Article

The Role of Peptones in Boosting Monoclonal Antibody Production

Source: Thermo Fisher Scientific

The utilization of peptones in Chinese hamster ovary (CHO) cell bioprocessing has emerged as a pivotal strategy for enhancing monoclonal antibody (mAb) production. Peptones — biologically-derived protein hydrolysates — have been shown to significantly improve productivity across various CHO cell lines, contributing to higher yields, enhanced protein quality, and robust cell growth. These improvements align with the biomanufacturing industry's goals of achieving high yields, efficient processes, and stable products.

Since 2008, the average mAb titer has increased from 1.95 grams per liter (g/L) to 3.09 g/L in 2024 — a testament to advancements in process optimization and raw material utilization. Peptones have played a crucial role in this progress by addressing key productivity challenges. However, variations in titer, protein quality, and cell growth often complicate process management. To mitigate these issues, strategic approaches, such as key driver identification methods and test-and-hold procedures, are essential for enabling consistent performance.

Real-world data highlights the specific effects of peptones on CHO cell line performance, underscoring their ability to enhance mAb yields and improve overall process efficiency. Yet challenges such as lot-to-lot variability in peptones can impact bioprocess consistency. The mechanisms by which peptones enhance CHO cell productivity, the impact of peptone variability on titer, protein quality, and cell growth, and strategies to mitigate these impacts are all crucial considerations for developers working to optimize their mAb. By leveraging these insights, biomanufacturers can harness the full potential of peptones to boost quality and yield while supporting process robustness and efficiency.

An Evaluation of Peptone Impact on Three Distinct CHO Cell Lines

Peptones are complex mixtures containing polypeptides, free amino acids, carbohydrates, salts, trace metals, and other components that provide vital nutrients for cell culture. They can be sourced from animal-based or animalfree origins, such as yeast or plant-based materials, offering flexibility to meet regulatory and ethical considerations.

Beyond their nutritive role, peptones provide protective effects for CHO cells through nutritional buffering and other mechanisms that help maintain cell health and delay apoptosis. These benefits are critical for optimizing monoclonal antibody production, as healthier cells contribute to higher titers and improved protein quality. In recent case studies, Thermo Fisher Scientific evaluated the impact of peptones on three distinct CHO cell lines.

Case Study 1: CHO K-1 and AOF Peptones

The first cell line, a CHO-K1 line expressing a mAb, was evaluated in a 14 day simple fed-batch study using only CD medium and peptones in duplicate Ambr15 bioreactors; 3 g/L or 6 g/L of each peptone was added on day 0 to the chemically defined basal medium. The following animal-origin free (AOF) peptones were evaluated as part of this study:

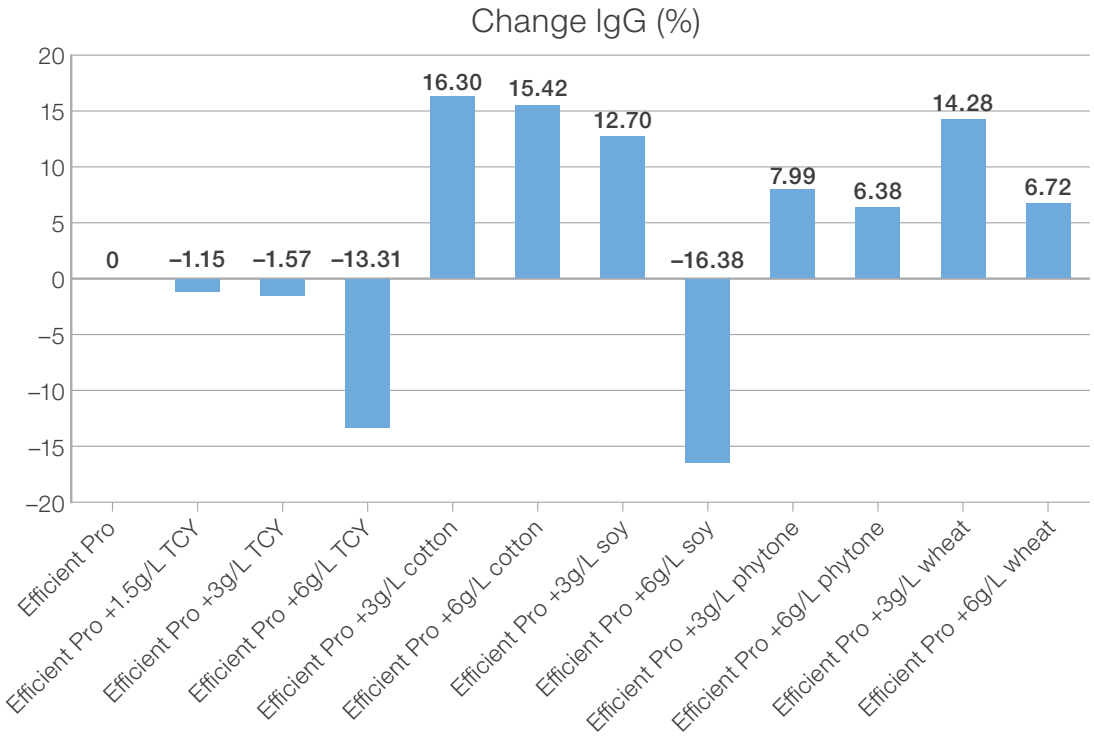
- Difco™ TC Yeastolate UF (TCY),
- Gibco™ Cotton Peptone 200 UF (Cotton),
- Gibco™ Soy 100 (Soy),
- Difco™ Phytone Supplement UF (Phytone),
- Gibco™ Wheat 100 UF (Wheat)

