Italian Journal of Animal Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

In vitro evaluation of sugar digestibility in molasses

Alberto Palmonari, Damiano Cavallini, Charles J. Sniffen, Luiza Fernandes, Phil Holder, Isa Fusaro, Melania Giammarco, Andrea Formigoni & Ludovica Maria Eugenia Mammi

To cite this article: Alberto Palmonari, Damiano Cavallini, Charles J. Sniffen, Luiza Fernandes, Phil Holder, Isa Fusaro, Melania Giammarco, Andrea Formigoni & Ludovica Maria Eugenia Mammi (2021) *In vitro* evaluation of sugar digestibility in molasses, Italian Journal of Animal Science, 20:1, 571-577, DOI: 10.1080/1828051X.2021.1899063

To link to this article: https://doi.org/10.1080/1828051X.2021.1899063

Published online: 27 Mar 2021.

ITALIAN JOURNAL OF ANIMAL SCIENCE
2021, VOL. 20, NO. 1, 571–577
https://doi.org/10.1080/1828051X.2021.1899063

Taylor & Francis
Taylor & Francis Group

PAPER

In vitro evaluation of sugar digestibility in molasses

Alberto Palmonari[a], Damiano Cavallini[a], Charles J. Sniffen[b], Luiza Fernandes[c], Phil Holder[c], Isa Fusaro[d], Melania Giammarco[d], Andrea Formigoni[a] and Ludovica Maria Eugenia Mammi[a]

[a]DIMEVET, Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Ozzano Emilia, Italy; [b]Fencrest L.L.C, Holderness, NH, USA; [c]ED&F Man Liquid Products, London, UK; [d]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy

ABSTRACT

Beet and cane molasses mainly contain mono- di-, and tri-saccharides, composed by hexoses, as well as pentoses in traces. However, rationing software consider sugars as only one entity, with a rate of digestion ~20% h^{-1}. The aim of this initial study was to investigate and evaluate the in vitro digestion dynamics and rates of the sugar fraction in molasses. Three beet and three cane molasses were randomly selected from a variety of samples collected world-wide and digested via in vitro rumen fermentation, at 1, 2, 3, 4, 6, 8, and 24 h. Samples were then analysed with a specific enzymatic kit to quantify residual sucrose, glucose, fructose, raffinose, galactose, and arabinose. Complete disappearance of sucrose happened within 3 hours of incubation. Glucose and fructose were completely digested within 4-6 h, showing variability among samples. Even if not so representative, galactose showed a similar trend of digestion (97% digestion within 3-4 h). Raffinose was quite slower in cane molasses, while it was completely digested within 1 h in beet molasses. Arabinose, a pentose, never reached a complete digestion, and its fermentation dynamic was different compared to other sugars. Calculated rates of digestion for sucrose, glucose and fructose, most representative sugars in molasses, were higher than 50% h^{-1} in both cane and beet. Obtained results showed that sugar fraction in molasses may vary, and different sugars are rapidly fermented by rumen microbes. Modern rationing models should consider a modification of sugar rates of digestion, since the actual one appears too slow than those observed in vitro.

HIGHLIGHTS

- Molasses are unique blends of several sugars
- Major sugars are digested in few hours
- Rationing software should consider a faster rate of digestion for different sugars

ARTICLE HISTORY
Received 26 November 2020
Revised 24 February 2021
Accepted 1 March 2021

KEYWORDS
Molasses; sugar fraction; fermentation dynamics; rates of digestion

Introduction

Beet and cane molasses are produced worldwide, as a by-product of sugar extraction. They are intensively used in animal nutrition due to their chemical composition and characteristics. From a chemical stand point, the main fraction in molasses is represented by sugars. Beet and cane molasses have their unique composition, but sucrose, fructose and glucose still count for 40-50% of molasses dry matter. Several studies reported that molasses addition would reduce cow sorting activity (DeVries and Gill 2012), stimulate DMI due to a sweetening effect (Murphy et al. 1997), increase butyrate production (DeFrain et al. 2004, 2006; Chibisa et al. 2015; Oba et al. 2015), and affect milk fat, FCM, ruminal ammonia, MUN, and fibre digestibility (Broderick and Radloff 2004; Brito et al. 2015). Overall, molasses could be added to the diets in substitution of starch sources, due to a different impact on rumen fermentations and pH (Oelker et al. 2009; Brito et al. 2017; De Ondarza et al. 2017), effect related to their sugar fraction composition. Molasses contain mono- di-, and tri-saccharides, composed by hexoses, as well as pentoses in traces (Palmonari et al. 2020). Thus, it could be assumed that each one of these compounds would have a different fermentation patter or rate, depending on the complexity of the molecule, and its structure. However, it should be noted that in the modern rationing software such CNCPS (Van Amburgh et al. 2015), sugars are considered as a sole entity, without any differentiation

CONTACT Dr Alberto Palmonari alberto.palmonari2@unibo.it Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Ozzano Emilia, Italy

among them. Besides, the digestion rate (Kd) of sugars in the rumen is considered as ~20% h^{-1}, despite higher rates were observed in previous studies (Weisbjerg et al. 1998; Petruzzi et al. 2002). The aim of this preliminary study was to evaluate the in vitro digestion dynamics and rates of the sugar fraction in molasses.

