

## Colostrum immunoglobulin G as a predictor for serum immunoglobulin G concentration in dairy calves

LW Coleman, RE Hickson, J Amooore, RA Laven and PJ Back\*

*Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.*

\*Corresponding author. Email: P.J.Back@massey.ac.nz

### Abstract

Feeding newborn calves high-quality colostrum is of well-recognised importance as calves that do not receive an adequate concentration of immunoglobulin-G (IgG) have an increased risk of morbidity and mortality. This experiment aimed to characterise IgG concentrations in colostrum from the first milking in New Zealand dairy cows, examine IgG status of calves, and to evaluate the use of a refractometer for estimating quality of colostrum in terms of IgG concentration. Heifer calves entered the calf shed at 0-24 hours of age and were fed pooled first-milking colostrum within eight hours of entering the shed. Blood samples were taken the following morning. Cows were individually milked and samples from individual and pooled colostrum were analysed. IgG concentration of serum and colostrum samples were determined by turbidimetric immunoassay. IgG concentration was adequate according to commercial reference ranges in 70.1% of individual colostrum, 80.0% of pooled colostrum and 82.5% of calf serum samples. There was no relationship between calf serum and IgG concentration in dam or pooled colostrum. The refractometer was a good predictor of IgG concentration in serum ( $r=0.64$ ,  $P<0.0001$ ) and colostrum both in dam ( $r=0.87$ ,  $P<0.0001$ ) and pooled ( $r=0.81$ ,  $P<0.0001$ ) samples. The relationship between IgG concentration and refractometer reading indicates that it could be a useful on-farm tool.

**Keywords:** colostrum; heifer; Brix refractometer; immunoglobulin; calf; dairy

### Introduction

Calves are born hypogammaglobulinemic (have a naïve immune system) due to no placental transport of maternal immunoglobulins (predominantly immunoglobulin G - IgG) (Faber et al. 2005; Godden 2008; Quigley 2004; Quigley et al. 2002; Weaver et al. 2000). Absorption of colostrum immunoglobulins is essential to establish passive immunity (Godden 2008; Quigley 2004; Quigley et al. 2002; Weaver et al. 2000). Immunoglobulin absorption occurs in the first 24 hours after birth, after which macromolecular absorption ceases (Quigley 2004). Feeding newborn calves an adequate volume of colostrum is of well-recognised importance (Elfstrand et al. 2002; Faber et al. 2005; Weaver et al. 2000).

Pooling of colostrum from several freshly-calved cows is used to minimise the effect of individual cows with low IgG concentrations (Weaver et al. 2000). However this is no longer suggested as best practice because cows producing high volumes of colostrum with a low concentration of IgG may dilute the IgG concentration of the whole pool (Godden 2008; Weaver et al. 2000).

Passive immunity is transferred to calves from maternal colostrum (Deelen et al. 2014; Quigley 2004; Quigley et al. 2002). Failure of passive transfer (FPT) is known to increase the risk of morbidity and mortality (Deelen et al. 2014; Quigley 2004; Quigley et al. 2002). FPT occurs when the concentration of calf serum IgG is less than 10 g/L after absorption of IgG ceases (Deelen et al. 2014; Quigley 2004; Quigley et al. 2002). As the quality of colostrum produced varies between individual cows, the encouraged practice is to feed four litres of colostrum within eight hours of being brought into the shed. Feeding two litres or less was shown to increase the risk of mortality

and morbidity (Faber et al. 2005). Furthermore, feeding calves within two hours of birth optimised the transfer of immunoglobulins (Morin et al. 2010).

Digital refractometers are an inexpensive means of approximating the total solid percentage in a sample (Brix%), and have been used previously to estimate IgG concentration in calf serum and colostrum samples from dairy cattle in USA and Canada (Bielmann et al. 2010; Deelen et al. 2014; Quigley et al. 2013).

The aim of this experiment was to characterise IgG concentrations in colostrum from the first milking in New Zealand dairy cows, the relationship between IgG concentration in serum of calves and colostrum fed to them, and to evaluate the use of a digital refractometer for estimating quality of colostrum from New Zealand cows in terms of IgG concentration.

### Materials and methods

This experiment was conducted with approval from the Massey University Animal Ethics Committee. This experiment was conducted at Massey University's dairy 1 farm in Palmerston North.

