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Nutritional intervention in early life to manipulate rumen microbial colonization and methane output by kid goats postweaning[1]

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ABSTRACT: The growing interest in reducing methane (CH_4) emissions from ruminants by dietary means is constrained by the complexity of the microbial community in the rumen of the adult animal. The aim of this work was to study whether intervention in early life of goat kids has an impact on methane emissions and the microbial ecosystem in the rumen and whether the effects persist postweaning. Sixteen doe goats giving birth to 2 kids each were randomly split into 2 experimental groups: 8 does were treated (D+) with bromochloromethane (BCM) after giving birth and over 2 mo, and the other 8 does were not treated (D-). In both groups of does, 1 kid per doe was treated with BCM (k+) for 3 mo, and the other was untreated (k-), resulting in 4 experimental groups: D+k+, D+k-, D-k+, and D-k-. Methane emissions were recorded, and ruminal samples were collected from kids at 2 mo of age (weaning, W) and 1 (W+1) and 4 (W+4) mo later. At W+1 mo, CH_4 emissions by k+ kids were 52% and 59% less than untreated kids (in D+ and D- groups, respectively). However, at W+4 mo, only D+k+ kids remained lower (33%) emitters and exhibited greater daily BW gain (146 g/d) compared with the other 3 groups (121.8 g/d). The analysis of the archaeal community structure by Denaturing Gradient Gel Electrophoresis (**DGGE**)showed a strong effect of BCM treatment on does and kids that persisted only in D+k+ kids. The study showed that the application of BCM during early life of kids modified the archaeal population that colonized the rumen, which resulted in decreased CH_4 emissions around weaning. The effect is influenced by the treatment applied to the doe and persisted 3 mo later in D+k+ kids.

Key words: bromochloromethane, early life, methane, methanogens, rumen, ruminal colonization.

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INTRODUCTION

Enteric fermentation in ruminants represents a loss of around 2% to 12% of the GE intake for the host (Johnson and Johnson, 1995) and accounts for 19% of anthropogenic sources of CH_4 emissions (Steinfeld et al., 2006). Numerous approaches have been studied to reduce CH_4 emissions by using nutritional antimethanogenic additives. However, plans for application appear premature as results are inconsistent, mainly because of the difficulty of modifying a well-established microbial ecosystem in the rumen of the adult animal (Hart et al., 2008).

In young ruminants and during rumen development, ingested microbes colonize and establish in a defined and progressive sequence. Methanogenic archaea have been found in the undeveloped rumen of lambs well before the ingestion of solid feed begins (2 to 4 d; Morvan et al., 1994). The use of metagenomics has shown the great diversity that soon develops in the immature rumen of calves with a core microbiome that includes 45 genera (Li et al., 2012).

The hypothesis to test here is that the physiological processes occurring in early life might include a "microbial impact" by which the microbial population that first establishes in the rumen would influence the microbial ecosystem postweaning and in turn the efficiency of ruminal fermentation. We have reported that feeding forage vs. concentrate around weaning

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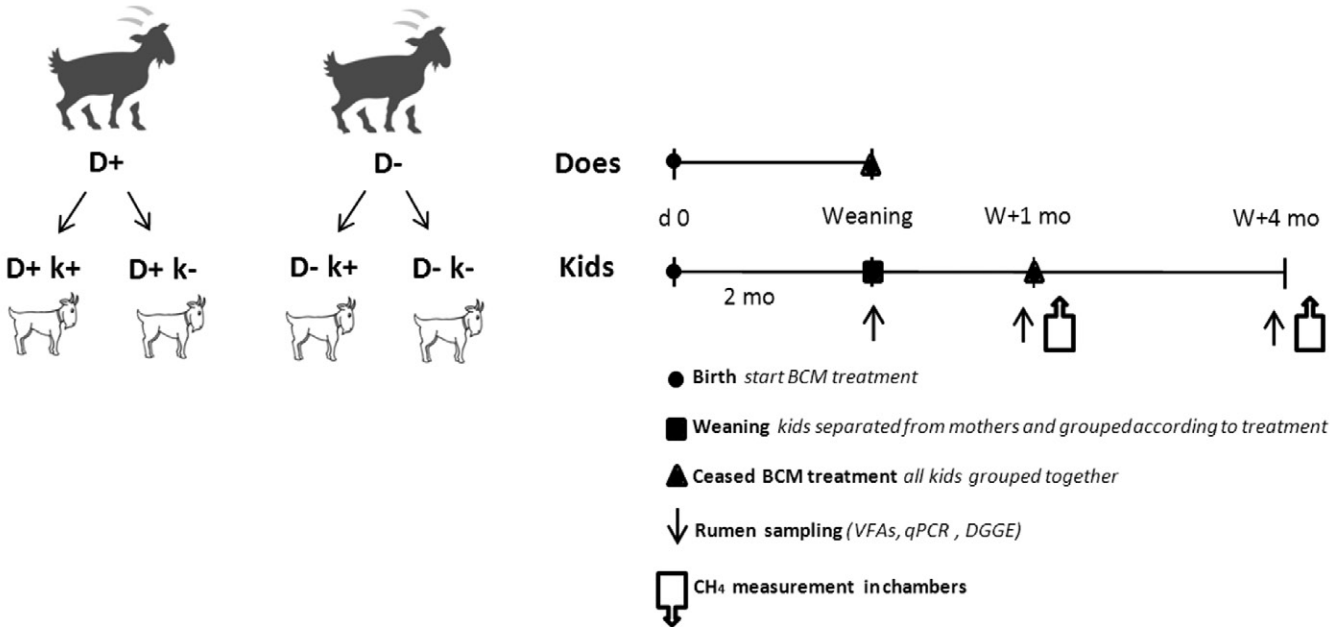

