Cultivated Meat: Investigating Media Dilution and Yeast Protein Supplementation for Cost-effective Culture Media

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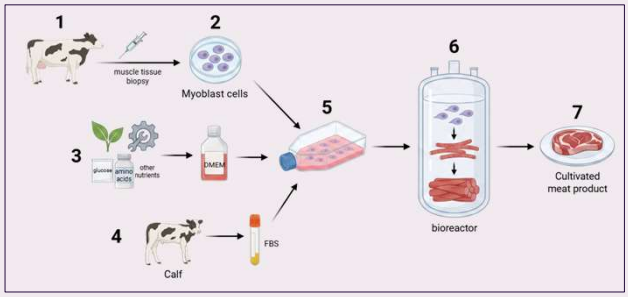
1. BACKGOUND

- Cultivated Meat (CM) refers to meat produced *in-vitro* using cellular agriculture techniques [1].



2. MOTIVATION

- Meat has a much larger climate footprint than plant-based foods [2].
- According to Life Cycle Assessments, CM is more sustainable than conventional livestock farming "when produced using renewable energy" [3].

Improves animal welfare | Increases food security | Reduces land use | Reduces disease risk

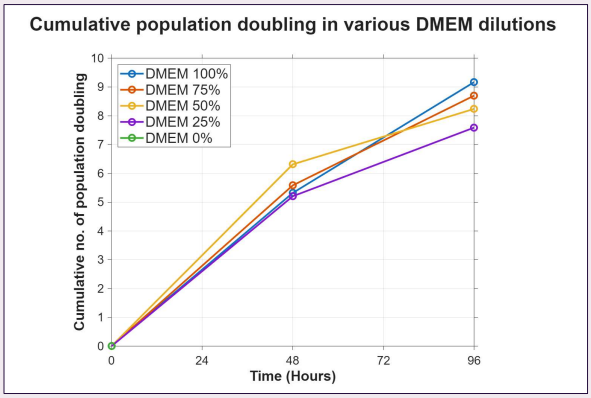
3. RESEARCH GAP

- The cost of culture media remains a major barrier to economically viable CM production.
- Although research has largely focused on serum alternatives, free amino acids also contribute significantly to media costs.
- Yeast extracts are particularly attractive because of high protein content, rapid biomass generation and established industrial-scale production.

4. METHODS

- **Yeast Protein Extraction**
  - Lysate: homogeniser, centrifuge
  - Hydrolysate: homogeniser, hydrolysis, centrifuge, neutralisation, dialysis
- **Media Preparation**
  - Sterile filtration
  - Dilution with saline buffer (PBS)
  - Glucose & osmolality adjustment
- **Cell Culture**
  - C2C12 myoblast cells
  - T-25 flasks, 12 well plates
  - 37°C, 5% CO_2
- **Measuring growth**
  - Microscopy at 4x
  - Nucleocounter
  - Trypan Blue Assay

5. RESULTS AND MAIN CONCLUSIONS



- 50% DMEM supported enhanced short-term cell proliferation than conventional 100% DMEM. However, beyond 48 hours, 100% DMEM showed higher cell growth (1000 cells cm^{-2}, n=1).
- When repeated with passage on day 2, no significant difference was observed in cell growth between 50% and 100% DMEM over 4 days (2000 cells cm^{-2}, n=4). Hence, DMEM can be diluted up to 50% without compromising cell growth, thereby reducing media costs significantly.
- Yeast lysate and hydrolysate resulted in cell death at 25%, 50%, and 100% replacement. Hence, in their present form, they cannot be used to replace DMEM.

6. LIMITATIONS AND FUTURE WORK

The specific component limiting growth in dilute media was not identified.	Morphological changes and stress caused by dilution were not quantified.	Yeast lysate and hydrolysate were highly variable. They caused cell death.
Spent media should be quantified for cultures of various dilutions. A Design of Experiments approach should be used.	Changes in cell shape should be quantified, and cellular stress, apoptosis and cell-cycle phase distribution should be assessed.	Protein extraction process should be optimised. The composition and cytotoxicity of the yeast formulations should be evaluated.

7. REFERENCES

[1] I. Pajčin *et al.*, 'Bioengineering Outlook on Cultivated Meat Production', *Micromachines*, vol. 13, no. 3, p. 402, Feb. 2022, doi: 10.3390/mi13030402.

[2] M. C. Parlasca and M. Qaim, 'Meat Consumption and Sustainability', Annu. Rev. Resour. Econ., vol. 14, no. 1, pp. 17–41, Oct. 2022, doi: 10.1146/annurev-resource-111820-032340.

[3] H. L. Tuomisto and T. Ryynänen, 'Environmental Impacts of Cultivated Meat', in *Cultivated Meat*, C. R. Soccol, C. F. M. Molento, G. G. Reis, and S. G. Karp, Eds, Cham: Springer Nature Switzerland, 2024, pp. 277–297. doi: 10.1007/978-3-031-55968-6_14.

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