Good Food Institute
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Dear Drs. Risner and Spang,

Thank you for posting your manuscripts that aim to assess the environmental impact of cell culture media and cultivated meat production to a pre-print server, enabling additional peer review of the studies and their conclusions. Cultivated meat is a nascent technology, and it is critical to have a variety of analyses that assess the techno-economics and environmental impact of production under various scenarios to ensure R&D and resources are spent on the most cost-effective and low-impact routes of production.

Although I do not believe it to be your intent, the negative news headlines and subsequent discussions online based on the worst-case scenarios in your study are a cause for concern because they sow confusion in educating the public and other industry stakeholders about the current and anticipated practices in cultivated meat production and by extension, its potential benefits or drawbacks. I am particularly concerned because my own research on the environmental impacts of cultivated meat over the past five years where I have been in close contact with many cultivated meat manufacturers and supply chain companies suggests that the highest environmental impact scenarios in your study are based on assumptions that are different from the current practices and long-term plans of the entire industry.

In the weeks since the studies were uploaded to *bioRxiv*, I have had discussions with cultivated meat manufacturers, media suppliers, and other scientists studying the environmental impact of cultivated meat. Below, I have summarized the key points from these discussions and had them reviewed for accuracy by the scientists I have spoken with.

I hope that you'll take the information below and other feedback and concern from scientists in the cultivated meat field seriously as your papers advance through the peer review process. Given the attention that has already been generated from these pre-prints, it is imperative that underlying assumptions are as accurate as can be and based on the best available information.

I would be happy to discuss these topics further with you at any point.

Best,
Elliot Swartz, Ph.D.
Principal Scientist, Cultivated Meat
The Good Food Institute

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Timothy Olsen, Ph.D.
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Major comments and critiques of the Essential 8 LCA (in relative priority):

1. **Assumptions about pharmaceutical-grade media do not align with reality.**
    a. The manuscripts as well as quotes in the press come off as authoritative on the use of pharmaceutical-grade media in the cultivated meat industry, but it's unclear where this authoritativeness is derived from. For example, in *New Scientist*:

    "This "pharmaceutical-grade" level of purification is required so that there are no contaminants such as bacteria or their associated toxins in the broth, says Risner"

        i. The DMEM/F12 carbon footprint calculated in this study (0.062 kg CO_2eq per liter) is only 8% higher than the carbon footprint of DMEM/F12 when back-calculating from the environmental impact of basal medium ingredients used in another model (personal communication with the co-authors of Sinke, 2023). Given this small difference in modeling of the complex basal media, almost all of the difference in the overall results of the study compared to Sinke, 2023 is due to applying an unnecessary 20x factor to these ingredients (discussed in #3 below), which is claimed to represent the impact of additional purification required for more refined pharma-grade media ingredients.
            1. The overall model for Essential 8 is still useful for the field, as LCA database inventories for cell culture media ingredients are currently incomplete.
        ii. Although the focus of the study is not on costs, costs and feasibility must also be considered when modeling different scenarios that are portrayed as being representative. Previous techno-economic models (Vergeer, 2021), including your own (Risner, 2020), demonstrate that using pharmaceutical-grade media results in costs that are several orders of magnitude higher than conventional meat costs. It is simply not possible to bring cultivated meat to market using pharmaceutical-grade inputs. This is known by everyone in the industry, so attempting to portray this as a realistic scenario is neither accurate nor beneficial to the analysis.
        iii. The UC Davis Cultivated Meat Consortium's external advisory board contains 10 individuals including myself and several cultivated meat startups and input suppliers that would have been happy to discuss this topic with you. It is unclear why you did not reach out to advisory board members to assess the current practices of the industry and to ensure the accuracy of your assumptions. As a

result, you've come to a conclusion — which is critical to the key findings — that does not represent the most recent science.

iv. **Media input suppliers are sourcing and selling food-grade ingredients to cultivated meat manufacturers *today.*** Food-grade materials are highly regulated and go through extensive testing by the raw material manufacturer/supplier with further validation at production/finishing facilities. They are often at the same or similar levels of purity as their pharma-grade counterparts (e.g., as in Kanayama, 2022 below) and thus are suitable for use in cell culture often with minimal differences in performance compared to pharma-grade counterparts. Food-grade amino acids are produced at scale by many large suppliers that are already plugged into the cultivated meat supply chain.

Supplementary Table 2. The table describing food ingredients, purity and quantity of raw materials.

