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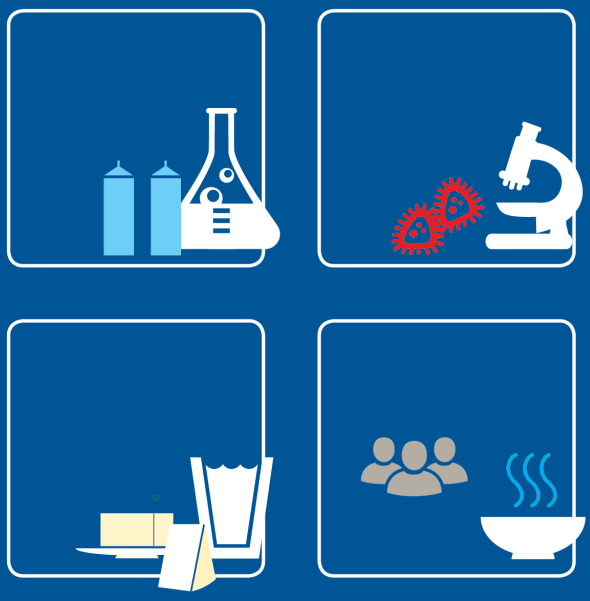
Katrine Lindholm Bøgh:

Mikroflora og komælkstolerance

Microbiota and cow's milk tolerance



Mejeribrugets
ForskningsFond

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Final report

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1. Title of the project

In Danish: Mikroflora og komælkstolerance

In English: Microbiota and cow's milk tolerance

2. Project manager

Head of Research Group, Senior Researcher PhD, Katrine Lindholm Bøgh, Research Group for Food Allergy, National Food Institute, Technical University of Denmark, Kemitorvet, Building 202, 2800 Kgs. Lyngby. Phone: +45 35 88 70 92, e-mail: kalb@food.dtu.dk

3. Other project staff

Professor Tine Rask Licht, PhD, National Food Institute, trli@food.dtu.dk

Senior scientist, PhD, Martin Iain Bahl, National Food Institute, mbah@food.dtu.dk

Professor, DMSci Susanne Halken, Hans Christian Andersen Children's Hospital, Odense University Hospital, Susanne.Halken@rsyd.dk

Food Scientist, Heidi Frahm Christoffersen, Arla Foods Ingredients, heidi.frahm.christoffersen@arlafoods.com

Pediatric Research Scientist, Lotte Neergaard Jacobsen, Arla Foods Ingredients, lotte.neergaard.jacobsen@arlafoods.com

PhD student, Katrine Bækby Graversen, National Food Institute, katgr@food.dtu.dk

4. Sources of funding

DTU Food, Milk Levy Fund and Arla Foods Ingredients

5. Project period

Project period with DDRF funding:	January/2016 - December/2018
Revised, if necessary:	January/2016 - October/2019

6. Project summary

In Danish:

FORMÅL OG METODER: Formålet med projektet var at tilføje viden om, hvordan forskellige proteiningredienser til modermælkserstatninger påvirker spædbørns mikrobiotasammensætning, samt at undersøge, hvordan mikrobiotasammensætningen påvirker de forebyggende effekt af forskellige proteiningredienser på komælksallergi. Dette blev undersøgt ved at give Brown Norway rotter valleprodukter med forskellige fysiokemiske egenskaber opnået ved hydrolyse eller varmebehandling. Sammenhængen mellem mikrobiotesammensætning og toleranceudvikling blev undersøgt ved at manipulere mikrobiota hos rotter med antibiotikumet amoxicillin. Desuden blev den direkte virkning af hydrolyserede valleprodukter på væksten af tarmbakterier, der stammer fra raske spædbarnsdonorer, evalueret i et *in vitro*-inkubationsforsøg.

