Dietary provision of omega-3 polyunsaturated fatty acids (PUFA), including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has received considerable attention, including in poultry nutrition. The latter has primarily focused on producing omega-3 enriched eggs and meat for human consumption. The current study, however, was designed to determine potential health benefits to laying hens receiving different sources of dietary omega-3 fatty acids, as assessed during an inflammatory challenge induced by lipopolysaccharide (LPS) administration. A total of 80 Lohmann LSL-Classic (white egg layer) were randomly assigned to 1 of 8 treatment diets (10 hens/treatment), with diets formulated to meet NRC requirements for laying hens. Treatments provided 0.2%, 0.4%, 0.6%, or 0.8% content of total dietary omega-3 fatty acids, provided as either flaxseed oil (ALA) or algal DHA for 56 days. Specific measures included weekly body weight, feed intake, egg production, egg weights and egg quality measures. On day 56, birds from each treatment were injected with either saline (Sham; n=5) or LPS (8 mg/kg i.v.; n=5), and blood and tissue samples (post-CO2 asphyxiation) were collected for subsequent analysis of fatty acids and oxylipins. In general, dietary omega-3 supplementation patterns yielded predictable responses in plasma, liver and yolk fatty acid concentrations in response to increasing dietary ALA or DHA. LPS challenge led to significant reductions in mean liver EPA (mg/g; Sham = 0.088 mg/g +/-0.04; LPS = 0.077+/-0.04 mg/g; P<0.05). There was a significant Diet×Challenge interaction for plasma EPA and DHA (mg/ml). Analysis of plasma oxylipins indicated significant main effects due to LPS and omega-3 supplementation for certain oxylipins (ng/ml), including a Challenge effect on the EPA-derived 8-hydroxy-eicosapentaenoic acid (HEPE) (Sham = 0.383+/-0.066 ng/ml; LPS = 0.19+/-0.066 ng/ml; P<0.05), 9-HEPE (Sham = 0.404+/-0.075 ng/ml; LPS = 0.148+/-0.075 ng/ml; P<0.05), 18-HEPE (Sham = 0.368+/-0.053 ng/ml; LPS = 0.592+/-0.053 ng/ml; P<0.05). In summary, LPS challenge modulated certain oxylipins, including those derived from EPA. Further studies will clarify the role of dietary omega-3 fatty acids in modulating responses to an inflammatory challenge in laying hens and the biological significance of changes in oxylipin profiles. (NSERC/Manitoba Egg Farmers/Egg Farmers of Canada)