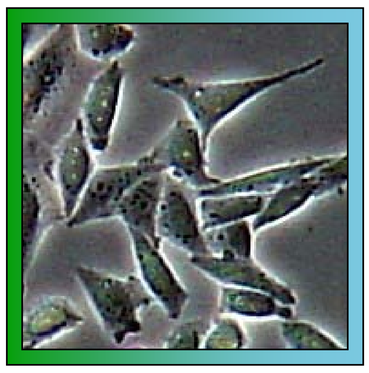

SHEFF-PULSE: AN ANIMAL COMPONENT FREE COMPLEX FEED SYSTEM THAT ENHANCES GROWTH AND PRODUCTIVITY OF CHO-K1 AND CHO DG44 CULTURES.

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Introduction

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. By focusing on the specific nutritive needs of common biopharmaceutical production CHO lines, the Sheff-Pulse systems provide a convenient and powerful tool for process development scientists tasked with delivering an efficient and robust fed-batch process. In order to evaluate the Sheff-Pulse system's utility vs. a competitor feed supplement, fed-bath shake flask experiments were performed in both CHO-K1 and CHO DG44 DHFR- cell lines, expressing secreted embryonic alkaline phosphatase (SEAP) and IgG respectively. Finally, the Sheff-Pulse system was tested at the 1L bioreactor scale.

Figure 1: Sheff-Pulse vs. Competitor in CD basal medium Viable Cell Density and Viability

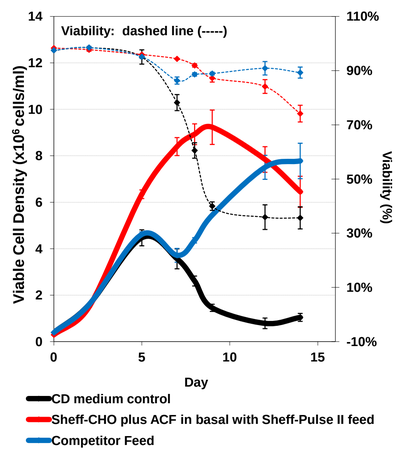


Figure 2: Sheff-Pulse vs. Competitor in CD basal medium SEAP Production Day 12

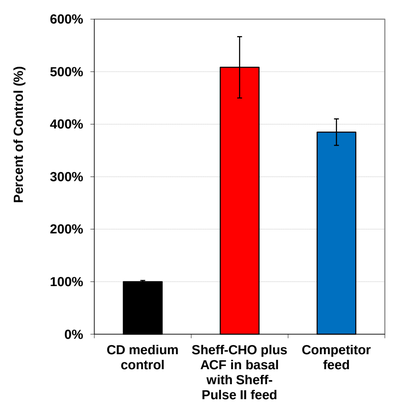


Figure 3: Sheff-Pulse vs. Competitor in DMEM Viable Cell Density and Viability

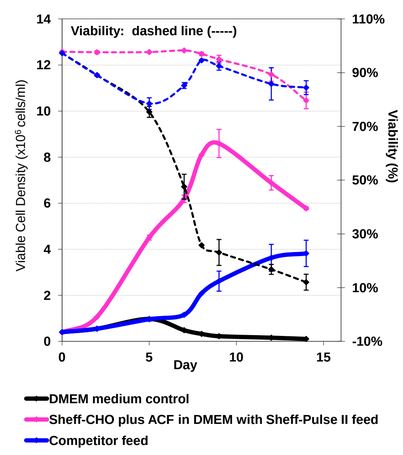
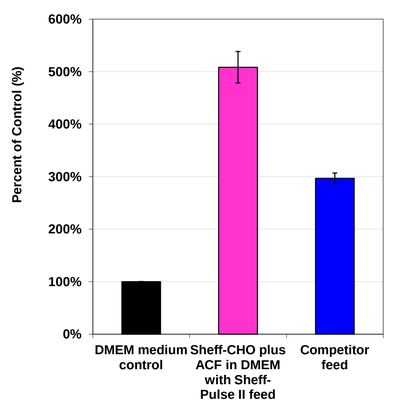


