Supplementing goat kids with coconut medium chain fatty acids in early life influences growth and rumen papillae development until 4 months after supplementation but effects on in vitro methane emissions and the rumen microbiota are transient

Sieglinde Debruyne,*,† Alexis Ruiz-González,* Einar Artiles-Ortega,*,§ Bart Ampe,† Wim Van Den Broeck,‡ Ellen De Keyser,# Leen Vandaele,† Karen Goossens,† and Veerle Fievez*,1

*Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653- Block F, 9000 Ghent, Belgium; †Department of Animal Sciences and Aquatic Ecology, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Animal Sciences Unit, Scheldeweg 68, Melle 9090, Belgium;§Department of Veterinary Medicine and Zootechnics, Faculty of Agricultural Sciences, Central University "Marta Abreu" de Las Villas, Carretera a Camajuaní km 5½, Santa Clara 54830, Cuba; ‡Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium; #Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Plant Sciences Unit, Caritasstraat 39, Melle 9090, Belgium

ABSTRACT: The aim of this study was to investigate the methane (CH_4) reducing potential of a combination of prenatal and/or postnatal treatment with coconut oil medium chain fatty acids (**CO MCFA**) in goat kids. The hypothesis is that influencing rumen function during early life has more chances for success than in the adult life, related to the resilience of the mature rumen microbiota. Forty-eight pregnant does were split into two experimental groups: treated does (**D+**) received 40 g/d of CO MCFA in a test compound feed, while control does (**D−**) received a control compound feed, during the last 3 wk of gestation. Twin kids from 10 does of each group were split up into a treated (**K+**) and nontreated (**K−**) group, resulting in four experimental groups: D+K+, D+K−, D−K+, and D−K−. The K+ kids received 1.8 mL/d of CO MCFA from birth until 2-wk postweaning (11 wk). Irrespective of treatment, the experimental rearing conditions resulted in absence of rumen protozoa at all sampling times, assessed by quantitative PCR (**qPCR**). In vitro incubations with rumen fluid at 4 wk old showed 82% lower CH_4 production of inoculum from D+K+ kids compared to D−K− kids ($P = 0.01$). However, this was accompanied by lower total volatile fatty acids (**tVFA**) production ($P = 0.006$) and higher hydrogen accumulation ($P = 0.008$). QPCR targeting the *mcrA* and *rrs* genes confirmed a lower abundance of total methanogens ($P < 0.02$) and total eubacteria ($P = 0.02$) in D+K+ kids at 4 wk old. Methanogenic activity, as assessed by *mcrA* expression by RT-qPCR, was also lower in these kids. However, activity did not always reflect methanogen abundance. At 11 and 28 wk old, prenatal and postnatal effects on in vitro fermentation and rumen microbiota disappeared. Nevertheless, lower milk replacer intake in the first 4 wk resulted in reduced BW in K+ kids, persisting until 28 wk of age. Additionally, differences assigned to postnatal treatment were found in papillae density, width, and length in

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[1]Corresponding author: Veerle.Fievez@ugent.be

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different areas of the rumen, recorded at 28 wk old. Conclusion: prenatal and postnatal supplementation with CO MCFA reduced in vitro CH_4 emissions until 4 wk old by depressing methanogen abundance and activity but at the expense of rumen fermentation and eubacterial abundance. Unfortunately, daily gain of K+ kids was suppressed. Some rumen papillae characteristics differed at 28 wk old due to postnatal treatment which ended at 11 wk old, indicating rumen papillary development can be affected by the early-life nutritional circumstances.

Key words: goat kids, in vitro enteric methane, medium chain fatty acids, prenatal/postnatal programming, (RT-) qPCR, rumen papillae

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INTRODUCTION

Nearly 50% of global greenhouse gas emissions coming from livestock farming is methane (**CH_4**) (Gerber *et al.*, 2013). Microbial fermentation in the rumen is the main contributor to these CH_4 emissions. Coconut oil (**CO**), rich in medium chain fatty acids (**MCFA**) (Hristov *et al.*, 2009), is a promising CH_4-reducing feed supplement (Machmüller 2006). In the rumen, it might act as a defaunating agent (Newbold and Chamberlain, 1988), affecting methanogens indirectly (Finlay *et al.*, 1994) but it also might affect methanogens directly (Machmüller *et al.*, 2003). However, the CH_4-reducing effects of CO have not yet been proven to be persistent, in part due to generally short-term studies, while microbial adaptation is likely to occur given the resilient microbial community in the mature rumen (Weimer 2015). Manipulating rumen function in early life (**EL**) could modify the microbiota in a targeted and permanent manner, using less supplement, while potentially resulting in long-lasting CH_4 reductions (Yáñez-Ruiz *et al.*, 2015). The rapid initial colonization of the rumen (Jami *et al.*, 2013; Rey *et al.*, 2014) illustrates the need for early interventions in very young animals for manipulation of these processes. Recent studies (Yáñez-Ruiz *et al.*, 2010; Abecia *et al.*, 2013) explored early postnatal nutritional interventions, while fewer studies also investigated the potential of prenatal programming (De Barbieri *et al.*, 2015a, 2015b). Accordingly, we hypothesized that supplementation with CO MCFA in EL could result in persistent lower CH_4 production, through suppressing effects on the rumen microbiota. As a change in rumen fermentation is expected, this suggests rumen papillae development could be affected as well (Malhi *et al.*, 2013). These changes could persist after ceasing the treatment.

MATERIALS AND METHODS

The experiment was approved by the ethical committee of the Faculty of Veterinary Medicine (Ghent University, Belgium, approval number EC2015/12) and the experiment was conducted at the experimental stables of the Laboratory for Animal Nutrition and Product Quality (Melle, Belgium). All persons involved with animal care and sampling had previously obtained the necessary FELASA (Federation of European Laboratory Animal Science Associations) certificates.

Animals, Treatments, and Experimental Design

Prenatal treatment. A flock of 48 synchronized pregnant dairy goats (Saanen) from a commercial farm (Zele, Belgium) was randomly split into two groups (**D−**, control and **D+**, treated) for the last 3 wk of gestation. Both groups were offered 2 × 0.5 kg/d of a compound feed, which only differed in type of oil included (at 40 g/ kg DM), i.e. palm oil for the D− group and unesterified MCFA from CO (AVEVE, Merksem, Belgium) for the D+ group. Both concentrates were iso-energetic. Table 1 shows the fatty acid composition of the palm oil and unesterified MCFA product from CO used in the compound feed. All does were fed the same roughage mixture: prewilted grass silage (ad libitum) from the beginning of the dry period (6 wk before parturition) until 2 wk before parturition, followed by a mixture of prewilted grass silage, maize silage, and sugar beet pulp (200/720/80 g/kg DM respectively, ad libitum) during the last 2 wk of gestation.

Postnatal treatment. Within each group, the objective was to obtain 10 does that gave birth to preferably two male kids. This objective could not be obtained within this flock, and hence four does with

Table 1. Fatty acid composition of palm oil (control product) and unesterified MCFA product from coconut oil used in compound feeds of does and supplemented to the K+ kids (MCFA from coconut oil only)

Item	MCFA from coconut oil	Palm oil
Fatty acid, g/kg		
C8:0	75	ND
C10:0	60	ND
C12:0	470	2
C14:0	185	10
C16:0	93	415
C18:0	30	43
C18:1 *n*−9	70	368
C18:2 *n*−6	17	99
C18:3 *n*−3	ND	34

ND = not detected.

one male and one female kid were used. Also, two individual kids from separate D+ does, but sharing the same sire, were used by lack of multiple kids being born from the group of 48 does available for this experiment. All 40 kids used in the experiment were born within a time frame of 7 days, and from a multiparous dam. The two kids from each doe were taken up into the trial and randomly assigned to a control (**K−**) or treated (**K+**) kids group. Four experimental conditions were created by combining prenatal and/or postnatal treatment: D−K− (n = 10), D−K+ (n = 10), D+K− (n = 10), D+K+ (n = 10). One kid of the D+K− group died at 21 d of age due to congenital heart failure. Postnatal treatment, initiated at the commercial farm, started within 6 hours after birth and continued until 2-wk postweaning (11 wk old; Figure 1). Dose of MCFA for the newborn kids was deducted from doe's dose, based on estimated BWs (doe: 40 g/d at 75 kg BW; Kid: 1.6 g or 1.8 mL/d at 3 kg BW). The coconut MCFA product (liquefied in glass pots using a hot water bath) (2 × 0.9 mL/d) was squirted with a small syringe into the back of the mouth of the kid, twice a day before milk feeding. BW was recorded before morning feeding every 2 wk until weaning (completed at 9 wk for all kids), at the end of the indoor stable period (13 wk) and at euthanasia (28 wk). Kids were sampled for rumen fluid at 4 wk, 11 wk (oesophageal tube), and 28 wk of age (after euthanasia). At 28 wk old, rumen wall samples were collected for analysis of papillae development.