Each batch was supplemented with 6 mML-glutamine and 1% Gibco™ Anti-Clumping Agent; cell counts and viability were measured using a Beckman Coulter Life Sciences Vi-CELL™ Cell Counter, and titers were measured using a Roche Cedex™ BioHT Analyzer. The primary goals of the study were to determine whether the selected peptones had a titration effect at certain concentrations, which would negatively affect titers, as well as to identify optimal peptones and their concentrations for increasing productivity while maintaining consistency and quality. The results of this evaluation showed that the cotton, wheat, and phytone peptones significantly enhanced mAb titer and cell viability under controlled conditions. The specific results, when compared to the unsupplemented control, were:

- **Wheat**—14% increase at 3 g/L and 7% increase at 6 g/L
- **Cotton** – 16% increase at 3 g/L and a 15% increase at 6 g/L
- **Phytone** – 8% increase at 3 g/L and a 6% increase at 6 g/L

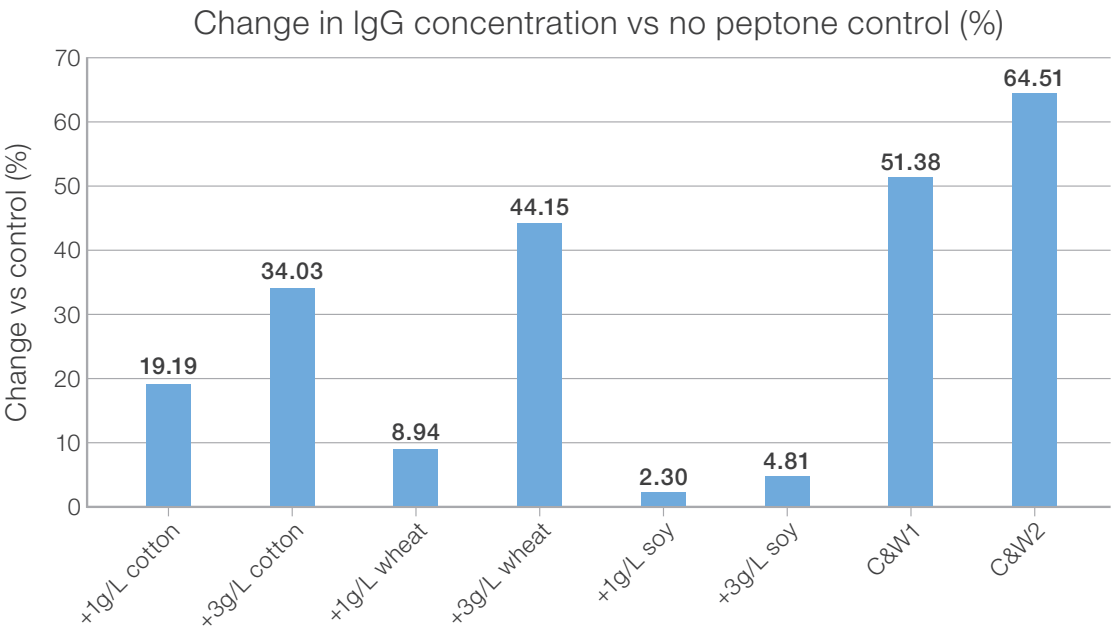
High cell viability and viable cell density (VCD) were also observed, making these three peptones promising candidates for further testing. Conversely, the TCY and soy peptones showed a negative impact on titer at one or both concentrations:

- **TCY**—loss of –2% was observed at 3 g/L and of -13% at 6 g/L
- **Soy**—increase of 13% occurred at 3 g/L, while a loss of –16% was observed at 6 g/L



In Case Study 1, 3 g/L cotton, soy and wheat showed the strongest positive titer impact with 13% to 16% relative titer improvement relative to control.

