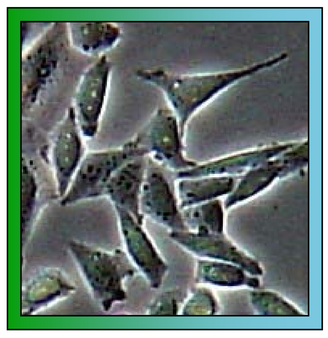

Comparison of Performance Enhancing Effects of Supplementation with a Complex Feed System when Applied to Multiple CHO Basal Media.

Karen A Benedict, Amanda L Perlman, John F Menton, Christopher P Wilcox and Hans Huttinga - ***Kerry, 3400 Millington Road, Beloit, WI 53511***
john.menton@kerry.com - http://www.SheffieldBioScience.com

Introduction

Feed systems are commonly utilized by the Biopharmaceutical industry during a production run in order to maximize the yield of recombinant proteins. Previous work has demonstrated synergies among plant-derived hydrolysates, yeast extracts and recombinant proteins for enhancement of CHO fed-batch cultures. Building on this knowledge, a CHO specific ACF (animal component free) feed system was developed with the intent to increase biopharmaceutical process yield, improve culture health and reduce media-related cost. A series of shake flask experiments were performed in a CHO-K1 cell line engineered to produce Secreted Embryonic Alkaline Phosphatase (SEAP). The efficacy of the developmental Sheffield ACF feed system was assessed when applied to both a classic medium (DMEM) and a commercially-available chemically defined CHO cell medium. In addition, performance of the Sheffield ACF Feed system was evaluated against two commercially available feed systems. Then effects of each feed system on viable cell density and protein titer was assessed to determine the best feed.

Figure 1: Sheffield ACF Feed System in DMEM and CD basal media
Viable Cell Density and Viability

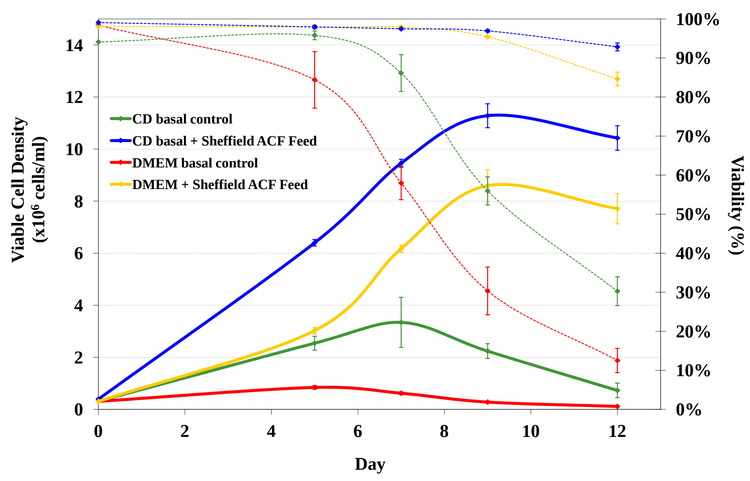

Figure 2: Sheffield ACF Feed System in DMEM and CD basal media
SEAP Production Day 12

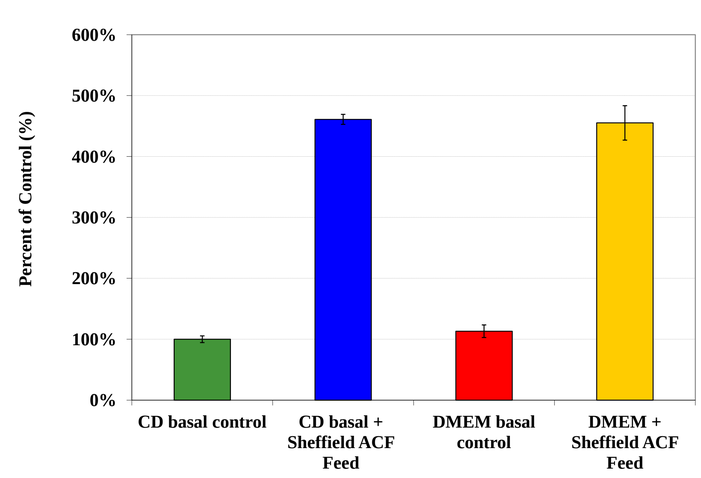

Figure 1 and 2. *Supplementation of DMEM basal media with the Sheffield ACF feed system resulted in double the peak cell density, and a 300% increase in SEAP production when compared to the commercially available, CHO specific CD medium. Application of the ACF feed system to the more expensive CD medium results in further growth improvements, but does not significantly increase SEAP production over the supplemented DMEM basal condition.*

