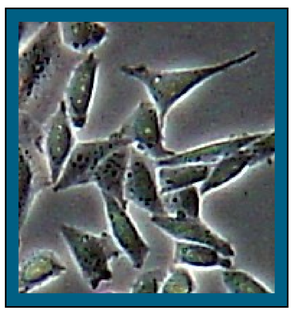

Development and application of a series of chemically defined and complex feed supplements for Extended Growth and Enhanced productivity in CHO-K1 and CHO DG44 Cultures

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Introduction

Fed-batch is currently one of the most attractive modes of operation for the large scale cultivation of CHO cells. With the introduction of QbD, a risk based approached to pharmaceutical development and manufacturing, new pressures have been placed on scientists formulating culture media for large scale, fed-batch processes. It is common for businesses to commit a great deal of time and cost to the development of an optimal feed formulation which fits the needs of both the process and the company. In response to customer requirements, Kerry has developed an animal component free (ACF) complex feed system, Sheff-Pulse I & II and a chemically defined (CD) feed system, Sheff-Feed CD. Along with the feeds, Kerry has also recently developed a completely chemically defined CHO media, Sheff-CHO CD Complete. This media has a low osmolality so that it can easily be combined with our various animal component free supplements.

The Sheff-Pulse system comprises a series of complex fed-batch supplements optimized to deliver consistent improvement of process performance parameters in a variety of CHO cell lines. The animal-component-free feed systems exploit known synergies among plant derived hydrolysates, yeast extracts and recombinant proteins to promote cell growth, optimal cell metabolism and enhanced protein production.

Sheff-Feed CD is a powdered, animal component free, peptide/protein free, chemically defined feed supplement that has demonstrated compatibility with multiple CHO cell lines and substantially improves product titers when used in fed-batch vs. batch culture.

Kerry's newly developed Sheff-CHO CD Complete media is an animal component free, peptide/protein free, chemically defined media that has been specifically formulated to increase protein production across a wide range of CHO cell lines.

The purpose of this study was to analyze the performance of all three of these products across a number of cell lines, comparing their performance using competitor controls.

Materials and Methods

CHO DG44: Data was collected using a transfected CHO DG44 DHFR- line engineered to express IgG and adapted to serum-free suspension culture. Cultures were seeded at 1.0 x 10^6 cells/ml and maintained at 37 °C from days 0-3, then shifted to 33 °C for the remainder of the experiment. 1 L Dasgip bioreactors were operated at a 500 ml working volume, 6.8-7.2 pH range controlled via Na_2CO_3 and CO_2 addition, 50 % DO with a 0.5-1.5 mL/min sparged gas flow rate and 150 rpm agitation rate. Cultures were grown in the Sheff-CHO CD Complete media supplemented with 10 mg/L Sheffield rInsulin ACF & or else a chemically defined, commercially available CHO specific basal media supplemented with 1X hypoxanthine/thymidine, 10 mg/L Sheffield rInsulin ACF, 0.1 % lutrol F-68 and 4 mM L-glutamine. The unsupplemented controls were fed with a 6 g/l glucose shot on day 3 and day 7.

CHO-K1: Data was collected using a transfected CHO-K1 line engineered to express SEAP and adapted to serum-free suspension culture. Cultures were seeded at 8.0 x 10^5 cells/ml and maintained at 37 °C from days 0-3, then shifted to 33 °C for the remainder of the experiment. 1 L Dasgip bioreactors were operated at a 500 ml working volume, 6.8-7.2 pH range controlled via Na_2CO_3 and CO_2 addition, 50 % DO with a 0.5-1.5 mL/min sparged gas flow rate and 150 rpm agitation rate. Cultures were grown in a chemically defined, commercially available, CHO specific basal media supplemented with 0.1 % pluronic, 4 mM L-glutamine, 0.75 mg/ml G-418 and 10 mg/L Sheffield rInsulin ACF.