RESULTATER: Først blev de tolerance- og allergifremkaldende egenskaber af valleprodukter, der var behandlet med forskellige grader af hydrolyse eller mild varmebehandling, karakteriseret. Dette viste, at et partielt hydrolyserede valleprodukt var mindre allergifremkalden end det intakte moderprodukt på trods af at evnen til at forebygge allergisk sensibilisering ikke var reduceret dvs. de tolerance-inducerende egenskaber var uændrede. Ligeledes reducerede mild varmebehandling de allergi-fremkaldende egenskaber, når valleprodukter blev injiceret ii bughulen, men havde ingen virkning på oral sensibilisering i den anvendte dyremodel. Dog resulterede oral provokation med varmebehandlet valle i mildere allergiske symptomer sammenlignet med umodificeret valle. Reduktionen af oral allergiske symptomer var sandsynligvis relateret til ændring af tarmoptaget af det varmebehandlede produkt. De tolerance-inducerende egenskaber blev ikke reduceret af mild varmebehandling.

Dernæst undersøgte vi samspillet mellem tarmmikrobiota og valleproteiningredienser samt den kombinerede effekt af disse på toleranceudvikling. For at undersøge direkte virkninger af valleingredienser på mikrobiotaen udviklede vi en *in vitro* inkubationsprotokol for at vurdere effekten på væksten af bakterier, der stammer fra afføringsprøver i et forenklet system. Inkubation af bakterier fra tre sunde spædbørns fæcesprøver med intakte og hydrolyserede valleprodukter afslørede, at den relative forekomst af *Enterococcus* var højere efter inkubationer med to partielt hydrolyserede valleproteinprodukter sammenlignet med det intakte produkt. For at undersøge virkningen af tarmmikrobiotaforstyrrelse på toleranceudvikling blev Brown Norway-rotter dagligt givet amoxicillin fra en uge før og under *ad libitum* indtag af de samme intakte og hydrolyserede valleprodukter. På trods af at administration af amoxicillin resulterede i øjeblikkelige og dramatiske ændringer i mikrobiotasammensætningen, så påvirkede det ikke udviklingen af tolerance. I én gruppe, der fik det højthydrolyserede valleprodukt, var de rotter med en amoxicillin-forstyrret mikrobiota faktisk bedre beskyttet mod allergiske reaktioner end dem med en konventionel mikrobiota.

KONKLUSION OG PERSPEKTIVER:

Samlet set fremhæver disse resultater både varmebehandling og moderat hydrolyse som potentielle metoder til fremstilling af effektive og sikre komælksbaserede produkter beregnet til at forebygge komælksallergi hos spædbørn i højrisiko for at udvikle komælksallergi, uanset deres mikrobiotasammensætning. Eventuelle effekter af hydrolyserede produkter på spædbørns mikrobiotasammensætning, som blev indikeret ved *in vitro* inkubationforsøge, bør dog undersøges nærmere.

In English:

AIM AND METHODS: The aim of the project was to add knowledge on how different protein ingredients for infant formulas influence the microbiota composition of infants and further to investigate how the microbiota composition influences the cow's milk allergy preventive effect of different protein ingredients. This was investigated by inducing tolerance in Brown Norway rat models through the administration of whey products with different physicochemical characteristics obtained by applying hydrolysis or heat treatment. The association between microbiota composition

and tolerance development was assessed by manipulating the microbiota of rats by the antibiotic amoxicillin. Finally, direct effect of hydrolysed whey products on the growth of gut bacteria derived from healthy infant donors was evaluated in an *in vitro* incubation study.

RESULTS: Initially, the tolerogenic and allergenic properties of whey products treated with varying degrees of hydrolysis or mild heat treatment were characterised. This showed that a partially hydrolysed whey product was less allergy inducing than the intact mother product despite the fact that the ability to prevent allergic sensitisation was not reduced i.e. the tolerance-inducing properties were unchanged. Likewise, mild heat treatment reduced the allergenic properties when whey products were injected in the abdominal cavity but had no effect on oral sensitisation in the animal model used. However, oral provocation with the heat-treated whey product resulted in milder allergic symptoms compared to the unmodified whey product. The reduction in oral allergic symptoms was probably related to changes in the intestinal uptake of the heat-treated product. The tolerance-inducing properties were not reduced by the mild heat treatment.