Materials and Methods

Data was collected using a transfected CHO-K1 line engineered to express SEAP and adapted to serum-free suspension culture. Cultures were grown in 125 ml shake-flasks containing a final 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 either 12 or 14 days. All basal media were supplemented with 0.2% pluronic and 2 mg/ml G-418. Sheffield ACF feed supplementation was achieved via the use of filter-sterilized 100 g/l stock solutions prepared in the basal medium. Sheffield ACF feed includes supplementation on day 0, 5, 7 and 9. Competitor supplements were used at the recommended dosages and times per manufacturer's instructions. Day 12 supernatant samples were analyzed by HPLC for SEAP concentration. On multiple days, culture samples were removed for assessing cell counts and viability. Samples were analyzed using the Nova Bioprofile FLEX.

Figure 3: Sheffield ACF Feed System vs. Competitor Feeds in DMEM
Viable Cell Density and Viability

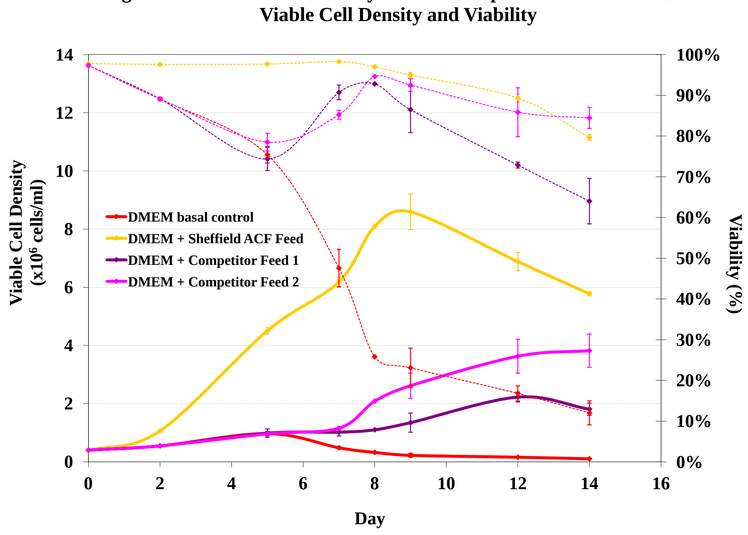

Figure 4: Sheffield ACF Feed System vs. Competitor Feeds in DMEM basal media
SEAP Production Day 12

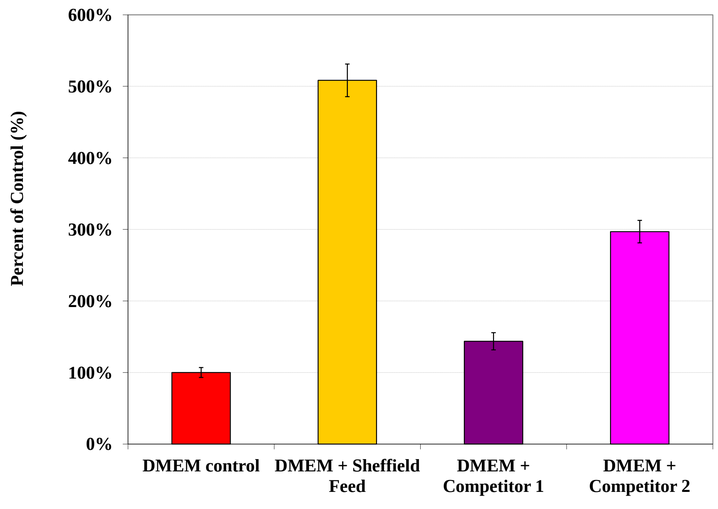

Figure 3 and 4: *When evaluated in DMEM basal medium, cultures supplemented with the Sheffield ACF feed system out performed both competitor feed systems with regards to growth and productivity. Cultures supplemented with the Sheffield ACF feed system reached the highest peak cell densities (8.5 x10^6 cells/ml) and produced 200% more SEAP than the next best competitor feed.*