Supplement/Sample Preparation:

Sheff-Pulse supplementation was achieved via the use of filter-sterilized 100 g/l stock solutions prepared in the basal medium. The basal media was supplemented with 6 g/l Sheff CHO Plus ACF. Sheff–Pulse I was added at 5 g/l on day 3, while Sheff-Pulse II was added at 2 g/l on day 5, 7, 9 & 11. Competitor supplements were used at the recommended dosages and times per manufacturer's instructions. Day 6, 8, 10, 12 & 14 supernatant samples were analyzed by HPLC for SEAP or IgG concentration. On multiple days, culture samples were removed and analyzed using the Nova Flex bioanalyzer yielding cell density/viability data along with nutrient and metabolite profiles.

Sheff-Feed CD supplementation was achieved via a 87.0 g/l stock solution, which was prepared by dissolving the powdered product in ccH_20 and sterilizing using a 0.2 µm PES filter. The CHO DG44 cell line A was fed at 10 % of initial working volume with Sheff-Feed CD stock solution. The CHO-K1 cell line was fed at 20 % of initial working volume with Sheff-Feed CD. The competitor feed supplements were prepared (as directed by the manufacturer) in ccH_20, filtered using a 0.2 µm PES filter and fed at the optimal concentration, which was found to be 20 % of the initial working volume for both cell lines. All culture conditions were fed on days 3, 5, 7, 9 and 11. Supernatant samples were collected on day 6, 8, 10, 12 and 14 and analyzed by HPLC for either SEAP or IgG concentration. On multiple days, culture samples were removed and analyzed using the Nova Flex bioanalyzer, yielding cell density/viability data along with nutrient and metabolite profiles (not shown).

Summary

The first step involved analyzing the performance of the complex Sheff-Pulse feed system in Sheff-CHO CD Complete basal media. It is clear from Figure 1 & 2 that the unsupplemented Sheff-CHO CD Complete media control greatly out performs the unsupplemented competitor CD media control with regard to titer, represented by the two-fold increase indicating the superiority of the Sheff-CHO CD Complete media. The addition of the Sheff-Pulse feed results in a 30% increase in titer when using the Sheff-CHO CD Complete basal media, and a 40% increase in titer when using the competitor CD basal media.

The use of Sheff-Feed CD in CHO-K1 & CHO-DG44 was also analyzed in this study. Supplemented into a chemically defined basal media, the Sheff-Feed CD system demonstrated an equivalent or enhanced ability to support both CHO-K1 and CHO-DG44 culture performance when compared to a chemically defined competitor feed as seen in Figures 3, 4, 5 and 6. Application of the Sheff-Feed CD supplement to a CHO specific, chemically defined basal media demonstrated performance improvements over an unsupplemented control in both CHO-K1 and CHO DG44 models. Results demonstrate that the Sheff-Feed CD is an effective chemically defined feed option for various CHO cell lines.

Conclusion

In this study, both the Sheff-Pulse & Sheff-Feed CD feeds have been shown to greatly improve productivity in CHO cells across a number of CHO cell lines. The newly designed Sheff-CHO CD complete media was also tested, which greatly improves titer when compared to a competitor CD media control. To conclude, Kerry now offers not only a high-performing and superior complete chemically defined media, but also both the complex Sheff-Pulse feed & chemically defined Sheff-Feed CD supplements (or systems) to cater to all aspects of customers' desires to significantly increase protein production in CHO cells.