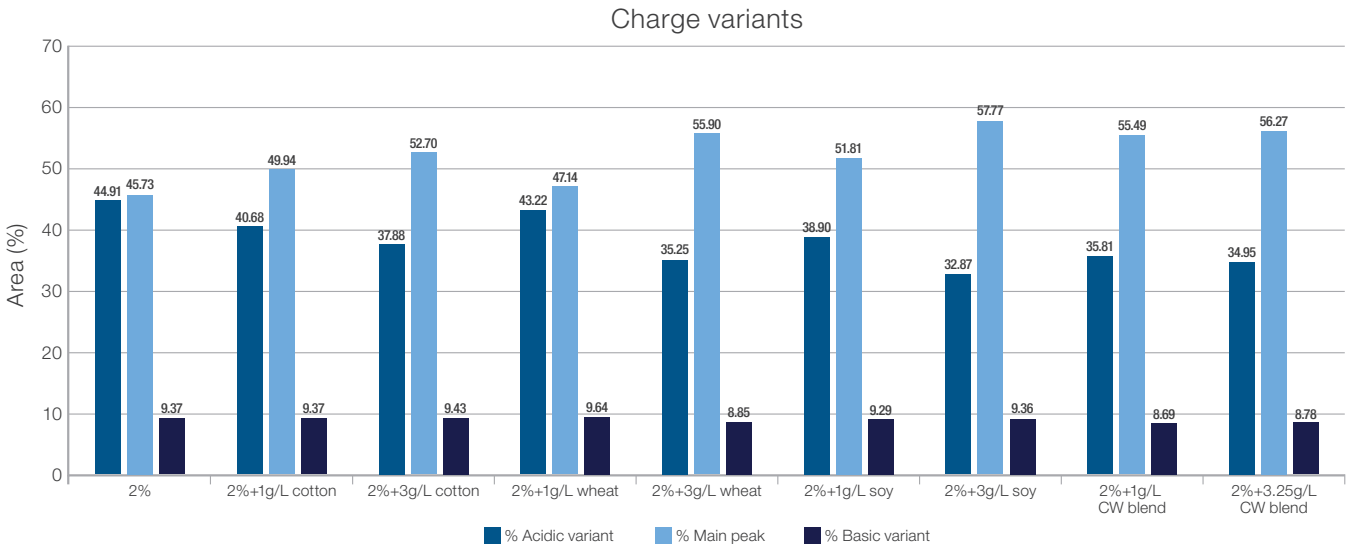
Building on these findings, fed-batch studies were conducted to evaluate the selected peptones in a more complex production environment. The addition of a proprietary blend of cotton and wheat peptones (C&W1 and C&W2), especially at 3 g/L with a subsequent boost on Day 11, yielded the highest titer increase, reaching up to 51% and 64.5% improvement over the control. These conditions maintained robust growth and high viability, establishing the cotton/wheat blend as a standout performer.



During Case Study 1, the cotton and wheat blends (C&W1 and C&W2) demonstrated the strongest titer enhancement, with 51% and 64% relative titer to control. Titer enhancement of 44% with 3 g/L wheat and 34% with 3 g/L cotton single peptones were also demonstrated.

