



Use of protein hydrolysates to reduce serum requirement in the production of veterinary vaccines by BHK cells

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INTRODUCTION

- Despite the benefits of animal serum for cell growth, its use in cell culture media shows many shortcomings, such as the potential risk of introduction of animal-derived contaminants, the lot-to-lot variability and the high costs. The replacement of animal serum can be achieved by supplementing culture media with protein hydrolysates, which are obtained by hydrolysis of proteins derived from plants, milk or meat.
- Modified Eagle's medium supplemented with peptone and casein hydrolysate could replace 10 amino acids and tryptose phosphate broth (TPB) while maintaining BHK-21 cells growth profile (Saha & Sen, 1989).
- Supplementation of chemically defined media with a cottonseed hydrolysate (HyPep™ 7504) facilitates a metabolic shift, allowing CHO-K1 cells to grow in such media and to utilize lactate as an energy source once the majority of glucose and glutamine are depleted from the media (Babcock et al., 2010).

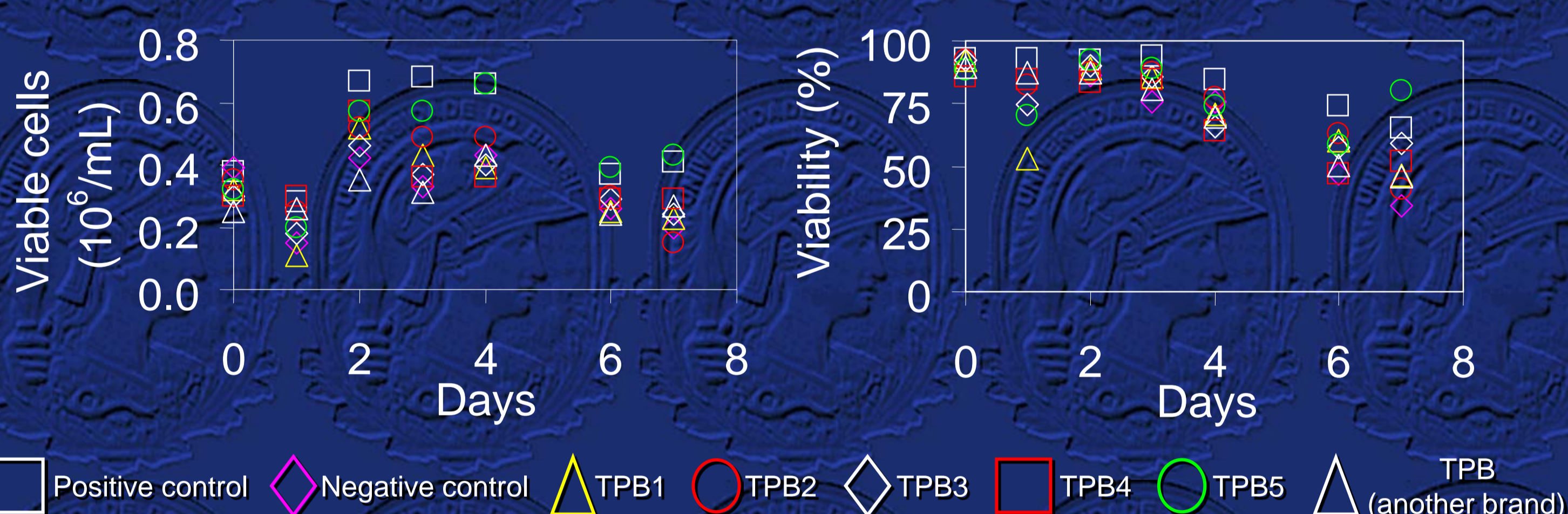
OBJECTIVE

Evaluate BHK-21 cell performance in media (w/ and w/out TPB) supplemented with protein hydrolysates and/or rHSA, derived from animal & animal-free sources, in order to reduce bovine serum concentration from 8% to 3%.

