Contaminated poultry meat products are considered as the major source of human cases of campylobacteriosis. Several strategies are considered to decrease the number of Campylobacter in the intestinal tract of chickens which in turn would reduce infections in humans. One of these strategies is vaccination. Our aim was to develop a flagellin-based subunit vaccine with intrinsic adjuvant activity. The immunostimulatory properties make bacterial flagellin a potent vaccine adjuvant, however C. jejuni flagellin is unable to activate TLR5, in contrast to the flagellin of most other bacterial species. We constructed a chimeric NHC flagellin that contains fragments from Salmonella enteritidis flagellin which is able to potently activate TLR5. To assess the potential of this flagellin-based vaccine, in ovo vaccination was performed at embryonic day 18. Additionally, one group of chickens was immunized with a C. jejuni 81116 total cell lysate. Serum samples were taken at 11 and 15 days after hatching to assess the specific immune response by ELISA. In ovo vaccination demonstrated the successful generation of IgG antibodies against flagellin-based subunit vaccine and total cell lysate of C. jejuni 81116. Our results indicate that in ovo vaccination with a protein subunit vaccine is an effective way to generate a specific antibody response against C. jejuni.