Summary

Supplementation with the Sheffield ACF feed system may be employed to reduce biopharmaceutical CHO based process costs by allowing for the substitution of proprietary CD media with a classic DMEM basal. The addition of the Sheffield ACF feed system to either basal formulation results in substantial culture growth and productivity improvements over un-supplemented media, while keeping the process free of animal derived components. Application of the Sheffield ACF feed system to commercially available CD media optimized for CHO cells, resulted in a 300% improvement in product yield when compared to competitor feed system 1 and a 200% increase over competitor feed system 2. Competitor feed system 2 demonstrates growth comparable to the Sheffield ACF feed system when supplemented into CHO specific CD basal, but is unable to achieve comparable densities when applied to DMEM. The versatility and performance enhancing properties of the Sheffield ACF feed system demonstrate its potential as a vehicle for media related cost reduction.

Figure 5: Sheffield ACF Feed System vs. Competitor Feeds in CD basal
Viable Cell Density and Viability

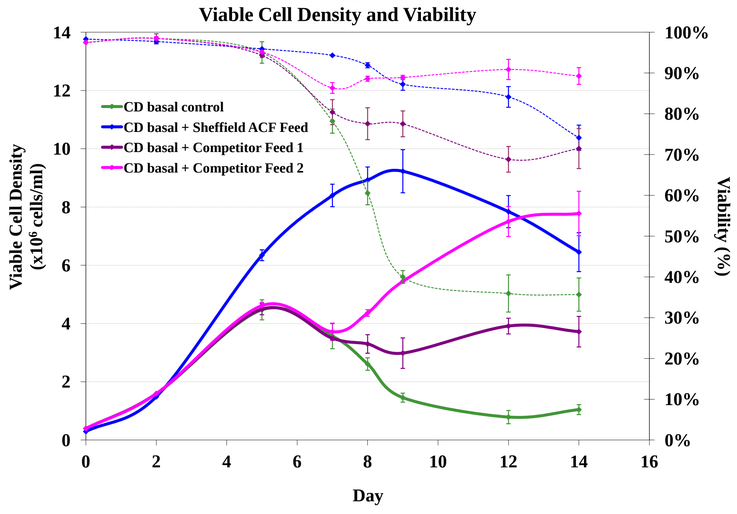

Figure 6: Sheffield ACF Feed System vs. Competitor Feeds in CD basal media
SEAP Production Day 12

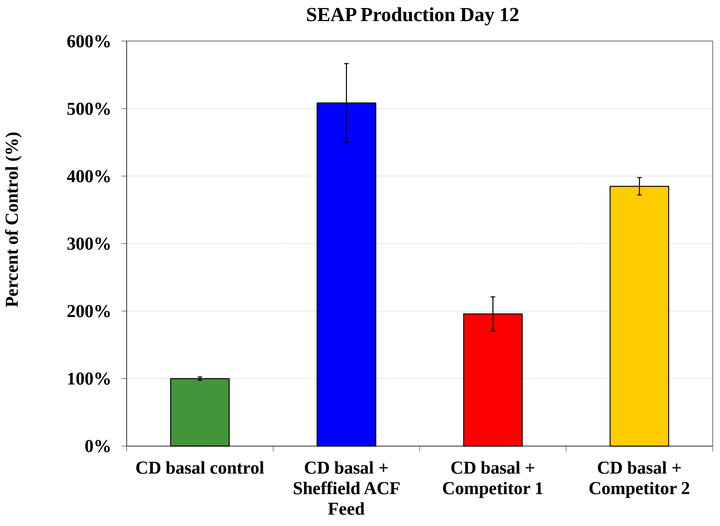

Figure 5 and 6: *When evaluated in commercially available CD basal medium, supplementation with the Sheffield ACF feed system resulted in 300% more SEAP on day 12 then competitor system 1, and a 200% increase over competitor 2. Cultures supplemented with the Sheffield ACF feed system peaked at the highest cell densities (9.0 x10^6 cells/ml), but was not able to support viability as well as competitor 2 when supplemented into commercially available CD basal medium.*