table 3), suggesting that values obtained were within a proper range for maintaining normal ruminal fermentation (Ørskov and Fraser, 1975). Although stomach tube is a suitable non-invasive technique for ruminal fluid sampling, it is prone to saliva contamination, which would increase the pH in the sample (Ramos-Morales et al., 2014) as we found for late-lactation with FCH diet and also FCL diet. A significant effect on NH_3-N was detected for F:C and stage of lactation, with values greater than 19 mg NH_3-N/dL at early-lactation with the FCM diet compared with feeding FCL at mid- and late-lactation. In cows, similar values were suggested to indicate poor protein utilization by ruminal microbes (Casper et al., 1999). Although, to our knowledge, there are no studies reporting an NH_3-N threshold in goats for optimal microbial protein synthesis in the rumen, our data could indicate a similar situation as in cows. Besides, the fact that goats have a greater degree urea recycling than cows, could indicates they might be less susceptible to dietary protein supply (Silanikove, 2000).

Despite the greater amount of concentrate used in the FCL diet, only FCM and FCL at mid-lactation caused differences (diet effect $P = 0.001$) in total VFA (83.3 vs. 68.9 mM). Total VFA also

Table 2. Apparent total tract digestibility (%) in Murciano-Granadina goats (n = 20) during early-, middle-, and late-lactation according to the F:C ratio[1]

	Early		Mid		Late					
	35:65	35:65	50:50	35:65	65:35	35:65		P-value		
Item[2]	FCL	FCL	FCM	FCL	FCH	FCL	SEM	Diet	Stage	Diet × stage
BW, kg	42.6	42.3	44.8	44.2	47.3	46.6	3.60	0.62	0.001	0.84
DMI, kg/d	1.83	1.86	1.63[a]	1.83[b]	1.28[a]	1.67[b]	0.254	0.001	0.001	0.17
DM	69.6	70.7	62.2[a]	70.1[b]	63.4[a]	71.5[b]	4.04	0.001	0.013	0.22
OM	72.1	71.8	63.2[a]	71.4[b]	65.7[a]	74.1[b]	4.23	0.001	0.014	0.26
CP	68.7	65.5	68.6	71.9	69.9	70.9	2.24	0.77	0.044	0.18
EE	49.0	50.4	53.9	61.0	41.8[a]	58.0[b]	6.88	0.08	0.024	0.51
NDF	55.5	51.7	41.6	49.1	52.9	57.1	5.53	0.070	0.048	0.52
ADF	45.5	42.2	29.7	37.4	36.8[a]	47.0[b]	6.43	0.10	0.037	0.51
GE	70.9	70.6	61.5[a]	69.9[b]	64.1[a]	72.9[b]	4.47	0.001	0.015	0.22

[1]FCL, forage-to-concentrate ratio low; FCM, forage-to-concentrate ratio medium; FCH, forage-to-concentrate ratio high.
[2]BW, body weight; DMI, dry matter intake; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber.
[a,b]Means, within each stage of lactation, with different superscript differ ($P < 0.05$).

decreased (stage of lactation effect $P = 0.003$) with advancing lactation (from 75.5 mM at early-lactation to 48.4 mM at late-lactation), but without significant differences between diets. Despite the greater amount of concentrate used in FCL, at late-lactation the total amount of VFA was low for both diets. This suggested that ruminal fluid collection through stomach tube did not yield a representative total VFA. Furthermore, Ørskov and Fraser (1975) reported that concentration of VFA could be affected by rate of carbohydrate degradability, passage rate, F:C ratio, and pH. Molar proportion of acetic acid was lower ($P < 0.05$) with FCL compared with other diets (61.7 vs. 66.1 mM, respectively), and the opposite was observed for propionic acid (17.2 vs. 12.9 mM). Acetic and butyric acids are considered lipogenic substrates and propionic acid is considered a glucogenic substrate (Van Knegsel et al., 2007). However, butyric acid was high, while propionic acid was low also in the FCL group at late-lactation. Only, differences ($P < 0.05$) were detected when the ratio of acetic to propionic acid was determined, being greater for FCM and FCH than FCL. Thus, based on Van Knegsel et al., (2007) study and, the ratio of acetic to propionic acid, we observed that lower F:C ratio was glucogenic diet and higher F:C ratio was lipogenic diet. Feeding FCL led to greater (diet effect $P < 0.05$) isovaleric, n-valeric, n-caproic, and heptanoic acid compared with diets with more than 50% of forage, although a reduction in isovaleric acid was detected with FCL at mid-lactation (diet × stage interaction $P < 0.05$). The greater amount of concentrate in the FCL diet and an increase in degradation of starch likely explains the greater concentrations of these VFA (Ørskov and Fraser, 1975; Casper et al., 1999).

In terms of effects due to stage of lactation, acetic acid increased (from 60.2 to 66.1 mM) and propionic acid decreased (from 18.6 to 12.9 mM) as lactation progressed ($P < 0.05$). Compared with mid and late-lactation, concentrations of minor VFA such as isovaleric acid, n-valeric and heptanoic acids were greater at early-lactation, while n-caproic acid followed the opposite trend. These VFA are mainly generated during degradation of branched-chain amino acids in the rumen, a process thought to indicate an inefficient use of amino groups for microbial protein synthesis (Casper et al., 1999). The possible asynchrony between protein degradation and F:C ratio was more pronounced when the FCL diet was maintained and lactation progressed.

