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Original Research Article

Suitability of n-alkanes and chromium (III) oxide as digestibility markers in calves at the end of the milk feeding period supplemented with a prebiotic

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ABSTRACT

Prebiotics reveal positive effects on the growth performance of pigs and poultry, and might influence intestinal microflora. This, in consequence, could alter recovery rates of digestibility markers. In the current study, we evaluated the suitability of chromium (III) oxide (Cr_2O_3) and the synthetic alkanes n-dotriacontane (C32) and n-hexatriacontane (C36) as external markers for digestibility estimation compared with the standard total collection method in calves supplemented with galacto-oligosaccharides. Eight male German Holstein calves (average age ± SD = 57 ± 8 days) were divided into 2 milk replacer feeding groups (group receiving galacto-oligosaccharides [A] and control group [B]). Each of 2 groups of 4 individually fed calves received a distinct milk replacer with added markers for 14 days. They were fed twice daily restrictively with milk replacer, concentrate and hay. After an adaptation period of 10 days, total faeces were collected. Faecal marker recoveries (FMR, means ± SD) for C32 were (72 ± 14)% for A and (80 ± 12)% for B. Faecal marker recoveries for C36 was (82 ± 15)% and (88 ± 13)% for groups A and B, respectively. The FMR for Cr_2O_3 was (102 ± 11)% and (100 ± 1)% for groups A and B, respectively. There were no significant differences between total collection organic matter digestibility and marker based organic matter digestibility when using Cr_2O_3 and C36. But, when utilizing C32 to calculate nutrient digestibilities, results differed from the total collection method for organic matter, crude protein and ether extract. The results indicate that Cr_2O_3 and C36 can be applied in digestibility studies with calves and give accurate estimates for OM and nutrient digestibilities without correction for FMR.

1. Introduction

Prebiotics are defined as feed ingredients, which are not digested by endogenous enzymes of the animal and are available as a substrate to be used by the intestinal microflora (Gibson and Roberfroid, 1995). Mammalian and avian endogenous enzymes are unable to hydrolyze the β-linkages between galactose monomers in trans-galacto-oligosaccharides as observed by Burvall et al. (1979). Therefore, they can be classified as prebiotics. The presence of prebiotics in the intestinal tract could lead to enhanced proliferation of beneficial microorganisms. Boehm et al. (2002) reported that growth of *Bifidobacteria* was promoted in infants supplemented with galacto- and fructo-oligosaccharides. This finding was confirmed by Ben et al. (2008), who observed increased growth of *Bifidobacteria* and Lactobacillaceae after supplementing infants with galacto-oligosaccharides. According to Vivatvakin et al. (2010) galacto- and fructo-oligosaccharides also act as suppressors for pathogens including Clostridiaceae in infants. Other studies, though not directly assessing the impact of prebiotics on gut microbes, have shown positive effects on fish (Buentello et al., 2010),

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poultry (Kim et al., 2011) and pigs (Hidaka et al., 1985; Hidaka et al., 1986; Katta et al., 1993).

The effect of non-digestible oligosaccharides (NDO) on calves during the milk-feeding period has so far been only little studied. No influence on growth performance of calves up to 6 weeks in age was reported by Heinrichs et al. (2003) and Silva et al. (2012) using mannan-oligosaccharides. However, Newman et al. (1993) and Dvorak et al. (1997) observed an increase in liveweight gain in calves receiving mannan-oligosaccharides. Determination of apparent digestibility might be helpful to evaluate whether prebiotics alter nutrient digestibility in calves, possibly by changing the population of gut microbiota. While the total collection technique is the most accurate method to determine digestibility, it is also very time-consuming and labor-intensive. An alternative is faecal spot sampling in combination with inert markers mixed into the diets.

