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News and Views

Letter to the editor: Comments on Schalich et al. (2021), Colostrum testing with Brix is a valuable on-farm tool. doi.org/10.193/jas/skab083

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Dear Editor-in-Chief of the *Journal of Animal Science*,

In a recent publication in the *Journal of Animal Science*, Schalich et al. (2021) concluded that "Bx values do not reasonably indicate IgG concentration to serve as a measure of 'colostrum quality'." Multiple papers have been published that refute this conclusion. Additionally, the study didn't include poor quality colostrum samples so there is no validity in stating the Brix was not able to differentiate poor from good quality colostrum. A valid study determining the utility of the Brix for on-farm colostrum quality evaluation needs to include good- and poor-quality colostrum which the Schalich et al., study did not. Numerous papers supporting the use of the Brix have included good- and poor-quality colostrum and are valid studies supporting its use as a valuable on-farm tool for producers.

The publication presented some new and interesting information on the secretion of IgG into colostrum and transition milk. The authors used Western blot technology to quantitate IgG in both colostrum and transition milk which the authors contend is a more accurate method of estimating IgG compared with established technologies such as single RID, which is often referred to simply as RID. Unfortunately, a comparison of Western blot and RID IgG values was not conducted in their study, so we don't know how well they are correlated. The study's findings on the amount of IgG in transition milk collected from 16 to 52 hours postpartum was interesting and expected to be lower based on previous studies. There have been other recent studies showing the value of feeding transition milk within the first 24 hours of life. This study adds to that body of knowledge suggesting that colostrum production and delivery in a natural setting where calves suckle from their dam has a temporal relationship, suggesting the specific constituents of colostrum, transition milk, and milk differ and are delivered at an appropriate time for the age of the calf. There's been a significant amount of work done recently on colostrum management and health of newborn calves and this paper is a good example.

Although we are excited to continue to see research in colostrum production and management, we have concerns with the Schalich et al. paper. We are concerned that their conclusion that measurement of colostrum quality by Brix has no utility was not substantiated and could, in fact be harmful to on-farm efforts to manage calves for improved health. On-farm methods of measuring colostrum quality need to be fast and inexpensive, and the key advantage of

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using Brix is not to quantitate colostrum quality in a linear fashion, but to exclude poor quality colostrum from use for first feeding.

Only 27 colostrum samples were tested in the Schalich study and all of them were good quality having Brix values above 22% and more than 50 g/L of IgG; the authors did not include poor quality colostrum in their study. Good- and poor-quality colostrum samples are necessary to assess the utility of Brix for on-farm management. Their evaluation of Brix and grams per liter of IgG showed a low R^2 of 0.127 although the P value showed a trend at 0.068. The small sample size and homogeneity amongst colostrum quality in all samples of this study are important limitations that should prevent the authors from making generalized extrapolations and recommendations on the use of Brix for evaluation of colostrum quality in dairy operations. The authors of this study state that the "classification of "good" and "poor" quality colostrum as interpreted by °BX values is unfounded …" We believe this statement is not supported by the design nor the results from this study.

The relationship between IgG (measured by RID) and Brix has been shown in many species and across the globe. The number of studies showing the value of Brix to give us a useable guideline for good vs. not so good colostrum is impressive. In those studies, the R^2 value has been much higher, ranging from 0.6 to 0.9 (Quigley et al., 2013). Buczinski and Vandeweerd (2016) conducted a meta-analysis evaluating data from 11 studies and 4,251 colostrum samples. Their study confirmed colostrum with a Brix of 22% or greater had a 94.3% probability of having >50 g/L of IgG while colostrum with a Brix of < 18% only had a 22.7% probability of having an IgG concentration of > 50 g/L. There are three likely reasons why the R^2 in previous studies was higher than in this study, including different methodology of measuring IgG (western blot vs. RID or ELISA), range in colostrum quality, and the large number of samples tested (>100) in most of the previous studies. Ignoring all these evaluations based on the results of this study is not prudent.

