Supplementation of DHA-Gold pre and/or postnatally to goat kids modifies in vitro methane production and rumen morphology until 6 mo old¹

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ABSTRACT: This study aimed to investigate the effect of pre and/or postnatal supplementation of a dry whole cell algae (**DHA-Gold**) to goat kids, on in vitro methane (**CH₄**) production, animal growth, and rumen morphology at the age of 6 mo. Furthermore, the in vitro retreatment effect of DHA-Gold was evaluated. Twenty pregnant Saanen goats giving birth to 2 male kids were used. Half of these does were supplemented (**D+**) with 18.2 g/d of DHA-Gold in the last 3 wk of pregnancy, whereas the other half was not (**D-**). After kidding, one goat kid per doe in both groups was supplemented daily with 0.28 g/kg of body weight of DHA-Gold (**k+**) until 12 wk, whereas the other goat kids were untreated (**k-**). This resulted in 4 experimental groups **D+k+**, **D+k-**, **D-k+**, and **D-k-**. In vitro incubations were performed at the ages of 4 wk, 11 wk, and 6 mo. At the age of 6 mo, goat kids were euthanized and additional incubations were performed supplementing 4 doses of DHA-Gold (0, 0.4, 0.8, and 1.6 mg/mL). Additionally, rumen tissue of the atrium ruminis, ventral rumen, and dorsal blind sac were collected to assess rumen morphology. Rumen inocula of 4-wk-old goat kids supplemented **D+** showed lower ($P < 0.05$) in vitro **CH₄** production, however,

this was mainly due to a reduction in the overall fermentation, while **CH₄** expressed relative to total volatile fatty acids (**VFA**) was higher when goat kids were treated **D+** or **k+**. The detrimental **D+** effect on **VFA** production diminished at 11 wk old but remained a tendency ($0.05 < P < 0.1$). As for 4 wk **D+** as well as **k+** supplementation of DHA-Gold stimulated rather than inhibited in vitro **CH₄** production expressed relative to total **VFA**. Supplementation of DHA-Gold either **D+** or **k+** decreased density, width, and surface area of the ruminal papillae. However, no effect on animal growth was observed. Moreover, detrimental effects of **D+** or **k+** treatment on **VFA** production or stimulation of relative **CH₄** production were no longer observed at 6 mo old. Nevertheless, direct exposure of DHA-Gold to 6-mo-old inoculum linearly ($P < 0.05$) decreased **CH₄** and **VFA** production, which tended ($P = 0.06$) to be greater when using **D**-rumen inoculum. Accordingly, neither **D+** nor **k+** DHA-Gold supplementation showed potential for reduction of rumen methanogenesis. Furthermore, this early life intervention could represent some risk for impaired rumen papillae development, which, however, did not impair animal performance.

¹The first author gratefully acknowledges CONACyT (The National Council for Science and Technology) for this PhD scholarship. Sieglinde Debruyne receives a PhD grant from the government agency Flanders Innovation & Entrepreneurship (VLAIO-Belgium). L.D. is financed by the Special Research Fund of Ghent University (BOF, Bijzonder Onderzoeksfonds) (BOF15/DOC/246). Financial support for this research has been provided within the frame of the FACCE JPI Multi-partner Call on Agricultural Greenhouse

Gas Research (RumenStability). The first author thanks Longhui Jing, Laura Rogge, and Dries Vermader for their help during the in vivo trial and Charlotte Melis for assistance in the in vitro incubations. We also acknowledge Yujie Liu and Nguyen Hai Quan for assistance during the internal review.

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Received February 9, 2018.

Accepted July 27, 2018.

Key words: DHA-Gold, goats, in vitro methane, pre/postnatal supplementation, retreatment, rumen papillae

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J. Anim. Sci. 2018.96:4845–4858

doi: 10.1093/jas/sky307

INTRODUCTION

Some micro-algae are particularly rich in docosahexaenoic acid [DHA, C22:6 *n*-3, 250 g/kg dry matter (DM)] (Barclay et al., 1998), a fatty acid which has been shown to increase newborn vitality and improve their cognitive and visual development (Leskanich and Noble, 1999). Furthermore, newborn supplemented piglets prenatally tended to contact the teat of the mother earlier (Rooke et al., 2001a). Moreover, DHA showed to decrease in vitro methanogenesis (Fievez et al., 2007). Hence, additional benefits might be linked to this pre or postnatal supplementation of DHA-rich products, since, early life (EL) methane (CH₄) mitigation strategies showed promising results (Yanez-Ruiz et al., 2010; Abecia et al., 2013). Early life mitigation might be of interest as the treatment takes place when rumen microbes are most susceptible to changes, compared to the more resilient microbes of a mature rumen (Weimer et al., 2015). Besides, De Barbieri et al. (2015) showed the possibility to alter rumen fermentation of newborn lambs by modifying the diet of the mothers prenatally with persistent effects beyond weaning. Nevertheless, persistency of EL CH₄ mitigation might decrease over time (Abecia et al., 2013): although CH₄ production was still reduced 3 mo after bromochloromethane supplementation was stopped, this inhibition was smaller than during supplementation. Hence, retreatment later in life could be an option to maintain permanent effectiveness of the EL intervention. Besides, effects on rumen microbes and their function, pre or postnatal treatment also has been shown to affect rumen wall morphology (Debruyne et al., 2018), as this can be influenced by a change in the rumen fermentation (Malhi et al., 2013). In the present study, it was hypothesized that DHA-Gold supply in the EL would affect the rumen's microbial CH₄ production capacity and rumen morphology, both or either during and after supplementation. The in vitro CH₄ production from inoculum of donor goat kids was used as a proxy to assess the effect on CH₄ production as done by, e.g., Fievez et al. (2003), Klop et al. (2017), and Debruyne et al. (2018).

Furthermore, this EL exposure could affect the microbial CH₄ production upon re-exposure to the same treatment. This was tested through in vitro DHA-Gold supplementation.

MATERIALS AND METHODS

Ethical Approval and Animal Handling

The in vivo experiment was carried out at the experimental farm of Ghent University (Agrivet, Proefhoevestraat 18, 9090 Melle), with the approval from the Ethical Committee of the Faculty of Veterinary Medicine and Bioscience Engineering of Ghent University, Belgium (EC-2016-16). Between the second and third week of age, goat kids were dehorned for safety of the care takers, to prevent injuries among goat kids and to avoid dominance behavior. The dehorning was done following a standard protocol used in practice and approved by the ethical committee of the local university. The in vitro set-up was developed and carried out at the Laboratory for Animal Nutrition and Animal Product Quality (LANUPRO) of Ghent University.