Figure 4: Sheff-Pulse vs. Competitor in DMEM SEAP Production Day 12



Figures 1-4: *The impact of the Sheff-Pulse feed system on CHO-K1 was assessed in both a classic medium (DMEM) and a commercially-available, chemically defined medium optimized for CHO cells. In addition, performance of the Sheff-Pulse feed system was evaluated against a commercially available feed supplement in both basal media. The optimized Sheff-Pulse feed system for CHO-K1 is comprised of Sheff-CHO plus ACF supplemented at 5 g/l in the basal medium, a 5 g/l dose of Sheff-Pulse I on day 5 and 2 g/l of Sheff-Pulse II on days 7, 9, and 12. Competitor feed was supplied to cultures at the recommended dosage. The Sheff-Pulse feed system outperformed the competition in an optimized CD basal media and DMEM. Supplementation of DMEM basal with the Sheff-Pulse feed system results in significant improvements over the control and provides a fully ACF DMEM supplementation strategy. Sheff-Pulse outperformed the competitor feed in both basal formulations with regards to productivity.*

Materials and Methods

CHO-K1: *Data was generated in a transfected CHO-K1 cell line engineered to express SEAP and adapted to serum-free suspension culture. Cultures were grown in 125 ml shake-flasks with an initial working volume of 35 ml. Triplicate cultures were seeded at 4.0 x10^5 cells/ml and incubated at 37°C in 5% CO_2 at 130 rpm for 14 days. All basal media were supplemented with 0.2% pluronic, 4mM L-glutamine and 0.75 mg/ml G-418.*

CHO DG44: *Data was collected using a transfected CHO DG44 DHFR- cell line engineered to express IgG and adapted to serum-free suspension culture. Cultures were grown in 125 ml shake-flasks with an initial working volume of 35 ml. Triplicate cultures were seeded at either 6.0 or 10.0 x10^5 cells/ml and incubated at 37°C from days 0-3 and 33°C from day 3 on. Shake flask experiments were maintained for up to 14 days in a 5% CO_2 environment with an agitation rate of 130rpm. 1 L Dasgip bioreactors were operated at a 600ml wv, 6.8-7.2 pH range controlled via Na_2CO_3 and CO_2 addition, 50% DO with a 2mL/min sparged air flow rate and 200rpm agitation rate. All basal media were supplemented with 0.2% pluronic, 4mM L-glutamine. 10mg/l Sheffield CC grade recombinant insulin and 25nM methotrexate.*

Supplement Preparation and Application: *Sheff-CHO plus ACF and Sheff-Pulse feed supplementation was achieved via the use of filter-sterilized 100 g/l stock solutions prepared in the basal medium. Sheff-CHO plus ACF was dosed at 5 g/l in the basal medium. A 5 g/l bolus dose of Sheff-Pulse I was administered on day 5 for CHO-K1, followed by 2 g/l doses of Sheff-Pulse II every other day. On day 3, 5 g/l of Sheff-Pulse II was administered to CHO DG44, followed by 2 g/l additions every other day. Competitor supplements were used at the recommended dosages and times per manufacturer's instructions.*

Sample Processing: *CHO-K1 supernatant samples were collected on day 12 and analyzed for SEAP with anion-exchange HPLC. CHO DG44 IgG titers were generated using the Nova Flex Bioanalyzer IgG module. On multiple days, culture samples were removed and analyzed for cell density, viability, nutrient and metabolite data on the Nova Flex bioanalyzer.*

