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MODIFICATION OF AN *IN VITRO* MODEL SIMULATING THE WHOLE DIGESTIVE PROCESS TO INVESTIGATE CELLULAR ENDPOINTS OF CARCINOGENESIS AND CHEMOPREVENTION

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Background

In vitro models simulating the digestive tract are commonly used to investigate degradation of nutrients or development of gut flora. Aim of this work was to modify an established model (Aura, 1999, J Cer Sci) to allow further studying mechanisms of carcinogenesis and chemoprevention using human carcinoma cells *in vitro*. Therefore the modified model, characterised by changing ox gall concentration to a physiological level, should be compared to the established model.

Methods

Fermentation supernatant (fs) of inulin was obtained by *in vitro* fermentation (Fig. 1). Bile acids, cholic (CA) and deoxycholic acid (DCA), were determined in fs by HPLC-MS/MS. Cell growth of HT29 cells treated with different concentrations of fs and corresponding synthetic mixtures of CA and DCA were determined by quantifying DAPI-labelled DNA.

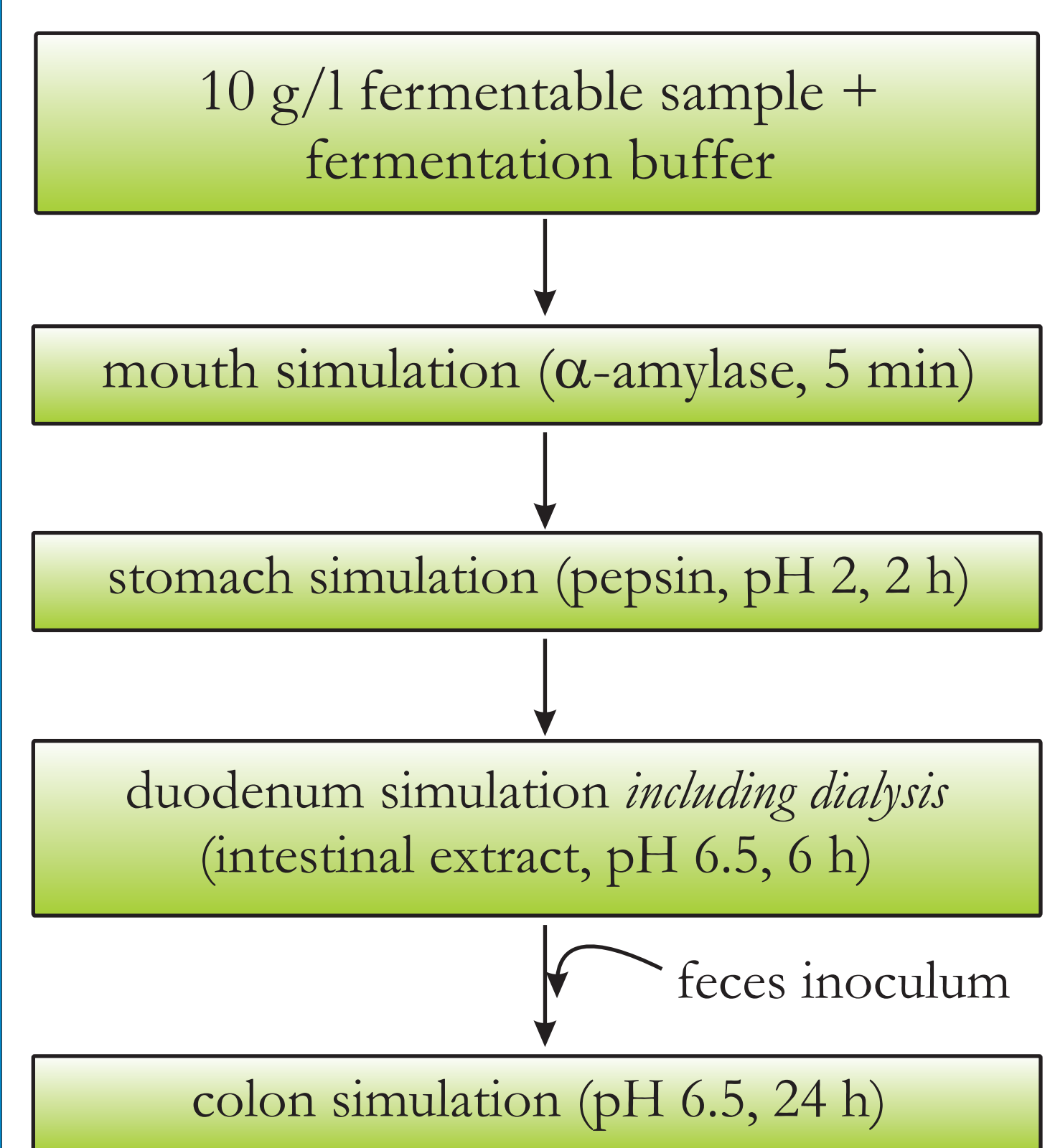


Figure 1: Process of *in vitro* fermentation.

Results

Established Model (high ox gall)

Using 41.6 g/l ox gall both fs inhibited cell growth whereas fs blank was more cytotoxic (Fig. 2).

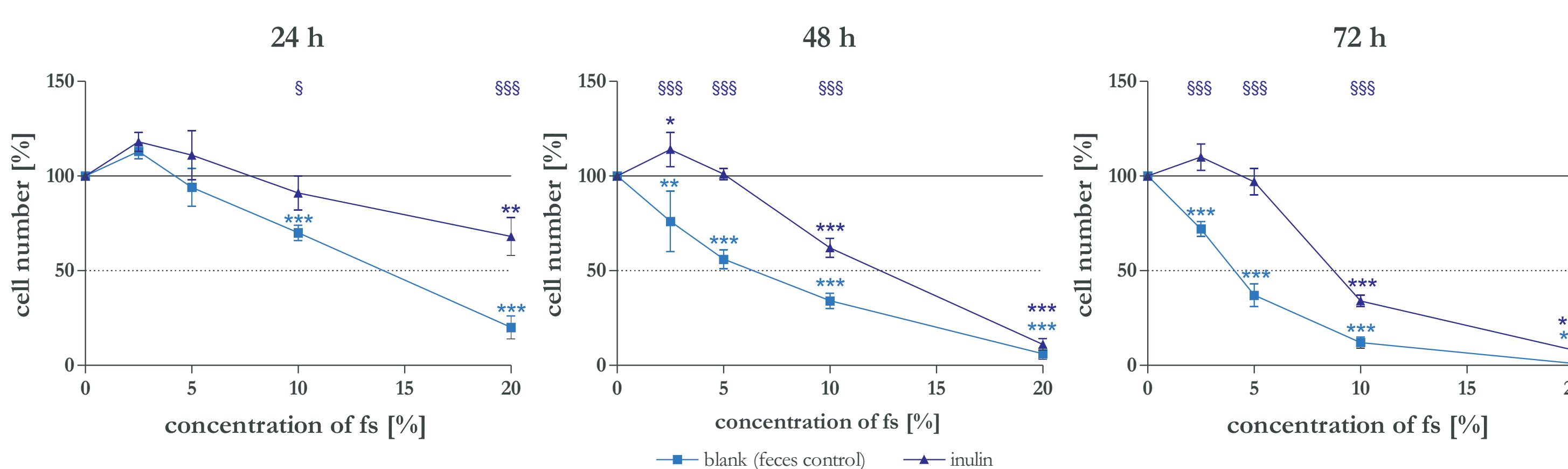


Figure 2: Modulation of cell growth after incubation of HT29 cells with fs obtained by fermentation using 41.6 g/l ox gall in intestinal extract. Shown are means \pm SD (n=3) and significant differences between fs blank and fs inulin (§ p<0.05, §§§ p<0.001) and differences of fs concentrations to medium control (* p<0.5, ** p<0.01, *** p<0.001).

