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Short communication: Quantifying postruminal starch fermentation in early-lactation Holstein-Friesian cows

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ABSTRACT

It has previously been shown that fermentation may contribute substantially to small intestinal carbohydrate disappearance. The fact that the energetic efficiency of starch fermentation is considerably less than that of enzymatic digestion of starch, makes it of nutritional importance to quantify the level of postruminal starch fermentation for dairy cows. Hence, we subjected six rumen-fistulated Holstein-Friesian dairy cows (48 ± 17 days in milk) to 5 d of continuous abomasal infusions of 0.0, 2.5, and 5.0 mol NH_4Cl/d, with and without 3 kg ground maize/d, followed by 2 d of rest in a 6 × 6 Latin square design. A total mixed ration (**TMR**) consisting of (DM basis) 70% grass silage and 30% concentrate was fed at 95% of ad libitum intake. Separation of postruminal starch disappearance into enzymatically digested starch and fermented starch was based on the measurement of natural ^{13}C enrichment of the TMR, abomasally infused ground maize, and resulting ^{13}C enrichment of faeces. Within each cow, 0.0, 2.5, and 5.0 mol NH_4Cl/d without ground maize served as control for the same levels of NH_4Cl with 3 kg ground maize/d. Abomasal infusion of ground maize was associated with increased total DM and starch intake, faecal starch excretion, and digestibility of starch, and with decreased digestibility of DM and N. The increased faecal volatile fatty acid (**VFA**) output and ^{13}C enrichment of the individual VFA indicate increased starch fermentation with abomasally infused ground maize. On average, 1 311 g starch/d was postruminally fermented, representing 60.8% of total starch intake. Overall, postruminal starch fermentation of early-lactation dairy cows abomasally infused with 3 kg ground maize/d is considerable and may result in substantial amounts of VFA rather than glucose production.

Implications

This study aimed to quantify the level of postruminal starch fermentation in early-lactation dairy cows. Abomasal infusion of 3 kg ground maize/d resulted in considerable postruminal starch fermentation, on average 60.8% of total starch intake. This level of postruminal starch fermentation indicates that glucose yield from bypass starch is likely substantially overestimated.

Introduction

In cattle, a part of ingested starch is fermented in the rumen with volatile fatty acids (**VFA**) as major end-products. Postruminally, starch may be digested via enzymatic hydrolysis producing glucose, or fermented to VFA and microbial biomass. According to Harmon et al. (2004), ruminal starch fermentation is 75–80% of starch intake, and starch that escapes ruminal fermentation flows into the small intestine where on average, 35–60% is degraded. Of the fraction that escapes small intestinal digestion, an additional 35–50% is fermented in the large intestine (Harmon et al. 2004). Gilbert et al. (2015) estimated, from ^{13}C excretion in faeces that total-tract starch fermentation was 89% in milk-fed calves and that 52% of the starch was fermented prior to the terminal ileum. This suggests that microbial activity may contribute substantially to the small intestinal carbohydrate disappearance. These results cannot be effortlessly extrapolated to dairy cows, because milk-fed calves may have different mechanisms of regulation than adult ruminants (Harmon and Swanson, 2020). This, together with the fact that energetic efficiency of starch fermentation is only 54–74% of that of enzymatic digestion of starch (Harmon and Swanson, 2020), makes it of nutritional importance to quantify the level of postruminal starch fermentation for dairy cows. van Gastelen et al. (2021) reported that abomasal infusion of ground maize in dairy cattle resulted in increased hindgut fermentation, mainly evidenced by increased faecal VFA concentra-

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tions. As a follow-up on the study of van Gastelen et al. (2021), the objective of the present study was to quantify the level of postruminal starch fermentation in early-lactation dairy cows that were abomasally infused with 3 kg ground maize/d based on natural ^{13}C enrichment contrasts.