Materials and methods

The study was conducted at the University of Bologna and all procedures that included animals were approved by the University of Bologna Institutional Animal Care and Use Committee.

In vitro *fermentations*

For the in vitro fermentations, three beet and three cane molasses were randomly selected from a group of 32 samples collected world-wide. Chosen cane molasses came from Central America, Asia and Europe, while beet molasses from Europe, North America and Africa. Samples were analysed for their chemical composition as described in a previous paper (Palmonari et al. 2020), and the results are reported in Table 1. In particular, DM was determined according to AOAC 934.01 official method. Crude Protein determination was carried out following the AOAC 990.03 method, while starch and other carbohydrates, such as dextran, levan and araban, with polarimetric procedure (IS0 10520: 1997E). For sugar determination, samples were clarified using a commercial kit based on Carrez reagents (Sigma-Aldrich S.r.l, Milan, Italy). After this procedure, glucose, fructose, sucrose, galactose, raffinose, arabinose and xylose were extracted and quantified using an enzymatic method, according to manufacturer manual (Megazyme International Ltd., Bray, Ireland). Ash content was calculated as reported in AOAC 900.02 method for this specific feed. Then, they were recovered to quantify Ca, Mg, Na and K by ICP, while organic acids (lactic, acetic, butyric, propionic, citric, malic, formic, aconitic, glycolic and oxalic) and other components (sulphates, phosphates, chlorides and nitrates) were measured using ionic HPLC (Metrohm Italiana Srl, Origgio, Italy), according to the methods UNI EN ISO 10304-1 and 14911-2001.

This analytical approach was able to characterise almost all the components of the samples. The remaining not-quantified compounds should be listed as lipids and nitrogen-free extracts. Moreover, some numerical differences occur among the different samples: sucrose was more concentrated in beet compared to cane molasses, while glucose and fructose showed the opposite. Besides variation in poorly represented sugars, such as raffinose, other variability was observed in lactic acid and pyrocarbonic acid. The first was numerically higher in cane molasses, while the latter in beet. Even dietary anion-cation difference (DCAD) showed numerical difference among beet and cane molasses, as well as within group, ranging from +129 (beet 2) and 18 (beet 3). *In vitro* fermentations were conducted following the procedure described by Palmonari et al. (2017), and few changes were made. Briefly, two lactating Holstein cows were selected as donors based on similar BW, parity, DIM, milk production, and milk composition (SCC, fat and protein, lactose, and urea). Animals were milked twice a day. Donor cows were fed a hay-based diet, containing alfalfa hay (45% aNDFom), grass hay (52% aNDFom), and corn grain (62% starch). Rumen fluid was sampled via oesophageal probe, pouring off the first volume collected to avoid saliva or mucous contamination, and immediately placed in a thermostatic bottle. Sampling was conducted 0300 h after feeding. Rumen

Table 1. Molasses composition.

