# Summary

This thesis describes the research to explore the feasibility of plants for oral vaccination. The research focussed on a model of LTB produced in potato tubers or ovalbumin (OVA) as antigen and tested in mice. A general introduction into the backgrounds of oral immunisation is given in Chapter 1. The next two chapters describe the optimisation of an immunisation protocol for edible vaccines by addressing the following questions: can the immune response be increased by more frequent doses of edible vaccines without negative effects on general health (Chapter 2)?; and can the immune status of the host be modified to react more efficiently to a subsequent oral boost (Chapter 3)?. Ovalbumin (OVA) was used as model antigen and administered via intragastric (IG) gavage to mice. The results of these studies led to a refined immunisation protocol in which one single subcutaneous, adjuvanted priming is followed three weeks later by oral boost immunisations on three alternating days (also referred to as the 'systemic prime/oral boost' protocol).

As a model for edible vaccines, the heat-labile enterotoxin subunit-B (LTB) of *Escherichia coli* was produced in potato tubers. This edible vaccine was either fed or administered orally to mice. Using the optimised immunisation protocol, local and systemic responses against LTB were induced (Chapter 4). Subsequently, the use of LTB as adjuvant for co-expressed antigens in edible vaccines was explored. A glycoprotein (E2) of the classical swine fever virus was co-expressed as fusion protein to LTB or expressed together with LTB in potatoes, resulting in E2-LTB and E2 + LTB potatoes, respectively. LTB fused to these antigens retained its biological activity (GM1-binding). The expression levels of LTB varied from 0.25% in LTB-transgenic plants to 0.01% LTB per total soluble protein in E2-LTB-transgenic plants. The levels of LTB as fusion-proteins were lower than those of LTB alone or co-expressed with E2. LTB, E2 + LTB, and CVP-LTB producing tubers were immunogenic upon subcutaneous immunisation and significant antibody responses against LTB were detected. The response towards the co-expressed antigens were low or undetectable. Probably, the antigen dose of the co-expressed antigen was too low and the adjuvant capacity of LTB was insufficient (Chapter 5). Further research is required to improve the carrier and adjuvant function of LTB. Another point of concern is that besides adjuvant activity, LT,CT and their B-subunits are also known to be capable to induce oral tolerance. Future research must concentrate on more effective carrier-molecules or adjuvants devoid of tolerating properties.

The chapters 4 and 6 clearly demonstrated that IG gavage of tuber or chow extracts induced higher antibody responses than similar doses of antigen taken up with feed or drinking water. It was concluded that the route of oral administration is at least as important than the vaccine composition. The difference between feeding and oral administration must be taken into account when the feasibility of edible vaccines is assessed (Chapter 6).

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In Chapter 7, the general findings of this thesis are discussed. The proposed systemic prime/'triple dose' oral boost protocol appeared to be applicable for oral administration and oral intake of edible vaccines in particular. However, significant adverse effects were observed after tuber intake. The ideal edible vaccine in plants has sufficient levels of antigen, is easy to propagate under a wide range of conditions and is not toxic when given the amounts required. In this respect, the use of potatoes has several drawbacks. Consumption of raw potatoes is not preferable and cooking might denature the antigen. For future edible vaccine studies, a more suitable plant with relatively high expression levels should be chosen (e.g. tomato).