Use of Chemically Defined Supplements in Optimizing the Performance of CHO Cells

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Introduction

Chinese Hamster Ovary (CHO) cells are widely used by the biopharmaceutical industry for the production of therapeutic recombinant proteins. The enhancement of cellular productivity can be achieved through the use of many techniques, including the delivery of nutritional supplements (feeds) during cell culture. Historically, undefined supplements, such as protein hydrolysates, have been used to increase both the cell growth and productivity in fed-batch processes. However, both regulatory concerns and variability associated with undefined components have discouraged the use of such additives, creating a need for chemically defined (CD) feeds. In response to this need, a platform of CD supplements has been developed using spent media analysis and historical data to understand the nutritional and physiological requirements of CHO cells in suspension culture.

In the present study, we have examined the effect of undefined and CD supplementation on the growth and productivity of multiple CHO cell lines cultured in various chemically defined media. We observed a significant increase in growth and productivity when cells were supplemented with feeds from the CD platform as compared to a glucose-only feed. This increase was comparable to the effect seen when cultures were supplemented with undefined protein hydrolysates, though the degree to which growth and productivity increases is dependent on cell line and media formulation.

Materials and Methods

Cell Lines and Media

Five recombinant IgG-producing test cell lines **(Table 1)** were maintained in suspension in a proprietary chemically defined, animal component-free medium (Medium Z) supplemented as necessary for individual cell lines. Stocks were maintained in 250 mL or 500 mL shaker flasks at 37 °C, 5% CO_2 and 120 rpm.

Cell Line	Parental	Media Supplement
CHO-A	CHOK1SV*	25 µM methionine sulphoximine (MSX)
CHO-B	CHO-S Derived	6 mM L-Glutamine
CHO-C	CHO-S Derived	6 mM L-Glutamine
CHO-D	CHOK1SV*	25 µM MSX
CHO-E	DHFR-	4 mM L-Glutamine and 200 nM methotrexate (MTX)

*Licensed from Lonza Biologics

Test media included three SAFC Biosciences formulations, two chemically defined media (Media X and Z) and one CD/protein free (PF) medium (Medium Y). Cells were fed with each of three CD feeds (CD Feed-1, -2, and -3) and each of three protein hydrolysates (soy, yeast, and wheat).

Cell Growth and Recombinant Protein Production Assays

To test the performance of each cell line in a fed-batch process, 50 mL TPP® tissue culture tubes were inoculated in duplicate at 3e5 cells/mL (CHO-A and CHO-D), 2e5 cells/mL (CHO-B and CHO-C), or 1e5 cells/mL (CHO-E) in 30 mL of supplemented test media. Cultures were maintained at 37 °C, 5% CO_2 and 200 rpm in a Multitron® II Incubated Shaker System (Infors HT). Supplementation of 3 g/L glucose with either 10% initial culture volume of CD Feeds or 1g/L hydrolysates was performed on days two and four of culture. Stock solutions of glucose and each hydrolysate were made so that the glucose control and all experimental conditions were fed with 10% initial culture volume on both days of supplementation. This strategy was determined by previous experiments to be optimal for cell growth and productivity.

Cell density and viability were determined for CHO-A and CHO-D using the Cedex® Cell Density Examination System (innovatis AG). The ViCell™ Cell Viability Analyzer (Beckman Coulter) was used to determine cell density and viability of CHO-B, CHO-C, and CHO-E. Beginning on day four, samples were collected for quantitation of IgG levels using the FortéBio™ OctetQK System (ForteBio) and for spent media analysis. Cultures were maintained for 10 days or until cell viability was less than 70%.

Results and Discussion

Growth and Productivity in Response to Feeding with Undefined or Chemically defined Supplements

Growth and Productivity of CHOK1SV Cell Lines in Medium X

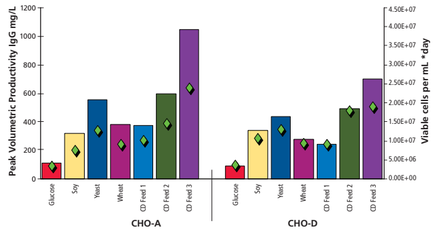

Figure 1

Growth and Productivity of CHO-S Derived Cell Lines in Medium X

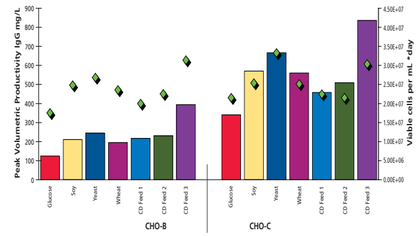

Figure 2

Peak Productivity
Cell Growth

Figures 1-2: Growth in integrated cell days (single points) and peak productivity (columns) in Medium X, a chemically defined media formulation.
Figure 1: The performance of CHOK1SV cell lines, CHO-A and CHO-D, showed an improvement in growth and productivity when supplemented with CD Feeds 2 and 3 as compared to feeding with hydrolysates. The two cell lines also showed similar performance with CD Feed 1 relative to other experimental conditions as well as comparable trends in the responsiveness to undefined supplements. **Figure 2:** Utilizing CD Feed 3 with CHO-B shows an improvement in productivity over hydrolysate-fed conditions. CD Feeds 1 and 2 increased specific productivity (qP) with this cell line compared to qP values achieved by conditions fed with undefined supplements. This is shown by comparable peak productivity of the CD Feeds-supplemented conditions achieved at a lower cell density. CHO-C showed increased growth and productivity over hydrolysates when fed with CD Feeds 3, though this cell line was not as responsive to CD Feeds 1 and 2. The CD Feeds did not significantly affect lactate production but did increase ammonium by 1.5-4 mM over conditions fed with undefined supplements (data not shown).