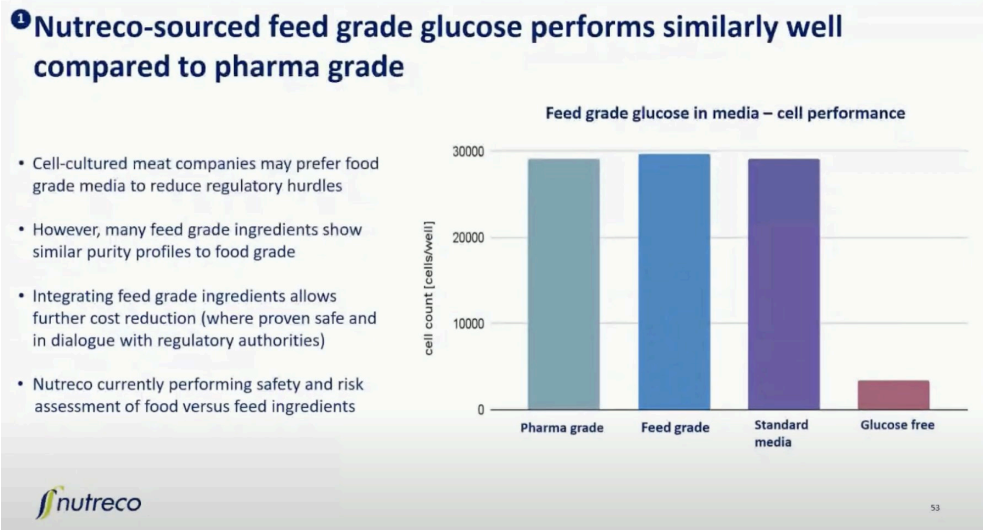
To add the same amount of raw material as the composition of DMEM, the amount added was calculated according to the lower value of the purity range. The values in the table are the amounts of raw materials needed to make 1 L of FG-DMEM.

	FG-DMEM			Manufacturer
Components	Purity(%)	Molecular Weight	(mg/L)	
Inorganic components				
CaCl2·0-2H2O	70.0 - 78.0	147.01	378.44	KANTO CHEMICAL, Japan
KCl	> 99.0	74.55	404.04	KANTO CHEMICAL, Japan
$MgSO_4 \cdot 7H_2O$	> 99.0	246.47	202.01	KANTO CHEMICAL, Japan
NaCl	99.5	58.44	5509.41	Nihonkaisui, Japan
$NaHCO_3$	> 99.0	84.01	3737.37	KANTO CHEMICAL, Japan
$NaH_2PO_4 \cdot 2H_2O$	98.0 - 103.0	156.01	144.23	KANTO CHEMICAL, Japan
Trace element				
$FeCl_3 \cdot 6H_2O$	>= 98.5	270.30	0.07	JUNSEI CHEMICAL, Japan
Amino acids				
L-Arginine·HCl	98.0 - 102.0	174.20	70.88	KANTO CHEMICAL, Japan
L-Cystine	98.0 - 102.0	240.30	49.33	Nippon Rika, Japan
L-Glutamine	98.0 - 102.0	146.14	595.88	Nippon Rika, Japan
Glycine	>= 98.5	75.07	30.46	JUNSEI CHEMICAL, Japan
L-Histidine	98.0 - 102.0	155.15	31.72	Nippon Rika, Japan
L-Isoleucine	98.0 - 102.0	131.17	107.14	Nippon Rika, Japan
L-Leucine	98.0 - 102.0	131.17	107.14	Nippon Rika, Japan
L-Lysine·HCl·H_2O	>= 98.0	182.65	148.98	Nippon Rika, Japan
L-Methionine	>= 98.5	149.21	30.46	Nippon Rika, Japan
L-Phenylalanine	98.5 - 102.0	165.19	67.01	Nippon Rika, Japan
L-Serine	98.0 - 102.0	105.09	42.86	FRONTIER FOODS, Japan
L-Threonine	98.0 - 102.0	119.12	96.94	Nippon Rika, Japan
L-Tryptophan	98.0 - 102.0	204.23	16.33	Nippon Rika, Japan
L-Tyrosine	98.0 - 102.0	181.19	73.05	Nippon Rika, Japan
L-Valine	98.0 - 102.0	117.15	95.92	Nippon Rika, Japan
Vitamins				
Ca Pantothenate	96.94 - 102.25	476.54	4.13	Kongo Yakuhin, Japan
α-GPC	98	257.00	7.51	Alpha GPC; Bio Actives Japan, Japan
Folic acid	98.0 - 102.0	441.40	4.08	DSM, Netherlands
i-Inositol	>= 97.0	180.16	7.42	TSUNO FOOD INDUSTRIAL, Japan
Niacinamide	>= 99.0	122.13	4.04	CHUGAI CHEMICAL INDUSTRIAL, Japan
Pyridoxine·HCl	>= 98.0	205.64	4.08	Kongo Yakuhin, Japan
Riboflavin	98.0 - 102.0	376.36	0.41	SEIWA, Japan
Thiamine·HCl	98.0 - 102.0	337.28	4.08	Kongo Yakuhin, Japan
Other component				
D-Glucose monohydrate	>= 99.0	198.17	4999.88	San-ei Sucrochemical, Japan