Figure 5: Sheff-Pulse vs. Competitor CHO DHFR- Shaker Flasks Viable Cell Density

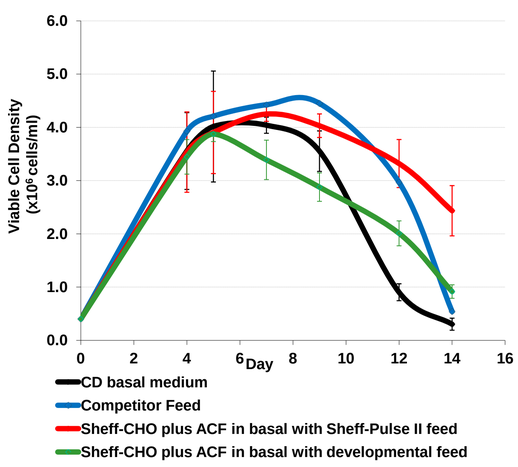


Figure 6: Sheff-Pulse vs. Competitor CHO DHFR- Shaker Flasks Viability

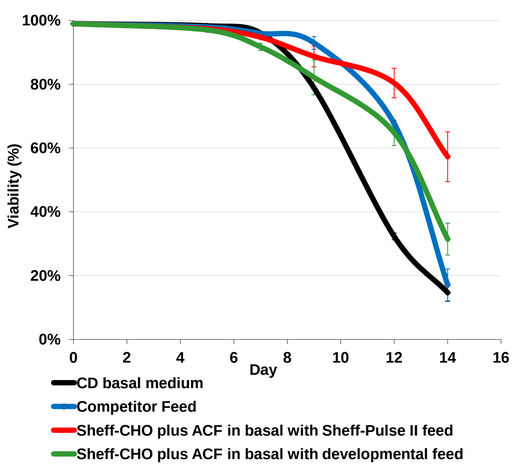
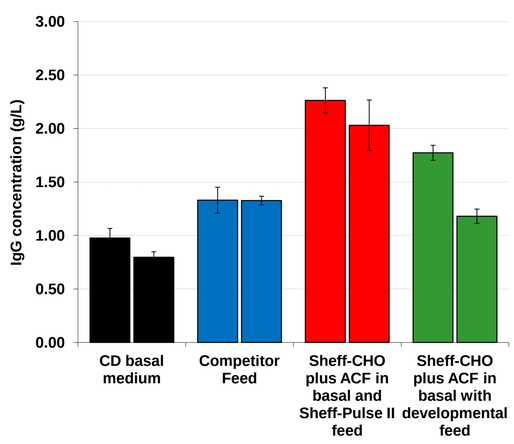


Figure 7: Sheff-Pulse vs. Competitor CHO DHFR- Shaker Flasks IgG Titer days 12 and 14



Figures 5-7: *The Sheff-Pulse feed system was evaluated against a competitor feed in an IgG expressing CHO DG44 line. The Sheff-Pulse feed system for CHO DG44 includes Sheff-CHO plus ACF in the basal medium and Sheff-Pulse II fed at 5 g/l on day 3 and 2 g/l on days 5, 7 and 9. It may be necessary to extend the feeding schedule at bioreactor scale. Sheff-Pulse outperformed the competitor feed with respect to viability and titer. A developmental feed formulation also demonstrated improvement in titer when compared to the competitor feed. Sheff-CHO plus ACF was supplemented into the basal medium at 5 g/l for both Sheff-Pulse feed conditions. The developmental feed, like Sheff-Pulse II, was dosed at 5 g/l on day 3 and 2 g/l on days 5, 7 and 9. Competitor supplements were supplied at the recommended dosages and times per manufacturer's instructions.*

Figure 8: Sheff-Pulse vs. Competitor CHO DHFR- Bioreactors Viable Cell Density and Viability