#### Animals

Two hundred and four Friesian ( $n=56$ ), Jersey ( $n=45$ ) or Friesian-Jersey crossbred ( $n=103$ ) primiparous ( $n=56$ ) and multiparous cows ( $n=148$ ) calving in spring 2014, and 63 heifer calves to be reared as replacement heifers were included in this study. Bull calves and calves born to primiparous cows were excluded.

Calves were removed from their mothers and placed in a calf shed at approximately 9 am each day, within 24 hours of birth, sometimes before suckling from their mothers. All

calves were offered two litres of first colostrum morning and afternoon for the first day in the calf shed (between 0 and 32 hours old). Colostrum offered on the first day was pooled colostrum from the cows which had entered the herd that morning, having calved in the previous 24 hours. If the calf would not drink in the morning, it was assumed the calf had drunk from its mother and it was left until the afternoon, at which time any calves not drinking were given two litres of first-milking colostrum by oesophageal tube.

#### Samples

Cows were milked once a day. At their first milking all cows were milked into individual test buckets, from which a sample was taken. Colostrum was pooled for feeding to the newborn heifer calves and a second sample was taken. Serum samples were collected from calves via jugular venipuncture the morning after entering the shed (approximately 24–48 hours old).

A refractometer (OPTi digital hand held refractometer, Brix 54, Bellingham + Stanley, Thermo Fisher Scientific) calibrated with distilled water, was used to estimate IgG concentration in both individual and pooled colostrum (warmed and stirred to distribute fat) and serum samples.

The IgG concentration in colostrum and serum was analysed at the New Zealand Veterinary Pathology lab, Palmerston North. Colostrum was homogenised and a dilution buffer was added prior to analysis. IgG concentration in the colostrum and serum was measured by an automated turbidimetric immunoassay (TIA) (Besser et al. 1988) on a Roche Modular P800 clinical chemistry analyser (Roche Diagnostics, Auckland, NZ). Reagents used were supplied by Midland BioProducts Corporation (Boone, IA, USA).

IgG concentration in calf serum was classified as being adequate ( $>1600$  mg/dl) or inadequate ( $\leq 1600$  mg/dl) (Wittum & Perino 1995). IgG concentration in colostrum from individual and pooled samples was classified as adequate ( $\geq 3000$  mg/dl), or inadequate ( $<3000$  mg/dl) based on Holmes et al. (1987) recommendation for cows in New Zealand.

#### Statistical Analysis

Data were analysed using SAS (Version 9.3, SAS Institute Inc., Carey, North Carolina, USA). The colostrum IgG concentration was analysed using a general linear model that included the fixed effects of breed, parity (primiparous versus multiparous) and the interaction of breed with parity.

Pearson correlation coefficients were calculated for serum IgG concentration against the concentration of IgG offered in colostrum from dam and pooled colostrum.

Colostrum IgG concentration and serum IgG concentration were also analysed using a general linear model that included the fixed effect of the Brix refractometer value. Pearson correlation coefficients were calculated for colostrum IgG concentration and serum IgG concentration against the Brix refractometer.

## Results

There were no differences among the concentration of IgG in colostrum from the different breeds, or from multiparous and primiparous cows (Table 1). There was also no interaction between breed and parity.