Diets and Housing

Does stayed at the commercial farm after kidding, while kids stayed there for 1 d, separated from the dam. Kids received mixed unpasteurized colostrum three times on day 1 from does that recently gave birth and were housed on straw under a heat lamp. Kids were transported at day 2 to the experimental stable. From birth until 13 wk old, kids were housed in pairs of the same experimental condition in pens equipped with rubber mats and bedded with straw (1.8 × 2.2 m; width × length). The pens were constructed to avoid physical contact between the neighboring kids during the first wk. Kids were fed colostrum from the second and third milking of all does that recently gave birth, twice a day during the first 2 d at the stable. Afterward, milk replacer (200 g/L, Denkamilk Capri Ovi, Denkavit, Voorthuizen, the Netherlands) was fed by bottle twice a day (08:30 h and 16:00 h) until kids were able to drink in pairs from a suckling bucket (per feeding max. 2 L/2 kids). From 6 wk onward, milk powder concentration was lowered from 200 g/L to 175 g/L. At 7 wk, the amount of milk replacer was reduced to 1 L/2 kids per feeding and at 8 wk, feeding frequency was lowered to once daily (08:30 h), until kids were weaned at 9 wk old. Concentrate (Capri 181, AVEVE, Merksem, Belgium) was provided twice a day from 1 wk of age onward (per feeding: 50 g/2 kids at week 1 up to max. 500 g/2

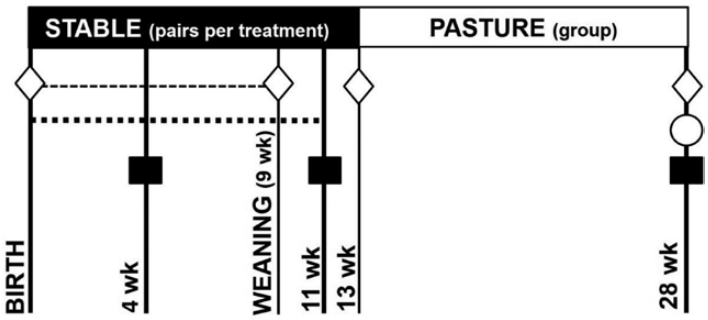

Figure 1. Sampling and data collection schedule of goat kids. Kids were weighed (◊) from birth until weaning every 2 wk (----), at the end of the stable period and at 28 wk old. The bold dashed line (▪ ▪ ▪ ▪) indicates postnatal treatment period. At three ages (4 wk, 11 wk, 28 wk), and rumen fluid were sampled (■). At 28 wk old, additional rumen wall samples (○) were collected after euthanasia to assess papillae development.

kids). Kids had ad libitum access to water and hay during the whole indoor stable period. The treatment period lasted until 2 wk post weaning (11 wk old), and kids were kept indoors until 13 wk old. Afterward, they were placed outside on a pasture (67 × 50 m) to graze in group. Female kids were physically separated from males during the pasture period. On the pasture, all kids were daily supplemented (08:30 h) with concentrate (max. 500 g/kid) until the end of the trial (28 wk). Hay was supplemented when fresh grass supply from the pasture was not sufficient.

Rumen Fluid Collection and Euthanasia

At the ages of 4 and 11 wk old, rumen fluid was sampled, after a night of fasting and before morning feeding through esophageal tubing using a self-made plastic transparent tube (outer diameter 15 mm) connected to a plastic erlenmeyer and a vacuum pump. A volume of 30-mL fluid and digesta was collected per kid for subsequent in vitro incubations the same day. Materials were rinsed with hot water between sampling different kids and a 70% ethanol in water solution was used to disinfect. At approximately 28 wk old, kids were brought back into the stable on four different days (in the afternoon) within a 2-wk period, in three groups of 10 and one group of 9 kids. Kids were fasted overnight in the stable, with access to water, and euthanized the next day between 08:30 h and 09:00 h. First Xylazine (4 mL/kid intravenously) was given to sedate the kids, then Nembutal (Release, 10 mL/kid) was used to euthanize. One group of nine kids (one D+K+, two D+K−, three D−K+, and three D−K− kids) was removed from the data set for the incubations at 28 wk because of a feeding error on the day of euthanasia, which resulted in two kids with clear signs of acute rumen acidosis (rumen pH: 4.15 and 4.65). As results of in vitro incubations are largely influenced by feed remainders in the rumen inoculum, the statistical analysis for these in vitro fermentation parameters was done with the remaining 30 kids which were correctly fasted overnight before euthanasia (D+K+ $n = 9$, D+K− $n = 7$, D−K+ $n = 7$, D−K− $n = 7$). Within 1.5 hours after euthanasia, kids were weighed and exsanguinated, the rumen was dissected and opened and approximately 100 mL of rumen fluid was collected by squeezing the fibrous contents. In accordance to sampling at 4 and 11 wk, polypropylene tubes (15 mL) were completely filled with rumen fluid before closing with a screw cap, to minimize air contamination. Tubes were stored in thermos flasks filled with warm water for transportation to the lab, at walking distance (3 min) from the stable. Additionally, for all kids at all three sampling times, three aliquots of approximately 1.5 mL of rumen fluid were pipetted into screw cap cryovials and immediately snap-frozen with liquid nitrogen (in the stable). The cryovials were then stored at −80°C until later microbial analysis.

In Vitro Incubations (CH_4 and Rumen Fermentation)

In vitro batch incubations with fresh rumen fluid (Vlaeminck *et al.*, 2014) were performed within 15 min after sampling. Rumen fluid was first sieved (1 mm pore size) and mixed with a phosphate-bicarbonate buffer at a ratio of 1:4, under constant CO_2 flushing. The pH was measured before and after addition of the buffer. Then, 14 mL (at 4 and 11 wk, due to a limited amount of inoculum which could be retrieved at these ages) or 24 mL (at 28 wk) of the mixture was injected into a glass penicillin flask (volume 120 mL), closed with a rubber stopper. Prior to inoculum-buffer addition, flaks had been supplemented with 150 mg (4 and 11 wk) or 250 mg (28 wk) of dried grass silage, wetted by 1 mL of distilled water and air-tight closed. Flasks were then flushed with CO_2 to create anaerobic conditions. Overpressure of gas was released and 1 mL of ethane (C_2H_6) was injected as an internal standard for later gas measurements. Flasks were incubated in duplicate for 4 h and 24 h (39°C) on a shaking platform to investigate differences in speed of fermentation processes by the different inocula (4 h) and total potential to ferment the added substrate (24 h) by the rumen micro-organisms in the inocula. After incubation, flasks were put for 5 min in an ice bath to stop fermentation processes. Built-up gas overpressure was released and gas composition (CH_4, H_2, CO_2, C_2H_6) was measured by micro gas chromatograph (3000 micro-GC, Agilent, USA) (Hassim *et al.*, 2010). A standard series of eight flasks, first flushed with CO_2 and injected with known volumes of H_2, CH_4, and C_2H_6, were also analyzed for each incubation run. Areas were recovered and regression curves were fitted based on CH_4/C_2H_6 and H_2/C_2H_6 ratios from the standard series flasks, which was used to calculate the net production of CH_4 and H_2 accumulation in each flask. Average values were calculated from duplicate flasks. Volatile fatty acid (**VFA**) analysis (C2:0, C3:0, iso-C4:0, C4:0, iso-C5:0, C5:0, C6:0) of incubated rumen fluid was performed (Gadeyne *et al.*, 2015). Also nonincubated fresh rumen samples were analyzed

(blanks) and these VFA concentrations were used to calculate net production of VFA in the incubated flasks.

Rumen Wall Sampling

The reticulum, rumen, and omasum were dissected together and weighed full, after which the rumen was separated. The rumen was opened with an incision of approximately 15 cm starting from the dorsal side of the esophagus crossing the dorsal sac. After sampling of rumen content for in vitro incubations, the remaining rumen content was discarded. The empty rumen was flushed with tap water to remove particles and then weighed. After this, the rumen was presented on a table with the esophageal opening pointing to the left. The inner rumen wall was exposed by further cutting the rumen open over the left lateral side. Four anatomical areas were selected for papillae development analysis: (1) the ruminal atrium, (2) the ventral sac, (3) the caudodorsal blind sac, and (4) the caudoventral blind sac (Figure 2). A patch of 3 × 3 cm was cut from each of the four areas and cleaned carefully with distilled water. Patches were immediately pinned on cardboard pieces with needles and immersed in glass pots (150 mL) filled with phosphate-buffered 4% formaldehyde solution (pH 7.4) for histomorphometric analysis.

