Development of capture ELISA for chicken cytokines using commercially available antibodies

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Cytokines are small proteins that are produced mainly by immune cells and play crucial roles in the communication within the immune system. The type of cytokine and their levels can inform about the current immune state of the organism. Cytokines may serve as biomarkers for infection, disease and inflammation. In poultry field, cytokines are routinely measured by qPCR. This method allows to check expression of cytokines on a mRNA level in cells. To quantify secreted cytokines on a protein level a method like capture ELISA should be used. Unfortunately, only for chicken IFN-y a capture ELISA is commercially available. Therefore, the aim of this project was to develop and validate capture ELISAs for chicken IL-2, IL-6, IL-10, IL-12p40 and IFN-y, using commercially available antibodies. We were able to develop capture ELISAs for the sensitive detection and quantification of all the mentioned cytokines. The detection range of the assays goes from a minimum detection level of 3.39 – 31.82 pg/ml up to a maximum detection level of 1250 – 5000 pg/ml (depending on the ELISA). The ELISAs perform well for the detection of recombinant cytokines in dilution buffer as well as in more complex matrixes, like plasma. Plate to plate variations for the assays are very low, indicating the high reproducibility of the ELISAs. Detection of native cytokines by these capture ELISAs was tested on chicken plasma and supernatant from stimulated chicken cells (monocytes, PBMCs and the HD11 macrophage cell line). We detected IL-6, IL-10 and IL-12p40 from culture supernatant from monocytes and HD11 macrophages and IL-2, IL-6, IL-12p40 and IFN-y from supernatants of stimulated PBMCs. From plasma of naive chickens, all of the cytokines were detected. In conclusion, we developed robust capture ELISAs for the quantification of native chicken cytokines, based on reagents that are all commercially available. The lack of tests to quantify chicken cytokine levels on a protein basis has hampered the development of the field of chicken immunology in the past. The cytokine capture ELISA developed during this project will be valuable research tools for a better assessment of the immune system of chickens and may stimulate progress in this research field.