Material and methods

Experimental design

This study was part of the experiment described by van Gastelen et al. (2021). In short, in a 6 × 6 Latin square design, six rumen-fistulated Holstein-Friesian dairy cows (48 ± 17 d in milk) were subjected to 5 d of continuous abomasal infusions of treatments followed by 2 d of rest. The infusion treatments were 0.0, 2.5, and 5.0 mol NH_4Cl/d, with and without 3 kg ground maize/d. A total mixed ration (**TMR**) consisting of 70% of grass silage and 30% concentrate (on DM basis) was fed at 95% of ad libitum intake of individual cows, where TiO_2 was included in the concentrate as an external digestibility marker. Cows were milked at 0500 and 1530 h. The experiment was conducted in climate respiration chambers, where the cows were fed using an automated feeding system that dispensed equal portions of feed every 2 h to promote metabolic steady-state conditions. For further details, see van Gastelen et al. (2021).

Sample collection and analytical procedures

Faecal samples were collected before each milking during the last 72 h of the infusion period by rectal grab sampling, samples of TMR components were collected three times weekly during feed preparation, and samples of the infusion ingredients were collected daily when new treatment solutions were prepared. All samples were stored at −20 °C until analysis, and subsequently thawed at room temperature, freeze-dried until a constant weight, and ground to pass a 1-mm screen. The DM, starch, and Ti content (latter in concentrate and faecal samples only) was analysed according to Nichols et al. (2018). For determination of VFA, fresh faecal samples were analysed as described by van Gastelen et al. (2021). The ^{13}C enrichment and C content were analysed according to Gerrits et al. (2012). The ^{13}C enrichment of grass silage and concentrate was 1.075 and 1.074 atom%, respectively. The ^{13}C enrichment of the infusion treatments (excl. TMR) was 1.067, 1.069, 1.076, 1.091, 1.092, and 1.092 atom% for 0.0, 2.5 and 5.0 mol NH_4Cl/d without ground maize, and 0.0, 2.5 and 5.0 mol NH_4Cl/d with 3 kg ground maize/d, respectively.

Calculations

The quantity of maize starch fermented was calculated by the method described by Gerrits et al. (2012) with some adjustments. First, we differentiated between starch of the TMR (i.e., wheat; low ^{13}C enrichment) and starch of the abomasal infusion treatments (i.e., ground maize; high ^{13}C enrichment). Second, each cow served as its own control, where 0.0, 2.5, and 5.0 mol NH_4Cl/d without ground maize served as control for the same levels of NH_4Cl with 3 kg ground maize/d. We assumed the results of the treatments without ground maize to represent the TMR fed to the same cows receiving the abomasal infusion treatments with ground maize. Third, non-starch, non-VFA faecal C output was calculated by the difference of the total faecal C output and faecal C output as starch and VFA. Fourth, the amount of C from maize starch incorporated into microbial biomass was calculated from measured ^{13}C enrichment in faeces, corrected for ^{13}C in faecal output of starch and VFA. Fifth, the quantity of starch fermented was calculated from starch incorporated into microbial biomass by assuming that 25% of fermented C is incorporated in microbial biomass. Kirchgessner et al. (1994) reported that faecal microbial protein excretion was 0.20 per g of fermented wheat bran. Thus, on a microbial organic matter (**OM**) basis, a ratio of 0.25 (i.e., 0.20/0.75, where protein is assumed to be 75% of the microbial polysaccharide-free DM) was adopted.

Statistical analysis

All data, except for starch fermentation characteristics, were analysed using the MIXED procedure in SAS (version 9.4, SAS Institute Inc., Cary, NC). The model contained ground maize, NH_4Cl, and ground maize × NH_4Cl as fixed effects, and cow and period as random effects. Differences were considered significant at $P \leq 0.050$. Multiple comparisons between treatment means were made using the Tukey-Kramer method when a NH_4Cl effect or ground maize × NH_4Cl interaction was detected. For starch fermentation characteristics, the MEANS procedure in SAS was used to determine the 95% confidence interval. The results discussed in this study focus specifically on the effect of abomasal infusion of 3 kg ground maize/d relative to 0 kg ground maize/d infusion, irrespective of the level of NH_4Cl infusion.

