Dairy and plant-based proteins as FBS alternative in muscle cell cultivation

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INTRODUCTION

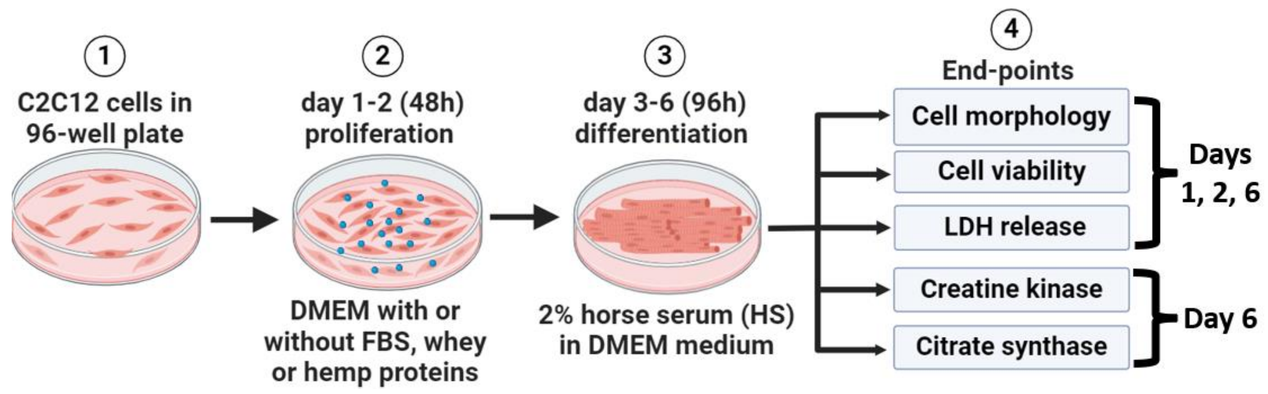
In-vitro meat cultivation, innovative technology in food science, offers a more sustainable and ethical source of alternative animal proteins to traditional meat (Lanzoni et al., 2023). Fetal bovine serum (FBS) is widely used in cell culture media to support proliferation. As FBS poses ethical challenges and batch-to-batch variability, there is a need to replace it with sustainable alternatives in in-vitro meat cultivation.

AIM

❖ To determine whether whey proteins (WP) and hemp seed proteins (HP) can substitute FBS in supporting C2C12 muscle cell proliferation and subsequently facilitate myotube formation under standard differentiation media.

❖ To determine cell response by morphology analysis, viability, lactate dehydrogenase (LDH) release, differentiation markers as creatine kinase (CK) and citrate synthase (CS) activities.

METHODS



RESULTS

❖ WP high (β-LG 1.25%, α-LA 1.25%, BSA 1.25%) and WP low (β-LG 0.07%, α-LA 0.15%, BSA 0.15%) mixtures **improved cell viability** on day 2 ($P < 0.05$), while HP (0.06-1 mg/ml) on days 1-6 compared to DMEM control ($P < 0.05$) (Figure 1A-D).

❖ Cell morphology analysis showed **myotube formation** on day 6 in both HP and WP mixture treated cells, while DMEM control showed poor differentiation (Figure 2A-E).

❖ WP mixtures **enhanced CK and CS** activities compared to DMEM control on day 6 ($P < 0.05$) (Table 1).

