Predicting colostrum and calf blood components based on refractometry

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SUPPLEMENTARY FILE

Supplementary Methods

Enzyme-linked immunosorbent assay for immunoglobulin G (IgG) concentration

The immunoglobulin G (**IgG**) concentration in the individual cow colostrum, pooled colostrum and bulk tank milk samples was quantified by enzyme-linked immunosorbent assay (**ELISA**) in 96-well plates (Coat Nunc F96 Maxisorp plates, Thermo-Fisher Scientific) using two bovine IgG specific antibodies (Life Technologies, USA), affinity purified goat anti-bovine IgG antibody unconjugated (Novex Cat. #A18753) and affinity purified goat anti-bovine IgG antibody conjugated with horseradish peroxidase (HRP Invitrogen Cat. #18751). The pooled colostrum samples and individual cow colostrum samples from days 0 and 1 were diluted in 0.05% Tween 20-PBS solution at a ratio of $1{:}10^6$, while the bulk tank milk samples and the individual cow colostrum samples from days 2 and 3 were diluted at a ratio of $1{:}10^5$. Bovine gamma globulin (Bio-Rad Laboratories, Inc., USA) was used as the standard, with serial dilutions (0; 6.3; 12.5; 25; 37.5; 50; 75; and 100 ng/mL). 3,3',5,5'-tetramethylbenzidine (**TMB**) substrate (Ultra TMB-ELISA, Thermo Fisher Scientific) was added and the plates were incubated at room temperature in the dark for 15 minutes before the reactions were stopped with the addition of 0.1 M H_2SO_4. Antibody binding was measured as the enzymatic colour change at 450 nm using a Benchmark Plus microplate spectrophotometer (Bio-Rad Laboratories, Inc., USA). The IgG concentrations in samples were calculated based on the standard curve. Calf serum IgG concentration was determined using the same ELISA as the colostrum samples, however, calf

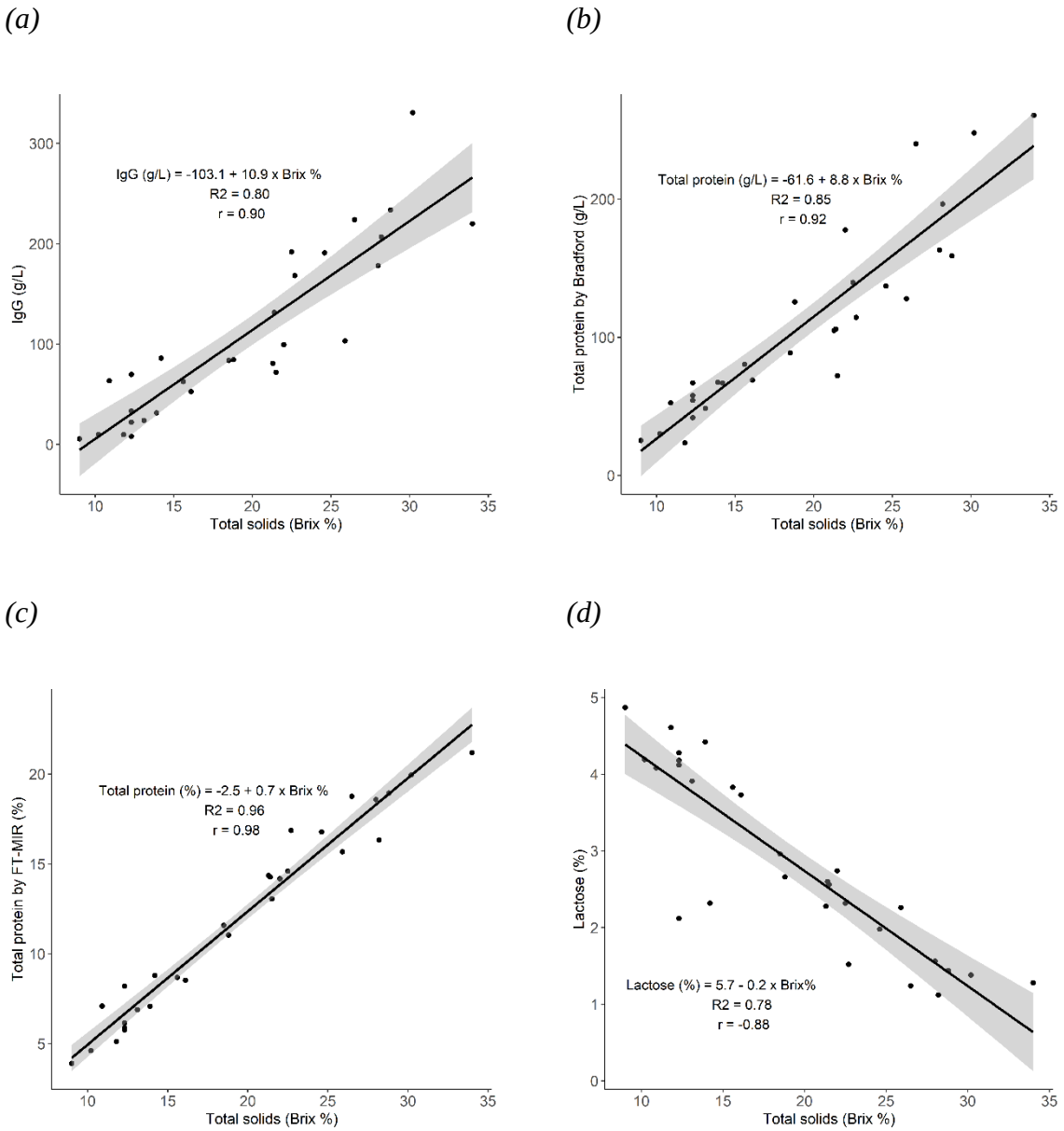
serum samples were diluted in 0.05% Tween 20-PBS solution with at a ratio of $1:10^4$ for day 0 and a ratio of $1:10^6$ for days 1 and 7.