Results

No ground maize × NH_4Cl interactions were observed for nutrient intake and apparent total-tract digestibility (**ATTD**) of nutrients (Table 1). Regardless of NH_4Cl, ground maize increased total DM and starch intake, and ATTD of starch, but decreased ATTD of both DM and N. No ground maize × NH_4Cl interactions were observed for the faecal output characteristics (Table 2). Regardless of NH_4Cl, ground maize increased ^{13}C enrichment of faeces, and faecal output of DM, starch originating from the abomasal infusion, and total starch. No ground maize × NH_4Cl interactions were observed for faecal output of VFA and their ^{13}C enrichment (Table 2). The exception was iso-butyrate with an overall higher ^{13}C enrichment for the abomasal infusion treatments with ground maize compared to those without ground maize, but within each level of ground maize ^{13}C enrichment was not affected by the level of NH_4Cl. Regardless of NH_4Cl, ground maize increased faecal output of acetate, butyrate, and total VFA, and decreased faecal output of iso-butyrate, valerate, and iso-valerate. Furthermore, ground maize increased ^{13}C enrichment of all faecal VFA, with the exception of valerate. Relative to the treatments without ground maize, abomasal infusion of 3 kg of ground maize/d resulted, on average, in an estimated 328 g faecal microbial biomass/d (95% confidence interval: 227–428 g) from fermented starch (Table 3). On average, an estimated 1 311 g starch/d was postruminally fermented upon ground maize infusion, representing 60.8% of total starch intake (confidence interval: 41.2–80.5%).

Discussion

The increased total intake of DM and starch upon ground maize infusion was a direct consequence of the treatment design. We did not observe a depressed voluntary DM intake upon ground maize infusion, likely because of the low DM intake of the TMR (i.e., 13.3 kg/d for 0.0 mol NH_4Cl/d without ground maize vs. the calculated feed intake capacity of 18.8 kg DM/d at 95% feeding level). The decreased ATTD of DM upon ground maize infusion may be the result of (1) reduced fermentation or digestion and absorption of the nutrients because the ground maize was infused to bypass the rumen, (2) the decreased hindgut pH (van Gastelen et al., 2021) potentially inhibiting fibrolytic bacteria, and (3) the

Table 1
Nutrient intake and apparent total-tract digestibility of nutrients of dairy cows abomasally infused with ground maize.[1]

	Ground maize infusion		SEM	*P*-value		
	0 kg/d	3 kg/d		Maize	NH_4Cl	Maize × NH_4Cl[2]
Number of observations	18	16				
Nutrient intake						
DM TMR (kg/d)	12.8	11.9	0.55	0.161	0.099	0.358
DM abomasal infusion (kg/d)	0.23	2.57	0.037	< 0.001	0.907	0.206
DM total (TMR + abomasal infusion; kg/d)	13.0	14.5	0.56	0.025	0.103	0.371
Starch TMR (kg/d)	0.58	0.54	0.027	0.174	0.077	0.270
Starch abomasal infusion (kg/d)	0.00	1.64	0.023	< 0.001	0.248	0.245
Starch total (TMR + abomasal infusion; kg/d)	0.58	2.18	0.036	< 0.001	0.031	0.393
Apparent total-tract digestibility						
DM (% of total intake)	71.7	69.1	0.86	< 0.001	0.123	0.633
N (% of total intake)	68.5	62.8	0.97	< 0.001	0.009	0.453
Starch (% of total intake)	93.6	95.0	0.42	< 0.001	0.029	0.466

[1] Least square means of 0 and 3 kg of ground maize/d, averaged over 0.0, 2.5, and 5.0 mol NH_4Cl/d.
[2] Interaction between two levels of ground maize (0 and 3 kg/d) and three levels of NH_4Cl (0.0, 2.5, and 5.0 mol/d).