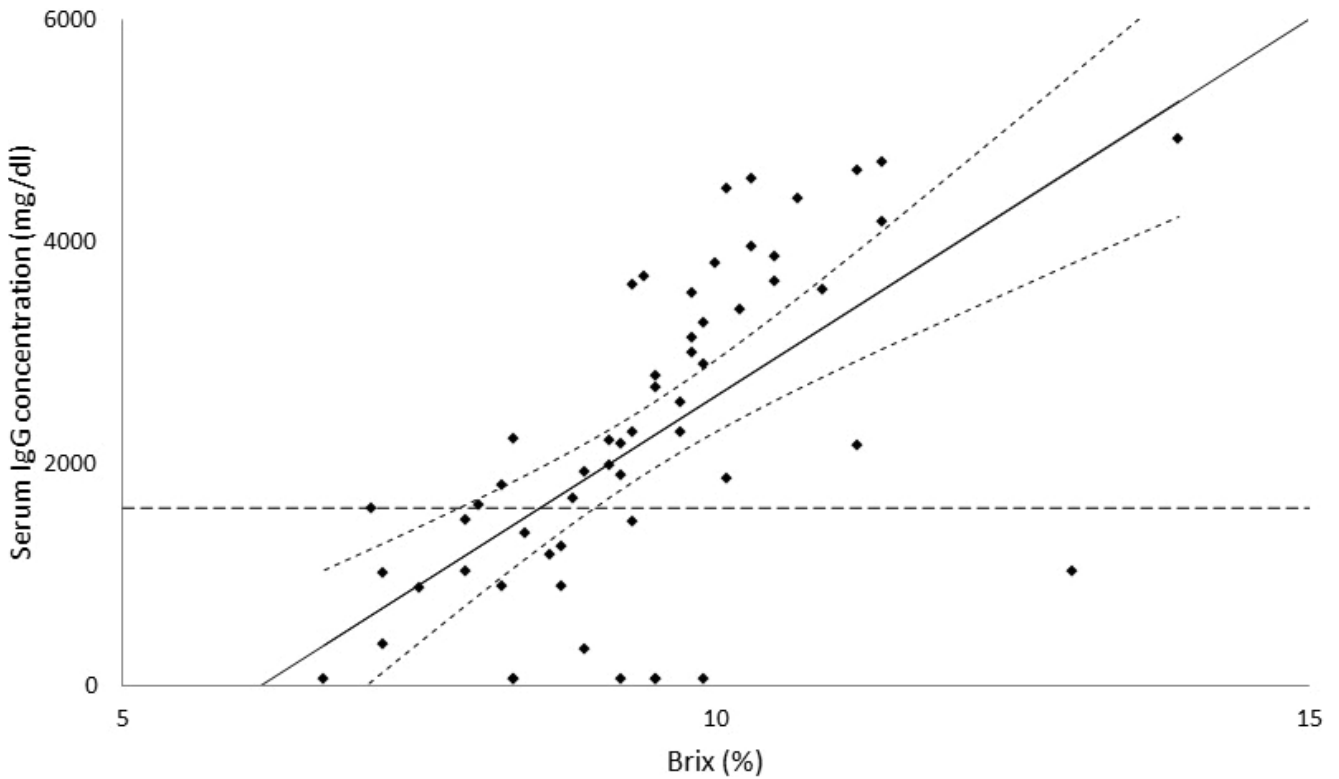
**Table 1** Concentration of IgG in colostrum (mg/dl) from individual cows of different breeds and parity. Values are least squares means  $\pm$  SEM.

	n	IgG concentration (mg/dl)
<b>Breed</b>		
Holstein Friesian	56	4345 $\pm$ 278
Jersey	45	3636 $\pm$ 298
Friesian – Jersey cross	103	3820 $\pm$ 268
P value (breed effect)		0.1881
<b>Parity</b>		
Primiparous	56	3828 $\pm$ 267
Multiparous	148	4039 $\pm$ 186
P value (parity effect)		0.5174

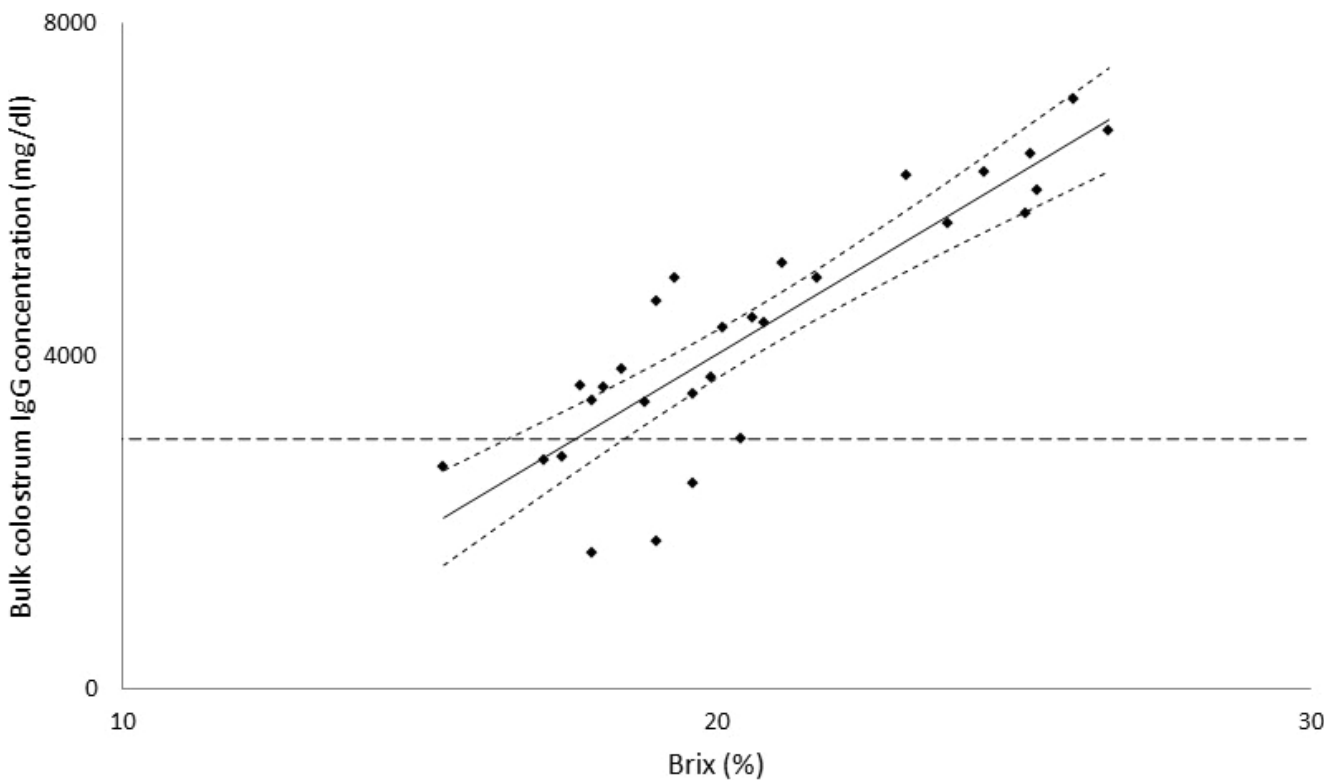
The colostrum concentration of IgG was adequate ( $\geq 3000$  mg/dl) in 70.1% of samples from individual cows and in 80.0% of samples from pooled colostrum. IgG samples from 82.5% of calves had adequate concentrations ( $>1600$  mg/dl).