Bradford assay for total protein concentration

Total protein in the individual cow colostrum, pooled colostrum, and bulk tank milk was assayed in a 96 well-plate using a Quick Start Bradford Protein assay kit following the manufacturer's instructions and bovine serum albumin for the standard curves (Quick Start, Bio-Rad Laboratories, Inc., USA). The individual cow colostrum samples from days 1, 2 and 3, pooled colostrum and bulk tank milk were diluted with ultrapure water at a ratio of 1:100 and the individual cow colostrum from day 0 was diluted with a ratio of 1:200 or 1:300. The diluted samples (5 µL) were mixed with Coomassie Brilliant Blue G-250 dye (250 µL) and the colour change was measured using a Benchmark Plus microplate spectrophotometer (Bio-Rad Laboratories, Inc., USA) at 595 nm (OD595). The total protein by Bradford assay (**TP-B**) in the samples was determined based on the standard curve. Calf serum total protein was measured by Bradford assay (TP-B) as described above except the serum was diluted with ultrapure water at a ratio of 1:100.

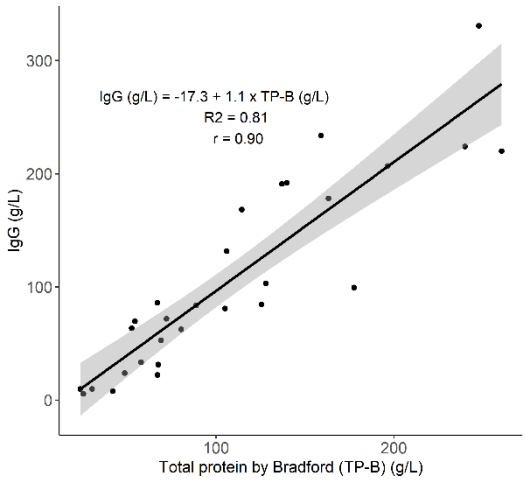
Supplementary Table S1. Estimated IgG, total protein and lactose in colostrum based on regression formulae from Brix refractometer measurements.