Modified Model (low ox gall)

Using 0.6 g/l ox gall fs blank was less cytotoxic whereas fs inulin was more effective in reducing cell growth (Fig. 3).

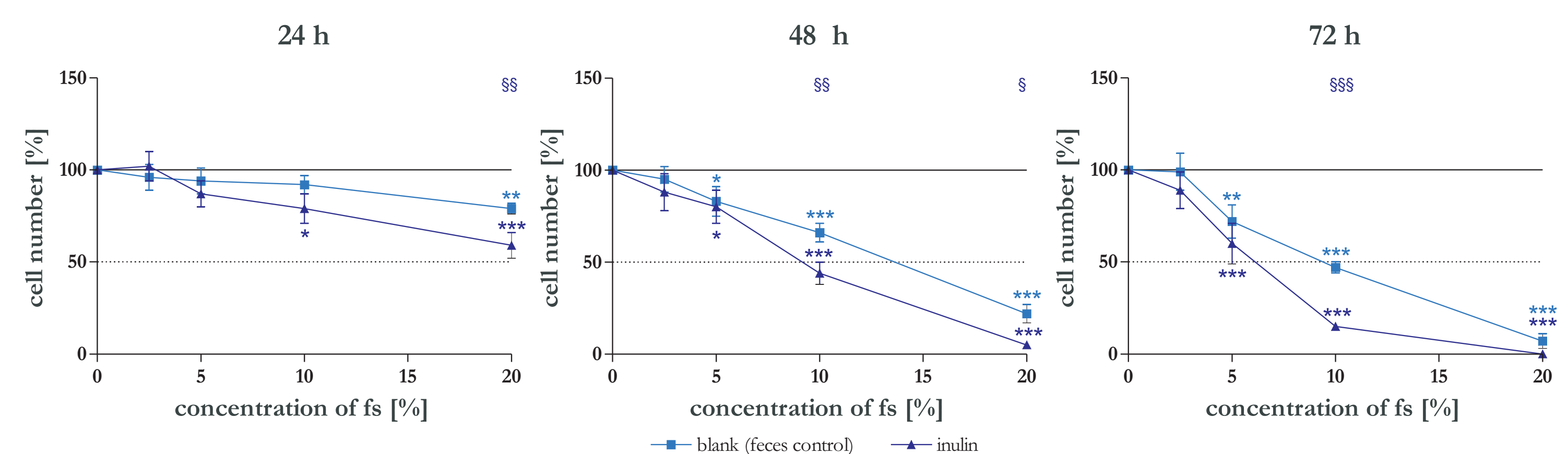


Figure 3: Modulation of cell growth after incubation of HT29 cells with fs obtained by fermentation using 0.6 g/l ox gall in intestinal extract. Shown are means \pm SD (n=3) and significant differences between fs blank and fs inulin (§ p<0.05, §§ p<0.01, §§§ p<0.001) and differences of fs concentrations to medium control (* p<0.5, ** p<0.01, *** p<0.001).

Use of 41.6 g/l ox gall resulted in unphysiological high amounts of CA and DCA in fs. Inulin decreased bile acid concentrations (Fig. 4).