The dams of 34.9% of calves had inadequate concentrations of IgG in colostrum. Although offered inadequate IgG colostrum from their dam, 17.5% were offered adequate IgG concentration from the pooled colostrum. Although 15.9% of calves were offered inadequate colostrum from both dam and pooled colostrum, these calves did not have inadequate serum IgG concentrations. In contrast, 17.5% of calves had inadequate serum IgG concentration despite being fed adequate quality colostrum from their dam and/or the pooled colostrum. There was no relationship between IgG concentration in dam ( $r=0.045$ ,  $P>0.7$ ) or pooled ( $r=0.019$ ,  $P>0.8$ ) colostrum available to the calf, and the IgG concentration in calf serum.

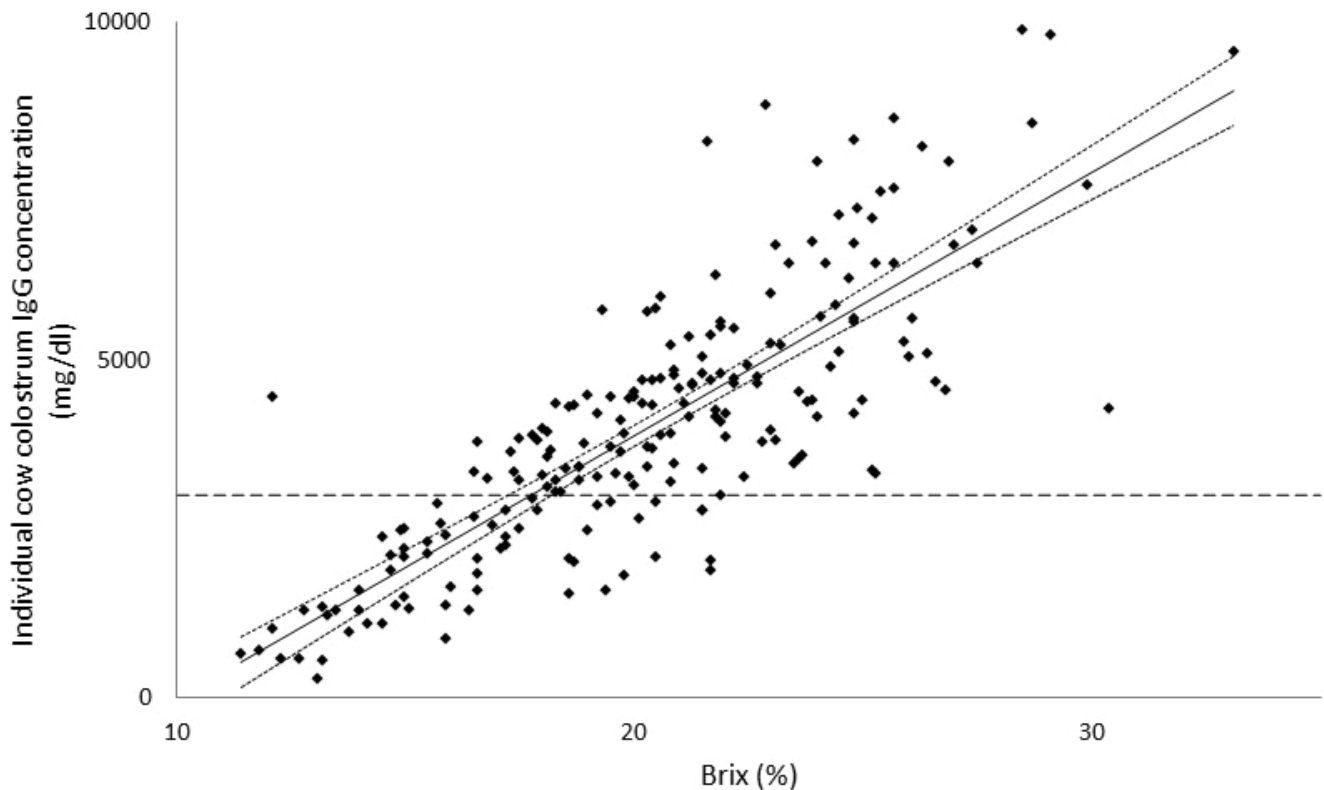
Use of the refractometer showed a high correlation between the reading and IgG concentration in calf serum (Figure 1;  $r=0.64$ ,  $P<0.0001$ ) and colostrum in individual samples (Figure 2;  $r=0.87$ ,  $P<0.0001$ ) and a high correlation in pooled colostrum (Figure 3;  $r=0.81$ ,  $P<0.0001$ ) samples.