Methane emissions

Differences in CH_4 emission were observed for both diet type and stage of lactation (Table 4). Reduction in CH_4 was due to the lower F:C ratio in FCL diet and not to its increase in the others groups. Compared with the FCL diet (24.8 g/d), goats fed FCM and FCH produced significantly ($P < 0.05$) greater amounts of CH_4 (30.2 and 28.5 g/d, respectively). Because ruminants lose 2% to 12% of their dietary GE as CH_4, a decrease in production of CH_4 represents an improvement in feed efficiency (Johnson and Johnson, 1995). The **Ym** (energy loss as CH_4 per unit of GEI) was 5.84% with FCL, 7.78% with FCL and 9.36% with FCH, thus, the greater concentrate and lower fiber content in the FCL diet likely did not favor methanogens, and the reduction of hydrogen as observed at late-lactation did not allow methanogens activity. Although CH_4 emission is most commonly expressed in the literature relative to GEI, the most meaningful expression is relative to DM or OM intake. In the present study, when CH_4 was expressed relative to DM and OM intake or digested, statistical differences ($P < 0.05$) remained. Thus, goats fed a diet with lower F:C ratio produced less CH_4 compared with higher fiber diets mainly because fiber is the main substrate for methanogens (Niu et al., 2018). Furthermore, compared with other diets feeding FCL reduced the amount of CH_4 by 7 g/kg of milk on average.

Energy balance

Differences ($P < 0.05$) in GEI were detected for F:C ratio and stage of lactation (Table 5). Although diets differed in the amount of forage, no differences were observed between diets for losses of energy in feces and urine due partly to the decrease in digestion when F:C increased. As reported previously (Knapp et al., 2014), there were greater ($P < 0.05$) energy losses in the form of CH_4 as F:C increased, 105 vs. 126 and 114 kJ/kg of $BW^{0.75}$ for FCL, FCM and FCH, respectively. Due to greater GEI in FCL diet and lower energy loss in CH_4, MEI was greater ($P < 0.05$) with FCL (1,120 kJ/

Table 3. pH, ammonia-N (NH_3-N), and VFA in Murciano-Granadina goats ($n = 20$) during early-, middle-, and late-lactation according to the F:C ratio[1]

	Early		Mid		Late					
	35:65	35:65	50:50	35:65	65:35	35:65		P-value		
Item	FCL	FCL	FCM	FCL	FCH	FCL	SEM	Diet	Stage	Diet × stage
pH	6.8	7.0	7.0	6.9	7.3	7.4	0.18	0.121	0.111	0.21
NH_3-N,[2] mg/dL	21.3	20.4	23.2[b]	15.9[a]	18.6	19.3	4.13	0.015	0.050	0.12
Total VFA, mM	79.0	72.0	83.3[b]	68.9[a]	47.9	48.9	15.75	0.001	0.003	0.15
Individual VFA, mol/100 mol										
Acetic acid	60.6	59.7	64.8[b]	61.8[a]	67.3[b]	64.9[a]	2.55	0.001	0.001	0.33
Propionic acid	18.0	19.1	13.6[a]	18.0[b]	12.2	13.6	2.26	0.001	0.001	0.14
Isobutyric acid	1.79	2.08	1.33	1.31	1.83	1.92	0.337	0.06	0.016	0.22
Butyric acid	16.0	13.8	17.4	15.8	15.5	15.6	2.53	0.011	0.33	0.35
Isovaleric acid	2.87	3.22	1.45[b]	1.40[a]	1.80[a]	2.42[b]	0.579	0.001	0.77	0.001
n-Valeric acid	1.44	1.68	1.01[a]	1.41[b]	1.11[a]	1.19[b]	0.243	0.001	0.001	0.15
n-Caproic acid	0.20	0.24	0.24[a]	0.27[b]	0.24[a]	0.35[b]	0.095	0.004	0.001	0.10
Heptanoic acid	0.04	0.16	0.05[a]	0.06[b]	0.01[a]	0.01[b]	0.038	0.001	0.001	0.31
Acetic/propionic ratio	3.37	3.12	4.76[b]	3.44[a]	5.51[b]	4.78[a]	0.115	0.001	0.15	0.17

[1]FCL, forage-to-concentrate ratio low; FCM, forage-to-concentrate ratio medium; FCH, forage-to-concentrate ratio high.
[2]NH_3-N, ammonia nitrogen.
[a,b]Means, within each stage of lactation, with different superscript differ ($P < 0.05$).

Table 4. Methane emission in Murciano-Granadina goats (n = 20) during early-, middle-, and late-lactation according to the F:C ratio[1]

	Early		Mid		Late			P-value		
	35:65	35:65	50:50	35:65	65:35	35:65				
Item[2]	FCL	FCL	FCM	FCL	FCH	FCL	SEM	Diet	Stage	Diet × stage
CH_4, g/d	25.9	25.9	30.2[b]	25.0[a]	28.5[b]	22.3[a]	3.29	0.018	0.055	0.15
Ym, %	5.96	5.92	7.78[b]	5.80[a]	9.36[b]	5.66[a]	1.491	0.013	0.40	0.06
CH_4/DMI, g/kg	14.2	13.9	18.5[b]	13.7[a]	22.2[b]	14.2[a]	3.49	0.028	0.043	0.38
CH_4/OMI, g/kg	15.3	15.1	20.0[b]	14.7[a]	24.1[b]	14.4[a]	3.96	0.026	0.048	0.38
CH_4/OMd, g/kg	21.2	21.0	31.7[b]	20.7[a]	36.7[b]	19.5[a]	7.24	0.014	0.022	0.16
CH_4/milk, g/kg	9.8	8.8	19.6[b]	15.9[a]	18.5[b]	12.0[a]	4.56	0.032	0.041	0.37

[1]FCL, forage-to-concentrate ratio low; FCM, forage-to-concentrate ratio medium; FCH, forage-to-concentrate ratio high.
[2]DMI, dry matter intake; OMI, organic matter intake; OMd, digested organic matter.
[a,b]Means, within each stage of lactation, with different superscript differ ($P < 0.05$).