	Beet			Cane		
Measure (% DM)	1	2	3	1	2	3
Dry matter	76.7	79.4	78.4	76.6	78.5	76.7
Crude protein	12.8	14.6	12.5	7.7	8.8	6.0
Sugar fraction						
Sucrose	66.1	60.3	60.4	49.3	55.2	43.5
Glucose	0.02	0.05	0.15	5.97	1.99	5.74
Fructose	0.05	0.12	0.28	10.02	5.03	7.9
Raffinose	2.18	0.28	0.2	0.03	0.02	0.03
Galactose	0.03	0.03	0.03	0.04	0.04	0.04
Arabinose	–	0.02	0.03	0.02	–	0.01
Xilose	0.01	–	–	–	–	–
Starch	0.09	0.03	0.1	0.54	0.18	0.32
levans	0.5	0.67	0.41	0.81	0.83	1.21
destrans	0.07	0.09	0.06	0.63	1.42	0.31
arabans	0.03	0.05	0.05	0.15	0.25	0.19
Organic acids						
Aconitic Acid	–	–	–	0.37	1.25	3.78
Lactic Acid	3.34	3.67	6.91	3.34	6.43	12.8
Malic Acid	0.02	0.11	0.13	0.03	0.11	0.21
Citric Acid	0.39	0.38	0.15	0.08	0.13	0.19
Pyrocarbonic Acid	3.1	2.96	2.59	0.18	0.29	0.2
Oxalic Acid	0.04	0.04	0.03	0.05	0.05	0.04
Glicolic Acid	0.22	0.26	0.23	–	–	–
Acetic Acid	0.59	0.28	0.49	0.23	0.29	0.2
Ash	10.3	11.9	13.3	12.9	12.8	12.2
Ca	1.24	0.06	0.54	1.43	1.3	1.55
Mg	0.03	0.02	0.02	0.57	0.33	0.58
Na	0.32	1.06	0.36	0.01	0.02	0.03
K	2.39	4.93	1.07	0.5	1.37	2.17
Sulphates	0.17	0.4	0.38	1.69	2.89	1.93
Sulfur	0.06	0.13	0.13	0.56	0.96	0.64
Phosphates	0.58	1.23	1.33	2.72	2.55	2.45
Nitrates (mg/kg)	35	36	18	211	784	688
Chlorides (mg/kg)	4450	797	5610	14	39	0.5
DCAD[a] (meq/100g)	47	129	18	−17	−20	13

Values are expressed as % DM, unless differently specified.
[a]Dietary cation-anion difference, calculated as: DCAD, meq/100g = (K, % DM/0.039 + Na, % DM/0.023) − (Cl, % DM/0.0355 + S, % DM/0.016).

Table 2. Descriptive statistics of sugar digestibility in cane molasses at different time points of fermentation.

time point (h)	Sucrose[a]			Glucose			Fructose			Raffinose			Galactose			Arabinose		
	cane 1	cane 2	cane 3	cane 1	cane 2	cane 3	cane 1	cane 2	cane 3	cane 1	cane 2	cane 3	cane 1	cane 2	cane 3	cane 1	cane 2	cane 3
1	94.2 (2.18)	92.3 (3.01)	95.3 (3.54)	85.6 (2.89)	75.1 (2.77)	65 (3.02)	92.7 (3.31)	63.8 (2.75)	28.3 (3.80)	74.8 (2.63)	72 (2.21)	73.8 (2.59)	77.2 (4.02)	52.6 (3.65)	94.5 (1.56)	71.3 (3.91)	ND	55.7 (0.62)
2	95.1 (3.52)	94.1 (2.77)	96.4 (2.93)	89.3 (2.43)	75.5 (2.31)	71.1 (2.76)	95.4 (3.13)	82.7 (2.78)	34.6 (3.91)	78.6 (2.22)	66.9 (2.03)	96.6 (1.94)	82.8 (3.85)	61 (3.91)	96 (3.23)	67.5 (3.62)	ND	72.2 (2.18)
3	98 (3.04)	95 (3.12)	95.4 (2.85)	91.1 (1.12)	75.5 (2.55)	77.3 (2.38)	96.5 (2.85)	83.6 (2.65)	52.4 (2.67)	93.5 (2.96)	67.7 (1.93)	97.9 (2.79)	96.9 (3.42)	95 (2.51)	96.7 (2.49)	71 (2.39)	ND	57.2 (2.04)
4	99.5 (4.12)	98.1 (2.31)	95.4 (2.33)	92.9 (1.56)	79.2 (2.01)	81.2 (1.89)	96.5 (2.55)	90 (2.38)	77 (1.43)	93.4 (1.93)	81.7 (1.26)	96.9 (1.86)	98.3 (2.76)	94.8 (2.85)	99.1 (2.28)	100 (2.84)	ND	80.4 (2.58)
6	99.8 (3.78)	99.8 (3.44)	100 (3.07)	99.3 (0.99)	99.4 (1.02)	99 (1.28)	99.7 (2.24)	97.7 (2.12)	98 (1.17)	95 (1.74)	97.6 (0.95)	100 (1.75)	100 (1.87)	97.9 (2.63)	100 (2.12)	100 (1.68)	ND	66.6 (2.27)
8	100 (3.54)	100 (1.81)	100 (2.74)	100 (1.01)	100 (0.79)	100 (1.15)	100 (2.09)	100 (1.97)	100 (0.95)	97.7 (3.59)	100 (2.57)	100 (3.05)	100 (2.54)	99.5 (1.54)	100 (3.27)	100 (2.96)	ND	70.9 (2.02)
24	100 (1.03)	100 (1.27)	100 (1.18)	100 (0.72)	100 (1.11)	100 (0.97)	100 (1.69)	100 (1.57)	100 (0.92)	100 (1.26)	100 (0.52)	100 (2.45)	101 (1.58)	100 (3.42)	100 (2.82)	100 (2.27)	ND	83.6 (2.89)