MATERIAL AND METHODS

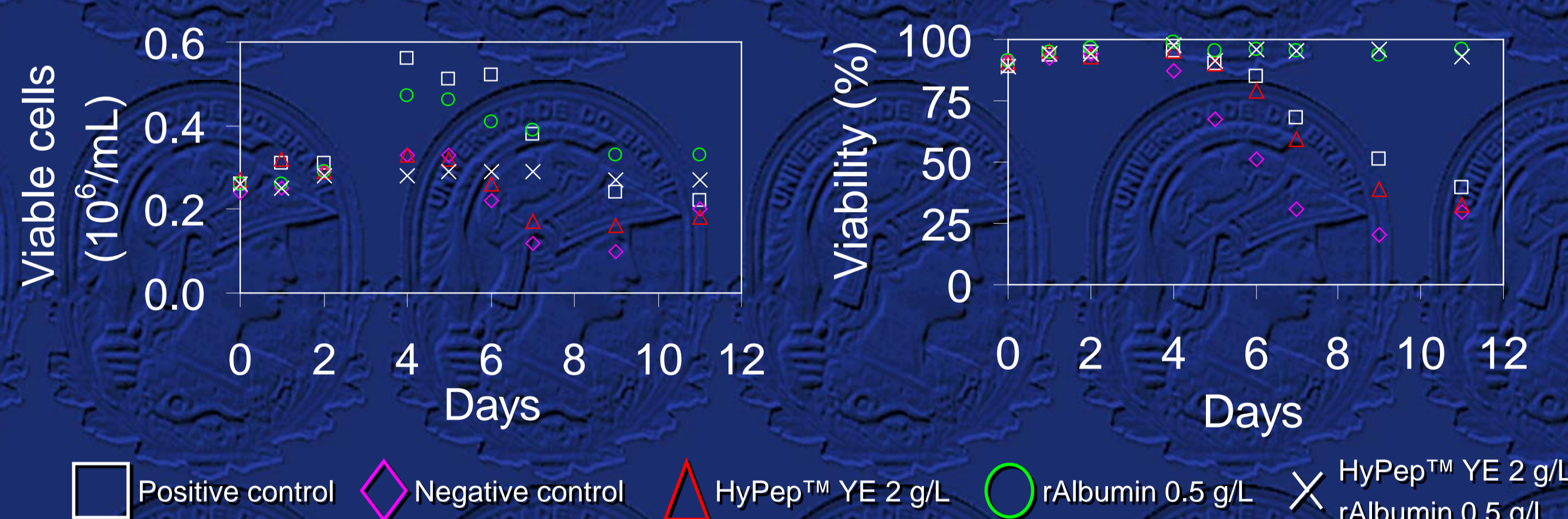
- Cells and media:** The cell line BHK-21, obtained from ATCC (catalogue nº CCL-10), was adapted to grow in suspension in Glasgow Minimum Essential Medium (GMEM) supplemented with 8% (v/v) adult bovine serum (ABS) (Cultilab). The GMEM medium tested were from LGC (without TPB) and Hyclone (with TPB). The cells were routinely maintained in spinner flasks (Techne) at 37°C and 5% CO₂.
- Protein hydrolysates and supplements (Sheffield Bio-Science):**
 - Pepticase™: low-salt source of amino acids and small peptides produced by the acid hydrolysis of casein;
 - HyPep™ 1512: ultrafiltered enzymatic digest of soy protein utilizing a unique enzyme blend;
 - HyPep™ 7504: ultrafiltered enzymatic digest of cottonseed;
 - Hy-Peptone PB 1-5: Sheffield's equivalent for tryptose phosphate broth (TPB)
 - HyPep™ YE: ultrafiltered yeast extracts, animal-component free, produced from primary grown yeast (*Saccharomyces cerevisiae*);
 - rAlbumin ACF: Sheffield's rHSA, expressed in plants
- Experimental set-up:** 125-mL disposable shake flasks (Corning) were inoculated with BHK-21 cells at 0.3 x 10⁶ cells/mL with 35 mL of working volume. Samples were taken every 24h for determination of viable cell density and cell viability with Vi-CELL® Analyser (Beckman Coulter) and glucose and lactate concentrations with YSI Biochemistry Analyser™ (YSI Life Sciences). Positive and negative controls had no supplements or protein hydrolysates addition but only 8% (v/v) and 3% (v/v) of ABS, respectively. All the other flasks tested had 3% (v/v) ABS.

