Short communication: Characterization of molasses chemical composition

(Article begins on next page)

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1 *Short communication:* **Characterization of Molasses chemical composition**

2 **Palmonari, A. et al.**

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4 **INTERPRETATIVE SUMMARY**

5 Molasses are widely used in ruminant nutrition. Despite their utilization in dairy cows' rations, their

6 characterization is not complete, and in literature they are partially described, reporting few

7 parameters (i.e., dry matter, total sugars, protein, and ash). Our aim was to properly characterize

8 cane and beet molasses, and to evaluate variability among different molasses. The results showed

9 that more detailed analyses of individual molasses sources could improve their use in ration

10 formulation.

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12 RUNNING HEAD: Short communication: Chemical composition of cane and beet molasses

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15 A. Palmonari[1]*, D. Cavallini[1], C.J. Sniffen[2], L. Fernandes[3], P. Holder[3], L. Fagioli[4], I. Fusaro[5], G.

16 Biagi[1], A. Formigoni[1], and L. Mammi[1].

17 [1] DIMEVET, Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, 40064 Ozzano

18 Emilia, Italy.

19 [2] Fencrest L.L.C., 03245 Holderness (NH), USA.

20 [3] ED&F Man Liquid Products, 3 London Bridge Street, LondonUK.

21 [4] Studio Associato Campi – Fagioli, 44123 Ferrara, Italy.

22 [5] Facoltà di Medicina Veterinaria, Università di Teramo, 64100 Teramo.

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24   *Corresponding author:

25   Alberto Palmonari, e-mail address: alberto.palmonari2@unibo.it

26   Present address: DIMEVET, Dipartimento di Scienze Mediche Veterinarie, Università di Bologna,

27   40064 Ozzano Emilia, Italy.

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ABSTRACT

30   Beet and cane molasses are produced worldwide, as a by–product of sugar extraction, and widely

31   used in animal nutrition. Due to their composition, they are fed to ruminants as an energy source.

32   However, molasses have not been properly characterized in the literature. Their description has often

33   been limited to the type (sugarcane or beet), or the sole amount of dry matter (DM), total or water

34   soluble sugars, crude protein (CP), and ash. Our objective was to better characterize cane and beet

35   molasses composition, examine possible differences, and obtain a proper definition of such feeds. For

36   this purpose, 16 cane and 16 beet molasses were sourced worldwide and analyzed for chemical

37   composition. The chemical analysis used in this trial was able to characterize 97.4% and 98.3% of

38   the compounds in the DM of cane and beet molasses, respectively. Cane molasses contained less DM

39   amount compared to beet molasses (76.8±1.02 vs 78.3±1.61%), as well as CP content (6.7±1.8 vs

40   13.5±1.4% of DM), with a minimum value of 2.2% of DM in cane to a maximum of 15.6% of DM

41   in beet molasses. The amount of sucrose differed among beet and cane molasses (60.9±4.4 vs

42   48.8±6.4% of DM), but with high variability even within cane molasses (67.3 max to 39.2 min, % of

43   DM) and beet. Glucose and fructose were detected in cane molasses (5.3±2.7 and 8.1±2.8% of DM,

44   respectively), showing high variability. Organic acid composition differed as well. Lactic acid was

45   more concentrated in cane compared to beet (6.1±2.8 vs 4.5±1.8% of DM), varying from 12.8%

46   maximum to 1.6% of DM minimum within cane molasses. Dietary cation-anion difference showed

47   numerical differences among cane and beet molasses (7±53 vs 66±45 meq/100g of DM, on average).

48   Within the cane group, it varied from +155 to -76 meq/100g of DM, while in beet from +162 to +0

49   meq/100g of DM. Data obtained in this study detailed source differences in molasses composition,

50   and suggested that a more complete characterization of them could improve their use in ration

51   formulation.