Histological Evaluation and Histomorphometry of Rumen Wall Samples

On the same day of sampling, rumen wall samples were analyzed for papillae density (number/cm^2) by stereomicroscopy (Olympus SZX7, Olympus, Berchem, Belgium) (power objective lens: 8×), using the "forbidden lines method" (Gundersen 1977). Samples were further left to fixate in phosphate-buffered 4% formaldehyde solution (pH 7,4) for 24 h. Afterward, 1 cm^2 was cut from the sample and divided into two subsamples of 0.5 cm^2. Subsamples were placed in fixating cassettes and flushed with tap water to remove formaldehyde from the tissue. Next, fixating cassettes were put in a tissue processor (Microm STP420D, Fisher Scientific, Loughborough, UK) for dehydrating, clearing, and paraffin infiltration (Böck and Romeis 1989). Sections (8 µm) were made from the paraffin-imbedded subsamples using a microtome (Microm HM 360 Cool-cut, Thermo Fisher Scientific, Waltham, MA, USA) and placed on a glass microscope slide. Paraffin was then removed from the section by immersing the glass slide in xylene, decreasing alcohol series and distilled water (Böck and Romeis 1989), and stained with hematoxylin and eosin (H&E). Tissue samples were further preserved by adding artificial resin (DPX [mixture of distyrene, a plasticizer, and xylene]) to the section, sealed with a glass coverslip and left to dry. Morphometric analysis of sections was performed using light microscopy (Olympus BX-OCB, Olympus, Berchem, Belgium) (power objective lens: 4 or 10× depending on the sample). For each rumen area of each goat, length from base to tip and width of the base from 10 papillae (e.g. 5 papillae from each subsample) were measured using image analysis software (Olympus CellSense Dimension, Olympus, Berchem, Belgium). Total surface area of papillae per square centimeter mucosa (**TSA**) was determined as length × width × 2 × papillae density (Ragionieri *et al.*, 2016).

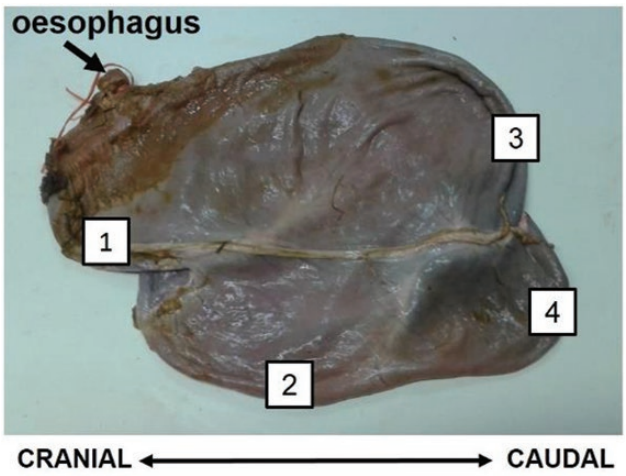

Figure 2. Rumen wall sampling areas. 1: bottom of the ruminal atrium 2: bottom of ventral sac, 3: caudal central part of dorsal blind sac, 4: caudal central part of ventral blind sac.

Total RNA and DNA Extraction From Rumen Fluid

Approximately 1 mL of frozen rumen fluid and digesta, snap-frozen and stored in cryotubes under −80°C until processing, was ground with a cooled pestle under addition of liquid nitrogen. For total RNA extraction, between 70 and 90 mg of ground sample was weighed and stored in Eppendorf tubes on liquid nitrogen, along with 0.3 g zirconia-silica beads (diameter 0.1 mm, Lab Services, Breda, The Netherlands). At the same time, between 200 and 220 mg of ground sample and 0.8 g zirconia-silica beads (diameter 0.1 mm) were stored at −80°C for subsequent DNA extraction. The RNA extraction protocol was adapted from the Qiagen RNeasy Plus Mini kit (Qiagen Benelux B.V., Antwerp, Belgium).

This consisted of initial lysis (lysis buffer and β-mercapto-ethanol) and homogenization (bead beating at 30 Hz for 2 × 1 min with 30 s of rest in between; Retsch Tissue Lyser, Qiagen Benelux B.V.), removal of genomic DNA by gDNA eliminator columns, binding of total RNA to RNeasy Mini spin columns, washing and elution in 30 µL of RNase-free water. The Nucleospin RNA Kit (Filter Service S.A., Eupen, Belgium), containing an on-column DNase incubation step, was subsequently used for further clean-up of RNA. Finally total RNA was eluted in 30 µL RNase-free water. Nucleic acid concentrations, 260/280 ratios, 260/230 ratios were measured and contamination assessment was done using Nanodrop (ND1000 V3.8.1, Wilmington, USA) and RNA quality was checked for a number of random samples by capillary gel electrophoresis (Experion RNA StdSens Analysis Kit, California, USA; Supplementary Table 2 and 3). A noRT PCR control with primers for the eubacterial *rrs* (16S rRNA subunit) gene (Table 2), was performed for a subset of RNA samples to confirm absence of DNA. The RNA samples were stored at −80°C until Reverse Transcription.

For total DNA extraction, a protocol based on CTAB/phenol-chloroform lysis with beat-beating and ethanol precipitation (Popova *et al.*, 2010) was followed. The DNA samples were eluted in 30 µl of RNase-free water. Nucleic acid concentrations, 260/280 ratio and 260/230 ratio of all samples were measured using Nanodrop (ND1000 V3.8.1, Wilmington, USA) and the Quantifluor dsDNA system (Promega Benelux, Leiden, The Netherlands). The DNA samples were stored at −20°C until quantitative PCR (**qPCR**).

Reverse Transcription

Reverse transcription was performed using the Promega RT system (Promega Benelux) and random hexamer primers (0.5 µg, IDT, Leuven, Belgium), starting with ca. 100 ng RNA. Concentrations of cDNA were measured using Nanodrop (ND1000 V3.8.1, Thermo Scientific, Wilmington, USA).

Quantitative PCR

The qPCR assays were conducted according to the MIQE guidelines (Bustin *et al.*, 2009).

Abundance of total methanogens, eubacteria, and protozoa. The abundance of these microbial groups was quantified using a LightCycler480 Real-time PCR System (Roche Diagnostics, Penzberg, Germany). Target genes were: *mcrA* (methyl coenzyme-M reductase) and methanogenic *rrs* (16S ribosomal RNA subunit) for methanogens, eubacterial *rrs* (16S ribosomal RNA subunit) for total eubacteria and protozoal *rrs* (18S ribosomal RNA subunit) for total protozoa. Samples of a 100-fold dilution of the DNA extracts were used. Amplicon detection was done using SYBR Green technology (SensiFAST SYBR No-ROX kit, Bioline, London, United Kingdom). Reactions were set up *in duplo* in a white 384 well plate (Bio-Rad Laboratories N.V., Temse, Belgium). Each reaction contained, in a total volume of 10 µL, 5 µL SensiFAST SYBR (Bioline), 0.3 µL of each primer (10 µM), 2.4 µL of water, and 2 µL of DNA. The primers, annealing temperatures, and PCR efficiencies are listed in Table 2. Amplification protocols for total methanogens and eubacteria started with a temperature of 95°C for 2 min, followed by 45 cycles of 95°C for 5 s, 60°C for 10 s, and 72°C for 20 s. At the end, melting curve analysis was performed as follows: 95°C for 5 s, 65°C for 1 min, and heating to 97°C with a ramp rate of 0.06°C/ s. Data acquisition occurred 10 times for every °C. For total protozoa quantification, the same protocol was used, but annealing temperature was set to 57°C. Within each run, a standard curve was constructed

Table 2. Primers used in this study

Primer pair	Target	Sequence ′5 → ′3	Annealing T (°C)	PCR efficiencies DNA|cDNA	Reference
qmcrA F/qmcrA R	*mcrA* total methanogens	TTC GGT GGA TCD CAR AGR GC GBA RGT CGW AWC CGT AGA ATC C	60	83%|83%	Denman *et al.*, 2007
Met630 F/Met803 R	*16S rRNA* total methanogens	GGA TTA GAT ACC CSG GTA GT GTT GAR TCC AAT TAA ACC GCA	60	81%|78%	Hook *et al.*, 2009
Bac338 F/Bac518 R	*16S rRNA* total eubacteria	ACT CCT ACG GGA GGC AGC AG ATT ACC GCG GCT GCT GG	60	82%	Ovreås *et al.*, 1997
Syl316 F/Syl539 R	*18S rRNA* total protozoa	GCT TTC GWT GGT AGT GTA TT CTT GCC CTC YAA TCG TWC T	57	74%	Sylvester *et al.*, 2004

using a 10-fold dilution series of specific gBlock DNA (IDT; Supplementary Table 1), containing a strain-specific sequence for each primer, in order to determine the PCR efficiency (Table 2). The number of gene copies was calculated by converting the quantification cycle values (Cq) to gene copy abundances, taking into account the PCR efficiency as determined by the standard curve. If Cq >36, this was considered as no amplification. Data were analyzed using the LightCycler480 software version 1.5.1.62 (Roche). The results are presented as (log_{10}) number of gene copies/µL DNA extract (eubacteria and protozoa), or number of target gene copies relative to total number of eubacterial *rrs* copies (for *mcrA* and methanogenic *rrs*).

Activity of methanogens (mcrA gene expression). The same general qPCR protocol specifications and primers (Table 2) were used for the methanogenic activity measurements as for measurement of total methanogen abundance. The abundance of *mcrA* and methanogenic *rrs* transcripts (cDNA, 5-fold diluted) from methanogens in all samples was quantified using LightCycler480 software (Roche, Vilvoorde, Belgium). Corresponding noRT samples were included within each run. The Cq values of noRTs differed at least in five cycles from the samples. Subsequent RNA expression analysis was performed using the program qBase+ (Biogazelle, Zwijnaarde, Belgium; Hellemans *et al.*, 2007). Expression levels of *mcrA* were normalized using *rrs* as an internal reference gene and calculated using the mathematical model of Pfaffl (Pfaffl 2001) with gene specific amplification efficiencies, as calculated by the LightCycler480 software (Table 2).