GMEM without TPB supplemented with HyPeptone PBs



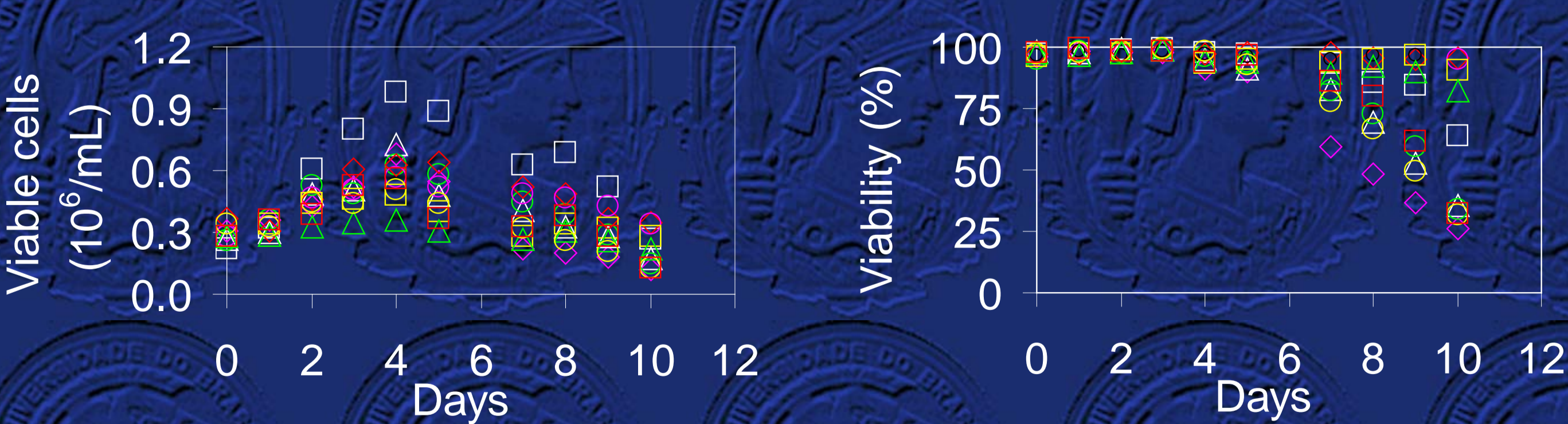
Amongst the five Hy-Peptone PB tested, # 5 promoted the best cell growth, comparable to positive control (8% ABS) and better than TPB from another brand