Figure 1. Experimental design and sampling schedule. W = Weaning; D+K+ = treated kids from treated does; D+K- = untreated kids from treated does; D-K+ = treated kids from untreated does; D-K- = untreated kids from untreated does; DGGE = Denaturing Gradient Gel Electrophoresis; CH4 = methane..

modified the bacterial population colonizing the rumen of lambs and that the effect persisted over 4 mo (Yáñez-Ruiz et al., 2010). However, no attempt has been made to date on the manipulation of the methanogenic Archaea in young animals and the effect on the digestive efficiency in the animal postweaning.

The aim of this work was to study whether feeding a methanogen inhibitor (bromochloromethane, **BCM**) in the early life of goat kids and to does has an impact on the microbial ecosystem colonizing the rumen, the effect on ruminal fermentation and CH_4 emissions, and to what extent the impact persists postweaning.

MATERIALS AND METHODS

All management and experimental procedures involving animals were performed by trained personnel in strict accordance with the Spanish guidelines (RD 1201/2005 of October 10, 2005) for experimental animal protection at the Estación Experimental del Zaidín. Experimental protocols were approved (October 1, 2010) by the Ethics Committee for Animal Research at the Animal Nutrition Unit.

Animals, Diets, and Experimental Design

Sixteen Murciano-Granadina goats (43 ± 1.7 kg BW) pregnant with 2 fetuses were acquired at 3 mo of pregnancy, kept in individual pens (1.7 × 1.2 m) with free access to water, and fed alfalfa hay ad libitum once per day (in g/kg of DM: OM, 880; CP, 214; ether extract,13.6; NDF, 419: ADF, 244; and ADL, 61) and a supplement 600 g/d fed twice per day (0900 and 1500 h) based on (g/kg) wheat shorts (350), corn shorts (100), corn grain (50), barley grain (160), soybean hulls (90), soybean meal (90), sunflower meal (120), CaO (22), NaCl (3.5), calcium salts (4.5), and trace minerals and vitamins supplement (10; in g/kg of DM: OM, 893; CP, 170; ether extract, 33.9; NDF, 342; ADF, 142; and ADL, 34.3).

The experimental period commenced when does gave birth, which happened within a period of 2 wk. After giving birth, each doe was randomly allocated to 1 of the 2 experimental groups: **D+**, treated daily with 30 g/kg BW of BCM divided in 2 equal doses, and **D-**, a control untreated group but receiving a placebo (10 g of ground oats in cellulose paper and sealed with molasses). Bromochloromethane (99.5%; Aldrich 13,526-7; Sigma - Aldrich Quimica, S.L., Madrid) is a halogenated aliphatic hydrocarbon entrapped in an α-cyclodextrin matrix (Alfa Aesar GmbH and Co., A18092; Alfa GmbH and Co KG, Karlsruhe, Germany; May et al., 1995). The BCM formulation was prepared as a dry white powder in 1 to 2 kg batches and contained 10% to 12% (wt/wt) BCM. The BCM complex was then wrapped in cellulose paper, mixed with 10 g of ground oats, and sealed with molasses. The BCM treatment was given orally twice a day at feeding times (0900 and 1500 h) to does.

All does gave birth to 2 kids, 1 of which remained untreated (**k-**) while the other was given a daily dose of 30 g/kg BW of BCM as above (**k+**), resulting overall in 4 experimental groups of kids: **D+k+**, **D+k-**, **D-k+**, and **D-k-** (n = 8), as illustrated in Fig. 1. During the first 2 wk of life of the treated kids, the BCM formulation was directly inserted in the mouth of the animal dissolved

in 10 mL of water twice a day. After 2 wk, BCM treatment was given orally twice per day at feeding times (0900 and 1500 h) to kids as described for does. The kids remained with does for 2 mo in the same pen with no physical contact with other animals to avoid touching and licking. Weights of kids were registered weekly.