In Vivo Animals, Diets, and Management

Prenatal supplementation. A group of 108 Saanen dairy goats were synchronized (insemination between 2nd and 25th September 2015) at a commercial farm (Zelee, Belgium). During the last 3 wk of pregnancy, the does were randomly divided into 2 experimental groups, 54 does for the control (D-) and DHA-Gold supplemented group (D+) (DSM, Nutritional Products, Deinze, Belgium) (Fig. 1). The DHA-Gold dose supplemented to the does was chosen based on a preliminary study with dairy cows (Debruyne et al., 2016). In this experiment, increasing doses of DHA-Gold were tested in consecutive periods. A daily dose of 0.28 g/kg body weight (BW) of DHA-Gold (corresponding to 0.041 g DHA/kg BW) reduced the in vitro methane production when incubating the rumen inoculum (similar approach as described in the

Figure 1. Experimental design and sampling schedule of the goat trial. 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.

current paper), without impairment of DM intake and *in vitro* VFA production. An equivalent dose (expressed on BW) was offered to the supplemented does (D+). Similarly, an equivalent dose, calculated on BW basis was chosen to supplement *in vivo* to the goat kids used in the present study. All does were fed grass silage (first week) or a roughage mixture (in g/kg of fresh material basis: 720/200/80 of maize silage/grass silage/fodder beet, during the last 2 wk) *ad libitum* which was supplemented with a compound feed (1 kg/d). Diets were offered as 2 equal meals at 0900 and 1500 h). Each group was housed in a pen with free access to fresh water. The compound feed differed in type depending on experimental group (Table 1), containing either 18.2 g/kg of rapeseed for the control group (D-) or 18.2 g/kg of DHA-Gold mixed in the concentrate for the supplemented group (D+). Both concentrates (Table 2) were equal in NE_L (958 VEM/kg, Van Es, 1978) and small intestine digestible protein (DVE = 119 g/kg; Tamminga et al., 1994) as well as proximate chemical composition (in g/kg of DM: organic matter, 954; crude protein, 205; crude fat, 36; neutral detergent fiber, 176). We relied on the Dutch NE evaluation system and the feed values of the different compounds in each concentrate (Blok and Spek, 2016), with VEM being the unit for the NE_L, which is also used to calculate NE requirements for maintenance and growth of goats (feed unit lactation; The Central Bureau Livestock Feeding; Van Es, 1978). Additionally, the Dutch true protein digested in the intestine system was used to calculate the small intestine digestible protein requirements for maintenance and growth of goats (true protein digested in the intestine; The Central Bureau Livestock Feeding; Tamminga et al., 1994).

Postnatal supplementation. In each group of does, the goal was to obtain at least 10 does kidding

Table 1. Chemical composition (g/kg of product) and fatty acid composition (g/kg of product) of rapeseed and DHA-Gold

	Rapeseed ¹	DHA-Gold ¹
Moisture	7.70	20.3
Crude protein	198	167
Crude fat	415	556
Starch	174	124
Crude ash	39.0	88.1
Crude fiber	97.0	45.0
Fatty acids		
14:0	26.0	30.7
16:0	2.8	83.8
18:0	0.6	2.0
18:2 <i>n</i> -6	7.0	0.5
22:0	n.d.	54.0
22:6 <i>n</i> -3	n.d.	148

n.d. = not detected.

¹Chemical composition according to CBV-table (Blok and Spek, 2016) and DSM for rapeseed and DHA-Gold, respectively.

of preferably at least 2 male kids. This goal could not be obtained, and hence 2 does with only 1 single male kid were retained in both the D+ and D- group as they did not give birth to male twins. All 40 goat kids used in the experiment were born within a time frame of 35 d. After kidding, the goat kids were immediately separated from their mother and each allocated to 1 of 2 experimental groups: nontreated (k-) or treated (k+, daily 0.28 g/kg of BW per day of DHA-Gold), thus resulting overall in 4 experimental groups each containing 10 goat kids: D+k+, D+k-, D-k+, and D-k- as illustrated in Fig. 1.

The treatment of the goat kids (k+) started within 6 h after birth and lasted until 3 wk post-weaning (12 wk old), during which goat kids were grouped in pairs of the same experimental condition in 20 separate pens with free access to fresh water and hay. The pens were constructed to avoid physical contact between the neighboring goat kids

Table 2. Ingredients (g/kg of product) and fatty acid composition (g/kg of product) of the concentrate of control (D–) group and DHA-Gold (D+) group

Ingredients	Concentrate	
Soy bean meal	260	
Rapeseed (D–) or DHA-Gold (D+)	18.2	
Maize	231.8	
Beet pulp	200	
Wheat	150	
Molasses	50	
Lignosulphonate	20	
Vitamin premix AD ¹	16	
Vitamin E ¹	1	
Feed phosphate	16	
Trace elements ²	16	
Salt	10	
MgO 93%	7	
Calcium carbonate	4	
DVE ³	119	
VEM (VEM/kg) ⁴	958	
FA composition	D–	D+
14:0	16.2	21.2
16:0	3.32	5.12
18:0	0.65	0.53
18:2 <i>n</i> -6	9.63	8.37
22:0	-	1.13
22:6 <i>n</i> -3	< 0.10	2.87

¹VIT A, D and E: (UI/g) A (312), D (62.5) and E (6,000).

²Premix of trace elements and minerals: (mg/g) Fe (281), Cu (62.5), Mn (116), Co (1.4), Zn (175), I (4.8), Ca (244.3), P (157), K (248), Mg (73.8) and Na (8.1).

³DVE = true protein digested in the small intestine (Tamminga et al., 1994).

⁴VEM = feed unit milk (1000 VEM = 6.9 MJ; Van Es, 1978).

during the first weeks of life (until 12 wk of age). Prior to each supplementation, a DHA-Gold emulsion was freshly prepared in water (0.333 g/mL) and was administered orally before the morning and afternoon feeding with a 10 mL syringe. The dose of DHA-Gold was raised every 2 wk according to the BW of the goat kids. Body weight was recorded before morning feeding every 2 wk until 12 wk for all goat kids and at euthanasia (6 mo of age). Goat kids received colostrum by individual bottles (mixture from all does kidding on the same day, irrespective of their treatment) on the first day after birth, on the second day they received mixed colostrum from the second and third milking, and from day 3 until weaning (9 wk) goat kids received artificial milk, prepared using goat milk powder (Denkamilk Capri Ovi, DenkaVit, Voorthuizen, the Netherlands) (containing g/kg DM: crude protein 380, crude fat 470 fat, and crude ash 70, respectively).

Concentrate and hay was at the disposal of the goat kids from week 2 of age onwards and goat kids were gradually weaned from 6 to 9 wk of age, replacing the milk powder by increasing the offer of hay (ad libitum) and concentrate (maximum 1 kg per pen/d) (Capri, AVEVE, Merksem, Belgium). From week 6 onwards, milk powder concentration was lowered from 200 to 175 g/liter. At week 7, the total amount of artificial milk was reduced to 1 liter per 2 goat kids per feeding time, for the last 2 wk (week 8 and 9), the milk feeding time was reduced to 500 mL per 2 goat kids once a day (0830 h). By the end of week 9, goat kids were totally weaned. Postnatal supplementation of DHA-Gold (k+) was ceased 3 wk after weaning (12 wk old). At this age, goat kids were moved to a pasture for the last 3 mo of the experiment (May to August, 2016) and goat kids were housed together.