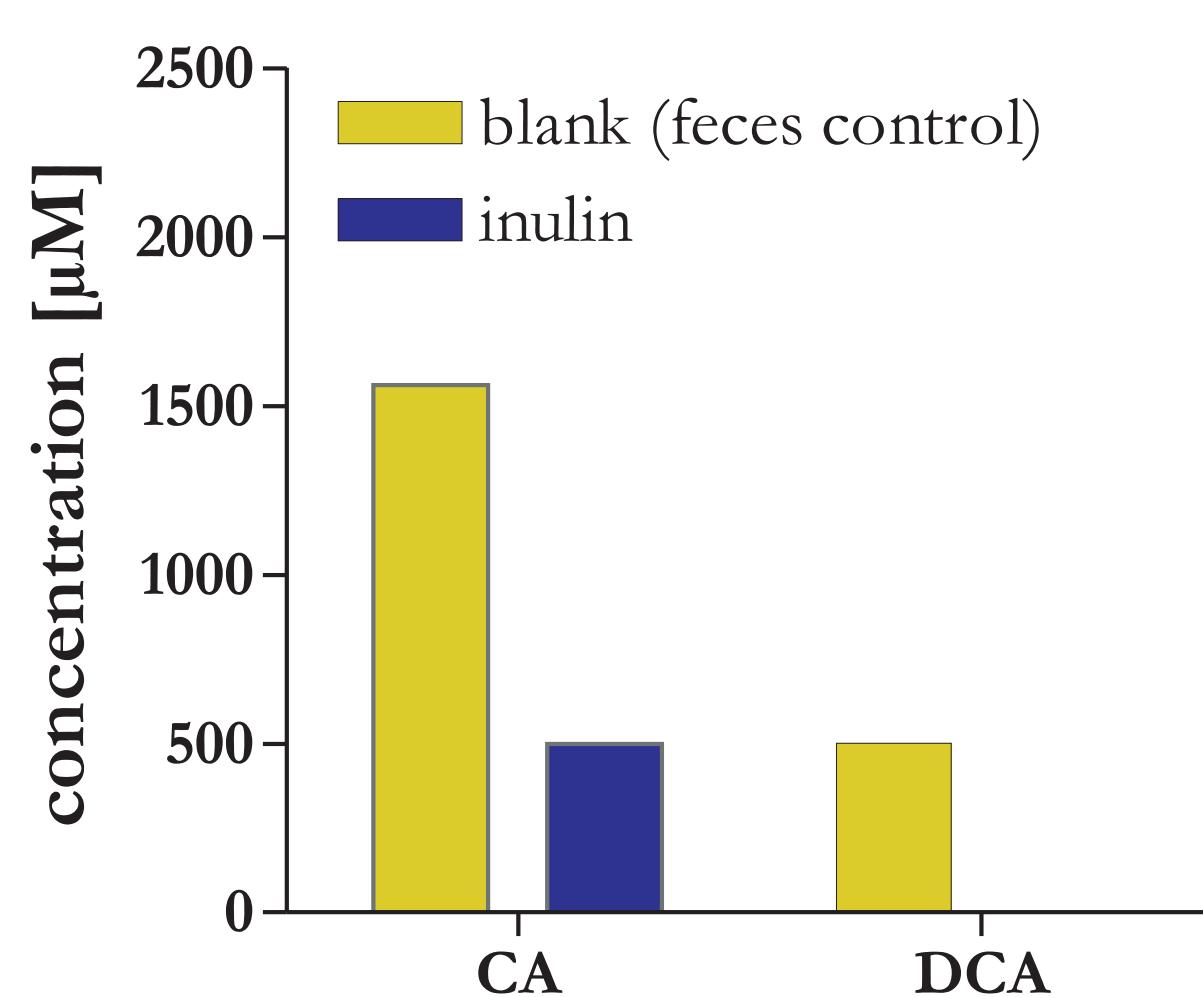


Figure 4: Concentrations of CA and DCA in fs after fermentation of inulin using 41.6 g/l ox gall in intestinal extract.

Use of 0.6 g/l ox gall resulted in considerable lower amounts of CA and DCA in fs. Inulin decreased bile acid concentrations (Fig. 5).