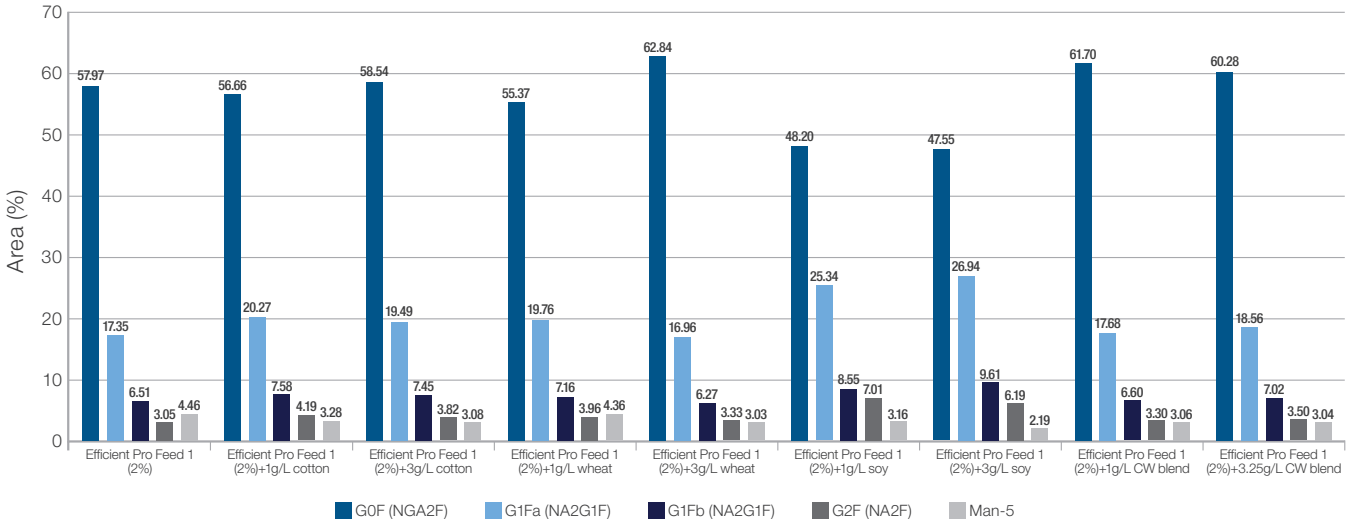
Protein quality was another critical factor assessed; charge variant analysis was conducted with cation exchange high-performance liquid chromatography (HPLC) coupled to a UV detector; protein aggregation analysis was performed using size exclusion HPLC with UV detection; and glycan profiles were analyzed with hydrophilic interaction liquid chromatography (HILIC), ultra performance liquid chromatography (UPLC) and fluorescence detection.

Across all peptones, a consistent decrease in acidic variance and an increase in the main peak were observed, indicating a positive impact on charge variance without significant changes in basic peaks. Additionally, evaluation of aggregation and fragmentation showed no change with the addition of a peptone.



In Case Study 1, the addition of peptones demonstrated increases in the main peaks and a decrease in the acidic peaks, while the basic peaks showed relatively little change from control.

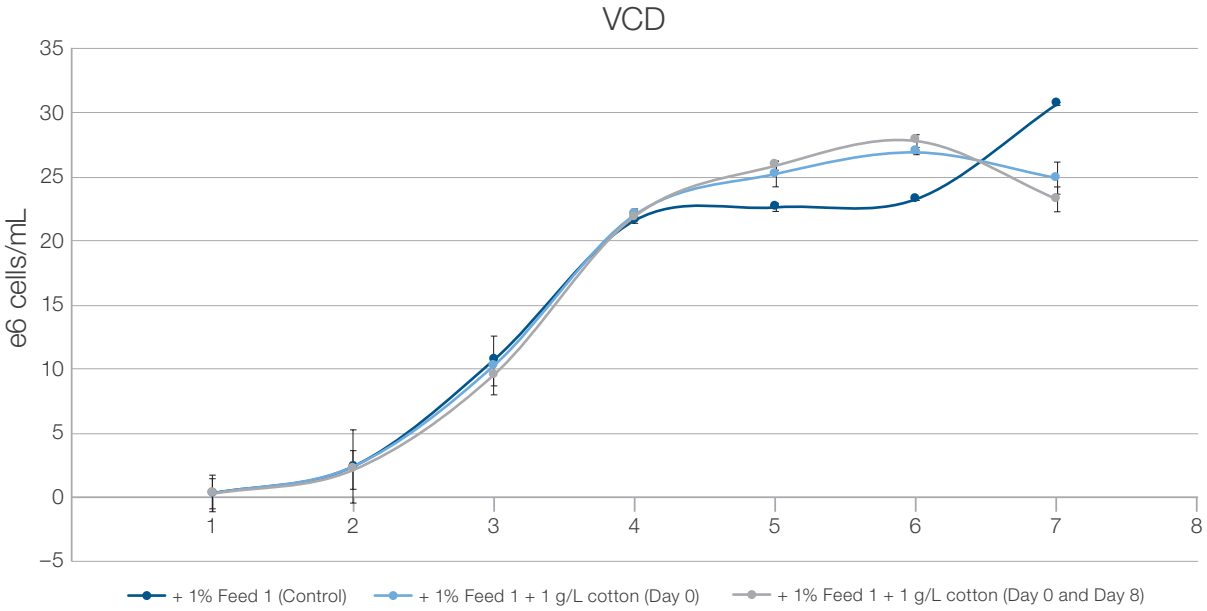
Glycan profiles remained stable across most peptones, with the soy peptone demonstrating a unique ability to modulate glycan levels by reducing G0F and increasing G1F and G2F. The addition of cotton and wheat peptones demonstrated comparable N-glycan profiles or the potential to shift glycans to less or more mature structures. Finally, immunogenic Man-5 glycans with peptones remained within acceptably low ranges of less than 5%.