The pasture (0.5 ha) surface was a permanent pasture with native species, i.e., perennial ryegrass (*Lolium perenne*), bent grasses (*Agrostis sp.*), and rough stalked meadow-grass (*Poa trivialis*). The pasture had been fertilized as follows: dairy cattle slurry was injected as soon as the bearing capacity of the land was sufficient (end February to mid March 2016). The average injected dose was 42.5 ton ha/yr with an average composition (kg/ton) of 4.0 kg N, 0.6 kg P, and 3.5 kg K, respectively, resulting in a slurry fertilization of 170 kg N ha/yr, 38.3 kg P ha/yr, and 149 kg K ha/yr on average. An additional 150 kg of N-P-K from artificial fertilizer in a ratio of 15/4/15 (w/w/w), respectively, was applied in 2 applications between May and August 2016. Pasture was provided with a shelter, water was offered ad libitum and a supplementation of 500 g/animal per day of goat concentrate was provided (Capri, AVEVE, Merksem, Belgium). The pasture surface had been grazed by goat kids during a previous experiment. The pasture had not been grazed for a period of 7 mo before the goat kids were moved to the pasture. Hay was offered throughout the time on the pasture in a fixed hay rack to complement the animal requirements, when weather conditions (excess or lack of rain) were not favorable to support grass growth.

Rumen Fluid Sampling and Euthanasia

At the ages of 4 and 11 wk, rumen inoculum from the 40 goat kids was collected before the morning feeding through esophageal tubing using a plastic tube of 15 mm diameter connected to a plastic erlenmeyer and a vacuum pump. Goat kids were fasted the night prior to sampling. Sampling

material was rinsed with hot water between sampling, from different goat kids and disinfected with a solution of 70% of ethanol diluted in water. At each sampling time, an aliquot of 30 mL of rumen inoculum was collected per goat kid in 2 different insulated flasks of 15 mL of volume. Rumen inocula per goat kid were kept anaerobically at 39 °C inside of a prewarmed thermos and were used to perform in vitro incubations which started within 1 h after collection of rumen inoculum.

At 6 mo of age, goat kids were brought back into the stable on 4 different days (in the afternoon) within a 2-wk period, in 4 groups of 10 goat kids (2 or 3 goat kids per group). Goat kids were euthanized by a standard protocol (Xylazine and Release Nembutal, 3 mL/10 kg BW, injected intravenously) after a previous fasting of 12 h. Total rumen content from each goat kid was collected after opening the rumen, from which an aliquot of 100 mL was kept anaerobically in 2 insulated flasks of 50 mL which were kept at 39 °C in a prewarmed thermos flask to perform in vitro incubations, within 1 h after sampling. For practical reasons, euthanasia and following rumen sample collection were performed for 4 different days. On each sampling day, animals from each experimental condition were included.

In Vitro Incubations at the Age of 4 wk, 11 wk, and 6 mo

Batch in vitro incubations were performed according to [Vlaeminck et al. \(2014\)](#), using fresh rumen fluid collected from 40 goat kids at 4 and 11 wk of age and at 6 mo of age. The insulated flasks containing the rumen fluid of the same goat kid were mixed, homogenized, and filtered through a sieve with a pore size of 1 mm. Rumen fluid was kept under continuous CO₂ flushing in a water bath at 39 °C. Dried grass silage 150 mg (at the age of 4 and 11 wk) or 250 mg (at the age of 6 mo) was added to the 120 mL incubation flasks, wetted with 1 mL of distilled water and flushed with 100% CO₂ or 100% H₂. Prior to flushing, 4 doses of DHA-Gold (0, 0.4, 0.8, and 1.6 mg/mL) were supplemented to the flasks (only to those with the goat kid's rumen inoculum at the age of 6 mo) and rumen inoculum was mixed with a phosphate-bicarbonate buffer at a ratio of 1:4, under constant CO₂ flushing. Then, 14 mL (at 4 and 11 wk of age) or 24 mL (at 6 mo of age) of the mixture was injected in the flask. The phosphate-bicarbonate buffer was prepared the day prior to the incubation ([Mould et al., 2005](#)), and continuously flushed with CO₂ overnight. Immediately prior to the start of

the in vitro incubation, 1 mL of ethane (C₂H₆) gas was injected in the flask as internal gas standard. Incubation flasks were maintained under anaerobic conditions at 39 °C during 24 h, with intermittent shaking in a batch culture incubator (Edmund Bühler GmbH, Hechingen, Germany). After 24 h of incubation, the flasks were removed from the incubator and the reaction was stopped by cooling the flasks for 5 min in an ice bath. All in vitro incubations were performed in duplicate, serving as an analytical replicate.

Sampling of Rumen Wall and Assessment of Rumen Wall Morphology at the Age of 6 mo Old

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 spread out 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. Three anatomical areas (adapted from [Lesmeister et al., 2004](#)) were selected for papillae morphology analysis: 1) the ruminal atrium, 2) the ventral sac, and 3) the caudodorsal blind sac. A patch of 3 × 3 cm was cut per area and cleaned carefully with distilled water. Patches were immediately pinned on cardboard pieces with needles and immersed in glass pots (150 mL) filled with 4% neutral formaldehyde solution for histomorphometric analysis.

On the same day of sampling, rumen wall samples were analyzed for papillae density (PD, number/cm²) by stereomicroscopy (Olympus SZX7, Olympus, Center Valley, PA, USA) (power objective lens: 8×), using the “forbidden lines method” ([Gundersen, 1977](#)). Samples were further left to fixate in 4% neutral formaldehyde solution for 24 h. Afterwards, 1 cm² was cut from the sample and divided into 2 subsamples of 0.5 cm². 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 sample 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 prepare by immersing the glass slide in decreasing alcohol series (Böck and Romeis, 1989), and stained with hematoxylin and eosin (H & E). Tissue samples were further preserved by adding artificial resin (DPX) 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, Center Valley, PA, USA) (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 through image analysis software (Olympus CellSense Dimension, Olympus, Center Valley, PA, USA). Total surface area (TSA) of papillae per cm² mucosa was determined as length × width × 2 × papillae density.

Chemical Analysis

In vitro methane production. Gas accumulation in the headspace of each incubated flask was estimated using a pressure transducer (Gems Sensors & Controls, Basingstoke, UK). Overpressure was released and the composition of the gas phase was analyzed for CH₄ and hydrogen (H₂) with a micro gas chromatograph (GC) equipped with 2 gas chromatographic modules and equipped with a thermal conductivity detector (3000 micro-GC, Agilent Technologies, Diegem, Belgium) according to Hassim et al. (2010). A standard series of 8 flasks with known volumes of H₂, CH₄, and C₂H₆ were injected to develop regression curves, fitted per incubation run, to calculate the net production of CH₄ and H₂ accumulation in the incubated flasks.