52   Key words: molasses, chemical composition, variability

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54   **SHORT COMMUNICATION**

55   Beet and cane molasses are produced worldwide, as a by–product of sugar extraction, and

56   they are widely used in animal nutrition. Due to their composition, they are fed to ruminants as an

57   energy source, and the interest in their utilization is still current (Martel et al., 2011; Siverson et al.,

58   2014). Previous studies showed positive effects of molasses addition on milk fat, FCM, ruminal

59   ammonia, MUN, and fiber digestibility (Broderick and Radloff, 2004; Brito et al., 2015; de Ondarza

60   et al., 2017). Moreover, they could be associated to nonnutritive and dietetic benefits: animals prefer

61   sweetened diets (Murphy et al., 1997), thus molasses generally stimulate DMI. Related to this, field

62   observations suggest that molasses, or molasses-based liquid feeds could impact animal sorting

63   behavior, with a positive impact on the consumption of longer particles in total mixed rations

64   (DeVries and Gill, 2012). A frequently used alternative is to add water whenever the diet is considered

65   too dry (i.e., where hay instead of silages represents the main forage source). During the warmer

66   months, however, water addition could lead to spoilage phenomena, decreasing the palatability of the

67   diet, and causing health problems to the animal. In such conditions, molasses would act positively,

68   since not associated with spoilage or molds. From a composition stand point, sugars represent the

69   main component of molasses. Sugars are rapidly fermented in the rumen, but the end products differ

70   from those obtained by starch fermentation (Penner and Oba, 2009). Previous studies indicate that

71   replacing starch with molasses or molasses-based liquid feeds would result in positive effects on

rumen pH (Broderick and Radloff, 2004; Oelker et al., 2009; Brito et al., 2017). However, molasses are in general not properly characterized in literature, and their description is related to the type (sugarcane or beet), or the sole amount of DM, total or water soluble sugars, CP and ash (Broderick and Radloff, 2004; Brito et al., 2015). Other authors made a better description of molasses, but the final results still lack in several parameters, such as organic acids or DCAD (Olbrich, 2006; Bortolussi and O'Neill, 2006). Consequently, by adding every single component cited in the characterization, a representative part of the DM of molasses remains unknown, since sugars, CP and ash are barely sufficient to reach 80% DM on average.

Objective of this study was to better characterize cane and beet molasses composition, underline possible differences, and obtain a proper definition of such feeds.

For this purpose, 16 cane and 16 beet molasses were sourced worldwide and analyzed for chemical composition. In particular, 7 cane molasses were sampled in Central / North America, 5 from Asia, 2 from Africa, and 1 in both Europe and Australia. Beet molasses were sampled in Europe (12), North America (2), and Africa (2). Dry matter was determined according to AOAC 934.01 official method (AOAC International, 1990), except for dried quartz sand which was added to each vessel. Ash content was calculated as reported in AOAC 900.02 method for this specific feed (AOAC International, 1990). Crude Protein determination was carried out following the AOAC 990.03 method (AOAC International, 1990), 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 was 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.

Statistical analysis was performed using the software JMP (version 12.0 pro, Statistical Analysis Systems Institute Inc., Cary, NC). Then, a principal component analysis was carried out using the FACTOR procedure of SAS (version 9.13, SAS Institute Inc., Cary, NC), as described by Gallo et al (2015). The analysis was conducted to evaluate variability among and across cane or beet molasses.

Overall, determinations of the different components were able to characterize, on average, 97.4% and 98.3% of the DM in cane and beet molasses, respectively. Analytical results are reported in Tables 1 and 2. Within the cane molasses group, DM ranged from 79.56% to 75.72%, with an average of 76.8%. An average 78.3% DM was observed in beet molasses, with a maximum of 78.9% and a minimum of 74.1%. Ash was numerically higher in cane (13.1% of DM) than beet (11.7% of DM) molasses, with a maximum value of 18.5% of DM and a minimum value of 6.5% of DM in beet molasses. The CP concentration differed among and within group, being $6.7 \pm 1.8\%$ and $13.5 \pm 1.4\%$ of DM in cane and beet molasses, respectively, and ranged from a minimum value of 2.2% of DM in cane to a maximum of 15.6% of DM in beet. This difference could be related to specific molecules occurring in sugar beet, such as betaine. Betaine is a nitrogen compound, widely used in the cosmetic, health and pharmaceutical industry as well as animal nutrition (Fernandez-Figares et al., 2002; Escudero and Ruiz, 2011), being able to promote growth and modulate lipid accumulation.