Statistical Analysis

All goats within one experimental condition were regarded as statistical replicates. Results in tables are shown as least square mean averages. In the case of log-transformed parameters, back-transformed LS means are presented together with the 95% confidence intervals. Duplicate flasks in the in vitro incubations as well as the 10 papillae measured from one area (per kid) were considered analytical repeats, and mean values of these measurements were used for statistical analysis. Analysis of qPCR data was performed on log-transformed values. Descriptive statistics (mean and SE) were used to create graphs. Values were analyzed by the MIXED procedure in SAS Enterprise (SAS Inst. Inc., Cary, USA) with prenatal treatment, postnatal treatment and the interaction as fixed main factors, and mother identity (kids coming from the same doe) and sampling day as random factors. If data was not normally distributed, then it was log transformed for statistical analyses (i.e. in vitro H_2 accumulation and propionate production). When the test showed significant differences, Tukey's post hoc tests were carried out. Statistical significance was assigned to $P < 0.05$. Statistical tendency was assigned to $0.05 < P < 0.10$. When interaction effects were not significant, they were removed from the final model and only the main effects were presented.

RESULTS

BW and Daily Weight Gain

Kids' weight upon arrival at the experimental stables (day 2 after birth) was not affected by prenatal treatment of does (Table 3), whereas postnatal supplementation with CO MCFA had a negative impact on total milk replacer intake ($P < 0.0001$) (Figure 3A), particularly in the first 4 wk of age. At the age of 1 wk, postnatally treated kids (K+) consumed 25% less milk replacer compared to K− kids, but the difference was largest in weeks 2 and 3, when K+ kids consumed 32% and 31% less milk replacer than K− kids, respectively (Figure 3B). After week 4 of life, the differences in milk replacer intake were declining. In the week prior to weaning, all kids consumed the same amount of milk replacer. Mean total concentrate intake until 13 wk of D−K+ kids was largely influenced by intake of two kids (same pen). Without these two kids total concentrate intake was 18.6 kg DM/ pen (compared to 15.0 kg DM/pen, as presented in Figure 3A). However, total concentrate intake was not different between experimental groups whether or not this pen was excluded from the analysis. The lower milk replacer intake of K+ kids during the first weeks resulted in a reduced daily weight gain and BW during the first 4 wks of life (Table 3). The K+ kids retained a lower BW compared to K− kids during the entire postnatal treatment period (birth until 11 wk) and beyond: until end of the indoor stable period (13 wk, $P = 0.009$) and at euthanasia (28 wk, $P = 0.09$). Overall, average daily weight gain from day 2 after birth until weaning remained lowest in K+ kids ($P = 0.001$). After the indoor period (wk 13), D+ kids tended to have an increased daily weight gain ($P = 0.07$). Although concentrate intake during the indoor stable period of two kids of the D−K+ group was extremely low compared to the other 8 kids in the group (0.757 kg/pen vs. 21.2 kg/pen group average), BW of these animals did not deviate from the group

Table 3. Effect of prenatal or postnatal treatment with MCFA from coconut oil on BW and daily weight gain of goat kids

	Treatments*					*P* value	
Item	D+	D−	K+	K−	SEM	Prenatal treatment	Postnatal treatment
BW, kg							
Day 2	3.96	3.76	3.89	3.82	0.132	0.33	0.64
Wk 2	5.53	5.55	5.00	6.07	0.184	0.94	0.0003
Wk 4	8.11	8.28	7.32	9.07	0.302	0.70	0.0007
Wk 6	11.5	11.3	10.5	12.3	0.38	0.82	0.002
Wk 8	14.3	14.6	13.3	15.5	0.46	0.70	0.004
Wk 9 (weaning)	16.4	16.4	15.3	17.6	0.51	0.99	0.004
Wk 13	23.2	22.3	21.3	24.2	0.67	0.39	0.009
Euthanasia (ca. 28 wk)	32.6	30.7	30.5	32.8	0.92	0.16	0.092
Daily weight gain, g/d							
0–2 wk	102	116	71.4	146	8.1	0.22	<0.0001
2–4 wk	186	195	166	215	13.0	0.61	0.02
4–6 wk	240	219	225	234	14.0	0.32	0.68
6–8 wk	203	229	204	227	13.7	0.19	0.26
0-weaning	196	196	177	216	7.1	0.98	0.001
Weaning-13 wk	249	218	227	241	11.1	0.07	0.35
13 wk-euth. (pasture)	86.4	75.2	83.0	78.6	5.33	0.16	0.54
ADG trial (0-euth.)	143	133	132	144	4.1	0.09	0.05

*Treatments consisted of four combinations of pre (D+ or D−) and/or postnatal (K+ or K−) treatment. Since interaction effects between D and K were not significant, only main effects are presented. Number of kids per treatment: D+K+ (n = 10), D+K− (n = 9), D−K+ (n = 10), D−K− (n = 10).

mean. Daily weight gain over the whole pasture period (13–28 wk) did not differ between treatment groups. Over the whole trial, K+ kids had the lowest average daily weight gain (Table 3, P = 0.05).

In Vitro Rumen Fermentation

Four wk old (4 and 24 h incubation). At 4 wk old, absolute CH_4 production after 4-h incubation was lower in rumen inoculum of D+ kids (−48%, P = 0.003) and of K+ kids (−43%, P = 0.01), compared with kids which did not receive any treatment prenatally or postnatally (Table 4). Associated to this CH_4 reduction, total VFA (**tVFA**) was lower in D+ kids and this decrease tended to be stronger for kids that also received a postnatal treatment (interaction, P = 0.06). These D+K+ kids also had the lowest acetate/propionate ratio (P = 0.04) and butyrate production (P = 0.03). Propionate production was increased in K+ kids (+40%, P = 0.01);

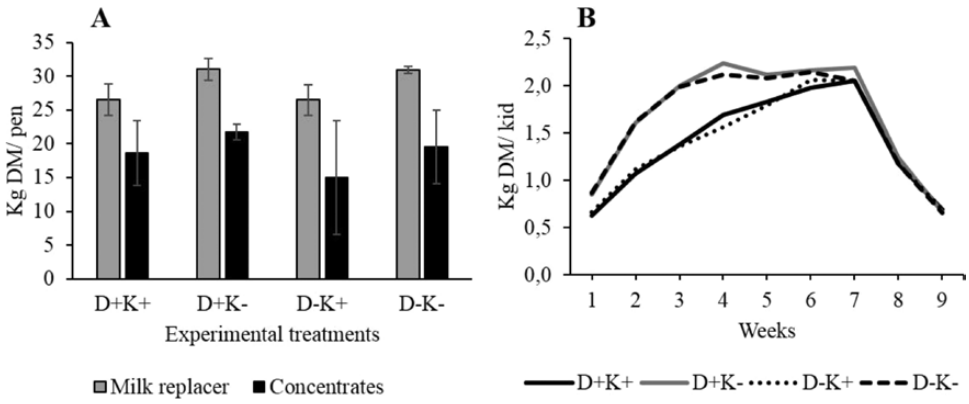

Figure 3. (A) Total milk replacer and concentrate intake (kg DM/pen) during the entire indoor stable period (0–13 wk). Values represent mean intake per pen and error bars represent SDs (n = 5). Total milk replacer intake of postnatally treated kids (K+) was reduced compared to the K− group (P = 0.004). Total concentrate intake of D−K+ kids was largely influenced by intake of two kids (same pen). Without these two goats total concentrate intake was 18.6 kg DM (compared to 15.0 kg DM, as presented in the figure). However, total concentrate intake was not different between experimental groups whether or not this pen was excluded from the analysis. (B) Mean milk replacer intake per week (kg DM/kid) during the preweaning period. Data were calculated as total intake per week for each experimental treatment group divided by the number of kids within that group.

