Enhancing Protein Titer in CHO Cells with a Novel Protein-Free Two-Part Feed System

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ABSTRACT

Chinese Hamster Ovary (CHO) cells are the most widely used mammalian host in biological research, particularly for the expression of human therapeutic proteins. By combining metabolomic analyses with high throughput processes, we have developed a novel product that consistently demonstrates enhanced titer performance in multiple CHO-K1 cell lines.

We have successfully developed a two-part feed that significantly raises the bar for expected performance in bioproduction cell lines to maximize titer.
There is growing demand for a feed system that can support the nutritional requirements of high cell density cultures as well as yield higher protein titer. Chemically-defined feeds can enhance protein titer in a broad range of cell lines ideal for industrial applications and can provide consistency, unlike non-chemically defined formulations which can cause variability between batches and affect protein quality.
Using metabolomic analyses, High Throughput Screening (HTS), and Design of Experiments (DOE), we have developed a two-part chemically-defined feed system to increase protein titer for CHO-K1 cell lines.

We evaluated performance of our two-part feed system with CHO-K1 and CHO-K1 GS cell lines that are producing industry-relevant monoclonal antibodies. Our results show up to 40% higher titer compared to internal feeds in benchtop bioreactors.
In addition, our two-part feed system increased cell specific productivity compared to commercially available feeds in multiple CHO-K1 and CHO-K1 GS cell lines.

Efficient-Pro™ Feed 3 and Efficient-Pro™ Feed Enhancer

- Animal component free
- Protein-free
- Efficient-Pro™ Feed 3 available as liquid and AGT
- Efficient-Pro™ Feed Enhancer available as liquid and DPM

MATERIALS AND METHODS

Cell culture: CHO-K1 and CHO-K1 GS cells expressing IgG antibodies were cultured in Efficient-Pro Medium as well as a commercially available medium from another supplier supplemented with 6mM L-glutamine and 1% Anti-Clumping Agent (ACA) from Gibco for CHO-K1 cells and 1x GS supplement from Sigma and 1% ACA from Gibco for CHO-K1 GS clone #1 and 1mg/L insulin for CHO-K1 GS clone #2 . Cultures were maintained at 37˚C, 8% CO_2, 125 rpm. IgG was measured using a Cedex™ BioHT Analyzer (Roche).

Shake flask study: Shake flasks were seeded at $0.3x10^6$ viable cells/mL in triplicate. Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer were fed at 3% and 0.3% for CHO-K1 cells, respectively, daily on days 3-13. The internal control was fed at 3% daily, on days 3-13. Glucose was fed to 6g/L when measured glucose dropped below 3g/L.

Fed batch ambr™ study: Ambr™15 bioreactors (Sartorius AG) were seeded at $0.3x10^6$ viable cells/mL in triplicate with the following conditions: pH 7.05 +/- 0.05, 50% DO, 37˚C, and 1200 rpm. Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer were fed at 2.6% and 0.26% for CHO-K1 GS clone # 1 and at 2% and 0.2% for CHO-K1 GS clone #2, respectively, daily on days 3-13. The internal control was fed at 2% for CHO-K1 GS clone # 1 and at 1.5% for CHO-K1 GS clone # 2 daily, on days 3-13. The other supplier's feed was a 2-part feed that was tested according to the manufacturer's recommendations. Glucose was fed to 6g/L when measured glucose dropped below 3g/L.

Fed batch 3L bioreactor study: 3L HyPerforma glass bioreactors were seeded at $0.3x10^6$ viable cells/mL in triplicate with the following conditions: pH 7.05 +/- 0.05, 30% DO, 37˚C, and 435 rpm. Carbon dioxide and sodium carbonate were used to control pH. Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer were fed at 2.6% and 0.26% respectively daily on days 3-13. The internal control was fed at 2% daily, on days 3-13. The other supplier's feed was a 2-part feed that was tested according to the manufacturer's recommendations. Glucose was continuously fed to maintain reactor concentrations of 2 g/L

RESULTS

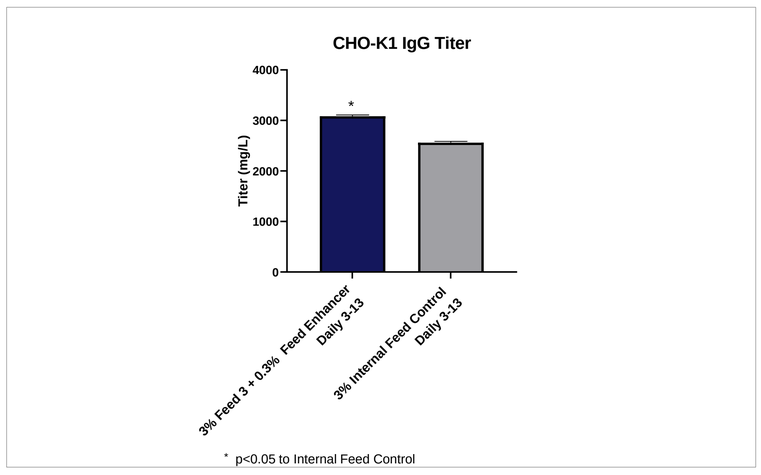


Figure 1. Efficient-Pro Feed 3 + Feed Enhancer outperformed Internal Feed Control in CHO-K1 Cell Line