Data are expresses as averaged %, with standard deviation in brackets.
[a]Individual sugars and their respective residue were analysed with enzymatic method, as described in the manuscript.

contents were filtered through 4 layers of cheese cloth under constant O_2-free CO_2. Once filtered, an equal amount of each liquor collected was sampled and equally mixed with the other. Selected time points to evaluate sugar disappearance were 1, 2, 3, 4, 6, 8, and 24 h. To ensure enough amount of residue at any given time point, the final volume in each fermentation flask was triplicate compared to the original procedure (150 mL), as well as the amount of sample (1.50 g). Because of its intrinsic characteristics, molasses was not weighted in each individual flask, but the proper amount was mixed into the buffer solution during the procedure. This adjustment was able to provide an equal amount of sample in each fermentation flask. Thus, 120 mL of buffer solution as described by Goering and Van Soest (1970) were added before 30 mL of rumen fluid were inoculated to each 250 mL Erlenmeyer flask, that had been placed in a heated (39.3 °C) water bath under CO_2 positive pressure to ensure anaerobiosis. Three replicates of each sample and two blanks were incubated per each time point. Blanks contained just buffer solutions (without molasses addition) and the rumen fluid. Once any given time point was reached, flask content was immediately poured into an aluminium vessel, and cold water (4 °C) was used to clean the flasks, in order to recover the residue. After that, labelled vessels were stored in refrigerator (−20 ± 2 °C). Once frozen, samples underwent freeze-drying, to facilitate recovery and sugar extraction.

This procedure was repeated, thus each one of the six samples, for each time point, was fermented in two different runs, to avoid any possible effect due to a single run. The two fermentations took place within 3 days, and selected donor cows were the same for both incubations.

Sugar extraction and analysis

Residue were weighted in 100 mL flasks and suspended in 40 mL of hot (50 °C) distilled water, then mixed for 30 minutes. After that, samples were stored again in a refrigerator (-20 °C) overnight. The following day, flasks were rinsed with 20 mL of hot water (50 °C) once thawed, prior absolute ethanol addition (30 mL). Samples were gently mixed for 30 minutes, then 2.5 mL of Carrez 1 and 2.5 mL of Carrez 2 solutions were added (Sigma-Aldrich S.r.l, Milan, Italy), followed by other 10 minutes mixing. Residue were finally filtered through Whatman filters #41 (GE Healthcare Life Sciences, Amersham, UK). Sugars are dissolved in the filtered solution, thus their recovery underwent

Table 3. Descriptive statistics of sugar digestibility in beet molasses at different time points of fermentation.

Time point (h)	Sucrose[a]			Glucose			Fructose			Raffinose			Galactose			Arabinose		
	beet 1	beet 2	beet 3	beet 1	beet 2	beet 3	beet 1	beet 2	beet 3	beet 1	beet 2	beet 3	beet 1	beet 2	beet 3	beet 1	beet 2	beet 3
1	92.1 (3.28)	88.2 (2.84)	99.7 (1.44)	52.4 (2.98)	85.3 (3.05)	68.8 (3.21)	38.9 (2.49)	82.4 (3.35)	62.6 (2.98)	95.9 (3.22)	98.2 (3.41)	99.3 (2.99)	76.4 (3.61)	87.3 (1.93)	96.9 (2.39)	ND	95.7 (1.54)	74.3 (4.06)
2	94.5 (2.93)	88.7 (2.49)	99 (2.59)	54.4 (1.65)	87.3 (2.93)	72.9 (3.94)	44.8 (1.94)	87.6 (2.82)	73.5 (1.54)	96.7 (2.50)	98.6 (2.92)	99 (2.58)	80.9 (2.39)	92.7 (2.94)	89.1 (3.44)	ND	97.5 (2.62)	100 (1.93)
3	97.8 (2.70)	94.1 (1.38)	98.4 (1.43)	74.5 (2.39)	97.9 (2.57)	73.3 (3.39)	67.1 (2.86)	97.1 (2.38)	79.8 (2.63)	97.8 (1.02)	99.7 (2.87)	99.7 (2.72)	100 (1.53)	95.3 (3.03)	88.1 (3.51)	ND	100 (3.46)	100 (2.49)
4	99.9 (2.54)	99.7 (2.27)	98.1 (2.37)	98.5 (2.83)	99.5 (1.89)	66.4 (2.54)	89.1 (2.29)	98.7 (1.64)	87.1 (2.84)	99.2 (3.29)	99.7 (2.74)	99.7(2.38)	99.6 (2.04)	94 (1.95)	89.5 (2.96)	ND	80 (2.65)	100 (2.27)
6	99.9 (1.15)	100 (3.77)	100 (2.59)	99.4 (2.51)	99.8 (2.78)	79.4 (2.51)	96.7 (2.36)	99.9 (1.45)	93 (2.43)	100 (2.72)	100 (2.95)	100 (2.91)	100 (2.68)	99.5 (3.11)	94 (2.68)	ND	100 (1.48)	100 (1.98)
8	100 (3.22)	100 (1.52)	100 (3.12)	99 (2.75)	100 (1.36)	100 (3.82)	100 (3.27)	100 (2.98)	100 (2.62)	100(2.33)	100 (3.26)	100 (1.35)	100 (2.48)	99.2 (1.53)	98 (2.59)	ND	91 (2.60)	100 (2.61)
24	100 (2.61)	100 (3.98)	100 (2.75)	100 (3.28)	100 (3.84)	100 (2.39)	100 (2.83)	100 (3.18)	100 (2.57)	100 (2.56)	100 (1.52)	100 (4.00)	100(2.69)	100 (2.73)	100 (1.63)	ND	100 (2.95)	100 (3.02)