Table 5. Energy balance (kJ/kg of $BW^{0.75}$) in Murciano-Granadina goats (n = 20) during early-, middle-, and late-lactation according to the F:C ratio[1]

	Early		Mid		Late			P-value		
	35:65	35:65	50:50	35:65	65:35	35:65				
Item[2]	FCL	FCL	FCM	FCL	FCH	FCL	SEM	Diet	Stage	Diet × stage
GEI	1,882	1,906	1,619[a]	1,812[b]	1,216[a]	1,589[b]	212.6	0.001	0.001	0.42
E_{feces}	558	551	590[a]	559[b]	432	433	111.1	0.58	0.001	0.81
DE	1,325	1,355	1,029[a]	1,252[b]	784[a]	1,156[b]	160.2	0.001	0.001	0.35
E_{urine}	49.9	45.3	50.7	55.6	41.9	36.5	12.32	0.46	0.005	0.13
$E_{methane}$	112	113	126[b]	105[a]	114[b]	90[a]	14.6	0.020	0.001	0.15
MEI	1,162	1,196	853[a]	1,092[b]	629[a]	1,029[b]	156.9	0.001	0.001	0.33
HP	790	789	568[a]	676[b]	415[a]	639[b]	99.1	0.001	0.001	0.28
E_{milk}	471	485	322[a]	403[b]	222[a]	355[b]	57.6	0.001	0.001	0.21
TE	−99	−77	−38[a]	13[b]	−8[a]	35[b]	6.6	0.001	0.001	0.18
k_{ls}	0.65	0.65	0.62[a]	0.64[b]	0.62[a]	0.65[b]	0.014	0.001	0.001	0.33
RQnp	0.60	0.61	0.68[a]	0.83[b]	0.61[a]	0.88[b]	0.031	0.001	0.12	0.15

[1]FCL, forage-to-concentrate ratio low; FCM, forage-to-concentrate ratio medium; FCH, forage-to-concentrate ratio high.
[2]GEI, gross energy intake; E_{feces}, energy losses in feces; E_{urine}, energy losses in urine; $E_{methane}$, energy losses in methane; MEI, metabolizable energy intake; HP, heat production; E_{milk}, recovered energy in milk; TE, recovered energy in tissue; k_{ls}, efficiency utilization ME for milk; RQnp, nonprotein respiration quotient.
[a,b]Means, within each stage of lactation, with different superscript differ ($P < 0.05$).

kg of $BW^{0.75}$) than other diets (714 kJ/kg of $BW^{0.75}$). HP and E_{milk} were also greater ($P < 0.05$) in FCL compared with FCM and FCH. The mean TE value between diets was negative (−32, −38, and −8 kJ/kg of $BW^{0.75}$ for FCL, FCM, and FCH, respectively).

For the FCL diet, a NEB was observed at the beginning of lactation, which became positive in the latter stages of lactation (TE balance for FCL diet: −88, 13, and 35 kJ/kg of $BW^{0.75}$). However, the TE with both the FCM (−38 kJ/kg of $BW^{0.75}$) and FCH (−8 kJ/kg of $BW^{0.75}$) diets was negative (both diets were fed at mid- and late-lactation, respectively). Due to an increasing F:C ratio, the bulk density of the diets FCM and FCH increased, the supply of ME diminished and increased BW due that probably more forage leads to heavier digestive tract. In lactating animals, lipogenic nutrients can increase partitioning of ME into milk (i.e., increasing milk fat yield), and consequently decrease partitioning of ME into body reserves (Van Knegsel et al., 2007). Thus, the present observations suggest that the lower content of glucogenic nutrients in both FCM and FCH (diets greater F:C ratio) did not favor body fat deposition and partitioning of ME into body tissue. This was likely because goats were in NEB and relied on fat mobilization to meet demands for milk synthesis. For diet FCL, the greater proportion of concentrate in the diet and the advanced stage of lactation reduced energy partitioning towards milk synthesis, suggesting that a greater amount of ME was retained in body tissues.

INRA (2018) defines efficiency of ME for milk and maintenance as k_{ls}; following this approach, the calculated efficiency for FCL and other diets was 0.65 and 0.62, respectively ($P < 0.05$). Differences in the F:C ratio between treatments and level of intake were responsible for the fact that use of ME was not constant. Aguilera et al. (1990) with Granadina goats and Tovar-Luna et al., (2010) with Alpine goats reported similar values, 0.67 and 0.63, respectively.

Assuming an ME for maintenance of 401 kJ/kg of $BW^{0.75}$ (according to Aguilera et al., 1990), the groups of goats receiving the FCL diet were fed at 3 times ME for maintenance, those fed the FCM diet at 2 times ME for maintenance, and those fed the FCH diet at 1.5 times ME for maintenance. Thus, differences among diets were detected for ME when expressed per kg of DM, and energy density was 11 and 9 MJ ME/kg DM for FCL and other diets, respectively.

When the stage of lactation was assessed, we observed significant differences ($P < 0.05$) for all energy balance variables. Greater GEI at early, followed by mid- and late-lactation were observed (1,894, 1,715, and 1,403 kJ/kg of $BW^{0.75}$, respectively).