As stated by Cellular Agriculture Europe, some companies are already using

basal media that consists of 99% food-grade ingredients. Meatable has stated they are using media that is 70% food-grade. **While some media components are still sourced pharma-grade, the statement, "At the moment, all cultivated meat is grown in pharmaceutical-grade nutrient broths," which appeared in the original *New Scientist* article, is incorrect.**

v. In the paper, it is stated, "Utilization of commodity grade growth medium components such as glucose for animal cell growth is unlikely unless the components undergo an endotoxin separation process." In fact, Nutreco has shown that feed-grade glucose can perform just as well in animal cell culture applications as pharma-grade glucose.



vi. If pharmaceutical-grade purification is required, how can this be reconciled with the numerous papers and other data points that show sufficient cell viability and growth in media that contain food-, fragrance-, or feed-grade ingredients, many of which are purified using simple protocols by students in a lab? Certainly, these studies weren't meeting pharmaceutical specifications for all media ingredients. Non-exhaustive examples include:

1. Plant protein isolates used to replace animal albumins
2. Food-grade methylcellulose can enhance the performance of serum-free media
3. Media derived from algae and fermented okara
4. Fragrance-grade oleic acid used in cultivated chicken production
5. Integriculture's food-grade basal media
6. Nutreco's use of food-grade amino acids and feed-grade glucose
7. The ShojinMeat project growing cells in ingredients acquired from a grocery store

2. The concern over endotoxin contamination is exaggerated.

a. Several cell culture media suppliers were puzzled by the focus on endotoxin removal as a challenge and why this concern over endotoxin was being used as a justification for including the [pharmaceutical-grade] purification factor (PF) scenarios in the full LCA.

i. First, it is important to clarify to the reader that endotoxin is not primarily a food safety concern, as the end product is ingested rather than injected into the bloodstream. Concern for endotoxin in the context of cultivated meat is therefore related to cell culture performance rather than the safety of the end product.

ii. Different cell lines and cell types are affected by endotoxin differently. As stated in the research papers cited in the study, "endotoxins do not act directly against cells or organs but through activation of the immune system, especially the monocytes and macrophages, thereby enhancing immune responses (Magalhães, 2007)." Accordingly, cell lines that are not derived from the immune system have been shown to tolerate much higher levels of endotoxin. For example, multiple cell lines, including widely used cells such as 3T3 and CHO, displayed no detectable effects on cell growth with endotoxin levels as high as 20 ng/mL (Epstein, 1990). These concentrations are far higher than the specifications for pharmaceuticals, which are measured on picogram scales — hence, **using pharmaceutical-grade purification processes to meet pharmaceutical specifications for endotoxin removal is not a requirement for successful animal cell culture.** This is especially true in cultivated meat, where the cells are not derived from the immune system.

iii. There are many ways to reduce endotoxin in the raw materials used for media production, but the simplest way is by seeking out raw materials that are low in endotoxin to begin with. This is standard practice for many key basal media ingredients such as glucose, salts, and trace elements. Furthermore, each raw material coming from a supplier is routinely tested for endotoxins as part of established quality management systems and the final milled dry powder media production batch can be tested upon request before it is released to the customer.

iv. Endotoxin removal is a byproduct of many purification processes, as the pre-print mentions. Ultrafiltration is often used in the production of materials such as hydrolysates, amino acids, and proteins, but ultrafiltration is not a requirement for cell performance. **Non**ultrafiltered hydrolysates have been shown to perform just as well as ultrafiltered ones. As stated in the study, "low endotoxin levels were detected in all hydrolysate samples that were used for testing, suggesting that ultrafiltration is not necessary as an endotoxin risk-mitigating activity."