Sugar profile differed among samples. Sucrose resulted as the most represented in both cane and beet molasses, although its concentration varied even within group. In cane molasses, an average of 48.8% of DM was observed, ranging from 67.3% to 39.2%. Beet molasses showed a numerically higher sucrose concentration, 60.9% of DM on average, with 66.1% max and 46.5% min. Glucose and fructose resulted in an average concentration of 8.1% for and 5.3% of DM, respectively, in cane molasses, while barely detectable in beet molasses (0.3% of DM on average, for both). The ranges

122 for cane molasses were wide, with maximum values of 14.3% and 12.1% DM and minimum values

123 of 2.3% and 1.3% of DM for fructose and glucose, respectively. Other analyzed sugars (galactose,

124 raffinose, arabinose and xylose) were almost undetectable, and even the sum of maximum values

125 resulted lower than 1% of DM in cane molasses. The only exception was raffinose in beet molasses,

126 which resulted 0.6% of DM on average, but with a maximum value of 2.2% of DM. This finding is

127 in line to what observed by Olbrich (2006), who identified sucrose and raffinose as the two major

128 sugars in German beet molasses. Reasons for these differences could be related to the extraction

129 process applied, as well as the origin of the molasses. Sucrose is a disaccharide, composed by glucose

130 and fructose. Uptakes of these two sugars are usually associated, and both represent a major substrate

131 for microbial fermentations. However, glucose and fructose could undergo different fermentation

132 pathways (Luick et al., 1957). Thus, considering the variability observed within group (cane or beet),

133 these data suggest that a more accurate analysis is required to properly characterize the molasses.

134   Differences were observed also in organic acids. Lactic acid was more concentrated in cane

135 compared to beet (6.1% and 4.5% of DM), varying from 12.8% max. to 1.6% min. of DM among

136 cane molasses. Aconitic acid was found only in cane molasses (1.4% of DM on average), while

137 glycolic acid in beet (0.25% of DM on average). Other analyzed acids (acetic, butyric, propionic,

138 citric, malic, formic, glycolic and oxalic) were poorly represented in both cane and beet molasses.

139 The total sum of acids ranged from 2.4% to 18.7% of DM in cane, while it was 4.1% as minimum

140 and 11.9% maximum of DM in beet molasses. Organic acids are not so frequently quantified when

141 molasses are added to a diet. However, considering their variability, it should be recommended to

142 determine such fraction, since organic acids could impact rumen metabolism, leading to different

143 consequences in terms of animal health and performances, as underlined by other authors in respect

144 to silages (Kung et al., 2018).

145   Starch, dextran, levan and araban were 2.2% of DM on average in cane molasses, while their

146 content was <1% of DM in beet molasses. Due to the low concentration, also the variability range

was narrow. Sulfates, phosphates, and chlorides had a higher concentration in cane molasses, which showed a numerically lower DCAD compared to beet (7±53 vs 66±45 meq/100g of DM). Within the cane group, DCAD varied from +155 to -76 meq/100g of DM, while in beet from +162 to 0 meq/100g of DM. The observed DCAD variability across samples underlines the importance of this determination when molasses are added to the diet. Even with a similar amount of total sugars, different molasses could have a completely different anion – cation ratio, with possible effects on animal health and performance. For example, given a ration for close-up cows (270dd pregnancy) formulated with corn silage, grass hay, corn meal, soybean meal and min. vit. supplement, such ration would result in a DCAD = ~39 meq/100g. Substituting corn meal with the molasses at opposite values (+155 and -76 meq/100g), final DCAD would result as +38 and +48 meq/100g. As reported in literature, a proper balance is required to avoid the occurrence of health disease in different stage of lactation (Block, 1984; Goff and Horst, 1997; Hu and Murphy, 2004) or in animals under stressful environmental conditions (West et al., 1991 and 1992; Sanchez et al., 1994).

