

IN VITRO CAECAL FERMENTATION AND VOLATILE FATTY ACIDS PRODUCTION FROM SUGARS IN RABBITS

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Abstract: The aim of this study was to evaluate the fermentability of selected sugar substrates using an *in vitro* gas production test and volatile fatty acid (VFA) analysis with a caecum inoculum from rabbits. The incubated substrates are glucose, fructose, sucrose, molasses and Molkolac® (high lactose content). In addition to determining the gas production of selected substrates, the VFA concentration was also determined after 8 h of incubation in the inoculum prepared from the caecal content of the rabbit. The gas production kinetics parameters were estimated using the Gompertz model and the maximum fermentation rate (MFR), time of maximum fermentation rate (TMFR), lag phase (Lag), amount of gas produced within 8 h (GAS8), maximum fermentation rate within 8 hours (MFR8), total amount of VFA, proportions of each VFA and the ratio between each VFA were determined. Sucrose and molasses yielded the highest gas and VFA production, with molasses showing a slower but intense early fermentation phase. Molkolac exhibited the lowest fermentability. Fermentation of sucrose and glucose was associated with a higher butyrate content, while molasses and Molkolac led to a higher acetate content. Although monosaccharides are normally absorbed in the small intestine, the results of this study confirm that if some sugars, particularly glucose, sucrose, and to a lesser extent fructose, reach the rabbit caecum, they can undergo intense fermentation comparable only to that of sugar beet pulp. In contrast, molasses and lactose (Molkolac) showed lower fermentation efficiency (lower total gas production), with molasses still making a notable contribution due to its rapid onset of fermentation, while lactose had minimal effects.

Key Words: carbohydrates, sugars, *in vitro* fermentation, caecal inoculum, rabbit.

INTRODUCTION

Current knowledge of the digestion of sugars, including mono- and disaccharides, in rabbits remains incomplete. Sugar is found in low concentrations in animal feed, although some feedstuffs, particularly sugar beet pulp and molasses, contain higher amounts. In rabbit feed, sugar is present either as monosaccharides or as the disaccharide sucrose (glucose + fructose).

Glucose and fructose are rapidly absorbed by the small intestinal mucosa, but Gidenne and Ruckebusch (1989) found that the sugar content (soluble in 80% ethanol) in the ileal contents of adult rabbits reached about 25 g/kg dry matter (DM). This could indicate that the amount of sugars that can enter the caecum is not negligible (Gidenne et al., 2020) and can be quickly fermented by the microbiota of the caecum (Slovakova et al., 2002). Monosaccharides can also be produced in the caecum by the microbial degradation of polysaccharides, such as pectins and hemicelluloses. Gidenne et al. (2020) assume that sugars in the caecum of rabbits can be completely digested by caecal microorganisms, but this has not yet been measured, and no results can be found in the available literature.

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Most of the sugar in rabbit feed comes from sugar beet pulp and molasses. In commercial feed mixtures for rabbits, sugar beet pulp represents 8-20% (Heuzé *et al.*, 2019) due to its high pectin content (15 - 18%), which has a protective effect against digestive disorders in rabbits (Gidenne and Licois, 2005) if correctly balanced with lignins and slowly fermented polysaccharides (Gidenne, 2015). Sugar beet molasses is a viscous liquid with an average of 60% to 63% total sugar and 14% crude protein in the dry matter (Maertens *et al.*, 2002; Heuzé *et al.*, 2019). In Spanish commercial feed mixtures for rabbits, it is used up to 3% to improve pellet hardness and durability (de Blas and Mateos, 2010).

Lactose is the primary carbohydrate in the milk of domestic mammals, typically accounting for 4.5-5.0% in cow milk and about 5.7% in sow milk. In contrast, rabbit milk contains only around 1.7% lactose, but significantly higher levels of protein and fat (Maertens *et al.*, 2006). For this reason, lactose is not used in the feeding of young rabbits (Blas and Gidenne, 2020) and there is little data on the digestibility of lactose in rabbits.