The same reduction in MEI (1,179, 972, and 829 kJ/kg of $BW^{0.75}$, at early-, mid-, and late-lactation) and E_{milk} (from 478 to 288 kJ/kg of $BW^{0.75}$) were observed as lactation progressed. Energy balance was negative for early- and mid-lactation (–88 and –12 kJ/kg of $BW^{0.75}$) and positive at late lactation (14 kJ/kg of $BW^{0.75}$). Thus, more efficiency of ME utilization for maintenance and milk (k_{ls}) were observed in early compared with mid- and late-lactation (0.65, 0.63, and 0.64, respectively). Assuming an ME for maintenance of 401 kJ/kg of $BW^{0.75}$, the level of intake at early lactation was 3 times ME for maintenance, at mid-lactation 2 times ME for maintenance, and 1.5 times ME for maintenance at late-lactation.

A significant difference ($P < 0.001$) was observed for RQnp, with FCM and FCH resulting in lower values than FCL (0.68, 0.61, and 0.86, respectively). These responses might have been the result of the NEB and fat mobilization. An RQnp value lower than 0.7 indicates predominance of fat mobilization (Chwalibog et al., 1997), as was observed in this study.

Carbon and nitrogen balance

The same trend was observed in C balance as in energy balance; greater intake with the FCL diet and, thus, greater C intake (Table 6). The greater intake with the FCL diet was accompanied by a greater amount of C in milk. Although it was expected that excretion of C would be greater with a higher fiber content in the diet (diet × stage interaction $P < 0.05$), as lactation progressed the intake in FCM and FCH decreased, thus, C excretion did not increase. Differences in C excretion were similar with FCL and FCM followed by FCH (32.0, 30.8 and 23.3 g/kg of $BW^{0.75}$ for FCL, FCM, and FCH, respectively). Differences were detected when C excretion was expressed as percentage of C intake (77% with FCL and 80% with FCM and FCH). In addition, greater efficiency of C in milk over C ingested was observed for FCL than other diets (22% vs. 19%, respectively). The C and N balance according to stage of lactation followed the observed pattern of ingestion, with increasing amount of dietary forage as lactation progressed leading to a reduction in intake. Thus, this reduction and their interaction with stage of lactation had an effect ($P < 0.05$) on both C and N balance.

Regarding diet and N balance, goats in the FCL group consumed more N, excreted more N in feces and retained more N in milk ($P < 0.05$). The ratio between milk N output and N ingested was 29% with FCL, 25% with FCM and 27% with the FCH diet. Amount of N excreted compared with ingested was 59% with FCL and 65% with both FCM and FCH. Thus, the trend was the same for N as for C, with the FCL diet being used with greater efficiency, i.e., there was less waste in feces, urine, and gases.

The values of N and C retained in the body were converted to g, protein, and fat retained were calculated. Increase $R_{protein}$ was observed ($P < 0.05$) as animals regained BW. Differences ($P < 0.05$) were detected between diets for R_{fat}. Differences in R_{fat} were also observed for stage of lactation; fat mobilization was reduced as lactation progressed (–41.5, –9.1, and 1.2 g/goat for early-, mid-, and late-lactation, respectively).

Milk production and FAs

Milk performance was influenced by both diet and stage of lactation and no interaction was observed (Table 7). Milk yield was greater with FCL compared with other diets (2.30 vs. 1.54 kg/d, respectively), and was greater in early lactation than mid- and late-lactation (2.90, 1.56, and 1.70 kg/d, respectively). We have observed higher values at late-lactation compared with mid-lactation. This is because the farrowing took place in autumn, the mid-lactation records were in winter and late-lactation was in spring time, where an increase in milk production is usually observed in temperate latitudes. Milk fat was greater ($P < 0.05$) when the F:C ratio increased and lower at late compared with early lactation ($P < 0.05$). Milk protein was not affected by dietary

Table 6. Carbon and nitrogen balance (g/kg of $BW^{0.75}$) in Murciano-Granadina goats ($n = 20$) during early-, middle-, and late-lactation according to the F:C ratio[1]

	Early		Mid		Late					
	35:65	35:65	50:50	35:65	65:35	35:65		P-value		
Item[2]	FCL	FCL	FCM	FCL	FCH	FCL	SEM	Diet	Stage	Diet × stage
C_{intake}	45.3	45.2	38.1[a]	39.9[b]	29.8[a]	36.0[b]	4.57	0.003	0.001	0.13
C_{feces}	15.1	14.1	15.4	14.6	11.7	12.0	2.95	0.35	0.002	0.59
C_{urine}	1.26	1.14	1.30	1.42	1.05	0.92	0.309	0.42	0.005	0.16
C_{CO2}	17.3	17.2	12.9[a]	15.0[b]	9.5[a]	14.0[b]	2.05	0.001	0.001	0.11
C_{CH4}	1.08	1.09	1.21[b]	1.01[a]	1.10[b]	0.87[a]	0.175	0.021	0.032	0.97
C excretion	34.7	33.5	30.8[a]	32.1[b]	23.3[a]	27.8[b]	4.18	0.043	0.001	0.33
C_{milk}	11.8	12.6	7.3[b]	7.0[a]	5.8[a]	6.7[b]	1.44	0.032	0.001	0.020
$C_{retained\ body}$	–1.19	–0.79	0.03[a]	0.81[b]	0.57[a]	1.46[b]	0.231	0.001	0.001	0.50
N_{intake}	2.95	3.00	2.46[a]	2.61[b]	1.86[a]	2.32[b]	0.402	0.005	0.001	0.08
N_{feces}	0.92	1.03	0.73[a]	0.80[b]	0.57[a]	0.71[b]	0.162	0.001	0.001	0.59
N_{urine}	0.79	0.70	0.87[a]	0.91[b]	0.65[b]	0.58[a]	0.209	0.34	0.006	0.032
N excretion	1.72	1.73	1.60[a]	1.71[b]	1.22[a]	1.29[b]	0.233	0.053	0.043	0.68
N_{milk}	0.90	1.03	0.61[a]	0.64[b]	0.50[a]	0.58[b]	0.128	0.002	0.001	0.34
$N_{retained\ body}$	0.33	0.24	0.25	0.26	0.14	0.45	0.410	0.34	0.89	0.11
$R_{protein}$, g/goat	34.3	24.9	26.9	27.7	16.3[a]	50.2[b]	11.45	0.046	0.10	0.46
R_{fat}, g/goat	–49.1	–33.9	–17.5[a]	–0.6[b]	2.4	0.0	21.13	0.035	0.031	0.10