Growth and Productivity of CHOK1SV Cell Line in Medium Y

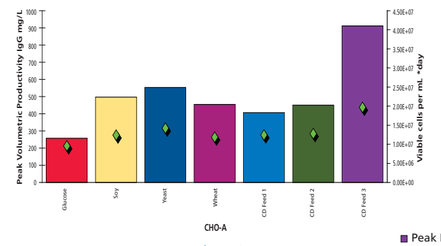

Figure 3

Growth and Productivity of CHO-S Derived Cell Lines in Medium Y

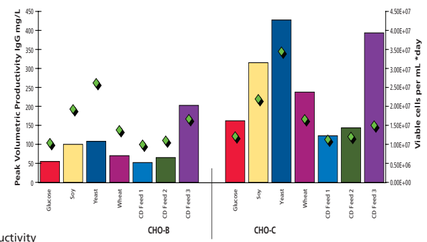

Figure 4

Peak Productivity
Cell Growth

Figures 3-4: Growth in integrated cell days (single points) and peak productivity (columns) in Medium Y, a chemically defined and protein free media formulation.
Figure 3: Results show cell line CHO-A has an increased responsiveness to CD Feed 3 as well as soy and yeast hydrolysates as compared to CD Feeds 1 and 2, which perform similarly to wheat hydrolysate. **Figure 4:** CHO-B has a similar response to the CD Feeds and hydrolysates; however, the qP of cell line CHO-B fed with CD Feeds 1 and 2 is comparable to feeding with any of the hydrolysates. The growth and productivity of CHO-C cells fed with hydrolysates are comparable to supplementing with CD Feed 3, but are increased in contrast to feeding with either CD Feed 1 or 2. These two CD Feeds show a negative effect on the productivity of CHO-C. Supplementation with the CD Feeds had no significant effect on lactate or ammonium in culture (data not shown).

Growth and Productivity of CHOK1SV Cell Lines in Medium Z

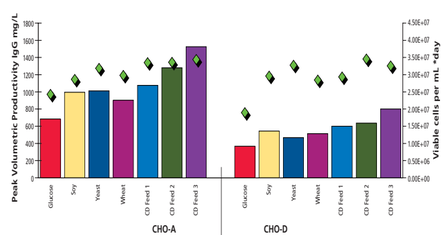

Figure 5

Growth and Productivity of CHO-S Derived Cell Lines in Medium Z

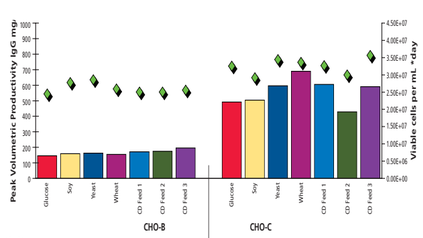

Figure 6

Peak Productivity
Cell Growth

Growth and Productivity of DHFR- Cell Line in Medium Z

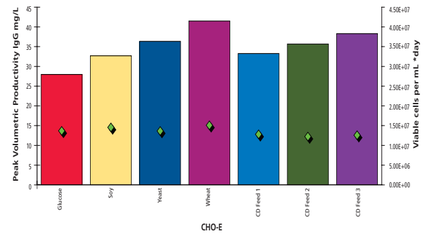

Figure 7

Figures 5-7: Growth in integrated cell days (single points) and peak productivity (columns) in Medium Z, a chemically defined media formulation.
We observed similar or improved growth and productivity of all cell lines fed with CD Feed 3 versus feeding with hydrolysates. **Figure 5:** Improved productivity was also seen with CD Feeds 1 and 2 for CHO-A and -D as compared to hydrolysates. This data combined with cell growth of these two lines indicates an increase in qP for conditions supplemented with the CD Feeds. **Figure 6:** CHO-B and CHO-C responded differently to supplementation with hydrolysates and the CD Feeds although both lines are derived from CHO-S. CD Feeds 1 and 2 performed similarly to CD Feed 3 when tested with CHO-B, but CHO-C showed a decreased responsiveness to CD Feed 2. **Figure 7:** CHO-E showed similar growth and productivity when fed with CD Feeds 1 and 2 when compared to feeding with hydrolysates. The CD Feeds did not significantly increase lactate accumulation in any cell line. Ammonium increased when supplementing with the CD Feeds as compared to soy and yeast hydrolysates. However, the ammonium levels are within acceptable ranges for cell culture (data not shown).

Conclusions

- We observed a significant increase in cell growth and up to a 300% increase in recombinant protein production when multiple CHO cell lines were fed with any of the three supplements in the CD Feed platform as compared to feeding with only glucose.
- Feeding with chemically defined supplements achieves similar cell growth and productivity as feeding with undefined supplements and can improve specific productivity, as observed in most of the tested cell lines.
- Responsiveness to feeding with individual undefined supplements and CD feeds is dependent on cell line and culture medium. Awareness of this is important for the development of platform systems.
- The findings reported here will aid in the future development and optimization of chemically defined supplements for multiple CHO cell lines.

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