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Understanding the Effects of Utilizing a Complete Feeding Supplement to Modulate Glycosylation Profiles

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ABSTRACT

Chinese Hamster Ovary (CHO) cells are the primary expression system used in the biopharmaceutical industry for the production of therapeutic glycoproteins. The glycosylation profiles of proteins in CHO cells are critical parameters that need to be extensively studied to ensure effective, consistent and high-quality protein products. N-linked glycans can display a wide range of heterogeneity, the degree of which is dependent on several factors including cell line, media, feeding supplements, and process. The effects of these variables have made it challenging to specifically target and maintain desired glycosylation profiles.

In order to address these challenges, we explored the effects of a complete glycosylation modulating feed supplement along with a unique fed-batch process that together have demonstrated the ability to modulate glycan profiles while maximizing protein titers.

We performed studies using an IgG-expressing CHO DG44 cell line. Utilizing glycosylation enhancing GlycanTune™ Total Feeds in combination with the complimentary EfficientFeed™ + Supplements, we demonstrated a progressive series of adjustments of G0F glycan proportion from 30% to 80% while still maintaining growth and productivity. Additionally we tested this ability to target specific glycosylation profiles with other commercially available media and feed supplements, confirming that GlycanTune can modulate glycan profiles, although with a narrower range of adjustability of 20% compared to 50% with the recommended EfficientFeed+ Supplements. GlycanTune when paired with EfficientFeed+ Supplements or other commercially available media and feed combinations can result in more specific and predictive glycan profiles compared to basic supplementation protocols.

INTRODUCTION

The glycosylation profile of a recombinant protein product is one of the most important attributes when defining product quality. Producing a protein with desired characteristics requires the ability to modify and target specific glycosylation profiles.

Traditionally the approach to modify the glycosylation profile of a protein involves supplementing a culture with components that can improve galactosylation. Experimentation using this supplemental approach resulted in a dramatic increase in terminal galactosylation, but with the disadvantage of lacking the ability to target specific glycosylation profiles through the entire spectrum (Figure 1).

Using novel and proprietary technology, we have developed a feed and a unique feeding process that will maximize growth and titer while being able to modulate glycan profiles. This new feed can be added as a stand alone process that can result in a significant shift from G0F to G1F and G2F (maximum galactosylation). Using a unique fed-batch process, GlycanTune can also be used with a standard feed to dial in a targeted glycosylation profile. Through process development testing, we have created a process where a transition point is used to switch from a standard feed to a glycan modulating feed. The timing of the transition point will determine the specificity of the glycan profile. A transition point early in culture will result in a greater shift from G0F to G1F and G2F. A transition midway or late in culture will result in a greater proportion of G0F compared to G1F and G2F. Glycosylation is only one facet of product quality. To further understand the broader effects of modulating glycan profiles with GlycanTune, we have included charge variant analysis of multiple glycan profiles, demonstrating minimal changes to charge variant profiles with increasing galactosylation.

MATERIALS AND METHODS

All materials were from Thermo Fisher Scientific unless otherwise indicated

Cell culture: CHO DG44 derived recombinant cells expressing an IgG molecule were grown in Dynamis™ media supplemented with 4mM L-glutamine and 1:100 Anti-Clumping Agent. Culture conditions were maintained at 37ºC, 8.0% CO2, 125 rpm. Cell densities and viabilities were measured using a Vi-CELL® counter (Beckman Coulter). Metabolites (glucose, ammonia, lactate) and IgG were measured using a Cedex® BioHT Analyzer (Roche).

Glycan Modulation Experiments With GlycanTune C+: 250mL flasks with 60mL starting volume inoculated at $0.3x10^5$ viable cells/mL in Dynamis™ medium. 2X EfficientFeed™ C+ AGT™ Supplement (EFC+) and/or 2X GlycanTune™ C+ Total Feed (GTC+) were supplemented at 1.7% on days 4 through 15 (20% total). Glycan modulation conditions involved transitioning from EFC+ to GTC+ on culture days 4, 5, 7, 9, 11, 13 and 15. Glucose was supplemented as required to maintain a concentration above 3g/L.

MATERIALS AND METHODS CONTINUED

Cells were grown in Cellvento™ CHO-200 or EX-CELL® Advanced™ Medium for at least three passages prior to the study to adapt cells. Cultures were seeded in duplicate at 3 x 10^5 viable cells/mL in 13mL working volume in 15mL Ambr® 15 micro bioreactors. The ambr 15 parameters were set at 37ºC, 50% DO, and a mixer speed of 800rpm. Glucose was fed at 3g/L when measured glucose was at or below 3g/L. Fed-batch strategies transitioning from either Cellvento™ Feed-200 or EX-CELL Advanced CHO Feed 1 to GlycanTune C+ were used as illustrated in Table 1. No further supplementation other than indicated was performed during culture.