CHO-K1 cells were evaluated in triplicate in shake flasks in Efficient-Pro Medium. Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer were fed at 3% and 0.3%, respectively. Internal control was fed at 3%. Supplementation with Enhancer feeds increased IgG titers by 20.5%.

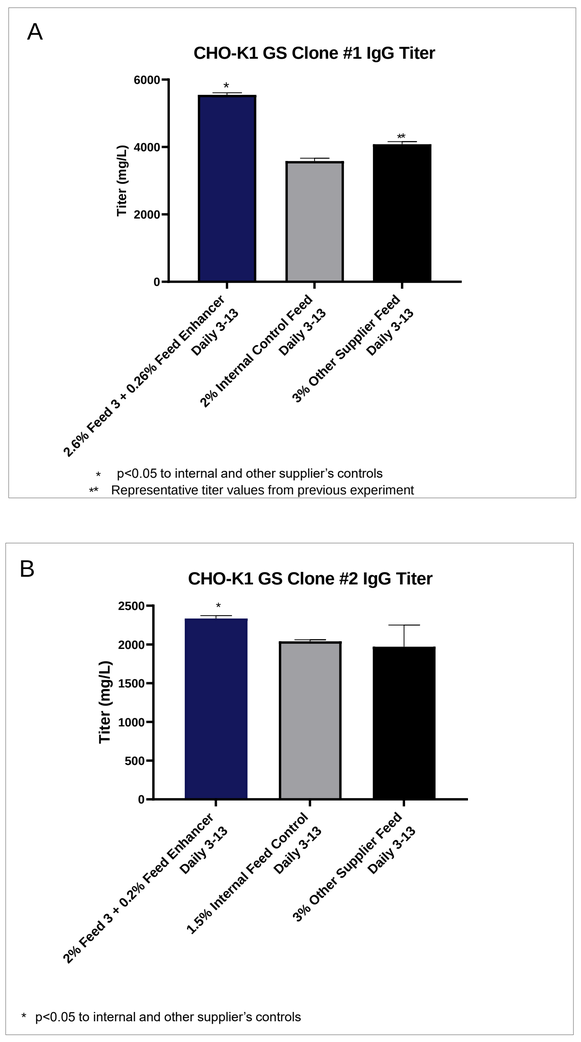


Figure 2. Efficient-Pro Feed 3 + Feed Enhancer outperformed Internal Feed Control and Other Supplier's Feed in CHO-K1 GS Clones #1 and #2

Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer in Efficient-Pro Medium were evaluated in an ambr™ 15 against internal control feed in Efficient-Pro Medium and medium and feed from another supplier in CHO-K1 GS cells producing different antibodies (clones #1 and # 2). A) Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer were fed at 2.6% and 0.26%, respectively. Efficient-Pro Feed 3 and Feed Enhancer increased IgG titers by 55% over internal control and by 36% over other supplier's Feed. B) Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer were fed at 2% and 0.2%, respectively. Efficient-Pro Feed 3 and Feed Enhancer increased IgG titers by 14.5% over internal control and by 18% over other supplier's control.

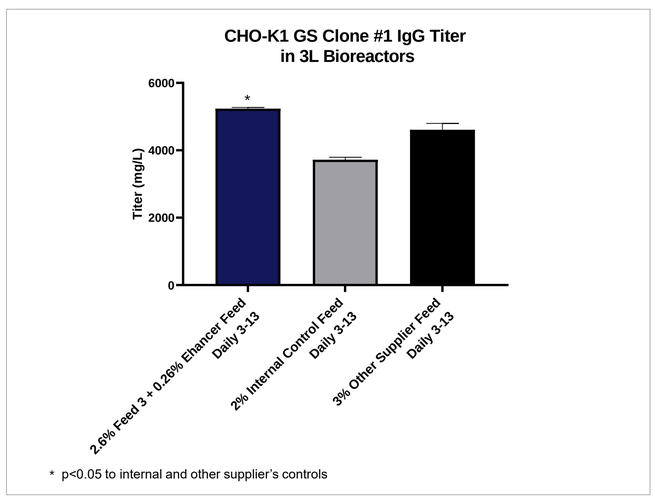
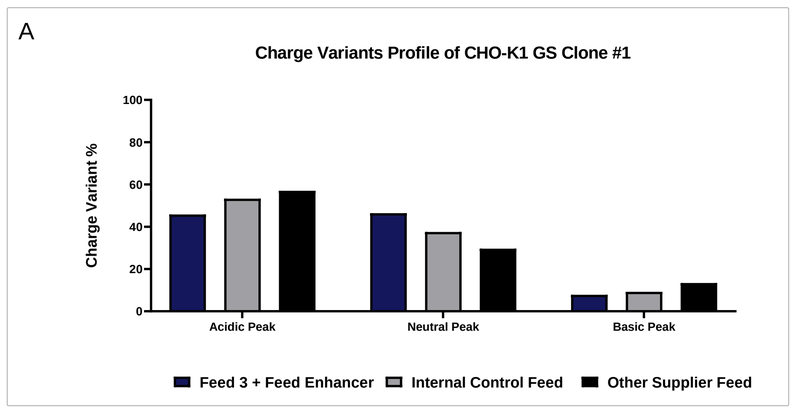