    1. It is true that in today's cultivated meat industry, many amino acids are supplied via individual microbial fermentation processes that could carry endotoxins. However, these amino acids are commonly being sourced at food-grade specifications today where they have not displayed issues with cell viability (see #1.a.iv above), and there is a strong push to develop media supply chains where the primary source of amino acids (and other vitamins and trace elements) are derived from hydrolysates

that inherently contain lower amounts of endotoxin than amino acids produced in bacteria.

v. Overall, it is unclear if the concerns regarding endotoxin relate to the raw materials themselves or to bottlenecks in the preparation of complete media, which may include various processing, filtration, and finishing steps. Could this be clarified? For example, for the former concern, a single raw material may be high in endotoxin, but by the time it is processed and combined with many other ingredients, endotoxin levels can be substantially diluted. Therefore, concern over endotoxin in raw materials has to be taken in the context of that specific raw material's concentration in the finished media. For the latter concern, many methods of filtration are available for use, and similarly to other industries (e.g., beer, wine), fit-for-purpose filtration technology and SOPs that balance the needs of the cultivated meat industry (e.g., cost, safety, performance) will be established with time.

vi. The justification for modeling TGFb production in CHO cells to avoid endotoxin is also questionable. There is a large negative incentive to manufacture proteins in animal cells, which is far more costly than microbial systems. While it is true that much of today's TGFb supply is produced in animal cells, it is also true that microbially-produced TGFb is sold today with low enough endotoxin levels to support cultivated meat companies and others performing animal cell cultures. Companies working on producing recombinant proteins in plants with no endotoxins are also planning to manufacture TGFb. Thus, researchers and companies can source microbially-produced TGFb with low endotoxin today, and the supply of TGFb manufactured in non-animal cells will only increase in the future.

3. **The use of Wernet, 2010 is not an accurate proxy for the environmental impact of cell culture media ingredients.**
    a. The study by Wernet *et al* is the basis for a 20x multiplication factor applied in various scenarios "to account for additional processing associated with active pharmaceutical ingredient production." This study looks at a 12-step chemical synthesis process to develop an active pharmaceutical ingredient (API). The relevance of this synthesis process to the majority of ingredients in Essential 8 is unclear, and **the justification for using this study as a proxy for a single media ingredient (let alone all of them in the worst-case scenario in the full LCA) is not adequately explained**. Furthermore, the energy mix used in this study as well as the actual purification processes used are not clear, making it even more difficult to assess its relevance to cell culture media and cultivated meat production.
        i. Overall, the environmental metrics for pharmaceutical products are very scarce, and information about processing steps is difficult to obtain. A similar problem currently exists in the cultivated meat industry, hence the impetus for this research. A discussion of these limitations, especially in the context of selecting

this single study as a proxy for the refinement of cell culture media ingredients, is warranted.

b. According to data we collected based on pharmaceutical-grade production of recombinant proteins produced in microbes (Sinke, 2023), downstream processing and purification make up about 33-50% of total facility energy use. Following your approach, this would lead to a factor of added energy use of 2x. A single data point can thus lead to a completely different model. This introduces great uncertainty and ignores the variety in upstream and downstream processing options leading to different environmental results.

c. In the Wernet 2010 study, it is stated that 65-85% of the impacts are energy-related (Fig 3). Given that this study was published 13 years ago and energy mixes have changed, this study is likely an overestimate of the actual impact of the production of the same API today or in the future, as the grid continues to reduce its reliance on fossil fuels. What would the impact be if predominantly renewable energy was used?

4. **Applicability of Essential 8 to cultivated meat production**
    a. In an article published in *Thin Ink*, it is stated, "He [Derrick Risner] also said they used E8 growth medium because that was identified by GFI as a growth medium which could be scaled." It is important to clarify that the media cost analysis published by GFI uses Essential 8 because it is a serum-free media formulation with a publicly-available composition. It is not stated in the analysis and should not be assumed that Essential 8 would be used for cultivated meat production. Rather, as described in Sinke *et al* 2023, the cell culture media composition will be based on the needs of the cells and is expected to deviate from commercially-available formulations designed for other purposes. Indeed, even the DMEM/F12 basal media formulation used in Essential 8 has been shown to contain ingredients that are nonessential or at suboptimal concentrations for pluripotent stem cell culture (Lyra-Leite, 2023). No cultivated meat companies are going to market using off-the-shelf Essential 8 or other common formulations. The decision to model Essential 8 with an off-the-shelf composition leads to inaccuracies in the downstream environmental impact model for cultivated meat production (discussed further in (B) below).
5. **Missing information related to energy use and media use calculations**
    a. Assumptions for energy mixes used in the study are not stated. More information is needed to validate the calculations presented in the study.