Samples distribution resulted from the principal component analysis, is reported in Figure 1. Range of variability is wide among samples, and even within the same group, especially in cane molasses. In conclusion the obtained results demonstrate that the differences in composition could occur among molasses.

Defining a molasses as "cane" or "beet" is important, but not sufficient to properly evaluate their potential nutritional role. As reported in several studies in which molasses are added to the diets, determination of ash, CP, total sugars and few other components represents a partial identification, and does not seem appropriate to characterize such feeds. Molasses are a good source of fermentable sugars, but other components are present as well, with potential impacts on animal health status or production performances. Moreover, from a scientific stand point, utilization of molasses which can be similar in terms of amount of total sugars or protein, but different in organic acids or in minerals could lead to different results across studies, as observed by other authors (Firkins, 2008; Baurhoo

172 and Mustafa, 2014; Ghedini et al., 2018). Thus, this study underlines that a more accurate description

173 and characterization of molasses is possible, and strictly required, especially if its use in animal feed

174 has to be fully optimized.

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290 Table 1. Descriptive statistic of the chemical composition of cane molasses. Values are expressed

291 as % D.M.

Measure, % D.M.	Cane Molasses			
	Avg.	Std. Dev.	Min. value	Max. value
DM	76.8	1.0	75.7	79.6
CP	6.65	1.79	2.22	9.31
Total Sugars	62.3	4.7	57.0	71.0
Sucrose	48.8	6.4	39.2	67.3
Glucose	5.29	2.69	1.30	12.07
Fructose	8.07	2.83	2.30	14.28
Raffinose	0.03	0.00	0.02	0.03
Galactose	0.04	0.00	0.04	0.04
Arabinose	0.01	0.02	0.00	0.04
Xylose	ND	ND	ND	ND
Starch	0.33	0.25	0.06	1.07
Levans	0.86	0.26	0.26	1.21
Dextrans	0.79	0.42	0.27	1.63
Arabans	0.20	0.05	0.06	0.28
Aconitic Acid	1.42	0.85	0.24	3.78
Lactic Acid	6.10	2.82	1.62	12.75
Malic Acid	0.10	0.05	0.03	0.21
Citric Acid	0.13	0.04	0.08	0.22
Pyrocarbonic Acid	0.34	0.13	0.18	0.62
Oxalic Acid	0.06	0.02	0.04	0.09
Glycolic Acid	0.00	0.00	0.00	0.00
Acetic Acid	0.44	0.28	0.16	1.04
Ash	13.1	1.5	10.2	16.3
Ca	1.39	0.55	0.82	3.13
Mg	0.43	0.14	0.19	0.63
Na	0.08	0.10	0.01	0.42
K	1.82	1.91	0.31	7.99
Sulphates	2.09	0.88	0.81	4.09
Sulfur[2]	0.69	0.29	0.27	1.36
Phosphates	2.03	0.77	0.70	2.97
Nitrates, mg/kg	464	337	17	999
Chlorides, mg/kg	60	86	1	340
DCAD[1], meq/100g	7	53	-76	155

