The gastrointestinal tract plays a major role in the processes of food decomposition and enzyme involved at the level of the digestive tract and its integrity is one of the most important systems in defense against ingested toxins. Nivalenol (Niv) is a trichotecene mycotoxin, found in cereals and processed grain, frequently associated with another widespread contaminant, Deoxynivalenol (Don). Following ingestion of contaminated food, intestinal epithelial cells are exposed to high concentrations of mycotoxins which could induce severe mycotoxicosis in the host. In this study we have investigated the effects of Niv and Don on intestinal epithelial cell line, IEC-6. Oxidative stress plays often an important role in maintaining intestinal mucosal integrity. In particular in IEC-6 cells, mitochondria are the major source of reactive oxygen species (ROS) and also a sensitive target for ROS-mediated damage. Increased generation of ROS causes oxidative stress and is responsible for intestinal mucosal injury. In this study we report that both Niv and Don induced ROS production, reflecting an induction of oxidative stress state. Mycotoxins-induced ROS in IEC-6 have been decreased in the presence of the NAD(P)H inhibitor, DPI. In addition NAC treatment, an antioxidant which is a precursor of reduced glutathione, was able to reduce ROS production induced by Niv and Don in IEC-6, although to a lesser extent respect to DPI inhibition. Moreover in mycotoxins treated IEC-6 variations in calcium homeostasis have been also observed. Therefore, our results highlighted the risks of Niv and Don associated in food intake for the intestinal homeostasis.