The treatment of kids lasted for 3 mo: 2 mo during their time with the doe and 1 mo after weaning, during which kids were grouped by treatments (D+k+, D+k-, D-k+, D-k-) in 4 independent pens separated from each other to avoid physical contact. After weaning, kids were offered ad libitum alfalfa hay and starter commercial compound (g/kg): wheat shorts (50), corn shorts (50), corn grain (150), oat grain (260), milk powder (190), soybean meal (172), sunflower meal (120), NaCl (3.5), and calcium salts (4.5; in g/kg of DM: OM, 925; CP, 162; ether extract, 35; NDF, 163; and ADF, 78).

At 3 mo, all kids from the 4 experimental groups were grouped together in a single pen and BCM treatment ceased (Fig. 1). They remained together for another 3 mo until the end of the experimental period.

Methane emissions (average of 2 consecutive days of measurements) from kids were recorded at 2 separate occasions: 1 mo after weaning (**W+1**), when kids were distributed by experimental treatment, and 3 mo later (**W+4**), when kids were all grouped together and the BCM treatment had stopped. Methane production was measured using 4 open circuit respiration chambers constructed of metal frame and polycarbonate panels as described previously (Abecia et al., 2012).

Ruminal content was collected 3 times from kids: at weaning (**W**) and 1 (W+1) and 4 mo after (W+4). Samples were taken before the morning feeding using a stomach tube, and aliquots were immediately stored at −80°C for further molecular and VFA analyses.

Chemical Analyses

Feed samples were ground through a 1-mm sieve before analysis, and DM and OM contents were determined using the AOAC official methods 7.008 and 7.010 (AOAC, 2005). Gross energy was determined in an adiabatic bomb calorimeter (Gallenkamp & Co. Ltd., London, UK) according to the methodology described by Prieto et al. (1990). The NDF, ADF, and ADL analyses were performed by the sequential procedure of Van Soest et al. (1991) using the Ankom 2000 Fiber Analyzer (Ankom Technology Corp., Macedon, NY). The NDF was assayed with sodium sulfite and without α-amylase. The ADF were expressed without residual ash. Total nitrogen was determined by Kjeldahl analysis (AOAC 2005; method 2.055).

The VFA were analyzed by gas chromatography as described by Isac et al. (1994).

Extraction of DNA, qPCR, and Denaturing Gradient Gel Electrophoresis analyses

Samples of rumen digesta were freeze-dried, genomic DNA were extracted, and DNA was used as a template to quantify the copy numbers of 16S rRNA (for bacteria), the *mcr*A gene (for methanogenic Archaea), and 18S rRNA (for protozoa) by real-time PCR (**qPCR**) as described by Abecia et al. (2012).

A fragment of the 16S rDNA gene of methanogenic Archaea was amplified from the extracted DNA by PCR-Denaturing Gradient Gel Electrophoresis (**DGGE**) as described by Cheng et al. (2009). The Shannon and evenness indices were used to estimate the bacterial diversity in each sample (Pielou, 1969).

Statistical Analyses

Data were analyzed using the SAS PROC MIXED procedure (SAS Inst. Inc., Cary, NC). The statistical model used included the effects of BCM treatment to does, kids, and the does × kids interaction as fixed effects, and animal effect was considered random. When does × kids interaction was significant ($P < 0.05$), differences between treatment means were evaluated using the pdiff option of the LS means statement in the MIXED procedure of SAS and declared significant at $P < 0.05$. A tendency was considered when P-values were <0.1.

The DGGE banding patterns were processed by Quantity One software (Bio-Rad; Hercules, CA , USA). To analyze the structural differences of archaeal community among the 4 kids groups, principal components analysis (**PCA**) of DGGE profiles was applied using GenStat 10th Edition (VSN International, Charleston, USA; Li et al., 2008).

RESULTS

Methane Production, Rumen Fermentation, and Animal Performance

No symptoms of ill health were observed in kids over the experimental period. One month after weaning (W+1), k+ produced 52% and 59% less methane than k- kids within D+ and D- groups, respectively (Table 1). At W+4, k+ remained lower (33%) emitters than k- kids ($P = 0.043$), although this difference only persisted in those raised by D+ (a tendency, $P = 0.066$, was observed for a significant interaction of doe × kid).