GMEM without TPB supplemented with HyPep YE and rAlbumin



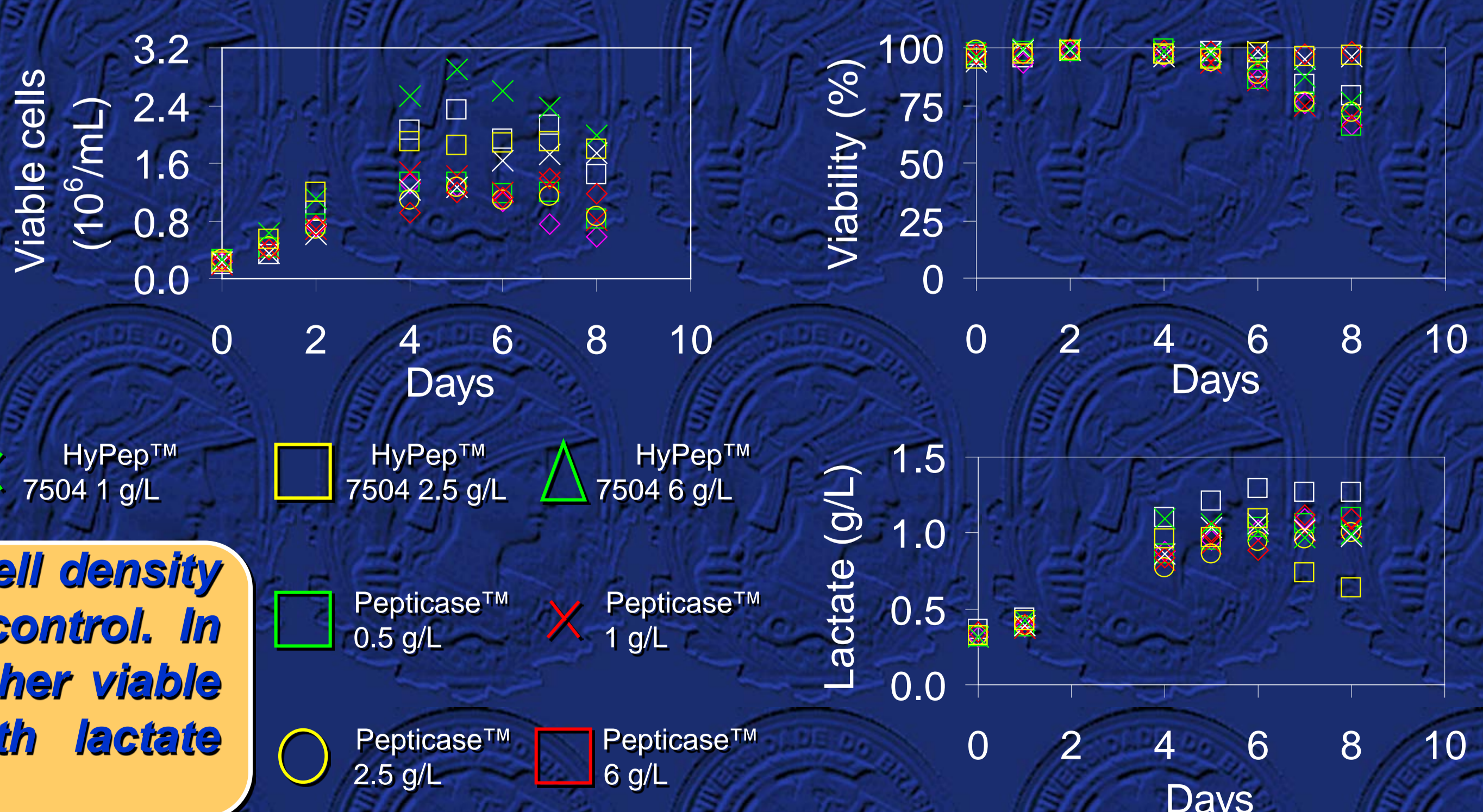
BHK-21 cells supplemented with recombinant albumin at 0.5 g/L presented a growth profile similar to positive control (8% ABS) and higher viability for longer period

GMEM without TPB supplemented with protein hydrolysates



In GMEM without TPB, the addition of HyPep™ 1512 led to higher viable cell density and viability compared to the other protein hydrolysates and negative control. In GMEM with TPB, supplementation with HyPep™ 7504 resulted in 25% higher viable cell density compared to positive control and a metabolic shift with lactate consumption was observed.

GMEM with TPB supplemented with protein hydrolysates



CONCLUSIONS

Hy-Peptone PB5, Sheffield rAlbumin ACF, and/or HyPep™ 1512 from Sheffield Bioscience can be effectively used in GMEM w/out TPB to reduce ABS from 8% to 3%. HyPep™ 7504 in GMEM w/ TPB with 3% ABS is able to increase cell growth in 25% compared to control with 8% ABS.

References:

Saha SN, Sen AK (1989) Vaccine 7:357
Babcock JF, Antosh A, Benedict K (2010) Cell Culture Engineering XII
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