**Figure 1** Relationship between IgG concentration (mg/dl) and Brix refractometer value (%) in serum of calves sampled between 24 and 48 hours old.  $r^2=0.41$ ,  $P<0.0001$ ;  $y=-4200\pm1058 + 681\pm111 \times \text{Brix}$ . Solid line represents the line of best fit; dashed line represents 95% confidence interval; horizontal dashed line represents adequate IgG concentration threshold (1600 mg/dl) as determined by Wittum and Perino (1995).



**Figure 2** Relationship between IgG concentration (mg/dl) and Brix refractometer value (%) in bulked cow colostrum.  $r^2=0.66$ ,  $P<0.0001$ ;  $y=-4529\pm955 + 427\pm45 \times \text{Brix}$ . Solid line represents the line of best fit; dashed line represents 95% confidence interval; horizontal dashed line represents adequate IgG concentration threshold (3000 mg/dl) as determined by Holmes et al. (1987).



**Figure 3** Relationship between IgG concentration (mg/dl) and Brix refractometer value (%) in individual colostrum.  $r^2=0.76$ ,  $P<0.0001$ ;  $y=-3907\pm 401 + 389\pm 19 \times \text{Brix}$ . Solid line represents the line of best fit; dashed line represents 95% confidence interval; horizontal dashed line represents adequate IgG concentration threshold (3000 mg/dl) as determined by Holmes et al. (1987).

## Discussion

### *IgG concentration*

Few published data exist on IgG concentrations found in colostrum produced by pasture-fed cows in New Zealand, particularly those milked once a day. Published mean concentrations range from 4100 to 9700 mg/dl in overseas studies (Bielmann et al. 2010; Elfstrand et al. 2002; Kehoe et al. 2011; Morin et al. 2010; Quigley et al. 2013). This range indicates cows in our study (Table 1) had low concentrations of IgG in colostrum, in some comparisons up to 50% less than previous reports (Bielmann et al. 2010; Elfstrand et al. 2002; Kehoe et al. 2011). Colostrum from different breeds has been reported to vary in IgG concentration (Godden 2008; Muller & Ellinger 1981; Villarroel et al. 2013; Weaver et al. 2000). Experiments in the United States have reported that Jersey cattle have higher IgG concentrations than Holstein Friesians (Godden 2008; Muller & Ellinger 1981; Villarroel et al. 2013; Weaver et al. 2000), but this was not observed in the present experiment. To the author's knowledge, this is the first study investigating IgG concentrations in colostrum from Friesian-Jersey crossbred cows. Similarly, there are reported differences between primiparous and multiparous cows in overseas studies, with primiparous cows having lower IgG concentrations than multiparous cows (Kehoe et al. 2011; Kessler et al. 2014; Muller & Ellinger 1981), which was also not observed in this experiment. There is a commonly reported recommendation to feed calves colostrum only from cows in their second or subsequent

lactation, and to discard that from first lactation cows (Weaver et al. 2000). However, this was not supported by the findings of the current experiment.