Chromium (III) oxide (Cr_2O_3) is an established marker (Borucki Castro et al., 2008; Delagarde et al., 2010) and has been successfully used in digestion trials with calves (Al Alami et al., 2014). However, Cr_2O_3 might be carcinogenic (Titgemeyer et al., 2001) and it alters the appearance of feed, which might result in a reduction of feed intake in *ad libitum* study designs. Consequently, it is favorable to establish an alternative marker to Cr_2O_3. In ruminants, alkanes have been widely used as markers because of the natural alkane occurrence in herbages. A disadvantage of alkanes is the incomplete faecal marker recovery (FMR), which has been observed in horses (O'Keefe and McMeniman, 1998; Ordakowski et al., 2001; Stevens et al., 2002) and pigeons (Hatt et al., 2001). Because, according to Yergeau et al. (2012) *Bifidobacteria* might be able to degrade hydrocarbons and consequently alkanes, the increased growth of these microorganisms caused by galacto-oligosaccharides might alter FMR of alkanes.

The main objective of this current study was to evaluate the suitability of Cr_2O_3 and 2 different even chain-length synthetic alkanes, n-dotriacontane (C32) and n-hexatriacontane (C36), as digestibility markers in pre-ruminant calves. The results can then be compared with the total collection method. We aimed to investigate whether feeding of galacto-oligosaccharides influences FMR, which might cause a bias in determination of nutrient digestibility, and also have an effect on nutrient digestibility itself.

2. Material and methods

The study was conducted at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI) in Brunswick, Germany. The experiment was carried out in accordance with the German Animal Welfare Act approved by the LAVES (Lower Saxony State Office for Consumer Protection and Food Safety, Germany).

The experimental design was a 2 × 3 factorial design with 2 different milk replacers (MR) and 3 different markers (Cr_2O_3, C32 and C36). Each of 2 groups of 4 individually fed calves received a distinct MR with added markers for 14 days. The first 10 days were an adaptation period and from day 11 until day 14, all faeces were collected completely.

2.1. Animals and diets

For this current study, 8 male German Holstein calves (average age ± SD at the beginning was 52 ± 8 days, ranging from 39 to 66 days) were used. The animals were weighed at the start and at the end of the study. Their average weight at the beginning was 83.6 ± 5.8 kg. Over the course of the experiment, the calves were housed individually in calf hutches with rubber floor in a non-climatized barn.

The animals were divided into 2 feeding groups (MR A and B) with 4 animals per group. Milk replacer A contained 6.7% galacto-oligosaccharides (non-commercially available, Norlac GmbH, Zeven, Germany) on a dry matter (DM) basis. Each animal was fed restrictively twice daily at 06:30 and 17:30 whereby each animal received 882 g/d concentrate and 88 g/d hay on DM basis. Additionally, each animal was given 677 g/d of MR A or B dissolved in 5 L water and 144.5 g/d of MR-marker-mix dissolved in 1 L water. The MR-marker-mix consisted of 137.85 g MR A or B, 3 g Cr_2O_3, 75 mg C32 and 75 mg C36. Before the start of the study the MR-marker-mix had been prepared by mixing MR and the markers homogeneously over a period of 5 min in a ploughshare laboratory mixer (Type M20 GR, Gebrüder Lödige Maschinenbau GmbH, Paderborn, Germany) with a capacity of 5 kg. The mix was then weighed into the amounts necessary per day for each calf. Shortly before each feeding time the MR and the MR-marker-mix were dissolved in water.

Table 1 shows the average chemical composition of the feedstuffs. The main component of the concentrate was soybean meal.

2.2. Sampling procedures and sample preparation

At the beginning of the study, hay and concentrate were mixed and samples of each feed were taken. The calves were fed the experimental diets for an adaptation period of 10 days. After that, faeces were collected for 4 consecutive days, whereby the first sampling day started at 12:00, and the last sampling day ended at 12:00, and the animals were observed continuously. Faeces were collected, labeled and weighed. After the determination of the dry

Table 1
Ingredients and chemical composition of concentrate, hay, milk replacer with 6.7% and without galacto-oligosaccharides during the experimental period (DM basis).