Next, we investigated the interplay between the gut microbiota and whey products, and further the combined effect of these on tolerance development. To investigate the direct effects of whey products on the microbiota, we developed an *in vitro* incubation protocol to assess the effect on the growth of bacteria derived from stool samples in a simplified system. Incubation of bacteria derived from three healthy infant faecal sample with intact and hydrolysed whey products, revealed that the relative abundance of *Enterococcus* was higher after incubations with two partially hydrolysed whey products compared to the intact parent-product. To investigate the effect of gut microbiota perturbation on tolerance development, Brown Norway rats were daily administered with amoxicillin from one week before and during *ad libitum* administration of the same intact and hydrolysed whey products. Administration of amoxicillin resulted in immediate and dramatic shifts in microbiota composition. However, this did not affect the development of tolerance. In one group administered with the extensively hydrolysed whey product, amoxicillin treated rats were actually better protected against allergic reactions than those with a conventional microbiota.

CONCLUSION AND PERSPECTIVES: Collectively, these results highlight both heat treatment and moderate hydrolysis as potential methods for producing efficient and safe cow's milk-based products intended to prevent cow's milk allergy in infants at high risk of cow's milk allergy (CMA), regardless of their gut microbiota composition. However, possible adverse effects of hydrolysed products on infant microbiota composition indicated by the *in vitro* incubation study warrants further investigation.

7. Project aim

Cow's milk allergy is a health problem of growing concern for which reason efficient strategies for the prevention of cow's milk allergy is urgently needed. In recent years, it has been shown that the composition of the gut microbiota influences the development of allergy as well as the induction of tolerance. However, our knowledge about how infant diet influences the microbiota constituent is basically undescribed, and how the microbiota composition influences the sensitising or tolerance inducing capacities of the food is only scarcely described.

In this project, we seeked to add knowledge on how different protein ingredients for use in infant formulas influence the microbiota composition of infants and further investigate how the microbiota composition influences the cow's milk allergy preventive effect of different protein ingredients and their hydrolysates. This may lead to new strategies for including microbial probiotics and/or their derivatives in future hypoallergenic infant formulas, as well as for improved personalised advise related to choice of hypoallergenic formulas.

8. Background for the project

The default immunological response to dietary antigen exposure via the gastrointestinal tract is oral tolerance, while failure to develop or breakdown of tolerance results in food allergy, but the mechanism leading to this outcome in some individuals is not fully understood. It has been suggested that failure to develop tolerance is linked to an imbalance in intestinal microbiota caused by environmental or lifestyle factors. Cow's milk allergy is the most common food allergy in infants and young children. The best way to prevent cow's milk allergy in infants is by exclusive breastfeeding until introducing solid foods. However, in many cases breast milk must be supplemented with or replaced by infant formula, which is most often based on cow's milk proteins. The recommendation for allergy prevention in non-exclusively breastfed infants at high risk of developing allergy is highly debated. Infant formula based on hydrolysed cow's milk have been suggested as a good option due to their reduced allergenicity, but concerns have been raised about the possible reduced preventive capacity of these products. Alternative types of processing, such as heat treatment, are being investigated with the aim of reducing allergenicity (the properties inducing allergy) while maintaining tolerogenicity (the properties inducing tolerance), with the ultimate goal of producing safe and efficient products for prevention and treatment of cow's milk allergy. To accomplish this, more knowledge on how tolerance development is influenced by both protein and host-related factors is essential.

9. Sub-activities in the entire project period

Sub-project 1

A: Protein-chemical characterisation of whey products.

B: Obtaining ethical approval for the Committee on Health Research Ethics (Videnskabsetisk komité).

C: Optimise, test and describe procedure for collection and storage of faecal samples for Odense University Hospital.

D: Collection of faecal samples from healthy infants.

E: Pilot fermentation study and media optimisation.

F: *In vitro* fermentation and microbiota profiling by quantitative PCR.

G: Paper describing the impact of different whey products, differing in the protein/peptide size distribution profiles, on the microbiota from healthy and food allergic individuals.

Sub-project 2

H: Establishing and optimising ELISA assays.