To support the reliability of these results, the study also investigated lot-to-lot consistency for peptones across multiple experiments. IgG titers, VCD, and protein quality metrics consistently exhibited variability below 15%, well within acceptable biological and analytical ranges. This demonstrated the reproducibility and reliability of peptone performance, a crucial factor for large-scale biomanufacturing.

Case Study 2: CHO-K1 GS and Cotton Peptone 200 UF

A second study was conducted to evaluate the impact of AOF peptones on CHO-K1 GS cells producing immunoglobulin G (IgG) mAb. Using a Sartorius Ambr® 15 system, a 14-day fed-batch assay was performed in duplicate.

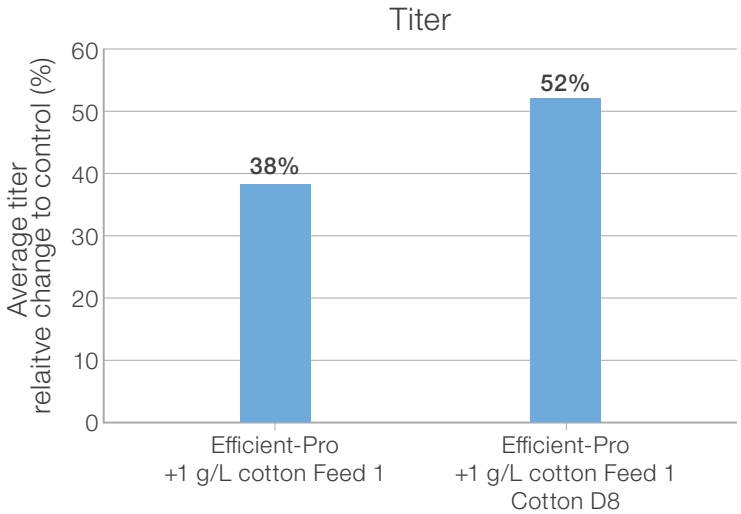


The cells were cultured in a chemically defined medium and the feed was added each day starting on day 3 through day 13 at 1.5% v/v. Viable cell density (VCD), cell viability, and mAb titers were measured using a Vi-CELL™ counter, alongside analyses of aggregation, charge variants, and N-glycans to assess product quality. The control for this study was a combination of the media and feed without any other additions.

There were two experimental conditions in this study:

- Experimental condition 1 involved the addition of 1g/L of Gibco Cotton 200 UF to the basal medium on day 0 while keeping the feeding schedule the same as the control.
- Experimental condition 2 involved the addition of 1g/L of Gibco Cotton 200 UF to the basal medium on day 0 and a subsequent addition on day 8 while keeping the feeding schedule the same as the control.

Experimental conditions 1 and 2 outperformed the control in terms of viable cell density and sustained higher viability than the control. For terminal IgG titers, both experimental conditions 1 and 2 outperformed the control by 38% and 52%, respectively.

