

## Abstract

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### **Development of a refined zebrafish feeding trial to evaluate food safety.**

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Before novel components are introduced into the food chain, a profound safety evaluation is required which generally includes a rodent feeding trial to assess potential toxicity. The development of a zebrafish feeding trial would advance existing testing strategies by reducing the cost, replacing the use of mammals by a lower vertebrate species, and facilitating reproductive and transgenerational studies.

When experimentally evaluating novel food components using an animal feeding trial, partial substitution of standard food ingredients by the component of interest is often required, possibly interfering with nutritional requirements. In our case, the extent of component substitution tolerable for the zebrafish metabolism should therefore be assessed prior to the evaluation trial. We determined the maximum tolerable percentage of maize, as maize is a well-known food product for human consumption, but not commonly used in fish feed. Fish were fed for 4 weeks with 6 experimental feeds ranging from 0% to 25% of maize. Growth slightly (2.5%) decreased when fish were fed with 0% or 25% of maize. The hepatosomatic index of males significantly increased when fed with 20% or 25% of maize. Feed digestibility analysis showed a decrease in carbohydrate uptake when fish were fed with an increasing maize substitution rate. Transcriptional regulation as a function of maize substitution rate was also studied using full genome Zebrafish Agilent Micro-arrays and qPCR analysis.

Furthermore, we tried to define the maximum tolerable percentage of maize for zebrafish larvae. Larvae fed with 20% of maize or less showed a significantly lower survival rate compared to the commercial control. Both length and swimming distance were significantly lower compared to the commercial diet, although this difference seems to be stabilising over time. Transcription levels of genes known to be involved in the digestive system (e.g. trypsin) were not affected by feeding with an increased maize percentage (0% versus 25%). However, we did observe a difference between feeding with the experimental diet and the commercial control, reflected by 3 distinct transcription patterns (aligned, convergent and divergent).

We suggest that our approach of determining component substitution rates could be a valuable asset to food safety evaluation trials.

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