Table 4. In vitro fermentation parameters of rumen fluid of goat kids, 4 wks old (4 and 24 h incubation)

	Treatments*					*P* value		
Item	D+K+ (*n* = 10)	D+K− (*n* = 9)	D−K+ (*n* = 10)	D−K− (*n* = 10)	SEM	Prenatal treatment	Postnatal treatment	Interaction
Absolute CH_4 production, µmol/flask								
4 h	6.78	26.6	28.7	35.8	3.33	0.003	0.01	NS†
24 h	23.5[b]	127[a]	103[a]	133[a]	12.9	0.004	<0.0001	0.01
Absolute H_2 accumulation‡, µmol/flask								
4 h	13.1	7.29	10.9	5.73		0.51	0.07	NS
24 h	32.3[a]	2.27[b]	2.51[b]	1.32[b]		0.0009	0.0001	0.008
Relative CH_4 production (CH_4/total VFA, µmol/µmol)								
4 h	0.0435	0.193	0.156	0.232	0.0200	0.02	0.001	NS
24 h	0.0648[b]	0.307[a]	0.263[a]	0.349[a]	0.0309	0.001	<0.0001	0.02
VFA production, µmol/flask								
Total VFA§								
4 h	113	153	181	154	16.5	0.05	0.71	0.06
24 h	285[b]	415[a]	388[a]	378[a]	22.1	0.15	0.02	0.006
Acetate								
4 h	48.0	62.1	71.2	60.3	7.38	0.31	0.97	NS
24 h	137[b]	215[a]	201[a]	207[a]	16.5	0.11	0.02	0.05
Propionate†								
4 h	36.5	25.6	36.6	26.6		0.87	0.01	NS
24 h	93.9	74.3	71.6	59.1		0.03	0.07	NS
Butyrate								
4 h	20.8[b]	50.9[a]	52.3[a]	50.9[a]	6.65	0.29	0.05	0.03
24 h	48.9[b]	99.3[a]	81.8[a]	85.0[a]	7.28	0.27	0.001	0.003
Acetate/propionate ratio								
4 h	1.16[b]	2.47[a]	2.07[a]	2.11[a]	0.322	0.44	0.04	0.04
24 h	1.46	2.89	3.02	3.80	0.279	0.006	0.01	NS

*Treatments consisted of four combinations of pre (D+ or D−) and/or postnatal (K+ or K−) treatment. Main and interaction effects are presented. Means with different superscripts differ ($P < 0.05$). Superscripts are only shown when an interaction effect was detected.

†NS: nonsignificant *P* values for interaction effects. Interaction effects were omitted from the mixed model when not significant, hence no *P* value is presented.

‡Log transformation was performed for this parameter. Back-transformed LS means are presented. No meaningful SEM can be presented for these parameters, instead confidence intervals (95%) per exp. group ([lower limit; upper limit]) are presented in same order as in the table. H_2 (4 h): [2.95; 11.1], [5.60; 21.1], [3.48; 15.3], [6.76; 25.4]; H_2 (24 h): [0.62; 2.82], [1.17; 5.37], [1.02; 5.03], [15.1; 69.0]; propionate (4 h): [20.7; 34.2], [28.4; 47.1], [19.3; 33.9], [27.9; 47.6], propionate (24 h): [47.1; 74.1], [57.1; 95.3], [58.5; 94.4], [74.0; 119].

§Total VFA consisting of seven short chain fatty acids: C2:0 (acetate), C3:0 (propionate), iso-C4:0 (iso-butyrate), C4:0 (butyrate), iso-C5:0 (iso-valerate), C5:0 (valerate), and C6:0 (caprionate).

however, acetate production was not different ($P > 0.05$) between groups. The production of CH_4 relatively to tVFA (CH_4/tVFA) was reduced in both D+ kids (−39%, $P = 0.01$) and K+ kids (−53%, $P = 0.001$), and a trend was observed for higher H_2 accumulation in flasks with rumen inoculum of K+ kids ($P = 0.07$). Results after 24-h incubation showed D+K+ kids' inocula had the lowest in vitro CH_4 production (−82%) accompanied by a very high H_2 accumulation (25-fold higher than D−K− kids) (interaction; $P = 0.01$ and $P = 0.008$, respectively). The rumen fluid of these kids further presented a decreased tVFA, acetate, and butyrate production (interaction; $P = 0.006$, $P = 0.05$, and $P = 0.003$, respectively). This resulted in a lower CH_4/tVFA ratio in D+K+ kids (interaction, $P = 0.02$). Production of propionate was increased in D+ kids (+28%, $P = 0.03$) and K+ kids (+24%, $P = 0.07$), and the acetate/propionate ratio was decreased (D+ kids, $P = 0.005$; K+ kids, $P = 0.01$). Nonincubated inocula of the fasted kids were also analyzed to provide insight into the in vivo concentrations of VFA (Supplementary Table 4). At 4 wk of age, no differences in tVFA concentrations or acetate were present in these nonincubated inocula; however, the amount of butyrate was higher in D−K+ kids compared to D−K− kids ($P = 0.02$) and the amount of propionate was higher in D+ kids ($P = 0.04$), an effect that was also visible after 24 h incubation.

Eleven wk old (4 and 24 h incubation). At 11 wk, incubation results of two D−K+ kids largely deviated from the eight other kids in the D−K+ group with regards to intake (lower concentrate intake and higher hay intake), and were

highly influencing the results of the D−K+ group (Table 5). Accordingly, it was decided to leave the two kids out of this analysis (n = 8). The treatment effects that were observed at 4 wk old had disappeared at 11 wk old. There were no differences observed in absolute CH_4 production, absolute H_2 accumulation or CH_4/tVFA after 4 or 24 h of incubation. There was a trend for lower tVFA (P = 0.06) and a significantly lower production of acetate (−15%, P = 0.005) in K+ kids after 4 h incubation. After 24-h incubation, there was no difference anymore in acetate production; however, there was still a trend for lower tVFA in K+ kids (P = 0.10) and also for lower acetate/propionate ratio in D+ kids (P = 0.06), corresponding to results at 4 wk after 24 h incubation. The fermentative capacity of D+K+ kids' inoculum had doubled compared to 4 wk old (reflected in tVFA production after 24 h incubation), together with a strong increase in absolute CH_4 production. For nonincubated rumen fluid samples, the only statistical difference (confirmed as a trend by Tukey's Post Hoc test) was found in acetate concentration, which was higher in D−K+ inocula compared to D+K+ inocula (Supplementary Table 4).

Twenty-four wk old (4 and 24 h incubation). There were no differences in absolute CH_4 production, H_2 accumulation, tVFA, individual VFAs, or CH_4/tVFA after 4 or 24 h incubation, nor for nonincubated samples (Table 6). In general, for inocula from all experimental conditions, tVFA and absolute CH_4 production values had doubled compared to results from 11-wk-old animals (24 h incubation).

Table 5. In vitro fermentation parameters of rumen fluid of goat kids, 11 weeks old (4 and 24 hours incubation)

	Treatments*					*P* value	
Item	D+	D−	K+	K−	SEM	Prenatal treatment	Postnatal treatment
Absolute CH_4 production, µmol/flask							
4 h	37.3	42.8	40.1	40.0	4.37	0.42	0.99
24 h	140	148	149	139	9.2	0.54	0.35
Absolute H_2 accumulation†, µmol/flask							
4 h	2.65	3.20	2.15	3.70		0.56	0.42
24 h	1.08	0.92	1.07	0.93		0.63	0.29
Relative CH_4 production (CH_4/total VFA, µmol/µmol)							
4 h	0.160	0.171	0.174	0.157	0.0178	0.67	0.46
24 h	0.266	0.276	0.287	0.255	0.0164	0.66	0.13
VFA production, µmol/flask							
Total VFA‡							
4 h	237	250	234	254	7.8	0.26	0.06
24 h	527	538	518	547	12.8	0.55	0.10
Acetate							
4 h	115	126	111	130	5.3	0.19	0.005
24 h	305	325	307	323	9.0	0.16	0.13
Propionate†							
4 h	83.0	79.3	79.4	82.9		0.85	0.44
24 h	149	137	134	152		0.25	0.16
Butyrate							
4 h	24.4	26.4	25.6	25.2	2.42	0.57	0.90
24 h	45.7	44.7	45.1	45.2	3.29	0.84	0.98
Acetate/propionate ratio							
4 h	1.50	1.64	1.51	1.62	0.095	0.31	0.40
24 h	2.10	2.54	2.42	2.22	0.150	0.06	0.30

*Treatments consisted of four combinations of pre (D+ or D−) and/or postnatal (K+ or K−) treatment. Since interaction effects between D and K were not significant, only main effects are presented. Number of kids per treatment: D+K+ (n = 10), D+K− (n = 9), D−K+ (n = 8), and D−K− (n = 10).

†Log transformation was performed for this parameter. Back-transformed LS means are presented. No meaningful SEM can be presented for these parameters, instead confidence intervals (95%) per exp. group ([lower limit; upper limit]) are presented in same order as in the table. H_2 (4 h): [1.44; 6.21], [1.60; 3.31], [1.35; 2.80], [1.67; 3.37], H_2 (24 h): [0.67; 1.28], [0.59; 1.13], [0.71; 1.22], [0.62; 1.05], Propionate (4 h): [68.0; 91.5], [65.8; 90.8], [64.1; 88.0], [69.7; 94.6], Propionate (24 h): [130; 165], [117; 151], [116; 149], [131; 167].

‡Total VFA consisting of seven short chain fatty acids: C2:0 (acetate), C3:0 (propionate), iso-C4:0 (iso-butyrate), C4:0 (butyrate), iso-C5:0 (iso-valerate), C5:0 (valerate), and C6:0 (caprionate).

