SUMMARY

Preterm infants have a delayed intestinal bacterial colonisation. Together with an immature gastro-intestinal tract and immature immune system, this puts preterm infants at risk to develop serious infectious morbidity. A mixture of prebiotics consisting of non-human neutral and acidic oligosaccharides may stimulate the growth of a bifidogenic intestinal microbiota, prevent adhesion of pathogens to the intestinal wall and positively stimulate the immune system. As a result of these effects, we hypothesised that neutral and acidic oligosaccharides decrease serious infections, increase feeding tolerance, improve the response to vaccinations, and decrease the risk to atopy and infections later in life. Most studies with prebiotics only focus on the colonisation of the intestinal microbiota. The influence on the immune system is not yet fully understood. However, studying the immune modulatory effects is complex because of the multicausal risk of infections in preterm infants. Increased insight in the effects of prebiotics on the developing immune system may help to identify possible therapies to decrease (infectious) morbidity and mortality in preterm infants.

This thesis describes a double-blind randomised controlled trial in 113 preterm infants on the effect of enteral supplementation of a prebiotic mixture of neutral and acidic oligosaccharides. The primary aim of this thesis is to determine the effect of enteral supplementation of a prebiotic mixture consisting of acidic and neutral oligosaccharides on serious infectious morbidity. Secondary aims are to determine the effect of acidic and neutral oligosaccharides on feeding tolerance and short-term outcome. In chapter 1 and 2 we describe the background and aims of the studies presented in this thesis.

In chapter 3, we determined the effect of enteral supplementation of the prebiotic mixture on serious infectious morbidity, feeding tolerance and short-term outcome in preterm infants. We found that enteral supplementation of the prebiotic mixture decreases the incidence of endogenous infections, if given in sufficient amounts. These results were not influenced by gestational age, birth weight, Apgar score <6 at 5 min, and exclusive breastfeeding during the 28d study period. We did not find an effect of enteral supplementation of the prebiotic mixture on feeding tolerance (time to full enteral feeding) or the incidence of necrotising enterocolitis. Enteral supplementation of neutral and acidic oligosaccharides decreased the incidence of mild BPD.

As part of the previously described randomised controlled trial on the effect of enteral
supplementation of a prebiotic mixture of neutral and acidic oligosaccharides on serious infectious morbidity, we performed several studies to elucidate the role of neutral and acidic oligosaccharides on postnatal adaptation of the gastrointestinal tract and in modulation of the immune response. The development of the intestinal microbiota and the gastrointestinal tract in preterm infants and the effect of a prebiotic mixture is described in chapter 4. In chapter 4A.1, we describe in a review of the literature that preterm infants have a delayed intestinal colonisation with health promoting bacteria compared to healthy breast-fed term infants and antibiotics further delay the intestinal bacterial colonisation. In chapter 4A.2, we found that enteral supplementation of the prebiotic mixture increased the intestinal colonisation at day 14 postpartum of all bacteria, but not at day 30 postpartum as measured with FISH. There was no significant increase of health promoting bacteria such as bifidobacteria and lactobacilli, nor a significant decrease of pathogenic bacteria, after enteral supplementation of a prebiotic mixture. In general the number of all faecal bacteria was low. In our study, treatment with broad-spectrum antibiotics substantially decreased the growth of all intestinal bacteria. We speculate that broad-spectrum antibiotic may (partly) diminish the beneficial effect of prebiotic supplementation on the intestinal microbiota and infectious morbidity. In chapter 4A.3, we found that enteral supplementation of the prebiotic mixture changed the intestinal microenvironment by decreasing stool pH and stool viscosity. There was a trend toward increased acetic acid concentration and increased stool frequency. This suggests an improved intestinal microenvironment that may favor the effect of a ‘bifidogenic’ microbiota and as a consequence has a beneficial effect on the intestinal wall and immune system. IL-8 and calprotectin are markers of intestinal inflammation. In chapter 4B, we found that enteral supplementation of a prebiotic mixture does not change f-IL-8 and f-calprotectin levels in preterm infants. The lower incidence of serious endogenous infections prebiotic mixture group is not directly related to a lower intestinal inflammatory response as measured by f-IL-8 and f-calprotectin. However, the intestinal inflammatory response may still play a role in the susceptibility to (endogenous) infections. In chapter 5, enteral supplementation of the prebiotic mixture of neutral and acidic oligosaccharides did not enhance the decrease in intestinal permeability in the first week of life, as measured by the sugar absorption test. Breast milk feeding during the first week of life decreased intestinal permeability.
In chapter 6, we focused on the effect of enteral supplementation of the prebiotic mixture on the postnatal modulation of the immune response. In chapter 6A, we show that enteral supplementation of the prebiotic mixture did not influence cytokine responses in preterm infants. There were major interindividual differences in cytokine levels. In chapter 6B, we show that the transplacental transport of IgG is significantly lower in preterm infants than in term infants. In term infants, low percentages of protective antibody concentrations were found in cord blood posing these infants at risk for vaccine preventable diseases in the first months of life. However, preterm infants with an immature immune system have even lower protective antibody concentrations, derived from their mothers, which predisposes them to higher risk for vaccine-preventable diseases. Enteral supplementation of a prebiotic mixture of neutral and acidic oligosaccharides did not increase IgG response to Diphtheria, Tetanus, acelullar Pertussis and Haemophilus influenzae type b and Pneumococcus vaccinations 1 month after the 3th and 1 month after the booster vaccination. Immunoglobulin free light chain (IgLC) may be involved in the development of allergic diseases and oral tolerance. In chapter 7, we show that IgLC is transferred over the placenta but levels are much lower in both preterm infants and term infants than in their mothers. In chapter 8, we present the long-term outcome of enteral supplementation of the prebiotic mixture on allergic and infectious diseases and neurological outcome in the first year of life. We found no effect of enteral supplementation of the prebiotic mixture on allergic and infectious diseases in the first year of life. Furthermore, we found that serious neonatal infections in preterm infants are associated with adverse neurodevelopmental outcome, as measured with a score used by physiotherapists.