Lactase activity in rabbits decreased with age (4 wk vs. 3-mo of age) and was much lower in the small intestine than in the caecum (Marounek *et al.*, 1995). Similarly, Gutiérrez *et al.* (2002) found that in early weaned rabbits the apparent faecal digestibility of lactose was almost 100%, whereas the apparent ileal digestibility of lactose was only 71 to 76%, and neither value was affected by the inclusion of lactose in the diet. Lactase activity in the jejunal mucosa decreased greatly with age and weaning (between 25 and 35 d of life) and was independent of dietary lactose intake, but mortality due to diarrhoea was significantly higher with the highest lactose content (Gutiérrez *et al.*, 2002). The authors assumed that undigested lactose entering the caecum could favour progressive colonisation of the intestine with pathogenic microorganisms leading to diarrhoea.

Fermentation in the caecum can be estimated by different methods (Cambra-López *et al.*, 2025). One of these is the gas production technique, which has recently been used in combination with volatile fatty acids (VFA) analysis to determine microbial activity in the caecum of rabbits (e.g. Lavrenčič, 2007; Kermauner and Lavrenčič, 2010; Kara, 2016).

There is little data on the *in vitro* fermentation of sugars in rabbits. Yang *et al.* (2010) compared the *in vitro* production of VFA from glucose and cellulose (microcrystalline cellulose and neutral detergent fibre (NDF) from lucerne hay) and Marounek *et al.* (2000) from glucose and xylose in an inoculum prepared from rabbit caecum.

Due to the very limited data available on the digestion and fermentation of sugars in rabbits (reviewed by Blas and Gidenne, 2020), the aim of the present study was to measure the gas production kinetics and VFA synthesis from sugars incubated in an inoculum prepared from the contents of rabbit caecum.

MATERIAL AND METHODS

Substrates

Five carbohydrate substrates were used for the *in vitro* gas test: three isolates (two monosaccharides and one disaccharide) and two feedstuffs with a high content of disaccharides:

1. Glucose (monosaccharide, Sigma G8270, Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).
2. Fructose (monosaccharide, Sigma F0127, Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).
3. Sucrose (disaccharide from glucose and fructose, Sigma S9378, Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).
4. Molasses (high sucrose content: 80.6% DM, in DM: 62.7% total sugars, 11.7% crude protein, 3.3% crude ash; provided by Jata-Emona d.o.o., Slovenia).
5. Molkolac® Instant (partially demineralized whey permeate powder, obtained from fresh sweet whey, with high lactose content: 96.0% DM, in DM: 86.5% lactose, 3.1% crude protein, max 1% crude fat, 6.3% crude ash; provided from Panvita kmetijstvo d.o.o., Slovenia).

***In vitro* fermentation**

The caecum content of two 78-d-old New Zealand white rabbits from the Slovenian meat line SIKA was selected to prepare the inoculum. The samples were taken at the slaughter line. Handling and selection of the animals, as well as preparation of the inoculum, were performed according to the methods used by Lavrenčič (2007). *In vitro* gas production was determined according to the procedure described by Menke and Steingass (1988). Two hundred milligrams of substrate were incubated anaerobically at 39°C in a 100 mL glass syringe with 30 mL inoculum. Four syringes were prepared for each substrate, two for gas production and two for VFA determination. The *in vitro* gas test was performed in two separate batches one week apart.

VFA determination

After 8 h, two of the four syringes of each substrate were withdrawn from the water bath; their contents were poured into 50-mL plastic tubes and stored in a freezer at –20°C until analysis of the VFA. The extracts for the VFA were prepared from the incubation fluid according to the modified method of Holdeman et al. (1977). The VFA were analysed using a gas chromatograph (Hewlett Packard 5890 A, Hewlett Packard, USA) equipped with a split injector and an FID detector. A 30 m NUKOL® TM, fused silica capillary column (SUPELCO, USA) was used for VFA separation.

Calculations and statistical analysis

The gas production data measured *in vitro* were corrected for the substrate DM content and for the gas produced by blank samples. The corrected values were then fitted with the Gompertz model (Lavrenčič, 2007), using the Marquard compromise of a nonlinear regression method with SAS software (Proc NLIN) (SAS Institute Inc., 2015) and the parameters “B” (total potential gas production), “C” (specific rate of gas production) and a constant “A” (often described as the decay of the specific gas production rate) were estimated. From these parameters, the maximum fermentation rate (MFR), the time of maximum fermentation rate (TMFR), the volume of gas produced in the first 8 h of incubation (Gas8), the fermentation rate after 8 h of incubation (FR8) and the lag phase (Lag) were calculated.