[1]FCL, forage-to-concentrate ratio low; FCM, forage-to-concentrate ratio medium; FCH, forage-to-concentrate ratio high.
[2]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; $C_{retained\ body}$, 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; $N_{retained\ body}$, recovered N in tissue; $R_{protein}$, protein retention; R_{fat}, fat retention.
[a,b]Means, within each stage of lactation, with different superscript differ ($P < 0.05$).

F:C ratio, and greater values ($P < 0.05$) were observed at mid and late compared with early-lactation. Milk lactose was not influenced by stage of lactation, but greater concentrations ($P < 0.05$) were observed with FCL compared with other diets. These results agreed with the notion that increasing F:C ratio is generally associated with greater milk fat (4.54%, 5.92%, and 4.62% with FCL, FCM, and FCH, respectively) and lower lactose content, and sometimes yield (Aguerre et al., 2011).

There was little effect of diet or stage of lactation on the FA profiles (Table 8). The content of FA with 16 or fewer carbon atoms derives from de novo synthesis, whereas those with 18 or more carbons atoms arise from the diet or lipid mobilization (Chilliard et al., 2003).

In our study, we have to be aware that bypass fat was added, and this protected fat was from palm which is rich in C16: 0 FA. Therefore, part of the increase in C16: 0 in milk could be of dietary origin and another part from synthesis by the mammary

Table 7. Daily milk production (g/kg) and chemical composition in Murciano-Granadina goats ($n = 20$) during early-, middle-, and late-lactation according to the F:C ratio[1]

	Early		Mid		Late					
	35:65	35:65	50:50	35:65	65:35	35:65		P-value		
Item	FCL[1]	FCL	FCM	FCL	FCH	FCL	SEM	Diet	Stage	Diet × stage
Milk production, kg/d	2.84	2.95	1.54[a]	1.57[b]	1.54[a]	1.85[b]	0.623	0.023	0.048	0.40
Composition, %										
Dry matter	13.6	13.4	15.6[b]	14.7[a]	13.7	13.2	1.02	0.017	0.75	0.34
Fat	4.86	4.44	5.92[b]	4.93[a]	4.62[b]	3.94[a]	0.774	0.001	0.047	0.37
Protein	3.41	3.57	4.37	4.38	3.86	3.71	0.357	0.92	0.001	0.18
Lactose	4.57	4.74	4.66[a]	4.81[b]	4.55[a]	4.90[b]	0.279	0.001	0.29	0.19

[1]FCL, forage-to-concentrate ratio low; FCM, forage-to-concentrate ratio medium; FCH, forage-to-concentrate ratio high.
[a,b]Means, within each stage of lactation, with different superscript differ ($P < 0.05$).

Table 8. FA composition (g/100 g of identified FA) of milk fat in Murciano-Granadina goats ($n = 20$) during early-, middle-, and late-lactation according to the F:C ratio[1]