Brix%	IgG (g/L)	Total protein (g/L)	Lactose (%)
	IgG (g/L) = -103.1 + 10.9 x Brix %	TP-B (g/L) = -61.6 + 8.8 x Brix %	Lactose (%) = 5.7 – 0.2 x Brix %
	R^2 = 0.80, P < 0.001	R^2 = 0.85, P < 0.001	R^2 = 0.78, P < 0.001
12	27.7	44.0	3.3
13	38.6	52.8	3.1
14	49.5	61.6	2.9
15	60.4	70.4	2.7
16	71.3	79.2	2.5
17	82.2	88.0	2.3
18	93.1	96.8	2.1
19	104.0	105.6	1.9
20	114.9	114.4	1.7
21	125.8	123.2	1.5
22	136.7	132.0	1.3
23	147.6	140.8	1.1
24	158.5	149.6	0.9
25	169.4	158.4	0.7

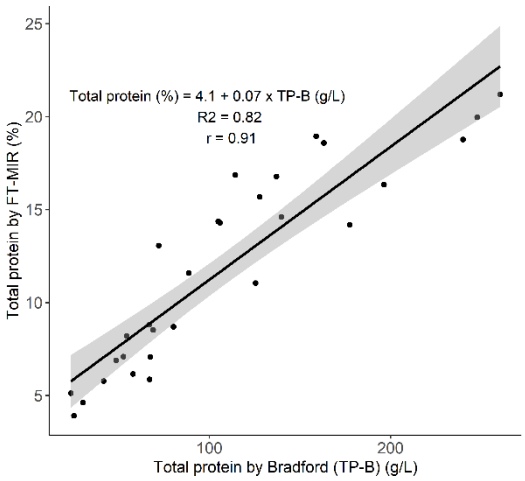


Supplementary Figure S1. Linear regression between total solids measured by Brix refractometry (Brix %) and IgG (g/L) *(a)*, total protein by Bradford assay (g/L) *(b)*, protein % *(c)*, and lactose % *(d)* in individual cow colostrum day 0 and 1 postpartum and pooled colostrum samples (n = 29). R^2 = coefficient of determination, r = correlation. P-value for all panels (a, b, c, d) < 0.001.

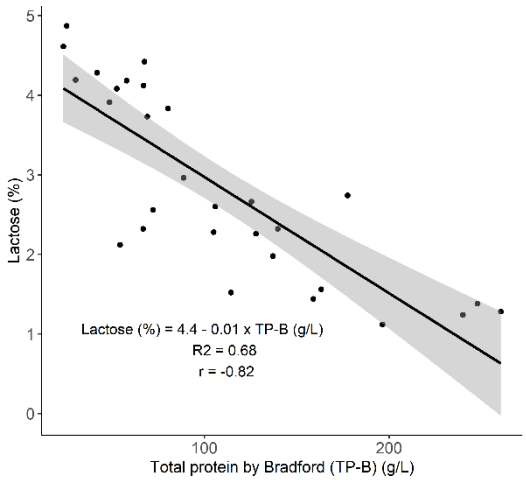
(a)



(b)

