Supplementary File

How total mixed ration particle size influences milk yield and quality, ingestive behavior, and methane emissions in high Holstein and lower yielding Girolando cows

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Material and methods

Maize silage harvesting and chopping

The maize silage was produced from a commercial maize hybrid [RB9308−flint type (RIBER KWS®, Patos de Minas, Brazil)] harvested approximately with 313 ± 5.0 g/kg of dry matter (DM), using a two-row forage chopped (Casale®, Totalmix 40, São Carlos, SP, Brazil) using a theoretical cut length of 30 mm. This adjustment resulted in 86.4% of the particles longer than 8 mm (Table S1). The large particle size (LPS) treatment was obtained using a no-adapted mixer with a capacity of 400 L and a speed of 25 rpm to mix the diet ingredients (Concrete mixer Motomil, model MB-400P, Navegantes, SC, Brazil) to ensure that particle sizes were not changed. SPS treatment was obtained using a mixer wagon (Casale®, São Carlos, SP, Brazil) equipped with three endless threads composed of circular knives of 10 cm in diameter, which cut the components of the diet. To ensure the uniformity of the total mixed ration (TMR) during the experimental period, a mixed time of 10 min with a rotation of 500 rpm was used in all treatment diets. The study consisted of two periods of 26 days each; the first 14 days of each experimental period were used to adapt the cows to the diets, and the last 12 days were used for data collection at cow level.

Particle size measurements

The evaluation of the diets particle size distribution was made using the Penn State Particle Separator using the 8- and 19-mm diameter screens and pan (Lammers *et al*., 1996) from the day 15 until the last day (day 26) of each period. Geometric mean particle length was measured according to the method S424 (ASAE, 2001).

Methane measurement

The methane (CH_4) yield and intensity measurements were performed using four open respiration chambers according to procedures described by Machado *et al*. (2016). The cows were allocated individually into the chambers immediately after the first milking. The measurements were performed in two consecutive periods of ~22 h and then extrapolated for 24 h, and the cows left the chambers only for milking (morning and afternoon). A correction factor was used for each period (average correction factor: 100 ± 24.04%) (Machado *et al*., 2016). The chambers remained at thermoneutrality (temperature: 23 ± 3.0 °C; relative humidity: 65 ± 5.0%). The calculation of the CH_4 emission was made using the air flux and the difference of the CH_4 in the air entering (outside air) and leaving the chambers (Machado *et al*., 2016).

Ruminal parameters

After the chamber evaluation period, ruminal fluid was collected from each cow using an esophageal probe coupled to a vacuum pump. The sample was filtered, the pH was immediately measured, and an aliquot was sampled to determine short-chain fatty acids (SCFA). Then, rumen fluid samples were centrifuged at 12,000 × g for 10 minutes, and the supernatants were treated according to Siegfried *et al*. (1984). The SCFA analyses were performed using HPLC (Ultimate 3000, Dionex Corporation, Sunnyvale, USA) equipped with a Shodex RI-101 refractive index (RI) kept at 40 °C, using Phenomenex Rezex ROA column, 300 × 7.8 mm kept at 45 °C (Tabaru *et al.*, 1988).

Chemical analysis

To estimate the crude protein (CP), a conversion factor of 6.25 was used to convert N values into CP. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined sequentially using Ankom 220 Fiber Analyzer (Ankom Technology, Macedon, New York, USA). For NDF analysis, a thermostable α-amylase was used without the addition. The non-fibrous carbohydrates (NFC; % of DM) was calculated by difference as follows:

$$NFC = 100 - (CP + NDF + EE + ash)$$

Passage rate

The passage rate (k_p) of wet forages was estimated according to NRC (2001) as follows:

$$\text{kp}\ (\%/\text{h}) = 3.054 + 0.614 \times \text{DMI}(\%\ \text{of BW})$$

where DMI is dry matter intake.

Apparent total tract digestibility

The apparent digestibility of the DM, organic matter (OM), and nutrients [CP, NDF, ADF, and NFC] were estimated by total feces collection during five consecutive periods (day 15 to day 20 of each period). Individual feces were collected and weighed twice daily (0900 e 1700). In addition, another sample (~ 1 kg; fresh matter basis) was collected for chemical composition directly from the rectum of the cows and then frozen at −20 °C until analysis. The feces used to analyze the chemical composition were grouped by day for each cow. The apparent digestibility (% of DM) was estimated using the intake and fecal excretion of each nutrient as follows:

$$\text{Apparent digestibility} = ((\text{kg DMI} \times \% \text{ of the nutrient}) - (\text{kg feces DM} \times \% \text{ of the nutrient})) / (\text{kg DMI} \times \% \text{ of the nutrient}) \times 100.$$

Where DMI is dry matter intake.

Milk yield and composition

Samples were placed into bottles containing Bromopol (D & F Control Systems Inc., San Ramon, CA, USA) and kept at 4 °C until analysis. The milk composition was analyzed by mid-infrared absorption spectrometry using the Bentley model FCM (Bentley Instruments). Fat-corrected milk (FCM) and energy-corrected milk (ECM) were calculated as follows, according to Tyrrell and Reid (1965) and NRC (2001), respectively:

$$\text{FCM(kg/d)} = \text{Milk yield} \times [0.4 + (0.15 \times \%\text{fat})]$$

$$\text{ECM (kg/d)} = \text{Milk yield} \times \left[\frac{(376 \times \%\text{ fat}) + (209 \times \%\text{ protein}) + 948}{3138}\right]$$

References

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Table S1. Ingredients and chemical composition of the experimental diets

Item[†]	Mean	SD[¶]
Ingredients, g/kg DM basis		
Maize silage	467.0	-
Tifton hay	51.0	-
Finelly ground corn	258.0	-
Toasted soybean meal	179.0	-
Suplement vit./mineral	14.0	-
Dicalcium phosphate	12.0	-
Sodium bicarbonate	10.0	-
Urea	4.0	-
Magnesium oxide	4.0	-
Chemical composition		
DM, g/kg	421.0	18.7

ash, g/kg DM	73.0	0.70
CP, g/kg DM	138.0	0.10
NDF, g/kg DM	340.0	12.8
ADF, g/kg DM	173.0	9.20
NFC‡, g/kg DM	434.0	14.4
EE, g/kg DM	26.0	2.20

†Item: DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract.

