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## Derivation of canine hepatocyte in vitro models to study Branched-Chain Amino Acid effects on liver functions.

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### Abstract

Branched chain amino acids (BCAA), have been shown to affect human gene expression, protein metabolism, apoptosis, and regeneration of hepatocytes. Furthermore, they have been demonstrated to inhibit proliferation of liver cancer cells in vitro, and to be essential for lymphocyte proliferation. In veterinary medicine, the use of BCAAs as integration of a normal dietary plan, is likely to be a valid choice for the same benefit found in human clinical nutrition, although this aspect is still debated. Indeed, long-term oral supplementation with BCAAs in the prevention of liver fibrosis and injury in the dog's liver is still unclear. Aim of the present study will be to determine how BCAAs preserve liver functions in vitro. To this purpose we have selected and set up three different in vitro models: hepatic dog cells and canine hepatocellular carcinoma cells plated in 2D monolayer and hepatic dog cells cultured onto 3D scaffolds, obtained from decellularized rabbit liver. All cells adhered and proliferated once plated. Cells grown in monolayer quickly entered G<sub>0</sub> end arrested growth, ELISA test confirmed their ability to produce albumin. Cells grown on scaffold vigorously replicated and showed their capability to recellularize ECM rabbit liver. These results, although preliminary, demonstrate that the culture conditions used well preserved the original phenotype and function and further support the possibility to use in vitro models to successfully study BCAA efficacy in dog.

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