

1 **SUPPLEMENTARY FILE**

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3 **Brix refractometer as useful on-farm tool for measurement of colostrum in Italian beef**
4 **and dairy cattle**

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9 **Materials and methods**

10 *Laboratory analysis*

11 Indirect evaluation of colostral IgG concentration was measured by the evaluation of total
12 solids measured with a digital Brix refractometer (MISCO Palm Abbe no. PA201, Misco,
13 Solon, OH). The scales on the refractometer were automatically temperature calibrated for
14 aqueous sucrose solutions at 20°C between the surrounding air temperatures from 0 to 50°C.
15 According to the manufacturer's recommendations, an aliquot of 0.4 mL of deionized water
16 was placed on the prism well of the refractometer to obtain a standardized reading.
17 Subsequently an aliquot of colostrum (0.4 mL) was placed on the refractometer prism. The
18 refractometer reports the Brix value in % with a low limit on the scale of 0% and an upper limit
19 of 85% ($\pm 0.1\%$).

20 Colostral immunoglobulin G concentration was directly measured by a commercial RID kit
21 (Bovine Ig Test Kit, Triple J Farms, Bellingham, WA). All colostrum samples were tested using
22 the manufacturer's directions. Briefly, the samples were diluted 1:4 with PBS (phosphate
23 buffered saline), mixed, then 5 μ l of diluted samples were put into the plate wells using the
24 reference sera provided by the manufacturer as controls. Plates were incubated for 24 h at 23°C
25 and the diameter of the precipitin ring was measured. Colostral IgG concentrations were
26 determined by comparing the diameter of the samples with standard straight line of best fit
27 between the three points corresponding to the diameter of the three standard sera. All samples
28 were analyzed in duplicate and the results multiplied by the dilution factor and reported in g/L.

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Cut-point Brix (%)	Sensibility (95% CI)	Specificity (95% CI)	VPP (95% CI)	VPN (95% CI)
<16	1.00 (0.85-1.00)	1.00 (0.85-1.00)	1.00 (0.85-1.00)	1.00 (0.85-1.00)
<17	1.00 (0.85-1.00)	0.92 (0.74-0.98)	0.50 (0.31-0.68)	1.00 (0.85-1.00)
<18	1.00 (0.85-1.00)	0.92 (0.74-0.98)	0.50 (0.31-0.68)	1.00 (0.85-1.00)
<19	1.00 (0.85-1.00)	0.84 (0.65-0.94)	0.33 (0.17-0.53)	1.00 (0.85-1.00)
<20	1.00 (0.85-1.00)	0.73 (0.52-0.87)	0.22 (0.09-0.42)	1.00 (0.85-1.00)
<21	1.00 (0.85-1.00)	0.65 (0.45-0.81)	0.18 (0.07-0.37)	1.00 (0.85-1.00)
<22	1.00 (0.85-1.00)	0.61 (0.41-0.78)	0.16 (0.06-0.36)	1.00 (0.85-1.00)
<23	1.00 (0.85-1.00)	0.61 (0.41-0.78)	0.16 (0.06-0.36)	1.00 (0.85-1.00)

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36 **Supplementary Table S1.** Sensitivity, specificity, positive predictive value (PPV) and
37 negative predictive value (NPV) for different digital Brix refractometer cut-points to screen
38 Chianina colostrum sample for low IgG concentration <50 g/L. CI = confidence interval.

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Cut-point Brix (%)	Sensibility (95% CI)	Specificity (95% CI)	VPP (95% CI)	VPN (95% CI)
<16	0.83 (0.63 -0.94)	1.00 (0.84-1.00)	1.00 (0.84-1.00)	0.95 (0.78-0.99)
<17	1.00 (0.84-1.00)	1.00 (0.84-1.00)	1.00 (0.84-1.00)	1.00 (0.84-1.00)
<18	1.00 (0.84-1.00)	1.00 (0.84-1.00)	1.00 (0.84-1.00)	1.00 (0.84-1.00)
<19	1.00 (0.84-1.00)	1.00 (0.84-1.00)	1.00 (0.84-1.00)	1.00 (0.84-1.00)
<20	1.00 (0.84-1.00)	0.95 (0.77-0.99)	0.85 (0.66-0.95)	1.00 (0.84-1.00)
<21	1.00 (0.84-1.00)	0.85 (0.66-0.95)	0.66 (0.46-0.82)	1.00 (0.84-1.00)
<22	1.00 (0.84-1.00)	0.81 (0.60-0.92)	0.60 (0.39-0.77)	1.00 (0.84-1.00)
<23	1.00 (0.84-1.00)	0.71 (0.50-0.86)	0.50 (0.30-0.69)	1.00 (0.84-1.00)