Minor comments and critiques:

- Phenol red is not food safe. It should be removed from the analysis as it will not be included in cell culture media for cultivated meat production. Furthermore, bioreactors are equipped with pH sensors, negating the need for a pH indicator in the media.
- Sinke, 2023 uses naphthalene sulfonic acid as a proxy for HEPES, which may also be considered for use in your model.
- Modeling the incorporation of antibiotics into the cell culture media is illustrative of how much of an environmental burden such a choice would carry. In this regard, its inclusion in this study

is useful. But it is misleading to present the use of antibiotics in Beefy-9 as the default, as this is not relevant to cultivated meat media that will actually be used in production. Beefy-9 contains antibiotics because the experiments in the corresponding paper (Stout, 2022) are performed in plastic dishes, which do not have the same degree of sterility control as a bioreactor. The manuscript fails to mention that antibiotics are not anticipated to be used for the production of cultivated meat, which is a major benefit of this way of meat production (McNamara & Bomkamp, 2022). There are two products from two companies in the United States that have received FDA clearance as well as multiple products from a single company that have been approved for sale in Singapore. None of these products are produced using antibiotics in the manufacturing process (only small amounts of antibiotics may be used during biopsy and initial cell isolation).

Major comments and critiques of the full LCA (in relative priority):

A. Media use calculations are not aligned with other studies, resulting in inefficient scenarios modeled for production

a. As discussed in #4 above, the amount/concentration of ingredients in Essential 8 is not optimized for cultivated meat production, and no companies would go to market using this off-the-shelf formulation. The consequences of assuming that Essential 8 would be used in the GCR and AAR scenarios in this study result in extremely poor yields of 0.87 to 3.43 grams of biomass per liter of media, corresponding to 292 liters to 1,148 liters of media per kilogram of meat (GCR to AAR scenarios, respectively). As discussed below, these scenarios represent very inefficient baselines from which to model.

i. **Despite the AAR scenario being inefficient, the carbon footprint of 19.2 kg CO_2eq/kg of meat is still 68% lower than the median carbon footprint of conventional retail beef (listed as 60 kg CO_2eq/kg in this study).** Indeed, the entire results section is misleading as it frames the findings as comparing worst-case and unrealistic scenarios for cultivated meat to best-case scenarios for conventional beef.

ii. The last sentence of the abstract states, “The results indicate that the environmental impact of near-term ACBM production is likely to be orders of magnitude higher than median beef production if a highly refined growth medium is utilized for ACBM production.”

1. As I’ve described above in #1-3, the highly refined growth medium scenario is reflective of an academic exercise, not near-term cultivated meat production. **The results actually indicate that models of inefficient cultivated meat production *still* have significantly lower carbon footprints than median conventional beef production.**

b. To further illustrate this inefficiency, we can compare the scenarios in this study to other published studies, as shown in Table D.9 from Sinke, 2023, which also contains the enhanced catabolism scenario from Humbird, 2021 — similar to the HGM scenario in this study (the difference being the amino acids sourced from hydrolysates as

opposed to fermentation).

Table D.9 – Comparison of amino acids and glucose mass balance between this study and other, recent studies (data from other studies recalculated to g/kg CM using publicly available information)

Aspect	This study			Tuomisto et al. (2022)			Humbird (2021) Scenarios for inclusion of hydrolysates	
	Low medium	Mid Medium	High medium	CMB	CMB128	CMC	Wild-type catabolism	Enhanced catabolism
Amino acids (g/kg CM)	200	283	400	448	197	196	453	388
Sugars (g/kg CM)	320	400	500	1270	557	557	816	360
Dry matter content	20%-30%	20%-30%	20%-30%	30%	30%	30%	30%	30%
Protein content	18%-25%	18%-25%	18%-25%	20%	20%	20%	21%	21%

We can then add the GCR and AAR scenarios from this study in this same format (based on the DMEM/F12 formulation containing glutamine), along with another study by your colleagues at UC Davis, O'Neill, 2023, which models an off-the-shelf mouse myoblast cell line called C2C12, the same cell line used in the CMB scenario above from Tuomisto, 2022.