With regard to the fermentation profile, acetic, propionic, butyric, and valeric proportions were affected by the treatment of the kid ($P < 0.01$), which resulted in a decreased ($P < 0.001$) acetic to propionic ratio in k+ compared to k- kids. These differences in the acetic to propionic ratio persisted only as a tendency ($P = 0.094$)

Table 1. Effect of bromochloromethane (BCM) treatment of does and kids on intake, BW gain, CH_4 emissions, and rumen fermentation pattern in kids at weaning (W) and 1 mo (W+1) and 4 mo later (W+4)

Item	Collection period	BCM treatment[1]				SEM	BCM *P*-value[2]		
		D+k+	D+k-	D-k+	D-k-		Doe	Kid	D × K
BW gain,[3] g/d		146	119	128	117[b]	8.9	0.20	0.049	0.30
Hay intake,[4] g DM/kg $BW^{0.75}$	W+1	37.1	35.4	40.0	27.8	5.02	0.32	0.93	0.69
	W+4	42.6	42.9	47.4	34.2	8.14	0.42	0.79	0.40
Supplement intake,[4] g DM/kg $BW^{0.75}$	W+1	61.1	68.6	75.0	76.8	6.40	0.33	0.059	0.86
	W+4	81.3	81.2	84.1	86.1	14.42	0.80	0.50	0.79
CH_4, L/kg DMI	W+1	12.7	26.6	10.9	26.9	5.57	0.79	0.001	0.71
	W+4	19.1	28.5	25.3	26.8	4.32	0.97	0.043	0.066
VFA profile, mol/100 mol									
Acetate	W	58.7	68.8	55.8	70.5	2.53	0.82	0.001	0.59
	W+1	62.1	65.8	59.9	63.4	2.15	0.28	0.097	0.95
	W+4	60.1	62.4	62.8	64.3	1.91	0.27	0.36	0.88
Propionate	W	20.6	15.3	19.1	14.9	1.62	0.59	0.017	0.74
	W+1	19.1	15.3	22.3	18.7	2.62	0.23	0.17	0.97
	W+4	20.8[a]	16.6[b]	18.5[b]	17.6[b]	3.96	0.45	0.52	0.001
Isobutyrate	W	3.68	2.98	4.47	3.10	0.786	0.57	0.22	0.68
	W+1	1.68	2.04	1.68	2.00	0.422	0.66	0.23	0.73
	W+4	2.16	2.43	3.26	1.89	0.355	0.68	0.098	0.065
Butyrate	W	9.90	7.43	11.7	5.65	1.778	0.99	0.040	0.34
	W+1	14.0	12.8	13.4	12.6	1.84	0.43	0.42	0.91
	W+4	12.7	13.9	11.1	12.7	1.82	0.89	0.46	0.41
Isovalerate	W	4.98	4.10	5.83	4.30	0.96	0.60	0.24	0.87
	W+1	1.85	2.60	1.50	1.84	0.498	0.28	0.28	0.69
	W+4	1.96	2.88	3.30	1.90	0.413	0.009	0.56	0.66
Valerate	W	2.20	1.48	3.00	1.65	0.296	0.13	0.007	0.32
	W+1	1.31	1.48	1.62	1.50	0.158	0.30	0.87	0.37
	W+4	1.60	1.75	1.37	1.56	0.337	0.62	0.59	0.95
Acetate/propionate, mol/mol	W	2.85[b]	4.50[a]	2.92[b]	4.73[a]	0.291	0.59	0.001	0.86
	W+1	3.25	4.30	2.69	3.39	0.372	0.084	0.094	0.97
	W+4	2.80	3.75	3.43	3.64	0.589	0.40	0.17	0.13

[a,b]Means with different superscripts differ ($P < 0.05$). Superscripts are only shown when D × K interaction ($P < 0.05$) was detected.

[1]D+K+: treated kids from treated does, D+K-: untreated kids from treated does, D-K+: treated kids from untreated does, D-K-: untreated kids from untreated does

[2]Effect of BCM treatment of doe (D), kid (K), and D × K interaction ($n = 8$).

[3]Average recorded over the first 10 wk of life.

[4]Average of 2-d measurements during time spent in the CH_4 chambers.

at W+1 as well as the effect of the treatment received by the doe ($P = 0.084$). At W+4, when all kids were grouped together and the treatment had ceased for 3 mo, the acetic to propionic ratio was not significantly different among the experimental groups.

The BW gain over the first 10 wk of life (Table 1) was greater ($P = 0.049$) in D+k+ kids (146 g/d) compared with the other 3 groups (119, 128, and 117 g/d for D+k-, D-k+, and D-k-, respectively), although no doe × kid significant interaction was observed ($P = 0.30$).