	Early		Mid		Late					
	35:65	35:65	50:50	35:65	65:35	35:65		P-value		
Item	FCL	FCL	FCM	FCL	FCH	FCL	SEM	Diet	Stage	Diet × stage
C4:0	0.43	0.39	0.35	0.34	0.39	0.39	0.034	0.08	0.07	0.94
C6:0	1.41	1.37	1.12	1.15	1.22	1.40	0.131	0.76	0.08	0.84
C8:0	2.70	2.80	2.15	2.31	2.22	2.75	0.294	0.90	0.06	0.73
C10:0	12.8	13.3	11.0	12.3	10.4	13.3	1.21	0.70	0.06	0.76
C12:0	6.68	7.46	6.84	8.87	5.12	7.14	1.216	0.26	0.38	0.92
C13:0	6.70	6.70	5.50	5.33	4.99	5.33	0.747	0.41	0.97	0.65
C14:0	13.5	13.4	14.6	14.3	13.0	12.7	0.726	0.21	0.12	0.93
C14:1	0.15	0.20	0.23	0.32	0.15	0.17	0.065	0.57	0.62	0.86
C15:0	1.11	1.21	0.69[a]	1.30[b]	0.83[a]	1.07[b]	0.230	0.009	0.14	0.48
C15:1	0.00	0.00	0.00	0.00	0.57	0.00	0.233	0.34	0.24	0.40
C16:0	37.3	37.1	41.4[b]	38.4[a]	45.0[b]	40.9[a]	3.03	0.007	0.011	0.05
C17:0	0.49	0.57	0.33[a]	0.63[b]	0.29[a]	0.38[b]	0.139	0.044	0.58	0.09
C17:1	0.30	0.27	0.32[b]	0.30[a]	0.25[b]	0.20[a]	0.043	0.020	0.29	0.05
C18:0	7.87	7.59	8.14[b]	7.37[a]	8.12[b]	6.81[a]	0.508	0.047	0.20	0.09
C18:1n9c	1.18	1.01	1.47	0.47	1.43	0.65	0.409	0.67	0.19	0.81
C18:2n6t	0.31	0.22	0.33	0.29	0.28	0.28	0.035	0.19	0.15	0.94
C18:2n6c	5.05	4.72	3.84	4.38	3.90	4.37	0.468	0.56	0.56	0.81
C20:0	0.15	0.13	0.16	0.17	0.20	0.15	0.024	0.16	0.17	0.36
C18:3n6	0.03	0.01	0.04	0.04	0.05	0.03	0.014	0.09	0.05	0.58
C20:1	0.08	0.07	0.06	0.08	0.06	0.07	0.007	0.99	0.29	0.84
C18:3n3	0.48	0.38	0.40[b]	0.35[a]	0.39[b]	0.32[a]	0.055	0.007	0.36	0.90
CLA 9c11t + 9t11c	0.84	0.81	0.66	0.86	0.71	1.10	0.152	0.31	0.05	0.53
C20:4n6	0.34	0.30	0.34	0.28	0.35	0.31	0.027	0.10	0.09	0.81
C22:0	0.04	0.02	0.08	0.07	0.09	0.09	0.028	0.49	0.42	0.99
Short-chain FAs	4.54	4.56	3.62	3.80	3.82	4.54	0.445	0.38	0.25	0.56
Medium-chain FAs	26.2	27.4	23.4	26.5	20.5	25.8	2.56	0.46	0.37	0.45
Long-chain FAs	69.3	68.0	73.0	69.7	75.6	69.7	2.86	0.40	0.33	0.33

[1]FCL, forage-to-concentrate ratio low; FCM, forage-to-concentrate ratio medium; FCH, forage-to-concentrate ratio high.
[a,b]Means, within each stage of lactation, with different superscript differ ($P < 0.05$).

gland, although we have not been able to discriminate the different origin. In this study, greater ($P < 0.05$) concentrations of long-chain FA were detected with FCM and FCH due mainly to the lower intake caused by the higher fiber content in the diet, which also led to more NEB and greater body fat mobilization compared with feeding FCL.

Concentration of C16:0 increased ($P < 0.05$) for diets higher in fiber; 38.44 g/100 g was the value obtained in goats fed FCL and, 41.37 and 45.00 g/100 g with FCM and FCH diets, respectively. Milk C16:0 results mainly from de novo FA synthesis in mammary tissue using acetate produced in the rumen during fiber digestion, with CH_4 output being positively correlated with milk C16:0 (Fievez et al., 2012). Milk concentrations of C15:0 and the sum of C17:0 and C17:1 were positively related to propionate concentration in the rumen as these are synthesized by microbial metabolism of propionate (Fievez et al., 2012; Vlaeminck et al., 2015). Differences ($P < 0.05$) between FCL and other diets were observed in the present study. When concentrations of FA C15:0, C17:0 and C17:1 were summed up, we detected greater concentrations with the FCL diet followed by FCM and FCH (1.96, 1.33, and 1.36 g/100 g, respectively), which corresponds to a gradual decrease in content of dietary concentrate. Further, propionate production is negatively related to CH_4 production suggesting a negative relationship between milk odd-chain FA concentration and CH_4 production (Van Lingen et al., 2014). Thus, both the tendency for lower milk fat content (Table 6) along with greater concentrations of C15:0, C17:0, and C17:1 in milk with the FCL diet can be related to the lower F:C ratio.

Milk ∑ > C16, C18, and C18:1n9c are associated with NEB, whereas milk ∑ C10:0 to 15:0, C16:0 and ∑ OBCFA < C16 are associated with positive energy balance (Fougère et al., 2018). In our study, ∑ > C16 in goats experiencing NEB were 16.38 g/100 g on average and 15.03 g/100 g when goats were in positive energy balance. Greater content of C18:1n9c (1.27 vs. 0.56 g/100 g) during negative compared with positive energy balance possibly reflected a larger contribution of mobilized fat (Fievez et al., 2012), although we have to be sentient the diet contribution because, diets had fat palm added that was rich in C18:0. For ∑ < C16, the concentrations observed averaged 43.42 and 45.29 g/100 g during negative and positive energy balance, respectively. Because of the known associations among NEB, lipomobilization and milk FA composition, these results were expected (Fougère et al., 2018; Billa et al., 2020).