I: Establishing and optimising methods to evaluate intestinal uptake of food proteins.

J: Design of animal study selection of end points.

K: Pilot animal studies.

L: Oral tolerance animal study.

M: Evaluation of tolerance inducing capacity of whey products.

N: Microbiota profiling by 16S rRNA gene sequencing ingredients.

O: Mechanistic studies of the influence of microbiota on the tolerance inducing capacity of whey products.

P: Paper describing the impact of microbiota on the tolerance inducing capacity of different whey products and the mechanisms behind.

	2016				2017				2018				2019		
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
A	x				x	x									
B		x	x												
C			x	x											
D							x					x			
E												x	x		
F													x		
G													x	x	x
H	x														
I		x	x												
J					x	x									
K		x				x	x								
L								x	x						
M											x				
N											x	x			
O											x	x			
P												x	x	x	x

Maternity leave indicated by grey shading.

10. Project results

Sub-project 1 – Whey protein hydrolysis and the gut microbiota

In recent years, evidence of the microbiota being involved in the pathogenesis of food allergy have accumulated [1,2]. In that light, the optimal infant formula for CMA prevention should support the development of a healthy gut microbiota. Considering specifically the protein fraction, which was the focus of the current project, this implies that the protein ingredients should have no adverse effects on the gut microbiota. However, only very few studies have yet investigated whether hydrolysed cow's milk protein-based infant formula affect the gut microbiota composition [3,4].

To address this, we developed an *in vitro* incubation protocol to screen the effect of ingredients for infant formula on the growth of bacteria derived from faecal sample in a simplified system. The protocol was used to investigate the effect of different whey-based protein ingredients for infant formula on growth of bacteria in stool samples from three healthy infant donors.

Four whey products were included in the study: One intact whey product, two non-filtered, partially hydrolysed whey products, and one filtered, extensively hydrolysed whey product with degree of hydrolysis of 7.2%, 22.4% and 27%, respectively. The products were thoroughly characterised in terms of degree of hydrolysis, amino acid composition, peptide size distribution and protein aggregation status. Our study revealed that the relative abundance of *Enterococcus* was higher after incubations with two partially hydrolysed whey products with degree of hydrolysis of 7.2% and 22.4%, compared to the intact parent-product [5]. Although species of *Enterococcus* are common gut bacteria in early life, high abundance of these has been associated with allergic conditions [6,7]. Thus, this observation warrants further investigation. The result should be confirmed by larger *in vitro* studies and/or *in vivo* studies.

Sub-project 2 – Whey protein processing and allergenicity versus tolerogenicity

The allergenicity and tolerogenicity of cow's milk proteins can be modified through processing such as hydrolysis and heat treatment [8,9]. In Sub-project 2, the capacity of different whey products to prevent cow's milk allergy was investigated by *ad libitum* administration of whey products to milk-naïve BN rats, that are high-IgE responders resembling atopy-prone (atopy refers to the genetic predisposition to develop allergic diseases) individuals in their predisposition to develop food allergy [10,11]. The preventive capacity was evaluated as the capacity to prevent whey-specific sensitisation and allergic reactions to intact whey after post-immunisations with intact whey.

In one animal study, the allergy preventive capacity of the same intact and hydrolysed whey products as described in Sub-project 1, were tested. The destruction of epitopes by hydrolysis is largely considered as a trade-off between allergenicity and tolerogenicity, which means: the higher degree of hydrolysis, the lower the allergenicity, but also the lower tolerogenicity. Interestingly, our results in contrast showed that although the sensitising capacity of partially hydrolysed whey products was reduced compared to the intact parent-product, the capacity to prevent whey-specific IgE sensitisation was not compromised [5]. To our knowledge, this study is the first to show that a partially hydrolysed whey product is superior to intact whey for preventing allergic sensitisation.

