## Characterization of variable related to high stability of raw cow milk

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## SUPPLEMENTARY FILE

## **Supplementary Materials and Methods**

Stability was analyzed following the method of Tronco (2010). The milk samples that precipitate in the alcohol presence were called unstable milk. Milk samples that did not precipitate in the alcohol presence were called stable milk. Milk samples tested on alcohol 86 °GL were classified as unstable at 88 °GL concentration.

Titratable acidity (g lactic acid/100 mL) was determined as described by Tronco (2010). Briefly, 10 mL of the milk sample was pipetted into an Erlenmeyer flask and four drops of the acid-base indicator phenolphthalein was added. Titration was performed with 0.11 N sodium hydroxide solution (Dornic solution) until the turning point when the color turns slightly pink. The titratable acidity was first measured as Dornic degrees and then transformed into grams of lactic acid/100 mL using the formula: titratable acidity (g lactic acid/100 mL) = titration value obtained \* correction factor of NaOH solution/10. Milk was classified as follows: normal = acidity of 0.14-0.18 g/100 mL considering a pH of 6.6-6.8; acid = acidity > 0.18 g/100 mL at pH less than 6.6; alkaline = acidity < 0.14 g/100 mL at pH higher than 7.0.

For the ionized calcium analysis, the potentiometer electrode was sensitized by immersion in a standard calcium chloride solution (1000 ppm) for at least 4 hours, with the electrode filling solution. For calibration of the electrode, standard solutions of calcium chloride were prepared at the following concentrations: 50 and 500 ppm. To each solution, 1 mL of ionic strength adjustment solution (ISA; 4 mol/L of KCl) was added. Next, 50 mL milk and 1 mL ISA were mixed and homogenized with a plastic rod. The electrode was immersed in the sample to obtain the milk ionized calcium concentration (Ribeiro *et al.*, 2010).

## **Supplementary Tables**

Variable	Ν	Mean ± standard deviation	Range
Ethanol stability (°GL)	814	$80 \pm 4.94$	72 - 88
Ionized calcium (mg/L)	813	111.81 ±40.35	29 - 540
Titratable acidity (g/100 mL)	813	$0.1582 \pm 0.0176$	0.11 - 0.28
Fat (g/100 g)	751	$3.77\pm0.38$	2.11-5.81
Protein (g/100 g)	756	$3.23\pm0.20$	2.56 - 4.06
Fat/protein ratio	749	$1.17\pm0.09$	0.59 - 1.8
Lactose (g/100 g)	756	$4.43\pm0.19$	2.76 - 5.46
Total solids (g/100 g)	756	$12.37\pm0.61$	9.07 - 14.41
DDE (g/100 g)	756	$8.62 \pm 0.30$	6.53 - 10.34
SCC ( $10^3$ cells/mL)	756	$528 \pm 410$	17 - 3,808
SPC (10 <sup>3</sup> CFU/mL)	757	$114 \pm 489$	2 - 9,999
MUN (mg/dL)	684	$12.23\pm4.05$	4.1 - 30.2
$T_{CT}$ (°C)	806	$3.31 \pm 0.44$	1.4 - 4.0
Milk volume (L/month)	801	$18,\!484 \pm 34,\!802$	270 - 336,797
Number of milkings	783	$2 \pm 0.25$	1 - 3
Number of lactation cows	764	$34.70\pm52.98$	2 - 500
Distance (km)	522	$102.21 \pm 48.97$	3 - 255
Transport time (min)*	524	$327.98 \pm 145.28$	60 - 720

**Table S1.** Descriptive statistics of the chemical composition and physicochemical characteristics of milk and of the dairy herds in the Paraná State

N: number of observations; DDE: dry defatted extract; SCC: somatic cell count; SPC: Standard plate count; MUN: milk urea nitrogen;  $T_{CT}$ : temperature of cooling tank. \*Transport form farm to milk processing plant.

**Table S2.** Description of the dairy farms

Variable	Frequency (%)
Type of milking	
Bucket milking	53.82
Piped milking	40.33
Manual milking	5.85
Breed	
Holstein	60.09
Jersey	16.05
Holstein x Jersey crossbreed	14.49
Girolando	9.38
Production system	
Pasture with supplementation	88.04
Feedlot	11.96