The net amounts of total and individual VFA produced after 8 h of incubation were calculated by subtracting the average amounts of total and individual VFA produced by blank samples at an appropriate incubation time and adjusting the total and individual VFA production to 1 g substrate DM.

Statistical analysis was performed with the GLM procedure using SAS software (SAS Institute Inc., 2015). Differences between substrates gas production and VFA production kinetic parameters, proportions and ratios were tested with the GLM model of SAS, using substrate as a fixed effect (SAS Institute Inc., 2015).

RESULTS AND DISCUSSION

Table 1 and Table 2 show the estimated and calculated parameters of gas production kinetics, while Figure 1 and Figure 2 show the progression of cumulative gas production for the substrates used in the inoculum prepared from the rabbit caecum.

Table 1: Estimated parameters of gas production kinetics of different sugars in inoculum from rabbit caecum

Substrate	B (mL/g DM)	C	A
Glucose	353 ^c	11.79 ^a	0.277 ^{bc}
Fructose	345 ^c	25.28 ^b	0.306 ^c
Sucrose	386 ^d	9.36 ^a	0.234 ^{ab}
Molasses (sucrose)	196 ^a	4.43 ^a	0.230 ^{ab}
Molkolac (lactose)	298 ^b	11.70 ^a	0.202 ^a
RMSE	13.1	7.302	0.0383
R ²	0.976	0.532	0.762

^{a,b,c,d}: means in columns with different superscripts are significantly different at the $P < 0.05$ level. RMSE: root mean square error; R²: coefficient of determination; “B”: total potential gas production; “C”: specific gas production fermentation rate as affected by the time of incubation, governed by a constant “A” (decay of the specific gas production rate); DM: Dry matter.

Table 2: Calculated parameters of gas production kinetics of different sugar in a rabbit caecum inoculum

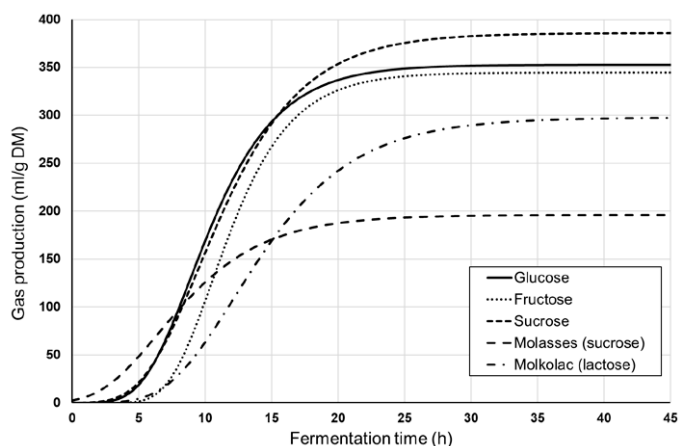
Substrate	MFR (mL/h)	TMFR (h)	Gas8 (mL/g DM)	FR8 (mL/h)	Lag (h)
Glucose	36.0 ^a	8.6 ^b	106.4 ^a	35.1 ^a	5.0 ^b
Fructose	38.9 ^a	9.6 ^b	66.2 ^b	31.9 ^a	6.2 ^{ab}
Sucrose	33.2 ^a	9.4 ^b	99.1 ^a	31.5 ^a	4.9 ^b
Molasses (sucrose)	16.7 ^b	6.4 ^c	98.1 ^a	15.6 ^b	2.1 ^c
Molkolac (lactose)	22.1 ^b	12.1 ^a	32.8 ^c	13.9 ^b	7.1 ^a
RMSE	4.97	0.97	15.58	4.24	0.93
R ²	0.850	0.905	0.846	0.883	0.878

^{a,b,c,d}: mean values in columns with different superscripts are significantly different at the $P < 0.05$ level; RMSE: root mean square error; R²: coefficient of determination; MFR: maximum fermentation rate; TMFR: time of maximum fermentation rate; Gas8: gas produced up to 8 h after incubation; FR8: fermentation rate after 8 h of incubation; Lag: lag phase.