Aspect	GCR (Risner)	AAR (Risner)	HGM (Risner)	O'Neill (2023)
Amino acids (g/kg CM)	1263	321	260	250-275
Sugars (g/kg CM)	3616	920	350	1100-1500
Dry matter content	30%	30%	30%	Not stated
Protein content	21%	21%	21%	Not stated

As illustrated by data from other studies, including those from your colleagues at UC Davis, the GCR scenario is an outlier that does not represent current or future cultivated meat production. It is highly questionable if such a scenario is even warranted for inclusion in the study, given that it requires 3 times as many amino acids and sugars to create 1 kg of meat compared to estimates from non-optimized, off-the-shelf cell lines. Despite this, at a carbon footprint of 75.4 kg CO_2eq/kg of meat, it is *still* only 25% higher than median conventional beef production and far lower than the worst forms of beef production.

B. Lack of discussion of more recent LCA studies

a. The paper has an entire section of its introduction called "The limitations of existing ACBM LCAs," but this section does not actually mention any of the more recent LCA studies, which are briefly described below and also referred to throughout this document. In fact, **none of these studies are cited at all in either of the papers**.

Notably, the conclusions (but not necessarily all underlying findings) in this paper — particularly the "PF" scenarios based on flawed assumptions — deviate significantly from every peer-reviewed study published to date.

- Tuomisto, 2022: This study uses bench-scale data and non-optimized cell lines and media to examine environmental impacts when cells are grown in hollow fiber bioreactors. This study is informative for understanding how some aspects of cultivated meat production may look today in early-stage startups.
- Kim, 2022: This study uses primary lab-scale and pilot-scale data from cultivated meat manufacturer SciFi Foods to examine the environmental impact of their hybrid beef burger.
- Sinke, 2023: This study uses data from over 15 different companies involved in the manufacturing and supply chain of cultivated meat to examine environmental impacts when cells are grown at a scale of 10,000 metric tons annually, set in the year 2030. This study is informative for understanding what cultivated meat production is anticipated to look like when it reaches commercial scale at the end of this decade.

C. The study does not model near-term cultivated meat production

a. The study claims to model "near-term" cultivated meat production, and this is used as a major justification for the inclusion of pharmaceutical-grade media scenarios. No definition of "near-term" is provided. Does "near-term" mean 3 years? 5 years? 10 years? 20 years? The production model used in the study is based on Humbird's techno-economic analysis (Humbird, 2021), which models cell growth in 20,000L bioreactors in a facility that outputs nearly 7,000 metric tons of meat per year. Additionally, the Humbird analysis assumes a market size of 100,000 tons of annual cultivated meat production. The cultivated meat industry is not operating at these scales today or in the next several years and thus it is difficult to reconcile how the study models "near-term" cultivated meat production. This seems to be a case of trying to have your cake and eat it too.

b. The Humbird analysis has higher energy use compared to other studies (i.e., Sinke, 2023) due to differing assumptions surrounding cleanroom infrastructure, and a discussion of these differences in the context of actual or anticipated practices in the cultivated meat industry is warranted.

D. Lack of any other comparison to conventional beef besides carbon footprint and fossil fuel depletion.

a. How does cultivated meat compare to conventional beef on other environmental indicators? Table 2 contains 10 different environmental metrics for cultivated meat, yet only two are discussed in the text of the paper, and none of the other corresponding metrics are listed for conventional beef. Why is this information and discussion omitted?

b. "Environmental impact" is discussed throughout the text of the paper, but in reality, only carbon footprints or fossil fuel depletion is discussed. Environmental impact is

much more than emissions. The text of the paper should be changed to reflect the actual comparisons being made.

Conclusions

In conclusion, my recommendation would be for major revisions to both manuscripts prior to publication. In particular, the major areas for focus in the full LCA are as follows:

- There is likely no market for cultivated meat produced with pharma-grade ingredients, and the value of modeling production in this way is dubious. Accordingly, the “PF” scenarios in the LCA should be excluded as they are highly misleading. These scenarios do not reflect “near-term” cultivated meat production, there is no requirement for pharma-grade ingredients or specifications to successfully grow animal cells, the justification based on concern for endotoxin is not sound, and it is highly questionable whether the proxy study used to estimate the environmental impact of pharma-grade purification is representative of cell culture media production.
- The GCR scenario should be excluded because even lab-scale data using non-optimized cell lines are far more (about 3x) efficient. The AAR and HGM scenarios are still useful and aligned with estimates from other published studies.

Because of these issues, the overall conclusions should be reconsidered. The analyses should be redone with scenarios that model near-term cultivated meat production based on current practices and the best available information.