Microbial Quantification

The effect of treating with BCM on the copy gene numbers of methanogenic archaea differed during the progress of the trial: at W, kids raised by treated does (D+k+ and D+k-) had greater ($P = 0.027$) numbers than kids from D- does, whereas the opposite pattern was observed at W+4 mo (Table 2). The BCM treatment applied to kids was only significant ($P = 0.016$) in samples collected at W+1, resulting in a lower concentration of methanogenic archaea in treated kids.

The concentration of bacteria tended to be less in kids raised by treated does (D+k+ and D+k-, $P = 0.066$) at W and in treated kids (D+k+ and D-k+, $P = 0.003$) at W+1. At W+4, no effects were observed ($P > 0.10$) of any treatment.

Protozoal numbers remained unaffected ($P > 0.10$) in rumen contents of kids during the experiment.

Table 2. Effect of bromochloromethane (BCM) treatment of does and kids on the abundance (log_{10} of gene copy number/g fresh matter sample) of bacteria, protozoa, and methanogens at weaning (W), 1 mo later (W+1), and 4 mo later (W+4) in the rumen of kids

Item	Collection period	BCM treatment				SEM	BCM *P*-value[1]		
		D+k+	D+k-	D-k+	D-k-		Doe	Kid	D × K
Methanogens	W	7.28	7.02	7.63	8.19	0.324	0.027	0.66	0.22
	W+1	6.72	7.81	7.31	7.96	0.306	0.36	0.016	0.64
	W+4	7.46	7.47	7.76	7.69	0.123	0.044	0.77	0.78
Bacteria	W	10.4	10.5	10.3	10.1	0.136	0.066	0.78	0.36
	W+1	9.97	10.4	9.93	10.4	0.13	0.83	0.003	0.92
	W+4	9.99	9.93	9.96	10.1	0.087	0.60	0.78	0.33
Protozoa	W	8.64	9.37	8.73	8.13	0.537	0.29	0.90	0.23
	W+1	9.68	9.79	9.68	9.74	0.167	0.89	0.59	0.84
	W+4	8.75	8.96	8.95	9.39	0.379	0.41	0.40	0.77

[1]D+K+ = treated kids from treated does; D+K- = untreated kids from treated does; D-K+ = treated kids from untreated does; D-K- = untreated kids from untreated does.
[2]Effect of BCM treatment of doe (D), kid (K), and D × K interaction (n = 8).

Methanogenic Archaea Community Structure

The PCA score plot of the DGGE band profile showed that the BCM treatment induced differences in archaeal community structure in the developing rumen of kids (Fig. 2)**.** The plot recognized 4 major groups on the basis of BCM treatment (applied to does or kids, D+k+, D+k-, D-K-, and D-k+) at W and W+1 (Figs. 2A and 2B); however, at W+4 the treatment applied to kids only promoted different archaeal community structure in kids raised by D+ does (Fig. 2C), whereas kids raised by D- does grouped together. The chi-squared probability test showed that along the 3 sampling times, the treatment received by the does was the most influential factor on the archaeal community structure (P = 0.049, 0.036, and 0.012 at W, W+1, and W+4, respectively).

The analysis of the richness, the Shannon diversity index, and the evenness of the archaeal community (Table 3) showed a reduction ($P \leq 0.036$) of values for D+k+ and D-k+ kids at W. This effect did not persist in later samplings (W+1 and W+4).

DISCUSSION

On the basis of the knowledge from previous studies (Yáñez-Ruiz et al., 2008, 2010), the hypothesis to test in this work is whether it is possible to promote different microbial populations establishing in the rumen of the young ruminant by manipulating the feeding management early in life. The final goal is to test the link between a differential microbial colonization of the offspring and the physiological response of the animal postweaning.

The dietary intervention used here consisted of a daily supply to does and kids of BCM, a compound that reduces CH_4 production by interfering with the cobamide-dependent methyl transferase step of methanogenesis (Wood et al., 1968; Chalupa, 1977). When BCM is entrapped in cyclodextrin (**CD**; **BCM-CD**) and supplied to ruminants, it induces a sustained inhibition of CH_4 production (up to 90%), as reported elsewhere (May et al., 1995; McCrabb et al., 1997; Tomkins et al., 2009). Although it is unlikely that BCM will be used commercially for CH_4 inhibition, as this compound has an ozone-depleting effect (Solomon et al., 2007), it represents a good model for effective anti-methanogenic treatment to test our hypothesis.

Methane Production and Rumen Fermentation

Methane is formed in the rumen as a mechanism to remove H_2 and allow the continued production of H^+ during the fermentation of feeds in the rumen. The reduction in CH_4 emissions as a result of BCM treatment is in agreement with findings in steers (Tomkins et al., 2009) and in batch- and continuous-culture fermenters (Goel et al., 2009) using the same compound.