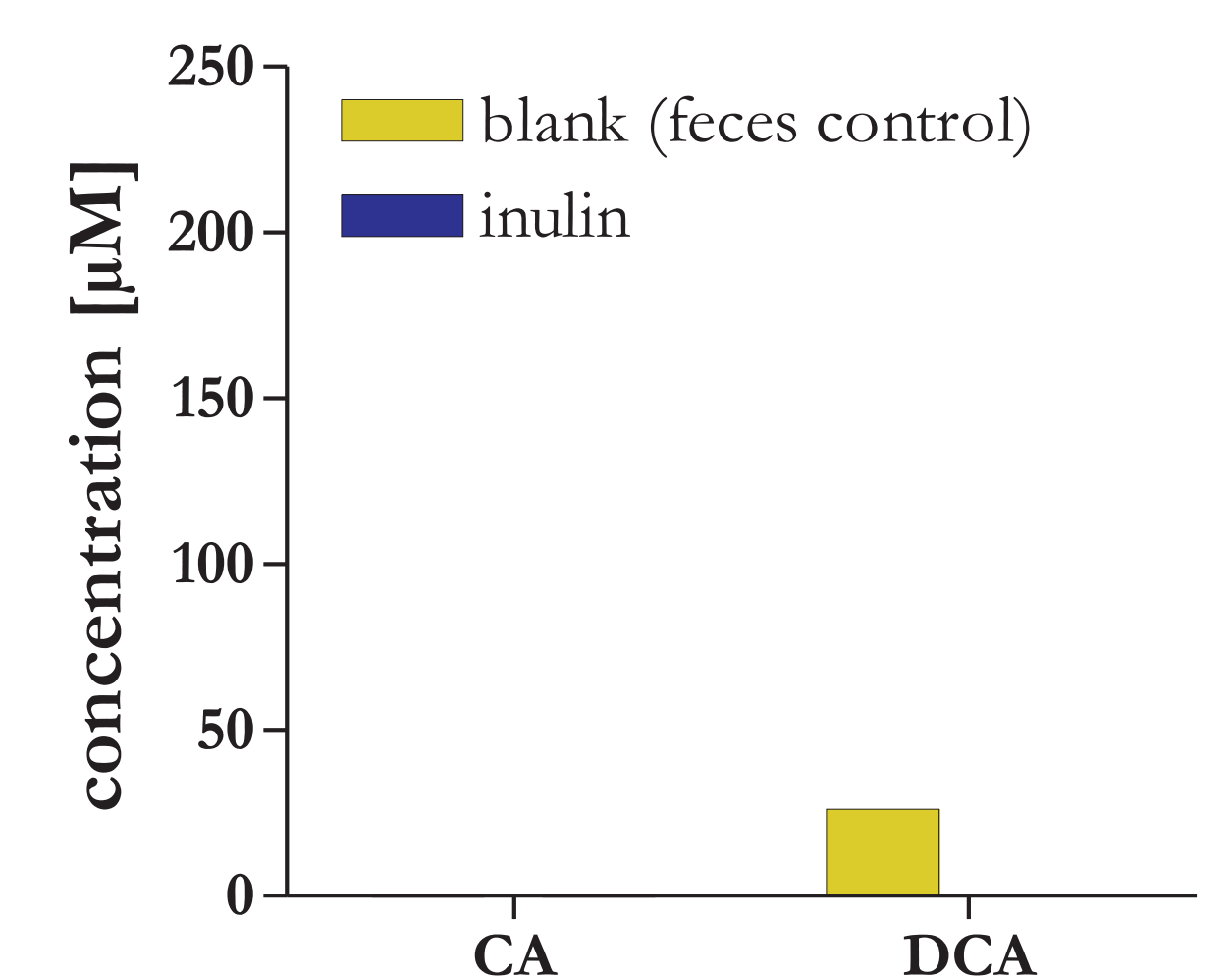


Figure 5: Concentrations of CA and DCA in fs after fermentation of inulin using 0.6 g/l ox gall in intestinal extract.

Cytotoxicity of fs blank is partly due to high amounts of CA and DCA (Fig. 6).