‡NFC = non-fibrous carbohydrates. NFC = 100 – (CP + NDF + EE + ash).

⁋SD = standard deviation.

Table S2. Particle size distribution of the whole-crop maize silage

Item	Mean	SD‡
Particle size, as-fed g/kg retained		
>19.0 mm	256.0	46.2
8.0 − 19.0 mm	608.0	31.0
< 8.0 mm	134.0	15.2
Bottom pan	3.0	0.30
X_{gm}†, mm	13.9	0.73

†X_{gm} = mean geometric length.

‡SD = standard deviation.

Table S3. Particle size distribution of the experimental diets

Item	Trataments†		SEM⁋	*P*-value
	SPS	LPS		
Particle size, as-fed g/kg retained				
>19.0 mm	119.0	206.0	2.38	<0.01
8.0 − 19.0 mm	415.0	411.0	3.60	0.44
< 8.0 mm	351.0	283.0	4.11	<0.01
Bottom pan	115.0	96.0	1.93	<0.01
X_{gm}‡, mm	8.5	10.0	0.048	<0.01

†SPS = short particle size; LPS = long particle size.

‡X_{gm} = mean geometric length.

⁋SEM = standard error of the mean.

Table S4. Effect of particle size and breed on ruminal fermentation parameters of lactating dairy cows

Item	Holstein		Girolando		SEM[§]	*P*-value[⁑]		
	SPS[⁋]	LPS[⸸]	SPS	LPS		PS	B	PS×B
Total SCFA[†], mmol/l	49.1	56.0	64.2	51.7	5.19	0.59	0.31	0.08
Acetic acid, mol/100 mol	62.8	60.3	60.7	59.4	1.36	0.19	0.29	0.64
Propionic acid, mol/100 mol	22.1	25.1	23.2	23.4	1.26	0.22	0.78	0.28
Butyric acid, mol/100 mol	15.0	14.6	16.1	17.1	0.75	0.72	0.02	0.33
A:P ratio[‡]	2.84	2.40	2.62	2.54	0.18	0.27	0.80	0.29
pH	6.7	6.6	6.5	6.6	0.05	0.96	0.28	0.35

[†]total SCFA = short-chain fatty acids.

[‡]A:P ratio = acetic to propionic acid ratio.

[⁋]SPS = short particle size.

[⸸]LPS = long particle size.

[§]SEM = standard error of the mean.

[⁑]PS = effect of particle size [short vs. long]; B = effect of breed composition [Holstein vs. Girolando]; PS x B = effect of interaction between particle size and breed.

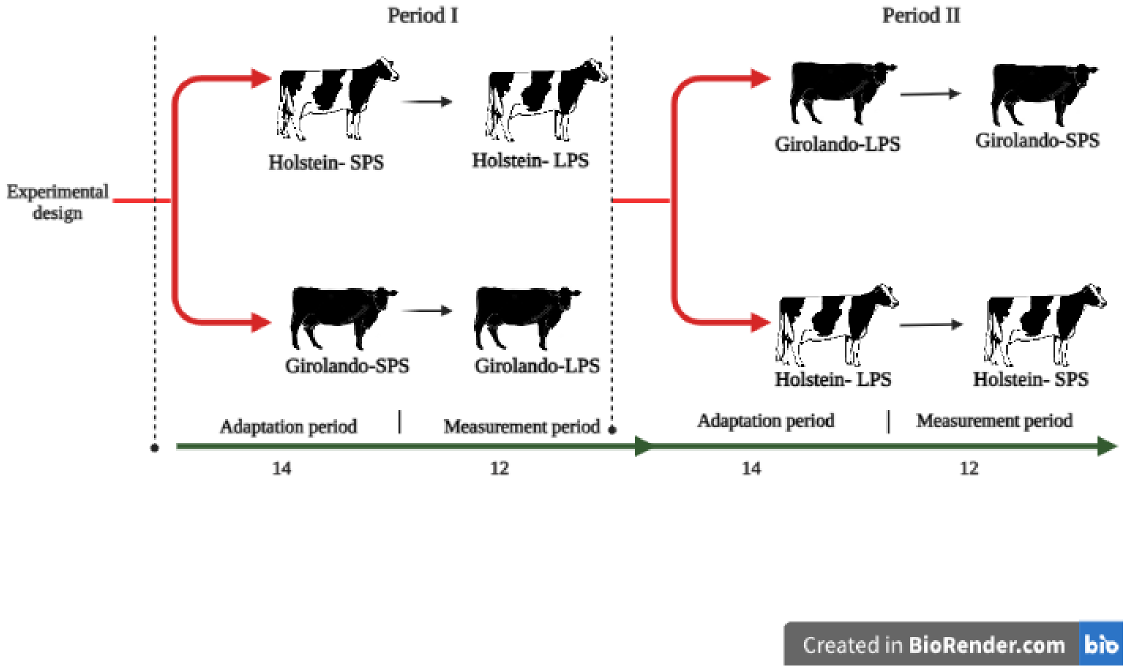

Figure S1. Experimental design and flowchart of the study.