The effects observed by Tomkins et al. (2009) in steers treated with BCM disappeared soon after the treatment ceased, which might be explained by the fact that when methanogenic archaea are no longer inhibited, the flow of hydrogen to other alternative electron acceptors is energetically less favorable than the reduction of CO_2 to CH_4 (McAllister and Newbold, 2008). Thus, it seems that the microbial ecosystem in the rumen of an adult animal tends to revert to the pretreatment situation once the treatment stops (Hart et al., 2008). However, to date it is unknown whether the effect of antimethonogenic intervention applied early in life would persist after the treatment is no longer applied as a result of a permanently modified ruminal ecosystem. In this experiment, the reduction of methane emissions in k+ kids compared with k- kids persisted 3 mo after the treatment ceased, but only in those raised by treated does (D+k+). This confirms the essential role of the doe as the main source of inoculation of methanogenic archaea as discussed be-

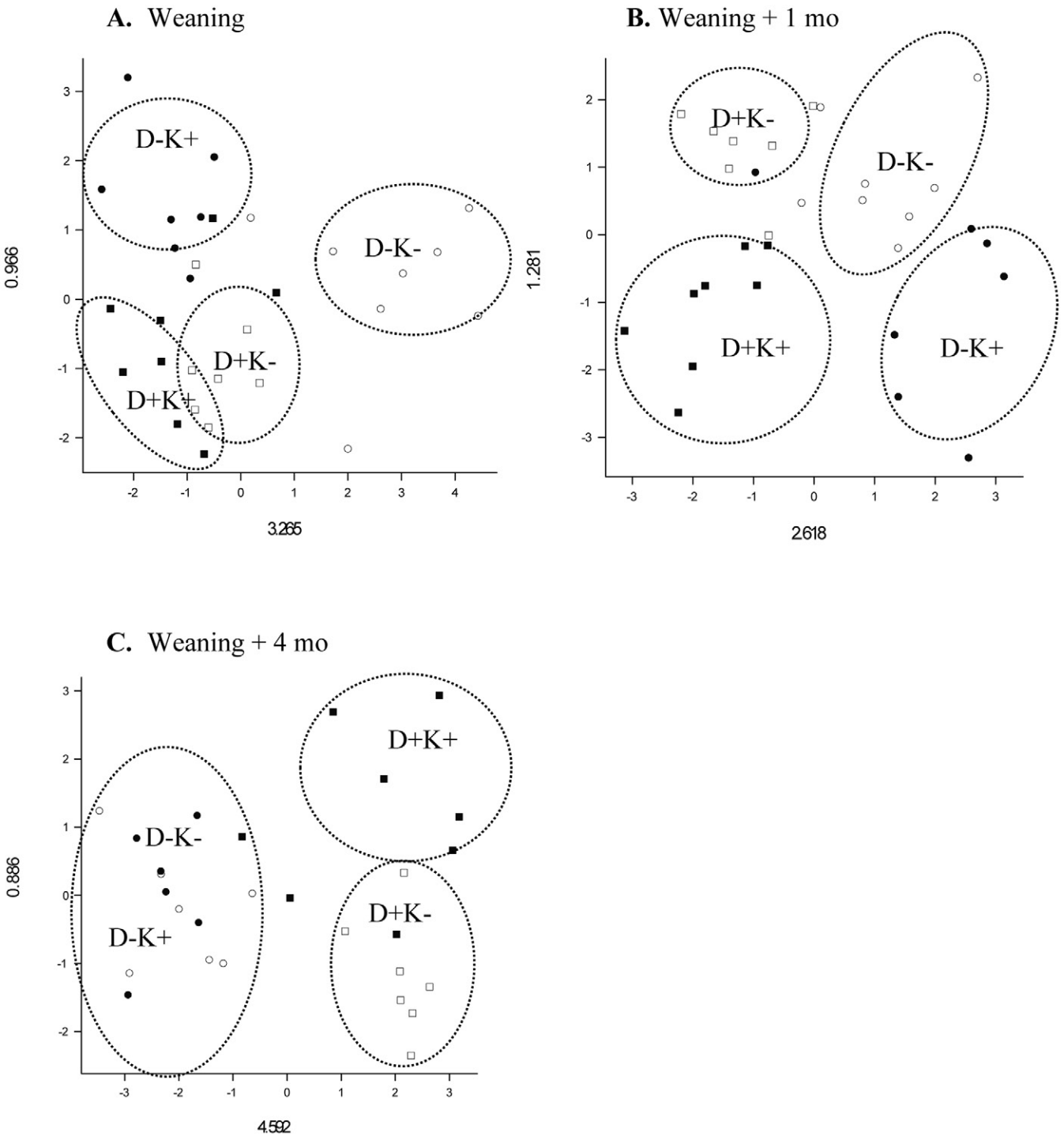

Figure 2. Principal component analysis of rumen methanogenic archaea profiles at (A) weaning, (B) 1 mo after weaning, and (C) 4 mo after weaning. Experimental groups = open circles; D-K- = solid circles; D-K+ = open squares; D+K- = solid squares; D+K+ = treated kids from treated does; D+K- = untreated kids from treated does; D-K+ = treated kids from untreated does; D-K- = untreated kids from untreated does.

low. However, the mechanisms involved in such doe-newborn interactions in relation to the transfer of microorganisms are still largely unknown.

The metabolic explanation of the reduction in methane synthesis is a shift in H_2 flow toward alternative electron acceptors such as propionate. With more H_2 being available, reductive processes involving propionate production and reductive acetogenesis become thermodynamically favorable (Ungerfeld and Kohn, 2006). This agrees with the reduction in the acetate to propionate ratio observed in the rumen contents of kids when CH_4 production is decreased. A reduction in the acetate:propionate molar ratio in the rumen has been described as a common feature of several antimethanogenic compounds, which indicates a concurrent decrease of CH_4 formation and redirection of H_2 from

Table 3. Effect of bromochloromethane (BCM) treatment of does and kids on diversity indexes in the methanogenic archaea community in the rumen of kids at weaning (W) and 1 mo (W+1) and 4 mo (W+4) later

Item	Collection period	BCM treatment[1]				SEM[1]	BCM *P*-value[2]		
		D+k+	D+k-	D-k+	D-k-		Doe	Kid	D × K
Richness	W	13.5	16.7	17.0	19.4	1.303	0.32	0.036	0.87