Table 2
Faecal output characteristics, faecal VFA output, and ^{13}C enrichment of faecal volatile fatty acids (VFA) of dairy cows abomasally infused with ground maize.[1]

	Ground maize infusion		SEM	*P*-value		
	0 kg/d	3 kg/d		Maize	NH_4Cl	Maize × NH_4Cl[2]
Number of observations	18	16				
Faecal output characteristics						
^{13}C enrichment faeces (atom%)	1.073	1.076	0.0002	< 0.001	0.678	0.588
DM (kg/d)	3.68	4.50	0.214	0.001	0.038	0.539
Starch						
From TMR (g/d)	37.3	37.4	2.77	0.983	0.003	0.852
From abomasal infusion (g/d)	0.0	71.5	7.65	< 0.001	0.157	0.174
Total (g/d)	37.3	108.9	7.78	< 0.001	0.019	0.137
Faecal VFA output						
Acetate (g/d)	60.2	74.9	4.95	0.045	0.222	0.533
Propionate (g/d)	11.6	17.3	2.32	0.067	0.169	0.562
Butyrate (g/d)	5.3	20.4	2.13	< 0.001	0.389	0.510
Iso-butyrate (g/d)	1.9	1.1	0.175	0.004	0.150	0.827
Valerate (g/d)	1.5	0.7	0.127	< 0.001	0.134	0.310
Iso-valerate (g/d)	1.5	1.0	0.163	0.003	0.204	0.423
Total (sum of all VFA; g/d)	82.0	115.3	8.87	0.011	0.204	0.574
^{13}C enrichment of faecal VFA						
Acetate (atom%)	1.078	1.082	0.0007	< 0.001	0.050	0.769
Propionate (atom%)	1.081	1.084	0.0005	< 0.001	0.198	0.962
Butyrate (atom%)	1.075	1.082	0.0003	< 0.001	0.080	0.105
Iso-butyrate[3] (atom%)	1.073	1.077	0.0004	< 0.001	0.653	0.024
Valerate (atom%)	1.073	1.075	0.0009	0.113	0.378	0.735
Iso-valerate (atom%)	1.074	1.078	0.0007	< 0.001	0.614	0.388

[1] Least square means of 0 and 3 kg of ground maize/d, averaged over 0.0, 2.5, and 5.0 mol NH_4Cl/d.
[2] Interaction between two levels of ground maize (0 and 3 kg/d) and three levels of NH_4Cl (0.0, 2.5, and 5.0 mol/d).
[3] Maize × NH_4Cl interaction; 0 kg/d ground maize + 0.0 mol NH_4Cl = 1.072[a], 0 kg/d ground maize + 2.5 mol NH_4Cl = 1.072[a], 0 kg/d ground maize + 5.0 mol NH_4Cl = 1.074[a], 3 kg/d ground maize + 0.0 mol NH_4Cl = 1.077[b], 3 kg/d ground maize + 2.5 mol NH_4Cl = 1.078[b], and 3 kg/d ground maize + 5.0 mol NH_4Cl = 1.076[b]. Different superscripts indicate a significant ($P < 0.05$) difference.

Table 3
Postruminal starch fermentation characteristics of dairy cows abomasally infused with 3 kg of ground maize/d.

	Lower CL mean[1]	Mean	Upper CL mean[2]
Faecal output of microbial biomass originating from starch fermentation (g/d)	227	328	428
Starch fermentation			
Quantity (g/d)	909	1 311	1 712
Relative (% of total starch intake)	41.2	60.8	80.5

[1] Lower bound of the confidence interval for the mean.
[2] Upper bound of the confidence interval for the mean.

increased outflow of microbial biomass synthesised from the fermentation of infused starch. The increased faecal DM output is the direct consequence of the increased DM intake in combination with a decreased ATTD of DM. The greater faecal output of starch is the result of the large increase in starch intake only partly compensated for by a small increase in ATTD of starch. The latter is related to the low starch content of the TMR leading to a lower ATTD of starch compared with the ATTD of the infused starch (van Gastelen et al., 2021).

