Abstract #2199 - Measuring IgG and Specificity in Milk Replacers

To view the presentation, scan here:

Roger L. Saltman[1], Shawn W. Jones [2], Michelle Tollefson [2]

[1] RLS Management Solutions LLC, Cazenovia, New York, USA
[2] Arkion Life Sciences, New Castle, Delaware, USA



EggTek® - C
A product of EgCel® Technology

See Poster #2495 - Improving Antibody Titers in Milk Replacers with IgY for related research

Introduction

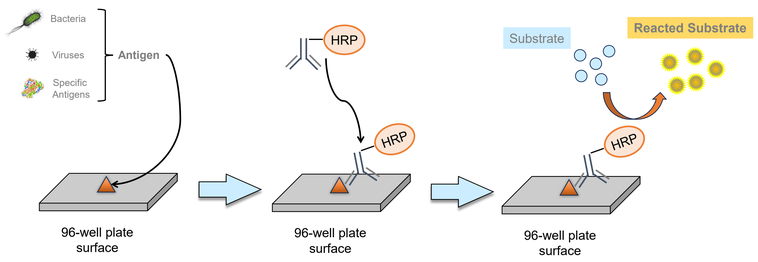
Calf Milk Replacers (CMRs) are formulated to provide calves an optimal nutritional source balancing protein, fat, and other nutrients. Protein is often derived from dried whey or other dried milk components, which can also contain IgG antibodies. These IgG, like those in colostrum, transition milk, or whole milk, can provide the calf with additional passive immunity, as the antibodies can bind recognized patohgens in the GI tract and cause them to be excreted in the calf's manure.

The Objective of this study was to determine the amount of IgG present in five commercial milk components-only CMRs (i.e. no plasma), and the specificity of these IgG to common causes of scours in pre-weaned calves.

Material and Methods

Five commercial CMRs were obtained from farm supply stores, randomized for the analysis, and reconstituted following manufacturer's instructions. First, the total IgG titers were found using a commercial ELISA kit. Second, IgG was purified from each CMR by: 1) acidifying the sample to remove casein, 2) precipitating the IgG with ammonium sulfate, 3) purification through a Protein A column, and 4) dialysis against PBS. The purified IgG were then conjugated to a horseradish peroxidase (HRP) and used in direct ELISAs to measure antigen specificity.

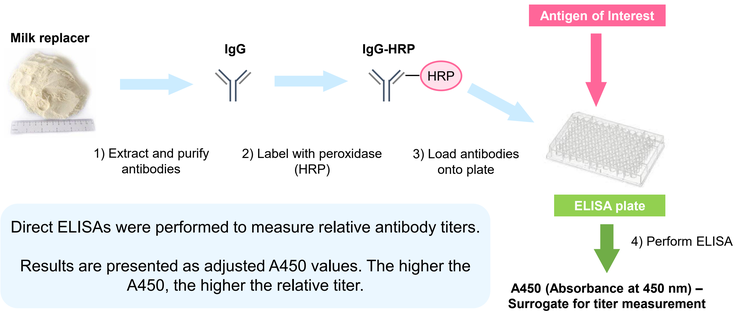
How ELISA Works



Antigens Tested via ELISA

Bovine rotavirus, Bovine coronavirus, *Cryptosporidium parvum*, *Escherichia coli* (mix of K88, K99, 987P, and F41), *Salmonella* Typhimurium, *Salmonella* Dublin, *Salmonella* Heidelberg, *Clostridium perfringens* (Type A & Type C/D)

How Analysis was Performed



Direct ELISAs were performed to measure relative antibody titers.

Results are presented as adjusted A450 values. The higher the A450, the higher the relative titer.

Results

For the assays, the amount of IgG used in each reaction was the equivalent to a 10 oz dose of CMR, which allows a comparison of each CMR based on equivalent masses. To determine this, first the total IgG concentration of each CMR was found and then scaled down proportionally.

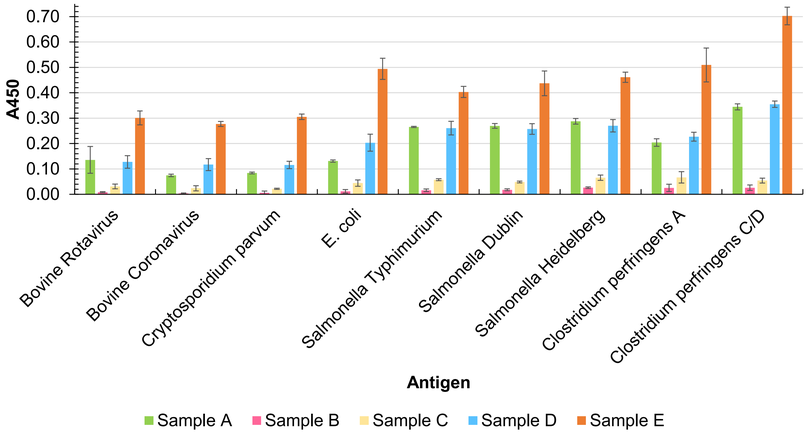
Total IgG Concentration		Standard Deviation
Sample A	11.1 mg/g	+/- 1.25 mg/g
Sample B	2.0 mg/g	+/- 0.11 mg/g
Sample C	3.8 mg/g	+/- 0.07 mg/g
Sample D	5.7 mg/g	+/- 0.24 mg/g
Sample E	12.2 mg/g	+/- 1.25 mg/g

Direct ELISAs were performed on nine antigens using the five CMR samples. The results are reported as the absorbance at 450 nm (A450). The A450 value is the raw output from the ELISA and is a measure of how much IgG is bound to the specific antigen of interest. Importantly, the A450 values cannot be compared between antigens, only between CMR samples for the same antigen. Each CMR sample was run in triplicate against each antigen, and the background absorbance was subtracted. The differences in A450 values between the different CMRs for each antigen were statistically significant (p-value: < 0.05) for all comparisons except for:

- Sample A vs Sample D for bovine rotavirus, *Salmonella* Typhimurium, *Salmonella* Dublin, *Salmonella* Heidelberg, *C. perfringens* Type A and *C. perfringens* Type C/D antigens
- Sample B vs Sample C for *C. perfringens* Type A antigen

Interestingly, though Sample A and Sample E have similar total IgG values, Sample E had significantly higher A450 values, while Sample A was more comparable to Sample D, which had about half the total IgG. Sample B, which had the lowest total IgG amount, also had the lowest A450 values for all the antigens tested.

Relative Titer per Dose of Milk Replacer



Conclusion

There was a large degree of variance between different CMR products both in terms of total IgG and the specificity of the IgG. The differences seen in total IgG values could reflect the different protein sources used in these milk component-only CMRs (e.g., whey protein concentrate vs. dried milk components). However, the total IgG value does not tell the whole story. Though Samples A and Sample E have comparative total IgG values (p-value = 0.354), Sample E had statistically greater A450 values for all antigens tested. Thus, it is important to know not only the total IgG of a CMR but also the antigen specificity of the IgG, because if the IgG has no specificity for potential pathogens, it cannot help protect the calf. For ways to improve antibody titers in the CMRs, see poster #2495 - Improving Antibody Titers in Milk Replacers with IgY.

RLS Management Solutions
Arkion Life Sciences

ETKC-0624-05