	W+1	13	11.4	12.8	13.6	1.882	0.76	0.43	0.37
	W+4	19.1	18	18.3	17.1	2.046	0.63	0.74	0.98
Shannon index	W	2.56	2.78	2.80	2.93	0.181	0.24	0.032	0.95
	W+1	2.54	2.37	2.54	2.56	0.153	0.49	0.38	0.37
	W+4	2.89	2.86	2.79	2.68	0.263	0.62	0.45	0.81
Evenness	W	0.55	0.6	0.61	0.63	0.029	0.24	0.032	0.95
	W+1	0.59	0.55	0.59	0.59	0.029	0.50	0.38	0.37
	W+4	0.66	0.65	0.64	0.61	0.070	0.62	0.45	0.81

[1] D+K+ = treated kids from treated does; D+K- = untreated kids from treated does; D-K+ = treated kids from untreated does; D-K- = untreated kids from untreated does.
[2]Effect of BCM treatment of doe (D), kid (K), and D × K interaction (n = 8).

methane to more propionic metabolic pathways (McAllister and Newbold, 2008). Because there was no change in acetate concentrations in kids 4 mo after weaning, we may conclude that reductive acetogenic bacteria did not contribute significantly to the consumption of the accumulated H_2. Only in the absence of methanogenesis can acetogens contribute significantly to hydrogen capture and sustain a functional rumen (Fonty et al., 2007).

The increased propionate production leads to a greater synthesis of glucose in the liver, as propionate from rumen fermentation is considered to be the major gluconeogenic precursor in ruminants, which may result in an increase in energy supply to the animal (Newbold et al., 2005). This agrees with the greatest BW gain observed in D+k+ kids, which links the shift in ruminal fermentation pattern with an increased animal efficiency. The greater growth rate observed by D+k+ kids might also be partly explained by the greater milk yield by D+ does, as reported in Abecia et al. (2012), although the fact that this was not reflected in the growth of D+k- kids suggests that the early life intervention played a role in such response (D+k+ vs. D+k-). Also, the link between modified microbiota colonizing the rumen and feeding behavior might be considered in early life. Although kids did not have different hay or supplement intakes during time spent in the chambers, there is evidence that rearing young calves with an older weaned companion causes earlier solid feed intake and, consequently, improved ruminal development and growth rate (De Paula Viera et al., 2012). These authors suggested, in line with our results, that manipulating microbial inoculation of the rumen in early life through feeding management may interact and trigger earlier rumen development. However, to further confirm this observation on the beneficial effect on animal productivity, conducting specifically designed trials to monitor feeding behavior with a larger number of animals and longer monitoring periods is envisaged.