Table 1. Fed-batch feeding strategies transitioning to GlycanTune C+

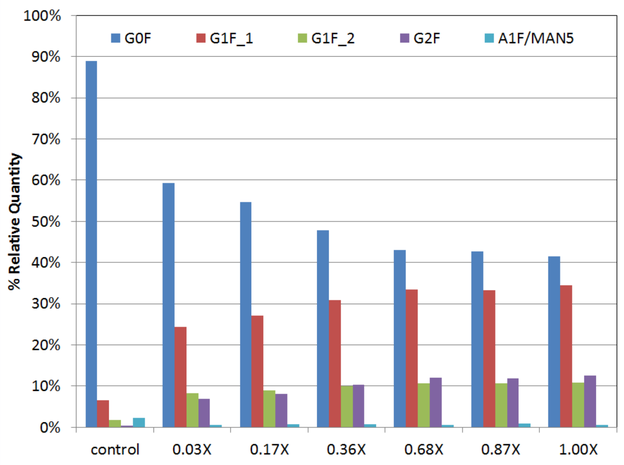
	Base Medium	Cellvento Feed-200	1X GlycanTune C+
Cellvento Condition #1	Cellvento CHO-200	3.6% Days 4-14	-
Cellvento Condition #2	Cellvento CHO-200	3.6% Days 4-12	3.6% Days 13-14
Cellvento Condition #3	Cellvento CHO-200	3.6% Days 4-9	3.6% Days 10-14
Cellvento Condition #4	Cellvento CHO-200	3.6% Days 4-6	3.6% Days 7-14
Cellvento Condition #5	Cellvento CHO-200	-	3.6% Days 4-14
SAFC Advanced Condition #1	EX-Cell Adv	10% Days 3,5,7,9,11	-
SAFC Advanced Condition #2	EX-Cell Adv	10% Days 3,5,7,9	10% Days 11
SAFC Advanced Condition #3	EX-Cell Adv	10% Days 3,5,7	10% Days 9,11
SAFC Advanced Condition #4	EX-Cell Adv	10% Days 3,5	10% Days 7,9,11
SAFC Advanced Condition #5	EX-Cell Adv	10% Days 3	10% Days 5,7,9,11
SAFC Advanced Condition #6	EX-Cell Adv	-	10% Days 3,5,7,9,11

Glycan Analysis: IgG was purified from supernatant samples using POROS MabCapture A resin. Purified protein samples were buffer exchanged into 20mM phosphate buffer using Zeba™ Spin Desalting Plates (7K MWCO). N-linked glycans were enzymatically removed with PNGase F and glycans purified carbon SPE. Glycans were labeled with 8-aminopyrene-1,3,6-trisulfonic acid (ATPS) using standard reductive amination (DMSO/15% acetic acid/1M sodium cyanoborohydride). Labeled glycans were purified by BioRad Biogel P-2 size exclusion. Glycans were identified by retention time relative to the LIZ 600 DNA size standard ladder. Glycans were quantified using 100pmole maltohexose and/or maltopentose internal standards labeled with ATPS as described by Laroy et al (1) or as described in the user guide for the Glycan Labeling and Analysis Kit (GlycanAssure user guide, Thermo Fisher Scientific). All CE separations were performed using the Applied Biosystems™ 3500xL.

Charge Variant Analysis: Purified IgG in 20mM phosphate were analyzed for charge variants on an UltiMate™ 3000 Rapid Separation Dual System with a UV detector using a MabPac SCX-10 column and a CX-1 pH gradient buffer.

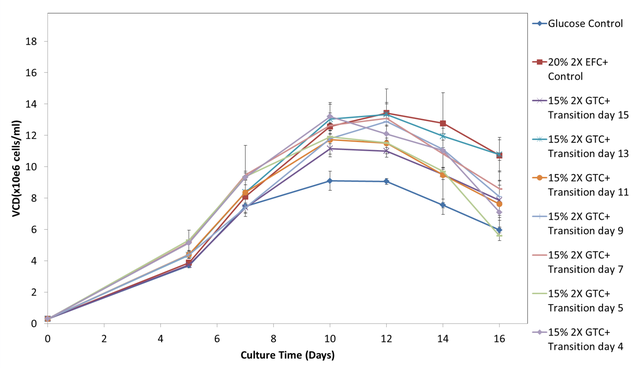
RESULTS

Figure 1. Using a supplement based approach to modulate glycosylation profiles results in only partial modulation