Heat treatment is an alternative processing method that can be applied to change the structure of proteins and thereby the allergenicity/tolerogenicity. However, heat treatment may not only affect the epitopes [12–14], but equally important may affect digestibility [15–17] and intestinal uptake [18]. In another animal study, we compared allergenic and tolerogenic properties of whey products subjected to mild heat treatment, which resulted in partial denaturation and aggregation of the proteins, to the same untreated product. Our results revealed that the capacity of whey to prevent sensitisation of naïve rats and to desensitise already sensitised rats was not reduced by the mild heat treatment [19]. Heat treatment reduced the intraperitoneal sensitising capacity but had no effect on oral sensitisation. However, oral provocation with heat-treated whey resulted in milder allergic symptoms compared to unmodified whey. Protein uptake studies showed that heat treatment changed the uptake route of whey with less being absorbed

through the epithelium but more into the Peyer's patches. This is in line with a few existing publications on this topic [18] and may likely explain why the oral eliciting capacity was reduced by heat treatment.

Sub-project 2 – Whey protein hydrolysis, the gut microbiota and allergy prevention

To investigate the effect of the gut microbiota composition on allergy prevention, the gut microbiota of BN rats was manipulated by daily oral administration of the antibiotic amoxicillin before and during *ad libitum* administration of whey products in BN rats. Amoxicillin is the most widely used β-lactam antibiotic in Europe [20], and is frequently prescribed for treatment of paediatric infections [21].

Initially, the effect of daily intra-gastric administration of amoxicillin on gut microbiota composition and immune regulation was characterised [22]. We found that amoxicillin administration resulted in immediate and dramatic shifts in microbiota composition, characterised by reduced diversity and increased relative abundance of Bacteroidetes and Gammaproteobacteria, with concurrent reduction of Firmicutes, compared to the water-administered control group. After one week, the total fecal IgA level, relative abundance of small intestinal regulatory T cells and goblet cell numbers were higher in the amoxicillin group compared to controls.

Next, we investigated the effect amoxicillin-induced perturbation of the gut microbiota one week prior to and during tolerance induction by *ad libitum* administration of intact and hydrolysed whey products to cow's milk naïve BN rats [5]. We found that despite amoxicillin dramatically affected microbiota composition, this did not affect the development of tolerance. In the group administered with the extensively hydrolysed whey product (the product with the weakest tolerance-inducing capacity), amoxicillin treated rats were actually better protected against allergic reactions than those with a conventional microbiota. In the light of epidemiological studies showing an association between early life antibiotic consumption and the development of cow's milk allergy [23–25], the observation that amoxicillin-induced perturbation of the gut microbiota promotes acute immune regulation and possibly tolerance development warrants further investigation.

Conclusion and perspectives

Collectively, these results highlight both heat treatment and moderate hydrolysis as potential methods for producing efficient products for CMA prevention (and desensitisation), as a safer alternative to conventional formula. Additionally, studies suggest that the presence of protein aggregates, which can be obtained by heat treatment but also by other processing methods, affects intestinal uptake of proteins, and thereby reduces their eliciting capacity. Knowledge on how protein structures (such as aggregates) affect intestinal uptake and thereby allergenicity may be exploited in the design of ingredients for infant formula intended for CMA prevention and desensitisation by specifically targeting uptake via Peyer's patches and preventing uptake via epithelial cells.

Despite the gut microbiota becoming increasingly appreciated in food allergy research, there is a huge knowledge gap in the understanding of how hypoallergenic infant formula affects the infant gut microbiota. Results from an *in vitro* incubation study revealed that moderately hydrolysed whey products promote the growth of *Enterococcus*, which may be associated with an increased risk of allergic diseases. Thus, this finding warrants further investigation to ensure that infant formula targeting CMA prevention does not negatively influence the gut microbiota.

Finally, results of the project suggest that amoxicillin-induced perturbation of the gut microbiota does not impair tolerance development. Actually, amoxicillin administration was found to promote acute immune regulation and tolerance development by extensively hydrolysed whey (the product with the poorest primary preventive capacity). In order to design infant formula targeting CMA prevention by supporting the development of a healthy gut microbiota, more studies are needed to elucidate which bacteria-derived signals promote tolerance development.