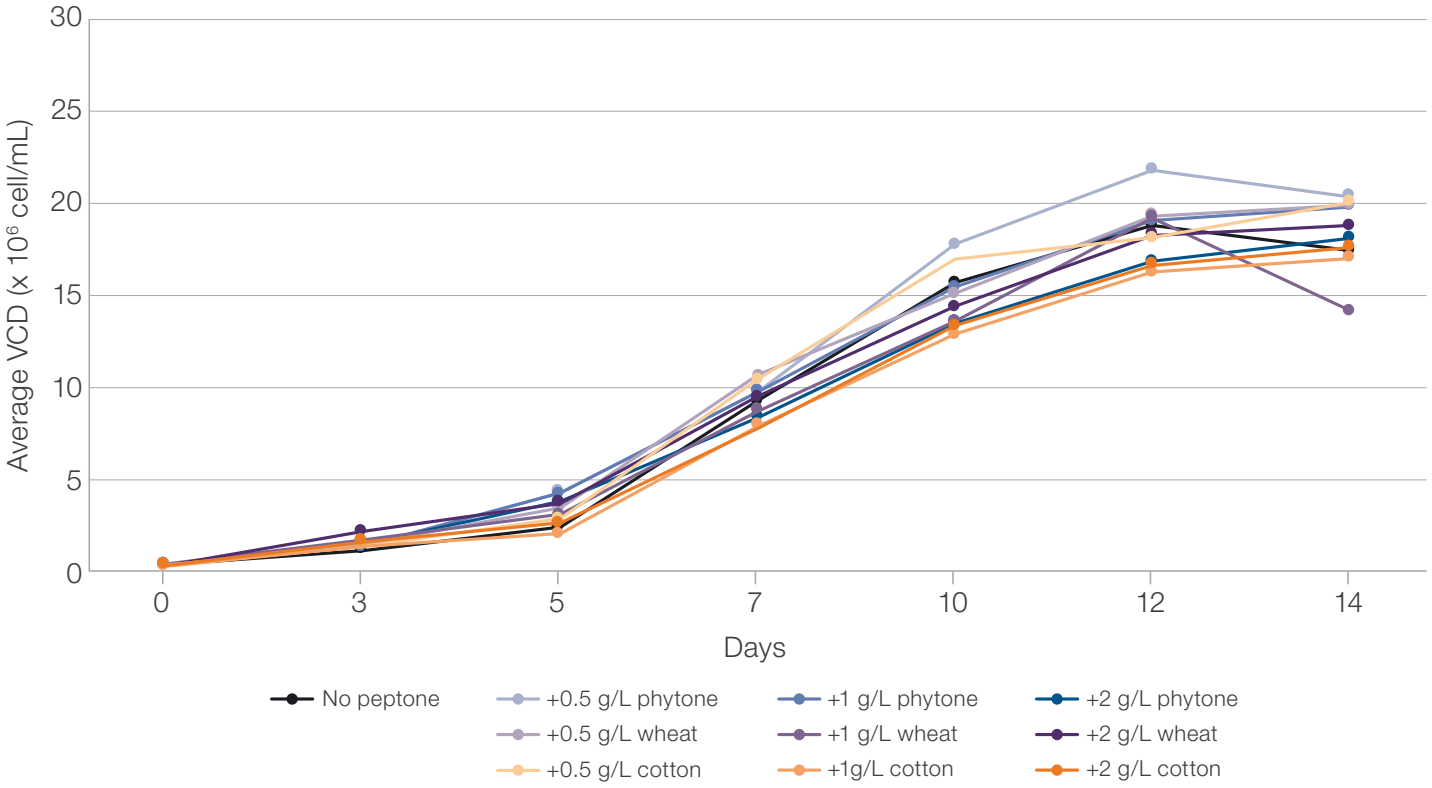


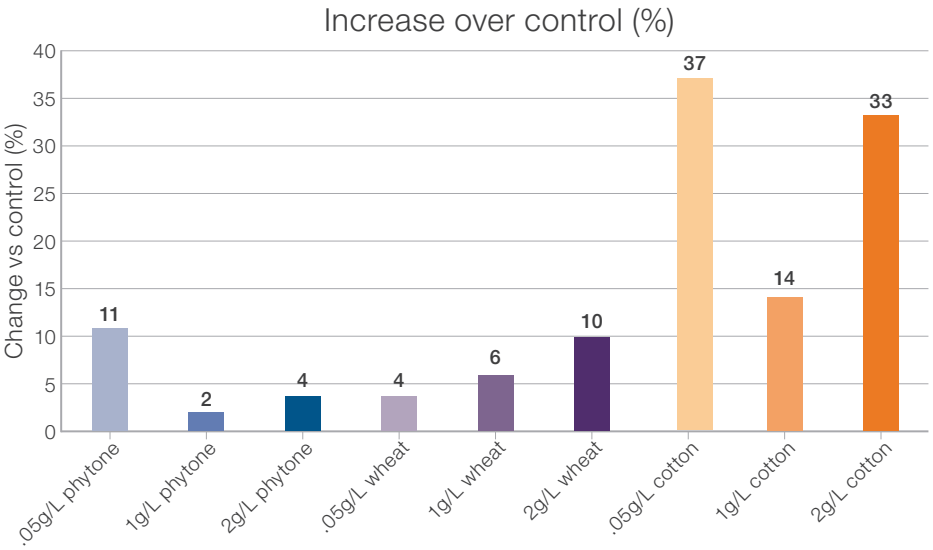
In Case Study 2, Feed 1 in conjunction with the Cotton peptone saw an increase in titer of more than 50 percent at Day 8 relative to the control.

Case Study 3: DG44 and Select Peptones

The third study evaluated the effects of three select peptones—Phytone, Wheat, and Cotton—on growth and titer of DG44 CHO cells cultured in CD basal medium. The objective was to identify lead candidate peptones and determine initial concentration limits for further evaluation. Peptones were added to the CD basal medium on day 0, alongside supplements of 4 mM Gibco™ GlutaMAX™ and 1:250 Gibco™ Anti-Clumping Agent. Cell growth and viability were measured using the Vi-CELL™ Cell Counter, while mAb titers were quantified using the Cedex™ BioHT Analyzer.