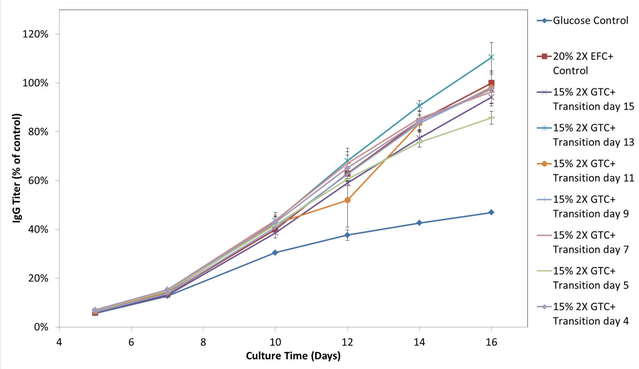
Glycan analysis data from DG44 cells measured as the percentage of total glycans. Using a glycosylation enhancing supplement (dilutions derived from a DOE experiment) added on days 3, 5 and 7, results in a shift from G0F to G1F and G2F. While there is an increase in terminal galactosylation, fine tuning the concentration of glycosylation enhancing components in the supplement to target specific glycan profiles throughout the entire G0F range (90-40%), left a 30% gap in G0F . Using the supplement based approach, it was not possible to target glycan profiles between 90-60% G0F.

Figure 2. GlycanTune C+ glycan modulation experiment viable cell densities



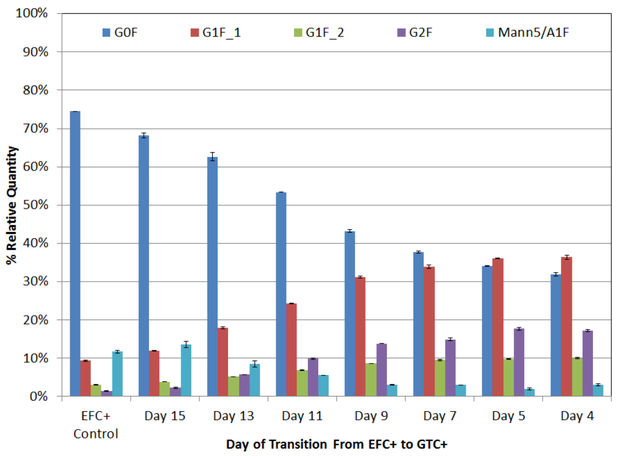
The growth profiles for the DG44 cell in Dynamis medium were similar with both EFC+ and with the use of or when transitioning to GTC+.

Figure 3. GlycanTune C+ glycan modulation experiment titer data



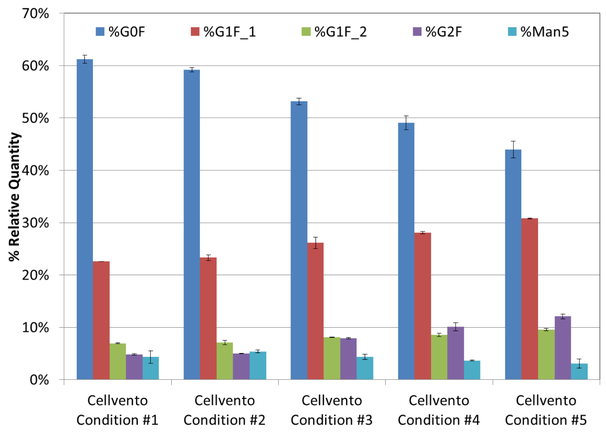
DG44 titer comparison between feeding conditions. 100% titer is the titer of 15% 2X EFC+ on day 16. Titer results indicate that the use of and transition to GTC+ does not negatively affect protein production.

Figure 4. Glycan analysis from modulating glycosylation with EfficientFeed C+ and GlycanTune C+



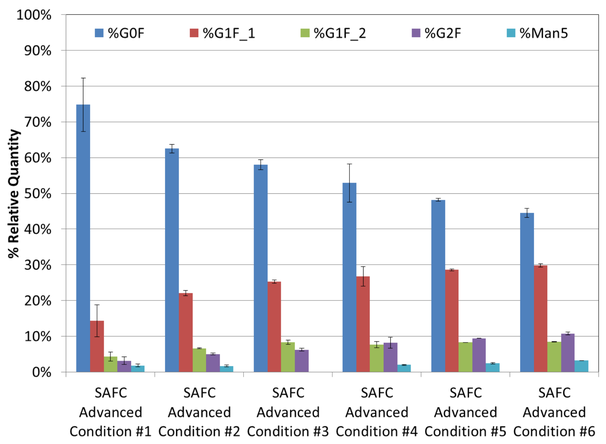
The timing of transition from EFC+ to GTC+ makes it possible to target specific glycosylation profiles. Modulating G0F from 75% down to 32%, while increasing G1F (1 and 2) and increasing G2F.