[a]Individual sugars and their respective residue were analysed with enzymatic method, as described in the manuscript.
Data are expresses as averaged %, with standard deviation in brackets.

purification in Rotavapor, under vacuum and hot water (75 °C) bath, to extract water and ethanol. The final concentrated sugar residue was then analysed with enzymatic kit (Megazyme Ltd, Wicklow, Ireland), following manufacturer's guidelines. These procedures were applied for the quantification of sucrose, fructose, glucose, raffinose, galactose and arabinose.

Statistical analysis, digestibility and rates calculation

Digestibility of each single sugar was calculated applying the following equation:

$$\text{IVSuD, \% sugar} = \left[1 - (\text{Sug}_R - \text{Sug}_B)/\text{Sug}_I\right] \times 100, \quad (1)$$

where Sug_R is the residual amount of single sugar, Sug_B is the blank correction, while Sug_I represents the initial amount of the given sugar.

The rate of digestion (kd, % h^{-1}) was calculated from the residual fractions at different time points using a first-order model (Mertens 2005):

$$\ln\ (Dt) = \ln\ (Di) - kd_t$$

where Dt is the digestibility at a given time point 't', Di is the potentially digestible residue and kd_t the fractional rate constant of digestion.

For the sugar digestibility data, the first comparison was made to evaluate the variance among the two different incubations. The ANOVA procedure of the software JMP (version 14.0 pro, Statistical Analysis Systems Institute Inc., Cary, NC) was adopted, using the following model:

$$Yij = \beta_1 x_1 + \beta_2 x_2 + \varepsilon_{ij}$$

where Yij is the digestibility value, x_1 the group fixed effect (cane and beet), x_2 represents the time points, and ε_{ij} the random effect (fermentation). Once run, no differences were observed among the two fermentations, thus the model was applied again to evaluate any possible difference among samples. Again, Yij is the digestibility value, x_1 the group fixed effect (cane and beet), x_2 represents the time points, while ε_{ij} stands for the random effect of sample ($n = 3$ for cane, $n = 3$ for beet molasses). Means were then compared using the Tukey post hoc test, and the significance was set at $p < .01$.

Results and discussion

The adopted procedure was able to extract residual sugars, even at longer fermentation time points.

Table 4. Comparison of averaged values of sugar digestibility in cane and beet molasses at different time points of fermentation.

Time point (h)	Sucrose[A]			Glucose			Fructose			Raffinose			Galactose			Arabinose		
	cane	beet	SEM	cane	beet	SEM	cane	beet	SEM	cane	beet	SEM	cane	beet	SEM	cane	beet	SEM
1	93.9	93.3	5.67	75.2	68.8	8.8	61.6	61.3	9.46	73.5^{b}	97.8^{a}	10.02	74.8^{b}	86.8^{a}	10.7	70.4	85.0	11.1
2	95.2	94.1	4.91	78.6	71.5	7.4	70.9	68.6	9.31	80.7^{b}	98.1^{a}	9.43	79.9^{b}	87.5^{a}	9.8	79.7	98.8	10.1
3	96.1	96.8	4.84	81.3	81.9	6.52	77.5	81.3	9.9	86.4^{b}	99.1^{a}	7.71	96.2	94.5	8.0	75.5	100	10.6
4	97.7	99.2	5.11	84.5	88.1	7.03	87.8	91.6	8.08	90.7	99.5	7.34	97.4	94.4	7.4	93.5	89.8	10.6
6	99.9	100	3.77	99.2	92.9	6.38	98.5	96.5	7.44	97.5	100	6.02	99.3	98.0	6.6	88.9	100	10.7
8	100	100	3.93	99.8	99.7	4.22	100	99.9	6.32	99.2	100	5.55	99.8	99.2	5.6	90.3	95.3	10.3
24	100	100	3.45	100	99.9	3.51	100	100	4.07	100	100	4.76	100	100	5.5	94.5	100	10.4

Data are expresses as %.
[A]Individual sugars and their respective residue were analysed with enzymatic method, as described in the manuscript. Least square means with different superscript letters within a row are significantly different ($p < .01$).