Item	Concentrate	Hay	Milk replacer	
			A[1]	B[2]
Ingredient, %				
Soybean meal	30.5			
Oat	30			
Barley	18			
Wheat	17			
Soybean oil	1.5			
Mineral premix[3]	2			
Lime	1			
Skimmed milk powder			37.8	37.8
Sweet whey powder			26	21.3
Galacto-oligosaccharides			6.7	–
Whey powder, partly desugared			15.3	19.5
Soybean protein			1	1
Wheat protein			1.5	1.5
Pre-gelatinized wheat starch			0.9	0.9
Plant oil			15.4	13.8
Mineral premix[3]			2.1	4.2
Chemical analysis				
Dry matter, g/kg	880	880	970	970
Nutrient, g/kg of DM				
Crude ash	62	62	90	87
Crude protein	226	82	230	233
Ether extract	45	12	168	167
Crude fiber	75	354	–	–
NDF_{OM}	238	703	–	–
ADF_{OM}	92	389	–	–

NDF_{OM} = neutral detergent fibre expressed exclusive of residual ash; ADF_{OM} = acid detergent fibre expressed exclusive of residual ash.
[1] Milk replacer with 6.7% galacto-oligosaccharides.
[2] Milk replacer without galacto-oligosaccharides.
[3] Ingredients per kg mineral feed: 160 g Ca, 80 g P, 100 g Na, 30 g Mg, 1,000 mg Fe, 800 mg Cu, 6,000 mg Zn, 50 mg I, 50 mg Se, 30 mg Co, 800,000 IU vitamin A, 80,000 IU vitamin D_3, 1,000 mg vitamin E.

matter content for the individual faeces samples, pooled samples were made for each calf and the dried samples then stored at −18 °C. Samples of the feed components did not need further drying for storage until analysis. The concentrate, hay and faecal samples were ground to pass through a 1 mm screen (Retsch Schneidwerkmühle, Retsch GmbH, Haan, Germany).

2.3. Analysis of feed and faecal samples

Feed and faeces samples were analyzed according to the standard methods defined by the Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschungsanstalten (VDLUFA, 1993; method numbers in brackets) for DM (3.1), ash (8.1), CP (4.1.1), EE (5.1.1), crude fiber (6.1), neutral detergent fiber (6.5.1, using heat-stable alpha-amylase) and acid detergent fiber (6.5.2).

2.4. Alkane extraction, gas chromatograph (GC) analysis and calculation of alkane concentrations

To extract the alkanes, we used the basic method described by Mayes et al. (1986) with several modifications described by Elwert et al. (2004). Ground samples of feed and faeces were weighed into McCartney-bottles (1 g for feed components and 0.5 g for faeces), and 0.15 g of an internal standard solution containing n-tetracosane and n-tetratriacontane dissolved in n-undecane (C24 and C34 in C11: 1,795 and 1,630 mg/g, respectively) were added. Furthermore, 10 mL of 1.5 mol/L ethanolic KOH were added to the samples. Then the samples were heated in a dry-block at 90 °C for at least 4.5 h and shaken several times to ensure complete saponification.

Before the beginning of the extraction the samples were heated to about 70 °C as this had been shown to be beneficial for extraction (Oliván and Osoro, 1999). After adding 8 mL of heptane to the samples, they were shaken intensively, next placed in the dry-block again and re-heated to 70 °C. Then, 5 mL of deionized water were added. Next, the samples were shaken again and placed into the dry-block. After that they were separated into aqueous and solvent layers. The solvent (top) layers were transferred to a scintillation vial using a Pasteur pipette. Again 5 mL of heptane were added to the original samples and the extraction procedure was repeated.

The scintillation vials were placed in the dry-block to evaporate at 80 °C. Heptane (2.5 mL) was added to the residues and then the solvent was pipetted onto columns filled with 10 mL silica gel (mesh size 63 to 200 mm). Both the scintillation vials and the pipettes were washed twice with 5 mL of heptane. Next, the solvent was pipetted twice onto the column and its eluate collected in scintillation vials. After the elution of solvent from the columns was completed, 1.5 mL of the eluate were transferred into vials suitable for gas chromatography. Lastly, the samples were stored in the refrigerator until analysis.