Metabolites

As treatments (diet and stage of lactation) affected energy balance, we hypothesized that milk metabolites could be used as indicators of nutritional status in the goats. Urine, plasma, and milk metabolites are reported in Table 9. We observed an interaction between the F:C ratio and stage of lactation for ammonium in urine, plasma glucose, and milk uric acid. In urine, compared with early- and mid-lactation, ammonium was lower at mid-lactation with both FCM and FCL diets. Urea and uric acid in urine followed the opposite tendency, greater in mid- than early- and late-lactation. Compared with other stages, plasma ammonium was also lower in mid-lactation, but plasma urea followed a different pattern. No effect of stage of lactation was observed, and greater ($P < 0.05$) values of plasma urea were detected when dietary F:C ratio increased (8.10, 8.62, and 9.01 mM for FCL, FCM, and FCH respectively). A similar trend was observed for milk urea with greater values when the F:C ratio increased (6.60, 7.50, and 8.39 mM for FCL, FCM, and FCH respectively), and greater values at late-lactation compared with other stages (7.74, 6.88, and 6.52 mM for late-, mid-, and

Table 9. Urine, plasma, and milk metabolites in Murciano-Granadina goats ($n = 20$) during early-, middle-, and late-lactation according to the F:C ratio[1]

	Early		Mid		Late					
	35:65	35:65	50:50	35:65	65:35	35:65		P-value		
Item	FCL	FCL	FCM	FCL	FCH	FCL	SEM	Diet	Stage	Diet × stage
Urine										
Ammonium, mM/L	568	581	109[b]	77[a]	574[a]	599[b]	252.4	0.039	0.047	0.050
Urea, mM/L	353	322	486	475	480	474	73.5	0.13	0.001	0.30
Uric acid, µM/L	396	406	955[a]	1150[b]	216	232	395.2	0.017	0.008	0.26
Plasma										
Ammonium, mM/L	198[a]	207[b]	111[a]	118[b]	165[a]	177[b]	40.3	0.003	0.002	0.26
Urea, mM/L	8.51	8.15	8.62[b]	7.95[a]	9.01[b]	7.79[a]	0.457	0.001	0.73	0.34
Protein, g/L	82.3	79.1	82.9[a]	85.6[b]	81.5	82.6	4.9	0.043	0.84	0.26
Glucose, mM/L	2.57	2.68	2.87[a]	3.49[b]	1.95[a]	2.61[b]	0.500	0.001	0.007	0.46
Fructosamin, µM/L	227	226	249	252	272	269	19.8	0.38	0.001	0.31
Fosfolipid, mM/L	1.62	1.48	1.65[b]	1.44[a]	1.75[b]	1.41[a]	0.137	0.001	0.66	0.22
BHB, mM/L	0.50	0.48	0.49[b]	0.45[a]	0.39[b]	0.32[a]	0.069	0.047	0.003	0.33
NEFA, mEq/L	375	305	1028[b]	348[a]	1070[b]	214[a]	385.7	0.001	0.98	0.47
Triglyceride, mM/L	0.17	0.18	0.13	0.14	0.13	0.13	0.024	0.44	0.001	0.43
Milk										
Urea, mM/L	6.5	6.6	7.5[b]	6.3[a]	8.4[b]	7.1[a]	0.80	0.001	0.033	0.63
Uric acid, mM/L	103	122	175[b]	147[a]	33[a]	36[b]	58.2	0.013	0.001	0.046
Free glucose, µM/L	124	128	137	141	149	151	10.7	0.72	0.06	1.00
Glucose-6 phosphate, µM/L	171	151	261[b]	207[a]	122[b]	73[a]	65.9	0.001	0.001	0.92
BHB, µM/L	185	172	159[b]	113[a]	85[b]	71[a]	47.8	0.027	0.001	0.34
Isocitrate, mM/L	99	101	116[b]	106[a]	106[b]	75[a]	13.7	0.017	0.003	0.46

[1]FCL, forage-to-concentrate ratio low; FCM, forage-to-concentrate ratio medium; FCH, forage-to-concentrate ratio high.
[a,b]Means, within each stage of lactation, with different superscript differ ($P < 0.05$).

early-lactation, respectively). Milk urea was positively correlated with urea concentration in blood plasma, as demonstrated by Spek et al., (2013).

Plasma and milk urea concentrations are currently used as nutritional indicators in ruminants because they are closely related to digestive tract activity and endogenous ammonia production, the latter being associated with gluconeogenesis (Giovanetti et al., 2019). Thus, milk urea was lower when increasing energy intake and content of the diets (11 and 9 MJ ME/kg DM for FCL and others) suggesting increases in the uptake of N by rumen microorganism and reductions in amino acid utilization for gluconeogenesis, both of which reduced N waste (Giovanetti et al., 2019; Girard et al., 2019). However, the low values of blood glucose at late-lactation and especially for FCH diet are difficult to explain. Kiani et al. (2015) compared blood glucose levels in adult, nonpregnant llamas, sheep, and goats and reported average values of 7.1, 3.5, and 3.6 mM/L, respectively. Therefore, it could be possible that there might have been issues related to sampling, handling, and/or storage. The greater dietary F:C ratio increased ($P < 0.05$) milk urea content. Overall, lower DMI and N intake ($P < 0.05$) were observed at late-lactation probably due to lower requirements associated with the gradual decrease in milk yield. High milk urea values at late-lactation were probably due to an excess of dietary protein concentration (16% CP on DM bases for all diets) relative to the animal's needs.

An interaction was also detected for milk uric acid, where greater values were obtained with FCM followed by FCL and FCH (175, 102, and 33 mM, respectively). Evaluating stage of lactation, greater uric acid was detected in mid followed by early and late lactation (161, 113, and 35 mM, respectively). Although some have questioned the predictive value of uric acid in milk (Timmermans et al., 2000), this compound is considered a potential biomarker of ruminal N flow and feed efficiency (Billa et al., 2020) as it represents degradation products from microbial-synthesized purines. However, at late-lactation a decrease in milk uric acid for both diets were found, and failed as milk biomarker of ruminal function at this stage of lactation.