The highest potential total gas production B was observed for sucrose, 8.5 to 10.6% lower for both monosaccharides, while Molkolac (lactose) produced about 23% less gas and molasses almost 50 % less gas. These results are slightly higher, but in agreement with those of Lavrenčič (2007), when pectin, starch and xylan were fermented in the caecal contents of 78-day-old rabbits (331, 314 and 307 mL/g DM, respectively), Kermauner and Lavrenčič (2008a; 2013) and Ferreira *et al.* (2019), when energy-rich feedstuffs were fermented, while protein- or fibre-rich feeds produced less gas (Kermauner and Lavrenčič, 2008b; 2009; 2013; Ferreira *et al.*, 2019). The total gas production of molasses was the lowest in the current experiment and comparable to the gas production of commercial rabbit feed (Mariezcurrena-Berasain *et al.*, 2018).

The specific gas production rate (parameter "C"), which ranges from 4.4 for molasses to 25.3 for fructose, but only the latter differs significantly ($P < 0.05$) from other substrates. The constant parameter "A", which describes the decrease in gas production rate, was similar for all substrates (from 0.202 to 0.306), with the only significant ($P < 0.05$) difference between fructose (0.306) and Molkolac (0.202).

The cumulative gas production curve (Figure 1) was similar for all isolated sugars, with a slightly higher gas production for sucrose, while Molkolac showed lower gas production. However, the gas production curve for molasses is very different from that of the other substrates. This difference can be seen more clearly in Table 2 and Figure 2, where the fermentation rate (MFR, TMFR, Lag etc.) is shown.

**Figure 1:** Kinetics of gas production in rabbit caecal content (DM = dry matter).

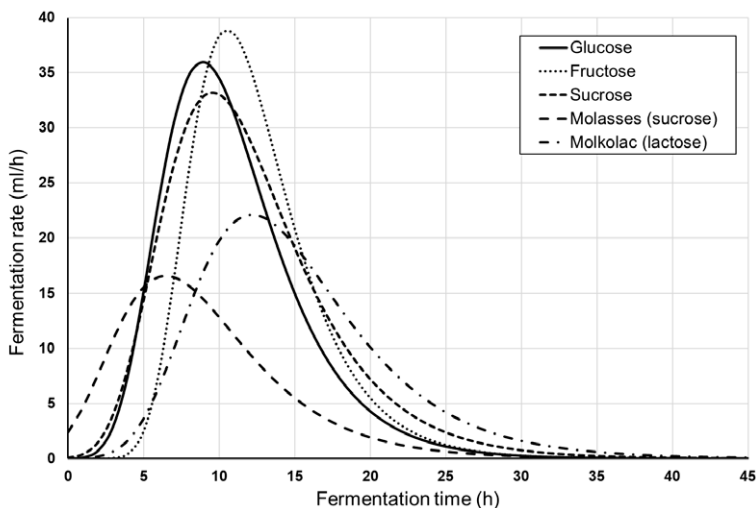


Figure 2: Gas production of fermentation rates in rabbit caecal content

While the maximum fermentation rate (MFR) was very similar between the isolated sugars, Molkolac and especially molasses had significantly ($P < 0.05$) lower MFR than fructose (for 43 and 57% resp.) (Table 2). There were also no differences in TMFR and Lag when isolated sugars were fermented, with Molkolac having the longest TMFR (126% of fructose) and Lag (142% of glucose) and molasses having the shortest TMFR (33% of fructose) and Lag (58% of glucose). Lag is the time needed for microbial colonisation of substrate, pH adjustment and hydration. However, under *in vivo* conditions, hydration is not needed, so lag time depends only on microbial colonisation, sugar hydrolysis from di- or polysaccharides and pH adjustment.

The MFRs in the current study are higher and the TMFR shorter than in our previous studies with different substrates (Lavrenčič, 2007; Kermauner and Lavrenčič, 2008a; 2008b; 2009; 2013; 2019). The only similar TMFR was observed when barley grain and sugar beet pulp (Kermauner and Lavrenčič, 2008a) or maize grain and wheat bran (Ferreira et al., 2019) were fermented. Similarly, Gas8 in the current study are much higher than in our previous studies (Kermauner and Lavrenčič, 2008a; 2008b; 2009; Lavrenčič and Kermauner, 2011) with only comparable Gas8 when sugar beet pulp was fermented (Lavrenčič and Kermauner, 2011). All of this indicates that caecal fermentation of sugar and molasses may also be important *in vivo* in rabbits.