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60 **Supplementary Table S2.** Sensitivity, specificity, positive predictive value (PPV) and
61 negative predictive value (NPV) for different digital Brix refractometer cut-points to screen
62 Podolica colostrum sample for low IgG concentration <50 g/L. CI = confidence interval.

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Cut-point Brix (%)	Sensibility (95% CI)	Specificity (95% CI)	VPP (95% CI)	VPN (95% CI)
<17	0.33 (0.17-0.53)	1.00 (0.85-1.00)	0.84 (0.65-0.94)	1.00 (0.85-1.00)
<18	0.50 (0.31-0.68)	0.95 (0.78-0.99)	0.75 (0.58-0.88)	0.87 (0.68-0.96)
<19	0.50 (0.31-0.68)	0.86 (0.67-0.95)	0.50 (0.31-0.68)	0.86 (0.67-0.95)
<20	0.83 (0.63-0.93)	0.77 (0.57-0.90)	0.50 (0.31-0.68)	0.94 (0.77-0.99)
<21	0.83 (0.63-0.93)	0.59 (0.39-0.76)	0.35 (0.19-0.55)	0.92 (0.75-0.98)
<22	0.83 (0.63-0.93)	0.45 (0.27-0.69)	0.29 (0.14-0.94)	0.90 (0.72-0.97)
<23	0.83 (0.63-0.03)	0.27 (0.13-0.47)	0.23 (0.10-0.44)	0.85 (0.66-0.95)
<24	1.00 (0.85-1.00)	0.22 (0.09-0.42)	0.26 (0.12-0.46)	1.00 (0.85-1.00)

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83 **Supplementary Table S3** Sensitivity, specificity, positive predictive value (PPV) and negative
84 predictive value (NPV) for different digital Brix refractometer cut-points to screen Holstein
85 Friesian colostrum sample for low IgG concentration <50 g/L. CI = confidence interval.

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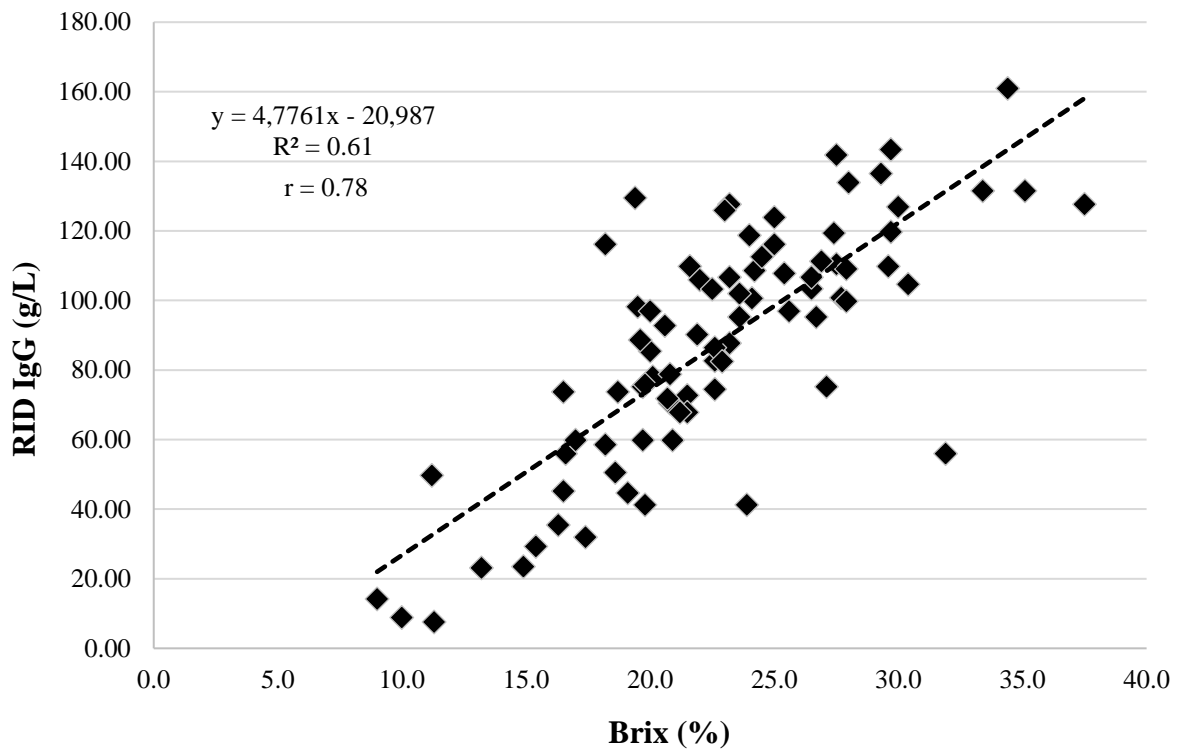
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108 **Supplementary Figure S1** Scatter plot of the correlation between Brix (%) and IgG
109 measurement (g/L) [by radial immunodiffusion (RID)] of first-milking colostrum. $R^2 =$
110 coefficient of determination; $r =$ correlation coefficient.