Plasma glucose is the obligatory precursor needed for milk lactose synthesis, while glucose-6 phosphate is an intermediate metabolite. Glucose content in plasma was greater with the FCL diet containing a greater content of cereal grain of greater fermentability, hence, explaining the greater lactose content in milk. As discussed in a previous section, and due to unknown reasons, plasma glucose concentrations were low in this study, particularly at late-lactation. A similar effect was reported by Cannas et al., (2013) when ewes were fed a diet with greater cereal grain than soybean hull content. They reported greater plasma glucose with the cereal grain diet and concluded it was due to greater gluconeogenesis in the liver (Cannas et al., 2013).

No effect of diet and stage of lactation were detected for milk glucose, and milk glucose-6 phosphate followed the opposite trend than plasma glucose. This response might have been related with lactose synthesis and milk fat synthesis because glucose-6 phosphate is an intermediate in glycolysis and also furnishes the pentose phosphate pathway to synthesize NADPH in mammary cells (Larsen et al., 2016). Plasma fructosamine is an indicator of medium-to-long-term glucose concentrations in the blood (Roche et al., 2013), but there was no relationship between these metabolites in the present study. Zamuner et al., (2020a) examined if differential productivity in dairy goats was related to differences in some aspects of energy metabolism. These authors found greater demand for glucose placed by the mammary gland of high milk yield goats at early-lactation compared with low milk yield goats.

High rates of fat mobilization (from −99 to 35 kJ TE/ kg of $BW^{0.75}$) led to marked increases in plasma concentrations of NEFA, BHB and accumulation of triacylglycerol in the liver, and also could increase milk fat content. Thus, it appeared that the greater NEFA observed with diets higher in F:C ratio and NEB was associated with fat mobilization. Plasma BHB are well known to be related to the incomplete β-oxidation of mobilized body fat and BHB could be used not only for energy but also for the synthesis of milk FA (Xu et al., 2020). As NEFA are particularly rich in long-chain FA, such as C18:1n9c and C18:0 (Hostens et al., 2012), concentrations in milk fat of those FA might be linked to NEB. The major NEFA released are C16:0, C18:0, and C18:1n9c (Table 7), and elevated concentrations in milk fat were identified as valuable early warning biomarkers of NEB (Vlaeminck et al., 2015). In the current study, a possible conversion of C18:0 to C18:1n9c in the mammary gland though the action of Δ9-desaturase (Toral et al., 2016) could explain the positive relationship between milk fat C18:1n9c concentration and blood plasma NEFA.

Under NEB, body fat mobilization and high requirements for glucose for lactose synthesis coincide with high plasma concentration and ketone bodies (Xu et al., 2020). In the present study, decreases in BHB content in plasma and milk were observed when goats received the FCL diet and when fat mobilization was lowest (at mid- and late-lactation). These effects in goats fed the FCL diet may reflect in part modifications of ruminal fermentation and VFA profiles because plasma BHB is derived in part from ruminal butyrate, which in this diet decreased in favor of propionate. Thus, the decrease in milk BHB in goats fed FCL and as lactation progressed (179, 136, and 78 μM for early, mid- and late-lactation, respectively) was in line with the differences in ruminal fermentation characteristics and the state of NEB. Zamuner et al., (2020b) examined the relationships between blood NEFA, BHB, and glucose during the transition period in dairy goats. They observed moderate to strong relationships between NEFA and BHB concentrations and between NEFA and glucose concentration throughout the transition period. Their results suggested that simultaneous BHB and glucose determination provide an improved indicator of the fat mobilization, energy status, and hyperketonemia in dairy goats. Our results show a similar trend; high NEFA and low glucose along with high BHB. Although NEFA has been proposed as a better predictor of NEB than BHB and glucose, testing for NEFA is expensive and challenging and, in contrast, glucose and BHB look promising.

In our study, we observed that when milk-free glucose decreased in a period of NEB isocitrate increased. The FCL diet led to lower isocitrate (95 vs. 111 mM for FCL and others diets, respectively) and lower values also were detected at late compared with mid- and early-lactation (91, 111, and 100 mM, respectively). Bernard et al., (2020) feeding a basal diet containing corn oil and wheat starch detected greater milk isocitrate in cows, and decreased isocitrate with the same diets in goats. Isocitrate is a substrate for NADPH synthesis via the isocitrate dehydrogenase pathway, which supplies the majority of NADPH used in milk fat synthesis in ruminants (Faulkner and Peaker, 1982). Thus, increased milk isocitrate content in cows may result from a decreased in de novo FA synthesis (Faulkner and Peaker, 1982). In contrast to goats, milk isocitrate concentration decreased in goats fed starchy diets without decreases in de novo synthesized FA concentrations (Fougère et al., 2018).

Further research is needed to determine whether isocitrate is a valuable biomarker in goats.

The choice of multiple milk metabolites and FA, as non-invasive method, to evaluate the degree of change in energy mobilization, or risk of diseases or in environments where food energy availability is scarce, would be useful as milk samples can be collected automatically. However, not in all cases the choice of multiple metabolites and FA as milk biomarkers has been successful (Larsen et al., 2016), thus, further investigation on this topic appears warranted.

Conclusions

This work was developed to find changes in nutrient balance in goats when fed different F:C ratio during lactation. When forage content in the diet increased from 35% to 65%, both CH_4 emission and fat mobilization increased, potentially to help the animal meet metabolic costs associated with lactation. However, the increase in NEFA contradicted the continuous rise of BW, besides the lower BHB values observed in late- compared with mid-lactation. Future research in this area with dairy goats should include sampling at various stages of lactation to confirm the utility of the milk biomarkers detected.

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Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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