11. Deviations

Scientific

From recent literature and in-house results, we learned that infant age and introduction of solid food both affect the intestinal microbiota composition significantly. Given the small number of potential participants that meet our inclusion criteria, the project's logistics and time frame we were not able to collect the required number of faecal samples from allergic infants at the exact same age. We believed that the expected variation in the age of participants would have affected the gut microbiota much more that a putative difference between allergic and healthy infants. Therefore, we decided, after agreement with the Milk Levy Fund and Danish Dairy Research Foundation, to terminate the collection of faecal samples from allergic individuals.

In vitro fermentations to evaluate how different milk protein ingredients, incl. hydrolysates, influenced the gut microbiota were thus conducted by bacteria derived from healthy infants only. These studies were supplemented with studies of the effects of the protein ingredients on growth of selected common infant gut bacteria with anticipated health effects. The influence of milk protein hydrolysates on growth of these bacteria were analysed by specifically targeted quantitative PCR after *in vitro* fermentations with bacterial isolates as well as complex bacterial communities from infant stool samples.

Financial

No derivations.

Timetable

The project was paused and therefore extended for 10 months due to Katrine Graversen being on maternity leave.

12. The relevance of the results, including relevance for the dairy industry

The results of the project have highlighted several points that, over time, might benefit the dairy industry to improve (hypoallergenic) infant formulas and thereby improving quality of life for food allergic individuals and their families and ultimately benefit society. However, more research is needed.

- All infant formulas, and especially those targeting allergy prevention and/or management, should support the development of healthy gut microbiota. This project has identified possible adverse effect of partially hydrolysed whey products on the gut microbiota composition, which warrants further investigation. If these effects are confirmed in future studies, it might be relevant to add other ingredients, such as for example synthetic human milk oligosaccharides, other prebiotic carbohydrates and/or live probiotic bacteria to specifically counterbalance possible adverse effects.

- This project has provided more knowledge on how protein structures (aggregates) affect intestinal uptake and thereby allergenicity. This may be exploited in the design of ingredients for infant formula intended for CMA prevention and desensitisation by specifically targeting uptake via Peyer's patches and preventing uptake via epithelial cells. In the current study, protein aggregation was induced by partial hydrolysis and mild heat treatment, but also other processes may possibly be applied to achieve these characteristics.

- Results from this project indicate that the same whey protein ingredient was superior in rats with amoxicillin-disturbed and conventional microbiota composition. Thus, this result could indicate that infants treated with amoxicillin, which is commonly used to treat paediatric infections, does not benefit from any special infant formula with regard to the protein ingredient.

13. Communication and knowledge sharing about the project

Papers in international journals:

Published:

Graversen KB, Ballegaard AR, Kraemer LH, Hornslet SE, Sørensen LV, Christoffersen HF, Jacobsen LN, Untersmayr E, Smit JJ, Bøgh KL. "Cow's milk allergy prevention and treatment by heat-treated whey-A study in Brown Norway rats" Clin Exp Allergy. 2020 Jun;50(6):708-721. doi: 10.1111/cea.13587.

Graversen KB, Bahl MI, Larsen JM, Ballegaard AR, Licht TR, Bøgh KL "Short-Term Amoxicillin-Induced Perturbation of the Gut Microbiota Promotes Acute Intestinal Immune Regulation in Brown Norway Rats" Front Microbiol. 2020 Mar 26;11:496. doi: 10.3389/fmicb.2020.00496.

Michalovich D, Rodriguez-Perez N, Smolinska S, Pirozynski M, Mayhew D, Uddin S, Van Horn S, Sokolowska M, Altunbulakli C, Eljaszewicz A, Pugin B, Barcik W, Kurnik-Lucka M, Saunders KA, Simpson KD, Schmid-Grendelmeier P, Ferstl R, Frei R, Sievi N, Kohler M, Gajdanowicz P, Graversen KB, Lindholm Bøgh K, Jutel M, Brown JR, Akdis CA, Hessel EM, O'Mahony L "Obesity and disease severity magnify disturbed microbiome-immune interactions in asthma patients" Nat Commun. 2019 Dec 13;10(1):5711. doi: 10.1038/s41467-019-13751-9.