The concentration of IgG in bovine colostrum decreases with increasing time after calving to first milking (Kehoe et al. 2011; Moore et al. 2005; Morin et al. 2010; Quigley et al. 2013). Therefore colostrum should be harvested as soon as possible after calving to ensure the greatest concentration of IgG (Morin et al. 2010). Colostrum pooling is used to minimise the effect of individual cows with low IgG concentrations (Weaver et al. 2000), however it is no longer recommended because of the risk of diluting the IgG concentration by including cows with a high volume of colostrum. The cows in this study were milked once a day so the time period from calving to first milking ranged up to 24 hours. The length of time until the first milking may be one of the variables that have contributed to the low IgG concentrations presented here.

Immunoglobulin absorption into the bloodstream of the calf occurs within approximately 24 hours of birth (Godden 2008; Quigley 2004; Weaver et al. 2000). Calves who are fed early within their first day of life will have higher serum IgG concentrations than those fed later due to the closure of the gut to macromolecular absorption (Godden 2008; Weaver et al. 2000). However, sampling the calves occurred 24 hours after them entering the shed (approximately within 48 hours of birth) which may have contributed to the lack of relationship between dam and calf IgG concentrations. Calves received their first feed after

removal from their dams by one of two different methods; but there are no reported effects of feeding technique on the concentration of serum IgG in calves fed via teat or oesophageal tube, with variation among calves more commonly due to the quality and amount of the colostrum fed (Weaver et al. 2000). The lack of relationship between serum and colostrum IgG concentrations indicated that there are other variables influencing successful colostrum transfer.

#### *Brix Refractometer*

No published data exists for the use of a digital Brix refractometer to estimate IgG concentration in colostrum from New Zealand pasture-fed dairy cows. Biemann et al. (2010) and Quigley et al. (2013) reported correlations of 0.73 and 0.75 respectively in North American Holstein cows, lower than that found in the current study (Figures 2 and 3).

Similarly there is no published data on the use of Brix refractometer to estimate IgG use in serum concentration of New Zealand dairy calves. Deelen et al. (2014) reported a correlation of 0.93, in Canadian dairy calves, higher than the correlations found in the current study (Figure 1).

Although the results from this experiment show a high correlation between IgG concentration and Brix percentage, using regression equations (Figures 1, 2, 3) to estimate colostrum or serum IgG concentration based on Brix refractometer reading will result in some samples falsely identified as being adequate or inadequate.

The North American studies used a different method of measuring IgG concentration, a radial immunodiffusion (RID) assay (Biemann et al. 2010; Quigley et al. 2013; Deelen et al. 2014) rather than TIA as used in the current study. The RID method, is considered a better estimate of IgG concentration in both colostrum and serum than the more rapid TIA method because the TIA measures the turbidity of the solution, and other components of colostrum can interfere with the assay (Quigley et al. 2013). The volume of colostrum produced and the proportion of colostrum protein can influence the accuracy of the refractometer as fat globules and casein molecules can change the refractive index of the milk (Biemann et al. 2010). Composition differences, especially a high-fat content and total solids can influence the refractive index (Biemann et al. 2010). As the cows were milked up to 24 hours post calving this is a possible source of variation in colostrum composition. Calves were aged between 0 and 24 hours old at the time of collection, and up to 48 hours old at the time of sampling and so creating variation in passive transfer of IgG from colostrum. Nevertheless, TIA is the method used commercially in New Zealand, so this is perhaps a more useful comparison.

## **Conclusion**

There was no difference in the colostrum IgG among cows of different breeds and different parity, which conflicts with previous reports from overseas. The findings presented here provide no evidence to discard colostrum from primiparous cows, or to discourage the process of bulking colostrum before feeding calves, however, further research is needed to confirm this. The relationships between IgG concentration and Brix % suggest that the refractometer is a good predictor of IgG concentration in serum samples and better predictor for colostrum samples. The relationship suggests that the refractometer could be a useful tool, to provide an inexpensive, real-time estimate of both colostrum quality and colostrum transfer to calves on New Zealand dairy farms.

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