Production of Low-Estrogen Goldfish Diet for \textit{in vivo} Endocrine Disrupter Test

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A low-estrogenic diet for goldfish \textit{Carassius auatus} was produced for an \textit{in vivo} estrogen activity test, because commercial fish feed has estrogenic activity and may affect the results of estrogen assays. The newly produced diet (FD5) was formulated with defatted rice bran and casein, and did not contain any soybean meal or fish meal. Phytoestrogen contents (genistein, daidzein, equol, and coumestrol) of FD5 were measured by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) and compared with those of the commercial trout diet (TD) and carp diet (CD). The genistein, daidzein, and coumestrol contents of TD and CD were much higher (5–2000 times) than those of FD5, but equol was detected only in FD5. Estrogenic activity of the fish diets was estimated \textit{in vitro} by the yeast estrogen-screen assay (YES assay). The estrogenic activity was detected in TD and CD, but not in FD5. The \textit{in vivo} estrogenic activity of the diets was examined by determining the production of vitellogenin in male goldfish. When male goldfish were fed TD or CD, plasma vitellogenin levels increased, but fish that were fed FD5 maintained low vitellogenin levels. These results indicate that FD5 produced in the present study has a low estrogenic activity, and FD5 would be suitable for the \textit{in vivo} estrogen activity test using goldfish.