In preparation/submitted:

Graversen KB, Larsen JM, Pedersen SS, Sørensen LV, Christoffersen HF, Jacobsen LN, Halken S, Licht TR, Bahl MI, Bøgh KL "Partially hydrolysed whey has superior allergy preventive capacity compared to intact whey regardless of amoxicillin administration in Brown Norway rats".

Locke AV, Larsen JM, Graversen KB, Licht TR, Bahl MI, Bøgh KL "The development of cow's milk allergy is not affected by concomitant antibiotic treatment in Brown Norway rats".

Easily read papers:

Graversen, K B and Bøgh, K L, "Forebyggelse af komælksallergi med modificeret valleprotein", Mælkeritidende, 2020, Issue 5, p. 10-11.

Bøgh K L "Tarmens bakterier og fødevareallergi", Miljø og Sundhed, 2017, Volume 23, Issue Suppl. 1, p. 17-21.

Graversen, K B and Bøgh, K L "Tarmens mikroflora og spædbørns komælkstolerance skal undersøges", Mælkeritidende, 2016, Issue 25-26, p. 6-7.

Student theses:

Signe Schultz Pedersen, The influence of the gut microbiota on induction of tolerance to a hydrolysed whey protein product for hypoallergenic infant formulas, master thesis, 2019.

Arielle Vallee Locke, The influence of the gut microbiota composition on the sensitising capacity of cow's milk proteins, master thesis, 2019

Gesika Jørgensen, The influence of microbiota composition on the tolerance inducing capacity of conventional, partially and extensively hydrolysed infant formulas, master thesis, 2019

Lise Lotte Eriksen, The influence of antibiotic treatment on the tolerance inducing capacity of three different infant formulas, bachelor thesis, 2018

Louise Kristensen, Effect of amoxicillin on the rat microbiota - Impact on allergy risk, bachelor thesis, 2018

Line Quist Stauersbøl, Longitudinal effect of amoxicillin on fecal microbiota af rats, bachelor thesis, 2018

Julie Wolpers Reholt and Chantida Asukowit, Comparison of the allergenic versus tolerogenic capacity of heat treated and untreated whey protein based ingredients, bachelor thesis, 2017

Oral presentations at scientific conferences, symposiums etc.:

Oral presentation at Paediatric Allergy and Anaphylaxis Meeting (PAAM) 2019, Florence, Italy.

Poster discussion presentation at Food Allergy and Anaphylaxis Meeting (FAAM) 2018, Copenhagen, Denmark.

Poster discussion presentation at the European Academy of Allergy and Clinical Immunology (EAACI) Congress 2017, Helsinki, Finland.

Oral presentation at Danish Society for Allergology Yearly Meeting, 201, Vejle, Denmark

Oral presentation at Danish Society for Allergology Yearly Meeting, 2017, Vejle, Denmark.

Oral presentations at the EU COST Action FA1402 ImpARAS Congress 2017, Helsingør, Denmark.

Oral presentations at the EU COST Action FA1402 ImpARAS Congress 2016, Warsaw, Poland.

Oral presentations at meetings:

Oral presentation at several meetings under the auspices of Danish Dairy Research Foundation.

Other:

Graversen K B "Mikrobiota and Cow's Milk Tolerance", PhD thesis, March 2020

14. Contribution to master and PhD education

PhD education of Katrine Bækby Graversen including external research stays at:
- Institute for Risk Assessment Sciences (IRAS), Utrecht University, The Netherlands, 17th Apr – 27th May 2016.
- The Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland, Nov 3rd 2016 – 28th Feb 2017.

Three Master students made their final project (Master's Thesis) as part of this project (see above).

15. New contacts/projects

The results obtained and especially the methods developed within this project, have been widely used in several other project, both related to dairy products (e.g. the IFD Grant Solution project ALLEVIATE) as well as related to other products/foods.

16. Signature and date

The project is formally finalised when the project manager and DDRF-representative (e.g. steering committee leader) have signed this final report.

Date: ___28.02.21____Signature, Project manager: _____

Date: 2 March 2021 Signature, DDRF-representative: 

References

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