The increased faecal ^{13}C enrichment upon ground maize infusion is caused by an increased faecal starch output originating from the infused ground maize with a high ^{13}C enrichment, an increased faecal VFA output with an elevated ^{13}C enrichment, as well as an increased faecal non-starch OM output including microbial biomass originating from fermented starch. Ground maize infusion resulted in increased postruminal fermentation, evidenced by the increased faecal total VFA output and ^{13}C enrichment of the individual VFA. The increased faecal output of acetate, propionate, and butyrate, assuming the absorption had not changed largely, suggests an increased production of these VFA postruminally. The decrease in faecal output of valerate, iso-valerate and iso-butyrate upon ground maize infusion may represent a decline in their net production, which embodies the gross production upon protein fermentation minus their incorporation in microbial biomass for amino acid synthesis (Andries et al., 1987). As microbial protein synthesis increases upon ground maize OM fermentation and net protein fermentation decreases due to increased protein incorporated, net production and thus faecal output of these VFA decreased. The increased ^{13}C enrichment of all faecal VFA (except valerate) with ground maize shows that the extra faecal VFA originates from postruminal fermentation of ground maize OM, corresponding to the high ^{13}C enrichment of maize.

Based on Harmon et al. (2004), one can expect that 20–33% of the abomasally infused starch is fermented in the large intestine. The amount of starch postruminally fermented in the current study was higher, on average 60.8%, likely caused by the high level of starch abomasally infused (i.e., 1.64 kg/d) relative to total DM intake (14.5 kg/d). Assuming a ruminal starch fermentation of 75% (Harmon et al., 2004), and given that abomasally infused starch represents bypass starch, daily starch intake would be 7.1 kg/d (i.e., 1.64 kg/d/(100% − 75%) + 0.54 kg/d starch in TMR). This results in a dietary starch content of 499 g/kg DM, a level generally not reached in practice. However, despite these conditions, the results indicate that a substantial part of the starch disappeared in the small intestine not by enzymatic digestion, but by fermentation. In agreement, Reynolds (2006) suggested that the capacity for starch digestion in the small intestine is limited and that incompletely degraded substrates, mostly starches, are fermented. Moharrery et al. (2014) however demonstrated no limited capacity for small intestinal starch digestion up to 2 kg/d, with an estimated small intestinal starch digestibility of 51% for maize starch. The initial phase of small intestinal digestion requires pancreatic α-amylase, and starch has a negative effect, whereas casein, certain amino acids, and dietary energy have a positive effect on pancreatic α-amylase secretion (Harmon and Swanson, 2020). No additional protein was abomasally infused in the present study, and Gilbert et al. (2015) did not add additional protein to the milk replacer for milk-fed calves, which may partially explain the rather high fermentation levels of starch in both studies. The large proportion of postruminal starch being fermented can have nutritional consequences, because nutritionist may overestimate glucose yield from bypass starch. First, the energetic efficiency of metabolisable energy utilisation of starch fermentation is lower compared with that of enzymatic digestion of starch. Second, dairy cows may need to produce glucose from other sources (e.g., propionate and amino acids). In conclusion, the results suggest that postruminal starch fermentation in early-lactation dairy cows abomasally infused with 3 kg ground maize/d is considerable, being on average 60.8% of total starch intake, and could result in substantial amounts of VFA rather than glucose production.

Ethics approval

The experiment was conducted under the Dutch Law on Animal Experiments in accordance with European Union Directive 2010/63, and approved by the Central Committee of Animal Experiments (The Hague, the Netherlands; 2018.D-0013.001).

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are confidential.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Sanne van Gastelen: conceptualisation, methodology, formal analysis, investigation, writing – original draft, project administration; **Jan Dijkstra:** conceptualisation, methodology, writing – review and editing, supervision, funding acquisition; **Walter J.J. Gerrits:** conceptualisation, methodology, writing – review and editing, supervision; **Myrthe S. Gilbert:** conceptualisation, methodology, writing – review and editing; **André Bannink:** conceptualisation, methodology, writing – review and editing, supervision, project administration, funding acquisition.

Declaration of interest

None.

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