Results of sugar disappearance in cane and beet molasses are reported in Tables 2 and 3, respectively.

For both groups, sucrose was the most digestible and the fastest in this process. After one hour of in vitro rumen fermentation, sucrose digestibility was higher than 90% (93.9 and 93.3% on average, for cane and beet molasses, respectively). A complete disappearance of this sugar appeared to happen within 4 hours of incubation (<99.0% on average). This intensive fermentation of sucrose was described also in previous studies (Kellogg and Owen 1969; Hall and Weimer 2007; Broderick et al. 2008), but without quantification of its dynamic. Sucrose is a di-saccharide, composed by glucose and fructose, which act as the major energy sources for rumen microbes (Russell 2002). The consequent rapid digestion of this sugar was related by other authors to an increased DMI (Broderick et al. 2000), and an improved rumen microbial protein synthesis (Khalili and Huhtanen 1991). In beet molasses, a numerically higher variability was observed among the analysed samples. One–hour digestibility ranged from 88.2 to 99.7%, while tent to decrease after 3 h of fermentation.

Glucose was completely fermented in 6 hours (99.0% on average) in cane molasses. Moreover, a numerically higher variability in glucose degradation compared to sucrose was found among cane molasses. This fact could depend on the molasses composition, and less likely on the proportion of this sugar in the molasses. Its content was indeed similar among samples, even if digestibility values differed for every time point up to 6 hours. A similar trend was observed for fructose, with numerically high variability across cane molasses, and a complete digestion after 6 hours of fermentation (98.5% on average). Unlike in cane, beet molasses have almost no glucose and fructose content. However, digestibility values were close to those obtained in cane molasses. Glucose and fructose reached a complete fermentation after 6 hours of incubation (99.2 and 92.9%, respectively). In general, the slower digestion observed for these two sugars, compared to sucrose, could be due to the breakdown of more complex carbohydrates, such as sucrose, lactose (for glucose), or others. Being the residual amount quantified, this process could lead to an underestimation of the digestion dynamics.

Raffinose was completely digested within 1 hour in beet molasses (97.8% on average), while it was slower ($p < .01$) in cane, for which a complete disappearance was obtained in 6 hours (97.5% on average). Galactose, a C-4 epimer of glucose, was rapidly digested in vitro in both groups, but digestibility values were statistically different ($p < .01$) at 1 and 2 hours of fermentation (74.8 vs. 86.8%, and 79.9 vs. 87.5% at 1 and 2 hours, for cane and beet respectively). Its complete disappearance was observed at 3 hours of fermentation in both groups (96.2 and 94.5% for cane and beet molasses, respectively).

Arabinose digestion was somehow different compared to the other sugars. An almost complete disappearance was reached in cane molasses only at the longest time point (24 hours), with a value of 94.5% on average. Moreover, digestion dynamic appeared to be linear until 4 hours, after which fermentation seemed to be on a negative trend. Even in beet molasses, the digestion of arabinose was not linear, with values of later time points (4 and 8 h) lower than the previous one. Arabinose is a pentose, and could be found in highly digestible fibrous compounds, such as feed rich in pectin or hemicellulose. In general, it must be noted that this pentose is slowly digestible compared to other 6 carbon monosaccharides, such as glucose or galactose. Moreover, this sugar was undetectable in one of the three samples of both cane and beet molasses, underlying numerical differences across samples once again. Together with arabinose, also galactose and raffinose were not particularly represented in any analysed sample, thus the impact of such sugars could be considered as irrelevant from a nutritional stand point. Another interesting point is related to some

Table 5. Descriptive statistics of digestion rates of individual sugars.