The GC used to analyze the alkanes was connected to an autosampler-autoinjector unit (Shimadzu GC2010 with AOC20i+s), a temperature programmable on-column injector and a flame ionization detector. The column used was a RTX-1 with Integra-Guard column (30 m column length, 0.53 mm inner diameter with a guard column length of 5 m). In the injector, helium was used as carrier gas, flowing with a linear velocity of 30 cm/s (column flow: 3.73 mL/s). The starting temperature was 80 °C, which was held for 6 sec. Then it was heated at a rate of 100 °C/min to 310 °C and held for 10 min with an injection volume of 0.5 mL. After being held at 80 °C for 6 s, the oven was heated at a rate of 50 °C/min to 240 °C and then held for 1 min. Heating continued at a rate of 6 °C/min to 264 °C, then at a rate of 4 °C/min to 284 °C and finally at a rate of 2 °C/min to 296 °C, which was held for 10 min. The temperature in the detector was 315 °C. A gas mixture consisting of helium (flow rate 30 mL/min), H_2 (flow rate 40 mL/min) and air (flow rate 400 mL/min) was used. One sample was injected every 35 min. A self-mixed standard sample was used to determine the retention time of the alkanes. The standard sample contained all alkanes from C24 to C36. Peak areas were calculated using the Shimadzu GC solution software.

Alkane concentrations were calculated by making use of peak area ratios (sample alkane: internal standard). Longer-chain alkanes can be discriminated by temperature. Therefore, the ratio of the peak areas of the internal standard was compared to the ratio of the internal standard alkane concentrations in the standard solution in order to correct the peak areas for any observed discrimination. For this correction, a linear discrimination depending upon the chain length was assumed.

2.5. Chromium analysis

For Cr_2O_3 analysis in feed and faeces, the respective samples were prepared according to Williams et al. (1962) and chromium content was quantified using an optical emission spectrometer with inductively coupled plasma (ICP-OES Quantima; GBC Scientific Equipment Pty. Ltd., Melbourne, VIC, Australia).

Solutions were prepared for the ICP analysis of chromium. Fifteen milliliters of the phosphorus manganese sulphate solution (7.579 g of $MnSO_4 \cdot H_2O$ in 100 mL deionized H_2O) were pipetted into a 500 mL flask filled with 85% H_3PO_4. For the blank solution needed for the chromium type curve, 30 mL phosphorus manganese sulphate solution and 40 mL potassium bromate solution (11.25 g $KBrO_3$ in 250 mL deionized H_2O) were heated, cooled again and then transferred into a 2 L flask with deionized water. In a next step, we added 250 mL of calcium-chloride-2-hydrate solution (29.344 g $CaCl_2 \cdot 2H_2O$) and deionized H_2O until the flask was filled up to the 2 L point.

First, 1 g of each freeze-dried sample was weighed into 45 mL ash crucibles and dried overnight at 105 °C in order to determine the DM. Samples were then ashed in the muffle furnace overnight at 550 °C. Next, 3 mL of phosphorus manganese sulphate solution were added, as well as the 4 mL of 4.5% potassium bromate solution. Samples were heated for 20 to 30 min until they stopped boiling and a blue color could be observed. After that, the samples were cooled and transferred into 250 mL flasks of deionized water, which contained 25 mL calcium-chloride-2-hydrate solution. The mixture was filtered and then measured by ICP and compared to a standard curve, in which 2, 4, 6, 8 and 10 mg/L chromium were plotted. Additionally, a blank value for faeces, which did not contain any chromium, was determined.

2.6. Calculations and statistical analysis

Apparent digestibility of the OM, CP and EE was calculated as the proportion of feed OM, CP and EE, which was not recovered in the faeces. Digestibility of OM and nutrients based on total excreta collection was calculated using the following formula:

$$\mathrm{DiT}\ (\%) = 100 \times \left(\frac{\mathrm{DI} - \mathrm{DE}}{\mathrm{DI}}\right)$$

where DiT is the apparent digestibility measured using the total collection method, DI is the daily intake (g) and DE is the daily faecal excretion (g).