Volatile fatty acid quantification. After gas analysis, pH was measured (Hanna Instruments, Temse, Belgium) after which the liquid content in the incubated flasks was collected (2 mL) and acidified with 200 µL of formic acid which contained an internal standard [10 mg 2 ethyl butyric acid (Sigma Aldrich, Diegem, Belgium) per 1 mL of formic acid) for analysis of volatile fatty acids (VFA). After 15 min of centrifugation (4 °C, 31,000×g), the supernatant was filtered and an aliquot was transferred into a 2 mL glass vial. Samples were stored at 4 °C until analysis using a gas chromatograph (HP 7890A, Agilent Technologies, Diegem, Belgium) as described by Gadeyne et al. (2016). Nonincubated rumen inoculum per goat kid was stored at 4 °C

and analyzed for VFA concentration to calculate the net production of VFA after 24 h in the incubated flasks by subtraction.

Long-chain fatty acids quantification in compound feed of does. Long-chain fatty acid quantification in the feeds was quantified using 25 mg of sample for DHA-Gold and rapeseed, and 250 mg of sample for both compound feeds (D− and D+). All samples were done in triplicate using fresh products by direct transesterification according to the method described by Vlaeminck et al. (2014). Analysis of fatty acid methyl esters was carried out by gas chromatography (HP7890A, Agilent Technologies, Diegem, Belgium) using a SP-2560 capillary column (75 m × 0.18 mm, i.d. × 0.14 µm thickness; Supelco Analytical, Bellefonte, PA, USA) and a flame ionization detector. The temperature program was initially set at 70 °C, increased at 50 °C/min to 175 °C, held at 175 °C for 13 min, increased at 5 °C/min to 215 °C and held at 215 °C for 20 min. Inlet and detector temperatures were 250 and 255 °C, respectively. The split ratio was 50:1. Hydrogen was used as the carrier gas at a flow rate of 1 mL/min. Peaks were identified using mixtures of FAME standards (C22:5 n-6 and GLC463; Nu-Chek-Prep., Elysian, MN, USA).

Hydrogen Recovery (2Hr, %)

Hydrogen recovery (2Hr) was calculated as $(2P + 2B + 4CH_4 + H_2)/(2A + P + 4B) \times 100$ with acetate (A), propionate (P), butyrate (B), and CH₄ expressed as net molar production and H₂, the net molar accumulation after 24 h of incubation. This recovery reflects the validity of the model of metabolic H₂ transfer in the rumen fermentation model (Marty and Demeyer, 1973).

Statistical Analysis

The effect of DHA-Gold supplemented pre and/or postnatally to goat kids, on in vitro rumen CH₄ production and fermentation pattern (at 4 and 11 wk of age) and animal growth (at 6 mo of age) were evaluated using the MIXED procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC, USA). Summarized, the statistical model can be presented as

$$y_{ijkl} = \mu + PR_j + EL_i + PR_j \times EL_i + A_l + \varepsilon_{ijkl}$$

in which y_{ijkl} is the response, PR_j the fixed effect of prenatal treatment with DHA-Gold (j = does supplemented (D+) or not (D−) with DHA-Gold), EL_i

early life treatment with DHA-Gold (i = goat kids supplemented (k+) or not (k-) with DHA-Gold), A_l the random effect of the animal (l = goat kids), and ε the residual error.

The effect of the in vitro doses supplementation of DHA-Gold was evaluated by multivariate ANOVA using the MIXED procedure in SAS (version 9.1; SAS Institute Inc., Cary, NC). Following the experimental model:

$$y_{ijkl} = \mu + PR_j + EL_i + D_k + PR_j \times EL_i + PR_j \times D_k + EL_i \times D_k + A_l + \varepsilon_{ijkl}$$

in which y_{ijkl} is the response, PR_j pregnancy treatment with DHA-Gold (j = does supplemented or not with DHA-Gold), EL_i early life treatment with DHA-Gold (i = goat kids supplemented or not with DHA-Gold), D_k in vitro doses of DHA-Gold (k = 0, 0.4, 0.8, and 1.6 mg/mL), A_l the random effect of the animal (l = goat kids), and ε the residual error.

Normality of data was checked and if necessary log transformation was performed (i.e., rumen/BW%, papillae base width in the ventral rumen side and papillae length in the ventral rumen side—presented in Table 3). Prior to statistical analysis, analytical replicates were averaged. Significance was declared at $P \leq 0.05$, whereas $0.05 < P < 0.10$ values were considered to be a trend toward significance.

RESULTS

Intake, Animal Performance, Rumen Characteristics, and Morphometric Properties of Ruminant Papillae

Neither pre nor postnatal supplementation of DHA-Gold affected ($P > 0.05$) the intake (mL/d) of colostrum and goat milk replacer (Table 3). From 7 wk until weaning (9 wk), all goat kids fully consumed the offered goat milk replacer. All goat kids started eating concentrate from the second week onwards, irrespective of the treatment (Table 3). In the second and third week, D+ goat kids consumed or tended to consume less concentrate (g/d) as compared to D- goat kids ($P = 0.01$ and $P = 0.06$, respectively) and this reduction was even stronger when combined with a postnatal supplementation k+ ($P_{D \times k} < 0.05$). However, this effect disappeared from the fourth week onwards (Table 3). From the seventh week onwards, all goat kids were eating at least 1 kg/pen per day of concentrate (Table 3). Goat kids without a prenatal supplementation of DHA-Gold (D-) showed a higher ($P < 0.05$) BW

at birth. However, this might be explained by the unintended larger number of goat kids born from the D+ mothers (D+: 3 twins; 6 triplets; 1 quad; D-: 6 twins; 4 triplets), which cannot be due to the prenatal treatment as this only took place during the last 3 wk of pregnancy. As the does were randomly divided into the 2 groups, no differences in fetal numbers had been expected. Hence, echographic checking prior to groups separation was not deemed necessary to compose both groups, but could have excluded this potential confounding factor. However, the effect on BW was not persistent, and did not differ between groups from week 4 onwards ($P > 0.05$) (Table 3). The daily growth (g/d) did not differ ($P > 0.05$) over the supplementation period. Nevertheless, a trend ($P = 0.08$) was observed for a greater daily growth (g/d) from birth until weaning in D- goat kids (Table 3). The BW (kg) of the goat kids at 6 mo of age did not show statistical differences ($P > 0.05$) between experimental groups (Table 3). However, k+ goat kids tended to slightly gain more BW ($P = 0.06$) as compared to k- goat kids (120 vs. 112 g/d, respectively; Table 3). Both empty rumen weight ($P_{D \times k} = 0.06$) as well as rumen digesta weight ($P_{D \times k} = 0.04$) tended to be greater for D+k+ goat kids (Table 3). Papillae density in the atrium ruminis ($P = 0.04$) and dorsal blind sac ($P = 0.08$) was greater for k- goat kids. Papillae width in the dorsal blind sac and total papillae surface area showed an interaction effect: the former was greater for D-k+ goat kids, whereas the latter was greater in the D-k- goat kids group ($P < 0.05$).