Rumen Microbiota

Total DNA yield increased with age of kids ($P < 0.0001$). The lowest DNA yield was observed in D+K+ kids at 4 wk old ($P = 0.05$; Table 7). In addition, total eubacterial abundance was lowest in these kids at 4 wk ($P = 0.02$, Table 7), as was total methanogen abundance (measured both as *rrs* or *mcrA* abundance relatively to eubacterial *rrs* abundance, $P = 0.01$ and $P = 0.002$, respectively; Figure 4). At 11 and 28 wk old, differences in eubacterial and methanogen abundance disappeared. The qPCR-based tests for total protozoal abundance revealed virtually no protozoa were present in the rumen fluid of kids up until 28 wk of age, irrespective of experimental treatment. Methanogenic activity (*mcrA* transcripts normalized against methanogen *rrs* transcripts), after a night of fasting was numerically lowest in D+K+ kids at 4 wk old, but this was not different from expression levels in D+K− or D−K− kids (Figure 5). However, taking into account the lower total methanogen abundance in D+K+ kids at 4 wk old, overall rumen methanogenic activity will be strongly reduced in these kids. Expression levels in D−K+ kids were higher than in D+K+ kids at this age (interaction, $P = 0.004$). At 11 wk old, there were no differences in *mcrA* expression detected, however at 28 wk old an interaction effect was detected ($P = 0.005$), showing highest levels of expression in rumen fluid of singularly treated kids (D+K− and D−K+ kids).

Table 6. In vitro fermentation parameters of rumen fluid of goat kids, 28 weeks old (4 and 24 hours incubation)

	Treatments*					*P* value	
Item	D+	D−	K+	K−	SEM	Prenatal treatment	Postnatal treatment
Absolute CH_4 production, µmol/flask							
4 h	98.5	98.0	99.0	97.5	4.45	0.93	0.82
24 h	341	347	349	339	15.0	0.80	0.56
Absolute H_2 accumulation†, µmol/flask							
4 h	1.83	1.56	1.79	1.61		0.28	0.46
24 h	0.533	0.560	0.559	0.533		0.47	0.49
Relative CH_4 production (CH_4/total VFA, µmol/µmol)							
4 h	0.257	0.265	0.264	0.259	0.0129	0.66	0.79
24 h	0.313	0.334	0.328	0.319	0.0143	0.31	0.68
VFA production, µmol/flask							
Total VFA‡							
4 h	386	375	380	381	14.0	0.62	0.94
24 h	1,085	1,077	1,093	1,069	21.6	0.80	0.45
Acetate							
4 h	189	176	178	186	7.2	0.20	0.47
24 h	631	616	634	612	17.7	0.57	0.33
Propionate†							
4 h	115	119	118	117		0.80	0.99
24 h	308	304	309	304		0.69	0.67
Butyrate							
4 h	54.4	54.0	56.0	52.4	4.70	0.87	0.50
24 h	100	99.3	100	99.5	4.86	0.88	0.93
Acetate/propionate ratio							
4 h	1.72	1.54	1.60	1.66	0.117	0.21	0.83
24 h	2.04	2.05	2.05	2.04	0.085	0.95	0.96

*Treatments consisted of four combinations of pre (D+ or D−) and/or postnatal (K+ or K− treatment. Since interaction effects between D and K were not significant, only main effects are presented. Number of kids per treatment: D+K+ ($n = 9$), D+K− ($n = 7$), D−K+ ($n = 7$), D−K− ($n = 7$).

†Log transformation was performed for this parameter. Back-transformed LS means are presented. No meaningful SEM can be presented for these parameters, instead confidence intervals (95%) per exp. group ([lower limit; upper limit]) are presented in same order as in the table. H_2 (4 h): [1.47; 2.03], [1.28; 1.81], [1.43; 1.99], [1.31; 1.85], H_2 (24 h): [0.47; 0.58], [0.49; 0.62], [0.49; 0.61], [0.46; 0.59], Propionate (4 h): [97.9; 129], [100; 134], [98.9; 132], [97.9; 133], Propionate (24 h): [286; 330], [279; 325], [285; 332], [277; 326].

‡Total VFA consisting of 7 short chain fatty acids: C2:0 (acetate), C3:0 (propionate), iso-C4:0 (iso-butyrate), C4:0 (butyrate), iso-C5:0 (iso-valerate), C5:0 (valerate), and C6:0 (caprionate).

Table 7. Abundance of total eubacteria (estimated by quantitative PCR) and DNA yield (estimated fluorometrically)

	Treatments*					*P* value		
Item	D+K+ (n = 10)	D+K− (n = 9)	D−K+ (n = 10)	D−K− (n = 10)	SEM	Prenatal treatment	Postnatal treatment	Interaction
DNA yield, ng/μL DNA extract								
4 wk	13.6[b]	42.9[ab]	62.8[a]	47.3[ab]	10.75	0.02	0.53	0.05
11 wk	73.2	83.9	81.3	73.5	10.73	0.91	0.46	NS
28 wk	85.1	111	114	124	22.55	0.34	0.42	NS
Total eubacteria, $\log_{10}$ gene copies of *rrs*/μL DNA extract								
4 wk	5.75[b]	6.30[a]	6.46[a]	6.31[a]	0.133	0.02	0.16	0.02
11 wk	6.76	6.77	6.83	6.78	0.056	0.52	0.58	NS
28 wk	6.67	6.80	6.82	6.85	0.093	0.28	0.38	NS

*Treatments consisted of four combinations of pre (D+ or D−) and/or postnatal (K+ or K−) treatment. Main and interaction effects are presented. Means with different superscripts differ ($P < 0.05$). Superscripts are only shown when an interaction effect was detected.

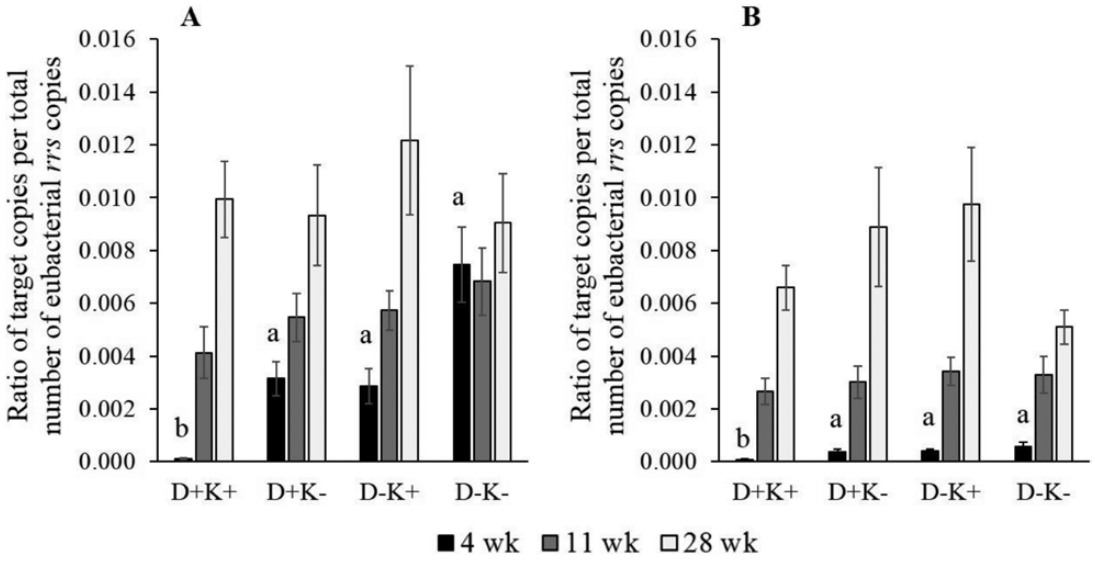

Figure 4. Relative abundance of rumen methanogens. Mean values per experimental group are presented and error bars represent SEs. Means with different letters differ ($P < 0.05$). (A) abundance of *rrs* of methanogens relatively to abundance of *rrs* of eubacteria; (B) abundance of *mcrA* relatively to abundance of *rrs* of eubacteria.

Rumen Characteristics and Morphometric Properties of Ruminal Papillae

The empty rumen weight of K+ kids was lower ($P = 0.05$) compared to K− kids (Table 8). Empty rumen weight expressed as a percentage of total BW was not different between kids, which suggests the lower empty rumen weight was probably caused by the general lower BW. The density of the papillae (number/cm^2 mucosa), observed by stereomicroscope in four different areas of the rumen, did not differ ($P > 0.05$) between the experimental groups for the ventral rumen, ventral blind sac, and dorsal blind sac. However, papillae density was greater in the ruminal atrium of K+ kids ($P = 0.04$) compared to their nontreated siblings. Anatomically, this rather small pouch is the first rumen area immediately following the end of the esophagus and was characterized by very dense, long, tongue-like, and dark-colored papillae compared to the rest of the rumen wall (Figure 6). Visually, papillae from the ventral rumen and dorsal blind sac were very similar in appearance (shorter and rounded, laying in roof tile formation), whereas papillae from ventral blind sac appeared less developed and bud-like compared to the other sampled areas, in all four experimental conditions. Papillae were small and seemed like they recently developed, hence only small tongue-like papillae were present. In the ruminal atrium, papillae base width of K+ kids ($P = 0.06$) and in particular of D+K+ kids ($P = 0.08$) tended to be smaller. Papillae length tended or was smaller in the ventral rumen and dorsal blind sac of K+ kids ($P = 0.08$, $P = 0.02$, respectively), but not different in ruminal atrium or ventral blind sac. Total papillae surface area (TSA) was not different between the four experimental conditions in any of the four areas.