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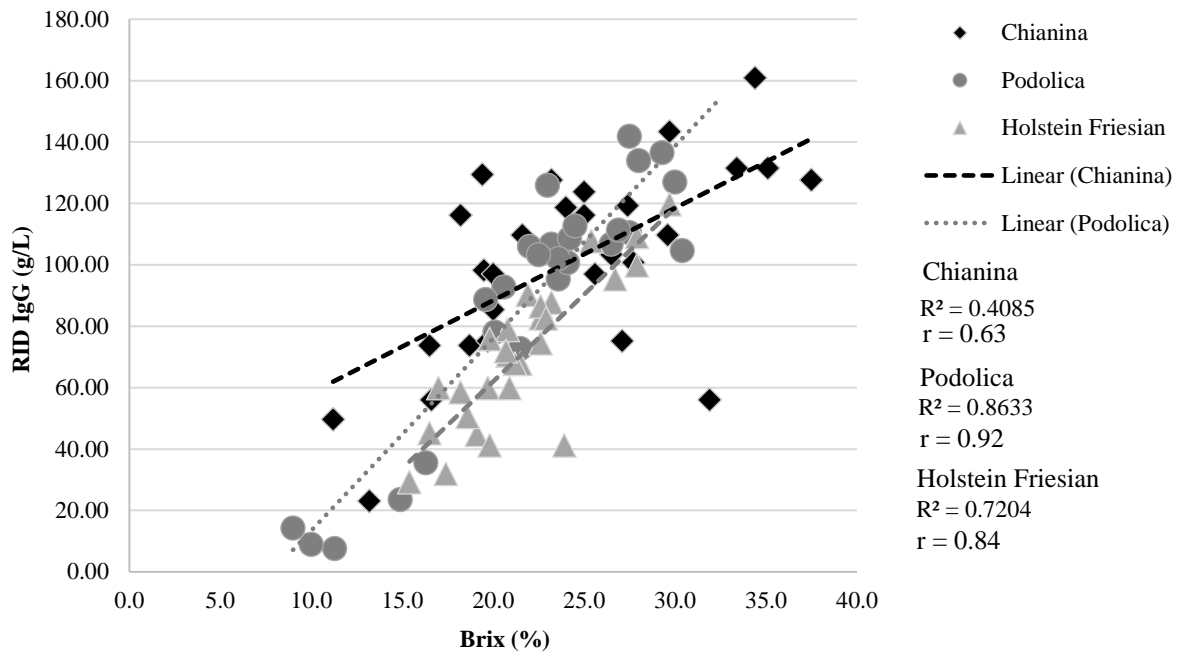
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129 **Supplementary Figure S2.** Scatter plot of the correlation between Brix (%) and IgG

130 measurement (g/L) [by radial immunodiffusion (RID)] of first-milking colostrum stratified by

131 breed. R² = coefficient of determination; r = correlation coefficient.

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