Microbial Ecosystem

The gastrointestinal tract of most animals is supposed to be sterile and germ free right after birth; then, microbes from other adult animals and the surrounding environment subsequently colonize the rumen until a very complex and diverse microbial population develops (Ziolecki and Briggs, 1961). Ample evidence now exists that a significant proportion of the strict anaerobes that become predominant in the mature rumen, including methanogens, are already present in the rumen 1 or 2 d after birth (Fonty et al., 1987; Morvan et al., 1994; Lukás et al., 2007). Our data showed that the biomass of methanogens in the rumen of kids at W was equivalent to that in adult goats (Abecia et al., 2012; Romero-Huelva and Molina-Alcaide, 2013). However, the impact of the BCM treatment on biomass was variable at different sampling times: significant effects due to the treatment applied to does at W and W+4 and of the treatment on kids at W+1 were observed, which did not correlate with the patterns observed for CH_4 production. Potentially, during rumen development the impact of antimicrobial treatment might be greater than in the adult animal as the different niches are still being occupied and established for the first time. The competition that exists between H_2 users in the developing rumen could explain the changes in archaeal biomass in response to BCM (Gagen et al., 2012). The information about the effect of BCM on the concentration of methanogenic archaea in ruminants is variable. Goel et al. (2009) reported a complete inhibition of methanogenic archaea as a result of adding BCM in batch cultures and continuous fermenters. However, Abecia et al. (2012) showed no effect in dairy goats treated over 2 mo, and Mitsumori et al. (2012) reported a slight increase and decrease in the abundance of methanogens for low and high doses of BCM, respectively, applied to Japanese goats. The disagreement among these results and the variation of the significance of the effects

in this work over the duration of the trial might be explained by the duration of the dietary intervention and therefore the time that the microbial ecosystem had to adapt to the treatment. Williams et al. (2009) reported that methanogens take longer than 4 wk to adapt to dietary changes, compared with only 10 to 15 d for the bacterial community. Nevertheless, our results from qPCR and DGGE are along the lines of the hypothesis that rather than the abundance, it is the distribution of different species of methanogens (community structure) that drives the synthesis of methane in the rumen as discussed below (Morgavi et al., 2010).

Recently, Gagen et al. (2012) reported that the diversity of methanogens in the rumen of young lambs at 17 h of life was not greatly different than in the rumen of conventional 2-yr-old sheep. This is particularly interesting as the bacterial population present in the rumen from a very young age is different from that found in the rumen of older animals (Fonty et al., 1987). Furthermore, Skillman et al. (2004) reported that the predominant methanogens in mature ruminants were established early, before the rumen is fully developed. This evidence makes our hypothesis of early intervention that specifically targets the Archaea group more feasible. The differences in the archaeal community structure revealed by PCA analysis from DGGE profiles suggest that at weaning the treatment that kids received (k+ vs. k-) was more important than the treatment applied to does. However, when the archaeal community was assessed 1 and 4 mo later, the effect of treating the doe became highly significant, even though all kids stayed grouped together for 3 mo with no treatment at all. Thus, our results agree with those from Skillman et al. (2004) and Gagen et al. (2012), who reported a link between the methanogens that are acquired by ruminants from a very young age throughout rumen development and those in the animal postweaning. In agreement with our work, very recent observations have confirmed that the abundance of some archaeal species and activity is key to explaining CH_4 production in the rumen (Poulsen et al., 2013). It appears then necessary to identify what species would be beneficial to colonize the rumen first to diminish the prevalence of highly active methanogens postweaning without compromising ruminal fermentation and animal performance. In addition, there is a need to establish the most effective time window for intervention after birth for practical feeding management.

In conclusion, these results show that the application of a dietary antimethanogenic compound (BCM) during the early life of kids modified the archaeal population that colonized the rumen, which resulted in lower CH_4 emissions around weaning. This effect persisted over 3 mo despite the treatment being removed after weaning, although only in kids that were raised by treated does, which could have important practical farming implications when designing early life dietary interventions.

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Errata

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In the article "Determination of endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs" (J. Anim. Sci. 2013.91:4807−4816), Equation [4] was published incorrectly in the original version of the article. The correct equation can be found below.

$$Ca_R = \{[Ca_{intake} - (Ca_{fecal} + Ca_{urine})]/Ca_{intake}\} \times 100 \quad [4]$$

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In the article "Nutritional intervention in early life to manipulate rumen microbial colonization and methane output by kid goats post-weaning" (J. Anim. Sci. 2013. 91:4832-4840), the daily dosage of bromochloromethane (BCM) in the section entitled 'Animals, Diets, and Experimental Design' is published incorrectly in the original version of the article. The correct dosage can be found below.

3 mg of BCM/kg BW