In Vitro Methane Production and Ruminant Fluid VFA

The effect of DHA-Gold supplemented to goat kids pre and/or postnatally on in vitro CH_4 and total VFA production using rumen inoculum at 4 wk, 11 wk, and 6 mo old is presented in Table 4. Under full CO_2 atmosphere in the in vitro incubation flasks, rumen inocula collected from 4-wk-old goat kids which had been prenatally exposed to DHA-Gold (D+) showed a lower ($P = 0.05$) CH_4 production. However, this effect disappeared by the end of the postnatal treatment period (12 wk) and at 6 mo of age. Postnatal supplementation of DHA-Gold during EL (k+) did not modify ($P > 0.05$) in vitro CH_4 production by rumen inoculum at the age of 4 wk, whereas at 11 wk of age, the rumen inoculum of k- goat kids showed a lower ($P = 0.05$) in vitro CH_4 production. Differences in CH_4 production at these 2 preweaning ages

Table 3. Body weight (gain), milk replacer and concentrate intake until weaning, as well as rumen weight and papillae histomorphometric parameters of goat kids supplemented with DHA-Gold pre and/or postnatally

		Early life treatment ¹					<i>P</i> -value ³		
	Period	D−k−	D+k−	D+k+	D−k+	SEM ²	D	k	D x k
Animal performance									
Start BW kg	2 d	4.4	3.5	3.9	4.1	0.61	0.02	0.96	0.09
BW kg	2 wk	6.8	5.8	6.3	6.8	1.56	0.03	0.85	0.23
BW gain b-2wk g/d ⁴	0–2 wk	172	169	180	182	10.80	0.88	0.21	0.94
Weaning BW kg	9 wk	19.9	18.4	19.4	19.8	1.12	0.21	0.32	0.31
BW gain 2wk-w, g/d ⁴	2–9 wk	316	292	307	314	22.0	0.10	0.21	0.42
BW slaughter	6 mo	36.2	33.5	36.4	35.9	1.32	0.50	0.18	0.15
BW gain w-s, g/d ⁴	9 wk–6 mo	115	110	123	118	35.2	0.53	0.06	0.32
Total MRI L⁵	0–9 wk	18.7	17.4	18.1	18.4	0.95	0.16	0.41	0.29
Concentrate intake (g/d)⁵									
	2 wk	24 ^b	5 ^c	3 ^c	53 ^a	20.7	0.01	0.45	0.03
	3 wk	286 ^{ab}	143 ^b	99 ^c	357 ^a	109.8	0.06	0.86	0.04
	4–12 wk	892	863	896	912	17.9	0.18	0.56	0.62
Slaughter									
	6 mo								
Rumen digesta (kg)		5.8 ^{ab}	5.3 ^b	6.4 ^a	5.7 ^{ab}	0.45	0.78	0.09	0.04
Empty rumen (g)		792	700	800	780	370	0.41	0.14	0.06
Empty rumen/BW (%) [*]		2.2	2.0	2.2	2.2	0.01	0.53	0.46	0.27
VFA (mmol/L)⁶									
	6 mo								
VFA		153	135	151	140	22.81	0.48	0.56	0.45
Acetate		114 ^a	101 ^b	110 ^a	100 ^b	10.68	0.19	0.18	0.05
Propionate		24.3 ^a	19.5 ^b	23.3 ^a	22.8 ^a	3.72	0.49	0.52	0.04
Butyrate		8.35 ^{ab}	7.51 ^b	9.01 ^a	8.60 ^a	1.31	0.65	0.41	0.05
Rumen morphology									
	6 mo								
PD (no/cm²)⁷									
Atrium ruminis		66.3	59.6	53.0	57.4	5.47	0.26	0.04	0.75
Ventral rumen		47.8	55.9	49.1	47.9	3.48	0.18	0.31	0.30
Dorsal blind sac		50.8	56.3	45.7	47.6	4.50	0.71	0.08	0.33
PW (mm)⁷									
Atrium ruminis		0.58	0.52	0.55	0.57	0.022	0.08	0.73	0.38
Ventral rumen [*]		0.52	0.49	0.50	0.49	0.014	0.67	0.62	0.31
Dorsal blind sac		0.48 ^{ab}	0.48 ^{ab}	0.47 ^b	0.53 ^a	0.013	0.09	0.21	0.05
PL (mm)⁷									
Atrium ruminis		4.8	3.9	4.4	4.4	0.26	0.21	0.86	0.06
Ventral rumen [*]		1.9	2.2	1.8	2.0	0.02	0.84	0.33	0.06
Dorsal blind sac		1.7	1.8	1.7	1.7	0.10	0.77	0.60	0.35
TSA (mm²/cm²)⁷									
Atrium ruminis		361 ^a	242 ^b	257 ^b	288 ^{ab}	25.8	0.01	0.15	0.03
Ventral rumen		96.5	122.6	93.3	95.5	9.07	0.20	0.11	0.13
Dorsal blind sac [*]		81.3	93.6	70.9	84.5	1.13	0.87	0.23	0.12

^{a,b,c}Means of the same parameter annotated with a different letter in the same row differ significantly ($P < 0.05$).

¹Early life treatments: D+k+ ($n = 10$): treated kids from treated does; D+k- ($n = 10$): untreated kids from treated does; D-k+ ($n = 10$): treated kids from untreated does; D-k- ($n = 10$): untreated kids from untreated does.

²SEM = standard error of the mean.

³P-value of the effect of DHA-Gold supplementation prenatally during pregnancy (D), during the first 11 wk of life (k) and interaction D x k.

⁴b = birth, w = weaning, s = slaughter, wk = week

⁵Milk replacer intake (MRI = colostrum + milk replacer intake) and concentrate intake are reported as the average intake over the whole period of 11 wk per pen (2 goat kids).

⁶Total and individual VFA (mmol/L) at slaughter.

⁷PD = papillae density, PW = papillae base width, PL = papillae length and TSA = total surface area.

*P-values of the statistical analysis using transformed data (Log). The means of each early life treatment were retransformed to be presented in the table.

BW = body weight; VFA = volatile fatty acids.

disappeared under 100% H₂ atmosphere. Total in vitro VFA production was lower ($P < 0.001$) for D+ goat kids at 4 wk of age, and this tended to persist ($P = 0.06$) up to 11 wk. As such, CH₄ reduction at 4 wk of age, induced by the prenatal treatment, particularly was caused by a reduction in the overall fermentation, CH₄ production proportional

to total VFA production was higher in inoculum from goat kids which had received DHA-Gold either pre or postnatally at both preweaning ages. In accordance with the increase in CH₄ production, in vitro acetate proportions (mol/100 mol) tended to increase ($P = 0.08$) when incubating rumen inoculum of k+ goat kids at 11 wk of age. In vitro

Table 4. In vitro CH₄ and total VFA production, acetate, propionate, and butyrate proportions (mol/100 mol) and hydrogen recovery (2Hr) after 24-h of in vitro incubation under 100% CO₂ atmosphere (100% H₂ atmosphere only for CH₄ production) using rumen inoculum of donor goat kids obtained at 4 wk, 11 wk, and 6 mo of age after in vivo supplementation with DHA-Gold pre and/or postnatally up to 11 wk