Figure 1: CHO DG44
Sheff-Pulse Fed-batch
Viable Cell Density and Viability

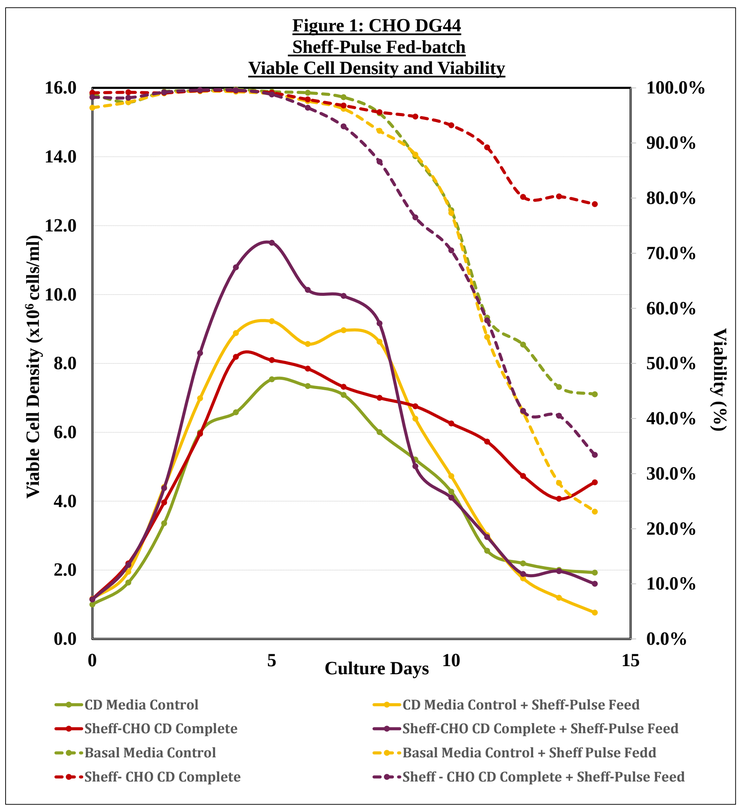
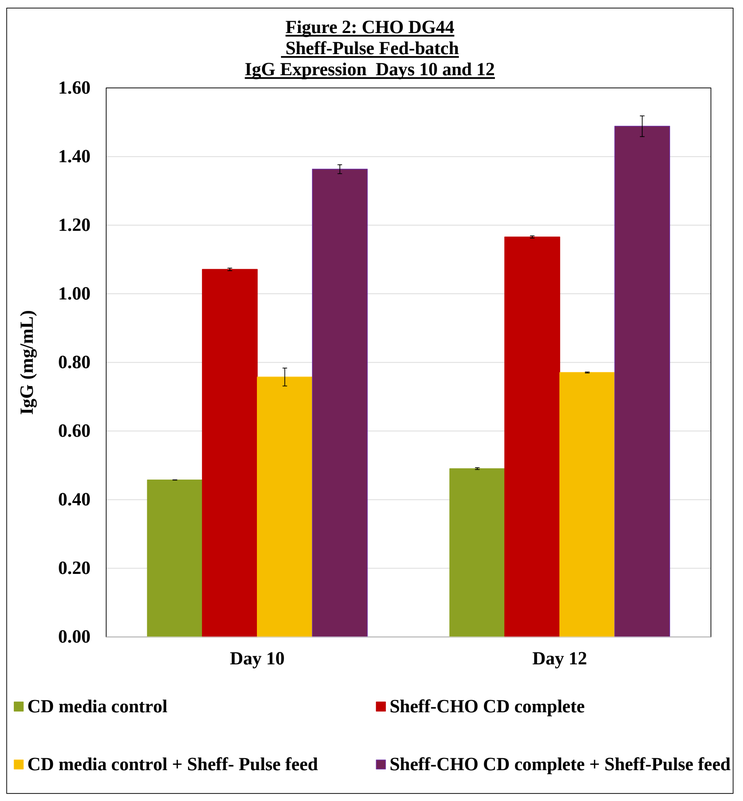


Figure 2: CHO DG44
Sheff-Pulse Fed-batch
IgG Expression Days 10 and 12



Figures 1 and 2: Sheff-Pulse I was administered at 5 g/l of the initial bioreactor working volume to a CD media control and to Sheff-CHO CD Complete. CHO DG44 cultures were supplemented with a 2g/l Sheff- Pulse II (of initial working volume) feed on days 5, 7, 9 and 11 of the experiment. An unsupplemented CD media control and unsupplemented Sheff-CHO CD complete were also included. The effects of the Sheff-Pulse Feed system on viable cell density, culture viability and protein titer was assessed to determine overall culture health and productivity. The unsupplemented Sheff-CHO CD Complete & the Sheff Pulse fed Sheff-CHO CD Complete cultures reached higher cell densities and produced higher IgG titer than the unsupplemented CD media control and Sheff-Pulse fed CD media control respectively.

Figure 3: CHO DG44
Sheff-Feed CD Fed-batch
Viable Cell Density and Viability

Figure 4: CHO DG44
Sheff-Feed CD Fed-batch
IgG Expression Days 6-14

Figures 3 and 4: Sheff-Feed CD was administered at 10 % of the initial bioreactor working volume and compared to a competitor feed supplement, which was fed at 20 % of initial working volume. CHO DG44 cultures were supplemented with a 4 % (of initial working volume) feed of the appropriate supplement on days 3, 5, 7, 9, and 11 of the experiment. The effects of each feed supplement on viable cell density, culture viability and protein titer was assessed to determine overall culture health and productivity. Despite a lower cell density later in the experiment, the Sheff-Feed CD supplemented culture was able to out perform the competitor product with respect to IgG production.