Figure 5. Glycan modulation with GlycanTune C+ and Cellvento Feed-200



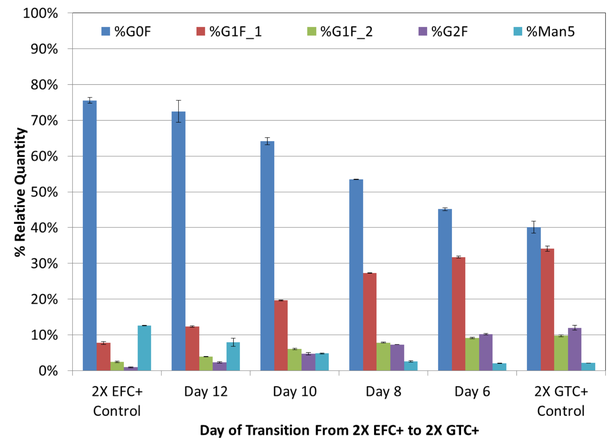
The timing of transition from Cellvento Feed-200 to GTC+ makes it possible to target specific glycosylation profiles. Modulating G0F from 61% down to 44%, while increasing G1F (1 and 2) and increasing G2F. Feeding conditions listed in Table 1.

Figure 6. Glycan modulation with GlycanTune C+ and EX-CELL Advanced CHO Feed 1



The timing of transition from EX-CELL Advanced CHO Feed 1 to GTC+ makes it possible to target specific glycosylation profiles. Modulating G0F from 75% down to 45%, while increasing G1F (1 and 2) and increasing G2F. Feeding conditions listed in Table 1.

Figure 7. Effects of GlycanTune C+ glycan modulation on charge variants



	2X EFC+ Control	Day 12	Day 10	Day 8	Day 6	2X GTC+ Control
Acidic	17.44%	16.02%	16.61%	17.75%	16.55%	16.42%
Neutral	50.50%	50.55%	50.46%	48.66%	46.75%	46.13%
Basic	32.06%	33.43%	32.94%	33.58%	36.71%	37.54%

Transitioning from EFC+ to GTC+ decreases G0F from 75% down to 40%, while increasing G1F (1 and 2) and increasing G2F. These changes to the glycosylation profile impacted the charge variant profile of each condition. Increasing galactosylation led to an increase in basic variants, a decrease to the neutral peak, while acidic variants remained similar throughout.

CONCLUSIONS

Supplementation based approaches using glycosylation modulating media components to modify and target specific glycosylation profiles proved to be difficult. These approaches were able to increase terminal galactosylation (G1F and G2F), but suffered from the inability to fine tune glycan profiles from 60-90% G0F. This could result in numerous rounds of titration experiments to target specific glycan profiles that would likely remain inconsistent between cell lines, culture media and feeds, and process scale.

The incorporation of GlycanTune™+ Total Feed into a fed-batch process as a stand alone feed was able to increase terminal galactosylation.

The development of a unique process made it possible to predictably target specific glycosylation profiles. Transition from standard feeding to GlycanTune™ allowed for precise targeting of glycan profiles. Transition to GlycanTune™ early in culture resulted in an increased shift from G0F to G1F and G2F. A transition late in culture resulted in increased G0F and decreased G1F and G2F.

GlycanTune™ can be highly concentrated to match EfficientFeed™+ products.

The use of GlycanTune™ C+ and EfficientFeed™ C+ enables maximum growth and titers.

Modulating glycosylation profiles is attainable with different media and feed supplement combinations. Transitioning from Cellvento Feed-200 to GlycanTune C+ in Cellvento CHO-200 Medium and transitioning from EX-CELL Advanced CHO Feed 1 to GlycanTune C+ in EX-CELL Advanced CHO medium resulted in a shift in glycan profiles, decreasing G0F by 17% and 30% respectively.

Increasing galactosylation using GlycanTune C+ resulted in a slight increase in basic charge variants, a slight increase in the neutral peak, and little to no change in the acidic charge variants.

REFERENCES

1. Laroy W. Contreras R and Callewaert N. Glycome mapping on DNA sequencing equipment, Nature Protocols, 206, 1(1), 397

TRADEMARKS/LICENSING

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