292 [1] = Dietary cation-anion difference, calculated as: DCAD, meq/100g = (K, % DM / 0.039 + Na, % DM / 0.023) – (Cl,
293 % DM / 0.0355 + S, % DM / 0.016).
294 [2] = Sulfur obtained from sulphates considering their respective molecular weights.
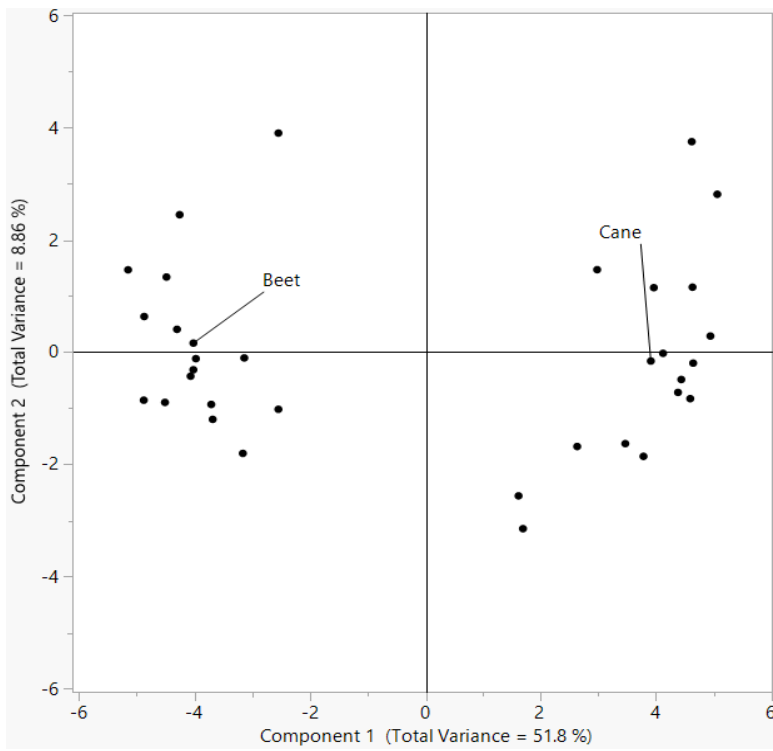
295 Table 2. Descriptive statistic of the chemical composition of beet molasses. Values are expressed

296 as % D.M.

Measure, % D.M.	Beet Molasses			
	Avg.	Std. Dev.	Min. value	Max. value
DM	77.6	3.2	67.0	80.9
CP	13.5	1.4	10.7	15.6
Total Sugars	62.1	3.9	50.6	68.4
Sucrose	60.9	4.4	46.5	66.1
Glucose	0.28	0.48	0.02	1.96
Fructose	0.29	0.30	0.01	0.87
Raffinose	0.60	0.56	0.12	2.18
Galactose	0.03	0.00	0.02	0.03
Arabinose	0.01	0.01	0.00	0.05
Xylose	0.01	0.00	0.00	0.01
Starch	0.08	0.04	0.02	0.17
Levans	0.47	0.16	0.15	0.71
Dextrans	0.09	0.04	0.02	0.19
Arabans	0.06	0.02	0.03	0.10
Aconitic Acid	ND	ND	ND	ND
Lactic Acid	4.51	1.83	1.77	7.13
Malic Acid	0.08	0.04	0.02	0.13
Citric Ac.	0.30	0.12	0.11	0.50
Pyrocarbonic Acid	2.77	0.52	1.74	3.76
Oxalic Acid	0.03	0.01	0.02	0.05
Glycolic Acid	0.25	0.04	0.18	0.32
Acetic Acid	0.42	0.12	0.20	0.60
Ash	11.7	2.5	6.5	18.5
Ca	0.30	0.35	0.02	1.24
Mg	0.02	0.02	0.00	0.09
Na	0.62	0.43	0.05	1.45
K	2.44	1.33	0.65	5.54
Sulphates	0.61	0.41	0.17	1.84
Sulfur[2]	0.20	0.14	0.06	0.61
Phosphates	0.76	0.38	0.31	1.65
Nitrates, mg/kg	55	29	16	116
Chlorides, mg/kg	3974	2236	411	8056
DCAD[1], meq/100g	66	45	0	162

297 [1] = Dietary cation-anion difference, calculated as: DCAD, meq/100g = (K, % DM / 0.039 + Na, % DM / 0.023) – (Cl,
298 % DM / 0.0355 + S, % DM / 0.016).
299 [2] = Sulfur obtained from sulphates considering their respective molecular weights.

Figure 1. Samples distribution. PCA results for molasses variability. Distance between dots is

inversely proportional to similarity among samples.