Figure 5: CHO-K1
Sheff-Feed CD Fed-batch
Viable Cell Density and Viability

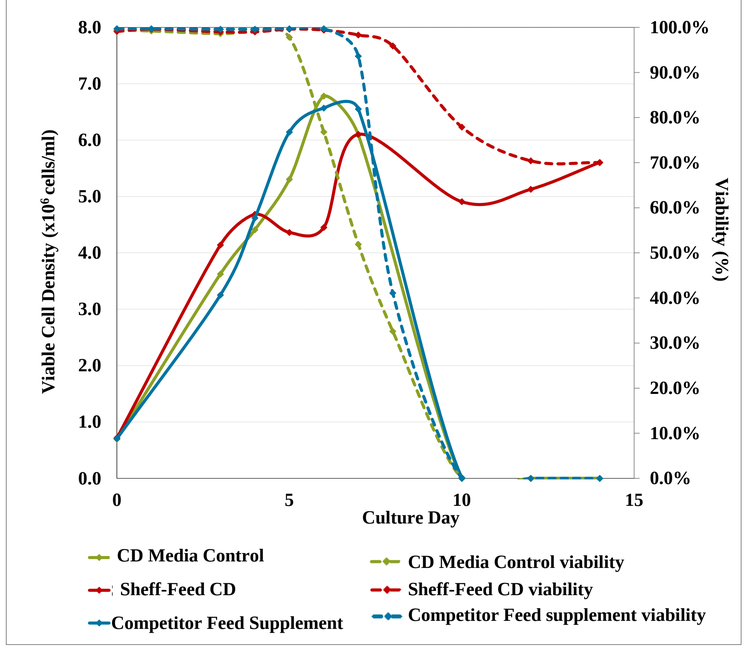
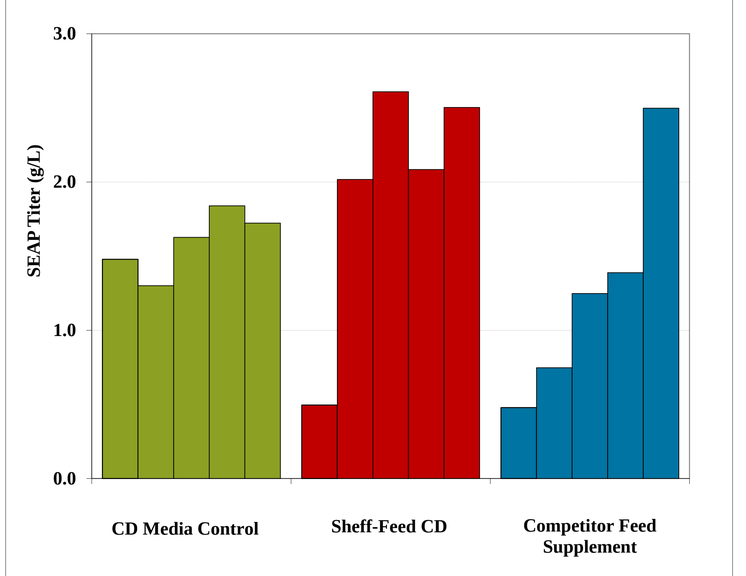


Figure 6: CHO-K1
Sheff-Feed CD Fed-batch
IgG Expression Days 6-14



Figures 5 and 6: Sheff-Feed CD was administered at 20 % of the initial bioreactor working volume and compared to a competitor feed supplement, which was also fed at 20 % of initial working volume. CHO-K1 cultures were supplemented with a 4 % (of initial working volume) feed of the appropriate supplement on days 3, 5, 7, 9, and 11 of the experiment. The effects of each feed supplement on viable cell density, culture viability and protein titer was assessed to determine overall culture health and productivity. While Sheff-Feed CD supplementation was able to support growth and steady SEAP production throughout the experiment, the competitor feed was unable to support cell density in the CHO-K1 line.