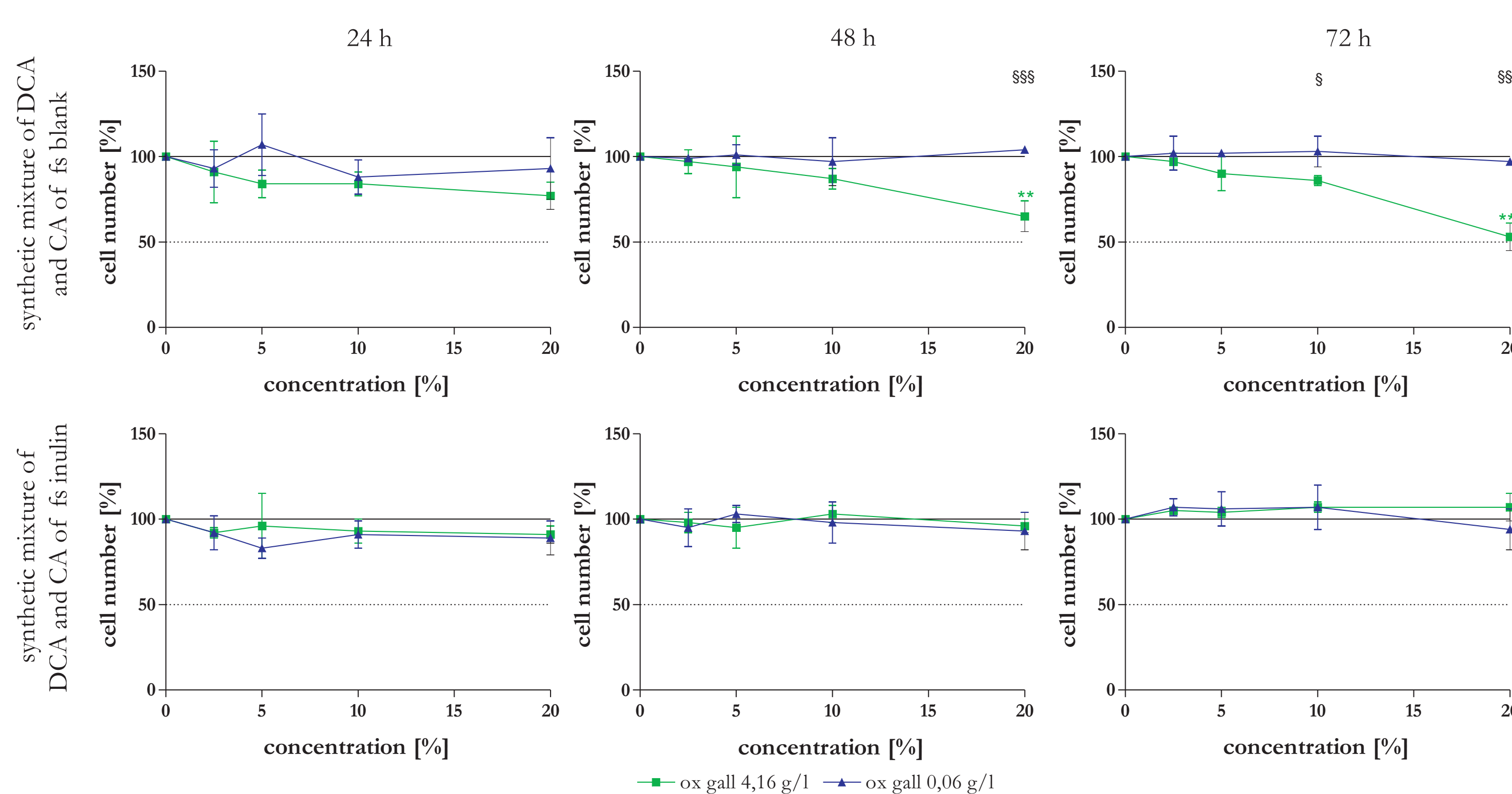


Figure 6: Modulation of cell growth after incubation of HT29 cells with synthetic mixtures of DCA and CA of corresponding fs yielded by fermentation using 41.6 or 0.6 g/l ox gall. Shown are means \pm SD (n=3) and significant differences between two synthetic mixtures of corresponding fs (§ p<0.05, §§§ p<0.001) and differences of synthetic mixtures to medium control (** p<0.01, *** p<0.001).

Conclusion

By decreasing ox bile concentration secondary cytotoxic side effects of fs are reduced. Moreover fermentation products of inulin suppress growth of HT29 cells. Fs obtained from the modified model (using 0.6 g/l ox gall in intestinal extract) can be used for *in vitro* investigations on chemopreventive properties of complex food ingredients.

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