Apparent digestibility of OM and nutrients was calculated by the marker method:

$$\text{DiM}\ (\%) = 100 \times \left(1 - \frac{\text{MF} \times \text{NFa}}{\text{MFa} \times \text{NF}}\right)$$

where DiM is the apparent digestibility collected using the marker method, MF is the concentration of marker in feed (g/kg DM); NFa is the concentration of OM or nutrient in faeces (g/kg DM); MFa is the concentration of marker in faeces (g/kg DM) and NF is the concentration of OM or nutrient in feed (g/kg DM).

The FMR of the alkanes and chromium were calculated as the proportion of the ingested markers, which were recovered with the faeces. The statistical analysis was carried out using SAS 9.3.

The statistical model was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where μ is the overall mean, α_i is the FMR, β_j is the marker, $(\alpha\beta)_{ij}$ is the interaction between FMR and marker and e_{ijk} is the residual.

A difference was considered significant at $P < 0.05$, and $P \leq 0.10$ was considered to be a trend. We used PROC MIXED to evaluate influences of MR, method of estimation and the interaction between them on the calculated digestibilities of OM and nutrients. Differences between methods of estimations and the MR groups for OM and nutrient digestibilities were evaluated using ANOVA. We used orthogonal contrasts to compare results found with total collection and markers.

3. Results

There was an observed difference in daily liveweight gain between the 2 MR groups ($P = 0.005$). The average daily gain during the 14-day study period for group A was 996 g (SD = 6 g), whereas the average daily gain for group B was 686 g (SD = 130 g).

The average amount of faeces collected per day was 325 g DM (SD = 68 g) for group A and 330 g DM (SD = 15 g) for group B. The amount of faeces per individual defaecation ranged from 14 to 601 g OM.

Dietary marker concentrations for Cr_2O_3, C32 and C36 were 932, 51 and 52 mg/kg DM in group A, and 843, 45, 46 mg/kg DM in group B, respectively. The analyzed faecal marker concentrations are presented in Table 2. The daily feed intake of the calves was 1,790 g/d DM without refusals. Calculating these marker concentrations resulted in a daily marker intake for Cr_2O_3, C32 and C36 were 1,668, 91 and 93 mg in group A and 1,509, 81 and 82 mg in group B, respectively.

Faecal marker recoveries of C32 was lower than that of Cr_2O_3 ($P = 0.034$). Feeding galacto-oligosaccharides did not affect the FMR of the 3 markers (Table 2).

Table 2
Concentrations of Cr_2O_3, n-dotriacontane (C32) and n-hexatriacontane (C36) (means ± SD) in faeces and faecal marker recoveries (FMR, means ± SD) for calves receiving a milk replacer with 6.7% ($n = 4$) and without ($n = 4$) galacto-oligosaccharides.

Marker	FMC, g/kg DM		FMR, %	
	A[1]	B[2]	A[1]	B[2]
Cr_2O_3	6.54 ± 0.74	5.76 ± 0.42	100 ± 1[a]	102 ± 11[a]
C32	0.20 ± 0.02	0.20 ± 0.04	80 ± 12[b]	72 ± 14[b]
C36	0.23 ± 0.02	0.22 ± 0.05	88 ± 13[ab]	82 ± 15[ab]

FMC = faecal marker concentrations.
[a,b] Within a column, means without a common uppercase superscript differ ($P < 0.05$).
[1] Milk replacer with 6.7% galacto-oligosaccharides.
[2] Milk replacer without galacto-oligosaccharides.