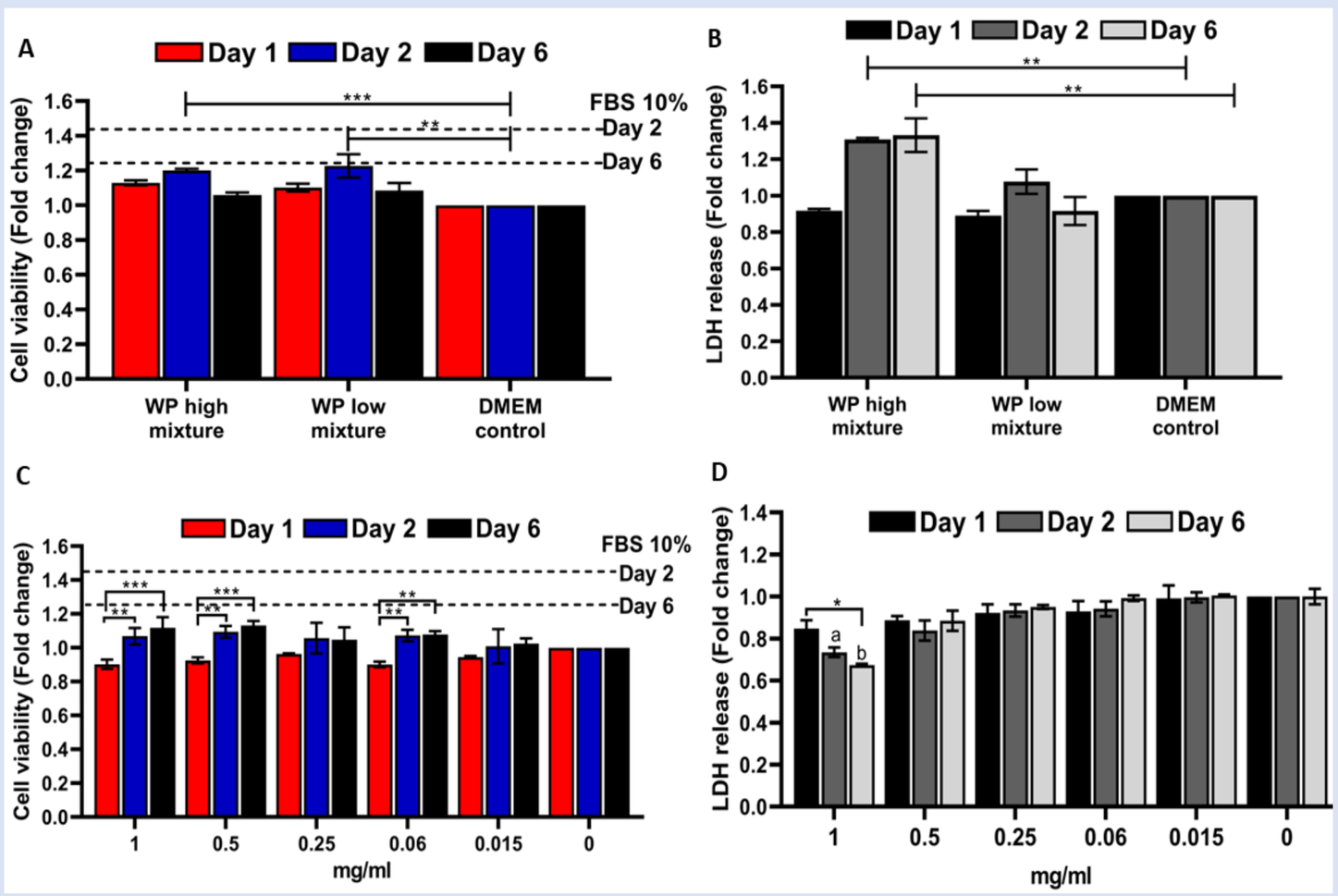


Figure 1. Viability and LDH release of C2C12 cells cultured in DMEM media comprising **(A-B)** WP high (β-LG 1.25%, α-LA 1.25%, BSA 1.25%) mixture, WP low (β-LG 0.07%, α-LA 0.15%, BSA 0.15%) mixture, or **(C-D)** HP (0-1 mg/ml). Day 1 and 2 represents cell proliferation with or without WP or HP and day 6 to differentiation with 2% HS media. 10% FBS that maximally improved viability is indicated with dashed lines for comparison. Data are normalized to control and shown as mean ± SEM (n=3, two-way ANOVA, **,***$P < 0.05$). Significant differences between same treatments at different time points are indicated with different letters ('a' for day 2; 'b' for day 2; $^{a,b}P < 0.05$).

Treatment groups	CK activity (U/L/mg protein)	CS activity (nmol/min/mg protein)
DMEM control (negative control)	$0^{\$}$	0.012 ± 0.0002
10% FBS (positive control)	20.24 ± 0.52^{a}	0.043 ± 0.001^{c}
WP low mixture (β-LG 0.07%, α-LA 0.15%, BSA 0.15%)	18.13 ± 1.23^{a}	0.042 ± 0.001^{c}
WP high mixture (β-LG 1.25%, α-LA 1.25%, BSA 1.25 %)	10.79 ± 0.52^{b}	0.059 ± 0.001^{d}

Table 1. CK and CS activities (day 6) were normalized against the total protein concentration. Within each assay, significant differences between the treatments are denoted by different letters, where $^{a,b,c,d}P < 0.05$. Groups with same letter do not differ significantly. $^{\$}$no change in colorimetric reading. (LG:lactoglobulin; LA:lactalbumin; BSA:bovine serum albumin)

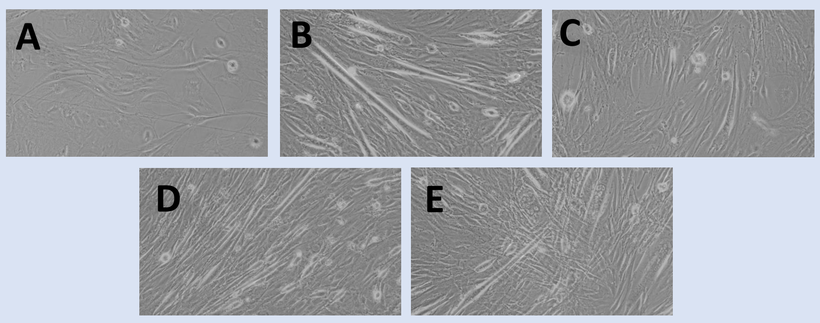


Figure 2. Morphology of C2C12 cells pre-cultured (48 h) in DMEM media alone **(A)** or supplemented with 10% FBS **(B),** 0.5% HP **(C),** WP low mixture **(D),** or WP high mixture **(E)** and then differentiated (96 h) in HS media. Images were acquired on day 6 post differentiation at 10x magnification.

Conclusions and future perspectives

Our study for the first time, showed that whey and hemp proteins can effectively replace FBS in establishing a serum-free muscle cell culture system. This study lays a groundwork for utilizing protein derivatives from plant and food industry by-products as FBS alternative in sustainable meat cultivation.

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