These differences between the fermentation patterns of the various types of sugar are illustrated even better in Figure 2. The fermentation pattern of glucose, fructose and sucrose was similar, while molasses fermented rapidly, but to a lesser extent. The shortest Lag may indicate that the microorganisms in the caecum were adapted to molasses, as it was present in the feed mixture throughout the fattening period. For this reason, microbial colonisation of molasses could be faster and the Lag shortest. Another reason for the earlier fermentation of molasses could be the relatively high content of crude protein in molasses (11.7% in DM), which begins to ferment rapidly and has shorter Lag (Kermauner and Lavrenčič, 2008a; 2008b; Ferreira et al., 2019). Longer Lag when sugars (glucose, fructose, sucrose) were fermented can be a consequence of high concentrations of sugars, since it is well known that sugars are used as food preservatives. But when fermentation started, the production of gas from sugars was high and fast (short TMFR, high Gas8 and the highest FR8).

The relatively high content of crude protein in molasses may also be the reason for the lowest total gas production (parameter B) of molasses, as proteins are fermented to a lesser extent (Marounek et al., 2000). Kermauner and Lavrenčič (2008a; 2008b; 2013) and Ferreira et al. (2019) found that high protein feeds (soya and sunflower meal) produced less than half of the total gas produced by fermentation of high energy feeds (maize, barley) and that the total amount of total gas was comparable to that of high fibre feeds (hay, dehydrated lucerne). The MFR of high protein feeds was only one third of the MFR of high energy feeds and was even lower than the MFR of high fibre feeds.

Molkolac fermented later and more slowly than the other substrates (the longest Lag and TMR) and reached an MFR that was significantly lower (for 33 to 43%) than that of the isolated sugars. The caecal microbiota is probably not adapted to lactose, as lactose is not used in the rabbit nutrition (Blas and Gidenne, 2020), so the microbial colonisation of Molkolac could be very slow (longest Lag) and the fermentation has a lower extent. This may be due to the relatively high crude ash content, which does not contribute to fermentation. In addition, it is possible that the processing conditions of the Molkolac preparation, e.g. temperature and pressure, can produce Maillard reaction products, which can affect the fermentation in caecum. Some of these products are biologically active and can alter microbiota, and thereby can also affect gas and VFA formation in the large intestine (Tuohy *et al.*, 2006). The fermentation rate at 8 h of incubation (FR8) and the gas production within 8 h after incubation (Gas8) describe the microbial fermentation and gas production within the caecum digesta retention time, which is on average less than 10 h (Gidenne, 1997). During this period, the highest ($P<0.05$) fermentation rate (FR8) was observed in the fermentation of isolated sugars, while molasses and Molkolac showed a 50-60% lower fermentation rate. The highest ($P<0.05$) gas production in 8 h of fermentation (Gas8) was observed in fermentation of glucose, sucrose and molasses; fructose produced two thirds and Molkolac only one third of the gas volume produced by glucose.

Lavrenčič and Kermauner (2011) found that the Gas8 of soya and sunflower meal is low and comparable to that of barley grain and dehydrated lucerne (20.5 to 28.5 mL/g DM) and lower than that of xylan, wheat bran (33-42 mL/g DM) and especially pectin and sugar beet pulp (81.6-92.8 mL/g DM); the latter is only comparable to the Gas8 of glucose, sucrose and molasses in the current trial. Similar results were obtained by Kermauner and Lavrenčič (2013) when the gas production in the first 10 hours of fermentation was considered, but with a much lower fermentation of sugar beet pulp.

As previously shown, molasses ferments to a lesser extent, but very rapidly, so that its fermentation can be significant within the normal retention time in the caecum. Our results indicate that fermentation of glucose, sucrose and molasses can also occur *in vivo*, whereas fructose and especially lactose (Molkolac) do not contribute significantly to *in vivo* caecal fermentation. The results indicate that elevated lactose concentrations in rabbit feed should not adversely affect digestive health.

The production of VFA and the molar proportion of the three main VFA (acetic, propionic and butyric acid) produced after 8 h of incubation are presented in Table 3. Significantly, the highest amount of VFA was produced when molasses was fermented, while the other substrates did not differ significantly, although the amount of VFA produced from glucose and sucrose was slightly higher (71% of molasses) than from fructose (66%) and Molkolac (62 % of molasses). VFA production was in accordance with the results of Kermauner and Lavrenčič (2011), who determined between 0.8 and 3.9 mmol VFA/g DM after 8-h of incubation of various feeds, with VFA production being highest from the fermentation of sugar beet pulp, while VFA production from starch, NDF from rabbit compound feed and cellulose was much lower (0.03-0.4 mmol/g DM). VFA production from molasses in the current experiment was comparable to VFA production from commercial rabbit feed (Mariezcurrena-Berasain *et al.*, 2018).