	Early life treatment ¹				SEM ²	<i>P</i> -value ³		
	D-k-	D+k-	D+k+	D-k+		D	k	D x k
4 wk								
CH ₄ (μmol/g DM)								
100% CO ₂	1,182	1,131	1,043	1,268	300.6	0.05	0.99	0.36
100% H ₂	7,885	8,059	7,351	8,982	1,738.2	0.19	0.72	0.11
Total VFA (μmol/g DM)	3,113	2,559	2,406	2,921	374.0	<0.001	0.15	0.87
Individual VFA (mol/100 mol)								
Acetate	69.6	70.0	69.1	67.7	3.99	0.48	0.27	0.69
Propionate	15.9	15.8	16.8	17.4	2.98	0.86	0.04	0.07
Butyrate	10.5	9.5	10.0	10.1	2.38	0.44	0.93	0.58
Ac:Prop (mol/mol)	4.65	4.62	4.33	4.01	1.33	0.75	0.33	0.75
CH ₄ :VFA (mol/mol)	0.37 ^c	0.44 ^a	0.43 ^b	0.43 ^b	0.09	0.08	0.76	0.03
2Hr (mol/mol)	1.0	1.1	1.1	1.1	0.13	0.34	0.75	0.56
11 wk								
CH ₄ (μmol/g DM)								
100% CO ₂	765	794	901	917	131.2	0.87	0.02	0.59
100% H ₂	4,821	5,244	5,598	5,533	990.2	0.27	0.61	0.71
Total VFA (μmol/g DM)	2,648	2,388	2,547	2,650	301.5	0.06	0.40	0.41
Individual VFA (mol/100 mol)								
Acetate	58.6	59.6	63.8	61.0	5.80	0.30	0.08	0.61
Propionate	24.4	22.0	21.9	23.8	3.96	0.09	0.77	0.87
Butyrate	9.0	9.7	9.1	8.7	2.26	0.76	0.24	0.67
Ac:Prop (mol/mol)	2.42	2.74	3.03	2.64	0.59	0.11	0.16	0.93
CH ₄ :VFA (mol/mol)	0.29 ^b	0.33 ^{ab}	0.35 ^a	0.34 ^a	0.04	0.05	0.07	0.04
2Hr (mol/mol)	1.0	1.0	1.0	1.1	0.12	0.85	0.23	0.42
6 mo								
CH ₄ (μmol/g DM)								
100% CO ₂	1,346	1,299	1,412	1,381	303.7	0.89	0.38	0.65
100% H ₂	4,235 ^c	4,781 ^{ab}	4,996 ^a	4,667 ^b	280.6	0.09	0.56	0.04
Total VFA (μmol/g DM)	5,338	5,291	5,521	5,576	334.9	0.63	0.03	0.97
Individual VFA (mol/100 mol)								
Acetate	64.7	66.3	67.7	66.7	1.54	0.01	<0.010	0.50
Propionate	23.8 ^a	22.8 ^a	20.5 ^b	22.3 ^{ab}	1.80	0.02	<0.001	0.04
Butyrate	6.91	6.27	6.72	6.22	0.67	0.75	0.56	0.07
Ac:Prop (mol/mol)	2.73 ^b	2.92 ^b	3.30 ^a	3.01 ^{ab}	0.29	0.01	0.00	0.04
CH ₄ :VFA (mol/mol)	0.25	0.24	0.25	0.24	0.04	0.97	0.80	0.59
2Hr (mol/mol)	0.90	0.87	0.82	0.86	0.96	0.56	0.42	0.11

^{a,b}Means of the same parameter annotated with a different letter in the same row differ significantly ($P < 0.05$).

¹Early life program treatments: D+k+ ($n = 10$): treated kids from treated does; D+k- ($n = 10$): untreated kids from treated does; D-k+ ($n = 10$): treated kids from untreated does; D-k- ($n = 10$): untreated kids from untreated does.

²SEM = standard error from the mean.

³P-value ($P < 0.05$) for the effect of Does (D), from birth until 2 wk after weaning (k) and interaction (D x k).

DM = dry matter; VFA = volatile fatty acids.

propionate proportions (mol/100 mol) were greater ($P = 0.04$) in the rumen inoculum of k+ goat kids at 4 wk of age, while rumen inoculum of D+ goat kids at 11 wk showed a trend ($P = 0.09$) to decrease propionate proportions (mol/100 mol). Using 6 mo rumen inoculum of k+ goat kids resulted in greater ($P = 0.03$) in vitro total VFA production ($\mu\text{mol/g DM}$). Moreover, a greater ($P < 0.001$) acetate proportion (mol/100 mol) was observed (67.2 vs. 65.5 mol/100 mol) for k+ vs. k- inoculum, respectively, whereas propionate proportions showed an interaction effect ($P = 0.04$) which resulted in the lowest propionate proportion for the double treated kids. Butyrate proportions (mol/100 mol) did not differ between the 4 experimental groups. Furthermore, a greater ($P_{D \times k} = 0.04$) acetate:propionate ratio (3.30) was observed for the D+k+ goat kids' rumen inoculum.

In Vitro Dose Response to DHA-Gold Supplementation of Goat Kid's Inoculum Which Had Been Exposed to a Similar Treatment Prenatally and/or Early in Life

The dose response of DHA-Gold supplemented in vitro on CH_4 and VFA production after 24 h of incubation with rumen inoculum of 6-mo-old goat kids supplemented pre and/or postnatally with DHA-Gold is presented in Table 5. Irrespective of the 4 experimental conditions, the in vitro CH_4 production ($\mu\text{mol/g DM}$) linearly decreased ($P < 0.05$) with increasing doses of DHA-Gold and hence, CH_4 inhibition (%) increased. Moreover, an interaction effect was observed ($P_{D \times k} = 0.04$), indicating a greater decrease in the in vitro CH_4 production for the rumen inoculum of D-k+ goat kids, supported by the biggest slope (-98.6). In vitro CH_4 inhibition by DHA-Gold tended to be smaller ($P_D = 0.09$) when using inoculum from D+ goat kids as compared with D- goat kids, which is reflected in a smaller slope (5.14 vs. 6.60, respectively).

Generally, in vitro supplementation of DHA-Gold linearly suppressed ($P < 0.05$) the total VFA production by rumen inoculum of 6-mo-old goat kids irrespective of the experimental condition. A significant effect of the prenatal condition ($P = 0.05$) was shown for total VFA production: in vitro supplementation of DHA-Gold on average suppressed fermentation more when supplemented to rumen inocula of goat kids which had not been exposed prenatally (D-) to DHA-Gold. Moreover, an interaction effect ($P_{D \times k} = 0.04$) was shown between the pre and postnatal condition. Accordingly, the response to in vitro DHA-Gold

supplementation depended on the rumen inoculum with more negative slopes for the D- inocula as compared with D+ inocula (-88.4 vs. -57.2 , respectively). Acetate proportions (mol/100 mol) showed an interaction trend ($P_{D \times k} = 0.06$) indicating that in vitro DHA-Gold supplementation decreased the acetate proportion more when supplemented to D-k+ goat kid's inoculum (slope = -1.01 , Table 5). Similarly, an interaction effect ($P_{D \times k} = 0.04$) was shown for propionate proportions (mol/100 mol), with the latter increasing more in D-k+ goat kid's rumen inoculum (slope = 0.44).