Results showed that all peptones supported growth and viability comparable to the CD control, except for 1 g/L wheat, which demonstrated reduced growth by day 14. Cotton peptone exhibited the strongest improvement in mAb production, with supplementation at 0.5 g/L and 2 g/L yielding a 37% and 33% increase in relative titer, respectively, compared to the control. Phytone and Wheat peptones also demonstrated moderate positive effects on productivity.

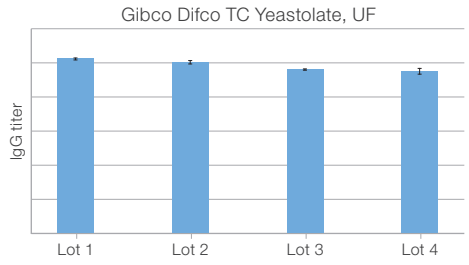


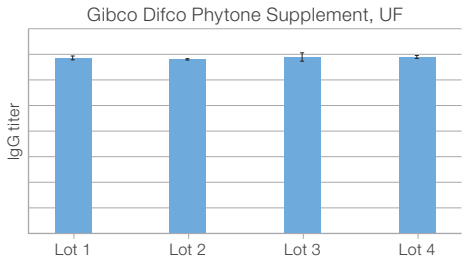


In conclusion, Gibco™ Cotton Peptone 200 UF emerged as the lead candidate, with optimal concentrations identified between 0.5 g/L and 2 g/L. Further evaluations will focus on optimizing its use in extended fed-batch cultures to improve productivity while maintaining robust cell growth and product quality.

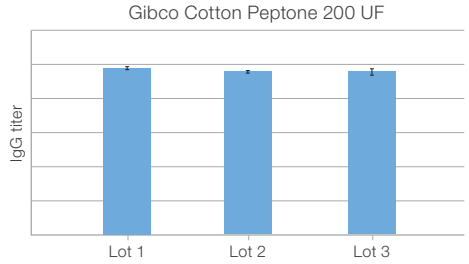
Peptones and Lot-to-Lot Consistency

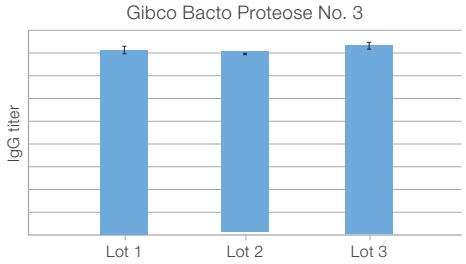
The support of lot-to-lot consistency of peptones is a critical factor for bioprocesses, particularly for the production mAbs. To establish the consistency of Gibco™ peptones for mAbs processes, a study was conducted to evaluate several AO and AOF peptone bases. The study involved multiple lots — four for Yeastolate and Phytone, and three for Bacto, Proteose, and Cotton — using a shake flask model with a single CHO cell line expressing an IgG mAb.



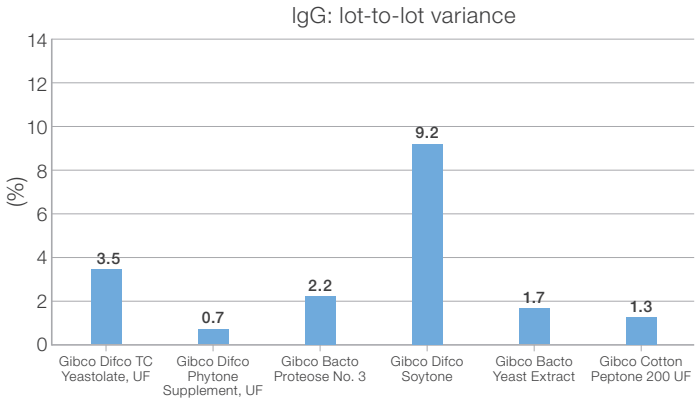


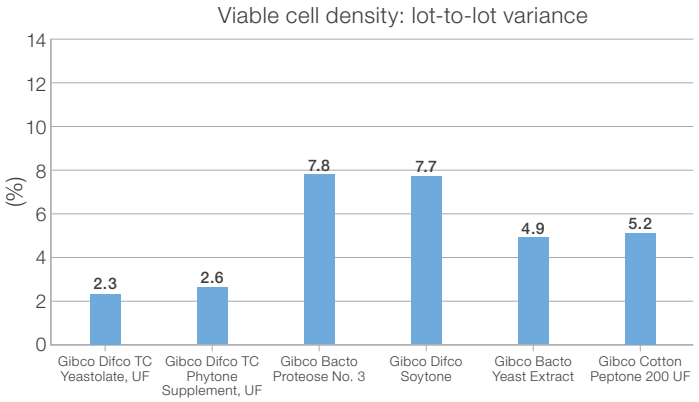
CHO-K1 cells expressing IgG molecules were grown in CHO Panel Medium #6 supplemented with 6 mM L-glutamine and 1% anti-clumping agent