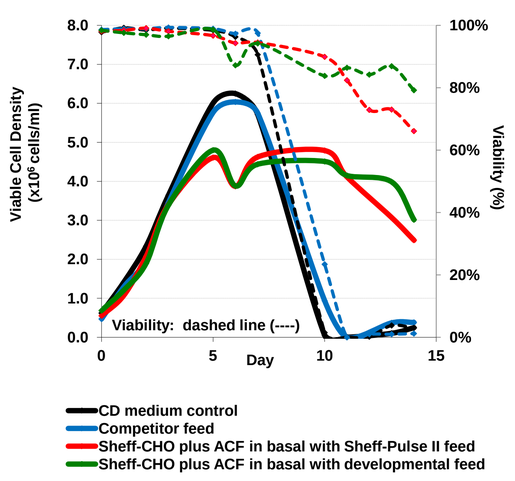


Figure 9: Sheff-Pulse vs. Competitor CHO DHFR- Bioreactors Lactate and Ammonium

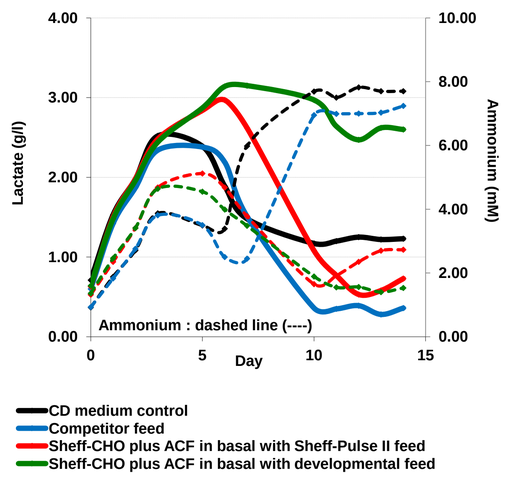
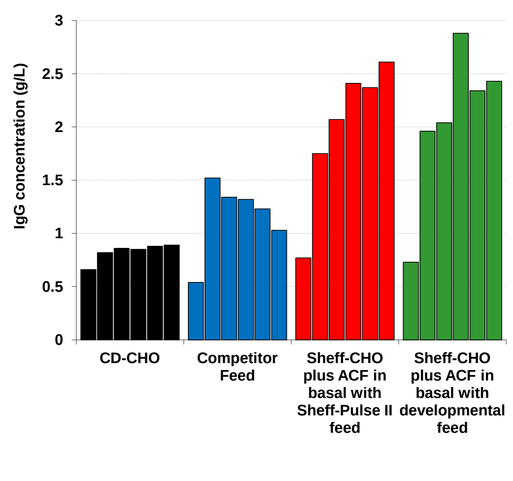


Figure 10: Sheff-Pulse vs. Competitor CHO DHFR- Bioreactors IgG Titer days 6 & 10-14



Figures 8-10: *The growth and productivity improvements observed when supplementing with the Sheff-Pulse feed system in a shake flask model were reproduced at the 1L bioreactor scale. The Sheff-Pulse feed system optimized for CHO DG44 consists of Sheff-CHO plus ACF at 5 g/l in the basal medium, Sheff-Pulse II dosed at 5 g/l on day 3 and 2 g/l on days 5, 7, 9, and 12. Feed dosage and timing was the same for the developmental Sheff-Pulse feed system, with an experimental formulation supplemented in place of Sheff-Pulse II. Competitor supplements were fed according to manufacturer's recommendations. Both Sheff-Pulse supplements tested were capable of extending the CHO DG44 growth curve beyond that of the commercially available, CHO specific, CD basal control. On day 14, Sheff-Pulse supplemented reactors maintained the highest cell density. Ammonium levels were healthy for this cell line with both Sheff-Pulse fed reactors. Osmolarity for all conditions did not exceed 340 mOsm. IgG titer levels were significantly higher in the Sheff-Pulse supplemented reactors throughout the experiment, leading to a more than 25% increase in IgG concentration on day 14, when compared to a competitor feed supplement.*

Summary

Supplementation with the Sheff-Pulse feed systems may be employed to reduce CHO-K1 based biopharmaceutical process costs by allowing for the substitution of CD media with a classic DMEM basal in combination with the feed system. Sheff-Pulse may also be supplemented into chemically defined basal formulations to improve culture growth and productivity in both CHO-K1 and CHO DG44 based processes. Sheff-Pulse improved product yield over a competitor feed supplement in both CHO-K1 and CHO DG44 shake flask experiments. Similar improvement in product yield was demonstrated at the 1L bioreactor scale in CD medium optimized for CHO cells. The flexibility and performance enhancing properties of the Sheff-Pulse feed systems deliver value by streamlining process development, increasing product yield and reducing time to market.