	Rate of digestion, % h^{-1}						
molasses	Sucrose	Glucose	Fructose	Raffinose	Galactose	Arabinose	Average
Cane							
average	54.49	51.34	50.48	54.11	52.06	49.92	52.07
std.dev.	2.85	2.92	3.31	3.06	3.77	3.12	–
c.v.	0.05	0.06	0.07	0.06	0.07	0.06	–
Beet							
average	57.88	53.01	53.77	56.86	54.83	51.75	54.68
std.dev.	3.14	3.43	2.97	3.61	3.55	3.72	–
c.v.	0.05	0.06	0.06	0.06	0.06	0.07	–

Rates were calculated from the residual fractions at different time points using a first-order model, as described in the manuscript.
c.v.: coefficient of variation.

digestibility values which are numerically higher in previous time points compared to later ones. As reported in Tables 2 and 3, this occurred in both cane and beet molasses for different sugars. This fact could be related to the breakdown of other compounds, which could release small amount of simple sugars in the incubation medium. Starch, levans, destrans and arabans are complex carbohydrates, thus their fermentation could act as described. However, only in cane 3 destrans were quantified at a concentration >1.0%, thus their impact on the overall sugar disappearance was not significant.

In conclusion, the comparison of digestibility average values of cane and beet molasses, showed no significant differences (Table 4) for the most representative sugars, despite numerical differences, while few were observed in raffinose and galactose. This result could be related to the variability across samples obtained in both groups. Although the minor relevance of these two sugars, related to their very low concentration, such overall differences could be considered when choosing a specific molasses to formulate the ratio. Results of averaged rates calculation are reported in Table 5.

Obtained digestion rate values were similar among cane and beet molasses, and higher than 50% h^{-1} for most of the evaluated sugars. In particular, sucrose showed a rate of 54.5 and 57.9% h^{-1} for cane and beet molasses, respectively. Glucose and fructose resulted in similar rates, being 51.3 and 50.5% h^{-1} in cane molasses, 53.0 and 53.8% h^{-1} in beet. Raffinose showed rates of 54.1 and 58.9% h^{-1}, while galactose 52.1 and 54.8% h^{-1} in cane and beet molasses, respectively.

However, it must be considered that the small amount of those sugars, could have led to analytical error for such calculations. Moreover, due to the same reason, these sugars do not appear to be particularly relevant from a nutritional stand point. Arabinose had a slower rate compared to the others (49.9 and 51.8% h^{-1} in cane and beet molasses, respectively). This result is not surprising, since arabinose has a different chemical composition. Nonetheless, observed values are particularly different from those considered in the CNCPS v6.55 based models. Rationing software uses an estimate rate of digestion of ~20% h^{-1}, without any differentiation across different sugars. Considering the values obtained in this preliminary study, a rate of ~55% h^{-1} on average for the major sugars (sucrose, glucose and fructose), seems more appropriate. By applying a proper rate for these carbohydrates, it would be possible to obtain a better estimation of the net energy of sugar sources, as well as their impact on the microbial mass and the rumen functions.

Conclusions

In conclusion, results obtained in this initial study showed that despite different sugars are rapidly fermented by rumen microbes, differences in their composition still occur. Thus, molasses should be viewed as a specific component of a ration, and not an 'unchangeable' feed, taking into account that their most representative fraction, sugars, may vary in composition and digestibility. Moreover, modern rationing models should consider a modification of sugar rates of digestion, since the actual rate (~20% h^{-1}) is too slow than those observed in this study.

Ethical approval

The study was conducted at the University of Bologna and all procedures that included animals were approved by the University of Bologna Institutional Animal Care and Use Committee.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Authors would like to acknowledge EDF&Man company for supporting the study.

ORCID

Alberto Palmonari http://orcid.org/0000-0003-3735-8826
Andrea Formigoni http://orcid.org/0000-0002-8109-2482

References

Brito AF, Petit HV, Pereira ABD, Soder KJ, Ross S. 2015. Interactions of corn meal or molasses with a soybean-sunflower meal mix or flaxseed meal on production, milk fatty acid composition, and nutrient utilization in dairy cows fed grass hay-based diets. J Dairy Sci. 98(1):443–457.

Brito AF, Soder KJ, Chouinard PY, Reis SF, Ross S, Rubano MD, Casler MD. 2017. Production performance and milk fatty acid profile in grazing dairy cows offered ground corn or liquid molasses as the sole supplemental nonstructural carbohydrate source. J Dairy Sci. 100(10): 8146–8160.

Broderick GA, Luchini ND, Reynal SM, Varga GA, Ishler VA. 2008. Effect on production of replacing dietary starch with sucrose in lactating dairy cows. J Dairy Sci. 91(12): 4801–4810.

Broderick GA, Luchini ND, Smith WJ, Reynal S, Varga GA, Ishler VA. 2000. Effect of replacing dietary starch with sucrose on milk production in lactating dairy cows. J. Dairy Sci. 83(Suppl. 1):248.

Broderick GA, Radloff WJ. 2004. Effect of molasses supplementation on the production of lactating dairy cows fed diets based on alfalfa and corn silage. J Dairy Sci. 87(9): 2997–3009.