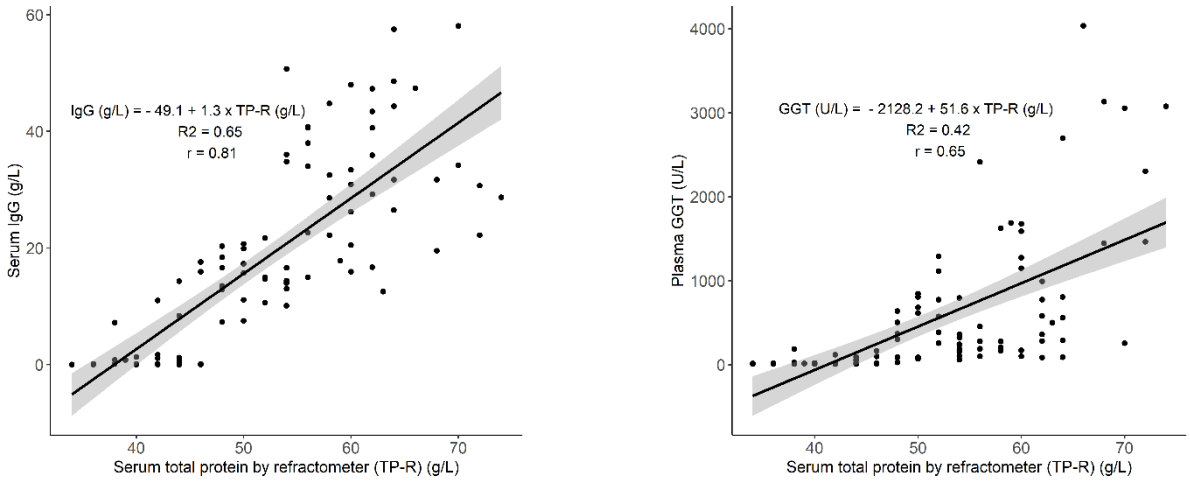


(c)



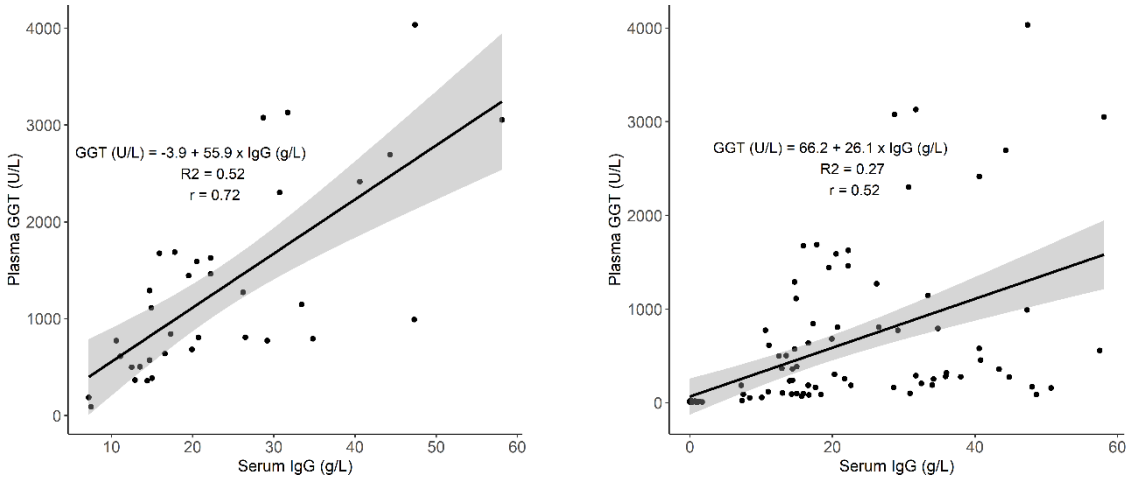
Supplementary Figure S2. Linear regression between total protein by Bradford assay (TP-B) (g/L) and IgG (g/L) *(a)*, protein % by FT-MIR *(b)*, and lactose % by FT-MIR *(c)* in colostrum collected within 1 day postpartum and pooled colostrum samples (n = 29). R^2 = coefficient of determination, r = correlation. P-value for all panels (a, b, c) < 0.001.

(a) *(b)*



Supplementary Figure S3. Linear regression between serum total protein by refractometer (TP-R) and serum IgG *(a)*, and plasma GGT *(b)* in calf blood measured within one week postpartum. R^2 = coefficient of determination, r = correlation. P-value for all panels (a, b) < 0.001.

(a) *(b)*



Supplementary Figure S4. Linear regression between serum IgG and plasma GGT in calf blood using data from day 1 (n=35) *(a)*, and data combined from days 0, 1 and 7 (n = 105) *(b)*. R^2 = coefficient of determination, r = correlation. P-value for all panels (a, b) < 0.001.