DISCUSSION

Supplementation of poly-unsaturated fatty acids (PUFA), such as DHA, to newborns has shown to increase vitality and growth, presumably through stimulation of dry matter intake (DMI) at young age, e.g., in piglets (Tanghe and De Smet, 2013). In neonatal and growing sheep improved health and performance through DHA or fish oil supplementation has also been reported (Lewis et al., 2008; Moreno-Indias et al., 2012). Additionally, supplementation of these long chain PUFA to young animals might be an interesting approach towards abatement of rumen methanogenesis. Indeed, in vitro supplementation of DHA-Gold decreased CH_4 production by 30% without depressing rumen fermentation (Fievez et al., 2007). Moreover, modification of rumen function early in life might have persisting effects at later age (Yañez-Ruiz et al., 2015). Accordingly, it was hypothesized that DHA-Gold supplementation pre and/or postnatally could induce changes in the rumen microbial functionality and particularly in rumen methanogenesis which could persist after supplementation ceased. As former studies on early life supplementation of dietary fat also indicated persistent effects on rumen morphology (Debruyne et al., 2018) and modifications in retreatment effectiveness (Ruiz-González et al., 2017), these parameters were also investigated in this study.

Effect of Pre and Postnatal DHA-Gold Supplementation on Rumen Methanogenesis and Fermentative Capacity: In Vitro Assessments

Former studies indicated in vitro supplementation of DHA, either as fish oil (Fievez et al., 2003) or as DHA enriched micro-algae (Fievez et al., 2007) reduced rumen methanogenesis. This in vitro effect further was confirmed in vivo through fish oil supplementation (Fievez et al., 2003). In line with this

Table 5. Effect of increasing in vitro doses of DHA-Gold on CH₄ inhibition and total VFA production after 24-h of in vitro incubation using rumen inoculum of 6-mo-old donor goat kids supplemented pre and/or postnatally with DHA-Gold

EL treatment ¹	<i>In vitro</i> DHA-Gold dose (mg/mL)					Slope ³	Contrast ⁴		<i>P</i> -value ⁵				
	0	0.4	0.8	1.6	SEM ²		L	Q	D	k	D x k	D x dose	k x dose
CH₄ (μmol/g DM)													
D−k−	1346	1316	1291	1324	40.2	-76.12 ^B	0.04	0.08	0.06	0.05	0.04	0.16	0.11
D+k−	1299	1280	1256	1202	82.6	-61.60 ^C	0.03	0.52					
D+k+	1412	1386	1346	1288	70.2	-79.08 ^B	0.03	0.23					
D−k+	1381	1343	1309	1223	52.4	-98.58 ^A	0.02	0.04					
CH₄ inhibition (%)													
D−k−	0	2	4	9	2.9	5.66 ^{AB}	0.04	0.45	0.09	0.42	0.24	0.06	0.35
D+k−	0	1	3	8	1.6	4.74 ^B	0.02	0.23					
D+k+	0	2	5	9	3.2	5.60 ^B	0.04	0.21					
D−k+	0	3	5	11	3.7	7.14 ^A	0.03	0.62					
Total VFA (μmol/g DM)													
D−k−	5338	5204	5324	5179	89.1	-74.44 ^B	0.08	0.09	0.05	0.07	0.04	0.13	0.21
D+k−	5291	5318	5290	5220	110.6	-51.75 ^C	0.09	0.03					
D+k+	5521	5592	5631	5437	120.8	-61.26 ^C	0.07	0.04					
D−k+	5576	5519	5418	5417	150.9	-101.50 ^A	0.06	0.04					
Individual VFA (mol/100 mol)													
Acetate													
D−k−	66.3	66.5	66.3	66.0	1.96	-0.24 ^B	0.82	0.63	0.16	0.08	0.06	0.94	0.97
D+k−	66.3	67.0	66.3	66.0	3.54	-0.34 ^B	0.12	0.56					
D+k+	67.6	68.0	67.5	67.0	2.12	-0.48 ^{AB}	0.52	0.23					
D−k+	64.7	65.8	64.6	63.5	1.42	-1.01 ^A	0.32	0.12					
Propionate													
D−k−	23.8	23.2	23.9	24.2	1.89	0.39 ^B	0.04	0.13	0.03	0.04	0.04	0.98	0.96
D+k−	22.8	22.7	22.8	23.0	0.86	0.15 ^C	0.13	0.25					
D+k+	20.5	20.3	20.7	21.0	1.23	0.38 ^{BC}	0.09	0.56					
D−k+	22.3	22.5	22.8	23.0	1.63	0.44 ^A	0.19	0.23					
Butyrate													
D−k−	6.9	6.6	7.1	7.3	1.98	0.34	0.15	0.12	0.21	0.56	0.08	0.99	0.97
D+k−	6.2	6.3	6.4	6.6	1.56	0.25	0.09	0.18					
D+k+	6.7	6.7	6.9	7.2	2.35	0.34	0.03	0.52					
D−k+	6.2	6.2	6.4	6.6	1.65	0.27	0.09	0.28					
Ace:Prop ratio													
D−k−	2.79	2.87	2.77	2.73	0.45	-0.06 ^B	0.52	0.41	0.08	0.05	0.11	0.06	0.96
D+k−	2.91	2.95	2.91	2.87	0.56	-0.03 ^B	0.21	0.25					
D+k+	3.30	3.35	3.26	3.19	0.23	-0.08 ^A	0.63	0.56					
D−k+	2.90	2.92	2.83	2.76	0.28	-0.10 ^A	0.21	0.32					
CH₄:VFA ratio													
D−k−	0.25	0.25	0.24	0.24	0.09	-0.01	0.03	0.41	0.45	0.65	0.46	0.56	0.89
D+k−	0.25	0.24	0.24	0.23	0.05	-0.01	0.04	0.32					
D+k+	0.26	0.25	0.24	0.24	0.03	-0.01	0.04	0.52					
D−k+	0.25	0.24	0.24	0.23	0.04	-0.01	0.02	0.47					
2Hr (mol/mol)													
D−k−	0.80	0.79	0.78	0.78	1.56	-1.39 ^B	0.02	0.23	0.03	0.72	0.79	0.08	0.45
D+k−	0.82	0.81	0.80	0.79	1.52	-1.89 ^A	0.03	0.12					
D+k+	0.83	0.81	0.81	0.79	1.89	-1.99 ^A	0.04	0.25					
D−k+	0.80	0.80	0.80	0.78	1.23	-1.29 ^B	0.01	0.19					

¹Early life (EL) treatment: D+k+ (*n* = 10): treated kids from treated does; D+k- (*n* = 10): untreated kids from treated does; D-k+ (*n* = 10): treated kids from untreated does; D-k- (*n* = 10): untreated kids from untreated does.

²SEM = standard error of the mean.

³Slope of the linear part of the curve relating the dependent variable (y-axis) to the in vitro DHA-Gold dose (x-axis). A, B capital letters annotated for the same parameter differ significantly (*P* < 0.05) between inocula origin.

⁴Orthogonal contrasts were used to assess a linear (Lin) or quadratic (Q) dose effect of DHA-Gold (*P* < 0.05).