Chibisa GE, Gorka P, Penner GB, Berthiaume R, Mutsvangwa T. 2015. Effects of partial replacement of dietary starch from barley or corn with lactose on ruminal function, short-chain fatty acid absorption, nitrogen utilization, and production performance of dairy cows. J Dairy Sci. 98(4): 2627–2640.

De Ondarza MB, Emanuele SM, Sniffen CJ. 2017. Effect of increased dietary sugar on dairy cow performance as influenced by diet nutrient components and level of milk production. Professional Animal Sci. 33(6):700–707.

DeFrain JM, Hippen AR, Kalscheur KF, Schingoethe DJ. 2004. Feeding lactose increases ruminal butyrate and plasma beta-hydroxybutyrate in lactating dairy cows. J Dairy Sci. 87(8):2486–2494.

DeFrain JM, Hippen R, Kalscheur KF, Schingoethe DJ. 2006. Feeding lactose toincrease ruminal butyrate and the metabolic status of transition dairy cows. J. Dairy Sci. 89(1):267–276.

DeVries TJ, Gill RM. 2012. Adding liquid feed to a total mixed ration reduces feed sorting behavior and improves productivity of lactating dairy cows. J Dairy Sci. 95(5): 2648–2655.

Goering HK, Van Soest PJ. 1970. Forage fiber analysis (Apparatus, Reagents, Procedures, and Some Applications). Agricultural Handbook No. 379. Washington (DC): USDA Agricultural Research Service.

Hall MB, Weimer PJ. 2007. Sucrose concentration alters fermentation kinetics, products, and carbon fates during in vitro fermentation with mixed ruminal microbes. J Animal Sci. 85(6):1467–1478..

Kellogg DW, Owen FG. 1969. Alterations of in vitro rumen fermentation patterns with various levels of sucrose and cellulose. J Dairy Sci. 52(9):1458–1460.

Khalili H, Huhtanen P. 1991. Sucrose supplements in cattle given grass silage-based diet. 2. Digestion of cell wall carbohydrates. Anim Feed Sci Technol. 33(3-4):263–273.

Mertens DR. 2005. Rate and extent of digestion. In: J. Dijkstra, J. M. Forbes, and J. France, editors. Quantitative aspects of ruminant digestion and metabolism. 2nd ed. Cambridge (MA): CABI Publishing. p. 13–47.

Murphy MR, Geijsel AWP, Hall EC, Shanks RD. 1997. Dietary variety via sweetening and voluntary feed intake by lactating dairy cows. J Dairy Sci. 80(5):894–897.

Oba M, Mewis JL, Zhining Z. 2015. Effects of ruminal doses of sucrose, lactose, and corn starch on ruminal fermentation and expression of genes in ruminal epithelial cells. J Dairy Sci. 98(1):586–594.

Oelker ER, Reveneau C, Firkins JL. 2009. Interaction of molasses and monensin in alfalfa hay- or corn silage-based diets on rumen fermentation, total tract digestibility, and milk production by Holstein cows. J Dairy Sci. 92(1):270–285.

Palmonari A, Canestrari G, Bonfante E, Fustini M, Mammi L, Formigoni A. 2017. Technical note: In vitro digestibility of amylase-treated, ash-corrected neutral detergent fiber, with addition of sodium sulfite, at 240 hours with or without rumen fluid reinoculation. J Dairy Sci. 100(2): 1200–1202..

Palmonari A, Cavallini D, Sniffen CJ, Fernandes L, Holder P, Fagioli L, Fusaro I, Biagi G, Formigoni A, Mammi L. 2020. Short communication: characterization of MOLASSES chemical composition. J Dairy Sci. 103(7):6244–6249.

Petruzzi H, Danfaer A, Nørgaard P. 2002. A dynamic simulation model of nutrient digestion in the rumen of dairy cows. J Anim Feed Sci. 11(3):367–397.

Russell JB. 2002. Rumen microbiology and its role in ruminant nutrition. Ithaca (NY): JB Russell Publishing Co.

Van Amburgh ME, Collao-Saenz EA, Higgs RJ, Ross DA, Recktenwald EB, Raffrenato E, Chase LE, Overton TR, Mills JK, Foskolos A. 2015. The Cornell net carbohydrate and protein system: updates to the model and evaluation of version 6.5. J Dairy Sci. 98(9):6361–6380..

Weisbjerg MR, Hvelplund T, Bibby BM. 1998. Hydrolysis and fermentation rate of glucose, sucrose and lactose in the rumen. Acta Agricultura Scandinavica, Section A, Animal Science. 48(1):12–18.