Digestibilities of OM and nutrients are presented in Table 3. The methods of digestibility calculation affected estimates of total tract digestibility of OM ($P = 0.002$), CP ($P < 0.001$) and EE ($P = 0.002$). For OM ($P = 0.035$), CP ($P = 0.012$) and EE ($P = 0.004$), calculated digestibility using C32 was lower compared to the 3 other methods. Feeding galacto-oligosaccharides reduced apparent digestibility of EE ($P < 0.001$). Apparent digestibilities of CF, ADF_{OM} and NDF_{OM} were not influenced by MR or markers.

4. Discussion

When evaluating the suitability of alkanes as a digestibility marker for calves, it is necessary to compare their characteristics to the 7 criteria for ideal markers defined by Kotb and Luckey (1972). Alkane markers meet several of these criteria. Toxic effects caused by them are not known, they do not add any bulk to the feed as they also occur naturally in plant cell walls, and they can be measured quantitatively. However, in this current study faecal recoveries for alkanes were lower than 100%. There are several possibilities for the lower recovery of alkanes observed in this current study. Alkanes could either have been absorbed in the gastrointestinal tract, metabolized by the enterocytes or microflora or chemically interacted with other substances in the digesta (e.g., nutrients or enzymes) and thus form new chemical compounds (Barrowman et al., 1989). There have been no investigations yet where and how alkanes may disappear during their gastrointestinal passage.

In this current study, faecal recovery of C32 was numerically lower than that of C36. This is in accordance with the literature regarding ruminants (Mayes et al., 1986; Dillon, 1993), where higher FMR for longer-chain alkanes were observed. O'Keefe and McMeniman (1998) as well as Ordakowski et al. (2001) could not find this relationship between alkane-chain-length and faecal recovery in horses. The same is true for the study of Hatt et al. (2001) who found recovery rates in pigeons of 0.81 and 0.80 for C32 and C36, respectively. However, this was the first study determining faecal recovery rates of alkanes in calves and future research is necessary to validate the faecal recovery rates observed in this current study.

Whereas recovery rates of both alkanes are numerically lower in calves receiving galacto-oligosaccharides, these differences were not significant. Therefore, we might conclude that galacto-oligosaccharides did not increase the population of *Bifidobacteria* up to a level, at which a breakdown of alkanes might occur at a significant amount. A possible explanation might be that soybean, which was used in the concentrate in this current study, does contain up to 1.95% galacto-oligosaccharides itself (Espinosa-Martos and Ruperez, 2006) and may mask the effect of the added galacto-oligosaccharides. However, another study would be necessary to investigate, whether gut microbiota are actually changed by galacto-oligosaccharides. For that purpose, samples of rumen and duodenal fluid need to be analyzed for their microbiota population.

Calves receiving galacto-oligosaccharides in this current study showed a lower EE digestibility, which could be attributed to enhanced bile acid excretion. An adverse relationship between bile excretion and fat digestibility in calves was reported by Xu et al. (1998). Although we did not investigate bile excretion with faeces in this current study, Levrat et al. (1994) observed increased fecal bile acid excretion in rats supplied with different NDO. Therefore, it could be hypothesized that calves supplemented with galacto-oligosaccharides excreted a higher level of bile acid, which in turn was not reabsorbed. Consequently, digestibility of fat might be impaired by galacto-oligosaccharides.

The apparent digestibility of OM and the other nutrients was not altered by galacto-oligosaccharides. Nevertheless, we observed a higher daily liveweight gain in calves receiving

Table 3
Organic matter and nutrient digestibilities calculated by total collection method (Di_T) and by using markers not corrected for faecal marker recovery (Di_M) (means ± SD; $n = 4$).

