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**In vitro models for biochar testing - evaluating its bioactivity according to 3R rules and translational studies**

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**Abstract**

**Background/Objective:** Biochar, derived from the pyrolysis of organic materials such as wheat straw or wood chips, is a feed additive used in livestock production. Nevertheless, the studies on biochar's role as a functional element of diet are poorly described and understood. In addition, the *in vitro* studies aimed at biochar cytotoxicity evaluation are limited, while could be beneficial for further translational research on the pro-health features of biochar e.g. in poultry.

**Methods:** A comprehensive literature review spanning a decade (2014-2024) was conducted (PubMed and Google Scholar databases) to summarize data considering biochar *in vitro* bioactivity. Simultaneously, a pilot-scale *in vitro* study was designed to assess the cytotoxicity of biochar, using the human Caco-2 cell line, which has proven to be a valuable model for evaluating various natural bioactive compounds like curcumin, resveratrol, and quercetin - though it has not previously been used for biochar testing.

**Results:** The review of the literature confirms insufficient data on *in vitro* studies related to biochar cytotoxicity and bioactivity. The Caco-2 cell line, an established and well-characterized in vitro model, possesses features beneficial for testing biochar and analyzing it as a feed additive in future livestock production. However, more in-depth studies are required.

**Conclusion:** There is a great need for biochar screening *in vitro*. The assays could help introduce novel functional feed additives affecting livestock productivity.

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**Keywords:** feed, additives, livestock, animal health, cytotoxicity, functional studies