Table 3: Total amount of volatile fatty acids (VFA) and molar proportions of acetate, propionate and butyrate produced in 8-hour fermentation in a rabbit caecum inoculum.

Substrate	VFA (mmol/g DM)	Molar proportions (mmol/mmol VFA)			Ratio	
		Acetate	Propionate	Butyrate	Ac: Pr	Pr: Bu
Glucose	2.25 ^a	0.558 ^a	0.129 ^b	0.313 ^{ab}	4.34 ^a	0.41 ^c
Fructose	2.09 ^a	0.564 ^a	0.119 ^b	0.318 ^b	4.76 ^a	0.37 ^{bc}
Sucrose	2.26 ^a	0.573 ^a	0.086 ^a	0.341 ^c	6.68 ^b	0.25 ^a
Molasses (sucrose)	3.15 ^b	0.639 ^b	0.083 ^a	0.278 ^a	7.76 ^c	0.30 ^{ab}
Molkolac (lactose)	1.94 ^a	0.622 ^b	0.088 ^a	0.290 ^a	7.12 ^c	0.30 ^{ab}
RMSE	29.750	0.2585	0.0661	0.1189	0.529	0.049
R ²	0.654	0.653	0.777	0.762	0.889	0.966

^{a,b,c}: mean values in columns with different superscripts are significantly different at the $P<0.05$ level; VFA: volatile fatty acid; RMSE: root mean square error; R²: determination coefficient; DM: dry matter.

Different studies in the field of ruminant nutrition found strong positive correlations ($r = 0.99$) between the volume of gas produced *in vitro* and the amount of VFA released during the corresponding times (Beuving and Spoelstra, 1992; Blümmel and Orskov, 1993). Lavrenčič and Kermauner (2011) also confirmed these results in rabbits with R^2 of 0.87 to 0.96, but with very variable regression coefficients and intercepts between two studies. The results of the current experiment are partially consistent with these findings, as the substrates with the highest Gas8 (glucose, sucrose and molasses) also had the highest VFA production, but only significant when the molasses was fermented.

In the fermentation of molasses and Molkolac, the molar proportion of acetic acid was the highest ($P < 0.05$) and the proportion of propionic acid and butyric acid the lowest ($P < 0.05$). Glucose, fructose and sucrose produced the lowest proportion of acetic acid, glucose and fructose the highest proportion of propionic acid and an average proportion of butyric acid. Sucrose not only had the highest proportion of acetic acid, but also the lowest proportion of propionic acid (similar to molasses and Molkolac) and the highest proportion of butyric acid.

The molar proportion between the three main VFAs is mainly influenced by the fibre content (Gidenne et al., 2020), while the effect of other nutrients remains unknown. Due to the lack of available literature dealing with the VFA production from sugars, we compared our results with those of feeds containing high levels of soluble carbohydrates. Kermauner and Lavrenčič (2011) found a higher proportion of acetate (between 0.84 and 0.65 mmol/mmol VFA) when different feedstuffs were incubated for 8 h, with the lowest proportion (0.58) found in the fermentation of maize grains. In another study (Lavrenčič and Kermauner, 2015) with the same feedstuffs, the acetate proportion after 10 h of incubation was similar (0.81 to 0.66), with the lowest proportion (0.66) found in the fermentation of maize and barley grain.

In the studies by Lavrenčič and Kermauner (2011, 2015), the propionate content was comparable (0.04-0.14 mmol/mmol VFA) and the butyrate proportion lower (0.11-0.32) than in the current study, with the most comparable results coming from maize grains fermentation (propionate 0.09, butyrate 0.32 mmol/mol VFA) (Lavrenčič and Kermauner, 2011). Among the pure nutrients, starch fermentation was most similar to the results of all used substrates in the current study, with the lowest proportion of acetate and the highest of propionate, while butyrate was about average (Lavrenčič and Kermauner, 2011). Similarly, Gidenne et al. (2000) found lower levels of acetic and butyric acid and higher proportion of propionic acid *in vivo* when rabbits were fed diets with higher starch content.