The Brix refractometer has been promoted and used on farm as a method of identifying poor quality colostrum that should not be fed as the first feeding of colostrum. Although the Brix refractometer is undoubtedly a crude measure of IgG concentrations in colostrum, it is the tool that producers currently have to quickly and inexpensively identify poor quality colostrum. The Brix refractometer was primarily promoted as identifying poor quality colostrum and it is suggested not to use colostrum if the Brix value is less than 18 – 22%, depending on the recommendation, for the first feeding of calves. Colostrum with a Brix value <18% shouldn't be fed as the first feeding but could be fed at later colostrum feedings or if colostrum availability is an issue, the lower quality colostrum could be fed but should be followed by a feeding of commercial colostrum supplement.

Another concern is that the colostrum samples analyzed in the study were collected after the administration of oxytocin. A study by Sutter et al. (2019) showed that colostrum quality (measured by Brix and ELISA) increased when collected with the assistance of oxytocin compared with colostrum collected without its use. A few studies have also shown that milk composition is changed by administration of oxytocin and, therefore, the composition of colostrum samples in this study, in addition to the increased IgG, might have also been altered by using oxytocin (Gorewit and Sagi, 1984; Faraz et al., 2020).

Numerous methods have been used to measure IgG in colostrum, as recently reviewed by Ahmann et al. (2021). The Western blotting technique used by Schalich et al. to measure IgG in bovine colostrum appears novel and no comparisons have been conducted (to our knowledge) with other methods such as RID or ELISA in complex media such as colostrum. The authors rightly point out variable relationships between methods such as ELISA and RID (e.g., Gelsinger et al., 2015; Dunn et al., 2018; Sutter et al., 2019), and suggested that non-specific binding by antibodies will be incorporated into estimated results, increasing error in assays. Differences among immunological methods such as ELISA and RID may indeed be due to non-specific binding; however, other aspects of methods may also contribute to lack of correlation among techniques. Dunn et al. (2018) opined that extensive dilutions required with ELISA may be associated with differences among assays. Exposure of IgG to low pH in some methods of purification may result in formation of aggregates, which affect fragment, crystallizable (Fc) region-binding and may influence quantitation in various assays (Lopez et al., 2019). The immunoglobulins used to construct standard curves are particularly important, and differences among sources may influence results. Li-Chan and Kummer (1997) reported that standard antigen and antibody specificity are important when measuring IgG in milk or colostrum and they recommended that quantification of IgG in milk requires standard curves based on IgG purified from this source. Further, underestimation of IgG using ELISA may be related to IgG isotypes (IgG1 vs. IgG2) in the serum standards typically used for quantification in commercial kits. Methods such as ELISA may be more affected by selection of source of standard. Many of the variables affecting other assays for IgG could also affect the validity of the western blot method so more details of the standardization work for this assay would be necessary to draw conclusions as to the reliability of the reported method. There are highly significant relationships between serum IgG measured RID and calf health (Urie et al., 2018); measures of IgG in colostrum by RID are highly related to transfer of passive immunity, morbidity, and mortality in newborn calves, piglets, foals, lambs, and kids. We recognize that, while it may be less precise than other methods of IgG analysis, RID remains the "gold standard" for veterinary practitioners, diagnosticians, and regulatory agencies around the world.

We applaud the authors for their continued work in this important area of colostrum production and delivery. We caution the authors that the conclusions from their studies must have internal and external validity. The conclusion that the "classification of good- and poor-quality colostrum as interpreted by °Bx values is unfounded …" with a sample size of 27 high quality colostrum samples is not valid, generates confusion, and its adoption could be detrimental for dairy-calf health. We believe previous literature shows the utility of the Brix refractometer in identifying poor quality colostrum and urge producers to keep using this management tool until a better tool becomes available or we have clear evidence that Brix refractometer values are not helpful in identifying poor quality colostrum.

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