Item	Di_T[1]	Di_M[2]			*P*-value					
		Cr_2O_3	C32[3]	C36[4]	MR	Marker	MR × marker	Di_T vs. markers	Cr_2O_3 vs. alkanes	C32 vs. C36
Organic matter										
A[5]	82.7 ± 3.8^{a}	83.1 ± 2.9^{a}	76.0 ± 2.3^{b}	80.9 ± 1.9^{ab}	0.759	0.002	0.835	0.042	0.004	0.035
B[6]	82.3 ± 0.8^{a}	82.3 ± 1.1^{a}	77.3 ± 4.8^{b}	79.3 ± 4.5^{ab}						
Crude protein										
A[5]	80.7 ± 1.7^{a}	81.0 ± 1.4^{a}	72.8 ± 3.6^{b}	78.3 ± 2.7^{ab}	0.174	<0.001	0.837	0.027	0.002	0.027
B[6]	78.9 ± 1.7^{a}	78.9 ± 1.9^{a}	72.9 ± 5.8^{b}	75.3 ± 5.2^{ab}						
Ether extract										
A[5]	81.6 ± 2.2^{aB}	81.3 ± 2.4^{aB}	73.2 ± 5.0^{bB}	78.6 ± 3.9^{abB}	<0.001	0.002	0.272	0.045	0.005	0.030
B[6]	89.3 ± 0.9^{A}	89.3 ± 1.0^{A}	86.2 ± 2.8^{A}	87.5 ± 2.6^{A}						
Crude fiber										
A[5]	72.6 ± 11.4	73.2 ± 9.6	74.0 ± 10.8	76.5 ± 9.7	0.985	0.604	0.985	0.872	0.948	0.742
B	72.7 ± 3.7	73.3 ± 4.1	73.3 ± 3.8	73.3 ± 3.8						
ADF_{OM}										
A[5]	74.0 ± 10.8	74.5 ± 9.2	72.6 ± 11.4	75.3 ± 10.3	0.985	0.805	0.985	0.870	0.912	0.740
B[6]	73.3 ± 3.8	72.6 ± 4.1	72.7 ± 3.8	72.7 ± 3.8						
NDF_{OM}										
A[5]	33.0 ± 23.8	42.2 ± 15.6	42.5 ± 16.4	45.3 ± 15.4	0.236	0.205	0.816	0.110	0.236	0.583
B[6]	28.0 ± 8.5	27.8 ± 9.7	38.3 ± 5.4	43.2 ± 5.7						

MR = milk replacer; NDF_{OM} = neutral detergent fibre expressed exclusive of residual ash; ADF_{OM} = acid detergent fibre expressed exclusive of residual ash.
[a,b] Within a row, means without a common lowercase superscript differ (ANOVA; $P < 0.05$).
[A,B] Within a column, means without a common uppercase superscript differ (ANOVA; $P < 0.05$).
[1] Digestibility calculated by total collection method.
[2] Digestibility calculated by marker method.
[3] *n*-dotriacontane.
[4] *n*-hexatriacontane.
[5] Milk replacer with 6.7% galacto-oligosaccharides.
[6] Milk replacer without galacto-oligosaccharides.

galacto-oligosaccharides. While the weight of calves in the current study was assessed only for 14 days, the difference in liveweight-gain due to galacto-oligosaccharides is in line with previous unpublished in-house studies (Meyer, unpublished). However, the mechanism behind the difference is unclear. Increased feed intake in calves supplied with mannan-oligosaccharides was observed by Dvorak et al. (1997). Research using NDO in pigs revealed that the enhanced growth performance is mainly attributable to an enhanced daily feed intake as well (Davis et al., 2002; Zhao et al., 2012). However, calves in our study were fed restrictively and it was assured that each calf did consume his individually measured ratio at each feeding time. Therefore, the daily feed intake between individual animals and hence the 2 groups did not differ.

5. Conclusion

When using markers, Cr_2O_3 as well as C36 can be employed to calculate OM digestibility and digestibility of nutrients without correction for FMR. However, without correcting for FMR there are differences between digestibilities of OM, CP and EE calculated using C32 compared to the total collection and using Cr_2O_3 and C36 in calves. Therefore, it is not advisable to use C32 to calculate digestibilities in calves without correcting for FMR. Because the reason for the lower recovery rate of C32 is unknown at present, further studies are warranted to establish a consistent FMR correction for C32, which might afterwards be used for correction. Therefore, in digestion studies with pre-ruminant calves, C36 and Cr_2O_3 appear to be more suitable markers.

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