After 72 h of glucose incubation, Yang et al. (2010) found a higher proportion of acetate and propionate (0.76 and 0.09, respectively) and a much lower proportion of butyrate (0.13) than in our study. The rabbit caecal bacterium *Bifidobacterium pseudolongum* produced more acetate when cultured on glucose than on pectin (Slovakova et al., 2002), while Marounek et al. (2000) found a lower proportion of acetate and propionate and a higher proportion of butyrate when the caecal microbiota of 3-month-old rabbits was grown on glucose rather than on xylose.

The ratio between acetate and propionate was the widest in the fermentation of molasses and Molkolac, the narrowest in the fermentation of glucose and fructose and in between in the fermentation of sucrose. However, all values in our experiment were much lower than the results obtained *in vitro* by Arce et al. (2022) (from 14.5 to 19.7:1): The use of feed containing 20% molassed sugar beet pulp (molasses supplementation) (SBP+M) instead of non-molassed sugar beet pulp (SBP) lowered the Ac:Pr ratio from 19.7 to 14.5:1, which was due to a higher proportion of propionic acid. When feed containing 40% SBP was used, the addition of molasses had no effect on the Ac:Pr ratio (SBP-40: 19.4 vs. SBP+M-40: 19.7:1) (Arce et al., 2022).

The ratio between propionate and butyrate is very important when it comes to the health of rabbits. The review by Gidenne et al. (2020) describes that the normal ratio of propionate to butyrate is 0.5 to 0.8:1 but changes to 1.5-8.0:1 in rabbits with digestive disorders or diarrhoea due to reduced butyrate production. In the current research, the ratio of propionate to butyrate was very narrow (normal) in all tested substrates, although there were some significant differences. This is in accordance with *in vivo* results (Gidenne et al., 2000) and *in vitro* results of different feedstuffs and pure nutrients (Lavrenčič and Kermauner, 2011; 2015) and in fermentation of glucose and cellobiose, while the ratio of propionate to butyrate was much wider in microcrystalline cellulose and NDF from lucerne hay (0.91 and 0.95: 1, respectively) (Yang et al., 2010). The ratio of propionate to butyrate was extremely wide when the caecal microbiota of 3-month-old rabbits was grown on glucose or xylose (1.1 and 2.0: 1, respectively) (Marounek et al., 2000). A similar ratio of propionate to butyrate as that in our study was also found in rabbits *in vivo* (Arce et al., 2022). When fed diets containing 20% SBP, the Pr:Bu ratio widened when molasses was added to the SBP (SBP-20:

0.32; SBP+M-20: 0.39 : 1), but narrowed in the case of feed containing 40% SBP compared to the non-molassed SBP feed (SBP-40: 0.30; SBP+M-20: 0.25 : 1), mainly due to the higher butyric acid content in the rabbit stomach and caecum samples (Arce *et al.*, 2022).

CONCLUSIONS

Monosaccharides (in feed or derived from sucrose digestion) are rapidly absorbed by the small intestinal mucosa, but there is some evidence that in rabbits some sugars can enter the caecum and be fermented by the caecal microbiota (Gidenne *et al.*, 2020). The results of the current experiment confirm this indication, as the *in vitro* fermentation of glucose, sucrose and partially fructose was generally more intense and to a greater extent than the fermentation of various substrates used in previous studies. The only substrate with similar fermentation was sugar beet pulp, which is known to ferment more rapidly and intensively than other feeds (e.g. grains, proteins feeds, hay, dehydrated lucerne) in the rabbit caecum. Molasses, despite its lower overall fermentation extent (the lowest B and MFR), which could be related to its relatively high protein content, exhibited the fastest onset of fermentation and the highest early gas and VFA production (shortest Lag and TMFR, and consequently a high Gas8), suggesting a potential contribution to *in vivo* caecal fermentation in rabbits. In contrast, Molkolac, as a source of lactose, fermented slowly and only to a limited extent, indicating poor microbial adaptation to this substrate. This likely reflects the fact that lactose is not regularly used in rabbit feed, and the caecal microbiota is therefore not adapted to it. The VFA concentration was also lowest during the first 8 hours of incubation, indicating that fermentation of lactose does not contribute significantly to *in vivo* fermentation in the caecum of rabbits.

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