Figure 3. Scale up of Efficient-Pro Feed 3 + Feed Enhancer to 3L Bioreactors in CHO-K1 GS Cell Line

Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer were evaluated in 3L bioreactors against internal control feed and other supplier feed in CHO-K1 GS in triplicate in 3L bioreactors. Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer were fed at 2.6% and 0.26%, respectively. Efficient-Pro Feed 3 and Feed Enhancer in Efficient-Pro medium increased IgG titers by 41% over internal control in Efficient-Pro medium and by 14% over control in another supplier's medium.



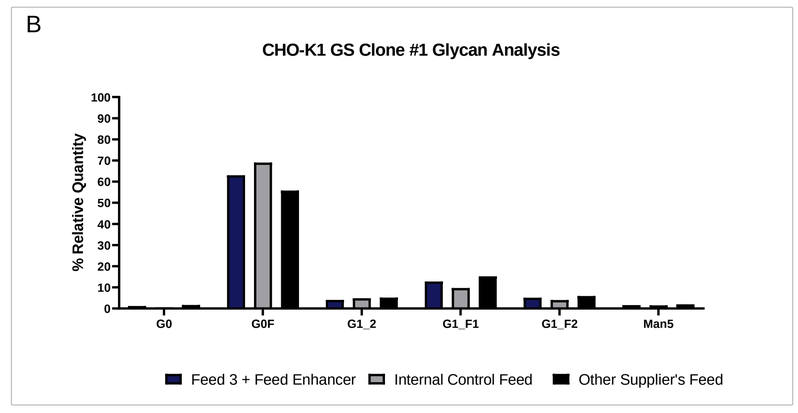


Figure 4. Charge Variant and Glycan profiles of IgG produced with CHO-K1 GS comparing Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer to Internal and Other Supplier's Feeds

A) CHO-K1 GS cells were evaluated in Ambr™ 15 bioreactors in triplicate. Day 14 charge variant profiles with Efficient-Pro Feed 3 and Efficient-Pro Feed Enhancer in Efficient-Pro medium were compared to an internal feed in Efficient-Pro medium and medium and feed from another supplier. Comparisons show that Efficient-Pro Feed 3 and Feed Enhancer in Efficient-Pro Medium have a higher neutral peak as desired for this IgG molecule. B) Feeding with Efficient-Pro Feed 3 and Feed Enhancer in Efficient-Pro Medium led to minor changes in glycosylation. Man-5 values were below 5%.

CONCLUSIONS

In effort to support CHO-based antibody therapies with improving effective culture and enhanced protein production, we designed a new robust 2-part feed system that is animal origin free and protein-free.

Our new formulations support consistent protein quality and high titer protein production and increased cell specific productivity compared to other commercially available feeds in multiple CHO-K1, and CHO-K1 GS cell lines which can facilitate downstream purification.

Efficient-Pro Feed 3 will be offered in liquid and Advanced Granulation Technology (AGT) formats and Efficient-Pro Feed Enhancer will be offered in liquid and Dry Powder Media (DPM) formats.

Efficient-Pro™ Feed 3 and Efficient-Pro™ Feed Enhancer – formulated with Animal Origin Free and Chemically-Defined components only

Performance
- Improved titer and specific productivity compared to internal feed controls and other supplier controls

Improved process
- Consistency and scalability
- Reduced risk of variability from non- defined components

Ease of use
- Multiple package volumes
- Multiple formats available

ACKNOWLEDGEMENTS

We would like to acknowledge Megan Pajak-Lee, Juliet Macharia, Neelanjan Sengupta, Christopher Brau, Mitch Maloy, Jeff Mulkin, Liam Gionis, Darius Rub, Jaime Goldfuss, Rula Abdallah, and Kayla Kerr for their support with this project.
We also would like to acknowledge Healsun Biopharm for providing CHO-K1 GS Clone #1.

TRADEMARKS

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