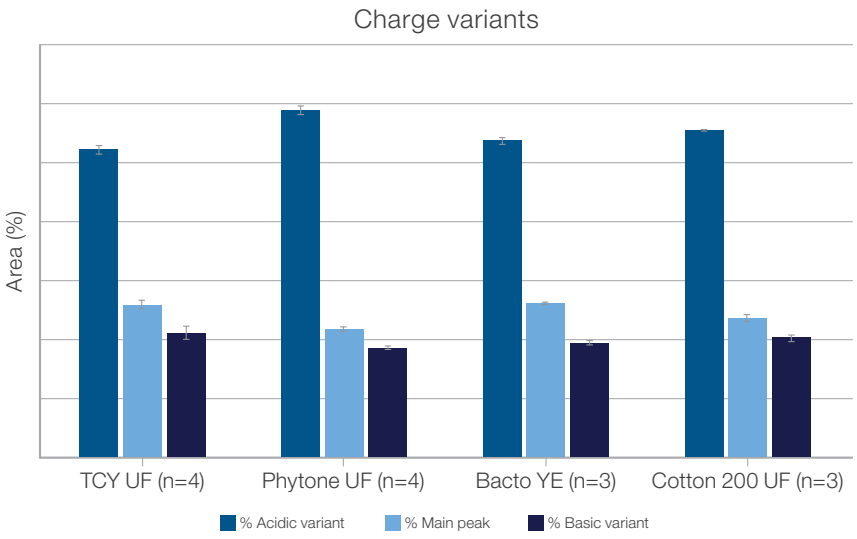


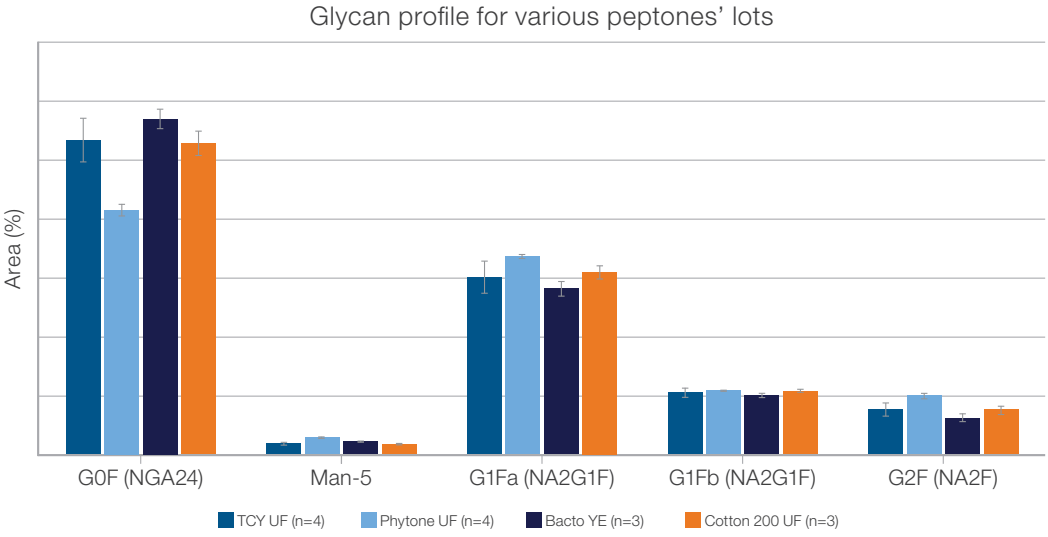
A growth performance assay was performed on multiple peptones to evaluate consistency between distinct lots and measure key cell culture metrics





Harvest IgG titers were assessed across all tested lots, revealing strong consistency with variance levels below 15%, a threshold accounting for biological and instrument variation. The coefficient of variance for IgG titer and viable cell density demonstrated minimal spread across multiple lots for each peptone, highlighting their reliability. Protein quality metrics, such as charge variants and glycan profiles, were also analyzed across the lots. Consistent results were observed for the acidic, main, and basic peaks of charge variants, as well as glycan species including G0F, G1F, G2, G2F, and Man-5.





This comprehensive study demonstrated the robust lot-to-lot consistency of Gibco™ peptones across key metrics — IgG titer, viable cell density, charge variants, and glycan profiles. These findings underscore the suitability of Gibco™ peptones for reliable and reproducible performance in mAb production processes, supporting consistent product quality and process outcomes.

Learn more at **thermofisher.com/peptones**