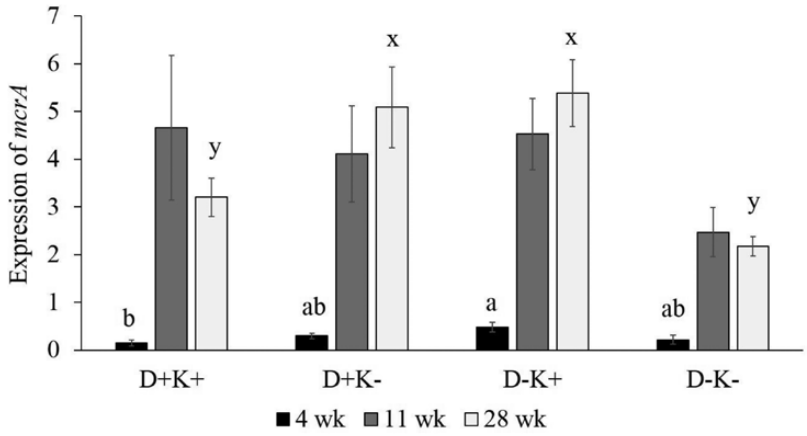

Figure 5. Expression levels of the *mcrA* gene in rumen methanogens. Mean Calibrated Normalized Relative Quantities (CNRQ) values per experimental group are presented and error bars represent SEs. Means with different letters differ ($P < 0.05$).

Table 8. Rumen weight and papillae histomorphometric parameters at 28 wks old

	Treatments*					*P* value	
Item	D+	D−	K+	K−	SEM	Prenatal treatment	Postnatal treatment
Empty rumen weight, kg	0.642	0.618	0.610	0.650	0.0199	0.44	0.05
Rumen/BW, %	1.96	1.98	1.98	1.96	0.046	0.67	0.64
Papillae density, number/cm^2							
Ruminal atrium	58.2	63.3	64.9	56.7	3.36	0.27	0.04
Ventral rumen	58.8	60.5	59.6	59.7	3.27	0.72	0.99
Ventral blind sac	55.2	58.1	57.1	56.2	2.72	0.49	0.74
Dorsal blind sac	51.5	51.9	51.6	51.9	2.57	0.92	0.91
Papillae base width, mm							
Ruminal atrium	0.671	0.638	0.625	0.684	0.0243	0.34	0.06
Ventral rumen	0.547	0.568	0.568	0.547	0.0133	0.28	0.25
Ventral blind sac	0.566	0.591	0.586	0.571	0.0206	0.44	0.45
Dorsal blind sac	0.593	0.556	0.580	0.569	0.0203	0.23	0.68
Papillae length, mm							
Ruminal atrium	3.05	3.08	2.98	3.15	0.144	0.88	0.45
Ventral rumen	1.50	1.50	1.39	1.60	0.084	1.00	0.08
Ventral blind sac	1.05	1.10	1.05	1.09	0.068	0.61	0.59
Dorsal blind sac	1.57	1.52	1.46	1.63	0.062	0.62	0.02
Total surface area†, mm^2/cm^2							
Ruminal atrium	237	244	244	236	15.5	0.75	0.72
Ventral rumen	92.1	99.1	93.5	97.7	8.07	0.53	0.55
Ventral blind sac	65.1	74.3	68.5	71.0	5.17	0.24	0.16
Dorsal blind sac	93.4	85.9	85.5	93.8	4.23	0.22	0.75

*Treatments consisted of four combinations of pre (D+ or D−) and/or postnatal (K+ or K−) treatment. Since interaction effects between D and K were not significant, only main effects are presented. Number of kids per treatment: D+K+ (n = 10), D+K− (n = 9), D−K+ (n = 10), D−K− (n = 10).

†Total surface area was determined as length × width × 2 × papillae density.

DISCUSSION

In this study, we tested whether it is possible to reduce enteric CH_4 production as well as abundance and activity of methanogenic archaea in the rumen of goat kids by supplementing CO MCFA in EL, and whether these effects can persist after supplementation stops (EL programming). In vitro CH_4 production from inoculum of the goat kids was used as a proxy to assess the effect on potential CH_4 production. Furthermore, effects on BW, DMI, as well as rumen papillae morphometry (at 28 wk old) were investigated.

Changes in Rumen Microbiota Leading to Differences in In Vitro Fermentation Parameters

The lack of rumen protozoa at all ages, irrespective of experimental treatment, is striking. To

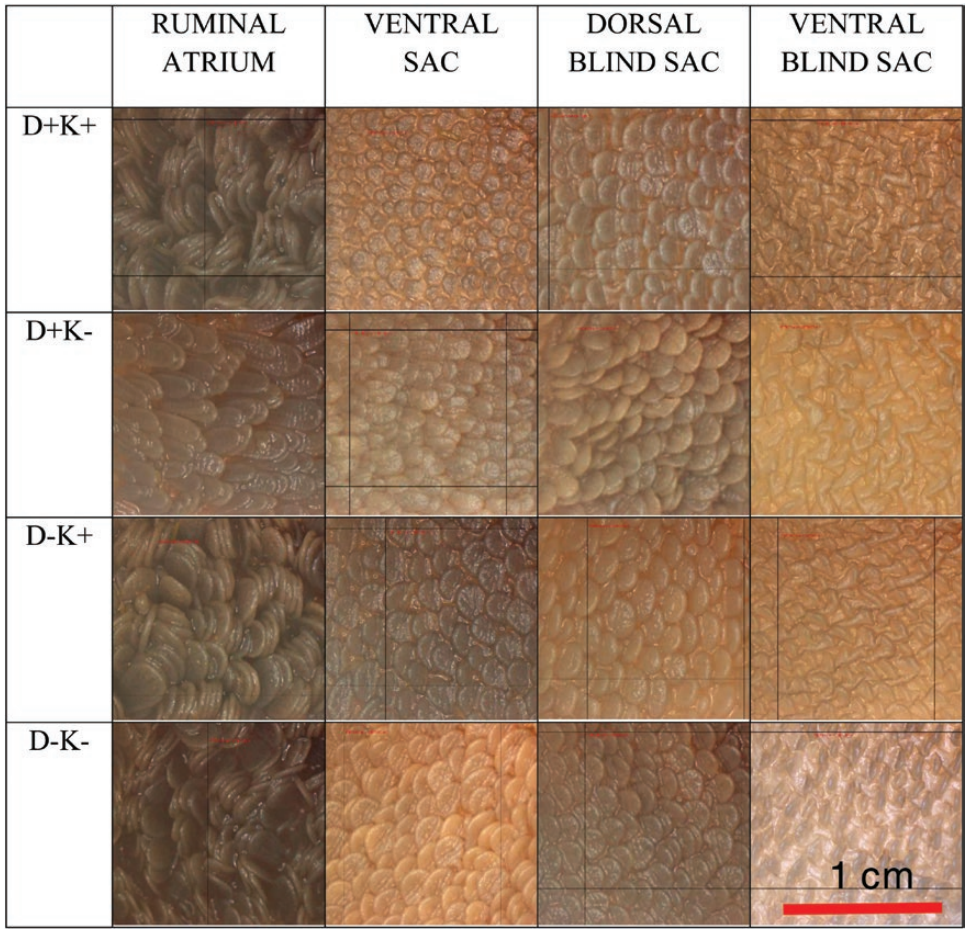

Figure 6. Representative pictures of each rumen area within each experimental condition. Number of kids per treatment: D+K+ (n = 10), D+K− (n = 9), D−K+ (n = 10), D−K− (n = 10). The bottom part of the ruminal atrium is characterized by long, tongue-like papillae. The bottom, central part of the ventral sac, and caudal part of the dorsal blind sac resemble each other most, with shorter and rounder papillae laying in a roof tile formation. The papillae of the caudal part of the ventral blind sac appeared to be less developed and bud-like; however, the density was not different from the ventral sac or dorsal blind sac.

verify that this observation was not a result of analytical errors, a number of positive control samples were added to the qPCR assay (i.e. DNA from rumen fluid of 1-, 4-, and 6-mo-old calves, prepared using the same extraction protocol). These tests confirmed the assay was indeed capable of quantifying the protozoal *rrs* gene in rumen fluid of young ruminants. Additionally, the primers used in the current study have already successfully been used for protozoal quantification in goat kids (Abecia *et al.*, 2014), lambs (Belanche *et al.*, 2010), and cattle (Boeckaert *et al.*, 2007). Probably this observation is an unintended side-effect of the rearing conditions in this trial. Indeed, several studies have shown that lambs and goat kids reared in isolation from adult ruminants have no or much less rumen protozoa compared to animals that have contact with older, faunated ruminants (Faichney *et al.*, 1999; Abecia *et al.*, 2014). Although CO is known to have strong negative effects on rumen protozoal numbers in adult, faunated ruminants (Machmüller *et al.*, 1998; Dohme *et al.*, 1999), defaunation of the prenatally and postnatally supplemented kids could not be attributed to CO MCFA supplementation used in this trial, since the control kids (D−K−) were also lacking rumen protozoa at the three ages.

