Development of a zebrafish feeding trial to evaluate food safety.

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The safety of food components, additives and contaminants is generally evaluated using rat feeding trials to assess (sub)chronic toxicity and reprotoxicity. The development and implementation of an alternative zebrafish feeding trial would refine these testing strategies by reducing the cost, replacing the use of mammals by a lower vertebrate species, and by facilitating reproductive and multigenerational studies. When testing food components (e.g. GMOs), a substantial part of the food is substituted by the component under evaluation, possibly interfering with nutritional requirements which has led to severe criticisms on rat feeding trials. Therefore, when developing a zebrafish feeding trial to investigate food components, the extent of component substitution still processable by the zebrafish metabolism (maximum tolerable percentage) should be assessed prior to the component evaluation trial.

Since we will conduct a feeding trial with GM maize in a later phase of our study, we defined the maximum tolerable percentage of maize. First, we formulated an experimental fish feed based on the composition of commercial fish feeds. The maize was introduced in the feed by a stepwise substitution of a wheat component (25% of the whole feed). In this way, 6 experimental feeds were formulated ranging from 0% to 25% of maize in steps of 5%. We compared these experimental feeds to three different commercial feeds in a one month zebrafish feeding trial. We investigated the following endpoints: relative condition factor and growth, hepatosomatic and gonadosomatic index (HSI and GSI respectively), reproduction, energy budget and feed digestibility. The growth of fish fed with either 25% wheat or 25% maize slightly decreased, possibly indicating that a combination of wheat and maize in the diet results in a more balanced carbohydrate composition for zebrafish. Next, we observed an increased HSI in males when the percentage of maize in the feed increased. The HSI was significantly increased when males were fed either 20% or 25% maize substitution compared to the control feeds. Measurement of feed digestibility showed a clear decrease in carbohydrate uptake when fish were fed with an increasing percentage of maize substitution, and this decrease was significant from a 15% maize substitution onward.

Based on these results, we selected an adequate percentage of 15% of maize substitution for conducting the feeding trial with GM maize. This selection was based on two criteria: (1) the biological limits of the maize component in the feed of zebrafish should be respected, (2) the amount of maize substitution should allow us to observe effects of GM maize if there are any. We suggest that our approach of first determining maximum tolerable food component substitution rates before carrying out feeding trials could be a valuable addition to existing protocols.