⁵Effect of DHA-Gold supplementation during pregnancy (D), from birth until 2 wk after weaning (k) and interaction D x k, D x dose and k x dose.

DM = dry matter; VFA = volatile fatty acids.

observation, prenatal DHA-Gold supplementation reduced CH_4 production at 4 wk of age, but this reduction particularly was the result of a reduction in the total fermentative capacity. As a consequence, pre and/or postnatal supplementation enhanced relative CH_4 production both at 4 and 11 wk. This is in contrast with our expectations, but in line with a study by Moate et al. (2013). There, dairy cows (fed a forage-based diet) supplemented with increasing doses of DHA-enriched algae (daily dose up to 375 g/cow; corresponding to 75 g of DHA/cow or approximately 0.131 g DHA/kg BW when assuming cows of 571 kg) did not affect total emissions of CH_4 but emissions expressed relative to total intake were increasing. Also, Klop et al. (2016) reported an increase in CH_4 /kg DMI through DHA supplementation (3 g/kg DM corresponding to 0.076 g DHA/kg BW when assuming cows of 650 kg) to lactating dairy cows. Nevertheless, in the present study, the prenatal stimulatory effect on CH_4 production was no longer observed at 6 mo of age. Similarly, when the postnatal DHA-Gold supplementation was ceased, the increase in CH_4 /VFA ratio disappeared. These observations coincided with the inhibitory effect of prenatal DHA-Gold supplementation on fermentative capacity (-17.7% , $P < 0.001$ in week 4, -6.9% , $P = 0.06$ in week 11 and $+4.4\%$, $P = 0.03$ at 6 mo of age). However, the enhanced in vitro VFA production at 6 mo of age was not reflected in a difference in VFA concentrations at slaughter, which might be related to the timing of sampling (slaughtering after overnight fasting).

Effect of Pre and Postnatal DHA-Gold Supplementation on Body Weight Gain and Papillae Development

The BW gain between weaning and slaughter tended to be increased for the postnatally treated goat kids ($P = 0.06$), which concurs with the stimulated fermentative capacity as suggested from the in vitro assessment with inoculum of 6-mo-old animals. However, it is uncertain whether the increased BW gain originated from an enhanced rumen fermentation in vivo, particularly as TSA of the papillae in the predominant absorption side (atrium ruminis) was smaller in DHA-Gold supplemented goat kids from which a reduced absorptive capacity of the rumen epithelium could be expected. Nevertheless, the correlation between rumen morphology and SCFA absorption has been questioned in recent studies (Dieho et al., 2017). Indeed, differences in papillae morphology induced by variation in concentrate intake did not affect the fractional absorption rate

of VFA (Dieho et al., 2016) suggesting that morphological changes cannot directly be interpreted at a functional level. This, could be the reason why a change in rumen papillae surface by the pre or postnatal DHA-Gold supplementation was not associated with differences in BW at slaughter. Moreover, the persistent prenatal (D+) effect of DHA-Gold at 6 mo of age is remarkable, as it is known that the rumen epithelium is continuously changing over time (Černík et al., 2014). Besides a potential direct effect of the prenatally supplemented DHA-Gold, an indirect effect could have played a role as physical stimulations, e.g., related to the intake of fiber by the young ruminants might affect rumen papillae development (Montoro et al., 2013). In the present study, the hay intake was not measured, and hence, it is unknown whether it was influenced by the prenatal supplementation of DHA-Gold. Additionally, concentrate intake which was reduced in D+ goat kids until 3 wk of age, could have influenced papillae development (Brownlee, 1956). Potentially, the lower concentrate intake might have been indicative for a generally lower intake of solid feed. However, it is uncertain whether a reduction which only took place during the second and third week of life, where papillae development is still limited, could have induced persistent effects on rumen morphology until 6 mo of age.

Effect of In Vitro Re-exposure to DHA-Gold Supplementation of Goat Kid's Inoculum Which Had Been Exposed to a Similar Treatment Prenatally and/or Early in Life

As the in vitro retreatment response of DHA-Gold depended of the supplementation of the donor goat kids' mothers during the last 3 wk of pregnancy, prenatal DHA-Gold supplementation was suggested to influence the microbial functionality in the offspring. Moreover, an interaction effect between pre and postnatal treatment was observed: in vitro supplementation of DHA-Gold suppressed absolute CH_4 production less in the rumen inocula of D+k+ goat kids as compared with D-k+ (slope = -99 vs. -79 , respectively), while the effect was most modest in inoculum of D+k- goat kids (slope -61.6). Similarly, prenatal supplementation might mitigate detrimental effects of PUFA supplementation on VFA yields (average slope = -59.5 vs. -88.0 $\mu\text{mol/g DM per mg/mL}$ of DHA-Gold for D+ vs. D-), while postnatal supplementation rather enhances these detrimental effects (average slope = 81.4 vs. 63.0 $\mu\text{mol/g DM per mg/mL}$ of DHA-Gold for k+ vs. k-). As such, a prenatal

supplementation diminished the detrimental effect of the postnatal treatment (slope = -61.26 vs. -101.5 $\mu\text{mol/g DM}$ per mg/mL of DHA-Gold for D+k+ vs. D-k+). Nevertheless, it has to be realized that the effect of “re-exposure” in the current study was smaller as compared with the ones observed in former studies from our research group with medium-chain fatty acids (A. Ruiz-González, unpublished data) and linseed (Ruiz-González et al., 2017).

The interaction between early life treatment of the offspring and their mothers also has been suggested in former studies, which even made Yañez-Ruiz et al. (2010) suggesting that persistent changes in the offspring only could be ensured when an intervention also considers applying the same treatment to the doe, e.g., the archaea community establishment in goat kids depended on the supplementation to the dam of the antimethanogenic agent bromochloromethane (Abecia et al., 2013). However, in the former studies, supplementation to the does took place during the suckling period of the goat kids, which differs from the prenatal supplementation in the present study. Persisting effects on the microbial functionality of a prenatal treatment might originate from differences in microbial colonization of the rumen. This might have been induced by an in-utero transfer of FA from DHA-Gold from the mother to the fetus, as many dietary compounds are known to cross the placenta and enrich the fetus (Provenzas et al., 1992). Alternatively, DHA-Gold supplementation could have modified the microbial population in the birth canal, which is the prior source of rumen microbes of newborns (Yeoman et al., 2018).

CONCLUSIONS

Supplementation of 0.28 g/kg BW of DHA-Gold prenatally or during early life of goat kids did not lower but rather enhanced in vitro CH_4 production during the supplementation period as compared with goat kids without DHA-Gold supplementation. Towards the end of the supplementation period, effects of DHA-Gold on rumen fermentation, including CH_4 production, disappeared, which could suggest some adaptation of the microbial population to DHA-Gold. Such microbial adaptation also could be responsible for smaller CH_4 inhibition in the D+ inoculum upon re-exposure to DHA-Gold in vitro. Furthermore, supplementation of 0.28 g/kg BW of DHA-Gold either pre or postnatally to goat kids decreased density, width and surface area of the ruminal

papillae at an age of 6 mo, which, however, did not impair animal performance.

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