Supplementing CO MCFA in preruminant goat kids led to similar effects on fermentation as for supplementing CO or MCFA in adult ruminants: a reduction of in vitro CH_4 production accompanied by an accumulation of hydrogen, associated with an increase in propionate production at the expense of acetate (Hristov *et al.*, 2009; Patra and Yu, 2013). Interestingly, these effects were enhanced by prenatal supplementation, but only until 4 wk after birth. The in vitro CH_4 reduction and hydrogen accumulation in D+K+ kids at 4 wk old corresponded to reduced methanogenic abundance and activity (Dong *et al.*, 1997; Machmüller *et al.*, 1998; Dohme *et al.*, 1999). A decrease in CH_4 production, associated with a lower methanogenic activity, has been described in sheep fed CO (Machmüller *et al.*, 2003). Furthermore, the reduced fermentative capacity (as measured by tVFA production after 24 h

incubation) of D+K+ kids corresponded to a lower total eubacterial abundance. The latter, in combination with the reduced acetate production, is in line with the high susceptibility of cellulolytic bacteria to MCFA and dietary fat in general (Machmüller *et al.*, 2003; Patra and Yu, 2013).

Despite the fact that K+ kids still received the CO MCFA at 11 wk old, treatment effects had disappeared. This is in contrast to previously mentioned studies, where animals showed effects as long as treatment lasted. In the current trial, initial CO MCFA dose at birth corresponded to a daily dose of ca. 0.53 g/kg BW. Given the major detrimental effect of the postnatal supplementation on DMI, growth and in vitro tVFA production as observed at 4 wk, the dose was not raised throughout the trial. Accordingly, the supplementation ratio was reduced to a daily dose of ca. 0.10 g/kg BW around weaning. A study in beef heifers, with different levels of CO (0 g/d, 125 g/d, 250 g/d, 375 g/d), showed a linear decrease in CH_4 production (−14%, −20%, −40%, respectively), and negative effects on DMI and digestibility only for the highest dose, corresponding to a daily supplementation of 0.78 g/kg BW (Jordan *et al.*, 2006). The two lower doses, corresponding to 0.26 and 0.52 g/kg BW, did not affect DMI or digestibility. Another study in 11-mo-old Tibetan sheep, supplementing daily 12 g CO or 0.48 g/kg BW, showed a strong CH_4 reduction in vivo (−61.2%) and a reduction of methanogen numbers and *Fibrobacter succinogenes* (Ding *et al.*, 2012), effects also observed by Liu *et al.* (2011) in 16-wk-old sheep receiving daily ca. 0.52 g/kg BW (25 g/kg concentrate). However, this did not result in negative effects on growth or a reduction of tVFA in these sheep (Liu *et al.*, 2011), as opposed to the goat kids in the current trial. Hence, the major impact on intake and performance of the CO MCFA, supplemented at a daily dose of about 0.5 g/kg BW in the beginning of the current trial, was unexpected. Supplementation of CO at this dose seemed safe based on former literature observations. The strong effects might be due to the younger age of the newborn preruminant animals used in the current study compared to the adult ruminants of the former studies. Additionally, the unesterified CO MCFA used in the current study might be more readily available than CO, which might have increased microbial toxicity. On the other hand, the lack of treatment effects at 11 wk old in the current trial could be explained by 1) a too low dose and possible dilution effect in the rumen, or 2) through an adaptation effect of the microbiota to the CO MCFA. Indeed, a recent study coupled to the current trial showed that, in vitro, a lower response in CH_4 reduction was found when re-supplementing CO MCFA to rumen fluid of goat kids that were previously exposed to this treatment, suggesting possible adaptation (Ruiz-González *et al.*, 2017).

Because no effects were observed at 11 wk, it is not surprising there were no (programmed) effects at 28 wk old. Abecia *et al.* (2013) did succeed in programming lower CH_4 emissions in goat kids, supplementing kids and/or their mothers postnatally from birth until 3 mo old with an antimethanogenic compound (bromochloromethane, 30 g/kg BW/d; Abecia *et al.*, 2013). The effects persisted until 3 mo after supplementation was ceased (particularly in double treated kids). In the current trial, the only effect at 28 wk old was a higher methanogenic activity—assessed by *mcrA* expression by RT-qPCR—in the singularly treated kids compared to the double or not supplemented kids, which did not correspond to methanogen abundance nor to the in vitro CH_4 production. Perhaps the sampling procedure with rumen samples at one single moment in time, after a night of fasting, was less appropriate for activity assessments.

Effects on Weight Gain

Additionally, CO MCFA supplementation negatively affected daily gain until 4 wk old, however no prenatal effect was found. It is unlikely that the growth delay in K+ kids results from the effects on fermentation in EL, since the kids were still preruminants at 4 wk of age. More likely nonrumen related effects played a role, such as EL milk replacer and concentrate intake, possibly caused by the irritating effect of the acid supplement (pH 3.23 at room temperature) on feed intake. Apparently, the K+ kids had not yet catched up in terms of weight gain at the end of the trial, possibly also due to a slower microbial development in the rumen due to the antimicrobial effects of the supplement. As mentioned before, concentrate intake during the indoor stable period of two kids of the D−K+ group was extremely low compared to the other eight kids in the group, but BW did not deviate from the group mean. This is probably because they compensated reduced concentrate intake by a larger hay consumption (no data on hay intake).

Papillae Development 4 mo After Postnatal Supplementation

Rumen development is a combination of anatomic development (growth, rumen papillae),

microbial colonization, and functional achievement (Li *et al.*, 2012; Jiao *et al.*, 2015) which can possibly be influenced by nutritional intervention in EL. Studies have shown that early intake, type and physical form of solid feed (Beharka *et al.*, 1998; Montoro *et al.*, 2013) and, related to this, the development of the microbial ecosystem, influences functional development of the rumen and therefore future animal performance. In short, rumen development is the result of microbial fermentation products and physical stimulation. Higher amounts of rumen VFA, through higher concentrate intake, could lead to more rapid rumen papillae development (Lesmeister *et al.*, 2004; Suárez *et al.*, 2007). Butyrate and propionate are assumed to positively influence papillary development (Sander *et al.*, 1959; Tamate *et al.*, 1962); however, the influence of butyrate is disputed (Zitnan *et al.*, 2005). Studies showed that suboptimal development of the rumen microbiota will negatively affect rumen development but also depress solid feed intake and papillae development (Tamate *et al.*, 1962; Lesmeister *et al.*, 2004). However, total concentrate intake until weaning or for the whole indoor stable period did not differ between kids. Also, tVFA concentrations in nonincubated rumen fluid were not different between treatment groups. On the other hand, the potential tVFA production, as well as individual VFA productions as assessed in vitro, were very different due to the combination of prenatal and postnatal treatment at 4 wk old. However, only postnatal treatment effects were found for papillae measurements. No clear causal relationship can thus be made between VFA in EL and the effects on rumen papillae. Despite the postnatal treatment effects on papillae density and length, the TSA was not different between experimental groups for any of the rumen areas. Since TSA reflects the absorption capacity of the papillae, we do not expect differences in papillary absorbing function between kids. Ragionieri *et al.* (2016) also supplemented MCFA to calves, however in the milk replacer (11g/d or 0.26 g/kg BW/d, from 14 until 56 d old). They did not find any effects on papillae density, length, width, or TSA at the age of 70 d old. The method of supplementation in their trial (in milk replacer, delivering MCFA into the abomasum and hence by-passing the rumen) most probably is the reason no effects were observed in the rumen.

Since the rumen epithelium is known to be subjected to continual changes, from regression to proliferation, like the gut lining (Černík *et al.*, 2014), it is remarkable that postnatal treatment effects have not completely disappeared after 4 mo. This suggests that an EL treatment, directly or indirectly, can influence rumen papillary development on the long term. Interestingly, Tamate *et al.* (1962) suggested that fetal papillary development is influenced by short chain fatty acids from maternal circulation, however no long-term effects of prenatal supplementation were observed in the present study. Next to the chemical stimulation of papillae development, hay provided physical stimulation during the developmental phases of the rumen. Earlier mentioned studies (Beharka *et al.*, 1998; Montoro *et al.*, 2013) indicated differences in forage intake levels (not measured here) might also account for variations in rumen development, which we cannot rule out based on our data.

In conclusion, supplementing CO MCFA both prenatally and postnatally to goat kids effectively reduced potential in vitro CH_4 emissions until 4 wk old, by depressing both methanogen and eubacterial abundance. However, the associated negative effects on rumen fermentation and the observed growth delay due to postnatal treatment indicate the initial dose was too high. A lower starting dose combined with step-wise increase of the dose could have led to longer-lasting effects with less negative impact on animal performance. Interestingly, some rumen papillary characteristics were influenced until 4 mo after postnatal treatment, but this did not lead to differences in total absorbing surface area.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

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